Int J Clin Exp Med 2017;10(9):14056-14062
www.ijcem.com /ISSN:1940-5901/IJCEM0058745

Original Article

Genotype-based anticoagulant therapy with warfarin for atrial fibrillation

Xiaolong Li, Yan Lu, Jianfeng Yin, Qi Zhang

Department of Cardiology, Changzhou Traditional Chinese Medicine Hospital, Changzhou, Jiangsu Province, China

Received June 3, 2017; Accepted August 27, 2017; Epub September 15, 2017; Published September 30, 2017

Abstract: Objective: To investigate the efficacy and safety of genotype-based anticoagulant therapy with warfarin in patients with atrial fibrillation. Methods: A total of 66 patients with non-valvular atrial fibrillation treated admitted to our hospital from December 2014 to May 2015 were enrolled in this study. The patients were randomly allocated to the control group (n=33) and the intervention group (n=33). Patients in the intervention group were given genotype-based (including VKORC1, CYP4F2, GGCX, and CYP2C9) anticoagulant therapy with warfarin, whereas patients in the control group received routine warfarin therapy according to the clinical practice. After 6 months of follow-up, the adjusted dose of warfarin, the time to achieve stable dose and International Normalized Ratio (INR) standard level, and the incidences of bleeding and thrombosis in both groups were recorded and compared. Results: Overall, 57 patients completed the follow-up, with 28 patients in the intervention group, and 29 patients in the control group. The intervention group received a daily dose of warfarin at 2.8 mg/d, which was lower than that of the empirical dose (3 mg/d). And the actual dose of warfarin used was not consistent with the empirical dose in 24 (85.7%) patients. What's more, there were 18 (64.3%) patients in the intervention group without dose adjustment, which was significantly more than that of the control group (10/34.5%). The time to stable dose and to the INR standard level in the intervention group were (15.1±5.1) d and (6.8±3.9) d, respectively, significantly shorter as compared to (27.6±6.6) d and (12.9±5.6) d, respectively in the control group. In addition, during the treatment and follow-up, the rate of bleeding and thrombosis was 0 in the intervention group and 5 (17.2%) in the control group, indicating that the adverse reactions in the intervention group was significantly lower than that of the control group (P=0.022). Conclusion: Genotype-based anticoagulant therapy with warfarin is safe and effective in the treatment of non-valvular atrial fibrillation, which can shorten the latency to achieve stable dose and to the INR standard level, reduce the risk of bleeding/thrombosis, and improve clinical prognosis.

Keywords: Genotyping, warfarin, non-valvular atrial fibrillation, clinical efficacy, safety

Introduction

Warfarin has been extensively applied in the prevention of venous thrombosis and cardiogenic cerebral embolism (valvular disease, non-valvular atrial fibrillation, cardioversion of atrial fibrillation) and antithrombotic therapy for prosthetic valve replacement as well [1]. Warfarin, a vitamin K antagonist, can affect the synthesis of coagulation factor II, VII, IX and X. In general, warfarin starts to work within 8-12 h after oral administration, maintains its anticoagulant effect for 2-5 d, and usually reaches a peak at 1-3 d. However, the dose of warfarin in clinical practice may vary by more than ten times, which may cause fatal bleeding if overdosed, or thrombosis if under-dosed [2, 3]. Therefore, it is of great significance to adopt appropriate strategies to balance and reduce the risk of hemorrhage and thrombosis in clinical practice.

The major function of prothrombin time (PT) is to monitor the effect of warfarin on anticoagulation. In most atrial fibrillation cases, the international normalized ratio (INR) standard level of PT should be maintained at 2.0-3.0 during the anticoagulation therapy with warfarin [4]. Conventionally, the standard dose of warfarin was given firstly, then the dose is adjusted until INR reaches the target range, which usually requires multiple adjustments and frequent testing of INR until the stable dose is achieved. All these limit the use of warfarin in clinical practice, especially for patients who are in urgent need

of anticoagulant therapy, such as, atrial fibrillation, venous thrombosis and replacement of prosthetic valves [5, 6].

Recent studies have demonstrated that genetic and environmental factors, as well as drug interactions affect the efficacy of warfarin on anticoagulation, including the genetic mutation in CYP2C9 and VKORC1 [7, 8, 9, 10]. In addition, race, age, height, weight, diet, smoking, hepatic disease, as well as medications (such as phenytoin, amiodarone) also have a great impact on warfarin dose variability. In 2007, the U.S. FDA revised the labeling standard for warfarin to warn doctors that the genetic factors in individual patients may affect their response to warfarin, and recommended the genotyping to calculate the accurate dose of warfarin. According to the Mayo Clinic Clinical Medical Center, the rate of hospitalization attributed to hemorrhage or thrombosis dropped by 28%, and the rate of overall hospitalization reduced by 31% after the genotype-based warfarin therapy [11]. Therefore, genotyping prior to warfarin therapy contributes to reducing the rate of adverse events, thereby improving the safety and efficacy of the therapy. However, the accurate dose of warfarin was still predicted in 20% patients in clinical practice, suggesting that additional studies are required to explore the genes are predictive of warfarin dose variability.

In the present study, we selected four genes for analysis, including VKORC1, CYP4F2, GGCX, and CYP2C9, most of which were closely associated with the individualized treatment with warfarin in Chinese population [9, 10, 12, and 13]. Warfarin dose was calculated using the formula of International Warfarin Pharmacogenomics Consortium (IWPC) with the parameters including genotype, race, age and body mass index (BMI), etc. The clinical efficacy, safety and feasibility of the protocol were analyzed, to optimize the clinical application of warfarin and tolerance of the patients.

Materials and methods

Participants

The Hospital Ethics Committee provided approval for this study, and each patient offered their own written informed consent before the initiation of the study. Patients with non-valvular atrial fibrillation treated in our hospital from December 2014 to May 2015 were enrolled in this study. Non-valvular atrial fibrillation is defined as atrial fibrillation without the occurrence of rheumatic mitral stenosis, mechanical/bioprosthetic valve, and mitral valve repairs [4]. The patients who were more than 18 years of age were eligible for inclusion if they were of Han nationality, refractory or persistent atrial fibrillation, a score of no less than 2 on the CHADS2 scale, no previous use of warfarin, INR 2.0-3.0 during hospitalization. Patients were excluded if they met any of the following conditions: a history of anticoagulant therapy with warfarin, intolerance to warfarin treatment, renal dysfunction, abnormal thyroid function or blood coagulation dysfunction, severe infection, severe heart failure, anemia, pregnancy, patients with malignancies, or hemorrhage. The criteria for evaluation of the CHA2DS2 scoring included 1 score for recent heart failure, 1 for diabetes mellitus, 1 for previous onset of stroke/TIA/thrombosis, and 1 for history of vascular diseases (including myocardial infarction, peripheral arterial disease, the presence of major artery plaque) [5].

A total of 66 eligible patients were enrolled in the study, including 46 patients with ischemic heart disease and 20 patients with dilated cardiomyopathy. They were randomly assigned to the intervention group (n=33) and the control group (n=33). Patients in the intervention group were given genotype-based anticoagulant therapy with warfarin, whereas those in the control group received routine warfarin therapy according to the clinical practice.

Genotype identification

The baseline data of the patients were pooled, including gender, age, body mass index (BMI), electrocardiogram, echocardiography, baseline biochemical indicators, the initial dose of warfarin (Shanghai Xinyi pharmaceutical company, 2.5 mg/tablet), and INR at baseline. A sample of 6 ml venous blood was collected from each patient after 8-h fasting and then poured into an EDTA-anticoagulant tube. The DNA was extracted from the sample with the use of a whole-blood genomic DNA extraction kit, and polymorphism genotyping for VKORC1 (rs9934438), CYP4F2 (rs2108622), GGCX (rs11676382), and CYP2C9 (rs1057910) was analyzed with the use of polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method [9, 10, 12, and 13].

Table 1. Clinical characteristics of patients in both groups

Clinical characteristic	Intervention (n=28)	Control (n=29)	*P* value
Age (year)	63.9±16.3	62.6±17.1	0.201
Gender (male/%)	13/46.4	16/55.2	0.599
BMI (kg/m^2)	25.9±3.3	26.1±3.1	0.390
ALT (IU/L)	25.69±6.10	25.30±5.91	0.610
AST (IU/L)	28.60±5.09	27.91±5.33	0.291
BUN (mmol/L)	5.26±0.71	5.98±0.66	0.671
Cr (μmol/L)	96.19±8.80	90.56±7.10	0.299
Platelet count (10^9/L)	189.9±8.91	176.10±10.31	0.410
INR baseline	1.02±0.30	1.01±0.29	0.516

Intervention

The patients in the intervention group were given genotype-based anticoagulant therapy with warfarin for 7 days. The daily dose of warfarin in the intervention group was calculated according to the warfarin dose calculation method using the website (www. Warfarindosing.org) [14]. The INR was monitored during the treatment, and the dose of warfarin was adjusted until INR reached the target range. The patients in the control group were treated with routine warfarin therapy in terms of the clinical practice as well as BMI. The initial dose of warfarin (3.0 mg/d) was adjusted based on the INR values.

In the course of the treatment, the INR value was detected twice per week. The adjusted dose of warfarin was at a minimum amount ranging 0.5 to 1 mg once daily till the INR reached the target range (2.0-3.0). After the target range was achieved, INR was detected once a week for the first month thereafter, and after that once a month until the end of the experiment. The coagulation indicators were detected at any time if bleeding or other adverse events occurred.

Follow-up

All the patients were followed up by telephone visits for 6 months after discharge, once a week for the first month, and every two weeks for the second and third months, and thereafter once a month from the 4th to the 6th month.

During the follow-up, the dose of warfarin, the adjustment dose, the time to achieve stable dose and to the INR standard level were recorded. The stable dose of warfarin is defined as the daily dose for each patient that can stabilize the INR at target range for at least 3 times. The target INR varied from 2.0 to 3.0.

The major adverse events occurred during the follow-up, including ischemic stroke/TIA cerebral hemorrhage, and other systemic embolism/hemorrhage were recorded. Stroke was validated as demonstrated on the CT or MRI findings.

Statistical analysis

All data analyses were performed using the SPSS software package, version 21.0. The measurement data were presented as mean ± standard deviation, and an independent t-test was used to analyze the differences between the two groups. The enumeration data were expressed as percentages, and a chi-square test was used to analyze the differences between the two groups. A *P* value less than 0.05 was deemed as statistically significant. In the present study, no intention to treat (ITT) analysis was made.

Results

Clinical characteristics of patients

A total of 57 patients (28 patients in the intervention group and 29 in the control group) completed the follow-up. Among the excluded patients, 6 did not follow the doctor's prescription, and 3 voluntarily withdrew from the study.

Table 1 shows there was no significant difference between the two groups in age, gender, BMI, serum ALT, AST, BUN and Cr levels, platelet counts and baseline INR value.

Genotyping and estimated initial dose of warfarin in the intervention group

The intervention group had 28 patients (13 males, and 15 females). The genotyping results showed that the maingenotype in CYP2C9*3 was AA; TT was the major genotype in VKORC1; CT was the main genotype in CYP4F2; the genotype in GGCX was all CC. Among these patients, one patient had both VKORC1 and CYP4F2 mutation, one had both CYP2C9*3 and CYP4F2 mutation. The maximum initial daily dose of warfarin was set at 4.3 mg/d, approximately

Table 2. Genotype and estimated initial dose of Warfarin in the intervention group

No	Gender	Age (year)	Height (cm)	Weight (kg)	CYP2C9 rs1057910	VKORC1 rs9934438	CYP4F2 rs2108622	GGCX rs11676382	Dose (mg/d)
1	Female	83	162	77	AA	TT	CT	CC	2.3
2	Female	26	160	65	AA	TT	CT	CC	4.3
3	Female	68	154	57	AA	TT	CT	CC	2.6
4	Female	78	162	62	AA	TT	CT	CC	2.5
5	Female	64	154	60	AA	CT	CT	CC	3.8
6	Female	69	155	42	AA	TT	CC	CC	2.4
7	Male	68	168	70	AA	TT	CC	CC	3.0
8	Female	63	163	70	AA	TT	CT	CC	2.9
9	Female	62	156	55	AA	TT	CC	CC	2.6
10	Female	76	155	51.5	AC	TT	CC	CC	2.3
11	Male	52	175	97	AA	TT	CT	CC	3.9
12	Male	65	180	54	AA	TT	CC	CC	2.9
13	Male	62	170	75	AA	TT	CC	CC	3.1
14	Male	70	158	55	AA	TT	CC	CC	2.3
15	Female	64	162	75	AA	TT	CT	CC	3.0
16	Female	82	152	50	AA	TT	CC	CC	1.9
17	Male	80	170	55	AA	TT	CT	CC	1.6
18	Male	77	158	56	AA	CT	CC	CC	3.4
19	Male	69	170	69	AA	TT	CT	CC	3.0
20	Male	52	180	91	AA	TT	CC	CC	2.9
21	Female	62	155	58	AA	TT	CC	CC	2.7
22	Male	82	177	46	AC	TT	CT	CC	2.1
23	Male	59	175	73	AA	TT	CT	CC	3.5
24	Male	63	165	67	AA	TT	CT	CC	2.3
25	Female	83	160	50	AA	TT	CC	CC	2.0
26	Female	78	162	92	AA	TT	CC	CC	3.0
27	Male	76	168	67	AC	CT	CC	CC	3.7
28	Female	59	158	80	AC	TT	CC	CC	3.4

3 folds of the minimum initial daily dose (1.6 mg/d). The intervention group was given an average daily dose of 2.8 mg/d, which was lower than the empirical dose of 3 mg/d. Among the patients in the intervention group, four (14.3%) patients had estimated initial dose, consistent with the empirical dose of 3 mg/d. Initial doses lower than the empirical dose were found in 17 patients (60.7%) whereas 7 (25%) had initial doses higher than the empirical dose (**Table 2**).

The adjustment of warfarin dose in both groups

Table 3 shows that in the intervention group, the estimated initial dose of warfarin was (2.83±0.65) mg/d, which was very close to the actual dose of (2.88±0.55) mg/d. Eighteen (64.3%) patients had no dose adjustment. On the other hand, in the control group, the initial dose of warfarin was 3 mg/d; the actual dose was (2.69±0.76) mg/d; 10 patients (34.5%) were not given dose adjustments. Therefore, the control group had significantly a lower incidence when compared with the intervention group (P=0.039).

The time toachievestable dose and to the INR standard level

Table 4 reveals that the time to achieve stable dose and to the INR standard level in the intervention group were (15.1±5.1) days and (6.8±3.9) days, respectively, substantially shorter than the time (27.6±6.6 d and 12.9±5.6 d, respectively) in the control group (P=0.033, and 0.019, respectively).

Table 3. The adjustment of warfarin in both groups

Group	Estimated dose (mg/d)	Actual dose (mg/d)	Patients without dose adjustment (n, %)
Intervention (n=28)	2.83±0.65	2.88±0.55	18/64.3*
Control (n=29)	3.00	2.69±0.76	10/34.5

Note: *Comparison with the control group, P=0.039.

Table 4. The time achieving stable dose and to the INR standard level in both groups

Group	The time to stable warfarin dose (d)	The time to standard INR (d)
Intervention (n=28)	15.1±5.1	6.8±3.9
Control (n=29)	27.6±6.6	12.9±5.6
P value	0.033	0.019

Table 5. Adverse reactions in both groups

Group	Thrombosis (n)	Mild bleeding (n)	Major bleeding (n)	Incidence (%)
Intervention (n=28)	0	0	0	0*
Control (n=29)	1	4	0	17.2

Note: *Comparison with the control group, P=0.022.

Adverse reactions in both groups

Table 5 shows that during the treatment and follow-up, no adverse reactions like thrombosis and bleeding were observed in the intervention group. In contrast, in the control group, thrombosis occurred in 1 patient and mild bleeding in 4 patients, with a total rate of 17.2%, which was significantly higher than that in the intervention group (P=0.022).

Discussion

Anticoagulant therapy is an effective strategy to prevent thromboembolic events in patients with atrial fibrillation. Clinically, warfarin is one of the most frequently-used agents for anticoagulation. Frequent monitoring of INR is needed during the treatment with warfarin due to the narrow therapeutic window and significant inter-individual dose variability. In China, nevertheless, most patients underwent warfarin treatment without INR guidance or INR monitoring. What's worse, they didn't even undergo a regular therapy for anticoagulation, leading to the high incidences of thrombosis and mortality [10].

The clinical efficacy of warfarin is impacted by many factors, including gene polymorphism, drug, food, age, concomitant diseases, lifestyle, and compliance [15]. Therefore, it is of particular importance to obtain accurate initial dose of warfarin and establish individualized treatment. When warfarin therapy starts at a high dose, the INR standard level will be achieved soon, which may increase the risk of bleeding or reduce patients' tolerance to warfarin, even if there is no serious fatal intracranial hemorrhage. Similarly, when warfarin dose is too low, it takes a long time to adjust the dose to the INR standard level, which may result in a high risk of thrombosis and poor compliance of patients. Currently, a hotspot in the medical world is an individualized therapy for anticoagulation with warfarin on the base of pharmacogenomics. VKORC1, CYP4F2, GGCX and CYP2C9 are the genes that are closely associated with warfarin-based individualized treatment. Among these genes, CYP2C9 and CYP4F2 are involved in the metabolic process of warfarin, and GGCX and VKORC1 are enzymes in the cycle of vitamin K [12, 13]. Thus, alteration in the genes encoding functional protein can affect the therapeutic dose of warfarin individually. According to previous studies, allelic mutation in CYP2C9 is associated with reduced enzymatic activity of CYP2C9, leading to the slow clearance of warfarin in vivo, and the increased risk of bleeding [16-18]. In addition, mutations in the gene of VKORC1 increase the sensitivity of VKOR to warfarin, thereby enhancing the efficacy of anticoagulants. Therefore, the accurate initial dosing of warfarin via warfarin dose prediction model, which considers both genetic and non-genetic factors, may contribute substantially to establishment of individualized treatment, and improvements in the clinical efficacy and safety of the treatment.

A wide range of literature has shown that, when the INR is maintained at 1.5-3.0, individuals with CYP2C9*1/*1 usually need a higher dose of warfarin than those with CYP2C9*1/*3; patients with VKORC1 containing TT need a markedly lower dose of warfarin than those with

CT; those with CC genotype in CYP4F2 need a significantly lower dose of warfarin than TT carriers; those with CC in GGCX need a significantly low dose of warfarin than GG carriers [19, 20]. However, the studies were mostly focused on the two genes of CYP2C9 and VKORC1, with few studies on other two genes. In the present study, we comprehensively considered the effects of the polymorphism of the 4 genes (VKORC1, CYP4F2, GGCX and CYP2C9), clinical parameters and individual factors to calculate the initial dose of warfarin by using the formula of International Warfarin Pharmacogenomics Consortium. We found that in the intervention group, 17 (60.7%) patients had initial doses lower than the empirical dose, and 7 (25%) patients had initial dose higher than the empirical dose. The maximum initial daily dose of warfarin in the intervention group was 4.3 mg/d, which was as approximately 3 folds many as that of the minimum initial daily dose (1.6 mg/d). Theoretically, insufficient anticoagulation or excessive anticoagulation can be observed if the empirical dose is employed in these 24 patients. Moreover, the intervention group showed the estimated dose of warfarin of (2.83±0.65) mg/d, which was very close to the actual dose of (2.88±0.55) mg/d, and there were 18 (64.3%) patients receiving no dose adjustment. In contrast, 10 (34.5%) patients had no dose adjustments in the control group, which was significantly lower than that of the intervention group, indicating that the accuracy of estimated dose of warfarin can reach more than 60%. Furthermore, we evaluated the time to achieve stable dose and to the INR standard level in both groups. The results showed that the intervention group had shorter time to achieve stable dose and to the INR standard level and a lower rate of bleeding/thrombosis events as compared to the control group. The results above suggest that the initial dose of warfarin used in the prediction model is more accurate. Notably, it is necessary to monitor the INR level and coagulation function regularly during the follow-up to ensure the long-term effectiveness and safety of anticoagulation therapy by timely adjustment of warfarin dose, even the stable dose and the INR standard level have been achieved. The initial dose of warfarin based on prediction model has been reported to contribute to earlier reach of the INR standard level, with higher clinical efficacy and lower risk of adverse events, which is of significance to guide the development and establishment of individualized protocols with warfarin [21, 22]. The above results in the present study were in accord with those of the previous studies.

There were still some limitations in this study, including the small number of participants enrolled, the short-term follow-up, and single-center study, which may cause some statistical bias. In the future work, it is of great need to improve the experimental design, expand the sample size and prolong the follow-up period, and conduct a multicenter, double-blind clinical study. If possible, a better genotype-guided, warfarin-based therapeutic strategy should be developed and established for the treatment of non-valvular atrial fibrillation.

In conclusion, genotype-based warfarin treatment plus INR monitoring can effectively shorten the dose adjustment time, achieve better therapeutic efficacy and reduce risk of adverse events when compared with conventional procedures.

Disclosure of conflict of interest

None.

Address correspondence to: Qi Zhang, Department of Cardiology, Changzhou Traditional Chinese Medicine Hospital, No. 25 Heping North Road, Changzhou 213161, Jiangsu Province, China. Tel: +86-0519-88124955; Fax: +86-0519-88124955; E-mail: qizhang68@126.com

References

[1] Zhou ZQ, Hu DY, Chen J, Zhang RH, Li KB, Zhao XL. An epidemiological survey of atrial fibrillation in China. Zhonghua Nei Ke Za Zhi 2004; 43: 491-4.

[2] Working Group on Thrombosis of Chinese Journal of Cardiology. Chinese experts consensus on new oral anticoagulant in the non-valvular atrial fibrillation. Zhonghua Xin Xue Guan Bing Za Zhi 2014; 42:362-9.

[3] Fuster V, Rydén LE, Asinger RW, Cannom DS, Crijns HJ, Frye RL, Halperin JL, Kay GN, Klein WW, Lévy S, McNamara RL, Prystowsky EN, Wann LS, Wyse DG, Gibbons RJ, Antman EM, Alpert JS, Faxon DP, Fuster V, Gregoratos G, Hiratzka LF, Jacobs AK, Russell RO, Smith SC, Klein WW, Alonso-Garcia A, Blomström-Lundqvist C, De Backer G, Flather M, Hradec J, Oto A, Parkhomenko A, Silber S, Torbicki A; Ame-

rican College of Cardiology/American Heart Association/European Society of Cardiology Board. ACC/AHA/ESC guidelines for the management of patients with atrial fibrillation: executive summary. A Report of the American college of cardiology/American heart association task force on practice guidelines and the European society of cardiology committee for practice guidelines and policy conferences (committee to develop guidelines for the management of patients with atrial fibrillation): developed in collaboration with the North American society of pacing and electrophysiology. J Am Coll Cardiol 2001; 38: 1231-66.

[4] January CT, Wann LS, Alpert JS, Calkins H, Cigarroa JE, Cleveland JC Jr, Conti JB, Ellinor PT, Ezekowitz MD, Field ME, Murray KT, Sacco RL, Stevenson WG, Tchou PJ, Tracy CM, Yancy CW; American College of Cardiology/American Heart Association Task Force on Practice Guidelines. 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: a report of the American college of cardiology/American heart association task force on practice guidelines and the heart rhythm society. J Am Coll Cardiol 2014; 64: e1-76.

[5] Camm AJ, Kirchhof P, Lip GY, Schotten U, Savelieva I, Ernst S, Van Gelder IC, Al-Attar N, Hindricks G, Prendergast B, Heidbuchel H, Alfieri O, Angelini A, Atar D, Colonna P, De Caterina R, De Sutter J, Goette A, Gorenek B, Heldal M, Hohloser SH, Kolh P, Le Heuzey JY, Ponikowski P, Rutten FH; ESC Committee for Practice Guidelines. Guidelines for the management of atrial fibrillation: the task force for the management of atrial fibrillation of the European Society of Cardiology (ESC). Europace 2010; 12: 1360-420.

[6] Wang L, McLeod HL, Weinshilboum RM. Genomics and drug response. N Engl J Med 2011; 364: 1144-53.

[7] Yang L, Han MH. The effects of VKORC1 and CYP2C9 gene polymorphisms on the warfarin dose in patients with atrial fibrillation. Med Rev 2012; 18: 1461-3.

[8] Hu J, Zhu JR, Yu F. Research on the individualized anti-coagulatant treatment of warfarin. Chin J Clin Pharmacol Therapeutics 2014; 19: 591-6.

[9] Gaikwad T, Ghosh K, Shetty S. VKORC1 and CYP2C9 genotype distribution in Asian countries. Thromb Res 2014; 134: 537-44.

[10] Chen XL. Current application of warfarin in patients with atrial fibrillation. Chinese Journal of Modern Drug Application 2014: 173-173.

[11] Mayo Clinic. Rheonix make warfarin genotyping test. Medical Device Daily 2011.

[12] McDonald MG, Rieder MJ, Nakano M, Hsia CK, Rettie AE. CYP4F2 is a vitamin K1 oxidase: an explanation for altered warfarin dose in carriers of the V433M variant. Mol Pharmacol 2009; 75: 1337-46.

[13] Tie JK, Stafford DW. Functional study of the vitamin K cycle enzymes in loive cells. Methods Enzymol 2017; 584: 349-394.

[14] International Warfarin Pharmacogenetics Consortium, Klein TE, Altman RB, Eriksson N, Gage BF, Kimmel SE, Lee MT, Limdi NA, Page D, Roden DM, Wagner MJ, Caldwell MD, Johnson JA. Estimation of the warfarin dose with clinical and pharmacogenetic data. N Engl J Med 2009; 360: 753-64.

[15] Gage BF, Eby C, Johnson JA, Deych E, Rieder MJ, Ridker PM, Milligan PE, Grice G, Lenzini P, Rettie AE, Aquilante CL, Grosso L, Marsh S, Langaee T, Farnett LE, Voora D, Veenstra DL, Glynn RJ, Barrett A, McLeod HL. Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin. Clin Pharmacol Ther 2008; 84: 326-31.

[16] Sanderson S, Emery J, Higgins J. CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: a HuGEnet systematic review and meta-analysis. Genet Med 2005; 7: 97-104.

[17] 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.

[18] Yin T, Miyata T. Warfarin dose and the pharmacogenomics of CYP2C9 and VKORC1 - rationale and perspectives. Thromb Res 2007; 120: 1-10.

[19] Zhu Y, Shennan M, Reynolds KK, Johnson NA, Herrnberger MR, Valdes R Jr, Linder MW. Estimation of warfarin maintenance dose based on VKORC1 (-1639 G>A) and CYP2C9 genotypes. Clin Chem 2007; 53: 1199-205.

[20] Pautas E, Moreau C, Gouin-Thibault I, Golmard JL, Mahé I, Legendre C, Taillandier-Hériche E, Durand-Gasselin B, Houllier AM, Verrier P, Beaune P, Loriot MA, Siguret V. Genetic factors (VKORC1, CYP2C9, EPHX1, and CYP4F2) are predictor variables for warfarin response in very elderly, frail inpatients. Clin Pharmacol Ther 2010; 87: 57-64.

[21] Scott SA, Patel M, Martis S, Lubitz SA, van der Zee S, Yoo C, Edelmann L, Halperin JL, Desnick RJ. Copy number variation and warfarin dosing: evaluation of CYP2C9, VKORC1, CYP4F2, GGCX and CALU. Pharmacogenomics 2012; 13: 297-307.

[22] Jorgensen AL, FitzGerald RJ, Oyee J, Pirmohamed M, Williamson PR. Influence of CYP2C9 and VKORC1 on patient response to warfarin: a systematic review and meta-analysis. PLoS One 2012; 7: e44064.