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Original Article

Correlation between GATA4 gene polymorphism and congenital heart disease

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Abstract: Objective: The correlation between GATA4 gene polymorphism and congenital heart disease (CHD) was analyzed. Method: Clinical data and blood samples were collected from 350 CHD patients who were treated at the Department of Cardiology in Beijing Anzhen Hospital. The control group consisted of 350 healthy subjects receiving physical examination at our hospital during the same period. Polymorphism was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis for locus rs11392441 of GATA4 gene. Results: Polymorphism of locus rs1139244 of GATA4 gene was detected in CHD patients. The distribution frequencies of GG genotype and G allele were significantly higher than those of the control group. Conclusion: Polymorphism of locus rs1139244 of GATA4 gene was correlated with CHD.

Keywords: Congenital heart disease (CHD), GATA4 gene, polymorphism

Introduction

Congenital heart disease (CHD) is the most common birth defect that seriously threatens the infants' health. Having the main manifestations of atrial septal defect (ASD), ventricular septal defect (VSD) and patent ductus arteriosus (PDA), CHD presents as the abnormality in the structure and function of heart and great vessels caused by embryonic development disorders [1-3]. The incidence of CHD in foreign reports is 5.4%-16.1% [4], while that of China is 2.51% [5]. The pathogenesis of CHD is highly complex and is not fully understood yet. It is currently accepted that CHD is a polygenic disease resulting from the interaction between environmental and genetic factors [6]. Connections have been established between normal development of heart and multiple transcription factors. Any mutation in transcription factor genes may lead to abnormal cardiac development and CHD. Researchers have found that CHD may be related to NKX2.5 [7], TBX5 [8] and JARID2 genes [9]. Zinc finger transcription factor GATA4 is the zinc finger domain that binds to specific DNA. GATA4 is highly conserved during evolution and implicated in cardiac stem cell differentiation, cyclization, heart block and conduction. GATA4 gene is localized to 8p23.1 with full length of cDNA being 3371 bp, consisting of 6 exons and encoding 422 amino acids [10]. GATA4 gene expression has a direct impact on the embryonic development of heart. Rat fetus with CHD dies at the gestational age of 13.5-16.5 d. GATA4 gene mutation has a close connection with ASD [11], an autosomal dominant disease. Some patients may be combined with VSD, pulmonary artery stenosis (PS), severe diastolic dysfunction and atrioventricular valve regurgitation, but not with contractile dysfunction or lesions of the cardiac conduction system and coronary heart disease [11-13].

We investigated the single nucleotide polymorphism (SNP) locus P66A (rs1139244) in the coding region of GATA4 gene and detected for its connections with CHD risk.

Materials and methods

General data

Three hundred and fifty CHD patients who were treated at Department of Cardiology at Anzhen Hospital from January 2005 to December 2014 were selected to constitute the CHD group.

Table 1. Primer sequences of locus P66A (rs1139244) in GATA4 gene

Locus	Primer sequence	Products (bp)	Tm (°C)	Endonuclease
Rs1139224	Sense: 5'-ACGCGGCCCCACCGGAGCTGCCGC-3'	314	56	SFaNI
	Antisense: 5'-TCGTTGTTGCCGTGGTTTTC-3'			

Table 2. Genotype frequencies and allele frequencies at locus rs1139244 in GATA4 gene

Groups	N	Genotype (n, %)			*P*	Allele (n, %)		OR (95% CI)	*P*
		CC	CG	GG		C	G		
Control group	350	51 (14.6)	165 (47.1)	134 (38.3)	0.002	267 (38.1)	433 (61.9)	1.33 (1.06-1.65)	0.011
CHD group	350	22 (6.3)	178 (50.9)	150 (42.8)		222 (31.7)	478 (68.3)		

Table 3. Frequencies of GG genotype in various types of CHD and in the controls

Groups	N	Genotype			*P*	Allele		*P*
		CC	CG	GG		C	G	
Control group	350	51 (14.6)	165 (47.1)	134 (38.3)		267 (38.1)	433 (61.9)	
ASD	121	10 (8.3)	75 (62.0)	36 (29.7)	0.015	95 (39.2)	147 (60.8)	0.759
VSD	132	8 (6.0)	78 (59.0)	46 (35.0)	0.013	94 (35.6)	170 (64.4)	0.468
PDA	68	2 (2.9)	15 (22.1)	51 (75.0)	<0.001	19 (13.9)	117 (86.1)	<0.001
PS	29	2(6.9)	10 (34.5)	17 (58.6)	0.089	14 (24.1)	44 (75.9)	0.034

They were 137 males and 213 females with an average age of 10.4±8.2 years old. The normal control group had 350 subjects, including 161 males and 189 females with an average age of 9.6±6.3 years old. All cases were matched for age and had no blood relationship with each other. The two groups did not differ in age and sex ratio significantly ($P>0.05$).

Inclusion criteria: (1) Presenting with clinical features of CHD and having echocardiographic evidences or surgical records; (2) Signing of informed consent.

Exclusion criteria: (1) Presenting with clinical features of CHD but having no echocardiographic evidences or other examination results; (2) Confirmed chromosomal abnormalities or syndrome-associated cardiovascular abnormality.

The protocol conformed to medical ethics and was approved by Medical Ethics Committee. Informed consent was obtained from all subjects.

Primer design

SNP database was searched for SNPs in the coding region of GATA4 gene. SNP locus P66A (rs11392441) was selected and primers were designed using Primer 5. The primer sequences are shown in **Table 1**.

Genome DNA extraction

For all cases 2-ml of venous blood was collected with citrate anticoagulation and preserved at -20°C. Genome DNA extraction was performed conventionally using Tris saturated phenol-chloroform method described previously [14].

PCR amplification of target fragment

PCR amplification was carried out conventionally. The 25 μl reaction system consisted of genome DNA (20 ng/μl) 2 μl, 10× buffer ($MgCl^{2+}$ 15 mmoL/L) 2.5 μl, dNTP 1.0 μl, upstream primer 0.5 μl, downstream primer 0.5 μl, TaqDNA polymerase 0.2 μl and sterilized water 18.3 μl. PCR reaction conditions: denaturation at 94°C for 5 min, 94°C for 45 s, 56-60°C for 45 s, 72°C for 45 s, 35 cycles, final extension at 72°C for 10 min. Genotyping was performed using PCR-RFLP. Restriction enzyme is SFaNI, the conditions and products of enzyme digestion for the genotyping of locus rs11392441 in GATA4 gene was described previously [15].

Statistical methods

Statistical analysis was carried out using SPSS software 17.0. Whether the genotype frequency satisfied Hardy-Weinberg equilibrium was examined with X^2 test. Differences in genotype

frequency and allele frequency were compared by using X^2 test between the groups, and $P<0.05$ was considered as statistically significant.

Results

Genotype frequency and allele frequency at locus rs1139244 in GATA4 gene

Genotype frequencies and allele frequencies at locus P66A (rs1139244) in GATA4 gene for CHD group and control group are shown in **Table 2**. It can be seen that the frequencies satisfied Hardy-Weinberg equilibrium. The frequency of homozygous GG genotype in GATA4 gene was 38.3% and 42.8% in the control group and CHD group, respectively, showing significant differences ($P<0.05$). The frequency of G allele in GATA4 gene was 61.9% and 68.3% in the control group and CHD group, respectively. A difference of statistical significance was found between the two groups ($P<0.05$). The CHD risk was significant increased 1.33 fold in the carriers of C allele compared to G allele carriers (OR=1.33, 95% CI: 1.06-1.65; $P=0.011$).

Frequencies of GG genotype in various types of CHD and in the controls

The frequencies of GG genotype at locus rs1139244 in GATA4 gene were significantly higher in cases with atrial septal defect (ASD), ventricular septal defect (VSD), and PDA than in the controls (**Table 3**).

Discussion

In the present study, we found rs1139244 in GATA4 gene were significantly associated with CHD in Chinese population, especially for ASD, VSD, and FDA.

GATA4 is a candidate gene for the occurrence of cardiovascular abnormalities, belonging to GATA transcription gene family. Containing zinc finger domain, GATA4 is a highly conserved transcription gene [16] and interacts with other transcriptional regulatory gene during early stage of heart development. Over 30 gene promoters in myocardial cells are directly regulated by GATA4. GATA4-related signaling transduction pathway plays a key role in specific differentiation and transport of cardiac stem cells after heart formation [17]. GATA4-knockout mice failed to develop primitive heart tube [18]. This indicates that GATA4 mainly controls the formation of cardiac progenitor cells in the endodermal layer. However, functional deficit of GATA4 may lead to abnormal differentiation of endodermal cells and hence heart defect. Some foreign studies have reported chromosome 8p deletion in syndromes associated with heart defect, and GATA4 deletion may be the pathogenic reason. Mutations in GATA4 can cause abnormal cardiac development, including E359dd (frameshift mutation), G296S (missense mutation) and 1074delC (frameshift mutation) [19]. But the correlation between GATA4 gene polymorphism and CHD has not been reported. We performed PCR-RFLP for SNP locus P66A (rs1139244) in the coding region of GATA4 gene among 350 CHD cases and 350 normal controls. SNPs and haplotypes were analyzed. Results showed that the frequencies conformed to Hardy-Weinberg equilibrium and were representative of the population. GG genotype frequency in CHD cases and normal controls was 42.8% and 38.3%, respectively, and G allele frequency was 68.3% and 61.9%, respectively, both frequencies showing significant differences between the groups. It was indicated that G allele is a susceptible factor of CHD, and its mutation can lead to CHD. Moreover, the genotype frequency at locus P66A (rsll39244) was significantly higher in cases with ASD, VSD, and PDA than in normal controls ($P<0.05$). No obvious differences were found for other types of CHD compared with the control.

In conclusion, the polymorphism at locus P66A (rs1139244) in GATA4 gene is related to congenital ASD, VSD, and PDA. Therefore, GATA4 genetic polymorphism can be useful marker for the diagnosis of CHD. The findings of the present study need to be further verified by large-sample trials.

Disclosure of conflict of interest

None.

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