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Original Article

Interactions of *HERG*, *ZFHX3*, *TBX5* and *ACE* gene polymorphisms associated with atrial fibrillation in Chinese Han population

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Abstract: Objective: To discuss how mutual effects of HERG rs1805120, ZFHX3 rs7193343, TBX5 rs3825214 and ACE rs4353 polymorphisms can affect atrial fibrillation (AF) risk in Han population of north China. Methods: The genotypes of HERG rs1805120, ZFHX3 rs7193343, TBX5 rs3825214, and ACE rs4353 polymorphisms in 90 AF patients and 90 healthy people were detected by PCR amplification and sequencing; and gene-gene interactions were analyzed with multifactor dimensionality reduction (MDR) method. Results: There existed obvious differences in genotype distributions of the four single nucleotide polymorphisms (SNPs) between the AF patients and the controls ($P < 0.05$). The MDR analysis results indicated that the rs1805120-rs7193343-rs3825214-rs4353 model was the optimal model among all the interaction models formed by the four SNPs (OR = 8.1850, 95% CI = 3.9128-17.1220, $P < 0.0001$). Conclusions: It is possible that interactions of HERG rs1805120, ZFHX3 rs7193343, TBX5 rs3825214, and ACE rs4353 polymorphisms can confer increased risk of AF.

Keywords: HERG, ZFHX3, TBX5, ACE, gene-gene interaction, atrial fibrillation, polymorphism

Introduction

Atrial fibrillation (AF) is the most frequent continuous arrhythmia in clinic. Among people aged over 30 in China, the incidence of AF is 0.77%; and among those aged over 80, the incidence can reach up to 7.5% [1, 2]. A large number of reports have revealed the existence of associations between mutations in various genes and AF [3-7], including the genes correlated with ion channel, structural remodeling and development of the heart, which mainly influence the occurrence or continuity of AF through changing structures and functions of various ion channels in the atrium [8-11].

TBX5 gene plays a key role in the early normal development of the heart, and mutations in the gene can lead to the abnormal formation and development of the atrial structure, thus increasing the risk of AF [12]. In addition, studies at home and abroad have also proved strong correlations of ACE [13]. HERG and ZFHX3 genes [14, 15] with AF susceptibility. In the present study, we performed an in-depth exploration into the relationships of HERG rs1805120, ZFHX3 rs7193343, TBX5 rs3825214 and ACE rs4353 polymorphisms with AF susceptibility through analyzing interactions of the four single nucleotide polymorphisms (SNPs).

Materials and methods

Study subjects

90 AF patients (case group) were selected from The First Affiliated Hospital of Chinese PLA General Hospital from April, 2013 to October, 2014, and were diagnosed with AF by 12-lead electrocardiogram, 24-hour electrocardiogram and medical history examinations. In addition, we recruited 90 healthy persons from the same hospital during the same period. All the participants had undergone routine electrocardiogram, blood pressure measurement, and transthoracic echocardiography examinations as well as tests of thyroid functions, electrolytes, kidney functions, and liver functions. Patients with the below-listed diseases were eliminated: secondary hypertension, rheumatic heart disease, dilated cardiomyopathy, hypertrophic car-

Table 1. PCR primers of the four SNPs

Gene	Locus	Primer	Length
HERG	rs1805120	F: CGCATCGCCGTCCACTACTT	210 bp
		R: GTAGTGGACGGCGATGCGGC	
ZFHX3	rs7193343	F: CCCACTCCTACAGCCAGATG	139 bp
		R: AGACTTCCTGTTTCCTTCCAA	
TBX5	rs3825214	F: TGGGTAGCTAAGAACTAAGATTGAG	160 bp
		R: GTTTCATTTTTCTTCACTTCTCCA	
ACE	rs4353	F: TCTTGGGGTGAATAAGTTTG	228 bp
		R: GAAGGTTCTACCGTCTCTCG	

diomyopathy, hyperthyroidism, electrolyte disturbance, viral myocarditis, malignancies, severe heart failure, severe hepatic and kidney dysfunction. Our study conformed to the ethic standards formulated by Institutional Review Board of The First Affiliated Hospital of Chinese PLA General Hospital, and all participators signed informed consents. The basic characteristics of all the subjects detected in this study included gender, age, body mass index (BMI), smoking, drinking, and coronary heart disease, hypertension, and diabetes histories.

Genomic DNA extraction and identification

2 ml of blood was extracted from each participator, and was then anticoagulated with sodium citrate. The extraction of genomic DNA was performed utilizing a DNA extraction kit (Shanghai Bioleaf Biotech Co., Ltd). Then the extracted DNA was preserved at -80°C for future use.

PCR system

The synthesis of PCR primers (**Table 1**) of HERG rs1805120, ZFHX3 rs7193343, TBX5 rs3825214 and ACE rs4353 polymorphisms was carried out by Shanghai Bioleaf Biotech Co., Ltd. In the PCR system, there were 2.5 μL 10 × Ex Taq buffer solution, 2 μL dNTP, 0.3 μL forward primer, 0.3 μL reverse primer, 2 μL template DNA, 0.2 μL Ex Taq, and 17.7 μL water. The reaction conditions were as follows: initial denaturation for 5 minutes at 94°C; 35 loops of 94°C for 40 seconds, 56°C for 40 seconds, and 72°C for 40 seconds; 72°C for 10 minutes; and final conservation at 12°C.

Sequencing reaction

The following conditions were for the sequencing reaction: 2 minutes of denaturation at 95°C, followed by 35 PCR cycles (95°C for 15 seconds, 50°C for 15 seconds, and 60°C for 90 seconds), and final preservation at 12°C. The sequence map was analyzed by Shanghai Bioleaf Biotech Co., Ltd.

Statistical analysis

We performed Hardy-Weinberg equilibrium (HWE) test to determine whether the genotype distribution of the subjects accorded with Mendelian genetic principles or not. The genotyping data of the four SNPs were input into SPSS 18.0 software, and χ^2 test or Fisher's exact test was executed to compare differences in the constitution ratio of genotypes of the four SNPs between the cases and the healthy controls. Gene-gene interactions were calculated by multifactor dimensionality reduction (MDR) 1.0 software package.

Results

Clinical data analysis

No obvious differences were observed to exist in such clinical materials as sex, age, BMI, smoking, alcoholism, and histories of coronary heart disease, hypertension, and diabetes between two groups ($P > 0.05$). The average left atrial diameters of the cases and the controls were respectively 39.31 mm and 35.27 mm, with the former being significantly larger than the latter; and the difference in the left atrial diameters between the case and control group was statistically significant ($P < 0.0001$).

Comparison of genotype distributions

The genotype frequencies of HERG rs1805120, ZFHX3 rs7193343, TBX5 rs3825214 and ACE rs4353 polymorphisms in the case and control groups passed through HWE test ($P > 0.05$). Differences in genotype distributions of the four SNPs between the AF patients and the healthy participators were observed with statistical significance ($P < 0.05$) (**Table 2**).

Correlation between the optimal MDR interaction model and AF

For HERG rs1805120, ZFHX3 rs7193343, TBX5 rs3825214 and ACE rs4353 polymorphisms, there were the following three interac-

Table 2. Genotype distributions of the four SNPs

Genotype CC		HERG rs1805120			ZFHX3 rs7193343			TBX5 rs3825214			ACE rs4343		
		CT	TT	TT	TC	CC	GG	AG	AA	GG	GA	AA	
Genotype frequency n (%)	Control	49	34	7	36	44	10	14	45	31	31	44	15
	Case	37	35	18	23	47	20	24	54	12	22	39	29
P		0.038			0.043			0.003			0.043		

Table 3. MDR analysis result

Model	X2-X3	X2-X3-X4	X1-X2-X3-X4
Training balance accuracy	0.6395	0.6753	0.7259
Testing balance accuracy	0.5333	0.5104	0.4712
CV consistency	5/10	8/10	10/10
χ^2 (P)	13.7411 (0.0002)	20.2928 (0.0001)	34.7175 (0.0001)
OR (95% CI)	3.5944 (1.8085-7.1438)	4.4794 (2.2951-8.7425)	8.185 (3.9128-17.1220)

tion models: rs7193343-rs3825214 (OR = 3.5944, 95% CI = 1.8085-7.1438, *P* = 0.0002); rs7193343-rs3825214-rs4353 (OR = 4.4794, 95% CI = 2.2951-8.7425, *P* < 0.0001); and rs1805120-rs7193343-rs3825214-rs4353 (OR = 8.1850, 95% CI = 3.9128-17.1220, *P* < 0.0001). The best interaction model determined by MDR software was HERG rs1805120-ZFHX3 rs7193343-TBX5 -rs3825214- ACE rs4353, revealing that the interactions of the four SNPs could probably confer susceptibility to AF (**Table 3**).

Discussion

The TBX5 gene can modulate the whole development process of the heart, and the accurate expression of the gene in the development of the embryonic heart has great significance in the normal development of the atrium. Mutations in TBX5 gene can result in abnormal expression of transcription factors and some downstream effectors of TBX5 gene, and thus cause cardiac malformation, abnormal structures and functions of atriums and electrophysiologies of the heart, which can increase the susceptibility to AF [16, 17]. Holm et al. have found through research that TBX5 rs3825214 polymorphism is apparently correlated with PR interval and QRS duration abnormalities as well as the occurrence of AF [18].

ZFHX3 gene mutations can influence the expression level and activity of ZFHX3 molecules [19]. However, the abnormal expression of ZFHX3 molecules can affect the transcription and expression of downstream angiotensin (ANG) II and CRP so that proliferation, edema, necrosis, apoptosis and interstitial fibrosis of atrial myocytes occur, which can increase the heterogeneity of electrical conduction of atriums, facilitate the forming of reentry, and provide substrates for the occurrence and development of AF [20, 21]. The close linkage between ZFHX3 rs7193343 polymorphism and AF has been proved by studies of Gudbjartsson and Kiliszek et al. [22, 23].

The ACE gene is located on the long arm of chromosome 17 (17q23), and its polymorphisms have been indicated to relate to heart diseases like sudden cardiac death, hypertrophic cardoiomyopathy, malignant arrhythmia and prolonged QT interval, indicating that ACE gene mutations may cause changed electrophysiological properties of the heart [24, 25]. ACE rs4353 polymorphism is located in intron 19 of the ACE gene. It can impact the blood circulation and the ACE level and activity in part of the myocardium so that the exposure level of ANG II in the myocardium may be changed. ANG II can activate the pathway of mitogen-activated protein kinase (MAPK), an important downstream regulator of ANG II which can change the structure and gap coupling of myocardial tissues and lead to AF onset [26].

HERG gene is a newly-found candidate gene for AF risk. It is located in 35-36 region of the long arm end of chromosome 7 (7q35-6), and encodes the α subunit of IKr of myocardial cells. In 2005, Hong et al. performed a study on

an AF pedigree and discovered that the N588K polymorphism of HERG gene could lead to both AF and short QT syndrome. The N588K mutation is located on 112247 position of intron 7, within 1 kb distance away from the position of rs1805120 (11225547); and the distance of mRNAs of the two polymorphisms is 212 kb. Thus, the two polymorphisms may be located in a same haplotype block and have a close linkage.

The AF occurrence has associations with mutual effects of multiple genetic polymorphisms, so we selected four genes that may have a correlation with the susceptibility to AF and analyzed their interactions using the MDR method. We found that the interactions of these SNPs might contribute to the increased risk of AF in Han population of north China. Nevertheless, only a preliminary investigation of gene-gene interactions was performed in our study because of limited time and funds. Therefore, future studies concerning this subject should be conducted with enlarged sample sizes.

Disclosure of conflict of interest

None.

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