TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2015) 45: 913-918
© TÜBİTAK
doi:10.3906/sag-1408-51

VKORC1 and *CYP2C9* genotypic data-based dose prediction alone does not accurately predict warfarin dose requirements in some Malaysian patients

Yung An CHUA[1,*], Wan Zaidah ABDULLAH[2], Zurkurnai YUSOF[3], Siew Hua GAN[4]

[1]Department of Pharmacology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia
[2]Department of Hematology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia
[3]Invasive Cardiology Laboratory, Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia
[4]Human Genome Center, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia

Received: 13.08.2014 • **Accepted/Published Online:** 11.11.2014 • **Printed:** 30.07.2015

Background/aim: *VKORC1* and *CYP2C9* genetic polymorphisms may not accurately predict warfarin dose requirements. We evaluated an existing warfarin dosing algorithm developed for Malaysian patients that was based only on *VKORC1* and *CYP2C9* genes.

Materials and methods: Five Malay patients receiving warfarin maintenance therapy were investigated for their *CYP2C9*2*, *CYP2C9*3*, and *VKORC1*-1639G>A genotypes and their vitamin K-dependent (VKD) clotting factor activities. The records of their daily warfarin doses and international normalized ratio (INR) 2 years prior to and after the measurement of VKD clotting factors activities were acquired. The mean warfarin doses were compared with predicted warfarin doses calculated from a genotypic-based dosing model developed for Asians.

Results: A patient with the *VKORC1*-1639 GA genotype, who was supposed to have higher dose requirements, had a lower mean warfarin dose similar to those having the *VKORC1*-1639 AA genotype. This discrepancy may be due to the coadministration of celecoxib, which has the potential to decrease warfarin's metabolism. Not all patients' predicted mean warfarin doses based on a previously developed dosing algorithm for Asians were similar to the actual mean warfarin dose, with the worst predicted dose being 54.34% higher than the required warfarin dose.

Conclusion: Multiple clinical factors can significantly change the actual required dose from the predicted dose from time to time. The additions of other dynamic variables, especially INR, VKD clotting factors, and concomitant drug use, into the dosing model are important in order to improve its accuracy.

Key words: Warfarin, dosing algorithm, *CYP2C9*, *VKORC1*, PCR-RFLP, clotting factor activities

1. Introduction

Warfarin is one of the most important drugs in personalized medicine. Many clinical conditions may affect the potency of warfarin over time, thus requiring patients to regularly visit a healthcare center for dose adjustment. Traditionally, warfarin dosage is determined by the international normalized ratio (INR) values, which are derived from prothrombin time (PT) (1). The difficulty of achieving the ideal warfarin dose is compounded by genetic variations that may affect the pharmacokinetics and pharmacodynamics of warfarin in different individuals.

Research has shown that cytochrome P450 2C9 (*CYP2C9*) and vitamin K epoxide reductase complex 1 (*VKORC1*) gene polymorphisms are significantly associated with warfarin sensitivity (2–5), including among Asian patients. Several dosing guidelines and algorithms based on genetic data have been proposed in response to reports that the addition of genetic data into a nongenetic dosing model may increase the predictability of the model by up to 16% (6–9). The strong association of these genes with warfarin sensitivity compelled the US Food and Drug Administration to recommend conducting genetic tests before determining the most suitable warfarin dose range (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2007/ucm108967.htm.) Although a few studies have established strong associations between genotypic data and warfarin dose requirements, the comprehensive use of these genotypic data in clinical practice is generally hindered by the lack of evidence of the overall efficacy of this approach (10), which suggests that there are other factors that play a role in warfarin dose variation. This situation is compounded by high

* Correspondence: yungan.chua@gmail.com

genetic variability across the world. Although Asians and Caucasians have different genetic backgrounds, many drug doses for Asians are still based on data from studies of Caucasian populations. Furthermore, the inclusion of additional laboratory tests, such as genetic screening, in addition to conventional INR is generally not preferred due to the extra cost involved (11).

In this study, we evaluated an existing warfarin dosing algorithm developed for Malaysian patients that was based only on the *VKORC1* and *CYP2C9* genes.

2. Materials and methods

Ethical approval was obtained from the Universiti Sains Malaysia Research Ethics Committee (USMKK/PPP/JEPeM [197.3{6}]). Written informed consent was acquired from all patients before the study. Blood samples (3 mL) were obtained from 5 Malay patients, between 44 and 81 years old, who had received warfarin treatment. DNA was extracted from the blood according to the DNA extraction kit manufacturer's standard guidelines (QIAGEN, Hilden, Germany). Each patient was tested for INR and the activity of the vitamin K-dependent (VKD) clotting factors II, VII, IX, and X using an STA Compact Benchtop Hemostasis Analyzer (Diagnostica Stago, Parsippany, NJ, USA). The *CYP2C9*2*, *CYP2C9*3*, and *VKORC1*-1639G>A genotypes were determined by using previously reported polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods (12,13).

The demographic data, medical records, mean daily warfarin doses, and INR values 2 years before and after the measurement of VKD clotting factor activities were acquired from both the patient's folder and the hospital database. The mean warfarin doses were considered to be the doses required by the patients to maintain their respective stable INR ranges (2.5–3.5 for atrial valve replacement or between 2 and 3 for other indications). The mean dose was then compared to the predicted daily warfarin dose calculated using an Asian population-based warfarin dosing model (7). In the previously developed dosing model for Asian patients (n = 108), age, weight, and *CYP2C9*3* and *VKORC1* 381 genotypes were processed by the algorithm and yielded a correlation coefficient of 0.73. In our study, we replaced the *VKORC1* 381 genotype with the *VKORC1*-1639G>A genotype because these genotypes have been confirmed to be closely linked with each other and therefore interchangeable (5). The percentage difference between the actual and predicted daily warfarin doses was then recorded.

3. Results

The induction of warfarin therapy and the concomitant drugs prescribed during a single hospital visit before the measurement of the current INR and the VKD clotting factor activities are shown in Table 1. Genotyping results for the *CYP2C9* and *VKORC1* polymorphisms are presented in gel electrophoresis images (Figures 1–3). In agreement with several findings that support *VKORC1* as having a stronger association with warfarin sensitivity than any other genetic or clinical data (4,14–17), all patients who had the *VKORC1*-1639 AA genotype had a lower warfarin dose requirement compared to patients with the GA genotype, with the exception of Patient C (Table 2). However, further investigation of the medication history of Patient C confirmed that he was also taking celecoxib, a nonsteroidal antiinflammatory drug that is also metabolized by *CYP2C9* and thus may interact with warfarin to cause an increase in warfarin sensitivity (18).

The purported "warfarin-sensitive" *CYP2C9*2* genotype was not proven to be the cause of warfarin sensitivity because Patient E, who had a "warfarin-sensitive" *CYP2C9*2* T allele, actually had the highest mean warfarin dose compared to other patients. Patient E also had the *VKORC1*-1639 G allele, which represents a "warfarin-resistant" allele. These differences further

Table 1. Related diseases and concomitant drugs prescribed to the patients during 1 hospital visit before the assessment of the INR and activities of the VKD clotting factors.

	Indication of warfarin prescription	Concomitant drugs
Patient A	AVR	Captopril, propranolol
Patient B	CCF, IHD	Aspirin, bisoprolol, digoxin, enalapril, pravastatin, ranitidine, trimetazidine
Patient C	AF, CCF, IHD	Aspirin, atorvastatin, celecoxib, digoxin, enalapril, furosemide
Patient D	AF, CCF, IHD	Aspirin, digoxin, furosemide, potassium chloride
Patient E	AF, CRHD, history of TIA	Digoxin, furosemide, potassium chloride, propranolol

AVR, Atrial valve replacement; CCF, congestive cardiac failure; IHD, ischemic heart disease; AF, atrial fibrillation; CRHD, chronic rheumatic heart disease; TIA, transient ischemic attack.

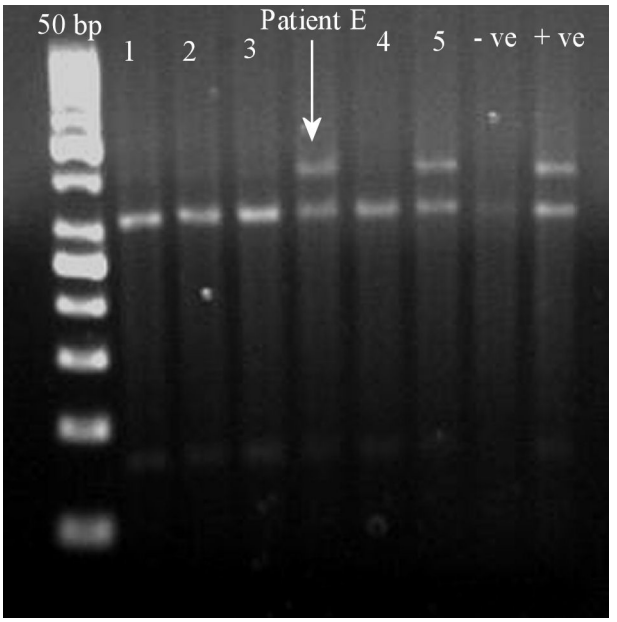

Figure 1. Representative gel electrophoresis image of PCR-RFLP analysis for *CYP2C9*2* for Patient E. The first lane contains a 50-bp DNA ladder, while lanes -ve and +ve are the negative and positive controls, respectively. The rest of the numbered lanes are the genotype results for patients not discussed in this study. The mutant allele (T) was resistant to restriction enzyme digestion; therefore, it is represented by the 375-bp fragment. The wild-type allele (C) was digested into 2 fragments of 76 and 299 bp. The presence of all 3 bands in a single lane indicates that the patient is heterozygous.

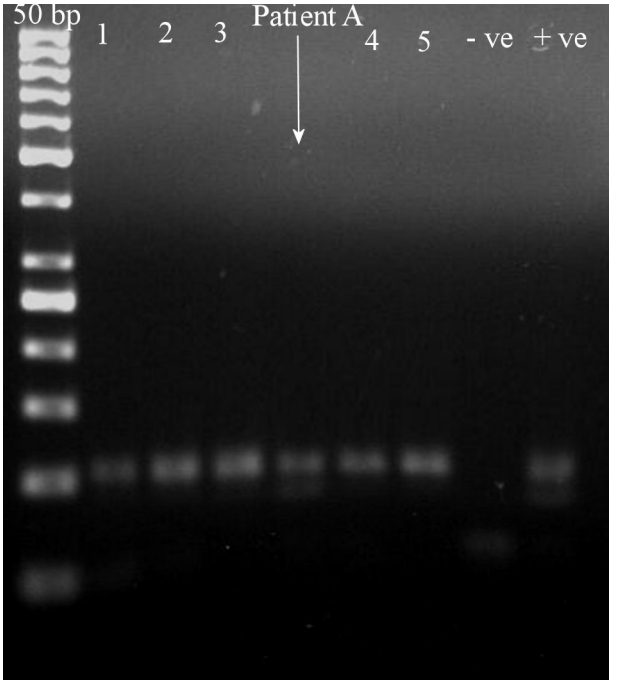

Figure 2 Representative gel electrophoresis image of PCR-RFLP analysis for *CYP2C9*3* for Patient A. The first lane contains a 50-bp DNA marker ladder, while -ve and +ve are the negative and positive controls, respectively. The rest of the numbered lanes are the genotype results for patients who are not discussed in this study. The wild-type allele (A) was resistant to restriction enzyme digestion; therefore, it is represented as a 105-bp fragment. The mutant allele (C) was digested into 2 fragments of 85 and 20 bp (the 20-bp band is not clearly visible in the gel electrophoresis image). The presence of 105- and 85-bp bands in 1 lane was sufficient to indicate that the patient is heterozygous.

confirm the fact that the *VKORC1* genotype is a stronger determinant of warfarin sensitivity among Asians than the *CYP2C9*2* genotype because the presence of the "warfarin-resistant" *VKORC1* allele can mitigate the effect of the *CYP2C9*2* alleles. These results also explain why the incorporation of the *CYP2C9*2* genotype into a dosing prediction model was not favored in a univariate analysis of warfarin dosing models developed for Asian patients as proposed by Tham et al. (7).

The opposite result was observed for the *CYP2C9*3* genotype. Patient A, who had the warfarin-sensitive *CYP2C9*3* C allele, received the lowest warfarin dose of all 5 patients. The low warfarin dose may be a result of the combined effects of the warfarin-sensitive genotypes of the *CYP2C9*3* AC and *VKORC1*-1639 AA alleles. All of the clotting factor activities in Patient A were also lower than in other patients. With the exception of Patient C, all patients with the *VKORC1*-1639 GA genotype had higher activities of clotting factors II, IX, and X when compared to patients who had the *VKORC1*-1639 AA genotype.

There were significant variations in the dose differences between actual and predicted mean daily doses among the 5 investigated patients. Patient D had the largest dose difference, with a predicted dose deviation of 54.24% from the actual mean dose. Apparently, Patient D would be overdosed if the warfarin dose was based on the recommended genetic-based dosing algorithm. Therefore, a dosing algorithm that is based solely on genetic data is inaccurate due to other confounding factors, such as environmental and clinical conditions. For example, drug–drug interactions, intake of supplements, intake of vitamin K, health status, smoking status, and compliance to warfarin may confound dosing predictions (19–22). Unlike genetic data, these factors are in a state of constant change and therefore should be incorporated in the dosing model. Therefore, genetic data alone can only serve as a guide, especially during the initiation of warfarin therapy, because patient response to this anticoagulant is dynamic and dependent on multiple factors.

4. Discussion

The dosing algorithm could not be applied to all patients. For example, administration of drugs that may interact

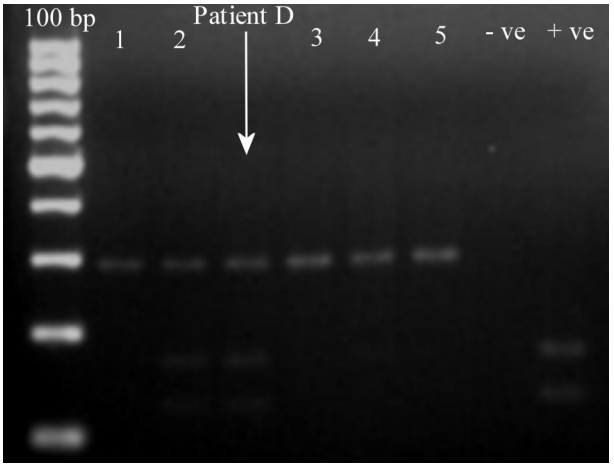

Figure 3. Representative gel electrophoresis image of PCR-RFLP analysis for *VKORC1*-1639G>A for Patient D. The first lane contains a 100-bp DNA marker ladder, while -ve and +ve are the negative and positive controls, respectively. The rest of the numbered lanes are the genotype results for patients who are not discussed in this study. The mutant allele (A) was resistant to restriction enzyme digestion; therefore, it is represented as a 290-bp fragment. The wild-type allele (G) was digested into 2 fragments of 168- and 122-bp. The presence of all 3 bands in a single lane indicates that the patient is heterozygous.

with warfarin's pharmacokinetics may render purported strong predictors of warfarin such as *VKORC1*-1639 GA less useful. Therefore, a clinical trial on a larger population is needed in order to validate the findings and to compare the algorithm with a conventional dosing method (23).

Because genetic tests are not routinely practiced by the local medical clinics in Malaysia, the measurement of VKD clotting factor activities can serve as a useful and relatively easy method to predict warfarin sensitivity in patients. Additionally, hemostatic tests can be performed simultaneously with other routine applications, such as INR tests. Each individual VKD clotting factor has a distinct diagnostic value. Factor VII is particularly sensitive to warfarin administration and may serve as an indicator for bleeding risk in addition to, or instead of, INR (24). Because the activity of factor IX is not detected by a PT test, it is determined independently of the INR result and can thus be used as a secondary measure for the determination of warfarin dosing, especially for clinical situations in which recommendations based solely on INR data are not effective (25).

A dynamic parameter such as INR must also be integrated into warfarin dosing models so that the dose can be adjusted for any changes in a patient's medical condition. Some studies also considered the use of drugs that may interact with warfarin, such as amiodarone, in the warfarin dosing model (6,9,26). Incorporation of concomitant drugs into the dosing algorithm is also advantageous for improved prediction accuracy, as evidenced by the effects of celecoxib on Patients C's warfarin dose requirement.

Although there are no studies that correlate *VKORC1* polymorphisms with VKD clotting factor activities, several studies have indicated that *VKORC1* polymorphisms may cause combined VKD clotting factors deficiency when plasma levels of VKD clotting factors are congenitally

Table 2. Comparison of the *CYP2C9*2*, *CYP2C9*3*, and *VKORC1*-1639 GA genotypes and the activity of VKD clotting factors.

	Patient A	Patient B	Patient C	Patient D	Patient E
*CYP2C9*2*	CC	CC	CC	CC	CT
*CYP2C9*3*	AC	AA	AA	AA	AA
VKORC1-1639	AA	AA	GA	GA	GA
Factor II (%)	9	16	16	30	32
Factor VII (%)	22	32	37	26	36
Factor IX (%)	20	48	50	59	51
Factor X (%)	9	13	11	18	23
Actual mean dose (mg daily)	2.00	2.30	2.02	3.30	3.48
Predicted dose based on algorithm (mg daily)	2.15	2.20	2.72	5.09	4.74
Dose difference (%)	7.50	−4.35	34.65	54.24	36.21

The *CYP2C9*2* C, *CYP2C9*3* A, and *VKORC1*-1639 G alleles are alleles with higher warfarin dose requirements, and their respective variants have lower dose requirement. A negative dose difference percentage indicates that the predicted warfarin dose is lower than the actual mean warfarin dose in the patient.

low (27–29). Future investigation of the role of *VKORC1* polymorphisms on the activity of VKD clotting factors may provide a better understanding of the association of individual VKD clotting factor activities with *VKORC1* polymorphisms.

Stratification of patients based on their genotypes at specific polymorphic loci may not be the only important factor when adjusting warfarin doses, despite the fact that genotypic data can help predict how a patient may respond. Clinical conditions such as INR and aberrant prothrombin time, decreased VKD clotting factor activities, and concomitant drug use can affect a patient's sensitivity to warfarin and have the potential to be integrated into the genetic-based dosing algorithm to better improve dosing predictions for Malaysian patients.

It is concluded that the current available warfarin dosing algorithms (6,7,9,30) are useful only if they are validated in the population for which they are intended to be used. Nevertheless, due to the limited sample size in this study, the risk for overdose found does not reduce the potential of the algorithm as a suitable predictor for warfarin dose; rather, its effectiveness can be diminished when some important clinical parameters are overlooked. In the future, a population-based study with a larger sample size should be conducted to successfully evaluate the effectiveness of the algorithm.

Acknowledgments

This study was funded by a Universiti Sains Malaysia Short Term Grant (304/PPSP/61312019), which had no influence on the outcomes of the study. The authors would like to thank Madam Che Rosnani binti Awang at the Specialised Medicine Clinic, Hospital Universiti Sains Malaysia, for her help with the sample collection.

References

1. Hirsh J, Fuster V, Ansell J, Halperin JL. American Heart Association/American College of Cardiology Foundation guide to warfarin therapy. Circulation 2003; 107: 1692–1711.
2. Hermans JJ, Thijssen HH. Human liver microsomal metabolism of the enantiomers of warfarin and acenocoumarol: P450 isozyme diversity determines the differences in their pharmacokinetics. Brit J Parmacol 1993; 110: 482–490.
3. Higashi MK, Veenstra DL, Kondo LM, Wittkowsky AK, Srinouanprachanh SL, Farin FM, Rettie AE. Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. JAMA 2002; 287: 1690–1698.
4. D'Andrea G, D'Ambrosio RL, Di Perna P, Chetta M, Santacroce R, Brancaccio V, Grandone E, Margaglione M. A polymorphism in the VKORC1 gene is associated with an interindividual variability in the dose-anticoagulant effect of warfarin. Blood 2005; 105: 645–649.
5. 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: 2285–2293.
6. Gage BF, Eby C, Milligan PE, Banet GA, Duncan JR, McLeod HL. Use of pharmacogenetics and clinical factors to predict the maintenance dose of warfarin. Thromb Haemost 2004; 91: 87–94.
7. 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: 346–355.
8. Caldwell MD, Berg RL, Zhang KQ, Glurich I, Schmelzer JR, Yale SH, Vidaillet HJ, Burmester JK. Evaluation of genetic factors for warfarin dose prediction. Clin Med Res 2007; 5: 8–16.
9. Lenzini P, Wadelius M, Kimmel S, Anderson JL, Jorgensen AL, Pirmohamed M, Caldwell MD, Limdi N, Burmester JK, Dowd MB et al. Integration of genetic, clinical, and INR data to refine warfarin dosing. Clin Pharmacol Ther 2010; 87: 572–578.
10. Limdi NA, Veenstra DL. Expectations, validity, and reality in pharmacogenetics. J Clin Epidemiol 2009; 63: 960–969.
11. Campos-Outcalt D. Personalized medicine: the promise, the reality. J Fam Practice 2007; 56: 621–626.
12. Ku CS, Gan GG, Vijaya Sangkar J, Phipps ME. Frequency of cytochrome P450 2C9 (CYP2C9) alleles in three ethnic groups in Malaysia. Asia-Pac J Mol Biol 2003; 11: 83–91.
13. Sconce EA, Khan TI, Wynne HA, Avery P, Monkhouse L, King BP, Wood P, Kesteven P, Daly AK, Kamali F. The impact of CYP2C9 and VKORC1 genetic polymorphism and patient characteristics upon warfarin dose requirements: proposal for a new dosing regimen. Blood 2005; 106: 2329–2333.
14. Veenstra DL, You JH, Rieder MJ, Farin FM, Wilkerson HW, Blough DK, Cheng G, Rettie AE. Association of vitamin K epoxide reductase complex 1 (VKORC1) variants with warfarin dose in a Hong Kong Chinese patient population. Pharmacogenet Genomics 2005; 15: 687–691.
15. Lee SC, Ng SS, Oldenburg J, Chong PY, Rost S, Guo JY, Yap HL, Rankin SC, Khor HB, Yeo TC et al. Interethnic variability of warfarin maintenance requirement is explained by VKORC1 genotype in an Asian population. Clin Pharmacol Ther 2006; 79: 197–205.
16. 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.
17. Yin T, Miyata T. Warfarin dose and the pharmacogenomics of CYP2C9 and VKORC1 - rationale and perspectives. Thromb Res 2007; 120: 1–10.

18. Mersfelder TL, Stewart LR. Warfarin and celecoxib interaction. Ann Pharmacother 2000; 34: 325–327.

19. Holbrook AM, Pereira JA, Labiris R, McDonald H, Douketis JD, Crowther M, Wells PS. Systematic overview of warfarin and its drug and food interactions. Arch Intern Med 2005; 165: 1095–1106.

20. Lin PJ. Reviewing the reality: why we need to change. Eur Heart J Suppl 2005; 7: E15–E20.

21. Nutescu EA, Shapiro NL, Ibrahim S, West P. Warfarin and its interactions with foods, herbs and other dietary supplements. Expert Opin Drug Saf 2006; 5: 433–451.

22. Ansell J, Hirsh J, Hylek E, Jacobson A, Crowther M, Palareti G. Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest 2008; 133: 160S–198S.

23. Kimmel SE, French B, Kasner SE, Johnson JA, Anderson JL, Gage BF, Rosenberg YD, Eby CS, Madigan RA, McBane RB et al. A pharmacogenetic versus a clinical algorithm for warfarin dosing. N Engl J Med 2013; 369: 2283–2293.

24. Benzon HT, Avram MJ, Benzon HA, Kirby-Nolan M, Nader A. Factor VII levels and international normalized ratios in the early phase of warfarin therapy. Anesthesiology 2010; 112: 298–304.

25. Scialla S, Rubin R, Liptock KM. Therapeutic management of a patient with a unique factor IX sensitivity to warfarin. Clin Adv Hematol Oncol 2006; 4: 73–75.

26. International Warfarin Pharmacogenetics Consortium, Klein TE, Altman RB, Eriksson N, Gage BF, Kimmel SE, Lee MT, Limdi NA, Page D, Roden DM et al. Estimation of the warfarin dose with clinical and pharmacogenetic data. N Engl J Med 2009; 360: 753–764.

27. Rost S, Fregin A, Ivaskevicius V, Conzelmann E, Hortnagel K, Pelz HJ, Lappegard K, Seifried E, Scharrer I, Tuddenham EG et al. Mutations in *VKORC1* cause warfarin resistance and multiple coagulation factor deficiency type 2. Nature 2004; 427: 537–541.

28. Weston BW, Monahan PE. Familial deficiency of vitamin K-dependent clotting factors. Haemophilia 2008; 14: 1209–1213.

29. Napolitano M, Mariani G, Lapecorella M. Hereditary combined deficiency of the vitamin K-dependent clotting factors. Orphanet J Rare Dis 2010; 5: 21.

30. Pavani A, Naushad SM, Rupasree Y, Kumar TR, Malempati AR, Pinjala RK, Mishra RC, Kutala VK. Optimization of warfarin dose by population-specific pharmacogenomic algorithm. Pharmacogenomics J 2012; 12: 306–311.