**Abstract.** Congenital heart defects (CHDs), a wide variety of developmental abnormalities in the structures of the heart and the great thoracic blood vessels, are the most common form of birth defect in humans worldwide. CHDs are accountable for substantial morbidity and are still the leading cause of birth defect-related deaths. Recent studies have demonstrated the pivotal roles of genetic defects in the pathogenesis of CHDs, and a great number of genetic mutations have been associated with CHDs. Nevertheless, CHDs are a genetically heterogeneous disorder and the genetic basis underlying CHDs in an overwhelming majority of cases remains unclear. In the present study, the coding exons and flanking introns of the heart and neural crest derivatives expressed transcript 1 (HAND1) gene, which encodes a basic helix-loop-helix transcription factor crucial for cardiovascular development, were sequenced in 158 unrelated patients with CHDs, and a de novo heterozygous mutation, p.K132X, was identified in a patient with double outlet right ventricle (DORV), as well as ventricular septal defect. The nonsense mutation, which was predicted to produce a truncated HAND1 protein lacking 84 carboxyl-terminal amino acids, was absent in 600 control chromosomes. Functional analyses revealed that the HAND1 K132X mutant had no transcriptional activity. Furthermore, the mutation disrupted the synergistic activation between HAND1 and GATA binding protein 4 (GATA4), another cardiac core transcription factor causally linked to CHDs. To the best of our knowledge, this is the first report on the association of HAND1 loss-of-function mutation with an enhanced susceptibility to DORV in humans. These findings expand the phenotypic spectrum linked to HAND1 mutations, suggesting potential implications for the development of novel prophylactic and therapeutic strategies for DORV.

**Introduction**

Congenital heart defects (CHDs), a series of developmental anomalies in the structures of the heart and the great endothoracic blood vessels, encompassing ventricular septal defect (VSD), atrial septal defect, tetralogy of Fallot, double outlet right ventricle (DORV), transposition of the great arteries, pulmonary atresia and persistent truncus arteriosus, are the most common form of birth defects in humans, with an estimated prevalence of 1% in live births worldwide (1). It was reported that in 2013, there were >34 million individuals living with CHDs worldwide (2). Severe CHDs may result in reduced exercise performance and quality of life (3-6), fetal brain injury and neurodevelopmental delay (7,8), pulmonary hypertension and Eisenmenger syndrome (9), cardiac enlargement and heart failure (10,11), cardiac arrhythmias and sudden cardiac death (12-14). In fact, CHDs are responsible for approximately 30% of all birth defect-related deaths (1). Globally in 2010, CHDs led to approximately 223,000 deaths (15). Although great advances in cardiac surgical techniques and intensive care have allowed the overwhelming majority of children with CHDs to survive into adulthood, unfortunately, the late morbidity and mortality rates are still high in the survivors (16-18). Therefore, CHDs have laid a heavy economic burden on society. To address this problem, a better understanding of the genetic factors involved in the pathogenesis of CHDs is crucial, especially for genetic counseling and risk stratification.
burden on patients and healthcare systems, and this burden is anticipated to be even heavier in the future due to an increasing number of CHDs in adults (16-18). Despite the important clinical significance, the causes of CHDs among most patients remain largely unclear.

It has been previously reported that non-inherited modifiable factors, including maternal illnesses, nutritional deficiencies, and exposure to drugs, toxicants or polluted air during the first trimester of pregnancy may confer an increased vulnerability to CHDs (19). However, a growing body of evidence strongly suggests that genetic defects are the predominant cause of CHDs, and mutations in a great number of genes, particularly those coding for transcription factors essential for cardiovascular morphogenesis, including NK2 homeobox (NKX2)-5, NKX2-6, GATA binding protein (GATA)4, GATA5, GATA6, T-Box (TBX)1, TBX5, TBX20, paired like homeodomain 2 (PITX2) and heart and neural crest derivatives expressed transcript (HAND)2, have been associated with various CHDs (20-57). Nevertheless, CHDs are a genetically heterogeneous disorder, and the genetic basis underlying CHDs in an overwhelming majority of cases remains to be elucidated.

The HAND subset of basic helix-loop-helix (bHLH) transcription factors is composed of two members, HAND1 and HAND2, which are required for the normal cardiovascular development in fish, chicks, rodents and humans (58). The HAND1 protein has a functionally important structural domain termed bHLH, which consists of a short stretch of basic amino acids followed by an amphipathic α helix, a loop and an additional α helix, and is required for binding to target gene DNA and protein-protein combinatorial interactions (59). A previous study demonstrated that HAND1 can directly activate the cardiac ANF promoter, alone or in synergy with transcriptionally cooperative partners, including GATA4, myocyte enhancer factor 2 (MEF2) and HAND2 (60). In chicks, HAND1 and HAND2 are both expressed in the bilateral heart primordia and subsequently throughout the primitive tubular heart, as well as its derivatives during embryonic genesis, and the treatment of chick embryos with HAND1 and HAND2 antisense oligonucleotides has revealed that either oligonucleotide alone has no effect on embryonic development, whereas together they arrest development at the looping heart tube stage (61). In mice, HAND1 is highly expressed in distinct regions of the linear heart tube during embryogenesis and, after looping, becomes localized to both primary heart fields, specifically in the outer curvature of the presumptive left ventricle and the developing outflow tract, and also at a lower level in the outer curvature of the right ventricle (62). Mice lacking Hand1 suffer from defective cardiac looping, failed chamber septation, anomalous ventricular myocardial differentiation and early embryonic lethality resulting from cardiac failure (63,64). Mouse embryos homozygous for the cardiac-specific Hand1-null allele present diverse cardiac deformations, including membranous VSD, overriding aorta and hyperplastic atrioventricular valves, and DORV (65). In humans, HAND1 is expressed in cardiac tissues within both ventricles (66), and mutations in HAND1 have been causally linked to hypoplastic hearts and cardiac septal defects (67-69). However, the prevalence and spectrum of HAND1 mutations in other cohorts of patients with various CHDs remain to be investigated.

Thus, in this study, the coding exons and flanking introns of the HAND1 gene, which encodes a basic helix-loop-helix transcription factor crucial for cardiovascular development, were sequenced in 158 unrelated patients with CHDs. We identified a de novo heterozygous mutation, p.K132X in a patient with DORV. Thus, this mutation may be associated with an enhanced susceptibility to DORV. Our findings may provide new insight into the pathogenesis of DORV and CHDs at the genetic level.

Materials and methods

Ethics. This study was conducted in conformity with the ethical principles for medical research outlined in the Declaration of Helsinki. The study protocol was approved by the local institutional Ethics Committee of Tongji Hospital, Tongji University, Shanghai, China [approval no. LL(H)-09-07], and written informed consent was obtained from the patients or their guardians prior to the study.

Study population. A cohort of 158 unrelated patients (87 males and 71 females, with an average age of 3.3 years) with non-syndromic CHDs was recruited from the Chinese Han population. The available relatives of the CHD cases were also enrolled. All patients underwent a comprehensive clinical evaluation, including medical history, complete physical examination, 12-lead electrocardiogram and two-dimensional transthoracic echocardiography with color flow Doppler. Cardiac catheterization, angiography and cardiac magnetic resonance imaging were carried out only when indicated. The cardiac phenotype was confirmed by echocardiography in all patients with CHDs. Patients with known chromosomal abnormalities or other recognized syndromic CHDs were excluded from the study. A total of 300 healthy subjects (162 males and 138 females, with an average age of 3.2 years), who were matched to the patients with CHD in age, gender and ethnicity, were enrolled as controls. All the control individuals underwent transthoracic echocardiography and their cardiac morphologic structures were shown to be normal.

Mutational analysis of HAND1. Peripheral venous blood samples were drawn from the study participants and the genomic DNA was isolated from leukocytes using the Wizard Genomic DNA Purification Kit (Promega Corp., Madison, WI, USA) according to the manual of procedure. The referential genomic DNA sequence of HAND1 was derived from GenBank (GenBank ID: NC_000005.10), an online nucleotide database at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/nucleotide/). With the aid of the online Primer-BLAST program (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?ORGANISM=9606&INPUT_SEQUENCE=NC_000005.10&LINK_LOC=nucleotide&PRIMER5_START=15474972&PRIMER3_END=15478264), the primers used to amplify the coding exons and splicing junction sites of HAND1 by polymerase chain reaction (PCR) were designed as shown in Table I. PCR was carried out using HotStar Taq DNA Polymerase (Qiagen, Hilden, Germany) on a Veriti Thermal Cycler (Applied Biosystems, Foster, CA, USA). The amplicons were fractionated by electrophoresis on a 2% agarose gel and then purified with the QIAquick Gel Extraction kit (Qiagen, Hilden, Germany). The purified amplicons were
PCR-sequenced under an ABI PRISM 3130 XL DNA Analyzer (Applied Biosystems) with BigDye® Terminator v3.1 Cycle Sequencing kits (Applied Biosystems). The DNA sequences were analyzed with the DNA Sequencing Analysis Software v5.1 (Applied Biosystems). For an identified sequence variance, the Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/), the 1000 Genomes Project (1000GP; http://www.1000genomes.org/data) database, the NCBI’s single nucleotide polymorphism (SNP; http://www.ncbi.nlm.nih.gov/snp) database and PubMed (http://www.ncbi.nlm.nih.gov/pubmed) database were consulted to confirm its novelty.

Alignment of multiple HAND1 protein sequences among species. The amino acid sequences of the HAND1 protein in humans were aligned with those in the chimpanzee, monkey, cattle, mouse, rat, fowl, fruitfly and frog using MUSCLE, an online program (http://www.ncbi.nlm.nih.gov/homologene), in order to show the evolutionary conservation for an altered amino acid.

Expression plasmids and site-directed mutagenesis. The recombinant expression plasmid, HAND1-pcDNA3.1, which contains the full-length cDNA of human HAND1, was constructed as previously described (69). The identified mutation was introduced into the wild-type HAND1-pcDNA3.1 plasmid by site-directed mutagenesis using a QuickChange II XL Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA, USA) and a complementary pair of primers, and was verified by sequencing. The expression plasmid GATA4-pSSRa and the ANF-luciferase reporter (ANF-luc) plasmid, which contains the 2600-bp 5'-flanking region of the ANF gene and expresses the Firefly luciferase, were kind gifts from Dr Ichiro Shiojima at Chiba University School of Medicine (Chiba, Japan).

Cell culture, DNA transfection and luciferase assays. HeLa and NIH3T3 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, and plated at a density of 1x10⁵ cells per well on 24-well plates 24 h prior to transfection. Transfection was performed using Lipofectamine® 2000 reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) following the manufacturer’s instructions. The internal control vector pGL4.75 (Promega Corp.), which expresses the Renilla luciferase, was co-transfected in transfection assays to normalize transfection efficiency. For the transfection of HeLa cells, 1.0 μg of empty pcDNA3.1 vector, 1.0 μg of wild-type HAND1-pcDNA3.1, 1.0 μg of mutant HAND1-pcDNA3.1, 0.5 μg of wild-type HAND1-pcDNA3.1, or 0.5 μg of wild-type HAND1-pcDNA3.1 plus 0.5 μg of mutant HAND1-pcDNA3.1 were used in combination with

Table I. Primers used for the amplification of the coding regions and splicing junction sites of the HAND1 gene.

<table>
<thead>
<tr>
<th>Coding exon</th>
<th>Forward primer (5'→3')</th>
<th>Reverse primer (5'→3')</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GAGCGGCGTTAATAGGCTG</td>
<td>TTCGACTACCTGATGCGCTT</td>
<td>666</td>
</tr>
<tr>
<td>2</td>
<td>GGAACCTCCCGGCATAAGGC</td>
<td>CGTGCGATCCAAAGTGTG</td>
<td>478</td>
</tr>
</tbody>
</table>

bp, base pairs.

Table II. Clinical characteristics of the patients with congenital heart defects.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No. or mean</th>
<th>% or range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>87/71</td>
<td>55/45</td>
</tr>
<tr>
<td>Age, years</td>
<td>3</td>
<td>0-12</td>
</tr>
<tr>
<td>Positive family history of CHDs</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Distribution of distinct forms of CHDs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolated CHDs</td>
<td>95</td>
<td>60</td>
</tr>
<tr>
<td>VSD</td>
<td>31</td>
<td>20</td>
</tr>
<tr>
<td>ASD</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>PDA</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>PS</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>DORV</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>TGA</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>PTA</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>HLV</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>PA</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>TAPVC</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>CoA</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>CAC</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Complex CHDs</td>
<td>63</td>
<td>40</td>
</tr>
<tr>
<td>TOF</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>VSD + ASD</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>DORV + VSD</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>VSD + PDA</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>PTA + VSD</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TGA + VSD</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Incidence of arrhythmias</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atioventricular block</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
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<tr>
<td>Surgical repair</td>
<td>92</td>
<td>58</td>
</tr>
<tr>
<td>Percutaneous closure</td>
<td>32</td>
<td>22</td>
</tr>
<tr>
<td>Follow-up</td>
<td>31</td>
<td>20</td>
</tr>
</tbody>
</table>

ASD, atrial septal defect; CAC, common atrioventricular canal; CHDs, congenital heart defects; CoA, coarctation of the aorta; DORV, double outlet right ventricle; HLV, hypoplastic left ventricle; PA, pulmonary atresia; PDA, patent ductus arteriosus; PS, pulmonary stenosis; PTA, persistent truncus arteriosus; TAPVC, total abnormal pulmonary venous connection; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect.
1.0 µg of ANF-luc and 0.04 µg of pGL4.75. For the transfection of NIH3T3 cells, the same amount (0.5 µg) of plasmid DNA (empty pcDNA3.1 vector, wild-type HAND1-pcDNA3.1, GATA4-pSSRa or mutant HAND1-pcDNA3.1) was used alone or in combination, in the presence of 1.0 µg of ANF-luc and 0.04 µg of pGL4.75. The transfected cells were incubated for 48 h at 37°C with 5% CO₂, then washed and lysed using 1X passive lysis buffer provided by the Dual-Glo luciferase reporter assay kit (Promega Corp.). The Firefly and Renilla luciferase activities were measured using the Dual-Glo luciferase reporter assay kit (Promega Corp.) according to the manufacturer’s instructions. The activity of the ANF promoter was expressed as the fold activation of the Firefly luciferase value relative to the Renilla luciferase value. At least 3 independent transfection experiments, all of which were conducted in triplicate, were performed to calculate average values and standard deviations.

Statistical analysis. Statistical analyses were performed using the SPSS version 17.0 software package (SPSS, Inc., Chicago, IL, USA). Data are expressed as the means and standard deviation, unless otherwise indicated. The numeric variables were compared between 2 groups using the Student’s unpaired t-test. A comparison of the categorical variables between 2 groups was made using Pearson’s χ² test or Fisher’s exact test where appropriate. A two-tailed value of P<0.05 was considered to indicate a statistically significant difference.

Results

Baseline clinical features of the study subjects. In this study, 158 unrelated patients with isolated CHDs (87 males and 71 females, with an average age of 3.3 years) were clinically evaluated in contrast with 300 ethnically-matched, unrelated healthy individuals (162 males and 138 females, with an average age of 3.2 years). All the patients had echocardiographically documented CHDs. Of the 158 patients with CHD, 11 (approximately 7%) had a positive family history of CHDs. The control individuals were physically and mentally healthy with no structural cardiac defects confirmed by echocardiogram, and they had a negative family history of CHDs. There were no significant differences between the patient and control groups as regards demographic characteristics, including age, gender and ethnicity. The baseline clinical characteristics of the 158 unrelated patients with CHDs are presented in Table II.

Discovery of a de novo HAND1 mutation. By PCR sequencing, a heterozygous mutation in HAND1 was identified in one of the 158 unrelated patients with isolated CHDs, with a mutational prevalence of approximately 0.63%. Specifically, a substitution of thymine (T) for adenine (A) in the first nucleotide of codon 132 (c.394A>T), predicting the conversion of the codon coding for lysine (K) into a stop codon (X) at amino acid position 132 (p.K132X), was discovered in a 5-month-old boy affected with congenital DORV, as well as VSD, who had no family history of CHDs. The nonsense mutation was neither found in the mutation carrier’s healthy parents, nor detected in the 300 unrelated control individuals, indicating it is a de novo mutation. The sequence chromatograms showing the heterozygous HAND1 mutation of c.394A>T, as well as its control sequence are shown in Fig. 1. A schematic diagram of HAND1 showing the bHLH structural domain and the location of the identified mutation is shown in Fig. 2. The identified HAND1 mutation c.394A>T has not been reported in the HGMD, 1000GP, SNP and PubMed databases (accessed on September 12, 2016), suggesting that it is a novel mutation.

Alignment of multiple HAND1 protein sequences across species. Multiple alignments of the HAND1 protein sequences among various species displayed that the altered lysine at amino acid position 132 (p.K132X) was completely conserved evolutionarily (Fig. 3).

No transcriptional activity of the mutant HAND1 protein. As shown in Fig. 4, dual-luciferase assays in the cultured HeLa cells revealed that the same amount (1.0 µg) of wild-type and
K132X-mutant HAND1-pcDNA3.1 plasmids transcriptionally activated the ANF promoter by approximately 10-fold and approximately 1-fold, respectively. When 0.5 µg of wild-type HAND1-pcDNA3.1 was used together with the same amount (0.5 µg) of 132X-mutant HAND1-pcDNA3.1, the induced activation of the ANF promoter was approximately 6-fold.

**Synergistic transactivational failure caused by the mutation.** As shown in Fig. 5, dual-luciferase assays in the cultured NIH3T3 cells revealed that the same amount (0.5 µg) of wild-type and K132X-mutant HAND1 activated the ANF promoter by approximately 4-fold and approximately 1-fold, respectively; while in the presence of 0.5 µg of wild-type GATA4, the same amount (0.5 µg) of wild-type and K132X-mutant HAND1...
activated the ANF promoter by approximately 22-fold and approximately 7-fold, respectively.

Discussion

In the current study, a novel heterozygous mutation, p.K132X, was identified in a patient with isolated DORV, as well as VSD. The nonsense mutation, which was absent in the 600 reference chromosomes, altered the amino acid that was completely conserved evolutionarily across species, and was predicted to generate a truncated protein with partial bHLH domain left. Functional tests revealed that the K132-mutant HAND1 protein had no transcriptional activation of the ANF promoter. Furthermore, the mutation abrogated the synergistic activation of the ANF promoter between HAND1 and GATA4. Therefore, it is likely that the identified HAND1 mutation predisposes to DORV, as well as VSD.

In humans, the HAND1 gene, as the eHAND gene, is located at chromosome 5q33, coding for a transcription factor protein of 215 amino acids. In this study, the HAND1 mutation identified in a patient with CHD was predicted to generate a truncated protein losing the partial bHLH domain; thus, it is reasonably anticipated to disable HAND1. Functional analyses substantiated that the mutant HAND1 lost the transcriptional activation of the ANF promoter. Furthermore, the mutation disrupted the synergistic activation between HAND1 and GATA4, another cardiac core transcription factor previously associated with CHDs in humans (70). These findings strongly suggest that haploinsufficiency caused by the HAND1 mutation is probably an alternative pathological mechanism of CHDs.

Somatic or germline mutations in HAND1 have been previously associated with various CHDs in humans (67-69). By direct PCR sequencing of the HAND1 gene in human heart tissues derived from 31 unrelated patients with hypoplastic hearts, Reamon-Buettner et al (67) identified a common frameshift mutation (p.A126fsX12) in 24 of 31 hypoplastic left or right ventricles. Luciferase assays revealed that the resulting mutant protein was unable to modulate the transcription of reporter genes, suggesting that functionally impaired HAND1 leads to hypoplastic human hearts. Subsequently, Reamon-Buettner et al (67) sequenced HAND1 in a cohort of 68 malformed hearts affected primarily by septation defects, and detected 32 different nonsynonymous mutations, of which 12 are in the bHLH domain of HAND1. Functional analyses using yeast and mammalian cells have revealed that the transcriptional activity of HAND1 is reduced or abolished by certain mutations, suggesting that genetically compromised HAND1 may also be responsible for septation defects of the human hearts. Chen et al (68) screened the coding regions of HAND1 in 498 unrelated individuals affected with non-syndromic CHDs, and found 2 novel non-synonymous mutations of p.G73S and p.K152N in 2 patients suffering from VSD, respectively. Yeast two-hybrid and liquid β-galactosidase assays indicated that both mutations increased the transcriptional activity of HAND1, probably by enhancing the capability of HAND1 to form homodimers. In this study, a de novo HAND1 mutation of p.K132X was discovered in a patient with DORV and VSD, thus expanding the clinical phenotypic spectrum linked to HAND1 mutations. Taken collectively, these findings highlight the exquisite sensitivity of the developing cardiovascular system to the function of HAND1, suggesting a key role of HAND1 in human heart development and CHDs.

In conclusion, this study firstly associates HAND1 loss-of-function mutation with enhanced susceptibility to DORV and VSD, which adds significant insight to the molecular pathogenesis underpinning CHDs, suggesting potential implications for precise diagnosis and genetic counseling of patients with CHD.

Acknowledgements

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References

A novel NKX2.6 mutation in human and mouse asso-

Sanchez-Castro M, Pichon O, Briand A, Poulain D, Gournay
David A and Le Caigenc C: Disruption of the SEMA3D gene in a
patient with congenital heart defects. Hum Mutat 36: 30-33, 2015.

Theis JL, Zimmermann MT, Evans JM, Eckloff BW, Wiesend EJ,
Quintero-Rivera F, Xi QJ, Keppler-Noreuil KM, Lee JH, Qureshi MY, O'Leary PW and Olson TM: Recessive MYH6

Kassab K, Hariri H, Gharibeh L, Fahed AC, Zein M, El-Rassi Y,
Nemer O, El-Rassi I, Bitar F and Nemer G: GATA5 mutation
potentially linked to a double outlet right ventricle phenotype

Perrot S, Schmitt KR, Roth EM, Stiller B, Posch MG,
Browne EN, Timmann C, Horstmann RD, Berger F and

Wang J, Mao JH, Ding KK, Xu WJ, Liu XY, Qiu XB, Li RG,
QX XK, YU JH, Huang RT, et al: A novel NKX2.6 mutation

investigation of somatic NKX2-5 mutations in Chinese children

Pan Y, Wang ZG, Liu XY, Zhao H, Zhou N, Zheng GF, Qiu XB,
Li RG, Yuan F, Shi HY, et al: A novel TBX1 loss-of-function

Zhao CM, Peng LY, Li L, Liu XY, Wang J, Zhang XL, Yuan F,
Li RG, Qiu XB and Yang YQ: PITX2 loss-of-function mutation
correlates to congenital endocardial cushion defect and

Li H and Ma X: Functional analysis of two novel mutations
in TWIST1 protein motifs found in ventricular septal defect patients. Pediart Cardiol 36: 1602-1609, 2015.

Pan Y, Geng R, Zhou N, Zheng GF, Zhao H, Wang J, Zhao CM,
Qiu XB, Yang YQ and Liu XY: TBX20 loss-of-function mutation

and Zou J: Whole-exome sequencing identify a new mutation

Sifrim A, Hitz MP, Wilson A, Breckpot J, Turki SH, Thiuyen T,
INTERVAL Study; UK 10K Consortium; Deciphering Developmental Disorders Study: Distinct genetic architectures for syndromic and nonsyndromic congenital heart defects

Boyle L, Wamelink MM, Salomons GS, Roos BS, Pop A,
Dauwer Hwa, Andrew M, Douglas J, Feingold M, et al;
mutations in TKT are the cause of a syndrome including short stature, developmental delay, and congenital heart defects. Am J Hum Genet 36: 1262-1266, 2015.

Zhao H, Wang J, Mao JH, Ding KK, Xu WJ, Liu XY, Qiu XB, Li RG,
QX XK, YU JH, Huang RT, et al: A novel NKX2.6 mutation

and Zou J: Whole-exome sequencing identify a new mutation