

The *HHEX* gene is not related to congenital heart disease in 296 Chinese patients

Xiao-Peng Deng, Li-Xi Zhao, Bin-Bin Wang, Jing Wang, Long-Fei Cheng, Zhi Cheng, Pei-Su Suo, Hui Li, Xu Ma

Shenyang and Beijing, China

Background: The *hematopoietically expressed homeobox (HHEX)* gene is an important determinant of mammalian heart development. This study aimed to identify the potential mutations of the gene in Chinese patients with congenital heart disease (CHD).

Methods: We collected 296 CHD patients and 200 controls, and classified the cardiac deformities. Then we conducted sequence analyses of the *HHEX* gene in those patients.

Results: In all the CHD patients, we did not find any causative mutations in the coding region of the *HHEX* gene.

Conclusion: To our knowledge, this is the first study to examine the *HHEX* gene in non-symptomatic CHD cases, and this has expanded our knowledge about its etiology.

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Key words: congenital heart disease; genetics; *HHEX*; transcription factor

Introduction

Congenital heart disease (CHD) is related to abnormal cardiac development and is a major cause of morbidity and mortality in human

newborns, affecting 1%-2% of live births.^[1] Transcription factors are known to play significant roles in the complex biological processes governing cardiac morphogenesis. Homeobox genes play critical roles in regulating the tissue-specific gene expression that is essential for tissue differentiation, as well as in determining temporal and spatial patterns of development. Mutations in the mouse *hematopoietically expressed homeobox (Hhex)* gene results in abnormal cardiac development and defective vasculogenesis.^[2]

The human *hematopoietically expressed homeobox (HHEX)* gene spans about 5.7 kb on chromosome 10q24, comprises four exons, and encodes a 270-amino-acid protein.^[3,4] Studies^[2,5,6] on the functions of the *HHEX* gene in avian, murine, *Xenopus*, zebrafish and human cells have shown that the essential roles of the *HHEX* gene are similar in all vertebrate species. In mice, *Hhex* is expressed in the developing blood island, contributing to the murine cardiovascular system. *Hhex* is also expressed in the endothelium of the developing vasculature, in the developing heart and, in the ventral foregut endoderm. The *HHEX* gene is a major cardiac determinant, responsible for mammalian heart development. It also functions as a transcriptional repressor that correlates with heart-inducing activities.^[2,7]

We hypothesized that the *HHEX* gene possibly contributed to the development of CHD in humans. We attempted to identify potential pathogenic *HHEX* mutations in 296 Chinese children with CHD, thereby providing insights into its etiology.

Methods

Study population

In this study there were 296 children with non-symptomatic CHD and 200 controls without cardiac phenotype. All of the children were recruited from Lanzhou University. Informed consent was obtained from patients' parents or guardians. The study protocol conformed to the ethical guidelines of the 1975 *Declaration of Helsinki* and was approved by the Ethics Committee of the National Research Institute for

Author Affiliations: Department of Obstetrics and Gynecology, Shengjing Hospital of China Medical University, Shenyang 110004, China (Deng XP, Li H); Graduate School of Peking Union Medical College, Beijing 100730, China (Zhao LX, Wang BB, Wang J, Cheng LF, Cheng Z, Suo PS, Ma X); National Research Institute for Family Planning, Beijing 100081, China (Zhao LX, Wang BB, Wang J, Cheng LF, Cheng Z, Suo PS, Ma X); World Health Organization Collaborating Centre for Research in Human Reproduction, Beijing 100081, China (Ma X)

Corresponding Author: Hui Li, Department of Obstetrics and Gynecology, Shengjing Hospital of China Medical University, No. 36 Sanhao Street, Heping District, Shenyang 110004, China (Tel: +86 024 83955179; Fax: +86 024 83955092; Email: FCKLIHUI@126.com)

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Family Planning. Clinical assessment of the patients included anthropometric measurement, physical examination for dysmorphism and malformation, and radiological evaluation. The patients also underwent chest X-ray examination, electrocardiogram, and ultrasonic echocardiogram.

DNA analysis

Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp blood kit (Qiagen, Hilden, Germany). The *HHEX* is located on 10q24 and is encoded by four exons. For mutational analysis, the four exons and nearby introns were amplified using polymerase chain reaction and three pairs of *HHEX*-specific primers (Table 1). Amplicons were sequenced using appropriate primers and a BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA). Sequencing was carried out on an automated sequencer (ABI 3730XL; Applied Biosystems).

Results

Clinical and molecular findings from patients with cardiac defects are shown in Table 2. Ventricular septal defects, atrial septal defects, patent ductus arteriosus, tetralogy of Fallot, pulmonary atresia or stenosis, double outlet right ventricle, pulmonary hypertension, and other complex cardiac malformations are all indicated. Sequence analysis of the *HHEX* did not show any non-synonymous variance in the coding regions.

Table 1. Oligonucleotide primers used in the amplification of the *HHEX*

Exon	Primer name	Sequence (5' to 3')	Amplicon size (bp)
1	<i>HHEX</i> -1F	CGC GGA TAA ATG TAG CGC	618
	<i>HHEX</i> -1R	ACC GTG ACT CAG ATT GGT TT	
2-3*	<i>HHEX</i> -2F	GGC CTA CCT TTG TCA TCC CT	538
	<i>HHEX</i> -2R	AGT GCA AGG CTG TTA TGG GT	
4	<i>HHEX</i> -3F	CCT TAT GTT CAT GAC TGG TTC TC	562
	<i>HHEX</i> -3R	CTG GAA GGC TAT ATA AGA AAG GG	

*: *HHEX*-2F and *HHEX*-2R were used to amplify exons 2 and 3.

Table 2. Cardiac deformities among the patients

Cardiac deformities	Total (n=296)
Ventricular septal defect	136
Tetralogy of fallot	16
Atrial septal defect	61
Patent ductus arteriosus	13
Pulmonary atresia or stenosis	4
Double outlet right ventricle	30
Aortic coarctation	6
Pulmonary hypertension	4
Other complex cardiac malformations	26

Discussion

Homeobox genes are an evolutionarily conserved class of transcription factors that are key regulators during developmental processes, such as regional specification, patterning and differentiation. Moreover, homeobox genes play crucial roles in specifying cell identity and cell positioning during embryonic development. Mutations in these genes can cause dramatic developmental defects.^[8,9]

Animal experiments have shown the significance of the *HHEX* in vertebrate cardiac development. Loss of the *HHEX* expression in the endoderm blocks heart development in *Xenopus* embryos. Similarly, knocking out *Hhex* in the mouse results in structural heart defects such as ventricular septal defect, valve hypoplasia, and thin myocardia.^[2] However, the cardiac phenotypes of CHD in our study included ventricular septal defects, but there was lack of evidence of other defects detected in mice with *Hhex* mutations. The absence of *HHEX* mutations could be caused by selection bias owing to different cardiac defects in our study. Antagonists of canonical Wnt/ β -catenin signaling have a vital role in the mesoderm during the initiation of cardiogenesis in mouse and *Xenopus* embryos.^[7,10,11] Transcriptional repression of the *HHEX* correlated with heart-inducing activities in tissues adjacent to the heart forming region in *Xenopus*, chick and mouse embryos, suggesting an evolutionarily conserved cardiogenic function for the *HHEX*.^[7,12-14] As the *HHEX* is highly conserved between humans and mice, we speculate that CHD patients might contain mutations in the *HHEX*.

In this study, we screened potential causative mutations in the *HHEX* in Chinese children with CHD. However, we did not find any pathogenic mutations. Previously, Balasubramanian^[15] analyzed the sequence of the *HHEX* in three unrelated patients with pancreatic agenesis and congenital heart defects by direct sequencing, also found no mutation.

The negative results of direct sequencing in this study did not suggest abnormal functions of the *HHEX* protein in CHD may be disregarded. Trans-acting factors and cis-acting elements, which are critical for gene transcription and protein synthesis, could affect the expression of the *HHEX*. Abnormal cardiac morphogenesis may occur when spatial and temporal transcription of the *HHEX* is altered, or when the mRNA is not correctly spliced. Over-expression of the *HHEX* in *Xenopus laevis* causes disruption to developing vascular structures and an increase in the number of vascular endothelial cells.^[16]

A 21-year study from the Czech Republic reported 1604 cases of prenatal diagnosis for CHD. The study showed that only 479 (29.9%) of the 1604 prenatally diagnosed fetuses were alive at the end of the study.^[17] In the present study, we did not find any diagnostic mutations

in the the *HHEX* coding regions. Possibly, there are two reasons for this finding. First, any mutation in the coding sequence of the *HHEX* results in early and severe cardiac defects, similar to those observed in *Hhex* knockout mice. Second, it may be simply due to the relationship between the *HHEX* and the risk of CHD in Chinese patients.

In conclusion, our understanding of the functions of the *HHEX* remains limited, and its exact role in heart development is still unknown. Therefore, further study is required to determine the important role of the *HHEX* during heart development, which may give an insight into the etiology of heart defects.

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Competing interest: There were no competing interests.

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