Correlation between CYP2C9 gene polymorphism and warfarin dose in Chinese Han population with coronary heart disease

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ABSTRACT

This study aimed to explore the correlation between CYP2C9 gene polymorphism and warfarin dose in Chinese Han patients with coronary heart disease. The selection was made to the analysis of blood coagulation test of Chinese Han ethnic group, 200 cases of coronary heart disease patients as target group, the patients were satisfied taking warfarin INR target (in the range of 2.0 ~ 3.0), the other to select 200 cases proved by physical examination health as health group. The warfarin dose and the clinical laboratory INR after warfarin administration were taken, and the DNA of the patients was extracted and tested. The CYP2C9 genotype and allele frequency of patients in the standard group were observed, and the basic information and gene test results of the healthy group and the standard group were compared. Patients in the standard group were grouped according to the CYP2C9 and VKORC1 genotype, and the warfarin dosage was compared respectively. The number of cases of anticoagulant overdose in patients with different genotypes in the target group at the initial stage of warfarin treatment was compared. Results showed that CYP2C9*1/*2, *2/*2, *2/*3, *3/*3 genotypes were not found by detecting CYP2C9 genotype and allele frequency in the standard group. There were no significant differences in genotype and allele frequency between the standard group and the healthy group (P BBB 0 0.05). The mean daily dose of the CYP2C9*1/*3 genotype was lower than the mean daily dose of warfarin required by the CYP2C9*1/*1 genotype, and the difference was statistically significant (P < 0.05). There were significant differences in the incidence of anticoagulation overdose at the initial stage of treatment among different CYP2C9 genotypes (P < 0.05), and the incidence of an anticoagulation overdose in group *1/*3 was significantly higher than that in group *1/*1. In general, There was a correlation between CYP2C9 gene polymorphism and warfarin dose in Chinese Han population with coronary heart disease. Compared with the wilD-type (*1/*1) enzyme, the metabolic ability of warfarin in mutant CYP2C9 enzyme (mainly *1/*3 in Chinese population) decreases, and the patients were sensitive to warfarin treatment, and the dose required decreases.

Introduction

The highest incidence rate of coronary heart disease (CHD) was 108.7/100 000 in Qingdao, Shandong province, and the lowest was 3.3/100 000 in Chuzhou, Anhui Province (1). There were significant regional differences, and the northern provinces were generally higher than the southern provinces. The prevalence of CHD was 1.59% in urban areas, 0.48% in rural areas, and 0.77% in total, showing an increasing trend. Coronary heart disease is the leading cause of death in the United States and many developed countries (2). However, the United States has seen a downward trend in CHD mortality since the 1960s. Efforts to reduce risk factors for CORONARY heart disease in the United States in the 1960s and 1980s focused on controlling risk factors and improving the treatment of myocardial infarction. In 2009, the death rate of coronary heart disease among Urban residents was 94.96 per 100,000, and that in rural areas was 71.27 per 100,000, higher in urban areas than in rural areas, and higher in men than in women (1). The treatment of coronary heart disease includes Lifestyle change: smoking cessation, alcohol restriction, low fat and low salt diet, appropriate physical exercise, weight control and so on; Drug therapy: antithrombotic (antiplatelet, anticoagulation), reduce myocardial oxygen consumption (β blocker), relieve angina pectoris (nitrate), lipid stabilization

plaque (statin lipid-regulating drugs); Revascularization therapy: including interventional therapy (endovascular balloon dilatation and stenting) and surgical coronary artery bypass grafting (1, 3). Medication is the basis of all treatment. Interventional and surgical treatment should also be followed by long-term standard medication. For the same patient, drugs are ideal for control at one stage of the disease, but at another stage drug therapy alone is often ineffective and requires a combination of drugs with interventional therapy or surgery (1, 4). The purpose of drug therapy is to relieve symptoms, reduce the onset of angina pectoris and myocardial infarction, delay the development of coronary atherosclerotic lesions, and reduce death from coronary heart disease (4). Standardized drug therapy can effectively reduce the mortality and reischemia events of patients with CORONARY heart disease, and improve the clinical symptoms of patients. For some patients with severe or even complete vascular obstruction, vascular reconstruction therapy can further reduce the mortality of patients on the basis of drug therapy (5, 6). Warfarin is an ideal drug for the treatment of coronary heart disease (7).

Warfarin is an indirect coumarin oral anticoagulant, which plays an anticoagulant role by inhibiting the synthesis of coagulation factors ii, vii, ix and x of vitamin K in the liver cells. Carboxylase in liver mitochondria can convert glutamate of the above clotting factors to γ -carboxylglutamate, which binds to calcium ions to exert its clotting activity (8). The pharmacokinetic parameters of warfarin are stable and superior to other oral anticoagulants, but the individual dose of warfarin varies greatly, and the dose required by different individuals to achieve the same anticoagulant effect can vary by more than 10 times (7).

The dose-response (international normalized ratio) relationship for warfarin is highly variable and is influenced by a number of factors, including genetics, age, weight, medication, and liver function (8). Cytochrome P450(CYP)2C9 is an important liver drug enzyme of Xie Warfarin, which has genetic polymorphism. It has two important single nucleotide polymorphisms (SNPs), CYP2C9.2 and CYP2C9.3, which encode 16%-20% and 4%-6% of the activity of the wild type enzyme in the homozygote and 80%-85% and 34%-37% of the wild type enzyme in the heterozygote, respectively (9). The mutant CYP2C9 enzyme has a reduced ability to metabolize warfarin, showing sensitivity to warfarin and an increased risk of bleeding (10). This study aims to investigate the correlation between CYP2C9 gene polymorphism and warfarin dose in Patients with coronary heart disease in Han Chinese population by comparing the warfarin maintenance dose required by patients with different genotypes to achieve the stability of international standardized ratio (INR), so as to provide an adjuvant basis for the clinical use of warfarin.

Materials and methods
General Information
A total of 200 Chinese Han patients with CORONARY heart disease who underwent coagulation analysis in our hospital from March 2020 to March 2021 were selected as the standard group. All the patients met the standard of receiving warfarin INR (within the range of 2.0-3.0), including 115 males and 85 females, aged 36-92 years. Another 200 healthy patients, including 102 males and 98 females, aged 36~80 years, were selected as the healthy group. There was no statistical significance in the general information of patients (P > 0.05). All patients and their families were fully informed of the contents of this study and signed informed consent. All subjects were not related to each other.

Inclusion and exclusion criteria
Inclusion criteria :(i) ethnic han; (ii) Patients have good compliance and can cooperate to complete the investigation; (iii) Complete clinical data of patients; (iv) The patients and their families are aware of this study and sign the consent form; (v) no consciousness or mental abnormality; (vi) warfarin treatment is acceptable; (vii) Patients in the standard group had stable anticoagulation for at least 3 months.

Exclusion criteria :(i) patients with other serious underlying diseases; (ii) Liver has serious organic lesions; (iii) with functional insufficiency of vital organs; (iv) Recent use of drugs or foods that have significant interactions with warfarin; (v) Severe alcohol abuse; (vi) merges the consciousness disorder, the mental disorder, the compliance is poor; (vii) Incomplete clinical data of patients; (viii) The patient was under 18 years of age.

Methods

(i) Specimen collection: 2 ml of EDTA anticoagulant venous blood was collected from selected subjects. Anticoagulant whole blood should be stored at room temperature for no more than 7 days, at 2-8 °C for no more than 1 month, and at -20 °C for no more than 7 weeks. The blood sample was freeze-thawed no more than 5 times. The electronic medical record system of Peking University People's Hospital was used to record the age, sex and other related information of the enrolled patients, warfarin dosage and clinical laboratory index INR after warfarin administration.

(ii) Detection method: DNA extraction Kit (TIANamp Blood DNA Kit DP318) was used for the extraction of Blood genomic DNA. The concentration and purity of extracted DNA should be determined by UV spectrophotometer, and the A260/280 of DNA should be between 1.8 and 2.0.

(iii) INR value: Automatic coagulation analyzer (East Asia, Japan) and transmission turbidimetric optical method (reagents human placental thrombin and CaCl2, etc.) were used to determine the INR value. Standard and quality control samples are attached to each batch of sample determination to ensure the accuracy and reliability of the measured INR value.

Observation Indicators

(i) CYP2C9 genotype and allele frequency were observed in the qualified group.

(ii) The basic situation and genetic test results of the healthy group and the standard group were compared.

(iii) Patients in the standard group were grouped according to CYP2C9 and VKORC1 genotypes, respectively, and warfarin dosage was compared.

(iv) The number of cases of anticoagulation overdose (INR≥ 4.0) in patients with different genotypes in the standard group during the initial stage of warfarin treatment (from the beginning of medication to the stabilization of INR value) was compared.

Statistical Analysis

In the form of an Excel datasheet, the number, sex, age and genotype results of the subjects were input one by one. After verification, the data were transferred to SPSSl0.0 statistical software for processing and analysis, and the frequencies of each genotype and allele were calculated. PearsonX2 test was used to verify whether the data complied with Hardyweinberg genetic balance law. The age of patients and healthy subjects were compared by a two-sample T-test, and the sex and genotype frequency were compared by X2 test. Bilateral $P < 0.05$ was considered statistically significant.

Results and discussion

CYP2C9 genotype and allele frequency in the qualified group

The CYP2C9 genotype and allele frequency of patients in the standard group are shown in Table 1. CYP2C9*1/*2, *2/*2, *2/*3 and *3/*3 genotypes were not found. According to Hardy-Weinberg genetic balance law, THE STATISTICAL software SPSSl0.0 was used to perform χ^2 test for CYP2C9 genotype frequency, and the P values were all greater than 0.05, indicating that it conforms to Hardy-Weinberg genetic balance law.

Table 1. Comparison of CYP2C9 genotype and allele frequency in the qualified group; A) Genotype, B) Genotype frequency (%), C) allele frequency (%), C) Hardy-Weinberglaw of genetic balance P

A	n	B		C	
*1/*1	183	91.61	*1	95.81	
*1/*2	0	0	*2	0	0.864
*1/*3	17	8.41	*3	4.21	
*2/*2	0	0			
*3/*3	0	0			
*2/*3	0	0			

Basic information and genetic test results of the healthy group and the standard group

There was no significant difference in genotype and allele frequency between the standard group and the healthy group (P > 0.05), as shown in Table 2.

Warfarin doses of patients in the standard group grouped by different genotypes

The average daily dose of patients with CYP2C9*1/*3 genotype was lower than that of patients with CYP2C9*1/*1 genotype, and the difference was statistically significant (P < 0.05), as shown in Table 3.

Table 2. Comparison of the basic situation and genetic test results between healthy group and standard group

Characteristics of the situation		Healthy group (n=150)	Standard group (n=150)	P
Age (years)		46.51 ± 1.32	60.35 ± 1.64	
Gender	Male	102	115	0.448
	Female	98	85	
CYP2C9 genotypes	*1/*1	181	183	0.633
	*1/*2	1	0	
	*1/*3	18	17	
CYP2C9 allele frequency	*1	95.2	95.8	0.635
	*2	0.2	0	
	*3	4.5	4.2	

Table 3. Warfarin dosage of patients with different genotypes in the standard group

CYP2C9 genotypes	*1/*1	*1/*3	t	P
n	183	17	18.453	0.023
Warfarin dose (mg)	3.90 ± 1.64	2.90 ± 1.31	16.457	0.031

Comparison of warfarin dose of different genotypes with that recommended by FDA

The correlation between the results of different genotypes and warfarin dosage in this experiment was basically consistent with the reference table of warfarin dosage for different genotypes recommended by THE US FDA, as shown in Figure 1.
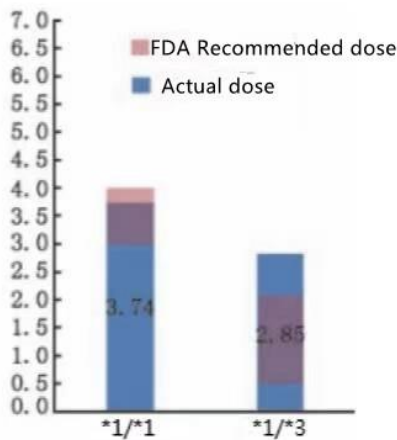

Figure 1. Comparison of warfarin dose of different genotypes with the recommended dose of FDA

Comparison of warfarin anticoagulation overdosage at the initial stage between different genotypes

The incidence of anticoagulation overdosage at the initial stage of treatment among different CYP2C9 genotypes was statistically significant (P < 0.05). The incidence of anticoagulation overdosage in *1/*3 groups was significantly higher than that in *1/*1 group, as shown in Table 4.

Table 4. Comparison of warfarin anticoagulation overdosage at the initial stage between different genotypes

CYP2C9 genotypes	Anticoagulation excess (N)	No excess anticoagulation (N)	t	P
*1/*1	15	168	19.652	0.013
*1/*3	13	12		
Total	28	180		

Coronary atherosclerotic heart disease is the coronary artery vascular atherosclerotic lesions caused by stenosis or obstruction of the vascular lumen, resulting in myocardial ischemia, hypoxia or necrosis of heart disease often called "coronary heart disease" (11). However, the scope of coronary heart disease may be broader, including inflammation, embolism and other causes of lumen stenosis or occlusion. The World Health Organization divides CORONARY heart disease into 5 categories: asymptomatic myocardial ischemia (occult coronary heart disease), angina pectoris, myocardial infarction, ischemic heart failure (ischemic heart disease) and sudden death (12). Clinically, it is often divided into stable coronary artery disease and acute coronary syndrome (13). Warfarin is a commonly used drug in the clinical treatment of coronary heart disease, but the dose-response (international standardized ratio) relationship of warfarin is highly variable and influenced by many factors, so it needs to be closely monitored (14). The dot-effect relationship of warfarin is affected by genetic and environmental factors, including the mutation of the cytochrome P450 gene site (15). Since the first report on the correlation between CYP2C9 gene polymorphism and the use of anticoagulant therapy, many studies have shown that CYP2C9 gene polymorphism is the main influencing factor of

effective warfarin dose, which can explain most of the individual warfarin dose differences (3, 14-16).

Polymorphism of CYP2C9 gene in open code region exists. As of 2007, more than 30 SNPs have been discovered, and the earliest, most studied and most common mutations are CYP2C9-2 and CYP2C9-3 (17). The CYP2C9·2 mutation has a 430C > T transition on exon 3, and the corresponding amino acid residue Ar9144 replaces Cysl44. In vitro, its homozygous activity is 12% of that of the wild type (18). The wild type CYP2C9·1 allele encodes Ava ii restricted sites, and CYP2C9·2 eliminated Ava ii recognized sites, so it could not be digested by Ava ii. In vitro, the homozygous activity of CYP2C9·3 was only 5% of that of the wild type with 1075A > C transformation on exon 7 and the corresponding amino acid residue ILe359Leu. Compared with the wild type, CYP2C9·3 causes a loss of NsiI restriction site and therefore cannot be digested by NsiI (19). In vivo studies of S-warfarin showed that cyp2C9.3 mutation significantly reduced the oral clearance rate of warfarin, and the metabolic capacity of mutant homozygotes and mutant heterozygotes decreased by 90% and 66%, respectively, compared with the wild type (18).

Oral warfarin has been shown to be effective in the treatment and prevention of venous or arterial embolic disease (15). However, due to the narrow therapeutic window, the response to the same dose of warfarin can vary greatly from individual to individual: some people have no significant anticoagulant effect, while others have severe adverse reactions due to excessive anticoagulation. In clinical practice, the dosage of warfarin is generally adjusted according to the detection results of PT-IRN value (19, 20). However, due to the mutation of coagulation factor ix, there are still a few patients who can cause bleeding even though PT has no obvious extension. Therefore, there is a certain lag in determining the dosage of warfarin only by measuring PT-IRN value (19). In recent years, it has been reported that CYP2C9 and other genes are highly correlated with the dose and sensitivity of warfarin in different individuals (21). Detection of CYP2C9 genotype to predict the maintenance dose of warfarin may be a means to reduce or avoid serious adverse reactions and improve patient compliance (22).

The results of this study showed that CYP2C9*1/*2, *2/*2, *2/*3, *3/*3 genotypes were not detected in CYP2C9 genotype and allele frequency detection of patients in the standard group, and they were consistent with Hardy-Weinberg law of genetic balance, which proved that the subjects included in this study were consistent with the existing investigation. With reference. The results in the standard set of genotype and allele frequency and health groups had no statistical difference, indicating that the distribution of genotypes and alleles and the growth of the age or the risk of thrombosis has no obvious correlation, standard group of patients with normal genotype distribution of the han Chinese population is consistent, guarantee the unbiasedness of sample selection. The results of this study showed that the average daily dose of CYP2C9*1/*3 genotype patients was lower than the average daily dose of warfarin required by CYP2C9*1/*1 genotype patients, and the difference was statistically significant, proving that the polymorphism of the CYP2C9 gene was related to the dose of warfarin in CHD patients in Chinese Han population. In addition, the correlation between the results of different genotypes and warfarin dosage in this study was basically consistent with the reference table of warfarin dosage for different genotypes recommended by THE US FDA, which proved the reliability of the data in this study and again verified the influence of CYP2C9 gene polymorphism on individual warfarin dosage. In other words, compared with the wild type (*1/* 1), the mutant CYP2C9 enzyme (*1/*3) had a weaker metabolic capacity of Warfarin, and the patients were more sensitive to warfarin treatment.

A study showed that the CYP2C9*2 mutation and CYP2C9*3 have similar effects on reducing warfarin maintenance dose, and the polymorphism of the CYP2C9 gene is of great significance for the precise use of warfarin (23). Since no allele cyp2C9.2 was detected in the 200 patients enrolled, only polymorphism of CYP2C9· L and CYP2C9·3 genes were included in the statistics. In this study, the CYP2C9 genotype explained about 4.2% of the individual dose differences. The average dose of *1/*3 patients was about 23.2% lower than that of *1/*1 patients, which was lower than the results of previous studies by domestic scholars. This may be due to the inconsistent ratio of the target value range

of the two selections. The value range selected in this study was mostly between 2.0 and 3.0, while the range of canthus selected in previous studies was mostly between 1.5 and 2.5. In this study, there was a statistically significant difference in the incidence of anticoagulant overdose among different CYP2C9 genotypes at the initial stage of treatment, and the incidence of anticoagulant overdose in the *1/*3 group was significantly higher than that in the *1/*1 group. It also suggested that the mutant CYP2C9 enzyme (mainly *1/*3 in the Chinese population) was compared with the wild type (*1/*1) enzyme, The metabolic capacity of warfarin decreased, the patients were sensitive to warfarin treatment, and the dose required was reduced.

The sample size included in this study is small, but the study results have suggested that the detection of related genotypes of patients before the prescription of warfarin will have a positive impact on clinical anticoagulant therapy. In the future, a large sample database can be constructed, the case information can be improved, the follow-up time can be extended, and the analysis can be combined with the clinical endpoint events to establish a warfarin administration model suitable for the Han Chinese population, so as to strengthen the treatment of coronary heart disease.

In conclusion, CYP2C9 gene polymorphism is correlated with warfarin dose in CHD patients in the Chinese Han population. Compared with the mutant CYP2C9 enzyme (*1/* 3 in the Chinese population) and the wild type (*1/*1) enzyme, patients with a reduced metabolic capacity of Warfarin are more sensitive to warfarin treatment and need a lower dose.

Acknowledgments

Interest conflict
The authors declare no conflict of interest.

References
1. Gao K, Wu Z, Liu Y et al. Risk of coronary heart disease in patients with periodontitis among the middled-aged and elderly in China: a cohort study. BMC Oral Health 2021; 21(1): 1-8.
2. Dorje T, Zhao G, Scheer A et al. SMARTphone and social media-based Cardiac Rehabilitation and Secondary Prevention (SMART-CR/SP) for patients with coronary heart disease in China: a randomised controlled trial protocol. BMJ Open 2018; 8(6): e021908.
3. Ercisli MF, Lechun G, Azeez SH, Hamasalih RM, Song S, Aziziaram Z. Relevance of genetic polymorphisms of the human cytochrome P450 3A4 in rivaroxaban-treated patients. Cell Mol Biomed Rep 2021; 1(1): 33-41.
4. Wang X, Jiang Y, Bai Y et al. Association between air temperature and the incidence of acute coronary heart disease in Northeast China. Clin Interv Aging 2020; 15: 47.
5. Bilal I, Xie S, Elburki MS, Aziziaram Z, Ahmed SM, Jalal Balaky ST. Cytotoxic effect of diferuloylmethane, a derivative of turmeric on different human glioblastoma cell lines. Cell Mol Biomed Rep 2021; 1(1): 14-22.
6. Lu W-H, Zhang W-Q, Sun F et al. Correlation between Occupational Stress and Coronary Heart Disease in Northwestern China: A Case Study of Xinjiang. Biomed Res Int 2021; 2021.
7. Pengo V, Denas G, Zoppellaro G et al. Rivaroxaban vs warfarin in high-risk patients with antiphospholipid syndrome. Blood, J Am Societ Hematol 2018; 132(13): 1365-1371.
8. Robinson AA, Trankle CR, Eubanks G et al. Off-label use of direct oral anticoagulants compared with warfarin for left ventricular thrombi. JAMA Cardiol 2020; 5(6): 685-692.
9. Sausville LN, Gangadhariah MH, Chiusa M et al. The cytochrome P450 slow metabolizers CYP2C9* 2 and CYP2C9* 3 directly regulate tumorigenesis via reduced epoxyeicosatrienoic acid production. Cancer Res 2018; 78(17): 4865-4877.
10. Maekawa K, Adachi M, Matsuzawa Y et al. Structural basis of single-nucleotide polymorphisms in cytochrome P450 2C9. Biochemistry 2017; 56(41): 5476-5480.
11. Zhu Y, Yang T, Duan J, Mu N, Zhang T. MALAT1/miR-15b-5p/MAPK1 mediates endothelial progenitor cells autophagy and affects coronary atherosclerotic heart disease via mTOR signaling pathway. Aging 2019; 11(4): 1089.
12. Xing J, Liu Y, Chen T. Correlations of chemokine CXCL16 and TNF-α with coronary atherosclerotic heart disease. Exp Ther Med 2018; 15(1): 773-776.

13. Zhang Y, Huang J, Yang X et al. Altered Expression of TXNIP in the peripheral leukocytes of patients with coronary atherosclerotic heart disease. Medicine 2017; 96(49).

14. Burn J, Pirmohamed M. Direct oral anticoagulants versus warfarin: is new always better than the old? Open Heart 2018; 5(1): e000712.

15. Coons JC, Albert L, Bejjani A, Iasella CJ. Effectiveness and safety of direct oral anticoagulants versus warfarin in obese patients with acute venous thromboembolism. J Human Pharmacol Drug Ther 2020; 40(3): 204-210.

16. Aziziaram Z, Bilal I, Zhong Y, Mahmod AK, Roshandel MR. Protective effects of curcumin against naproxen-induced mitochondrial dysfunction in rat kidney tissue. Cell Mol Biomed Rep 2021; 1(1): 23-32.

17. Ahmed S, Altaf N, Ejaz M et al. Variations in the frequencies of polymorphisms in the CYP2C9 gene in six major ethnicities of Pakistan. Sci Rep 2020; 10(1): 1-8.

18. Daly AK, Rettie AE, Fowler DM, Miners JO. Pharmacogenomics of CYP2C9: functional and clinical considerations. J Person Med 2018; 8(1): 1.

19. Al-Eitan LN, Almasri AY, Khasawneh RH. Impact of CYP2C9 and VKORC1 polymorphisms on warfarin sensitivity and responsiveness in Jordanian cardiovascular patients during the initiation therapy. Genes 2018; 9(12): 578.

20. Kazemi E, Zargooshi J, Kaboudi M et al. A genome-wide association study to identify candidate genes for erectile dysfunction. Brief Bioinformatics 2021; 22(4): bbaa338.

21. Saberi M, Ramazani Z, Rashidi H, Saberi A. The effect of CYP2C9 genotype variants in type 2 diabetes on the pharmacological effectiveness of sulfonylureas, diabetic retinopathy, and nephropathy. Vasc Health Risk Manag 2020; 16: 241.

22. Al-Eitan LN, Almasri AY, Khasawneh RH. Effects of CYP2C9 and VKORC1 polymorphisms on warfarin sensitivity and responsiveness during the stabilization phase of therapy. Saudi Pharma J 2019; 27(4): 484-490.

23. Yang F, Liu L, Chen L et al. OATP1B3 (699G> A) and CYP2C9* 2,* 3 significantly influenced the transport and metabolism of glibenclamide and glipizide. Sci Rep 2018; 8(1): 1-9.