Int J Clin Exp Pathol 2020;13(11):2831-2839
www.ijcep.com /ISSN:1936-2625/IJCEP0116659

Original Article

Personalized warfarin treatment based on the PITX2 single nucleotide polymorphism rs6843082

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Received June 20, 2020; Accepted September 1, 2020; Epub November 1, 2020; Published November 15, 2020

Abstract: Objective: To explore the effect of PITX2 gene rs6843082 single nucleotide polymorphism on the efficacy and adverse reactions of warfarin in patients with atrial fibrillation and hypertension, and to provide a theoretical basis for individualized warfarin treatment. Methods: Data on 97 patients with atrial fibrillation and hypertension treated in our hospital were collected from September, 2018 to December, 2019. PCR and SNP genotyping techniques were used to measure the genotype at the rs6843082 locus (pituitary homeobox 2, PITX2) using DNA from the peripheral blood cells of all patients. We compared the efficacy of warfarin and the incidence of adverse reactions in patients of different genotypes. Results: (1) Among 97 subjects, 58 cases (59.79%), 32 cases (32.99%) and 7 cases (7.22%) of PITX2 (rs6843082) genotypes GG, GA and AA were identified respectively. The G and A allele frequencies were 76.29% and 23.71%, respectively. (2) After all patients took warfarin to achieve the standard, the GA group and AA group's time to achieve the standard was significantly longer than that of the GG group ($P<0.05$). The difference was not statistically significant among groups ($P>0.05$). Compared with the GG group, the maintenance dose of the AA group was increased ($P<0.05$). (3) Compared with the GG and the GA group, the probability of bleeding events was higher in the AA group ($P<0.05$). (4) There was no difference in left ventricular end diastolic volume (LVEDV) and left ventricular end systolic volume (LVESV) group among GG, GA and AA groups ($P>0.05$). Compared with the GG group, left ventricular ejection fraction (LVEF) of the AA group was significantly reduced ($P<0.05$). (5) The mortality rates of the GG, GA, and AA groups were 15.51%, 12.50% and 22.57%, respectively, at the end of 120 d follow-up. Conclusion: Our findings show that rs6843082 SNP leads to the warfarin dose response differences that were observed in patients with atrial fibrillation and hypertension. Genotyping patients for rs6843082 before initiating warfarin treatment may optimize the treatment response and reduce bleeding incidence.

Keywords: PITX2, single nucleotide polymorphism, warfarin

Introduction

Atrial fibrillation is the most common type of persistent arrhythmia. With an increase in age, the risk of heart fibrillation increases [1], and is twice as common in men compared to women [2]. Hypertension is a risk factor for atrial fibrillation and is considered an aggravating factor in existing atrial fibrillation. Early studies have shown that in 50%-85% of patients, atrial fibrillation is associated with hypertension [3, 4]. Atrial fibrillation combined with hypertension increases the risk of cardiac fibrillation complications such as ischemic stroke [5], heart disease, and bleeding events.

Despite the increased hemorrhage risk, the benefits of anticoagulation therapy for stroke risk management in atrial fibrillation patients are well-recognized. The recent European Heart Rhythm Association [6] and the American guidelines for the management of patients with atrial fibrillation [7] both point out the need for anticoagulant therapy in patients with atrial fibrillation. Warfarin is currently the most commonly used oral anticoagulant.

Warfarin has a long history of clinical use. It is well established that warfarin has a very narrow therapeutic window and excess dosage can easilycause bleeding. Moreover, individual responses to warfarin treatment are highly variable. In order to improve the effectiveness of warfarin anticoagulant therapy and reduce adverse drug reactions, it is necessary to better understand the causes of thisdrug response variation. Gene polymorphism is considered the primary reason for individual differences in

warfarin response [8]. A recent genome wide association study (GWAS) showed that, a single nucleotide polymorphism (SNP) near the pituitary homeobox 2 (PITX2) locus-rs6843082-was closely related to the sensitivity of atrial fibrillation [9, 10]. At present, there are no data on rs6843082 and the anticoagulant effect of warfarin in patients with heart fibrillation and hypertension. Our studycompared warfarin efficacyin elderly patients with atrial fibrillation and hypertension genotyped for the rs6843082 SNP. We hypothesized that there would be differences in the warfarin response and/or adverse reaction rate among the three rs6843082 genotypes.

Materials and methods

Study population

Clinical data were collected on n=97 patients with atrial fibrillation and hypertension who were treated in our hospital from September 2018 to December 2019. Inclusion criteria were: (1) over 60 years old; (2) clinically diagnosed atrial fibrillation and hypertension; and (3) no recent treatment with warfarin.

Exclusion criteria were: (1) active bleeding or history of bleeding: intracranial hemorrhage, active ulcer, bronchiectasis, etc.; (2) suffering from malignant tumor, severe liver, or kidney dysfunction, blood diseases, or presence of serious infection; (3) Non-cooperation with treatment; and (4) incomplete clinical data. The selected patients were all of Han Chinese ethnicity and had no genetic relationships with each other. According to rs6843082 genotyping results, three genotypes at the locus were GG, GA, and AA Written informed consents were obtained from all participants and the study was approved by the Institutional Review Board of Shidong Hospital, Yangpu District, Shanghai.

Warfarin administration regimen

All patients take warfarin sodium tablets (Qilu Pharmaceutical, China) orally with an initial dose of 2.5 mg. The preset target value of international normalized ratio (INR) is 1.5-2.5. On the 3rd day after taking the drug, INR is measured and the dose is adjusted according to: INR<1.5, dose is increased; INR>2.5, dose is reduced. Subsequently, the dose is gradually increased or decreased according to the range of (0.625-1.25 mg/d), until target INR value is attained. The patient does not need to adjust the dose during the second review and the INR can reach the preset value which can be considered to reach a stable state of anticoagulation. Only patients who reach a stable state (target INR) can be discharged. After discharge, patients continue to take warfarin to maintain anticoagulation therapy.

Genotype detection

Blood collection was performed on all patients who fasted in the early morning of the second day of admission, and about 2 ml of venous blood was collected from the antecubital space of the arm into a vacuum tube containing anticoagulant. The blood sample was then centrifuged at 3000×g for 10 min, then placed at 4°C for 30 min, and stored at -20°C. Genomic DNA was extracted from the buffy coat using a genomic DNA extraction kit (AmyJet, China). The primer design and primer synthesis for genotyping at the rs6843082 site was performed by Shanghai Bioengineering Technology Service Co., Ltd. (China), and the genotype determination step was performed by Beijing Bomiao Biotechnology Co., Ltd. (China). An ABI-3730 DNA Analyzer (Thermofei, USA) was used for genotyping. Briefly, the main steps were undertaken as follows: (1) Primer design: forward primer: 5'-ACGTTGGATGGGTGTCCTGGGATTTGTATG-3', reverse primer: 5'-ACGTTGGATGACTGTTGGTGATGAGTGGTG-3', single base extension primer: 5'-CCCCCTGGTGCATAACAGCC-3'; (2) PCR amplification: PCR reaction system (25 μL) with PCR amplification solution, PCR reaction solution and DNA template; (3) PCR product purification reaction: 3 μL of PCR product was drawn and exo/sap treatment was used for purification. The main purpose was to remove the remaining primers and free dNTPs in the reaction product; (4) extension reaction and; (5) Chip deposition, detection and genotype determination. In order to control the quality of the test, 10% of DNA samples were randomly selected for repeat testing. The compliance rate reached 100%.

Observation indicators

Patient's age, gender, and BMI were recorded. A calibrated mercury column sphygmomanometer was used to measure systolic blood pressure (SBP) and diastolic blood pressure (DBP)

Table 1. Clinical information about the patients

Characteristics	Participants (n=97)
Age (year)	65.32±7.16
Sex (%)	
male	51 (52.58)
female	46 (47.42)
BMI (kg/m^2)[1]	22.46±1.18
Smoker n (%)	22 (22.68)
systolicblood pressure (mmHg)	141.52±7.86
diastolicblood pressure (mmHg)	93.15±4.19

[1]BMI is the Body Mass Index.

at the patient's right arm. The patient's anticoagulant stable maintenance dose, INR change, INR value, and time to reach the standard after taking warfarin were recorded. Adverse drug reaction events, such as bleeding or embolism were also recorded at the beginning of anticoagulation therapy. Six months after taking warfarin, color Doppler ultrasound diagnosis (ALOKA-a-7, Japan) was used to measure the left ventricular function of patients. The left ventricular end diastolic volume (LVEDV), left ventricular end systolic volume (LVESV), and left ventricular ejection fraction (LVEF) were selected as parameters. Patients were followed up for 120 days and survival status was recorded.

Statistical analysis

The measurement data were expressed as mean ± standard deviation. If the data distribution satisfied the normal distribution, single-factor analysis of variance was used for comparison between the 3 groups. Non-parametric test was used for inter-group analysis; count data were recorded in the form of rate (%), and comparison between the two groups was analyzed by χ^2 test. The significance level was defined as $P<0.05$. In addition, the χ^2 test was used to confirm that the PITX2 (rs6843082) genotype and allele frequency distributions were in Hardy-Weinberg equilibrium. SPSS 20.0 was used for statistical analysis. GradpadPrism 6.0 software was used for creating figures.

Results

Subject characteristics

A total of 97 patients were recruited in this study, including 51 males (52.58%) and 46 females (47.42%). The patients were 60-81 years old (65.3±7.2 years old) with an average BMI of (22.5±1.2) kg/m^2. Twenty-two of them had a history of smoking. The patients all had blood pressures that were higher than 140/90 mmHg (**Table 1**).

PITX2 (rs6843082) genotype and allele frequency distribution

From the genotyping results, 58 (59.79%), 32 (32.99%) and 7 (7.22%) of the sample had the PITX2 (rs6843082) genotypes GG, GA and AA, respectively. Frequencies of G and A alleles were 76.29% and 23.71%; the expected genotypes GG, GA, and AA were 56 (57.73%), 35 (36.08%) and 6 (6.46%), respectively. The distribution of genotype and allele frequencies were in accordance with Hardy-Weinberg equilibrium ($\chi^2=0.753$, $P=0.385$) (**Table 2**; **Figure 1**).

The effect of different genotypes on warfarin anticoagulation

Warfarin was used for anticoagulation treatment in all patients. When the stable anticoagulation state was reached, the INR time of the GG, GA and AA group were found to be (10.15±2.05, 11.42±2.9 and 13.20±2.00) d. The GA and AA group INR times were significantly longer than GG group ($P<0.05$). INR values among the three groups were not statistically different ($P>0.05$). Maintenance doses of warfarin in the GG, GA, and AA groups were 2.92±0.36, 3.07±0.39 and 3.30±0.31 mg/d, respectively. Compared with the GG group, the maintenance dose of AA group was higher ($P<0.05$) (**Table 3**; **Figure 2**).

Comparison of adverse drug reaction incidence in patients with different genotypes in the early stage of anticoagulant therapy

In the early stages of anticoagulation treatment with warfarin, patients were prone to have adverse reaction. Due to the low INR preset standard, the total number of patients with bleeding was relatively small, mostly minor bleeding. Also, intracranial bleeding and other serious bleeding events were uncommon. Total bleeding events in the GG, GA, and AA groups were 8 (13.79%), 4 (12.50%) and 4 (57.14%), respectively. Compared with GG and GA group, the AA group had a higher rate of bleeding

Table 2. PITX2 (rs6843082) genotype and allele frequency distribution

Gene	SNP	genotype	frequency n (%)	allele	frequency n (%)	P_{HWE}[3]
PITX2	rs6843082	GG	58 (59.79)	G[1]	148 (76.29)	0.385
		GA	32 (32.99)	A[2]	46 (23.71)	
		AA	7 (7.22)			
		Total	97 (100.00)	Total	194 (100.00)	

[1]G is the major allele, [2]A is the minor allele, [3]P_{HWE} is the Hardy-Weinberg equilibrium test.

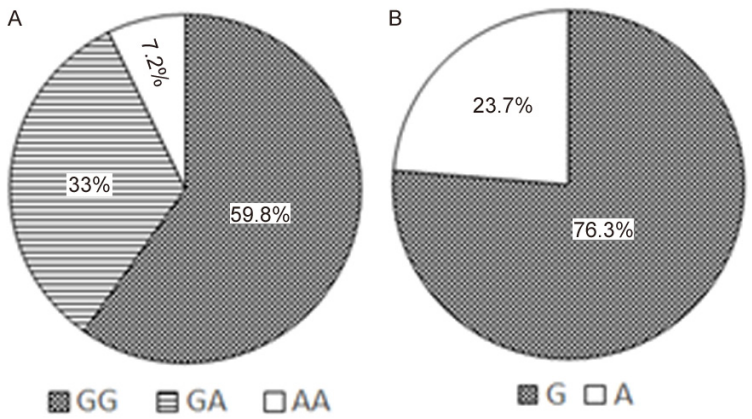

Figure 1. PITX2 (rs6843082) genotype and allele frequency distribution A: PITX2 (rs6843082) locus genotype frequency distribution. B: PITX2 (rs6843082) locus allele frequency distribution.

Table 3. Comparison of warfarin compliance time and stable standard dose of patients with different genotypes

Genotype	Time of target INR (d)	INR value	Stable standard dose of warfarin (mg/d)
GG	10.15±2.05	1.83±0.21	2.92±0.36
GA	11.42±2.93[a]	1.89±0.30	3.07±0.39
AA	13.20±2.00[a]	2.01±0.47	3.30±0.31[a]

Compare with the GG group, [a]P=0.04.

events (P=0.04). Other drug adverse reactions such as rash and embolism were not statistically significantly different among groups (**Table 4**; **Figure 3**).

Effect of different genotypes on left ventricular function parameters

Patients who continued to take warfarin for half a year after discharge were followed up to measure left ventricular function and evaluate the effect of different genotypes on left ventricular function. There were no differences in the LVEDV and LVESV among GG, GA, and AA group. Compared with the GG group, the LVEF of the AA group was significantly reduced (P=0.04) (**Table 5**; **Figure 4**).

Comparison of survival curves of patients with different genotypes

At the end of the follow-up (120 days), the case fatality rates of GG, GA, and AA group were 15.51%, 12.50%, and 22.57%, respectively. The case fatality rate of AA group was higher than that of GG and GA group, but there was no statistically significant difference between groups (**Figure 5**).

Discussion

We explored the effect of the PITX2 SNP rs6843082 on the efficacy and adverse reactions of warfarin in patients with atrial fibrillation and hypertension. Our findings suggest that the rs6843082 SNP may lead to the warfarin dose response differences in patients with atrial fibrillation and hypertension. Genotyping patients for rs6843082 before initiating warfarin treatment may optimize the time to reach INR and reduce bleeding incidence.

The PITX2 gene is a transcription factor belonging to the Bicoid family. It is located on chromosome 4q25 and is a neural crest expression gene. It plays an important role in determining heart structure and is related to problems such as left and right asymmetry [11]. There are four isoforms, namely PITX2a, PITX2b, PITX2c and PITX2d [12], among which PITX2c plays the most significant role in heart development. Only a small amount of PITX2a expression can be detected in the early embryo. As the embryo develops, Pitx2b expression is gradually lessened. The expression of PITX2d can only be

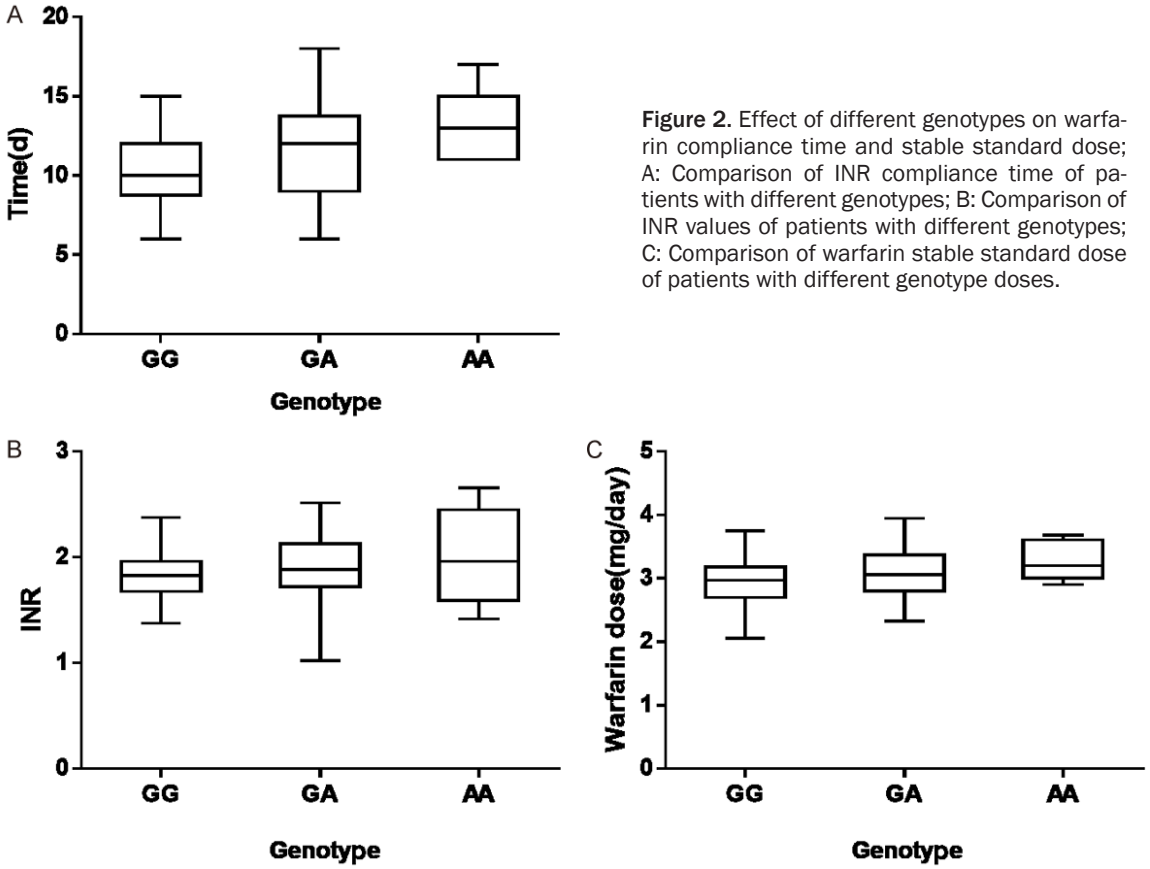

Figure 2. Effect of different genotypes on warfarin compliance time and stable standard dose; A: Comparison of INR compliance time of patients with different genotypes; B: Comparison of INR values of patients with different genotypes; C: Comparison of warfarin stable standard dose of patients with different genotype doses.

Table 4. Comparison of adverse drug reaction incidence in patients with different genotypes in the early stage of anticoagulant therapy

Adverse drug event		GG group (n=58)	GA group (n=32)	AA group (n=7)
Minor bleeding	Dermatorrhagia	2	1	0
	Fundus bleeding	2	1	1
	Gums bleeding	1	1	2
	Gastrointestinal bleeding	2	1	1
Major bleeding	Intracranial hemorrhage	1	0	0
Total bleeding n (%)		8 (13.79)	4 (12.50)	4 (57.14)[a,b]
Other adverse drug events	rash	3	2	1
	embolism	2	1	0
Total adverse drug event incidence, n (%)		5 (8.62)	3 (9.37)	1 (14.28)

Compared with GG group, [a]*P*=0.04. Compared with GA group, [b]*P*=0.04.

detected in human heart tissue [13]. Data increasingly support a role for PITX2 in the pathogenesis of heart fibrillation. The results of a large-scale replication and meta-analysis of several loci on the 4q25 locus in the European population showed that genetic polymorphisms on 4q25 are strongly associated with atrial fibrillation [14]. At the entrance of the pulmonary vein, myocardial cells extend into the blood vessel like a cuff to form a myocardial sleeve. The myocardial sleeve of the pulmonary vein is prone to abnormal excitement and triggers heartbeat. Studies have shown that, mice cannot form the original lung cardiomyocytes after the specific knockout of PITX2c gene, and then cannot form pulmonary vein myocardial sleeves which may reduce the risk of heart failure [15].

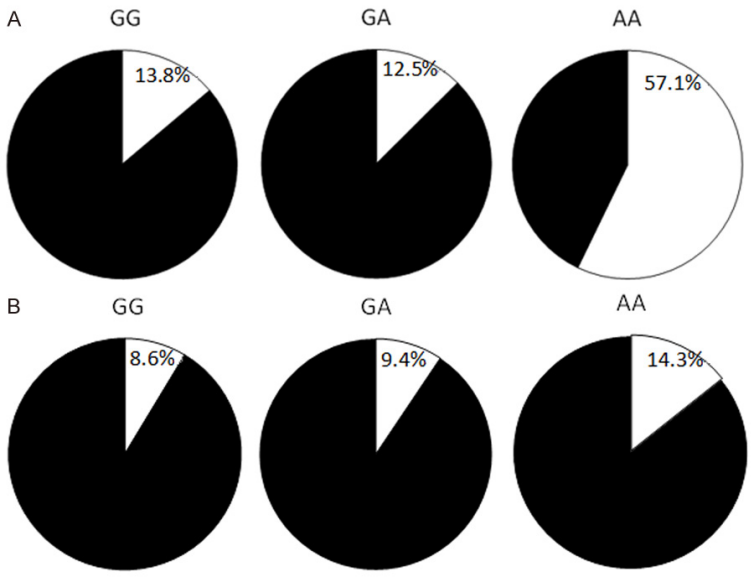

Figure 3. Comparison of adverse event proportions in the early stage of anticoagulation treatment among groups. A: Proportion of total bleeding events in the early stage of anticoagulation treatment of groups. B: Reaction ratio of other adverse events besides bleeding occurring in the early stage of anticoagulation treatment among groups.

Table 5. Effect of different genotypes on left ventricular function

Gene	LVEDV (ml)	LVESV (ml)	LVEF (%)
GG	130.76±13.54	73.60±10.39	63.16±4.92
GA	133.35±15.79	70.16±12.62	61.38±5.75
AA	131.73±16.62	71.74±10.34	57.12±4.71[a]

Compared with GG group, [a]P=0.05.

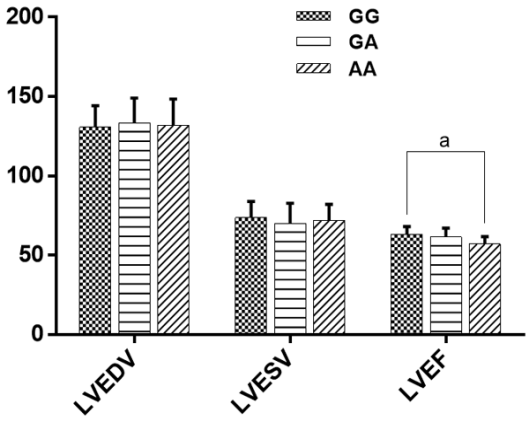

Figure 4. The influence of different genotypes on left ventricular function. After taking warfarin for half a year, the left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV) and left ventricle ejection fraction (LVEF) were detected by color Doppler ultrasound. Compared with GG group, [a]P<0.05.

The SNP rs6843082 is located very close to the PITX2 gene. In a 2012 GWAS meta-analysis [16] including data from 15 ischemic stroke cohorts, all of which were of European ancestry polymorphism of the rs6843082 gene near the PITX2 gene was associated with increased the risk of cardiogenic stroke (P=2.8×10^{-16}). The study found [17, 18] that NPPA expression was up-regulated in Pitx2c mutant rats, and NPPA can encode atrial natriuretic peptide (ANP). ANP can regulate the function of various ion channels located in the atrial muscle. In addition, ANP can also inhibit the proliferation of myocardial fibroblasts and the synthesis of collagen induced by transforming growth factor-β (TGF-β) [19]. Rs6843082 may protect the heart from remodeling and fibrosis by mediating the PITX2/NPPA/TGF-β pathway through regulating the expression of PITX2. The effect of PITX2 (rs6843082) SNP on antiarrhythmia and anticoagulation therapies in patients with heart fibrillation is not clear. This study focused on the association of rs6843082 with the effects of the anticoagulant drug warfarin.

Warfarin is a coumarin anticoagulant. It is a recommended drug for patients with atrial fibrillation. It can inhibit the synthesis of coagulation factors in the liver by vitamin K, thereby achieving anticoagulation. It has low price, efficacy, and continuous use by patients can prevent thrombosis. However, the treatment window for warfarin is extremely narrow, and the individual dose vary greatly, that is, the dose of drugs taken to achieve the same therapeutic effect can vary greatly among patients. Prothrombin time (PT) measured by INR was used to monitor the anticoagulant effect. In the early stage of anticoagulant therapy, patients need to monitor the INR value regularly, and according to that, continuously adjust the dosage to achieve safe and effective results. When the dosage is not adjusted properly, the body will enter a state of excessive anticoagulation or insufficient anticoagulation, and patients will be prone to adverse drug reactions such as bleed-

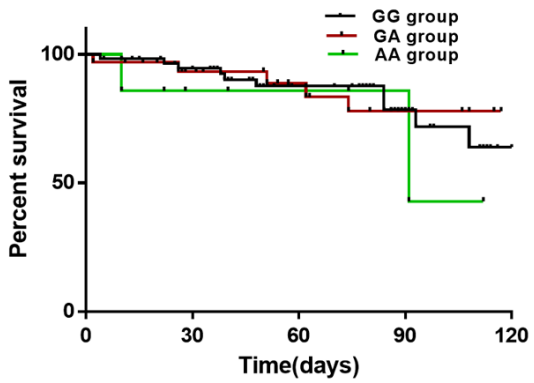

Figure 5. Comparison of 120 day survival curves of patients with different genotypes.

ing or thrombosis and thereby placing the patient's life at risk. In recent years, with the continuous development of pharmacogenomics, it has been shown that genetic polymorphism is the main reason for individual differences in warfarin responses. Among them, CYP2C9 and VKORC1 variants are the most frequently studied.

In this study, the genotyping technique was used to analyze the SNP of PITX2 (rs6843082) in 97 Chinese patients with atrial fibrillation and hypertension. The study results showed that the frequencies of the GG, GA, and AA genotypes were 58 (59.79%), 32 (32.99%), and 7 (7.22%), respectively. The frequency of the main allele G was 76.29%. The distribution of genotype frequency and allele frequency of PITX2 (rs6843082) loci in the 97 patients was in accordance with Hardy-Weinberg equilibrium. (The results of another study on the Han population in southern China [20] showed that the frequency of major alleles was 75.3% which was consistent with the results of this study. In this study, warfarin was used for anticoagulation treatment in all patients. When the INR value reached the preset 1.5-2.5 standard, the INR value of GA and AA group was significantly larger than that of the GG group. The INR was not different between groups (P>0.05). The maintenance doses of warfarin in GG, GA, and AA group was 2.92±0.36, 3.07±0.39 and 3.30±0.31 mg/d, respectively. Compared with GG group, the maintenance dose of the AA group was higher It was found that compared with GG and GA groups, the rate of bleeding events was higher in the AA group when comparing the incidence of adverse reactions such as bleeding at the beginning of treatment. The results showed that the maintenance doses of warfarin were higher in the homozygous mutant AA group patients: the risk of bleeding increased which affected the anticoagulant activity of warfarin.

In summary, this study obtained the genotype and allele distribution frequency of PITX2 (rs6843082) in Chinese patients with atrial fibrillation and hypertension. Our findings show that the rs6843082 SNP influences the warfarin anticoagulation response differences observed in patients with atrial fibrillation and hypertension. Genotyping patients for rs6843082 before initiating warfarin treatment may help to optimize the treatment response and reduce bleeding incidence. More research is needed to replicate these findings and identify other polymorphisms that may help to guide individualized warfarin treatment.

Acknowledgements

Special scientific research project of Shanghai Yangpu District Science and Technology Commission (No. YP18M07). I would like to extent my heartfelt thanks to the Medical Laboratory of Nantong ZhongKe, without whose assistance the accomplishment of this thesis would have been impossible.

Written informed consent was obtained from all participants and the present study was approved by the Institutional Review Board of Shidong Hospital, Yangpu District, Shanghai.

Disclosure of conflict of interest

None.

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References

[1] Lloyd-Jones DM, Wang TJ, Leip EP, Larson MG, Levy D, Vasan RS, D'Agostino RB, Massaro JM, Beiser A, Wolf PA and Benjamin EJ. Lifetime risk for development of atrial fibrillation: the Framingham Heart Study. Circulation 2004; 110: 1042-1046.

[2] Zoni-Berisso M, Lercari F, Carazza T and Domenicucci S. Epidemiology of atrial fibrillation: European perspective. Clin Epidemiol 2014; 6: 213-220.

[3] Carlsson J, Miketic S, Windeler J, Cuneo A, Haun S, Micus S, Walter S and Tebbe U; STAF Investigators. Randomized trial of rate-control versus rhythm-control in persistent atrial fibrillation: the Strategies of Treatment of Atrial Fibrillation (STAF) study. J Am Coll Cardiol 2003; 41: 1690-1696.

[4] Van Gelder IC, Van Veldhuisen DJ, Crijns HJ, Tuininga YS, Tijssen JG, Alings AM, Bosker HA, Cornel JH, Kamp O, Veeger NJ, Volbeda M, Rienstra M, Ranchor AV, TenVergert EM and Van den Berg MP. Rate control efficacy in permanent atrial fibrillation: a comparison between lenient versus strict rate control in patients with and without heart failure. Background, aims, and design of RACE II. Am Heart J 2006; 152: 420-426.

[5] Kim TH, Yang PS, Yu HT, Jang E, Shin H, Kim HY, Uhm JS, Kim JY, Sung JH, Pak HN, Lee MH, Joung B and Lip GYH. Effect of hypertension duration and blood pressure level on ischaemic stroke risk in atrial fibrillation: nationwide data covering the entire Korean population. Eur Heart J 2019; 40: 809-819.

[6] Gorenek B, Pelliccia A, Benjamin EJ, Boriani G, Crijns HJ, Fogel RI, Van Gelder IC, Halle M, Kudaiberdieva G, Lane DA, Larsen TB, Lip GY, Løchen ML, Marín F, Niebauer J, Sanders P, Tokgozoglu L, Vos MA, Van Wagoner DR, Fauchier L, Savelieva I, Goette A, Agewall S, Chiang CE, Figueiredo M, Stiles M, Dickfeld T, Patton K, Piepoli M, Corra U, Marques-Vidal PM, Faggiano P, Schmid JP and Abreu A. European Heart Rhythm Association (EHRA)/European Association of Cardiovascular Prevention and Rehabilitation (EACPR) position paper on how to prevent atrial fibrillation endorsed by the Heart Rhythm Society (HRS) and Asia Pacific Heart Rhythm Society (APHRS). Europace 2017; 19: 190-225.

[7] January CT, Wann LS, Calkins H, Chen LY, Cigarroa JE, Cleveland JC Jr, Ellinor PT, Ezekowitz MD, Field ME, Furie KL, Heidenreich PA, Murray KT, Shea JB, Tracy CM and Yancy CW. 2019 AHA/ACC/HRS Focused Update of the 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 Clinical Practice Guidelines and the Heart Rhythm Society in Collaboration With the Society of Thoracic Surgeons. Circulation 2019; 140: e125-e151.

[8] Stępień E, Wypasek E, Branicka A and Undas A. Optimalisation of treatment with vitamin K antagonists - the role of gene polymorphisms. Kardiologia Polska 2010; 68 Suppl 5: S428-35.

[9] Huang H and Darbar D. Genotype influence in responses to therapy for atrial fibrillation. Expert Rev Cardiovasc Ther 2016; 14: 1119-1131.

[10] Low SK, Takahashi A, Ebana Y, Ozaki K, Christophersen IE, Ellinor PT; AFGen Consortium, Ogishima S, Yamamoto M, Satoh M, Sasaki M, Yamaji T, Iwasaki M, Tsugane S, Tanaka K, Naito M, Wakai K, Tanaka H, Furukawa T, Kubo M, Ito K, Kamatani Y and Tanaka T. Identification of six new genetic loci associated with atrial fibrillation in the Japanese population. Nat Genet 2017; 49: 953-958.

[11] Kirchhof P, Kahr PC, Kaese S, Piccini I, Vokshi I, Scheld HH, Rotering H, Fortmueller L, Laakmann S, Verheule S, Schotten U, Fabritz L and Brown NA. PITX2c is expressed in the adult left atrium, and reducing Pitx2c expression promotes atrial fibrillation inducibility and complex changes in gene expression. Circ Cardiovasc Genet 2011; 4: 123-133.

[12] Cox CJ, Espinoza HM, McWilliams B, Chappell K, Morton L, Hjalt TA, Semina EV and Amendt BA. Differential regulation of gene expression by PITX2 isoforms. J Biol Chem 2002; 277: 25001-25010.

[13] Schweickert A, Campione1 M, Steinbeisser H and Blum M. Pitx2 isoforms: involvement of Pitx2c but not Pitx2a or Pitx2b in vertebrate left-right asymmetry. Mech Dev 2000; 90: 41-51.

[14] Kääb S, Darbar D, van Noord C, Dupuis J, Pfeufer A, Newton-Cheh C, Schnabel R, Makino S, Sinner MF, Kannankeril PJ, Beckmann BM, Choudry S, Donahue BS, Heeringa J, Perz S, Lunetta KL, Larson MG, Levy D, MacRae CA, Ruskin JN, Wacker A, Schömig A, Wichmann HE, Steinbeck G, Meitinger T, Uitterlinden AG, Witteman JC, Roden DM, Benjamin EJ and Ellinor PT. Large scale replication and meta-analysis of variants on chromosome 4q25 associated with atrial fibrillation. Eur Heart J 2009; 30: 813-819.

[15] Mommersteeg MT, Brown NA, Prall OW, de Gier-de Vries C, Harvey RP, Moorman AF and Christoffels VM. Pitx2c and Nkx2-5 are required for the formation and identity of the pulmonary myocardium. Circ Res 2007; 101: 902-909.

[16] Mommersteeg MT, Brown NA, Prall OW, de Gier-de Vries C, Harvey RP, Moorman AF and Christoffels VM. Genetic risk factors for ischaemic stroke and its subtypes (the METASTROKE collaboration): a meta-analysis of genome-wide association studies. Lancet Neurol 2012; 11: 951-962.

[17] Hodgson-Zingman DM, Karst ML, Zingman LV, Heublein DM, Darbar D, Herron KJ, Ballew JD, de Andrade M, Burnett JC Jr and Olson TM. Atrial natriuretic peptide frameshift mutation in familial atrial fibrillation. N Engl J Med 2008; 359: 158-165.

[18] Maekawa K, Saito Y, Ozawa S, Adachi-Akahane S, Kawamoto M, Komamura K, Shimizu W, Ueno K, Kamakura S, Kamatani N, Kitakaze M and Sawada J. Genetic polymorphisms and haplotypes of the human cardiac sodium channel alpha subunit gene (SCN5A) in Japanese and their association with arrhythmia. Ann Hum Genet 2005; 69: 413-428.

[19] Li P, Wang D, Lucas J, Oparil S, Xing D, Cao X, Novak L, Renfrow MB and Chen YF. Atrial natriuretic peptide inhibits transforming growth factor beta-induced smad signaling and myofibroblast transformation in mouse cardiac fibroblasts. Circ Res 2008; 102: 185-192.

[20] Hasegawa K, Wurita A, Minakata K, Gonmori K, Nozawa H, Yamagishi I, Watanabe K and Suzuki O. Postmortem distribution of MAB-CHMINACA in body fluids and solid tissues of a human cadaver. Forensic Toxicol 2015; 33: 380-387.