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Original Article

A complex insertion/deletion polymorphism in the compositionally biased region of the *ZFHX3* gene in patients with coronary heart disease in a Chinese population

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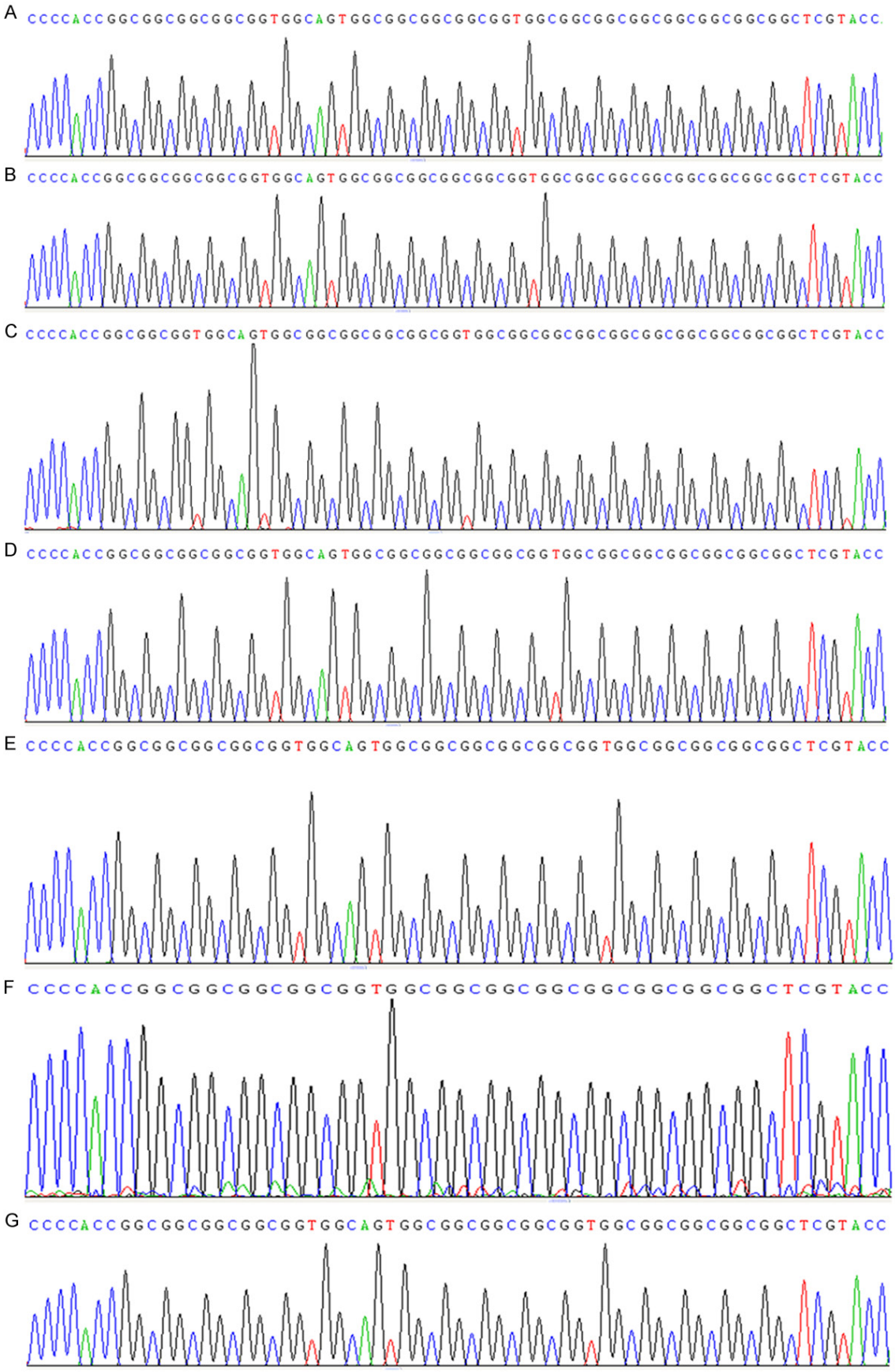
Abstract: Coronary heart disease (CHD) is a leading cause of morbidity and mortality around the world and has both genetic and environmental precipitants. Genetic factors are significant in determining the level of risk factors in individuals. Variants in *ZFHX3* gene are associated with atrial fibrillation in individuals of European ancestry. The aim of this study was to analyze the polymorphisms in the compositionally biased region of the *ZFHX3* gene in patients with coronary heart disease in a Chinese population, and to explore their associations with coronary heart disease. We recruited 278 CHD patients and 358 age and sex matched healthy controls in a Chinese Han population, polymorphisms in the compositionally biased region of the *ZFHX3* gene were determined by polymerase chain reaction followed by DNA sequencing. The genotype frequencies were calculated, and statistical analysis was performed using the non-parametric mood median test. A complex insertion/deletion polymorphism was identified in the compositionally biased region of the *ZFHX3* gene in a Chinese population. Six common genotypes $(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_8$, $(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_8$, $(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_7$, $(GGC)_2GGTGGCAGT(GGC)_5GGT(GGC)_{10}$, $(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_5$, and $(GGC)_4GGT(GGC)_8$ were found in both CHD patients and healthy controls, there was no significant difference in the six genotype frequencies between CHD patients and healthy controls. Rare genotypes $(GGC)_4GGTGGCAGT(GGC)_2GGT(GGC)_2GGT(GGC)_6$, $(GGC)_4GGTGGCAGT\ (GGC)_8$, $(GGC)_4GGTGGCAGT(GGC)_3GGT(GGC)_8$, and $(GGC)_6GGT(GGC)_8$ were only identified in healthy controls. Rare genotypes $(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_5$, $(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_4$, and $(GGC)_4GGTGGCGGT(GGC)_6$ were only found in CHD patients. The compositionally biased region of the ZFHX3 gene contains a poly-Gly sequence. A complex insertion/deletion polymorphism exists in this region in a Chinese population, clinical significance of some rare genotypes should be explored for CHD in the future.

Keywords: *ZFHX3* gene, polymorphism, coronary heart disease

Introduction

Coronary heart disease (CHD) is a leading cause of morbidity and mortality worldwide. Although, pathogenesis of CHD is still not fully understood, it is considered that vascular endothelial cell injury, lipid infiltration, platelet reactivity, vascular smooth muscle cell proliferation, and increased synthesis of connective tissue are the main pathological processes of CHD [1]. It is well established that CHD is a complex polygenic disease resulting from the interaction between genetic factors and environmental factors [2]. Although the exact genetic mechanism is unclear, genetic variations are estimated to account for about 30~60% of the CHD risk [3]. The gene zinc finger homeobox 3 (*ZFHX3*) encodes a transcription factor with multiple homeodomains and zinc finger motifs, and regulates myogenic and neuronal differentiation [4]. The *ZFHX3* gene is reported to function as a tumor suppressor in several cancers, and its genetic variants are associated with atrial fibrillation in individuals of European ancestry [5]. Study of polymorphisms in the *ZFHX3* gene showed that two SNPs (rs2106261, rs6499600) conferred a significant association with atrial fibrillation risk in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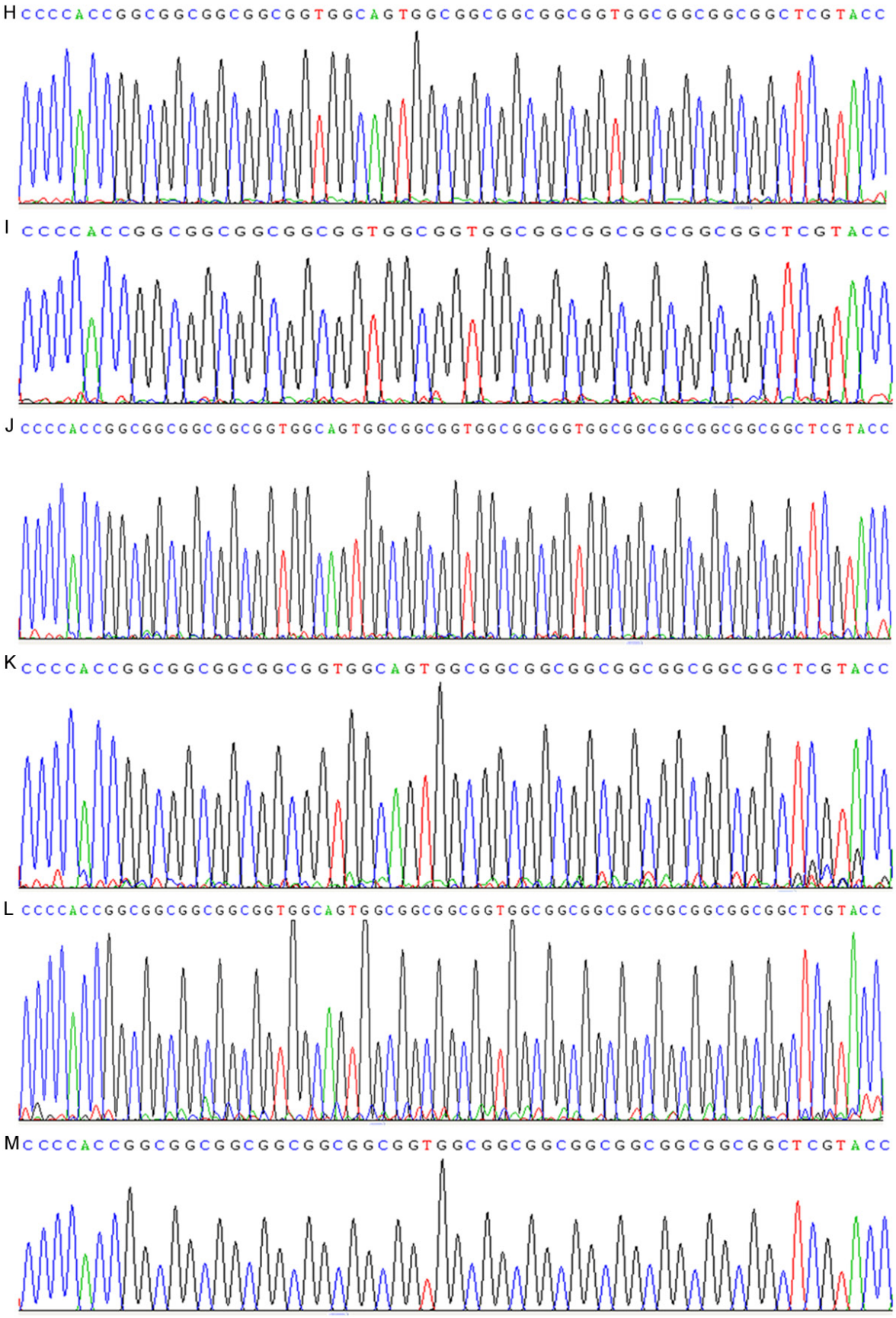
H CCCCACCGGCGGCGGCGGCGGTGGCAGTGGCGGCGGCGGCGGTGGCGGCGGCGGCTCGTACC
I CCCCACCGGCGGCGGCGGCGGTGGCGGTGGCGGCGGCGGCGGCGGCTCGTACC
J CCCCACCGGCGGCGGCGGCGGTGGCAGTGGCGGCGGTGGCGGCGGTGGCGGCGGCGGCGGCTCGTACC
K CCCCACCGGCGGCGGCGGCGGTGGCAGTGGCGGCGGCGGCGGCGGCGGCGGCTCGTACC
L CCCCACCGGCGGCGGCGGCGGTGGCAGTGGCGGCGGCGGTGGCGGCGGCGGCGGCGGCGGCGGCTCGTACC
M CCCCACCGGCGGCGGCGGCGGCGGCGGTGGCGGCGGCGGCGGCGGCGGCGGCTCGTACC

Figure 1. Polymorphisms in the compositionally biased region of the ZFHX3 gene. A: $(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_8$ sequence; B: $(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_8$ sequence; C: $(GGC)_2GGTGGCAGT(GGC)_5GGT(GGC)_{10}$ sequence; D: $(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_7$ sequence; E: $(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_5$ sequence; F: $(GGC)_4GGT(GGC)_8$ sequence; G: $(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_5$ sequence; H: $(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_4$ sequence; I: $(GGC)_4GGTGGCGGT(GGC)_6$ sequence; J: $(GGC)_4GGTGGCAGT(GGC)_2GGT(GGC)_2GGT(GGC)_6$ sequence; K: $(GGC)_4GGTGGCAGT(GGC)_8$ sequence; L: $(GGC)_4GGTGGCAGT(GGC)_3GGT(GGC)_8$ sequence; M: $(GGC)_6GGT(GGC)_8$ sequence.

Table 1. Frequency of different polymorphisms in the compositionally biased region of the *ZFHX3* gene in Chinese Han population

Polymorphisms	CHD	Controls	*P*-value
$(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_8$	64.39%	67.04%	*P*>0.05
$(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_8$	23.92%	23.18%	*P*>0.05
$(GGC)_2GGTGGCAGT(GGC)_5GGT(GGC)_{10}$	2.70%	2.93%	*P*>0.05
$(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_7$	2.16%	2.23%	*P*>0.05
$(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_5$	2.16%	1.82%	*P*>0.05
$(GGC)_4GGT(GGC)_8$	0.72%	0.56%	*P*>0.05
$(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_5$	2.52%		
$(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_4$	1.26%		
$(GGC)_4GGTGGCGGT(GGC)_6$	0.18%		
$(GGC)_4GGTGGCAGT(GGC)_2GGT(GGC)_2GGT(GGC)_6$		1.12%	
$(GGC)_4GGTGGCAGT(GGC)_8$		0.70%	
$(GGC)_4GGTGGCAGT(GGC)_3GGT(GGC)_8$		0.28%	
$(GGC)_6GGT(GGC)_8$		0.14%	

Chinese Han populations [6]. The *ZFHX3* gene contains a poly-Gly sequence in the compositionally biased region [7]. There was still no research data on the relation between the *ZFHX3* gene polymorphisms with CHD. In this study, we utilized a case-control study to reveal the relationship between the *ZFHX3* gene polymorphism and CHD in a Chinese Han population. We subsequently identified a complex insertion/deletion polymorphism existed in this region in a Chinese population. However, clinical significance of some rare genotypes in CHD remains to be determined in the future.

Materials and methods

Subjects

A total of 278 hospitalized CHD patients (mean age: 57.79 ± 13.64) were enrolled in the Department of Cardiology, Shenzhen Baoan Hospital, Southern Medical University from December 2011 to May 2013. The patients were diagnosed CHD according to WHO issued ischemic heart disease diagnostic criteria in 1979 or the presence of stenosis of more than 50% luminal diameter in at least one significant coronary artery on coronary angiography [8]. In addition, patients with coronary artery bypass surgery were considered as CHD cases. All these CHD patients were unrelated Han Chinese people. Three hundred and fifty-eight healthy volunteers from medical center of the same hospital were selected as the healthy control group (mean age: 56.68 ± 12.35) during the same period. The disease history, physical examination, laboratory tests (serum, urine, stool routine tests, serum lipids and glucose), ECG and other preliminary tests were performed for control subjects. These control subjects have no CHD history or any symptom of CHD. All healthy control individuals were Han Chinese people whose age and sex were matched with the case group. The study was approved by the ethics committee of Shenzhen Baoan Hospital, Southern Medical University, and was kept in accordance with the Helsinki Declaration of 1975 as revised in 1983. Written consent was obtained from all participants.

Polymorphism analysis

Genomic DNA was extracted from peripheral blood using a standard phenol-chloroform extraction procedure after proteinase K digestion for all participants, and it was quantified by using a spectrophotometer [9]. An absorbance ratio of 1.8:2.0 or greater was considered and the final solution was stored at -70°C. The compositionally biased region of the ZFHX3 gene was amplified by polymerase chain reaction using the following primers: 5'-gcagcaaaaagtgcagcag-3 (forward), 5'-gaggtagatgcggtgctagg-3 (reverse). PCR was performed using 100 ng of genomic DNA in 50 ml reactions containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 200 μM of each dNTP, 2 U Taq polymerase, and 0.5 μM each primer. Following

Table 2. Amino acid sequences of the polymorphic segment of ZFHX3 protein

Polymorphisms	Amino acid sequences
$(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_8$	ggggggsgggggggggggg
$(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_8$	ggggggsggggggggggggg
$(GGC)_2GGTGGCAGT(GGC)_5GGT(GGC)_{10}$	ggggsgggggggggggggggg
$(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_7$	ggggggsgggggggggggg
$(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_5$	ggggggsgggggggggg
$(GGC)_4GGT(GGC)_8$	gggggggggggg
$(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_5$	ggggggsgggggggggg
$(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_4$	ggggggsggggggggg
$(GGC)_4GGTGGCGGT(GGC)_6$	ggggggggggggg
$(GGC)_4GGTGGCAGT(GGC)_2GGT(GGC)_2GGT(GGC)_6$	gggggsgggggggggggg
$(GGC)_4GGTGGCAGT(GGC)_8$	gggggsgggggggg
$(GGC)_4GGTGGCAGT(GGC)_3GGT(GGC)_8$	gggggsgggggggggggg
$(GGC)_6GGT(GGC)_8$	ggggggggggggg

denaturation at 94°C for 5 minutes, amplification was performed using 35 cycles at 94°C for 50 seconds, annealing for 45 seconds at 60°C, and 72°C for 50 seconds. PCR products were visualized on 2% agarose gel. After purification, amplified PCR fragments were subjected to bidirectional direct and cycle sequencing. Direct sequencing was performed using the BigDye™ Terminator on ABI3730 Genetic Analyzer. Cycle sequencing was performed using Stratagene's RoboCycler Gradient 96 temperature cycler with Hot Top Assembly. The genotype frequencies were calculated, and statistical analysis was performed using the non-parametric mood median test.

Statistical analysis

Statistical analyses were performed using SPSS Statistics 13.0 for Windows. Differences between categorical variables were analyzed using Pearson's chi-square test. A P value<0.05 was considered statistically significant.

Results

A complex insertion/deletion polymorphism was identified in the compositionally biased region of the *ZFHX3* gene in a Chinese Han population (**Figure 1**). Sequencing analysis of PCR products revealed 13 different polymorphisms in the compositionally biased region of the *ZFHX3* gene, the allelic frequency of each genotype was calculated as shown in **Table 1** for CHD patients and healthy controls. The amino acid sequences of the polymorphic segment of ZFHX3 protein were composed of 13~20 glycine residues with or without one serine residue at the compositionally biased region, the difference between these polymorphic segments was mainly repeat numbers of glycine residues (**Table 2**). In the studied population, genotypes of $(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_8$, and $(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_8$ were two main polymorphic segments in CHD patients and healthy controls. Six genotypes of $(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_8$,$(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_8$, $(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_7$, $(GGC)_2GGTGGCAGT(GGC)_5GGT(GGC)_{10}$, $(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_5$, and $(GGC)_4GGT(GGC)_8$ were found in both CHD patients and healthy controls, there was no significant difference in the these genotype frequencies between CHD patients and healthy controls. Rare genotypes $(GGC)_4GGTGGCAGT(GGC)_2GGT(GGC)_2GGT(GGC)_6$, $(GGC)_4GGTGGCAGT(GGC)_8$,$(GGC)_4GGTGGCAGT(GGC)_3GGT(GGC)_8$, and $(GGC)_6GGT(GGC)_8$ were only identified in healthy controls; while, rare genotypes $(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_5$, $(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_4$, and $(GGC)_4GGTGGCGGT(GGC)_6$ were only found in CHD patients.

Discussion

CHD is the most common form of cardiovascular disease with high morbidity and mortality, and is common among patients with atrial fibrillation. Atrial fibrillation is the most common abnormal heart rhythm. Several family studies have identified common genetic variants to be associated with atrial fibrillation in the general population [10]. Recently, a genome wide association study has allowed improved scanning of the genome with greater statistical power to detect susceptibility alleles for atrial fibrillation. Two variants, rs2106261 and rs7193343, in the *ZFHX3* gene on chromosome 16q22 were associated significantly with atrial fibrillation in multiple populations of European ancestry [11]. In the study, a complex insertion/deletion polymorphism was identified in the compositionally biased region of the *ZFHX3* gene. A total of 13 different polymorphisms were found in a

Chinese Han population including healthy controls and CHD patients. A poly-Gly sequence was contained in the compositionally biased region in the *ZFHX3* gene [12]. The polymorphic segment of ZFHX3 protein was composed of 13~20 glycine residues with or without one serine residue at the compositionally biased region, the mainly difference was repeat numbers of glycine residues.

The *ZFHX3* gene, also called AT motif-binding factor 1 (*ATBF1*) on chromosome 16q22 has been reported to be a tumor suppressor gene in multiple cancers, was first described as an enhancer of the human alpha-fetoprotein gene expression in the liver [13]. The *ZFHX3* gene was the largest DNA binding protein reported and the protein shown to contain multiple homeodomains and multiple zinc finger motifs at the time of its discovery [14]. The *ZFHX3* gene has been associated with regulation of growth and differentiation of several tissues, including neuronal and skeletal muscle differentiation [15]. Although the function of *ZFHX3* in cardiac tissue is unknown, it was expressed in mouse hearts [16]. A study indicated that rs2106261 in the *ZFHX3* gene conferred a significant risk of atrial fibrillation in a Chinese Han population, the study expanded the relationship between the *ZFHX3* gene and atrial fibrillation to a non-European population [6]. CHD is common among patients with atrial fibrillation, this suggests that CHD may be associated with the *ZFHX3* gene. Therefore, we performed a case-control study to explore the association between polymorphisms in the compositionally biased region of the *ZFHX3* gene and CHD in a Chinese Han population.

In the study, Genotypes of $(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_8$, and $(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_8$ were common in CHD patients and healthy controls in a Chinese Han population. There was no significant difference in the two genotype frequencies between CHD patients and healthy controls. Four rare genotypes of $(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_7$, $(GGC)_2GGTGGCAGT(GGC)_5GGT(GGC)_{10}$, $(GGC)_4GGTGGCAGT(GGC)_5GGT(GGC)_5$, and $(GGC)_4GGT(GGC)_8$ were no significant difference in CHD patients and healthy controls. Therefore, above-mentioned six genotypes of the *ZFHX3* gene have no association with CHD. Four rare genotypes of $(GGC)_4GGTGGCAGT(GGC)_2GGT(GGC)_2GGT(GGC)_6$, $(GGC)_4GGTGGCAGT(GGC)_8$, $(GGC)_4GGTGGCAGT(GGC)_3GGT(GGC)_8$, and $(GGC)_6GGT(GGC)_8$ were only identified in 358 healthy controls, and not found in CHD patients. We can't therefore conclude that individuals carrying genotypes of $(GGC)_4GGTGGCAGT(GGC)_2GGT(GGC)_2GGT(GGC)_6$, $(GGC)_4GGTGGCAGT(GGC)_8$, $(GGC)_4GGTGGCAGT(GGC)_3GGT(GGC)_8$, or $(GGC)_6GGT(GGC)_8$ are not susceptible to CHD because of low genotype frequencies and inadequate subject numbers. Rare genotypes $(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_5$, $(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_4$, and $(GGC)_4GGTGGCGGT(GGC)_6$ were only found in CHD patients. Similarly, this study can't infer that rare genotypes $(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_5$, $(GGC)_4GGTGGCAGT(GGC)_4GGT(GGC)_4$, and $(GGC)_4GGTGGCGGT(GGC)_6$ in the compositionally biased region of the *ZFHX3* gene are associated with CHD.

The *ZFHX3* gene encodes a transcription factor with multiple homeodomains and zinc finger motifs, and regulates myogenic and neuronal differentiation [17]. The encoded protein has been shown to negatively regulate c-Myb, and transactivate the cell cycle inhibitor cyclin-dependent kinase inhibitor 1A, and suppresses expression of the alpha-fetoprotein gene by binding to an AT-rich enhancer motif. Multiple transcript variants expressed from alternate promoters and encoding different isoforms have been found for the *ZFHX3* gene [18]. Although, the function of ZFHX3 protein in cardiac tissue is unknown, Variants in the *ZFHX3* gene are associated with atrial fibrillation in individuals of European ancestry and Chinese population [6]. In the study, we found that some rare genotypes in the compositionally biased region of the *ZFHX3* gene was only presented in CHD patients, others only presented in healthy subjects in Chinese population. More studies should be focused on the function of the compositionally biased region of the *ZFHX3* gene.

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Disclosure of conflict of interest

None.

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