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TCF21 rs12190287 Polymorphisms Are Associated with Ventricular Septal Defects in a Chinese Population

Liping Yang,[1,*] Xiaobo Gao,[2,*] Haiyan Luo,[2] Qiuyu Huang,[1] Dongmei Su,[2] Xinyu Tan,[3] and Cailing Lu[2,4]

Aims: *TCF21* knockout mice display cardiac defects, including ventricular septal defects (VSDs). Functional rs12190287 polymorphisms located within the 3′ untranslated region (3′-UTR) of *TCF21* were associated with a risk of coronary heart disease in the European and Eastern populations. However, whether rs12190287 polymorphisms in the *TCF21*-3′UTR confer predisposition to congenital heart disease (CHD) is unclear.
Methods: A case–control study was designed consisting of 781 nonsyndromic VSD patients and 867 non-CHD control subjects. The genotype frequency of rs12190287 polymorphisms was determined by real-time polymerase chain reaction.
Results: There were significant differences in the genotype and allele frequencies of rs12190287 between the cases and controls in a Chinese population. Allele G of rs12190287 was significantly associated with an increased risk of VSD in a Chinese population.
Conclusions: Our results demonstrate that rs12190287 polymorphisms confer predisposition to VSDs in the Chinese population studied here.

Keywords: TCF21, rs12190287, ventricular septal defect, susceptibility

Introduction

Congenital heart disease (CHD) is the most common type of birth defect, accounting for one-third of all major congenital disorder anomalies (van der Linde *et al.*, 2011). CHD may occur as part of chromosomal and Mendelian syndromes. However, most instances occur sporadically as isolated nonsyndromic defects and may result from interactions between genetic and environmental factors (Blue *et al.*, 2012). Ventricular septal defects (VSDs) are the most common form of CHD, occurring in more than 1 in 300 live births, as well as a frequent component of more complex lesions, such as DiGeorge syndrome (DGS) (Hoffman, 1995; Hoffman and Kaplan, 2002). Some human syndromic and sporadic cases of VSD have been associated with mutations in the transcription factors *NKX2.5*, *TBX5*, and *GATA4* (Gruber and Epstein, 2004).

TCF21 is a member of the basic helix-loop-helix (bHLH) transcription factor family, which plays crucial roles in cell fate specification and differentiation during organ development (Massari and Murre, 2000). Recent studies have found that *TCF21* knockout mouse embryos display Tetralogy of Fallot (TOF), including a VSD, overriding aorta, and pulmonic stenosis (Harel *et al.*, 2012). Genome-wide association studies found that the single nucleotide polymorphism (SNP) rs12190287 located in the 3′ untranslated region (3′-UTR) of *TCF21* is associated with coronary heart disease in both Caucasian and East Asian populations (Schunkert *et al.*, 2011; Nyegaard *et al.*, 2012). We hypothesized that rs12190287 polymorphisms may play a role in the pathogenesis of CHD. In this study, we investigated the association of rs12190287 with patients with VSDs in a Chinese population. Our data showed that allele G at variant rs12190287 is a contributing risk factor for VSDs.

Materials and Methods

We enrolled 781 isolated patients with VSDs with a mean age of 8.57 ± 11.71 years (Table 1) and 867 control subjects from the Fujian Medical University. Cases that had clinical features of structural malformations involving another organ system or known chromosomal abnormalities were excluded.

[1]Department of Cardiovascular Surgery, Union Hospital, Fujian Medical University, Fuzhou, China.
[2]Department of Genetics, National Research Institute for Family Planning, Beijing, China.
[3]CAS Key Laboratory of Genome Sciences and Information, Chinese Academy of Sciences, Beijing Institute of Genomics, Beijing, China.
[4]Graduate School of Peking Union Medical College, Beijing, China.
*These authors contributed equally to this work.

Table 1. Characteristics of Ventricular Septal Defect Cases

Characteristic	*Cases (n = 781)*
Age, years (mean)	8.57 ± 11.71
Sex	
Males	447
Females	334
VSD	
Conoventricular defect	587
Conal defect	162
Muscular defect	6
Atrioventricular canal defect	26

VSD, ventricular septal defects.

Exclusion criteria also included a positive family history of CHD in a first-degree relative (parent, sibling, or child). Informed consent was obtained from the patients or their guardians. The patients underwent routine clinical examination and ultrasonic echocardiogram, which were further confirmed by open heart surgery.

The control subjects were non-CHD adult outpatients in the same geographic region. Given that neither age nor sex has been shown to have associations with VSD, we did not match the age or sex between cases and controls. This study was approved by the Ethics Committee of Fujian Medical University and adhered to the tenets of the Declaration of Helsinki. For each participant, ~2 mL whole blood was obtained to extract genomic DNA for genotyping analysis.

A genotype analysis of rs12190287 in the cases and the control subjects was performed using TaqMan-based real-time polymerase chain reaction (PCR) (forward primer sequence: 5′-CAGCAATAGTGACCTCATTA-3′; reverse primer for allele G: 5′-CAAATAGACAGGTGGATGAAC-3′; reverse primer for allele C: 5′-AAATAGACAGGTGGAT GAAG-3′). The TaqMan probe, TGGCGACCAC ATTACC AAGC, was labeled at the 5′ end with 6-carboxyfluoroscein (FAM) and at the 3′ end with Black Hole Quencher 1 (BHQ-1). PCR amplification was performed using an ABI Prism 7,500 Sequence Detection System (Applied Biosystems) under the following conditions: one cycle 95°C for 3 min, followed by 40 cycles of 95°C for 5 s and 61°C for 30 s. The ΔCt value was used to differentiate alleles G and C. Quality control of real-time PCR for genotyping was validated by DNA sequencing. Ten percent of the samples were randomly selected for direct sequencing.

The comparisons of genotype and allele frequencies were evaluated using the χ^2 test. The association of the SNP genotype with CHD risk was estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) from logistic regression analysis. The Hardy–Weinberg equilibrium was evaluated using the χ^2 test in the control subjects. All the statistical analyses were performed with the computer software Statistical Package for Social Sciences (SPSS) for Windows (version 18.0).

Results

The frequency distributions of the different genotypes for rs12190287 (C/G) in patients with VSDs and those in the control group are shown in Table 2. The observed genotype frequencies of the controls were in the Hardy–Weinberg equilibrium ($p = 0.21$), suggesting that there was no population stratification and no sampling bias. To evaluate the risk of VSDs associated with the rs12190287 genotype, logistic regression analyses were conducted. As shown in Table 1, there were significant differences in genotype and allele frequency of rs12190287 between the VSD cases and controls. Individuals carrying allele G of rs12190287 showed a 21.0% (OR = 1.21; 95% CI = 1.057–1.391; $p = 0.0061$) increase in VSD risk. Using the CC genotype as the reference genotype, the GG genotype increased the risk of VSD by 1.446-fold (OR = 1.446; 95% CI = 1.101–1.899; $p = 0.0079$), but the GC genotype did not increase VSD risk (OR = 1.206; 95% CI = 0.964–1.508; $p = 0.101$). We also observed that the rs12190287 variant homozygote GG was associated with a significant increase in VSD risk in both the recessive genetic model (OR = 1.598; 95% CI = 1.248–2.047; $p = 0.000205$) and the dominant genetic model (OR = 1.351; 95% CI = 1.095–1.668; $p = 0.005$).

Discussion

In the present study, we provide evidence of a significant association between the genotype and allele frequencies of rs12190287 C/G polymorphism in patients with VSDs but not in those of the control group.

TCF21 rs12190287 was associated with coronary heart disease in both Caucasians and Asians (Schunkert *et al.*, 2011; Nyegaard *et al.*, 2012; Bastami *et al.*, 2016). Wang *et al.* (2014) showed that rs12190287 polymorphisms are

Table 2. Distribution of rs12190287 in Ventricular Septal Defect Cases and Controls and its Effect on Ventricular Septal Defect Risk

Genotypes	*Cases (N = 781), N (%)*	*Controls (N = 867), N (%)*	*OR (95% CI)*	p
CC	224 (28.68)	294 (33.91)	1.00 (reference)	
GC	373 (47.76)	406 (46.83)	1.206 (0.964–1.508)	0.101
GG	184 (23.56)	167 (19.26)	1.446 (1.101–1.899)	0.0079
CC/CG	597 (76.44)	700 (80.74)	1.00 (reference)	
GG	184 (23.56)	167 (19.26)	1.598 (1.248–2.047)	0.000205
CC	224 (28.68)	294 (33.91)	1.00 (reference)	
CG/GG	557 (71.32)	573 (66.09)	1.351 (1.095–1.668)	0.005
C allele	821 (52.56)	994 (57.32)	1.00 (reference)	
G allele	741 (47.44)	740 (42.68)	1.21 (1.057–1.391)	0.0061

OR, odds ratio; CI, confidence interval.

significantly associated with myocardial infarction in a Chinese population. Fujimaki *et al.* (2015) reported that rs12190287 polymorphisms are significantly associated with hypertension in Japanese populations. In the current study, we found that rs12190287 polymorphisms are associated with VSDs in a Chinese population. It is noteworthy that allele C represented a risk factor for disease in previous studies, while our data showed that allele G increases the risk of VSD in our study population. Together, these data suggest that rs12190287 is a disease-related susceptibility locus implicated in cardiovascular disease that has different polymorphic effects on different tissues and in different stages of development. *TCF21* knockout mouse embryos displayed the TOF phenotype, a type of severe outflow tract (OFT) development and alignment defect, including a VSD, overriding aorta, and pulmonic stenosis (Harel *et al.*, 2012). This raises the possibility that rs12190287 polymorphisms may confer susceptibility to TOF.

Pharyngeal mesoderm cells are a subset of the head mesoderm contributing to broad regions of the heart and head musculature (Tzahor and Evans, 2011). Recent studies found that TCF21, TBX1, and LHX2 are genetically linked in the pharyngeal mesoderm transcriptional network, which controls cardiac and head muscle morphogenesis (Harel *et al.*, 2012). It has been reported that the mesoderm-specific deletion of *TBX1* causes severe pharyngeal patterning and cardiovascular defects, including VSDs and cardiac OFT defects (Zhang *et al.*, 2006). *TBX1* haploinsufficiency is probably a major contributor to human DGS and to murine models of the syndrome (Lindsay *et al.*, 2001; Merscher *et al.*, 2001). Mutations in *TBX1* have been found in some patients showing a DGS-like phenotype (Yagi *et al.*, 2003). Approximately 80% of DGS patients are born with CHD, including VSDs and OFT malformations (McDonald-McGinn and Sullivan, 2011). Mesodermal expression of MOZ, a member of the MYST family of histone acetyltransferases, is essential for the normal activation of the TBX1 gene and is necessary for cardiac septum development (Vanyai *et al.*, 2015). The mesoderm-specific deletion of MOZ results in a high penetrance of VSDs and overriding aorta (Vanyai *et al.*, 2015). Miller *et al.* (2013) identified a cis-acting mechanism by which the *TCF21* G allele at variant rs12190287 disrupts an atypical activator protein 1-like enhancer element to decrease the transcriptional control of *TCF21* gene expression. Further study showed that the rs12190287 allele G results in a mismatch that disrupts miR-224 binding and accessibility of this region of the *TCF21* 3′-UTR. The minor allele leads to dysregulated *TCF21* gene expression (Miller *et al.*, 2014). This implies that rs12190287 polymorphisms may influence the mesodermal expression of TCF21, which disrupts cardiac septum and OFT development.

Recent studies have shown that TCF21 is expressed in the epicardium and the proepicardial organ of embryonic zebrafish, chick, and mouse hearts (Hidai *et al.*, 1998; Serluca, 2008; Braitsch *et al.*, 2012). Loss of *TCF21* in mice leads to epicardial blistering, increased smooth muscle differentiation on the surface of the heart, and a paucity of interstitial fibroblasts, with perinatal lethality (Braitsch *et al.*, 2012). The epicardium is a mesothelial cell layer essential for vertebrate heart development and relevant for cardiac repair postinjury in adults (Limana *et al.*, 2010; von Gise and Pu, 2012). Tandon *et al.* (2013) reported that *TCF21* associates with cofactors involved in transcriptional regression, such as Pbx1 and Ctbp2, in the epicardium. Stankunas *et al*. (2008) reported that embryos with different combinations of *Pbx* mutations display a spectrum of cardiac malformations that include persistent truncus arteriosus (PTA), TOF, overriding aorta with a VSD, and bicuspid aortic valves. Each of the observed cardiac defects represents developmental abnormalities affecting distinct stages of cardiac OFT development, correlating with *Pbx* gene dosage (Stankunas *et al.*, 2008). Arrington *et al.* (2012) showed that nonsynonymous variants in *Pbx* genes are associated with CHD, including OFT malformations. *Ctbp2*-null embryos exhibit defects in heart morphogenesis characterized by a dilated pericardium and failure of cardiac looping (Chinnadurai, 2003). Glessner *et al.* (2014) reported that *Ctbp2* may be a pathogenic gene in CHD cases with 10q subtelomeric deletions. This implies that rs12190287 polymorphisms may disrupt cardiac development by influencing the expression of TCF21 and its interaction with other proteins in the epicardium.

In summary, our study demonstrates that rs12190287 polymorphisms confer genetic susceptibility to VSDs within the study population. However, the exclusion criteria for the cases did not include environmental factors, such as maternal diabetes mellitus and maternal teratogen exposures, which may result in selection bias and a false-positive correlation. In addition, our study represents a relatively small sample size; therefore, a larger population-based study should be conducted in the future.

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Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Cailing Lu, PhD
Department of Genetics
National Research Institute for Family Planning
12 Dahuisi, Haidian District
Beijing 100081
China

E-mail: lucailing@sina.com

Xinyu Tan
CAS Key Laboratory of Genome Sciences and Information
Beijing Institute of Genomics
Chinese Academy of Sciences
No. 1 Beichen West Road
Chaoyang District
Beijing 100101
China

E-mail: tanxy@big.ac.cn