Original Article

Analysis of the *CHD7* gene mutations in patients of congenital heart disease with extracardiac malformations

Xianghui Huang[1#], Han Gao[1#], Weicheng Chen[1#], Zhiyu Feng[1], Chaozhong Tan[1], Quannan Zhuang[1], Jinxin Wang[1], Yuan Gao[1], Shaojie Min[1], Qinyu Yao[1], Jingwei Sun[2], Weili Yan[1], Xiaojing Ma[1], Feizhen Wu[1], Wei Sheng[1,3,4], Guoying Huang[1,3,4]^

[1]Institute of Pediatrics, Children's Hospital of Fudan University, Shanghai, China; [2]Bengbu First People's Hospital, Bengbu, China; [3]Shanghai Key Laboratory of Birth Defects, Shanghai, China; [4]Research Unit of Early Intervention of Genetically Related Childhood Cardiovascular Diseases (2018RU002), Chinese Academy of Medical Sciences, Shanghai, China

Contributions: (I) Conception and design: G Huang, W Sheng; (II) Administrative support: G Huang; (III) Provision of study materials or patients: X Huang, H Gao, W Chen; (IV) Collection and assembly of data: X Huang, H Gao, W Chen, Z Feng, C Tan, Q Zhuang, J Wang, Y Gao, S Min, Q Yao, J Sun, W Yan, X Ma, F Wu; (V) Data analysis and interpretation: X Huang, H Gao; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Dr. Guoying Huang, MD, PhD. Children's Hospital of Fudan University, Shanghai Key Laboratory of Birth Defects, No. 399 Wanyuan Road, Shanghai 201102, China. Email: gyhuang@shmu.edu.cn; Dr. Wei Sheng, MD, PhD. Children's Hospital of Fudan University, Shanghai Key Laboratory of Birth Defects, No. 399 Wanyuan Road, Shanghai 201102, China. Email: Sheng4616@126.com.

Background: Congenital heart disease (CHD) is a common birth defect, and is frequently accompanied with extracardiac malformations (ECM). Uncovering the genetic etiology of CHD may have a meaningful impact on disease management. De novo variants have been proven to be associated with CHD.

Methods: Whole exome sequencing was performed for 4 unrelated CHD families with extracardiac malformations, candidate genes were screened by using stringent bioinformatics analysis, and the obtained variants were confirmed by Sanger sequencing. RT-PCR and Sanger sequencing were used to investigate the influence of a splice variant on pre-mRNA splicing. Further targeted sequencing was conducted to investigate the association of *CHD7* variants with sporadic CHD.

Results: Four novel heterozygous loss-of-function *CHD7* mutations were found by using stringent bioinformatics analysis: the frameshift mutation c.1951_1952delAAinsT (p.L651X) in family #1, the nonsense mutations c.2913C>G (p.Y971X) in family #2 and c.3106C>T (pA1036X) in family #3, and the splicing mutation c.4353+4_4353+12delinsGCCCA in family #4. Sanger sequencing confirmed that these were all de novo mutations and were absent in the healthy parents and siblings of the probands. Further studies revealed that the splice mutation c.4353+4_4353+12delinsGCCCA influenced CHD7 mRNA splicing *in vivo*. Targeted sequencing found 23 rare mutations in 1,155 sporadic CHD patients.

Conclusions: The findings here confirm that de novo loss-of-function variants of the *CHD7* gene are the genetic cause of familial CHD with extracardiac malformations and the spectrum of pathogenic *CHD7* variants in sporadic CHD is expanded.

Keywords: Congenital heart disease (CHD); extracardiac malformation (ECM); CHD7 gene variant; CHARGE syndrome

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^ ORCID: 0000-0003-0505-5839.

Introduction

Congenital heart disease (CHD) is the most common type of birth defect, with an incidence rate of 8.98‰ live births in China (1). The phenotypes of CHD patients are highly variable, ranging from single, localized structural defects, such as ventricular septum defect and atrial septum defect, to complex structural malformations, like tetralogy of Fallot and transposition of the great arteries. Moreover, complex congenital heart malformations are frequently accompanied with congenital extracardiac malformations, which are present in approximately 13% of newborns with CHD, including 2% of cases that are associated with a genetic syndrome such as neurodevelopmental disabilities and CHARGE (coloboma, heart defects, choanal atresia, growth retardation, genital abnormalities, and ear abnormalities) syndrome with the identification code of 214800 on Online Mendelian Inheritance in Man (OMIM) (2). These complex malformations are associated with severe CHD subtypes, and are the leading causes of death among newborns, accounting for approximately 10% of cases of fetal demise (3,4). Uncovering the cause of CHD may have a meaningful impact on the clinical outcomes and prognosis of patients.

The causes of CHD development are complex, involving environmental and genetic factors (5). Based on recurrent risk and familial inheritance, strong evidence indicates the genetic etiology of CHD (6). With advances in next-generation sequencing (NGS), the number of research on gene variants and CHD is rapidly increasing. *De novo* monogenic variants are identified in approximately 10% of CHD cases, and rare inherited variants and copy number variants account for approximately 1% and 25% of affected cases, respectively (7). More than 400 genes have been reported to be associated with CHD. These known genes encode heart development-related transcription factors (TF), structural proteins, cell-signaling molecules, chromatin modifiers, cilia-related proteins (8), suggesting the genetic mechanisms of CHD are complex (7). Hence, CHD patients with the same variants may exhibit different phenotypes, or even showing complete non-penetrance (no disease) (8). NKX2-5 is a TF regulating cardiomyocyte differentiation and proliferation (9,10). Damaging mutations in *NKX2-5* are associated with a wide spectrum of CHD (11), and the phenotype and penetrance of these mutations may be dependent on the genetic background of the individual or interaction with other proteins (12). GATA4 and TBX5 are another TF of T-box family. Deleterious variations in TBX5 are known to cause isolated septal defects or other types of CHD in the setting of Holt-Oram syndrome (13,14).

CHD7 (chromodomain helicase DNA-binding protein 7) is a transcriptional regulator that plays an important role in the development of the central nervous system, the inner ear, and the pharyngeal arches of the neural crest (15). Mutations in this gene have been shown to be the main pathogenic cause of CHARGE syndrome (16). Approximately 85% of CHARGE syndrome patients exhibit CHD phenotypes, such as tetralogy of Fallot, double outlet of right ventricle, and atrioventricular septal defect (17). In this study, whole exome sequencing (WES) was performed in four unrelated CHD families with extracardiac malformations, and four novel *de novo* deleterious variants of the *CHD7* gene were found, which may be the genetic cause of the development of familial CHD with extracardiac malformations. These findings expand the spectrum of pathogenic *CHD7* variants. We present this article in accordance with the MDAR reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-22-634/rc).

Highlight box

Key findings

- Four novel heterozygous loss-of-function *CHD7* mutations were found in four congenital heart disease (CHD) families with extracardiac malformations and 23 rare mutations were found in 1,155 sporadic CHD patients.

What is known and what is new?

- *CHD7* is a transcriptional regulator that plays an important role in the development of the central nervous system, the inner ear, and the pharyngeal arches of the neural crest.
- De novo loss-of-function variants of the *CHD7* gene are the genetic cause of familial CHD with extracardiac malformations and the spectrum of pathogenic CHD7 variants in sporadic CHD is expanded.

What is the implication, and what should change now?

- *CHD7* variants are associated with increased risk of CHD and extracardiac malformations development and may be served as an important susceptible genetic event for CHD.

Methods

Study participants and clinical evaluation

This study was approved by the ethics committee of Fudan University Children's Hospital (No. 2016121), and was conducted in accordance with the Declaration

Table 1 Primers for amplifying fragments including variants identified in the CHD families with extracardiac manifestations

No. patient (family)	Position	Variant	Primers	Length (bp)
1 (F1)	chr8:61693844	c.1951_1952delAAinsT	Forward: CATTCCCCGTCGGAGCCCTTTC	516
			Reverse: CACCTCATTTCATAGGCTGTAAC	
2 (F2)	chr8:61734660	c.2913C>G	Forward: AGGACATAGATCAAGCAAAGATC	469
			Reverse: ACACACTTATATCAATCTCTGTA	
3 (F3)	chr8:61735210	c.3106C>T	Forward: ATATTTTGTTGAATCTAAGAG	550
			Reverse: TTCATAGACACAGAAAGATAAT	
4 (F4)	chr8:61750398	c.4353+4_4353+12 delinsGCCCA	Forward: GCCACCTCCAAATACCATCAC	574
			Reverse: CATAGGCCCCTTTTCGTAGAAG	

CHD, congenital heart disease.

of Helsinki (as revised in 2013). Written informed consent was obtained from the guardians of the affected individuals. Four unrelated CHD families with extracardiac malformations and 1,155 patients with sporadic CHD were recruited from the Children's Hospital of Fudan University. The CHD patients and their siblings were subjected to a series of clinical evaluations, and their blood samples were collected for trio-WES, NGS, and Sanger sequencing. Reference control data of 9,197 healthy individuals from East Asian populations were collected from GnomAD v2 (Genome Aggregation Database, https://gnomad.broadinstitute.org/).

Whole exome sequencing

Genomic DNA samples were extracted from the peripheral venous blood of patients and their family members by using the QIAamp DNA Blood Mini Kit (Qiagen), according to the manufacturer's protocol. WES of the genomic DNA samples was performed by Shanghai Gemple Biotech Co. Ltd., to screen for deleterious genetic variants via stringent bioinformatics analysis. Allele frequencies were obtained from GnomAD v2. Genetic variants were predicted to be deleterious by using SIFT (Sorting Intolerant from Tolerant, https://sift.bii.a-star.edu.sg/), PolyPhen2 (prediction of functional effects of human nsSNPs, http://genetics.bwh.harvard.edu/pph2/), MutationTaster (https://www.mutationtaster.org/), and CADD (Combined Annotation Dependent Depletion, https://cadd.gs.washington.edu/snv). Unique or rare variants (absence or minor allele frequency <0.01% in gnomAD_exome_EAS) were also screen, and deleterious candidates (damaging variants as predicted by the SIFT, PolyPhen2, and MutationTaster prediction programs, and CADD score >20) were predicted.

Sanger sequencing

The selected candidate variants screened by WES were validated using Sanger sequencing in the patients and their family members. Primer pairs were designed using the online software Primer3 (https://bioinfo.ut.ee/primer3/), and were used to amplify fragments including the variants. The primers used in this study are listed in *Table 1*. For targeted sequencing, the sequencing of *CHD7* was performed to an average depth of 100×. Mutation filtering was conducted using the same criteria used for the trio-WES analysis. Polymerase chain reaction (PCR) assays were performed using genomic DNA samples following the PrimeSTAR® Max DNA Polymerase (Takara, Shiga, Japan) standard protocol. The PCR products were sequenced by Shanghai Jie Li Biotechnology Co. Ltd. (Shanghai, China). The sequencing results were analyzed using Mutation Surveyor software (SoftGenetics, Nittany Valley, Pennsylvania, USA).

Reverse transcription PCR and cDNA sequencing

Fresh blood samples were obtained from the members of the four families during their return visit. Total RNA was extracted from peripheral blood mononuclear cells in the fresh blood samples by using TRIzol reagent (Thermo Fisher Scientific, USA). Next, cDNA was synthesized from 500 ng total RNA by using PrimeScript™ RT Master Mix (Takara, China)

Table 2 Clinical characteristics of the affected individuals with *CHD7* mutations

Characteristics	Patient (family)				
	F1	F2	F3	F4	Total
Sex	Female	Female	Male	Male	NA
Age	30 days	5 hours	4 days	10 months	NA
Congenital heart disease	CoA, ASD, PDA	ASD, VSD, PDA, BAV	ASD, PDA	ASD, PDA	4/4
Structural brain anomalies	Lateral ventricular enlargement (left)	Lateral ventricular enlargement (left)	Lateral ventricular enlargement (both)		3/4
Hypothalamo-hypophyseal dysfunction and genital anomalies			Cryptorchidism, Small penis	Cryptorchidism	2/4
Renal /Skeletal/limb anomalies	Syndactyly		Micrognathia		2/4
Cranial nerve dysfunction including hearing loss		Hearing loss	Hearing loss		2/4
Developmental delay			+	+	2/4
Abnormal external, middle, or inner ears				Semicircular canal defect	1/4

NA, not available; CoA, coarctation of aorta; ASD, atrial septal defect; PDA, patent ductus arteriosus; VSD, ventricular septal defect; BAV, bicuspid aortic valve.

according to the manufacturer's protocol. With reverse transcription (RT)-PCR primer pairs (forward primer: 5'-GGCTCAGGCTAGATGTCATAGA-3' and reverse primer: 5'-TAAACGATTCCGGTCAAAACAAC-3') designed using the online software Primer3 (https://bioinfo.ut.ee/primer3/), the targeted cDNA fragments were amplified and the mRNA sequence of CHD7 were analyzed by using PCR. The PCR products were subjected to Sanger sequencing. The cDNA nucleotide sequence analysis was based on the reference sequence of *CHD7* (NM_017780).

AI predictions

RoseTTAFold was accessed via the RosettaCommons web-interface server Robetta (https://robetta.bakerlab.org/submit.php) to predict the possible structure of CHD7. Subsequently, the conserved score was rated using Consurf (https://consurf.tau.ac.il/).

Statistical analysis

The association of rare deleterious CHD7 mutations with CHD was analyzed using Fisher exact test (two-sided) with GraphPad Prism 9.0 (GraphPad Software). $P<0.05$ was considered indicative of statistical significance.

Results

Clinical features of affected individuals in CHD families with extracardiac malformations

The clinical characteristics of the affected individuals from the four CHD families are summarized in *Table 2*. Patient #1 in family #1 was a 30-day-old girl who was sent to the hospital because of a heart murmur detected after birth. The patient was delivered at full term, and had a birth weight of 2,800 g. The parents of the proband were not consanguineous and manifested normal phenotypes. Echocardiography revealed atrial septal defect, patent ductus arteriosus, bicuspid aortic valve, increased left pulmonary artery flow velocity, and pulmonary hypertension. Computed tomography angiography showed coarctation of the aorta and descending right arch, aberrant left subclavian artery, atrial septal defect, patent ductus arteriosus, and pulmonary hypertension. Computed tomography results indicated left lateral ventricular enlargement. The proximal bronchus of the right upper lobe showed stenosis. Laryngoscopy suggested congenital laryngeal dysplasia. The 4th and 5th toes on the left foot

were deformed (syndactyly), and the pulse of dorsal artery of the foot was unclear. A fundus examination revealed two focal masses below the right eye, approximately 1 pupillary distance apart, with clear borders; no exudation or retinal hamartoma was detected.

Proband #2 in family #2 was a baby girl who was sent to Children's Hospital 5 hours after birth because of congenital esophageal atresia. Her clinical data were as follows: G2P1; birth weight, 2,050 g; birth length, 45 cm; head circumference, 34 cm; gestational age, 36^{+6} weeks; and cesarean delivery due to intrauterine distress and hydramnios. Echocardiography revealed ventricular septal defect, atrial septal defect, patent ductus arteriosus, bicuspid aortic valve, tricuspid regurgitation, and pulmonary hypertension. Magnetic resonance imaging (MRI) showed a premature infant with occipital subdural hemorrhage, posterior horn of the left lateral ventricle hematocele, and a left lateral ventricular enlargement. The pelvis of the left kidney was slightly widened. Laryngoscopy suggested softening of the larynx. A hearing test revealed severe hearing loss in both ears.

The patient in family #3 was a 4-day-old boy who was referred to our hospital because of congenital malformations. He had been delivered at full term after his mother experienced premature rupture of membranes, and had a birth weight of 2,800 g. His parents and sister all showed normal phenotypes. Echocardiography revealed patent ductus arteriosus, atrial septal defect, and pulmonary hypertension. Brain MRI showed subdural hemorrhage, left choroid plexus hemorrhage with left lateral ventricle hematocele, left frontal and bilateral top scalp hematoma, and both-side lateral ventricular slight enlargement. B-ultrasound scans revealed that liquid occupied the space from the head of the choroidal nucleus to the head of the caudate nucleus on both sides. The middle cerebral artery resistance index increased. Brainstem auditory evoked potential testing revealed extremely severe hearing loss in the left ear and moderate hearing loss in the right ear. Laryngoscopy showed congenital hypoplasia of laryngeal cartilage. A fundus examination showed a large coloboma of choroid under both eyes. The following deformities of the external genitalia were also present: cryptorchidism and small penis.

The affected individual in family #4 was a 10-month-old boy who presented with atrial septal defect, patent ductus arteriosus, and developmental delay. His clinical manifestations were as follows: G2P2, weight 7,400 g, choking while drinking milk, not crawling, left ptosis, dysplasia of the external genitalia (cryptorchidism), normal auditory brainstem responses and otoacoustic emissions, and severe hearing loss in both ears. Computed tomography scanning of the temporal bone revealed bilateral vestibular and semicircular deformities. A plain MRI scan of the inner ear canal revealed the absence of semicircular canals. Laryngoscopy showed congenital hypoplasia of laryngeal cartilage.

Identification of de novo pathogenic variants of the CHD7 gene

To determine the potential genetic cause of CHD with extracardiac malformations in the four unrelated families, WES was performed on the four probands and their siblings or parents. Considering the normal phenotypes of the probands' parents, we focused on *de novo* homozygous or compound heterozygous disease-causing variants. By using a stringent filtering strategy, we identified four novel deleterious variants of the *CHD7* gene, and confirmed by Sanger sequencing that all these mutations were *de novo* and absent in the healthy parents and siblings of the probands. All the variants were loss-of-function mutations: the frameshift mutation c.1951_1952delAAinsT(p.L651X) in family #1, two nonsense mutations c.2913C>G (p.Y971X) in family #2 and c.3106C>T(pA1036X) in family #3, and the splicing mutation C.4353+4_4353+12delinsGCCCA in family #4 (*Table 3*).

In family #1, the proband was found to harbor a novel heterozygous frameshift mutation in the *CHD7* gene (*CHD7*: NM_017780, exon 3, c.1951_1952delAAinsT;p.L651X), which led to the deletion of two AA bases and the insertion of one T base, producing a stop codon and resulting in a protein that was prematurely truncated at acid amino position 651. This loss-of-function variant was absent in the public database gnomAD_genome_EAS, and was confirmed to be a *de novo* mutation by Sanger sequencing of the proband and his parents (*Figure 1*). The altered residue was located in an unknown domain of the CHD7 protein and was highly conserved across many species, except for zebrafish (*Figure 2*).

Patient #2 in family #2 was a newborn girl who carried a novel heterozygous nonsense variant in the *CHD7* gene (*CHD7*: NM_017780, exon 11, c.2913C>G;p.Y971X). Sanger sequencing of the patient and her parents confirmed this variant to be a *de novo* mutation (*Figure 1*). The variant was located in exon 11 of the *CHD7* gene and involved a stop codon, resulting in the premature truncation of the CHD7 protein at acid amino position 971 in an

Table 3 Variants identified in the CHD families with extracardiac manifestations

No. patient (family)	Position	Variant	Protein change	SIFT[a]	PolyPhen2[a]	MutationTaster[a]	CADD_phred score	ExAC_EAS[b]	Pattern of inheritance
1 (F1)	chr8:61693844	c.1951_1952delAAinsT	p.L651X	NA	NA	NA	52.3	0	*De novo*
2 (F2)	chr8:61734660	c.2913C>G	p.Y971X	NA	NA	NA	39.1	0	*De novo*
3 (F3)	chr8:61735210	c.3106C>T	p.A1036X	NA	NA	NA	41	0	*De novo*
4 (F4)	chr8:61750398	c.4353+4_4353+12 delinsGCCCA	Splicing	NA	NA	NA	49.6	0	*De novo*

[a], mutation assessment by SIFT, PolyPhen-2 (PPH2), and MutationTaster. [b], frequency of corresponding mutations in all populations of the ExAC Browser. NA, not available; CHD, congenital heart disease.

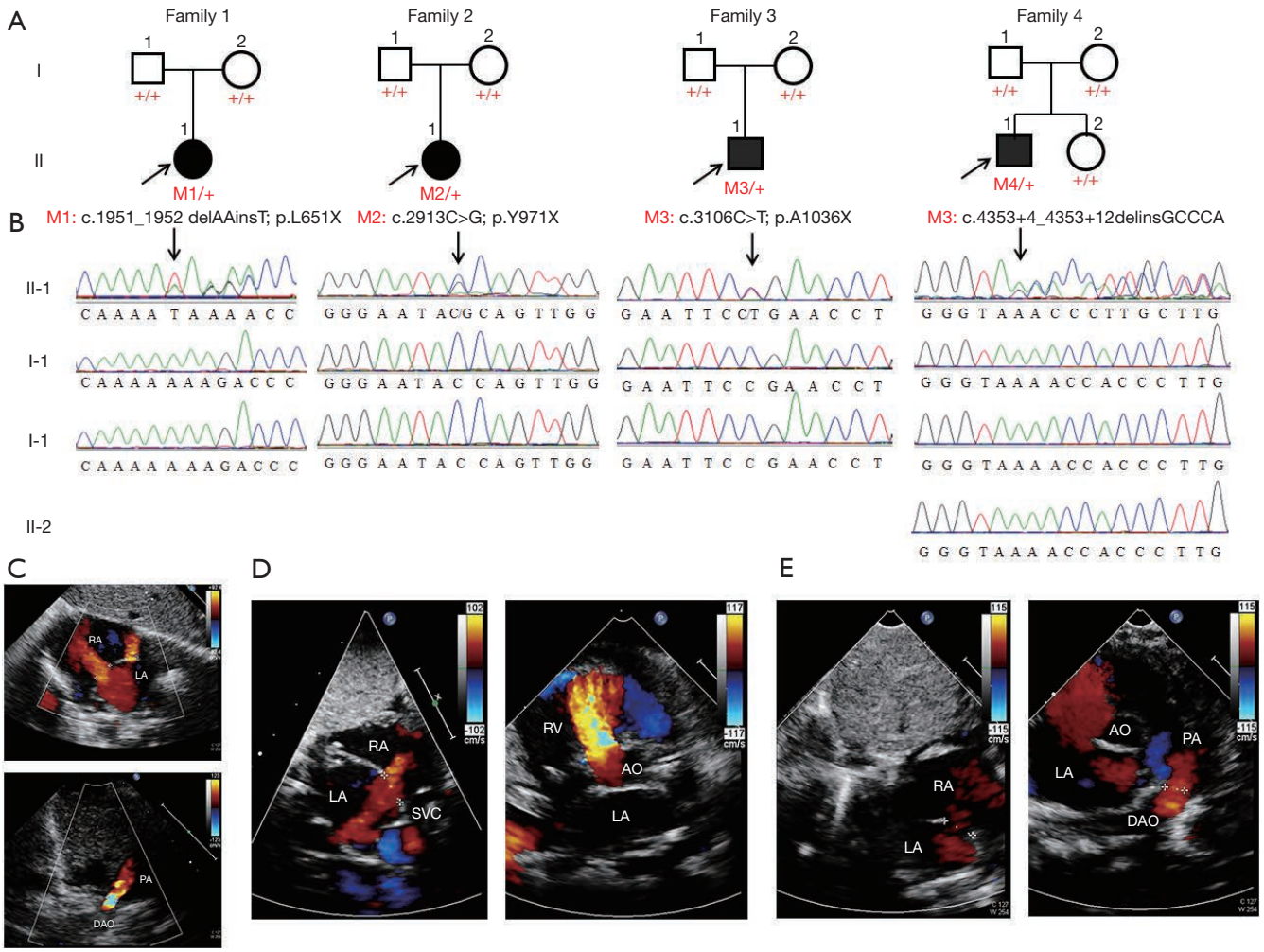

Figure 1 Identification of *CHD7* variants in CHD families with extracardiac malformations. (A) Pedigrees are shown for the 4 affected families. The oblique arrows indicate the probands. The black circles and squares represent the affected individuals. (B) Sanger sequencing confirmation of the variants is shown below the pedigrees. The vertical arrow indicates the location of the variant. (C) Echocardiography of II-1 in family #1 revealed ASD (top) and PDA (bottom). (D) Echocardiography of II-1 in family #2 indicated ASD (left) and VSD (right). (E) Echocardiography of II-1 in family #3 showed ASD (left) and PDA (right). LA, left atrium; RA, right atrium; PA, pulmonary artery; DAO, descending aorta; SVC, superior vena cava; RV, right ventricle; AO, aorta.

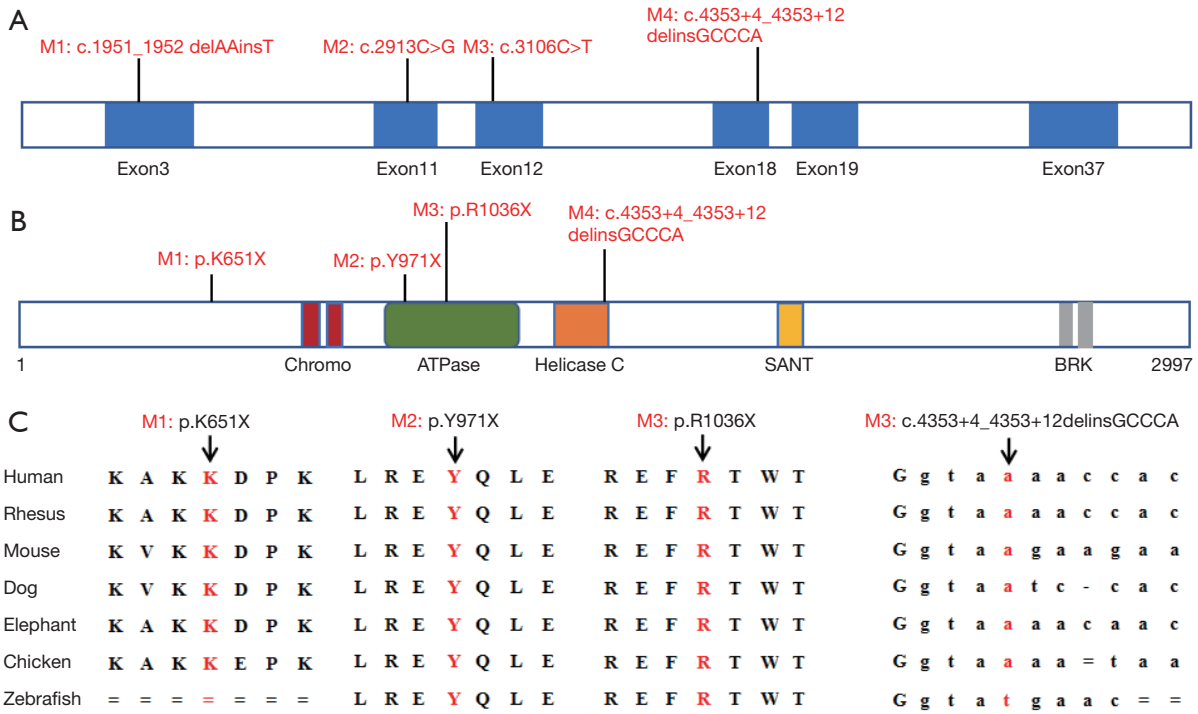

Figure 2 Locations and conservation of variants of *CHD7*. (A) The positions of the 4 variants are indicated in the genomic structure of *CHD7*. (B) Schematic representation of the structure of CHD7 and the position of the affected residues of CHD7. (C) The affected residues are highly conserved among different species (prepared from UCSC genome browser).

ATPase-related domain. This variant was predicted to be potentially deleterious. The affected residue was highly conserved among all seven species (*Figure 2*), supporting its pathogenicity.

In family #3, the proband was a 4-day-old boy with congenital malformations who harbored a heterozygous nonsense variant of the *CHD7* gene (*CHD7*: NM_017780, exon 12, c.3106C>T;p.A1036X). Sanger sequencing of the proband and his parents confirmed that this variant was only present in the proband (*Figure 1*). The variant was located in exon 12 and produced a stop-gain change, which resulted in the premature truncation of the CHD7 protein at acid amino position 1,036 in the SNF2-related domain. The affected residue was highly conserved across many species (*Figure 2*). This variant was absent in the public database and was predicted to be pathogenic.

The affected individual in family #4 was a 10-month-old boy who harbored a damaging heterozygous variant affecting normal splicing (*CHD7*: NM_017780, exon 18, C.4353+4_4353+12delinsGCCCA). The variant was located in exon 18 and has not been reported previously. The variant was absent in the patient's healthy parents and other family members, and was validated to be a *de novo* mutation by Sanger sequencing (*Figure 1*).

Evidence of the splice variant leading to a splicing abnormality

To verify the influence of the damaging *CHD7* variant in family #4 on splicing, RT-PCR was used to synthesize cDNA from total RNA samples collected from the patient and a healthy control subject. The obtained cDNA was then used as a template and subjected to Sanger sequencing of the region spanning exons 18 and 19; finally, the results were aligned to the genomic sequence (*Figure 3A*). The normal control subject was found to exhibit normal precursor mRNA (pre-mRNA) splicing that only included exons 18 and 19 (*Figure 3B*). For the patient with the splice variant, a 57-bp fragment was retained between exons 18 and 19 in the cDNA sequence, producing a stop codon and leading to a truncated CHD7 protein (*Figure 3C*). These findings revealed that the damaging *CHD7* variant in family #4 influenced pre-mRNA splicing, supporting the pathogenic role of this splice mutation.

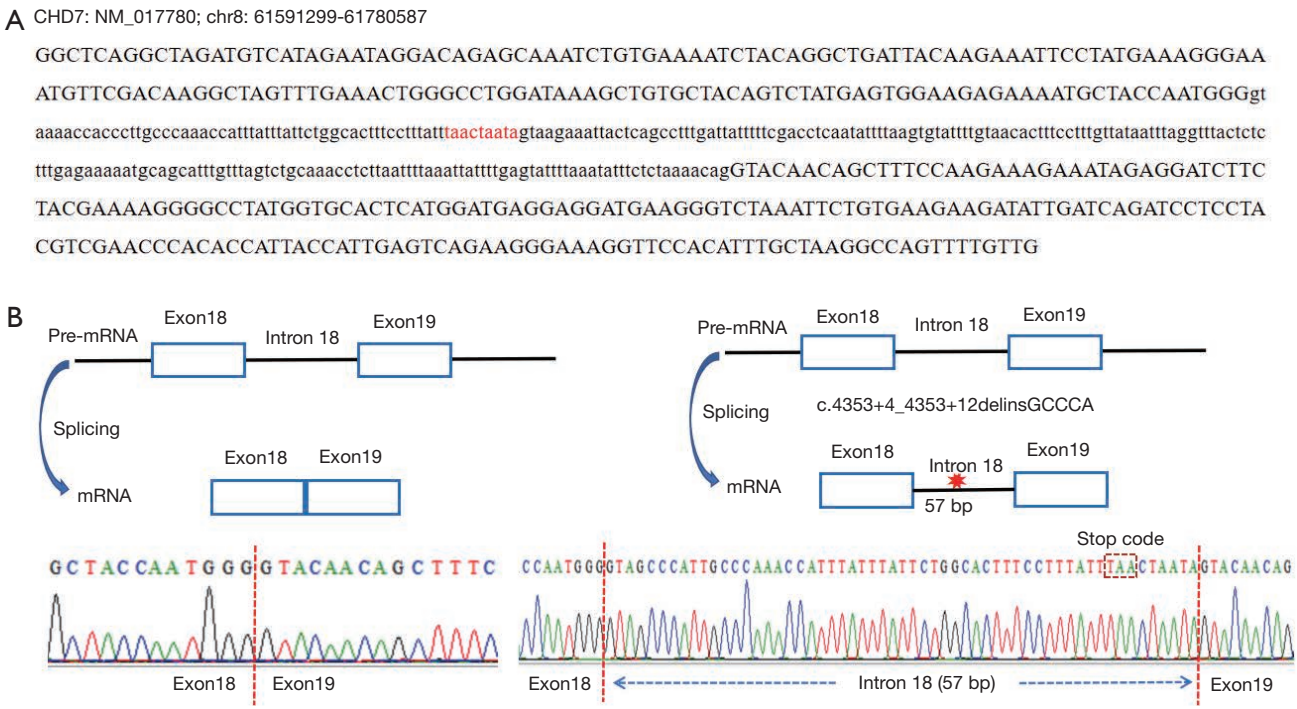

Figure 3 The splice variant in family #4 leads to a splicing abnormality. (A) The region of the *CHD7* sequence spanning exons 18 and 19 is shown aligned to the genomic sequence. The upper capital letters represent exon 18 and 19. The middle lower letters represent intron 18, and the red small letters indicate the affected nucleotides. (B) Schematic illustrating the normal splicing of the *CHD7* gene; there are no retained intron sequences between exons 18 and 19 on Sanger sequencing. (C) Schematic illustrating the effect of the splice variant in family #4 on the splice. A 57-bp intron-18 fragment is retained between exons 18 and 19 in the cDNA sequence, producing a stop codon (TAA in the red dotted box).

Association of rare deleterious CHD7 mutations with sporadic congenital heart disease

Further targeted sequencing was performed to investigate the prevalence of the disease-causing *CHD7* mutations in patients with sporadic congenital heart disease. In 1,155 patients, 23 novel or rare, possible disease-causing mutations were found after filtering with the following criteria: absence on GnomAD v2 or gnomADv2_exome_EAS frequency ≤0.01% and CADD score >25. The detailed characteristics of the above mutations are listed in *Table 4*. These mutations were found to be equally distributed in *CHD7*. Through RoseTTAFold and Consurf, the conserved profile and basic structure of *CHD7* were predicted (*Figure 4*). The majority of the rare mutations exhibited conserved profiles among different species (16 of 23 mutations were rated equal to or over 5 grades out of 10 grades).

To further evaluate the association of rare deleterious *CHD7* mutations with CHD, we compared the prevalence of the mutations in our sporadic CHD cohort with the control group in GnomAD v2-East Asians (CHD7 mutated allele frequency: 23/2,310 in our sporadic CHD cohort *vs.* 90/18,394 in control group). The association was considered statistically significant (P=0.0039, Fisher exact test; *Table 4*).

Discussion

In this study, to understand the genetic cause of CHD accompanied by extracardiac malformations, the WES data of four unrelated CHD families with extracardiac malformations were analyzed. Although there were many potentially predicted damaging variants in the affected individuals, considering the normal phenotypes of the probands' parents and siblings, we focused on *de novo* homozygous or compound heterozygous disease-causing variants. Four *de novo* damaging variants were identified in the the *CHD7* gene, including the frameshift mutation c.1951_1952delAAinsT (p.L651X) in family #1, the

Table 4 Variants identified in sporadic CHD patients

No. patient	Position	Variant	Protein change	SIFT[a]	PolyPhen2[a]	MutationTaster[a]	CADD_phred score	ExAC_EAS[b]	Consurf score
B680	chr8:61654020	c.29T>A	p.Phe10Tyr	D	D	D	25.8	0	9
NO_0040	chr8:61654731	c.740C>T	p.Pro247Leu	D	B	D	28.2	0	4
NO_0641	chr8:61693840	c.1953delA	p.Asp652fs	NA	NA	NA	NA	0	2
B357	chr8:61707684	c.2236C>G	p.Gln746Glu	D	P	D	32	0	8
NO_3333;B859;NO_0068	chr8:61713055	c.2347C>T	p.Pro783Ser	T	B	D	26.4	0.001	1
NO_0097	chr8:61734409	c.2758C>T	p.Arg920Trp	D	P	D	29.4	0	6
NO_2730	chr8:61736421	c.3224A>G	p.Tyr1075Cys	D	D	D	27.7	0.0001	5
NO_2421	chr8:61736495	c.3298C>T	p.Arg1100Cys	D	D	D	27.4	0	9
NO_2344	chr8:61742956	c.3598C>T	p.Pro1200Ser	D	D	D	25.4	0	9
NO_0285	chr8:61748842	c.3989G>A	p.Arg1330Gln	D	D	D	28.2	0	9
NO_2217	chr8:61757441	c.4869C>A	p.Asp1623Glu	T	B	D	37	0	9
B166	chr8:61761133	c.5270C>T	p.Ala1757Val	T	B	D	28.3	0	4
NO_1856	chr8:61764789	c.5877A>G	p.Ile1959Met	T	B	D	32	0	1
NO_3057	chr8:61765082	c.5920T>A	p.Phe1974Ile	D	D	D	26.4	0	9
NO_2541	chr8:61765615	c.6331C>T	p.Arg2111Trp	D	D	D	27	0	9
NO_1023	chr8:61765619	c.6335C>T	p.Thr2112Met	D	D	D	28.4	0	9
B508	chr8:61765622	c.6338A>T	p.Asp2113Val	D	D	D	35	0	9
B506	chr8:61765667	c.6383A>G	p.His2128Arg	T	B	D	31	0	7
NO_3252	chr8:61767013	c.6867G>C	p.Met2289Ile	T	B	D	27.6	0	1
NO_2930	chr8:61768742	c.7145C>T	p.Thr2382Met	T	B	D	28.2	0.0003	2
B459	chr8:61769146	c.7307A>G	p.Asn2436Ser	T	P	D	25.2	0	3
NO_1561	chr8:61777857	c.8359G>A	p.Gly2787Ser	D	D	D	27.4	0	6
B471	chr8:61777918	c.8420C>T	p.Pro2807Leu	D	D	D	27.1	0	8

[a], mutation assessment by SIFT, PolyPhen-2 (PPH2), and MutationTaster. [b], frequency of corresponding mutations in all populations of the ExAC Browser. CHD, congenital heart disease; T, tolerant; P, probably damaging; D, disease causing; B, benign; NA, not available.

nonsense mutations c.2913C>G (p.Y971X) in family #2 and c.3106C>T (pA1036X) in family #3, and the splicing mutation C.4353+4_4353+12delinsGCCCA in family #4. The above findings suggest that loss-of-function *CHD7* variants may be responsible for the development of familial CHD with extracardiac malformations.

CHD7 belongs to the SNF2/RAD54 helicase family, and is a type of ATP-dependent chromatin remodeling factor. During early human embryonic development, CHD7 is highly expressed in many tissues and organs, such as the heart, neural tube, undifferentiated neuroepithelium, and stroma, and controls the formation of multipotent neural crest cells by cooperating with PBAF (18). The cardiac neural crest cells play an important role in the development of the cardiac outflow tract; these cells migrate ventrally to form the third, fourth, and sixth pharyngeal arch arteries, and give rise to the septum that divides the truncus arteriosus, a single great vessel arising from the embryonic heart, into the aorta and pulmonary trunk (19). Mutations in the *CHD7* gene have been proven to be associated with multiple developmental disorders, such as CHARGE syndrome (20,21). The phenotype spectrum of CHD in CHARGE syndrome varies greatly and includes tetralogy of Fallot, double outlet of right ventricle, and atrioventricular septal defect (22). In this study, all of the four patients had different CHD phenotypes. Patient #1 in family #1 showed

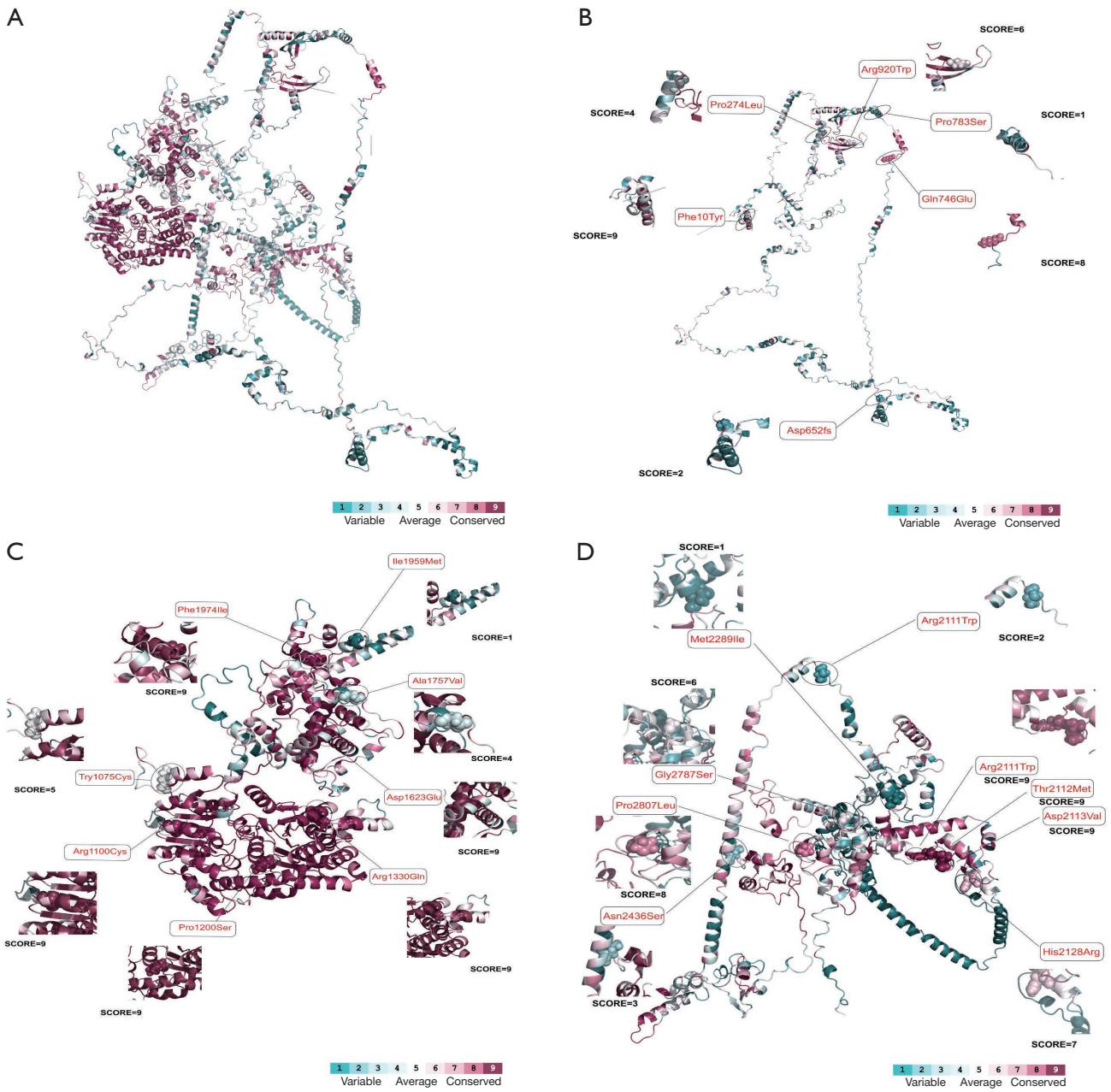

Figure 4 The predicted structure and conserved profile of CHD7. (A) The entire CHD7 protein is divided into 3 parts (1–962, 963–2016, and 2017–2997) for structure prediction, and colored blue (variable) and red (conserved) based on the conserved profile from Consurf. (B-D). Images of 3 parts of the CHD7 protein: part 1 (A) is representative of amino acids 1–962; part 2 (B) is representative of amino acids 963–2016; and part 3 (C) is representative of amino acids 2017–2997. Information about the 23 mutations is magnified and indicated.

atrial septal defect, patent ductus arteriosus, bicuspid aortic valve, and pulmonary hypertension. Patient #2 presented with atrial septal defect and patent ductus arteriosus. Proband #3 in family #3 had ventricular septal defect, atrial septal defect, patent ductus arteriosus, two-leafed aortic valve, tricuspid regurgitation, and pulmonary hypertension. The patient in family #4 had atrial septal defect, patent ductus arteriosus, and pulmonary hypertension. We analyzed the WES data of these 4 CHD families with extracardiac malformations, and found 4 loss-of-function

variants of the *CHD7* gene that were potentially associated with CHD development. However, some studies have suggested that *CHD7* mutations are not a major cause of congenital heart defects, even when patients are selected for extracardiac features (23,24). A series of studies showed that the incidence of CHD was higher in patients of CHARGE syndrome with *CHD7* mutations (66–92%) than in those without *CHD7* mutations (71%) (21,25-27). Moreover, in patients with pathogenic *CHD7* variants, the incidence of isolated or other disease-related atrioventricular septal defects and patent ductus arteriosus was higher (27), which is consistent with the CHD phenotype in our study.

De novo variants have been proven to play a key role in CHD development. Their contribution has been estimated to be 8% in sporadic CHD and approximately 28% in CHD patients with extracardiac malformations (28,29). In the present study, we conducted Sanger sequencing of the probands and their healthy parents and siblings, and confirmed that all the identified variants were *de novo* mutations and were absent in the healthy parents and siblings. Several types of mutations have been detected in the *CHD7* gene, including frameshift, nonsense, splice site, and missense mutations (30). Heterozygous mutations and loss-of-function mutations of *CHD7* are found in 60–70% of patients with CHARGE syndrome (21,31). For example, in a family with CHARGE syndrome, trio-WES analysis of the proband and their parents identified the *de novo* pathogenic variant c.4379_4380del (p.Ile1460Argfs*15) in exon 19 of the *CHD7* gene, which resulted in a premature translational stop signal (32). Wang *et al.* reported the *de novo* splice-site mutation c.3523-2A>G in the *CHD7* gene in a Chinese patient with typical CHARGE syndrome; this mutation activated a cryptic splice site that resulted in 172 missing base pairs in exon 15, leading to the premature truncation of the CHD7 protein (p.V1175Afs*11) (33).

In our study, all of the four identified variants in the *CHD7* gene were loss-of-function mutations and were predicted to affect the function of the CHD7 protein. *CHD7* is extremely intolerant to loss-of-function mutations, as demonstrated by a pLI score of 1 on DECIPHER data, which supports of the pathogenesis of the loss-of-function *CHD7* variants in our study. This could explain the association of *CHD7* with sporadic CHD, suggesting its function as a susceptibility gene.

Conclusions

In summary, four *de novo* loss-of-function mutations in the CHD7 gene in four unrelated families with CHD and extracardiac malformations are reported in this study. These findings confirm that loss-of-function *CHD7* variants are responsible for the development of familial CHD with extracardiac malformations. Furthermore, the missense mutations identified in patients with sporadic CHD expand the spectrum of *CHD7* variants associated with the development of CHD.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tp.amegroups.com/article/view/10.21037/tp-22-634/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tp.amegroups.com/article/view/10.21037/tp-22-634/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of Children's Hospital of Fudan University (No. 2016121) and informed consent was obtained from all the

affected individuals' guardians.

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