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SPECIAL ISSUE ARTICLE

DNA sensors to assess the effect of *VKORC1* and *CYP2C9* gene polymorphisms on warfarin dose requirement in Chinese patients with atrial fibrillation

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Abstract The optimal dose of warfarin depends on polymorphisms in the *VKORC1* (the vitamin K epoxide reductase complex subunit (1) and *CYP2C9* (cytochrome P450 2C9) genes. To minimize the risk of adverse reactions, warfarin dosages should be adjusted according to results from rapid and simple monitoring methods. However, there are few pharmacogenetic-guided warfarin dosing algorithms that are based on large cohorts from the Chinese population, especially patients with atrial fibrillation. This study aimed to validate a pharmacogenetic-guided warfarin dosing algorithm based on results from a new rapid electrochemical detection method used in a multicenter study. Three SNPs (*CYP2C9* *2, *3 and *VKORC1* c.-1639G > A) were genotyped by electrochemical detection using a sandwich-type format that included a 3′ short thiol capture probe and a 5′ ferrocene-labeled signal probe. A total of 1285 samples from four clinical hospitals were evaluated. Concordance rates between the results from the electrochemical DNA biosensor and the sequencing test were 99.8%. The results for gene distribution showed that most Chinese patients had higher warfarin susceptibility because mutant-type and heterozygotes were present in the majority of subjects (99.4%) at locus c.-1639G > A. When the International Warfarin Pharmacogenetics Consortium algorithm was used to estimate therapeutic dosages for 362 patients with AF and the values were compared with their actual dosages, the results revealed that 56.9% were similar to actual dosages (within the 20% range). A novel electrochemical detection method of *CYP2C9* *2, *3and *VKORC1* c.-1639G > A alleles was evaluated. The warfarin dosing algorithm based on data gathered from a large patient cohort can facilitate the reasonable and effective use of warfarin in Chinese patients with AF.

Keywords Warfarin dosing · Electrochemical DNA sensor · Frequency · Atrial fibrillation

Introduction

Warfarin is an oral anticoagulant that is widely used to treat and prevent thromboembolic conditions, including myocardial infarction, venous thrombosis, and ischemic stroke, as well as after heart valve replacement and atrial fibrillation [1]. Especially in patients with atrial fibrillation (AF), warfarin has been widely used. More and more new methods

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have made drug development easier [2], however, the therapeutic window of warfarin is narrow and the dosage varies across individuals and populations. While suboptimal doses can increase the risk for stroke, high doses can increase the risk of bleeding [3]. In order to clarify warfarin dosage algorithms based on genetic polymorphisms, a growing number of studies attempted to analyze these genetic variations [4–6], especially polymorphisms occurring in *VKORC1* and *CYP2C9* [7–9]. SNPs in *VKORC1* reportedly significantly affect sensitivity to warfarin, and can account for 6–37% of the inter-individual variability in warfarin doses [10]. Furthermore, the two most common *VKORC1* SNPs, c.1173C > T (rs9934438) and c.-1639G > A (rs9923231), can increase warfarin sensitivity [11], and they were in complete linkage disequilibrium [12]. *CYP2C9* gene SNPs can result in slower metabolism of warfarin and longer drug half-life that may translate into bleeding complications [13]. Individuals who are homozygous for these *CYP2C9* SNPs may require 35–75% lower doses than homozygous wild-type patients. *CYP2C9* *2 (rs1799853) and *3 (rs1057910) are the most common polymorphisms, while others such as *4 (rs56165452), *5 (rs28371686), and *11 (rs28371685) are very rare. Although many reports addressed the relationships between genetic polymorphisms and warfarin dosing in China and some other countries [14–17], these studies involved small sample sizes. As such, validation of these associations with larger patient cohorts is needed.

Based on previous studies, more methods that rely on molecular recognition, including sequencing [18] and real-time PCR [19], have been developed for warfarin genetic tests. However, sequencing is time-consuming and expensive, and real-time PCR assays may have low threshold [20]. ARMs PCR [21] is found high efficient in detecting SNPs, but fewer fluorescence channels lead to more ARMs PCR reactions if multiplex biomarkers are needed in one test. New tools such as DNA biosensors are now being used. DNA biosensors are devices that combine DNA molecular analysis method and a detector component, and exploit the hybridization dynamics of nucleic acids [22, 23], an electrochemical DNA biosensor that represents one innovative type of recently developed biosensor is applied in this study [24–27]. Compared with traditional nucleic acid assays, electrochemical biosensors offer fast detection, simplicity, good selectivity, sensitivity, and experimental convenience at low cost [28]. Moreover, electrochemical biosensors have great significant value for gene expression studies, genotyping, pharmacogenomics, pathogen classification, screening of DNA–protein or DNA-drug interactions, sequencing, and molecular diagnostics [29–31]. In view of those advantages, electrochemical biosensors are a good approach for use in genetic testing for warfarin dosages.

A novel electrochemical biosensor to detect three SNPs in two genes (*CYP2C9* *2, *CYP2C9* *3, and *VKORC1* c.-1639G > A) that affect warfarin drug response were developed in this study, and also determined a dosage regime based on results obtained using this DNA sensor in a large cohort of Chinese patients with AF.

Materials and methods

Chemicals

All reagents used were of the highest quality available. Trizma base, EDTA, tris(2-carboxyethyl) phosphine hydrochloride (TCEP), and 6-hydroxy-1-hexanethiol (MCH) used in electrochemical buffer preparation were purchased from Sigma–Aldrich (USA). Ferrocene-dT-CE phosphoramidite (FC1) [32] was purchased from Glen Research (cat No./ID: 10-1059-02, USA), and Ferrocene-containing phosphoramidite (FC2) [33] was synthesized by Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences. The electrode array (PCB) was purchased from Guangzhou Fastprint Circuit Tech Co., Ltd. (China).

Reagents for PCR using Hot Start Taq DNA polymerase were purchased from Applied Biosystems (USA). All synthetic oligonucleotides were purchased from Sangon, Inc. (Shanghai, China). The base sequences of capture probes and signal probes are summarized in Table 1.

Samples

A total of 1285 blood samples (Table 2) were collected between August 2012 and June 2013 from patients ranging in age from 1 to 88 years-old (male/female ratio: 0.865:1) who were treated at four hospitals: The Third Affiliated Hospital of Sun Yat-sen University, The First Affiliated Hospital of Sun Yat-sen University, Linyi People's Hospital, and Luan Affiliated Hospital of Anhui Medical University. The four hospitals are located in different regions of China, and 362 patients with AF among the 1285 had received at least one warfarin treatment. These 362 patients met the following criteria for inclusion in this study: (1) undergoing continuous warfarin therapy for at least 3 months; (2) did not smoke or drink; (3) absence of liver, kidney, or gastrointestinal diseases; and (4) were not taking any medications (including amiodarone, statin, azole, sulfamethoxazole) that may interfere with warfarin therapy. Clinical information for each patient, including age, gender, weight, mean daily warfarin dosage, and mean International Normalized Ratios (INRs) was noted. Acquisition of blood samples was approved by the Research Ethical Committee, Sun Yet-sen University and all patients gave

Table 1 Primers and probes for the electrochemical DNA assay

Names	Sequence (5′-3′)
Type-specific primers	
430-F	GGATCTCCCTCCTAGTTTCG
430-R	CTTGGTTTTTCTCAACTCCTCC
1075-F	AGTTTTTACTTGTGTCTTATCAG
1075-R	AAACAAACTTACCTTGGGAAT
1639-F	AGGGTAGGTGCAACAGTAA
1639-R	CCTGACCTCAAGTGATCCACCC
Capture probes	
430-CP	CCACAAGGCAGAGGGCTTCCTC-SH
1075-CP	ACAGGTCACTGCATGGGTCAGGCTGGTGGG-SH
1639-CP	CCTCTGTTCCCCGACCTCCCATCCTA-SH
Type-specific signal probes	
430WT-SP	3×Fc1-GAACACGGTCCT
430MUT-SP	3×Fc2-GAACACAGTCCTCA
1075WT-SP	3×Fc1-GAAGGTCAATGTATC
1075MUT-SP	3×Fc2-GAAGGTCAAGGTATC
1639WT-SP	3×Fc1-GCACCCGGCCAA
1639MUT-SP	3×Fc2-GCACCTGGCCAA

Table 2 Characteristics of the 1285 patients

Demographics	
All of patients	
Age (year)	51.73 ± 19.68
Gender ratio (male:female)	0.865:1
Major diagnostic category	Tumor (168) Cardiac disease (273) Valvular heart disease (115)
Patients with AF and used warfarin (n = 362)	
Age (year)	59.72 ± 12.37
Gender ratio(male:female)	1.017:1
Body weight (kg)	63.04 ± 13.10
Body length (cm)	162.42 ± 9.26
INR value	2.11 ± 0.92
Maintenance dose (mg/day)	3.13 ± 0.59

INR international normalized ratio

*Values represented as mean ± SD

informed consent prior to donation. All samples were stored at −20 °C within 1 h of collection for later use.

Genomic DNA extraction and PCR assay

Genomic DNA was extracted from blood specimens using a DNA extraction kit (Qiagen, cat No./ID: 51206) carried out according to the manufacturer's instructions. Asymmetric multi-PCR was applied to simultaneously amplify three different DNA sequences in one PCR tube. The 50 µL reaction mixture for the three SNP variations consisted of 10 mM Tris–HCl (pH 8.3), 20 mM KCl, 200 mM dNTPs, 3.5 mM $MgCl_2$, forward primers (10 µM each), reverse primers (1 µM each) and 50 ng extracted DNA. PCR was carried out using an ABI9700 Thermal Cycler (Applied Biosystems, USA) with the following protocol: 95 °C for 2 min, 45 cycles of 94 °C for 30 s, 56 °C for 35 s and 72 °C for 40 s; final 72 °C for 7 min.

Capture probe immobilization and electrochemical detection

The DNA sensor works by a three-electrode system consisting of 10 gold electrodes as the working electrode, the auxiliary electrode, and a reference electrode (Ag/AgCl). Pretreatment of electrodes and capture probe immobilization was performed according to a previously reported protocol [34]. First, gold electrodes were polished with a microcloth (Buehler) and gamma MicroPolish deagglomerated alumina suspension (0.05 µm) for 5 min. Then, the electrodes were sonicated in ethanol and Milli-Q water for 5 min each. Finally, the electrodes were electrochemically cleaned to prepare for immobilization [35]. After drying with nitrogen, electrodes were exposed to oligonucleotides at 6mM for 14–16 h followed by a 2 h deposition in 1 mM MCH in water. The SH-DNA-modified electrodes were then further treated with 2 mM MCH for 1 h to obtain a well-aligned mixture of SAMs. PCR products (50 µl) were mixed with 100 µl 0.1 M PBS (pH 7.0) containing signal probes (0.4 µM each) and $NaClO_4$ (0.6 M). Voltmeter

measurements were acquired with a CHI 660 C Electrochemical Workstation (CH Instrument, USA) and used for data analysis. The entire procedure, including DNA hybridization and electrochemical detection, required only 30 min to complete.

Sequencing assay

All specimens were analyzed using DNA sanger sequencing method as a controlled trial General PCR was used to propagated individual the three different DNA fragments. The 50 µL reaction mixture for the sequencing method consisted of 10 mM Tris–HCl (pH 8.3), 20 mM KCl, 200 mM dNTPs, 3.5 mM $MgCl_2$, primers (10 µM each) and 20 ng extracted DNA. The following protocol were the same with the above Asymmetric multi-PCR. DNA sequencing methods that was performed by Genewiz, Inc.

Predicted dose calculation

The International Warfarin Pharmacogenetics Consortium (IWPC) is composed of 21 pharmacogenomics research centers located in nine countries on four continents. The IWPC team collected data for about 5700 patients related to warfarin dosing in order to define a consensus model for dosing. Researchers can access this database at http://www.pharmgkb.org. A study by *Klein* [36] filtered 4043 datasets from the IWPC database and set up a prediction algorithm with a warfarin predicted dose that was used to test 1009 patients. The results were used to establish the IWPC algorithm. The IWPC algorithm is convinced and widely accepted for the high number of cases analyzed. In this study, we used the IWPC algorithm and online warfarin dosing software (http://www.warfarindosing.org) to calculate the predicted dose for 362 patients and to analyze differences between actual and predicted doses.

Statistical analysis

Continuous variables were expressed as mean ± SD. Hardy–Weinberg equilibrium for the 3 target SNPs were analyzed by Chi square test. Population frequencies of alleles are given together with their 95% confidence limits. Kappa analysis was used to evaluate the consistency between electrochemical DNA sensor and sequencing results. A *p*-value of less than 0.050 was considered statistically significant. All statistical analyses were performed using SPSS 19.0 software.

Results

A total of 1282 patients were recruited in this study, and 362 patients of them with AF were treated with warfarin. Demographic and clinical features, including age, gender ratio, body weight, and body length are summarized in Table 2. INR and predicted dose were also collected for patients using warfarin (Table 2).

Three targets alleles: *CYP2C9* *2, *3, and *VKORC1* c.-1639G > A were tested using an electrochemical DNA sensor. The results from the electrochemical test to detect each individual SNP were consistent with those from the sequencing test in 100% of 1282 cases, while the remaining 3 cases could not be interpreted by sequencing. Based on Kappa test results (kappa = 1.000, $p < 0.001$), the electrochemical DNA sensor was in good agreement with sequencing. The above-referenced 3 cases were positive by electrochemical DNA biosensor testing, but the sequencing results were unclear due to an interference peak that overlapped with the normal peak in *CYP2C9* *3 (Fig. 1).

In the 1282 samples in which the electrochemical DNA sensor and sequencing results were in agreement, the observed genotypic frequency (Fig. 2a) showed no deviation from Hardy–Weinberg equilibrium ($P > 0.05$).

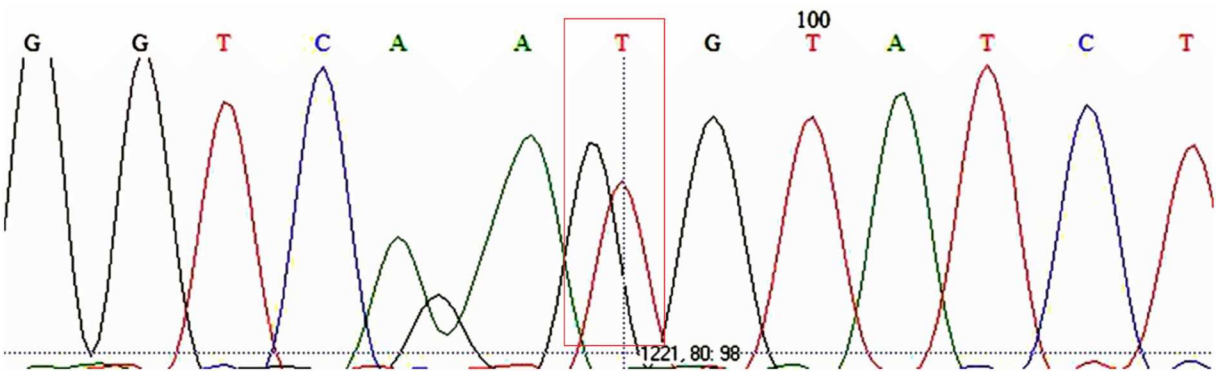

Fig. 1 The Sequence diagram of locus 1075 which was unable to be interpreted. The locus 1075 is shown by *dotted lines*. Overlap between two signal figure of bases intervened interpretation of the results

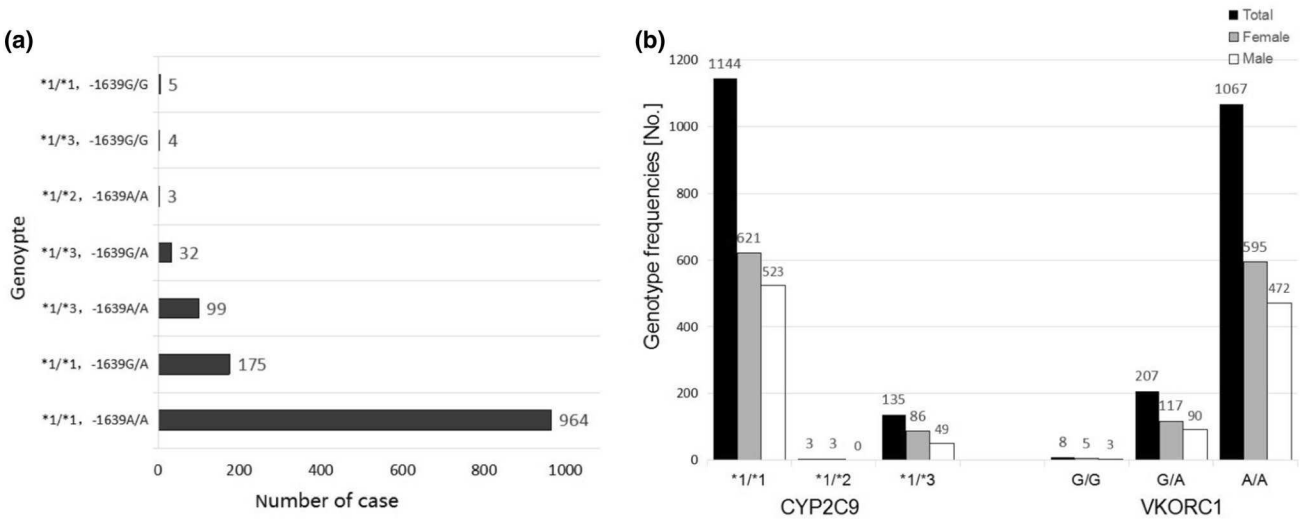

Fig. 2 Genotype and allelic frequencies in Chinese population. **a** Genotype distribution in Chinese population *1/*1, 1639 A/A was the most common genotype in Chinese, up to 75.2%. **b** genotype frequencies in the study cohorts. Wildtype *CYP2C9* gene and Mutant *VKORC1* gene was preponderantly in Chinese population. There was no significant difference found between female and male

Although Chinese patients showed significant differences from Caucasian patients at all three loci, the differences were especially apparent at *VKORC1* c.-1639G > A (Fig. 2b). Notably, the frequency of warfarin metabolism capacity linked *VKORC1* -1639A allele in our patient group was 91.3%, which was similar to studies involving East Asian patients (91.6−91.8%) [37, 38], but was much higher than that reported in other areas [39–43]. The *CYP2C9* *2 allele is rarely found in East and South East Asia, and there were only 3 patients who carried the *2 allele in our study. The frequency of the *2 allele is higher in Caucasian (14.3%), Indian (0.6−5.3%), and Central Asian (2%) patients. Meanwhile, the frequency of the *CYP2C9* *3 allele is similar in Asian patients (2.4–6, 5.3% in this study), except for Indian populations (9.1−10.5%). In our study, the patients with *CYP2C9* *3 allele were present in 10.45% of the total, while the ones with *3 allele accounted for just 0.08%.

Among all alleles examined, eight different genotypes were detected (Fig. 2a). Patients carrying wildtype *CYP2C9* (*1/*1), mutant homozygote *VKORC1* (1639 A/A) were the most common in Chinese, and were present in 964 (75.1%) of the 1282 patients in this study. Meanwhile, Patients carrying wild type *CYP2C9* (*1/*1), wild type *VKORC1* (1639G/G) genotypes appeared in only 5 cases (0.39%). The above results indicate that 99.6% of Chinese individuals carry at least one mutation in warfarin-sensitive genes. And there was no difference in frequency of *CYP2C9* and *VKORC1* gene deletion among four hospitals. As such, most Chinese are predicted to be sensitive to warfarin based on genetic pharmacology.

Among the 1282 patients in this study, 362 adult patients for whom complete clinical information was available were selected for follow up to compare differences between predicted dose and actual dose. The results of the calculation were limited to the 4 common genotypes because information for patients carrying the 4 rare genotypes was not available (Table 3). In China, the recommended dose of warfarin is 2.5–3.5 mg/day in clinical treatment, and 80.71% of the 362 follow up patients were in this range, further 72.37% were used 2.5 mg a day which led to the boxplot showed a straight line. Using the online IWPC pharmacogenetic dosing algorithm to calculate the predicted dose, the median daily predicted dose was 3.13 mg (first to third quartiles, 2.72 to 3.4 mg/day) in all 362 adult patients, which was lower than that for Caucasian patients (5 mg/day). The comparison for daily maintenance dose among 362 patients bearing different genotypes were shown in Fig. 3. Patients with mutational homozygote AA of *VKORC1* c.-1639G > A and wild homozygote *1/*1 of *CYP2C9* required a significantly lower maintenance dose than those with heterozygotes GA and *1/*3. However, comparing predicted dose with actual dose, there also were some difference (Fig. 4), the median daily actual dose was 2.76 mg which was about 0.4 mg lower than the predicted dose. Thus analysis of each patient's results showed that the maintenance dosages of 56.9% of patients were within 20% of the actual dose, which is in agreement with earlier reports (Table 3).

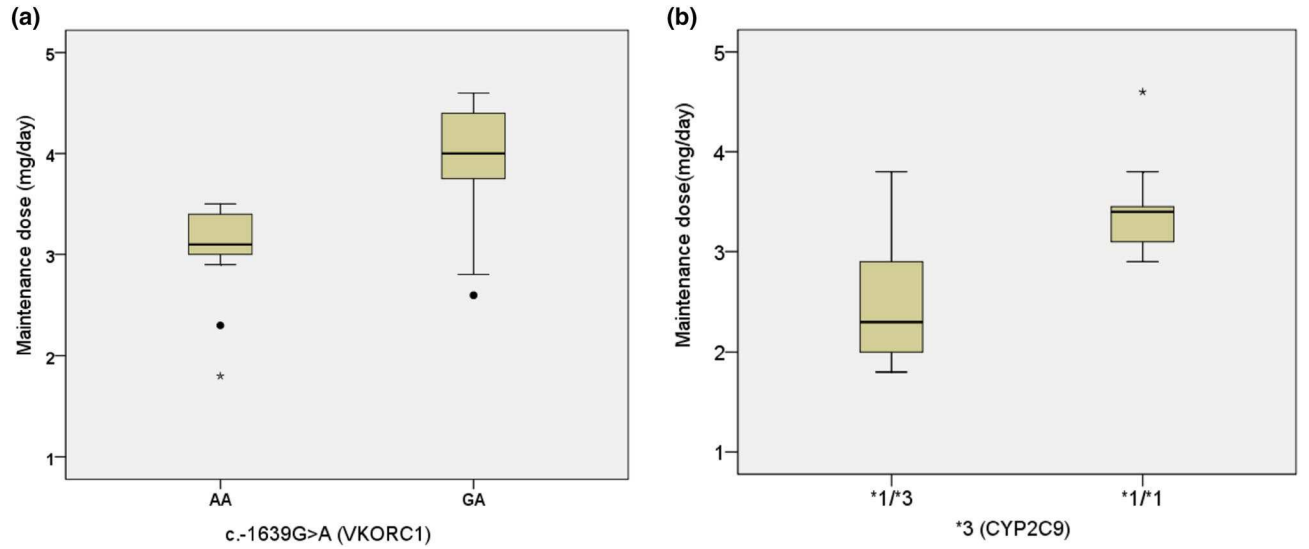

Fig. 3 Relation between the maintenance dose and the tested genes. *Panel A: VKORC1* c.-1639G > A, *Panel B: CYP2C9* *3. Each *box* indicates 25 to 75 percentile of values, and the *horizontal lines* repersent the median value of maintenance dose

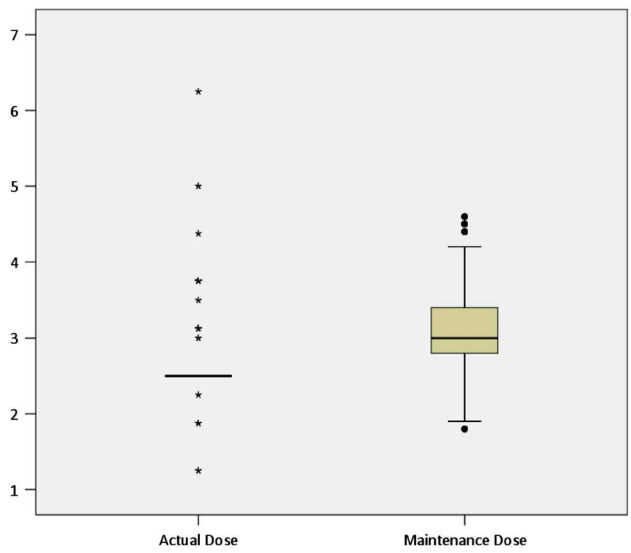

Fig. 4 Relation between the actual dose and the maintenance dose. Maintenance dose repersents calculated dose by IWPC algorithm. Actual Dose of 72.37% of the total 362 patients were 2.5 mg/day, led to the boxplot showed a *straight line*

Discussion

The clinical performance of a novel electrochemical DNA biosensor was developed and validated in this study, which was designed to assess three SNPs (*CYP2C9* *2 and *3, *VKORC1* c.-1639G > A) that affect warfarin drug responses in a Chinese population. Compared with traditional sequencing methods, the DNA sensor showed better turnaround time and cost which entire procedure required only 30 min and cost about $3, so it was more convenient in clinical practice. Electrochemical DNA biosensors have attracted increasing attention [44–46]. Electrochemical DNA biosensors consist of a solid electrode that is immobilized with ss-DNA probes and redox-active hybridization indicators. Among the three common probe-immobilization schemes, self-assembly, biotin-avidin interaction, and electro-polymerization, the self-assembly method is increasingly used due to its simple deposition process. Moreover, several electrochemical approaches [47–49] for DNA biosensors have been studied, including direct or indirect detection, enzyme-amplified labels, and amplification with

Table 3 Warfarin predicted dose compared with actual dose in different genotypes

Genotype	−20 to 20%	>+20%	<−20%	Total
CYP2C9 *1/*1, *VKORC1* c.-1639G/A	7	30	0	37
CYP2C9 *1/*1, *VKORC1* c.-1639A/A	186	101	5	292
CYP2C9 *1/*3, *VKORC1* c.-1639G/A	3	7	0	10
CYP2C9 *1/*3, *VKORC1* c.-1639A/A	10	0	13	23
Total	206 (56.9%)	138 (35.1%)	18 (4.97%)	362

nanomaterials. Additional indicators such as ferrocene [50] and MB [51] have also been evaluated. To avoid shortcomings associated with a single-target protocol, a new method, "sandwich", has been adopted [52]. Asymmetric multi-PCR was used to get large number of single-stranded DNA instead of double-stranded DNA so that following testing did not need the process of heat denature the double-stranded DNA which might cause aerosol pollution. Here 1285 samples were analyzed to validate novel detection method. Results obtained with the electrochemical DNA biosensor agreed well (up to 99.7% concordance, Kappa value = 1, $P < 0.001$) with those obtained from a sequencing assay to screen clinical samples. The only 3 disagreements were caused by a failure to interpret sequencing results.

A comparative analysis demonstrated that there is a significantly different allele distribution of warfarin sensitive polymorphisms in areas surrounding China. In general, the frequency among patients in East and South Asian was similar, but there were differences between Central Asian and Indian patients (Table 4). For India, these variable distributions may have arisen due to the large geographical area (e.g., 0.65 and 5% in Southern and Northern Indians, respectively) and high degree of ethnic variation attributable to past invasions and immigration. Meanwhile, the population in Central Asia is more homogeneous, which may have contributed to the different frequencies observed in this study. However, due to the vast territory and transnational ethnic groups in China, there may be large differences in different regions or nationalities, especially in areas that border India or Central Asia. In our Chinese patient population, the *CYP2C9* *1/*1 and *VKORC1* 1639 A/A genotypes were the most common. Samples with simultaneous mutations in *CYP2C9* and *VKORC1* accounted for 10.4% of patients, and those patients having no mutations represented just 0.4% of the total. Therefore, warfarin dosing must be carefully monitored in Chinese patients to avoid bleeding events.

No researches were reported on the IWPC algorithm tested for Chinese, but Takeuchi's [53, 54] showed it had clinical application in Japanese, and Zhao 's [55] study showed IWPC algorithm performed similarly with other Han-Chinese pharmacogenetics-based warfarin dosing algorithms in their cohort. So we still chose IWPC algorithm to calculate the predicted dose for the 362 follow-up patients, the IWPC algorithm result showed that most members of the Chinese population who were not taking enzyme inducer and amiodarone needed a low (≤3 mg/day) warfarin dose. Notably, about 7% of the population with *CYP2C9* *1/*3 or -1639A/A genotypes needed a lower warfarin dose (≤2.3 mg/day).

The mutational allele of c.1639G > A in *VKORC1* could increase warfarin sensitivity and *3 in *CYP2C9* reduced metabolism of warfarin in the SNPs linked to warfarin metabolism. Thus, individuals carrying the allele require lower daily maintenance doses than homozygous wildtype. Further study found this dosage difference brought by the two SNPs. However, there were some differences between predicted dose and actual dose, which suggests that other SNPs and non-genetic factors were at work. Analysis of the predicted dose and the actual dose in each patient showed that the predicted dosages for 56.9% of patients with AF were similar to the actual dosages, which reflected high inosculation with each dosage and fits well with other studies that showed that the genetic algorithm can explain 54.1−76.8% [56–58] of individual differences in warfarin daily dose requirements. According to Huang's study, gene-directed warfarin-therapy pattern can be very effective in helping the clinicians to prescribe warfarin with greater

Table 4 Allelic frequencies of *CYP2C9* *2, *CYP2C9* *3, and *VKORC1* c.-1639G > A obtained in this study and in other references of Chinese and neighboring countries

Area	*CYP2C9* *2 allele [No. (%)]		*CYP2C9* *3 allele [No. (%)]			*VKORC1* c. -1639G > A allele [No. (%)]			Ref
	*1	*2	*1	*3	95% CI (*3)	G	A	95% CI (A)	
Our data	2561 (99.9)	3 (0.1)	2429 (94.7)	135 (5.3)	4.41–6.13	223 (8.7)	2341 (91.3)	90.21–92.39	Present study
China	1016 (100)	0 (0)	866 (96.7)	30 (3.3)		17 (7.4)	213 (92.6)		[60, 63]
Japan	4554 (100)	0 (0)	4444 (97.6)	110 (2.4)		72 (8.4)	780 (91.6)		[37, 64]
Korea	932 (100)	0 (0)	673 (94.0)	43 (6.0)		42 (8.2)	458 (91.8)		[38, 61]
Malaysia	666 (98.8)	8 (1.2)	639 (94.8)	35 (5.2)		335 (37.1)	569 (62.9)		[39]
Thailand	178 (100)	0 (0)	173 (97.2)	5 (2.8)		40 (22.5)	138 (77.5)		[40]
Kazakhstan	856 (97.8)	18 (2.2)	863 (97.2)	25 (2.8)		142 (27.4)	376 (72.6)		[41]
Russia	217 (97.8)	5 (2.2)	210 (94.6)	12 (5.4)		148 (66.7)	74 (33.3)		[42]
South Indian	163 (99.4)	1 (0.6)	149 (90.9)	15 (9.1)		141 (86.0)	23 (14.0)		[43]
North Indian	396 (94.7)	22 (5.3)	374 (89.5)	44 (10.5)		340 (81.3)	78 (18.7)		
Caucasian	197 (85.7)	33 (14.3)	205 (89.1)	25 (10.9)		133 (57.8)	97 (42.2)		[54]

safety and efficiency. So, compared with traditional dosing (starting dose 2.5 or 3 mg/day), in conjunction with other indicators such as PT and INR, a better guide for determining dosage could be provided. Nonetheless, 43% of follow-up patients fell outside the 20% range, which serves as a reminder that a new algorithm that is better tailored to Chinese populations is needed. Different form Gu 's and Li 's study, additional SNPs such as EPHX1 rs4653436 should be detected in order to meet requirements of the newest genetic algorithm explain higher ratio of individual differences in warfarin daily dose requirements, and predict optimal therapeutic warfarin doses in this study. New IWPC algorithm suggested to increase more SNP detected sites include *GGCX* rs11676382 and *CYP4F2* V433M, we 'll study in the further. In addition, pill dose sizes of warfarin in China were 2.5 mg/piece or 3 mg/piece, and this size is not convenient to people to take warfarin with the recommendation dosage calculated by IWPC algorithm in the study. Therefore, a pill dose size such as 2 mg corresponding to the result calculated by IWPC algorithm is needed.

In summary, the present study assessed the analytic and clinical performance of an electrochemical warfarin biosensor, a DNA test that provides rapid and simple genotyping of three SNPs that affect warfarin dosing. However, as with PCR, the accuracy of results can be affected by human operation error and sample contamination. For this study, electrode quality also can influence results, as evidenced by the two false negative results produced by the electrochemical DNA biosensor. Therefore, novel electrode systems need to be improved to accomplish the detection task. In those cases, the presence of scratches in gold electrode likely damaged the circuit lead to overload, and no electrochemical signal was detected. Meanwhile, novel signal data analysis tools [59] can be deployed on biosensor systems in the further study lead to better in suppressing the impact of background. Automation can be introduced to reduce the workload of detection process [60, 61]. Despite these concerns, the electrochemical DNA biosensor has great development potential. More SNPs associated with warfarin or other drugs, as well as other pathogens or disease genomics, can be detected using electrochemical DNA biosensors. Combining biosensors with other technologies such as branch DNA and isothermal nucleic acid amplification can simplify detection further such that target DNA can be quantitatively analyzed without the need for PCR amplification.

Conclusion

The present study assessed the analytical and clinical performance of an electrochemical biosensor to provide the rapid and simple method genotyping of three SNPs that affect warfarin dosing. A warfarin dosing algorithm based on data gathered from a large patient cohort can facilitate the reasonable and effective use of warfarin in Chinese patients with atrial fibrillation.

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Compliance with ethical standards

Conflict of interest The authors declared that there is no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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