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Original Article

Next-generation sequencing identified genetic variations in families with fetal non-syndromic atrioventricular septal defects

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Abstract: Atrioventricular septal defects (AVSDs) account for approximately 5% of all congenital heart disease (CHD). About half of AVSDs are diagnosed in cases with trisomy 21 (Down's syndrome, DS). However, many AVSDs occur sporadically and manifest as non-syndromic. The pathogenesis is complex and has not yet been fully elucidated. In the present study, we applied two advanced applications of next-generation sequencing (NGS) to explore the genetic variations in families with fetal non-syndromic AVSDs. Our study was mainly divided into two steps: (1) low-pass whole-genome sequencing (WGS) was used to detect the genome-wide copy number variations (CNVs) for included subjects; (2) whole-exome sequencing (WES) was used to detect the gene mutations for the subjects without AVSD-associated CNVs. A total of 17 heterozygous de novo CNVs and 19 heterozygous de novo gene mutations were selected, and 15 candidate genes were involved in these variations. Among these heterozygous de novo variations, most have potential pathogenicity for AVSDs, but the others require further investigation to confirm their pathogenicity. Our study not only shows the genetic diversity and the etiological complexity of AVSDs but also shows the rationality and practicability of this sequential genetic detection and analysis strategy.

Keywords: AVSD, copy number variation, gene mutation, whole-genome sequencing, whole-exome sequencing

Introduction

Congenital heart disease (CHD) is the most common congenital malformation seen at birth as well as the most common congenital defect contributing to death in the first year. The prevalence of CHD is approximately 8 per 1000 births worldwide [1]. Atrioventricular septal defects (AVSDs) or atrioventricular (AV) canal defects account for approximately 5% of all CHD [2, 3]. This group of defects is caused by abnormal development of endocardial cushions. Meanwhile, endocardial cushions are involved in the formation of the atrial septum, the ventricular septum, and the mitral and tricuspid valves during embryonic development. Therefore, AVSDs are manifested by varying degrees of AV valvular and septal abnormalities, including atrial septal defect (ASD) and ventricular septal defect (VSD), and are classified as "partial, intermediate, or complete". AVSDs are often associated with other cardiac defects, such as Tetralogy of Fallot (TOF), double outlet right ventricle, and transposition of the great arteries [4, 5]. The clinical presentation and prognosis in AVSDs depend on the specific morphology of the defects and the associated anomalies. Untreated patients with AVSDs may present with cyanosis, breathlessness, recurrent respiratory infection, growth retardation, variable heart murmur, or even congestive cardiac failure, pulmonary hypertension, and death in early life [3, 4]. Many parents who are diagnosed with fetal AVSDs may choose to terminate the pregnancy to reduce the economic and psychological burdens of their families. At the same time, some parents may seek genetic counseling to assess the genetic defects of the malformed fetuses and the risk of recurrence in their next pregnancies.

About half of AVSDs are diagnosed in cases with trisomy 21 (Down's syndrome, DS) [6, 7].

Deletions on chromosome 21 on a trisomic background may reduce the risk for AVSDs [8]. Some genes may act as susceptibility factors for AVSDs in DS patients, such as *CRELD1* gene [9]. However, many AVSDs occur sporadically and manifest as non-syndromic. The pathogenesis is complex and has not yet been fully elucidated.

In recent years, a relationship between subchromosomal anomalies and CHD has been strongly suggested [10]. These subchromosomal anomalies are known as copy number variations (CNVs) and defined as copy number changes, including deletions, duplications, or multiallelic variation events of genomic regions ranging from 1 kilobase (Kb) to several megabases (Mb). CNVs can be identified using chromosomal microarray analysis (CMA), which is based on gene chip technology and limited by probe spacing and density. Recently, several studies have demonstrated the possibility of using low-pass whole-genome sequencing (WGS) to detect CNVs [11, 12]. Low-pass WGS is an application of next-generation sequencing (NGS) that can detect genome-wide CNVs, even those beyond the probe's range of CMA [13]. However, there is very little research on the detection of CNVs in AVSD cases without DS [14]. As another approach of NGS, whole-exome sequencing (WES) has been more and more used to explore the gene mutations of some diseases. However, this research is only just beginning for AVSDs [15].

In our study, we applied NGS to explore the genetic variations in fetuses with non-syndromic AVSDs and normal chromosome karyotypes. Our study was mainly divided into two steps: (1) Low-pass WGS was used to detect the genome-wide CNVs for included subjects; (2) WES was used to detect the gene mutations for the subjects without AVSD-associated CNVs. To exclude benign family genetic factors and to analyze the sources of the meaningful genetic variations, we applied family study, and the same steps were completed on the healthy parents.

Materials and methods

Subject enrollment

The study subjects were fetuses with non-syndromic AVSDs diagnosed by fetal echocardiography and confirmed by post-mortem autopsy in Beijing Obstetrics and Gynecology Hospital, China. Fetuses with identified chromosomal karyotype abnormalities or extracardiac malformations were excluded. Umbilical cord blood samples were collected from prenatal samples, and fetal tissues were collected from abortuses. Meanwhile, peripheral blood samples were collected from the parents. All samples sent to the MyGenostics medical laboratory (Beijing, PRC) for analysis. The study was approved by the ethics committee of the hospital. Informed consent for storage and subsequent analysis was obtained from all parents.

DNA library construction

The RelaxGene Blood DNA System (Tiangen Biotech, Beijing, PRC) and the Universal Genomic DNA Kit (CWBiotech, Beijing, PRC) were used to extract genomic DNA from the blood and tissue samples, respectively. The quality and concentration of the genomic DNA were evaluated by Nanodrop 2000 (Thermo Fisher, MA, USA). The ratio of A260/280 was between 1.8 and 2.0, and the concentration was greater than 30 ng/μL. The genomic DNA was broken into fragments of 100-500 base pairs (Bp) using the Covaris S220 DNA sonication system (Covaris, MA, USA). The fragments were end-polished, adenylated, and ligated with adaptors in turn. Proper reaction systems and cycles of polymerase chain reaction (PCR) amplification were carried out using the GeneAmp PCR System 2720 (Applied Biosystems, CA, USA) for enrichment of ligated DNA fragments. All enzymes and buffers were from MyGenostics (Baltimore, MD, USA). All operations were carried out according to the manufacturers' recommendations. The final DNA library products were quantitatively detected using NanoDrop 2000 and 1% agarose gel electrophoresis. The concentration of normal DNA library products was greater than 30 ng/μL, and the ratio of A260/280 was between 1.8 and 2.0. The main bands of the DNA library fragments were about 280-400 Bp.

Low-pass WGS and data analysis

NGS was carried out on the Nextseq 500 system (Illumina, CA, USA) to generate 150 Bp paired-end reads (a target depth of 0.6×) for each prepared DNA library according to the manufacturer's recommendations. Reads were

Table 1. Phenotypic characteristics of the 50 AVSD fetuses

Sex	n (%)
Male	28 (56.0)
Female	22 (44.0)
AVSD type	n (%)
Partial	21 (42.0)
Intermediate	11 (22.0)
Complete	18 (36.0)
Associated cardiac defects	n (%)
Yes	27 (54.0)
No	23 (46.0)

Abbreviations: AVSD, atrioventricular septal defect.

aligned to the National Center for Biotechnology Information human reference genome build 37 (HG19) using Burrows-Wheeler Aligner (version 0.7.10) [16]. Quality control and removal of duplicated reads were carried out using Picard (picard-tools-1.119). Finally, the mapped reads were produced. The exact CNV breakpoint sequences were calculated using the binary segmentation algorithm to determine candidate CNV regions and the copy ratio. A CNV was defined as a deletion or a duplication when its average copy ratio did not exceed 0.75 or was not less than 1.25, respectively. To assess the clinical importance of the detected fetal CNVs and the potential relationship with AVSDs, we selected the CNVs containing the AVSD-associated genes (described in detail below). Finally, the selected CNVs were compared to the databases of known pathogenic or likely pathogenic variations and the general population databases of CNVs (Database of Genomic Variants, DGV) [11, 17].

WES and bioinformatics analysis

For exome capture of the prepared DNA library, a GenCap Enrichment Kit (Baltimore, MD, USA) was used according to the manufacturer's recommendations. NGS was performed using the Nextseq 500 system (Illumina, CA, USA) to generate 150 Bp paired-end reads and cover at least 98% of the exome (an average depth of 200×) for each sequenced sample. A Burrows-Wheeler Aligner was used to align the raw data to HG19 and Picard was used to sort and mark the duplicated reads. Then, local realignment, base quality score recalibration, single nucleotide polymorphism calling, and short insertion/deletion calling were performed using the Genome Analysis Toolkit (version 3.7) software tools [18]. Variants were first prioritized based on their frequency in the 1000 Genomes Project (1000 g 2015aug_all), Exome Sequencing Project (ESP6500, ExAC_ALL, ExAC_EAS) and an inhouse database of 800 healthy Chinese Han adults, with rare (minor allele frequency < 0.05) variants receiving priority [19, 20]. Variants in AVSD-associated genes (described in detail below) were selected for further analysis and annotated by different bioinformatics tools: The Sorting Intolerant Form Tolerant (SIFT), PolyPhen-2, Mutation Taster, GERP++ [21-24].

AVSD-associated gene list

In order to identify potential candidate CNVs and gene mutations associated with AVSDs, we compiled a list of 375 human genes with a putative role in the development of AVSDs using Phenolyzer. We used the disease or phenotype terms "heart septal defect", "heart ventricular septal defect", "heart atrial septal defect", "atrioventricular canal/septal defect", "endocardial cushion defect", and selected the "seed genes" sorted by Phenolyzer (Supplementary Table 1) [25]. Also, we added 21 other genes by consulting the related published literature (including human and animal studies) on the candidate genes associated with AVSDs (Supplementary Table 2).

Variation validation

The selected CNVs were validated using quantitative real-time PCR (qPCR), and amplification levels were calculated with the 2-ΔΔCT method. The selected gene mutations were validated using Sanger sequencing. Primer pairs were designed by the Realtime PCR tool from Integrated DNA Technology or Primer3 (v.0.4.0), verified by primer BLAST or UCSC In-Silico PCR. The SYBR Premix Ex Taq II PCR reagent kit (TaKaRa Bio, Dalian, PRC) was used for qPCR reactions, and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA) was used for the Sanger sequencing reactions. All operations were done according to the manufacturers' recommendations.

Results

Basic characteristics of the study subjects

We recruited 50 non-syndromic AVSD families from Beijing Obstetrics and Gynecology Hos-

Table 2. De novo CNVs containing AVSD-associated genes

Candidate gene	Fetus ID	AVSD type	Associated cardiac defects	Cytoband	Start-end	Length	Type
NOTCH2	4	Partial	TA, PA	1p12p11.2	120524783-120904419	379.64 Kb	Dup, het, intragenic
	17	Complete	TGA, PS	1p12p11.2	120563920-120936695	372.78 Kb	Dup, het, intragenic
	45	Intermediate	DORV	1p12p11.2	120597708-120904419	306.71 Kb	Dup, het, intragenic
COL11A1	16	Complete	None	1p21.1	103361276-103582736	221.46 Kb	Dup, het, intragenic
	30	Partial	None	1p21.1	103319157-103743271	424.12 Kb	Del, het, whole gene
	32	Partial	None	1p21.1	103403979-103856289	452.31 Kb	Del, het, intragenic
NIPBL	1	Partial	IAA	5p13.2	36891413-37044984	153.57 Kb	Del, het, intragenic
	8	Intermediate	None	5p13.2	36891312-37054895	163.58 Kb	Del, het, intragenic
EHMT1	12	Complete	TGA, RAA	9q34.3	140203637-141023198	819.56 Kb	Dup, het, whole gene
	15	Intermediate	None	9q34.3	140481413-140707091	225.68 Kb	Del, het, intragenic
NR2F2	9	Complete	TGA, DORV, PS, PA	15q26.2	96777378-96923621	146.24 Kb	Dup, het, whole gene
	21	Complete	None	15q26.2	96786595-97311581	524.99 Kb	Dup, het, whole gene
COL6A1/2	2	Partial	TGA, PA	21q22.3	47389758-47576705	186.95 Kb	Dup, het, whole gene
	49	Partial	None	21q22.3	47378452-47612768	234.32 Kb	Del, het, whole genes
TBX1	38	Complete	TGA, DORV, PS	22q11.21	18939748-21721712	2.78 Mb	Del, het, whole gene
SHANK3	5	Complete	CAT	22q13.31q13.33	46933489-51219152	4.29 Mb	Del, het, whole gene
SMC1A	29	Partial	AS	Xp11.22	53363770-53490937	127.00 Kb	Dup, het, whole gene

Abbreviations: CNV, copy number variation; AVSD, atrioventricular septal defect; TA, tricuspid atresia; PA, pulmonary valve atresia; TGA, transposition of the great arteries; PS, pulmonary stenosis; DORV, double outlet right ventricle; IAA, interruption of aortic arch; RAA, right aortic arch; CAT, common arterial trunk; AS, aortic stenosis; Dup, duplication; Del, deletion; het, heterozygous.

pital in China; each family comprised one non-syndromic AVSD fetus and two healthy parents. All the couples were non-consanguineous and terminated the pregnancy at midterm. All the fetuses had normal chromosomal karyotypes, and were without extracardiac malformations. The phenotypic characteristics of these AVSD fetuses are presented in **Table 1**.

CNVs detected by low-pass WGS

In total, 1,736 CNVs were detected from the 50 AVSD fetuses. Seventeen de novo CNVs containing 10 AVSD-associated genes (candidate genes) were selected from these CNVs. These CNVs were derived from 17 AVSD fetuses, and none of them were carried by the healthy parents and included in the DGV. These CNVs ranged from 127 Kb to 4.29 Mb. Two CNVs larger than 1 Mb were the known pathogenic CNVs (pCNVs) for chromosome 22q11.2 deletion syndrome and chromosome 22q13.3 deletion syndrome respectively (Supplementary Figure 1). The others were smaller than 1 Mb and validated by qPCR (Supplementary Table 3). All the 17 CNVs contained whole or a part of the exons of their candidate genes (**Table 2**).

We retrieved the 10 candidate genes in DECIPHER. Seven genes (*NOTCH2*, *NIPBL*, *EHMT1*, *NR2F2*, *TBX1*, *SHANK3*, *SMC1A*) are contained in the CNVs (including deletions and duplications) detected in patients with septal defects (including AVSD, ASD, VSD) (Supplementary Table 4). In our study, 8 de novo deletions and 9 de novo duplications were selected. Among these duplications, 5 duplications contained the whole candidate genes, and the others contained part of exons of the candidate genes (intragenic duplications).

Gene mutations detected by WES

Thirty-three AVSD fetuses and their healthy parents were included for WES. A total of 6,713 high-quality, rare, and nonsynonymous variants were detected from these AVSD fetuses, and there were 138 variants in the AVSD-associated genes. Among them, 7 candidate genes (*C5ORF42, COL11A1, COL6A2, GATA6, GLI3, HSPG2, LRP2*) were relatively enriched for de novo variants at least 2 AVSD fetuses carried the de novo variants in the same gene). Nineteen de novo heterozygous variants in these genes were selected (**Table 3**), and these variants were derived from 14 AVSD fetuses. All variants were validated by Sanger sequencing (Supplementary Table 5).

Two genes, *COL11A1* and *COL6A2*, were contained in de novo CNVs derived from another 3 and 2 AVSD fetuses, respectively. In the WES

Table 3. Rare nonsynonymous de novo variants in 7 AVSD-associated genes

Fetus ID	AVSD type	Associated cardiac defects	Candidate gene	Nucleotide changes	Amino acid changes	dbsnp147	Damaging predict*	GERP++
23	Partial	None	COL11A1	652-5->TT	Splicing	rs749687230	-	-
40	Complete	TGA	COL11A1	3266C>T	P1089L	rs373734529	Yes	Conserved
13	Complete	CAT	COL6A2	499G>A	G167S	rs115957676	Yes	Conserved
3	Partial	None	COL6A2	679G>A	D227N	rs35881321	No	Conserved
43	Intermediate	TGA, PS, AS	COL6A2	2798G>A	R933H	rs374384263	Yes	Nonconserved
20	Partial	TGA, PA	C5ORF42	8746G>A	A2916T	rs369585190	Yes	Conserved
25	Partial	None	C5ORF42	6443A>G	N2148S	rs150999024	No	Nonconserved
28	Complete	TGA, PS	C5ORF42	608A>G	Y203C	rs144969169	Yes	Conserved
3	Partial	None	GLI3	169G>A	A57T	rs775586921	No	Conserved
3	Partial	None	GLI3	164G>A	R55K	rs764332121	Yes	Conserved
20	Partial	TGA, PA	GLI3	169G>A	A57T	rs775586921	No	Conserved
20	Partial	TGA, PA	GLI3	164G>A	R55K	rs764332121	Yes	Conserved
7	Complete	TGA, PS	LRP2	9937G>A	D3313N	-	Yes	Conserved
41	Complete	TGA	LRP2	9914G>A	R3305H	rs3213760	Yes	Conserved
10	Intermediate	None	GATA6	43G>C	G15R	rs116262672	Yes	Conserved
44	Intermediate	DORV, TAPVC	GATA6	551G>A	S184N	rs387906816	No	Nonconserved
14	Partial	TGA, IAA	HSPG2	2008G>A	V670I	rs147810145	No	Conserved
27	Intermediate	None	HSPG2	10589G>A	R3530Q	rs200062985	Yes	Conserved
44	Intermediate	DORV, TAPVC	HSPG2	2057T>C	L686P	-	Yes	Conserved

Abbreviations: AVSD, atrioventricular septal defect; TGA, transposition of the great arteries; PA, pulmonary valve atresia; PS, pulmonary stenosis; CAT, common arterial trunk; AS, aortic valve stenosis; DORV, double outlet right ventricle; TAPVC, total anomalous pulmonary venous connection. *Yes: at least 2 bioinformatics tools suggest damaging or probably damaging or possibly damaging (SIFT, PolyPhen-2, Mutation Taster); No: 2 or 3 bioinformatics tools suggest benign (SIFT, PolyPhen-2, Mutation Taster).

group, 2 AVSD fetuses had rare nonsynonymous variants in the *COL11A1* gene; one was an exonic splicing variant, and the other (P1089L) was highly conserved and predicted to be damaging. Three AVSD fetuses had rare nonsynonymous variants in the *COL6A2* gene, 2 variants (G167S and R933H) were predicted to be damaging.

Two AVSD fetuses had the same highly conserved, de novo, compound heterozygous mutations in *GLI3* gene; however, only one variant (R55K) was predicted to be damaging. The other variant (A57T) was predicted to be benign.

Three AVSD fetuses had rare nonsynonymous variants in the *HSPG2* gene, and 2 variants (R3530Q and L686P) were highly conserved and predicted to be damaging, and one of them (L686P) was a novel variant. Three AVSD fetuses had rare nonsynonymous variants in the *C5ORF42* gene, and 2 variants (A2916T and Y203C) were highly conserved and predicted to be damaging.

Rare nonsynonymous variants in another 2 genes (*GATA6* and *LRP2*) were carried by 2 AVSD fetuses for each gene. Except for one de novo variant (S184N) in the *GATA6* gene, the other 3 de novo variants were highly conserved and predicted to be damaging. Among the 4 variants, one variant (D3313N) was novel.

Discussion

Embryologically, human cardiac septation takes place in the first 8 weeks of pregnancy. After primary heart tube looping, endocardial cushions (superior, inferior, and two lateral cushions) are formed at the AV junction as a result of a critical process, endothelial to mesenchymal transition. Subsequently, the two lateral endocardial cushions develop and divide the AV canal into two separate AV orifices and contribute to the formation of the mitral valve and tricuspid valve. A deficiency in these processes will lead to a common AV annulus and a common AV valve. Meanwhile, the superior and inferior endocardial cushions extent and close the atrial septum primum and the interventricular foramen, but a deficiency in these processes will lead to an ostium primum defect and an inlet VSD just below the AV valves (membranous VSD). In partial AVSD, there is an isolated ostium primum defect or an inlet VSD, and two separate AV orifices and AV valves. In complete AVSD, besides an isolated ostium primum

Table 4. The association of gene variations with AVSD

Candidate gene (OMIM ID)	CHD-associated syndromes caused by heterozygous or haploinsufficient variations	Association with AVSD
NOTCH2 (600275)	Alagille syndrome 2; Hajdu-Cheney syndrome	Chick *Notch2* initiates the signaling cascades that delimits the non-chamber AV canal regions, causes the progressive restriction of *Bmp2* and *Tbx2* expression to within the developing AV canal [31].
COL11A1 (120280)	Stickler syndrome, type II	Murine *Col11a1* can express in AV valve and involved in AV valve development and maintenance [32, 33].
NIPBL (608667)	Cornelia de Lange syndrome 1	30% Cornelia de Lange syndrome patients have CHD, including AVSD, ASD, VSD [34]. *Nipbl* ± mice can exhibit the phenotypes of Cornelia de Lange syndrome 1, septal defects were especially common [35].
EHMT1 (607001)	Kleefstra syndrome 1	41% Kleefstra syndrome patients have CHD, including VSD, ASD; *EHMT1* de novo mutation was reported in an AVSD patient [36, 37].
NR2F2 (107773)	Congenital heart defects, multiple types, 4	*Nr2f2* is expressed in the endocardium and the epicardium; *Nr2f2* mutant mice exhibit a spectrum of cardiac defects (including AVSD) resulting from the disruption of endocardial cushion development in a dosage-sensitive fashion [38]. Rare variants in *NR2F2* gene were reported in AVSD patients [39].
COL6A1 (120220)	-	Collagen VI is expressed in the AV cushions in human and mouse heart, plays a role in valve and septal differentiation; overexpression or insufficient expression of *COL6A1* could cause AVSD formation [2, 40].
COL6A2 (120240)	-	Collagen VI is expressed in the AV cushions in human and murine heart, plays a role in valve and septal differentiation; overexpression or insufficient expression of *COL6A2* could cause AVSD formation [2, 40].
TBX1 (602054)	Chromosome 22q11.2 deletion syndrome	Tbx1 regulates SHF progenitor cell status during heart tube elongation, its failure results in a spectrum of morphological defects affecting the cardiac poles, including AVSD [41,42].
SHANK3 (606230)	Chromosome 22q13.3 deletion syndrome	Patient 253,900 with 86.55 Kb duplication containing *SHANK3* gene at 22q13.33 has AVSD in DECIPHER.
SMC1A (300040)	Cornelia de Lange syndrome 2	30% Cornelia de Lange syndrome patients have CHD, including AVSD, ASD, VSD [34].
C5ORF42 (614571)	-	*C5orf42* -/- mice exhibit multiple CHD, including AVSD, VSD; its mutation disrupts ciliogenesis and cilia transduced Hedgehog signaling, and the Hedgehog signaling is required in the SHF for AV septation [43, 44].
GLI3 (165240)	Pallister-Hall syndrome; Greig cephalopolysyndactyly syndrome	*GLI3* is a transcription factor that functions in the Hedgehog signaling [44].
LRP2 (600073)	-	*LRP2* acts as a receptor of Hedgehog signaling, *Lrp2* -/- mice result in abnormal development of the SHF [45].
GATA6 (601656)	Atrioventricular septal defect 5; Atrial septal defect 9; Tetralogy of Fallot	Gata6 is expressed in the endocardial cushions, atrial and ventricular myocardium, atrioventricular valve leaflets, and a heterozygous missense mutation in the gene was identified in an AVSD patient [49].
HSPG2 (142461)	-	*HSPG2* is expressed in the basal surface of myocardium and endocardium, plays a role in the earliest stages of formation of the endocardial cushions [50].

Abbreviations: AVSD, atrioventricular septal defect; CHD, congenital heart disease; AV, atrioventricular; ASD, atrial septal defect; VSD, ventricular septal defect; SHF, second heart field.

defect and an inlet VSD, there is a common AV annulus and a common AV valve. Intermediate AVSD refers to the situation between the partial type and complete type, in which there is an atrial septum primum and an inlet VSD, but two separate AV orifices [26, 27].

This study was designed to detect the genetic variations associated with non-syndromic AVSDs. To cover the meaningful variations as far as possible, we used two applications of NGS to achieve it, low-pass WGS for the genome-wide CNVs, and WES for the gene mutations. NGS is an advanced technology used to detect genetic variations with unprecedented resolution. Although the application of low-pass WGS is not widely used for CNV detection, it was confirmed to have an equivalent effectiveness for detection of pCNVs compared with CMA, and besides, it can detect CNVs beyond the probe's range of CMA [11]. The specificity of detected deletions and duplications larger than 100 Kb was 100%, even using a read depth of 0.2× [28]. In our study, we chose a more accurate read depth (0.6×) to detect CNVs. We finally selected 17 de novo CNVs containing AVSD-associated genes, and all small CNVs (larger than 100 Kb but smaller than 1 Mb) were validated by qPCR, with a very high credibility. WES is a cost-effective, high-

depth DNA sequencing strategy to detect DNA variations in the coding regions that may alter protein function. Not only can it detect common variations, it can also find low frequency variations, and rare variations. In our study, we used WES to detect gene mutations with an average sequencing depth of 200× and finally selected 19 de novo, high-quality, rare, and nonsynonymous variants in 7 AVSD-associated genes.

In the low-pass WGS group, 10 AVSD-associated genes were involved in 17 de novo CNVs derived from 17 AVSD fetuses. All CNVs contained the whole or a part of the exons of their candidate genes, causing the dosage changes of the genes or functional changes of the proteins. Among these CNVs, there were 8 deletions and 9 duplications (4 duplications were intragenic duplications). In humans, deletion (such as *COL11A1*, *NIPBL*, *EHMT1*, *COL6A1*, *COL6A2*, *TBX1*, *SHANK3* gene in our study) can lead to haploinsufficiency and a loss-of-function change of an important gene, and this is very similar to those caused by heterozygous mutations within the coding region of the gene. Duplication of the whole gene (such as *EHMT1*, *NR2F2*, *COL6A1*, *COL6A2*, *SMC1A* in our study) can cause triplication of the gene that could cause a similar but milder clinical phenotype resulting from the deletion [29]. However, intergenic duplication (such as the *COL11A1*, *NOTCH2* gene in our study) may lead to gene disruption or fusion, resulting in loss of gene function, and then cause a similar clinical phenotype to the deletion [30]. Except for 3 genes (*COL11A1*, *COL6A1*, *COL6A2*), the other 7 genes are contained in the CNVs detected in patients with septal defects (including AVSD, ASD, VSD) in DECIPHER. Also, 8 genes (*NOTCH2*, *COL11A1*, *NIPBL*, *EHMT1*, *NR2F2*, *TBX1*, *SHANK3*, *SMC1A*) are dominant pathogenic genes, and heterozygous or haploinsufficient variations of these genes can cause syndromes which have CHD phenotypes, including AVSD, ASD and VSD, suggesting a potential relationship between these CNVs with phenotypes. Seven genes (*NOTCH2*, *COL11A1*, *NIPBL*, *NR2F2*, *COL6A1*, *COL6A2*, *TBX1*) have been thought to play a role in the normal development of the AV canal, endocardial cushions, or AV valves, according to some molecular studies and animal models. Mutations in these genes could cause AVSD formation (**Table 4**) [31-42]. The other 3 genes (*EHMT1*, *SHANK3*, *SMC1A*) have not been reported to play a direct role in heart development or CHD formation, but the variations of them have been reported in some AVSD cases, suggesting the need for more research in this area [34, 36, 37].

In the WES group, 7 AVSD-associated genes were involved in 19 de novo variants derived from 14 AVSD fetuses. Interestingly, the variations of 2 genes (*COL11A1* and *COL6A1*) were detected both in the low-pass WGS group and the WES group, and there were total of 10 variations (including CNVs and gene mutations), suggesting the important roles of collages in heart development [2, 32, 33, 40]. Notably, the *COL6A1* gene is mapped to the DS's obligate region of chromosome 21, the same as the *COL6A2* gene, and AVSD is a common feature of DS. These 2 genes encode the collagen VI a1 and a2 chains, respectively. The collagen VI a3 chain is encoded by the *COL6A3* gene which is located at chromosome 2. Normally, these 3 chains are assembled in a 1:1:1 stoichiometric ratio. Overexpression or insufficient expression of one gene could result in an inappropriate collagen VI chain secretion and a functional abnormality of collagen VI, and may have a role in the pathogenesis of AVSDs [2, 40]. Three genes (*C5ORF42*, *GLI3*, *LRP2*) are involved in hedgehog signaling, and hedgehog signaling is required in the second heart field (SHF) [43-45]. Molecular events (such as Hedgehog signaling, BMP signaling, and T-box gene family signaling) in the SHF cardiac progenitors, which are located dorsal to the primary heart tube, can drive the processes of heart tube elongation and AV septation [31, 41, 42, 44-46]. Failure of these processes could result in a spectrum of morphological defects affecting the cardiac poles, including outflow tract defects and AVSDs [41]. In our study, a total of 9 de novo variants were detected in the *C5ORF42*, *GLI3* and *LRP2* genes, and most of them were predicted to be damaging and highly conserved. Among these heterozygous variants, 2 AVSD fetuses had the same compound heterozygous mutations (R55K and A57T) in the *GLI3* gene. Although only one variant (R55K) was predicted to be damaging, *GLI3* is a dominant pathogenic gene for Pallister-Hall syndrome and Greig cephalopolysyndactyly syndrome, both of which have CHD phenotypes, and heterozygous mutations in the *GLI3* gene may be associated with AVSDs [47, 48]. Both the

C5ORF42 and *LRP2* genes are recessive pathogenic genes, and the contribution of the heterozygous variants in the two genes to AVSD phenotypes is uncertain and needs further study. Another 2 AVSD-associated genes, *GATA6* and *HSPG2*, were involved in 5 heterozygous de novo variants. Both of them play a role in the development of endocardial cushions [49, 50]. The *GATA6* gene has been identified as a dominant pathogenic gene for multiple CHD, including AVSD5, ASD9, and TOF. The heterozygous variants in *GATA6* gene are likely to be the cause of the fetal phenotypes. Although haploinsufficient variations of *HSPG2* gene have been considered a possible cause of heart defects in patients with chromosome 1p36 deletion syndrome, heterozygous mutant mice did not exhibit significant heart defects [50-52]. The potential pathogenicity for AVSDs of the heterozygous variants in *HSPG2* gene is not yet clear.

In this study, we applied NGS to explore the genetic variations in 50 non-syndromic AVSD families. For a more comprehensive exploration of genetic variations associated with non-syndromic AVSDs, we conducted an advanced detection and analysis strategy. First, we applied family study which was helpful in finding out the meaningful de novo genetic variations deriving from the AVSD fetuses, and in better understanding the potential causes of these sporadic, non-syndromic AVSDs. Second, we compiled an AVSD-associated gene list of 396 human genes by retrieving Phenolyzer and by reviewing the literature, and these genes are thought to have a potential relationship with septal defects or AVSDs. The genetic variations in these genes are more likely to be associated with AVSDs. Third, we applied two excellent applications of NGS to detect the genetic variations. Low-pass WGS was used to detect the genome-wide CNVs for 50 non-syndromic AVSD families, and WES was used to detect whole-exome mutations for 33 non-syndromic AVSD families without AVSD-associated CNVs. Both of the two methods are beneficial to the discovery of more meaningful genetic variations. Fourth, we systematically searched the related databases (such as DECIPHER, OMIM) and the published literature to explore the relationship between these candidate genes and AVSDs and to assess the potential pathogenicity of these de novo heterozygous genetic variations. As far as we know, there is no similar study.

There are two important findings from our study. First, it shows the genetic diversity and the etiological complexity of AVSDs. Although half of the AVSDs are associated with trisomy 21, many AVSDs occur sporadically and without a clear cause. So, we chose the fetuses with non-syndromic AVSDs and normal chromosome karyotypes as our study subjects, applied a reasonable and comprehensive strategy to explore the genetic variations associated with the phenotypes in addition to chromosomal karyotype abnormalities. In the low-pass WGS group, we ultimately selected 17 heterozygous de novo CNVs. According to the American College of Medical Genetics standards and guidelines for interpretation and reporting of CNVs, 2 CNVs are associated with the known syndromes, and can be defined as pCNVs; the other CNVs can be defined as likely pCNVs, because the heterozygous/haploinsufficient variations or overexpression of the candidate genes in these CNVs have been reported to be associated with AVSDs or CHD-associated syndromes which have septal defect phenotypes [17]. In the WES group, we finally selected 19 de novo mutations, and all of the candidate genes are important functional genes in the normal development of the heart, especially for endocardial cushions. The heterozygous variants in 4 genes (*COL11A1*, *COL6A2*, *GLI3*, *GATA6*) have the potential pathogenicity that lead to the occurrence of AVSDs. The pathogenicity of the other mutations is uncertain and needs further study. Second, our study shows the rationality and practicability of this sequential genetic detection and analysis strategy, especially for the diseases with undefined pathogenic mechanisms and genetic bases. In clinical work, when the traditional genetic testing methods (such as chromosomal karyotype analysis) can't determine the genetic defects associated with diseases, meaningful CNVs and gene mutations should be considered. We can choose some cost-effective detection methods (such as low-pass WGS and WES) to get more genetic information about the diseases, and we can use the related databases and published literature to select the pathogenic or likely pathogenic variations. The strategy can help us to make a more accurate genetic diagnosis, providing a theoretical basis for individualized prenatal diagnosis and genetic counseling.

In summary, we applied two advanced applications of NGS, low-pass WGS and WES, to

explore the genetic variations in families with fetal non-syndromic AVSDs. A total of 17 heterozygous de novo CNVs and 19 heterozygous de novo gene mutations were selected by using a sequential genetic detection and analysis strategy. Fifteen candidate genes were involved in these variations, and all of them have demonstrated an association with AVSDs. Among these heterozygous de novo variations, most have potential pathogenicity for AVSDs, but the others require further investigation to define their pathogenicity. The functional validation of these genetic variations wasn't the focus of our study, and the number of included subjects was somewhat small, so these were the shortcomings of our study to be improved on in the future.

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Disclosure of conflict of interest

None.

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References

[1] Bernier PL, Stefanescu A, Samoukovic G, Tchervenkov CI. The challenge of congenital heart disease worldwide: epidemiologic and demographic facts. Semin Thorac Cardiovasc Surg Pediatr Card Surg Annu 2010; 13: 26-34.

[2] Gittenberger-de Groot AC, Bartram U, Oosthoek PW, Bartelings MM, Hogers B, Poelmann RE, Jongewaard IN, Klewer SE. Collagen type VI expression during cardiac development and in human fetuses with trisomy 21. Anat Rec A Discov Mol Cell Evol Biol 2003; 275: 1109-1116.

[3] Malik M, Khalid Nuri M. Surgical considerations in atrioventricular canal defects. Semin Cardiothorac Vasc Anesth 2017; 21: 229-234.

[4] Craig B. Atrioventricular septal defect: from fetus to adult. Heart 2006; 92: 1879-1885.

[5] Oshima Y, Yamaguchi M, Yoshimura N, Oka S, Ootaki Y. Anatomically corrective repair of complete atrioventricular septal defects and major cardiac anomalies. Ann Thorac Surg 2001; 72: 424-429.

[6] Christensen N, Andersen H, Garne E, Wellesley D, Addor MC, Haeusler M, Khoshnood B, Mullaney C, Rankin J, Tucker D. Atrioventricular septal defects among infants in Europe: a population-based study of prevalence, associated anomalies, and survival. Cardiol Young 2013; 23: 560-567.

[7] Barlow GM, Chen XN, Shi ZY, Lyons GE, Kurnit DM, Celle L, Spinner NB, Zackai E, Pettenati MJ, Van Riper AJ, Vekemans MJ, Mjaatvedt CH, Korenberg JR. Down syndrome congenital heart disease: a narrowed region and a candidate gene. Genet Med 2001; 3: 91-101.

[8] Rambo-Martin BL, Mulle JG, Cutler DJ, Bean LJH, Rosser TC, Dooley KJ, Cua C, Capone G, Maslen CL, Reeves RH, Sherman SL, Zwick ME. Analysis of copy number variants on chromosome 21 in down syndrome-associated congenital heart defects. G3 (Bethesda) 2018; 8: 105-111.

[9] Asim A, Agarwal S, Panigrahi I, Sarangi AN, Muthuswamy S, Kapoor A. CRELD1 gene variants and atrioventricular septal defects in Down syndrome. Gene 2018; 641: 180-185.

[10] Digilio MC, Marino B. What is new in genetics of congenital heart defects? Front Pediatr 2016; 4: 120.

[11] Dong Z, Zhang J, Hu P, Chen H, Xu J, Tian Q, Meng L, Ye Y, Wang J, Zhang M, Li Y, Wang H, Yu S, Chen F, Xie J, Jiang H, Wang W, Choy KW, Xu Z. Low-pass whole-genome sequencing in clinical cytogenetics: a validated approach. Genet Med 2016; 18: 940-948.

[12] Xia S, Huang CC, Le M, Dittmar R, Du M, Yuan T, Guo Y, Wang Y, Wang X, Tsai S, Suster S, Mackinnon AC, Wang L. Genomic variations in plasma cell free DNA differentiate early stage lung cancers from normal controls. Lung Cancer 2015; 90: 78-84.

[13] Stavropoulos DJ, Merico D, Jobling R, Bowdin S, Monfared N, Thiruvahindrapuram B, Nalpathamkalam T, Pellecchia G, Yuen RKC, Szego MJ, Hayeems RZ, Shaul RZ, Brudno M, Girdea M, Frey B, Alipanahi B, Ahmed S, Babul-Hirji R, Porras RB, Carter MT, Chad L, Chaudhry A, Chitayat D, Doust SJ, Cytrynbaum C, Dupuis L, Ejaz R, Fishman L, Guerin A, Hashemi B, Helal M, Hewson S, Inbar-Feigenberg M, Kannu P, Karp N, Kim R, Kronick J, Liston E, MacDonald H, Mercimek-Mahmutoglu S, Mendoza-Londono R, Nasr E, Nimmo G, Parkinson N, Quercia N, Raiman J, Roifman M, Schulze A, Shugar A, Shuman C, Sinajon P, Siriwardena K, Weksberg

R, Yoon G, Carew C, Erickson R, Leach RA, Klein R, Ray PN, Meyn MS, Scherer SW, Cohn RD, Marshall CR. Whole genome sequencing expands diagnostic utility and improves clinical management in pediatric medicine. NPJ Genom Med 2016; 1: 15012.

[14] Priest JR, Girirajan S, Vu TH, Olson A, Eichler EE, Portman MA. Rare copy number variants in isolated sporadic and syndromic atrioventricular septal defects. Am J Med Genet A 2012; 158A: 1279-1284.

[15] D'Alessandro LC, Al Turki S, Manickaraj AK, Manase D, Mulder BJ, Bergin L, Rosenberg HC, Mondal T, Gordon E, Lougheed J, Smythe J, Devriendt K, Bhattacharya S, Watkins H, Bentham J, Bowdin S, Hurles ME, Mital S. Exome sequencing identifies rare variants in multiple genes in atrioventricular septal defect. Genet Med 2016; 18: 189-198.

[16] Li H, Durbin R. Fast and accurate short read alignment with burrows-wheeler transform. Bioinformatics 2009; 25: 1754-1760.

[17] Kearney HM, Thorland EC, Brown KK, Quintero-Rivera F, South ST; Working Group of the American College of Medical Genetics Laboratory Quality Assurance Committee. American college of medical genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. Genet Med 2011; 13: 680-685.

[18] DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, Philippakis AA, del Angel G, Rivas MA, Hanna M, McKenna A, Fennell TJ, Kernytsky AM, Sivachenko AY, Cibulskis K, Gabriel SB, Altshuler D, Daly MJ. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet 2011; 43: 491-498.

[19] 1000 Genomes Project Consortium, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, McVean GA. An integrated map of genetic variation from 1,092 human genomes. Nature 2012; 491: 56-65.

[20] Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG; Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016; 536: 285-291.

[21] Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res 2003; 31: 3812-3814.

[22] Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. Nat Methods 2010; 7: 248-249.

[23] Schwarz JM, Rodelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods 2010; 7: 575-576.

[24] Davydov EV, Goode DL, Sirota M, Cooper GM, Sidow A, Batzoglou S. Identifying a high fraction of the human genome to be under selective constraint using GERP++. PLoS Comput Biol 2010; 6: e1001025.

[25] Yang H, Robinson PN, Wang K. Phenolyzer: phenotype-based prioritization of candidate genes for human diseases. Nat Methods 2015; 12: 841-843.

[26] Calkoen EE, Hazekamp MG, Blom NA, Elders BB, Gittenberger-de Groot AC, Haak MC, Bartelings MM, Roest AA, Jongbloed MR. Atrioventricular septal defect: from embryonic development to long-term follow-up. Int J Cardiol 2016; 202: 784-795.

[27] Anderson RH, Spicer DE, Brown NA, Mohun TJ. The development of septation in the four-chambered heart. Anat Rec (Hoboken) 2014; 297: 1414-1429.

[28] Li X, Chen S, Xie W, Vogel I, Choy KW, Chen F, Christensen R, Zhang C, Ge H, Jiang H, Yu C, Huang F, Wang W, Jiang H, Zhang X. PSCC: sensitive and reliable population-scale copy number variation detection method based on low coverage sequencing. PLoS One 2014; 9: e85096.

[29] Zarrei M, MacDonald JR, Merico D, Scherer SW. A copy number variation map of the human genome. Nat Rev Genet 2015; 16: 172-183.

[30] Newman S, Hermetz KE, Weckselblatt B, Rudd MK. Next-generation sequencing of duplication CNVs reveals that most are tandem and some createfusion genes at breakpoints. Am J Hum Genet 2015; 96: 208-220.

[31] Rutenberg JB, Fischer A, Jia H, Gessler M, Zhong TP, Mercola M. Developmental pattern-

ing of the cardiac atrioventricular canal by notch and hairy-related transcription factors. Development 2006; 133: 4381-4390.
[32] Yoshioka H, Iyama K, Inoguchi K, Khaleduzzaman M, Ninomiya Y, Ramirez F. Developmental pattern of expression of the mouse alpha 1 (XI) collagen gene (Col11a1). Dev Dyn 1995; 204: 41-47.
[33] Peacock JD, Lu Y, Koch M, Kadler KE, Lincoln J. Temporal and spatial expression of collagens during murine atrioventricular heart valvedevelopment and maintenance. Dev Dyn 2008; 237: 3051-3058.
[34] Chatfield KC, Schrier SA, Li J, Clark D, Kaur M, Kline AD, Deardorff MA, Jackson LS, Goldmuntz E, Krantz ID. Congenital heart disease in Cornelia de Lange syndrome: phenotype and genotype analysis. Am J Med Genet A 2012; 158A: 2499-2505.
[35] Kawauchi S, Calof AL, Santos R, Lopez-Burks ME, Young CM, Hoang MP, Chua A, Lao T, Lechner MS, Daniel JA, Nussenzweig A, Kitzes L, Yokomori K, Hallgrimsson B, Lander AD. Multiple organ system defects and transcriptional dysregulation in the Nipbl(+/-) mouse, a model of Cornelia de Lange Syndrome. PLoS Genet 2009; 5: e1000650.
[36] Vargiami E, Ververi A, Al-Mutawa H, Gioula G, Gerou S, Rouvalis F, Kambouris M, Zafeiriou DI. Multiple coronary artery microfistulas in a girl with Kleefstra syndrome. Case Rep Genet 2016; 2016: 3056053.
[37] Priest JR, Osoegawa K, Mohammed N, Nanda V, Kundu R, Schultz K, Lammer EJ, Girirajan S, Scheetz T, Waggott D, Haddad F, Reddy S, Bernstein D, Burns T, Steimle JD, Yang XH, Moskowitz IP, Hurles M, Lifton RP, Nickerson D, Bamshad M, Eichler EE, Mital S, Sheffield V, Quertermous T, Gelb BD, Portman M, Ashley EA. De Novo and rare variants at multiple loci support the oligogenic origins of atrioventricular septal heart defects. PLoS Genet 2016; 12: e1005963.
[38] Lin FJ, You LR, Yu CT, Hsu WH, Tsai MJ, Tsai SY. Endocardial cushion morphogenesis and coronary vessel development require chickenovalbumin upstream promoter-transcription factor II. Arterioscler Thromb Vasc Biol 2012; 32: e135-146.
[39] Al Turki S, Manickaraj AK, Mercer CL, Gerety SS, Hitz MP, Lindsay S, D'Alessandro LC, Swaminathan GJ, Bentham J, Arndt AK, Louw J, Breckpot J, Gewillig M, Thienpont B, Abdul-Khaliq H, Harnack C, Hoff K, Kramer HH, Schubert S, Siebert R, Toka O, Cosgrove C, Watkins H, Lucassen AM, O'Kelly IM, Salmon AP, Bu'lock FA, Granados-Riveron J, Setchfield K, Thornborough C, Brook JD, Mulder B, Klaassen S, Bhattacharya S, Devriendt K, Fitzpatrick DF; UK10K Consortium, Wilson DI, Mital S, Hurles ME. Rare variants in NR2F2 cause congenital heart defects in humans. Am J Hum Genet 2014; 94: 574-585.
[40] Klewer SE, Krob SL, Kolker SJ, Kitten GT. Expression of type VI collagen in the developing mouse heart. Dev Dyn 1998; 211: 248-255.
[41] Cortes C, Francou A, De Bono C, Kelly RG. Epithelial properties of the second heart field. Circ Res 2018; 122: 142-154.
[42] Rana MS, Théveniau-Ruissy M, De Bono C, Mesbah K, Francou A, Rammah M, Domínguez JN, Roux M, Laforest B, Anderson RH, Mohun T, Zaffran S, Christoffels VM, Kelly RG. Tbx1 coordinates addition of posterior second heart field progenitor cells to the arterial and venous poles of the heart. Circ Res 2014; 115: 790-799.
[43] Damerla RR, Cui C, Gabriel GC, Liu X, Craige B, Gibbs BC, Francis R, Li Y, Chatterjee B, San Agustin JT, Eguether T, Subramanian R, Witman GB, Michaud JL, Pazour GJ, Lo CW. Novel Jbts17 mutant mouse model of Joubert syndrome with cilia transition zone defects and cerebellar and other ciliopathy related anomalies. Hum Mol Genet 2015; 24: 3994-4005.
[44] Hoffmann AD, Yang XH, Burnicka-Turek O, Bosman JD, Ren X, Steimle JD, Vokes SA, McMahon AP, Kalinichenko VV, Moskowitz IP. Foxf genes integrate tbx5 and hedgehog pathways in the second heart field for cardiac septation. PLoS Genet 2014; 10: e1004604.
[45] Baardman ME, Zwier MV, Wisse LJ, Gittenberger-de Groot AC, Kerstjens-Frederikse WS, Hofstra RM, Jurdzinski A, Hierck BP, Jongbloed MR, Berger RM, Plösch T, DeRuiter MC. Common arterial trunk and ventricular noncompaction in Lrp2 knockout mice indicate a crucialrole of LRP2 in cardiac development. Dis Model Mech 2016; 9: 413-425.
[46] Bai Y, Wang J, Morikawa Y, Bonilla-Claudio M, Klysik E, Martin JF. Bmp signaling represses Vegfa to promote outflow tract cushion development. Development 2013; 140: 3395-3402.
[47] Hall JG, Pallister PD, Clarren SK, Beckwith JB, Wiglesworth FW, Fraser FC, Cho S, Benke PJ, Reed SD. Congenital hypothalamic hamartoblastoma, hypopituitarism, imperforate anus and postaxial polydactyly--a new syndrome? Part I: clinical, causal, and pathogenetic considerations. Am J Med Genet 1980; 7: 47-74.
[48] Schulz S, Volleth M, Muschke P, Wieland I, Wieacker P. Greig cephalopolysyndactyly (GCPS) contiguous gene syndrome in a boy

with a 14 Mb deletion in region 7p13-14 caused by a paternal balanced insertion (5; 7). Appl Clin Genet 2008; 1: 19-22.
[49] Maitra M, Koenig SN, Srivastava D, Garg V. Identification of GATA6 sequence variants in patients with congenital heart defects. Pediatr Res 2010; 68: 281-285.
[50] Costell M, Carmona R, Gustafsson E, González-Iriarte M, Fässler R, Muñoz-Chápuli R. Hyperplastic conotruncal endocardial cushions and transposition of great arteries in perlecan-null mice. Circ Res 2002; 91: 158-164.
[51] Jordan VK, Zaveri HP, Scott DA. 1p36 deletion syndrome: an update. Appl Clin Genet 2015; 8: 189-200.
[52] Zaveri HP, Beck TF, Hernández-García A, Shelly KE, Montgomery T, van Haeringen A, Anderlid BM, Patel C, Goel H, Houge G, Morrow BE, Cheung SW, Lalani SR, Scott DA. Identification of critical regions and candidate genes for cardiovascular malformations and cardiomyopathy associated with deletions of chromosome 1p36. PLoS One 2014; 9: e85600.

Supplementary Table 1. AVSD-associated gene list from Phenolyzer

Gene	OMIM ID	CHD-associated syndromes or diseases	CHD phenotypes
ABCD3	170995	Zellweger syndrome	Heart septal defect
ABCD4	603214	Methylmalonic aciduria and homocystinuria cblj type	Heart septal defect
ACF	603375	Cayler cardiofacial syndrome	Heart septal defect
ACTC1	102540	Atrial septal defect 5; dilated cardiomyopathy 1r; hypertrophic cardiomyopathy 11; left ventricular noncompaction 4	Heart septal defect; heart atrial septal defect
ACVR2B	602730	Situs ambiguus; heterotaxy visceral 4 autosomal	Heart septal defect; atrioventricular septal defect
ADAMTS10	608990	Weill marchesani syndrome	Heart septal defect
ADAMTSL2	612277	Geleophysic dysplasia	Heart septal defect
ADK	102750	Hypermethioninemia due to adenosine kinase deficiency	Heart septal defect
AGGF1	608464	Klippel trenaunay weber syndrome	Heart septal defect
AHI1	608894	Acrocallosal syndrome; joubert syndrome	Heart septal defect
ALG1	605907	Congenital disorder of glycosylation	Heart septal defect
ALG11	613666	Congenital disorder of glycosylation	Heart septal defect
ALG12	607144	Congenital disorder of glycosylation	Heart septal defect
ALG13	300776	Congenital disorder of glycosylation	Heart septal defect
ALG2	607905	Congenital disorder of glycosylation	Heart septal defect
ALG3	608750	Congenital disorder of glycosylation	Heart septal defect
ALG6	604566	Congenital disorder of glycosylation	Heart septal defect
ALG8	608103	Congenital disorder of glycosylation	Heart septal defect
ALG9	606941	Congenital disorder of glycosylation	Heart septal defect
ANK1	612641	8p11.2 deletion syndrome	Heart septal defect
ANKRD11	611192	16q24.3 microdeletion syndrome	Heart septal defect
ARHGAP31	610911	Adams oliver syndrome	Heart septal defect
ARID1A	603024	Coffin siris syndrome	Heart septal defect
ARID1B	614556	Coffin siris syndrome	Heart septal defect
ARL13B	608922	Acrocallosal syndrome; joubert syndrome	Heart septal defect
ARVCF	602269	22q11.2 deletion syndrome	Heart septal defect
ARX	300382	Lissencephaly x linked 2	Heart septal defect
ASXL1	612990	Bohring opitz syndrome; c like syndrome	Heart septal defect
ATIC	601731	Aicar transformylase imp cyclohydrolase deficiency	Heart septal defect
ATP6V0A2	611716	Cutis laxa; wrinkly skin syndrome	Heart septal defect
ATRX	300032	Atr x syndrome	Heart septal defect
B3GALTL	610308	Peters plus syndrome	Heart septal defect
B3GAT3	606374	Multiple joint dislocations short stature craniofacial dysmorphism and congenital heart defects	Heart septal defect
B4GALT1	137060	Congenital disorder of glycosylation	Heart septal defect
BAZ1B	605681	Williams syndrome	Heart septal defect
BCL7B	605846	Williams syndrome	Heart septal defect
BCOR	300485	Microphthalmia syndromic 2	Heart septal defect
BMP2	112261	20p12.3 microdeletion syndrome	Heart septal defect
BMP4	112262	Microphthalmia syndromic 3	Heart septal defect
BPIFA1	607412	Fetal alcohol syndrome	Heart septal defect
BRAF	164757	Cardiofaciocutaneous syndrome; leopard syndrome; noonan syndrome	Heart septal defect; atrioventricular septal defect
BRCA2	600185	Fanconi anemia	Heart septal defect
BRIP1	605882	Fanconi anemia	Heart septal defect
BUB1	602452	Mosaic variegated aneuploidy syndrome	Heart septal defect
BUB1B	602860	Mosaic variegated aneuploidy syndrome	Heart septal defect
BUB3	603719	Mosaic variegated aneuploidy syndrome	Heart septal defect
C5ORF42	614571	Acrocallosal syndrome; joubert syndrome	Heart septal defect
CACNA1D	114206	Primary aldosteronism seizures and neurologic abnormalities	Heart septal defect
CANT1	613165	Desbuquois syndrome	Heart septal defect
CC2D2A	612013	Acrocallosal syndrome; joubert syndrome	Heart septal defect
CCBE1	612753	Hennekam lymphangiectasia lymphedema syndrome	Heart septal defect
CD96	606037	Bohring syndrome; opitz trigonocephaly syndrome	Heart septal defect
CDKN1C	600856	Williams beuren syndrome	Heart septal defect

CEP290	610142	Acrocallosal syndrome; joubert syndrome 18; meckel syndrome type 4	Heart septal defect
CEP41	610523	Acrocallosal syndrome; joubert syndrome	Heart septal defect
CEP57	607951	Mosaic variegated aneuploidy syndrome	Heart septal defect
CFC1	605194	Conotruncal heart malformations; double outlet right ventricle; situs ambiguus; heterotaxy visceral 2 autosomal	Heart septal defect; atrioventricular septal defect
CHD7	608892	Charge syndrome	Heart septal defect
CHST14	608429	Ehlers danlos syndrome musculocontractural type	Heart septal defect
CHST3	603799	Multiple joint dislocations short stature craniofacial dysmorphism and congenital heart defects	Heart septal defect
CITED2	602937	Tetralogy of fallot; ventricular septal defect 2; atrial septal defect 8	Heart septal defect; atrioventricular septal defect
CKAP2L	616174	Filippi syndrome	Heart septal defect
CLIP2	603432	Williams syndrome	Heart septal defect
COG1	606973	Congenital disorder of glycosylation	Heart septal defect
COG4	606976	Congenital disorder of glycosylation	Heart septal defect
COG5	606821	Congenital disorder of glycosylation	Heart septal defect
COG6	606977	Congenital disorder of glycosylation	Heart septal defect
COG7	606978	Congenital disorder of glycosylation	Heart septal defect
COG8	606979	Congenital disorder of glycosylation	Heart septal defect
COL11A1	120280	Stickler syndrome, type II	Heart septal defect
COL11A2	120290	Otospondylomegaepiphyseal dysplasia	Heart septal defect
COL1A1	120150	Ehlers danlos syndrome classic type	Heart septal defect
COL2A1	120140	Otospondylomegaepiphyseal dysplasia	Heart septal defect
COL5A1	120215	Ehlers danlos syndrome classic type	Heart septal defect
COL5A2	120190	Ehlers danlos syndrome classic type	Heart septal defect
COMT	116790	22q11.2 deletion syndrome	Heart septal defect
CREBBP	600140	Rubinstein taybi syndrome 1	Heart septal defect
CRELD1	607170	Atrioventricular septal defect partial	Heart septal defect; heart ventricular septal defect; atrioventricular septal defect
CRKL	602007	Digeorge syndrome	Heart septal defect
CSGALNACT2	616616	Hirschsprung disease	Heart septal defect; heart ventricular septal defect
CTCF	604167	Mental retardation autosomal dominant 21	Heart septal defect
DDOST	602202	Congenital disorder of glycosylation	Heart septal defect
DDX11	601150	Warsaw breakage syndrome	Heart septal defect
DHCR7	602858	Smith lemli opitz syndrome	Heart septal defect; atrioventricular septal defect
DHFR	126060	Smith lemli opitz syndrome	Heart septal defect; atrioventricular canal defect
DLG4	602887	Williams syndrome	Heart septal defect
DOCK6	614194	Adams oliver syndrome	Heart septal defect
DOLK	610746	Congenital disorder of glycosylation	Heart septal defect
DPAGT1	191350	Congenital disorder of glycosylation	Heart septal defect
DPM1	603503	Congenital disorder of glycosylation	Heart septal defect
DPM2	603564	Congenital disorder of glycosylation	Heart septal defect
DPM3	605951	Congenital disorder of glycosylation	Heart septal defect
DSE	605942	Ehlers danlos syndrome musculocontractural type	Heart septal defect
DTNA	601239	Left ventricular noncompaction	Heart septal defect
ECE1	600423	Hirschsprung disease	Heart septal defect; heart ventricular septal defect
EDN3	131242	Hirschsprung disease	Heart septal defect; heart ventricular septal defect
EDNRB	131244	Hirschsprung disease	Heart septal defect; heart ventricular septal defect
EFTUD2	603892	Growth and mental retardation mandibulofacial dysostosis microcephaly and cleft palate; mandibulofacial dysostosis guion almeida type	Heart septal defect
EHMT1	607001	Kleefstra syndrome; kleefstra syndrome due to 9q34 microdeletion	Heart septal defect
EIF4H	603431	Williams syndrome	Heart septal defect
ELN	130160	Williams syndrome	Heart septal defect

EOGT	614789	Adams oliver syndrome	Heart septal defect
EPO	133170	Heart septal defects ventricular	Heart septal defect; heart ventricular septal defects
ERBB3	190151	Lethal congenital contracture syndrome 2	Heart septal defect
ERCC4	133520	Fanconi anemia	Heart septal defect
ESCO2	609353	Roberts syndrome	Heart septal defect
EVC	604831	Ellis van creveld syndrome	Heart septal defect; atrioventricular septal defect
EVC2	607261	Ellis van creveld syndrome	Heart septal defect; atrioventricular septal defect
EYA1	601653	Cayler cardiofacial syndrome	Heart septal defect
FAM58A	300708	Syndactyly telecanthus anogenital and renal malformations	Heart septal defect
FANCA	607139	Fanconi anemia	Heart septal defect
FANCB	300515	Vacterl association; fanconi anemia	Heart septal defect
FANCC	613899	Fanconi anemia	Heart septal defect
FANCD2	613984	Fanconi anemia	Heart septal defect
FANCE	613976	Fanconi anemia	Heart septal defect
FANCF	613897	Fanconi anemia	Heart septal defect
FANCG	602956	Fanconi anemia	Heart septal defect
FANCI	611360	Fanconi anemia	Heart septal defect
FANCL	608111	Fanconi anemia	Heart septal defect
FANCM	609644	Fanconi anemia	Heart septal defect
FBN1	134797	Aortic aneurysm familial thoracic 4; marfan syndrome; shprintzen goldberg syndrome; geleophysic dysplasia; weill marchesani syndrome	Heart septal defect
FGF8	600483	Digeorge syndrome	Heart septal defect
FGFR1	136350	Apert syndrome; encephalocraniocutaneous lipomatosis	Heart septal defect
FGFR2	176943	Acrocephalosyndactyly type I; apert syndrome	Heart septal defect
FGFR3	134934	Apert syndrome; thanatophoric dysplasia	Heart septal defect
FIG4	609390	Yunis varon syndrome	Heart septal defect
FKBP6	604839	Williams syndrome	Heart septal defect
FKTN	607440	Fukuyama congenital muscular dystrophy	Heart septal defect
FLNA	300017	Cardiac valvular dysplasia x linked; frontometaphyseal dysplasia; melnick needles syndrome; otopalatodigital syndrome type II	Heart septal defect; atrioventricular septal defect
FLNB	603381	Larsen syndrome	Heart septal defect
FOXC1	601090	Axenfeld rieger syndrome type 3	Heart septal defect
FOXC2	602402	Lymphedema distichiasis syndrome	Heart septal defect
FOXE3	601094	Anterior segment mesenchymal dysgenesis	Heart septal defect
FOXG1	164874	Acrocallosal syndrome	Heart septal defect
G6PC3	611045	Dursun syndrome	Heart septal defect; heart atrial septal defect
GAS1	139185	Holoprosencephaly	Heart septal defect
GATA1	305371	Diamond blackfan anemia	Heart septal defect
GATA4	600576	Atrial septal defect 2; atrioventricular septal defect 4; tetralogy of fallot; ventricular septal defect 1; 8p23.1 microdeletion syndrome	Heart septal defect; heart atrial septal defect; heart ventricular septal defect; atrioventricular septal defect
GATA6	601656	Conotruncal heart malformations; persistent truncus arteriosus; tetralogy of fallot; atrioventricular septal defect 5; pancreatic agenesis and congenital heart defects; atrial septal defect 9	Heart septal defect; atrioventricular septal defect
GDF1	602880	Conotruncal heart malformations; double outlet right ventricle; right atrial isomerism; tetralogy of fallot	Heart septal defect; atrioventricular septal defect
GDF3	606522	Isolated klippel feil syndrome	Heart septal defect
GDF6	601147	Isolated klippel feil syndrome	Heart septal defect
GDNF	600837	Hirschsprung disease	Heart septal defect; heart ventricular septal defects
GH1	139250	Turner syndrome	Heart septal defect
GJA1	121014	Atrioventricular septal defect 3; hypoplastic left heart syndrome; palmoplantar keratoderma; oculodentodigital dysplasia	Heart septal defect; atrioventricular septal defect
GLA	300644	Fabry disease	Heart septal defect
GLI3	165240	Acrocallosal syndrome; pallister hall syndrome	Heart septal defect
GP1BB	138720	22q11.2 deletion syndrome	Heart septal defect
GPC3	300037	Simpson golabi behmel syndrome	Heart septal defect

GPC4	300168	Simpson golabi behmel syndrome	Heart septal defect
GPC6	604404	Omodysplasia 1	Heart septal defect
GPX4	138322	Spondylometaphyseal dysplasia sedaghatian type	Heart septal defect
GTF2I	601679	Williams syndrome	Heart septal defect
GTF2IRD1	604318	Williams syndrome	Heart septal defect
HCCS	300056	Microphthalmia syndromic 7	Heart septal defect
HDAC8	300269	Cornelia de lange syndrome; de lange syndrome	Heart septal defect
HIRA	600237	22q11.2 deletion syndrome	Heart septal defect
HOXA13	142959	Hand foot genital syndrome	Heart septal defect
HRAS	190020	Costello syndrome	Heart septal defect
HSD17B4	601860	Zellweger syndrome	Heart septal defect
HSPG2	142461	Dyssegmental dysplasia silverman handmaker type	Heart septal defect
HYLS1	610693	Hydrolethalus syndrome	Heart septal defect; atrioventricular septal defect
IMPAD1	614010	Catel manzke syndrome	Heart septal defect
INPP5E	613037	Acrocallosal syndrome; joubert syndrome	Heart septal defect
IRX5	606195	Hamamy syndrome	Heart septal defect
JAG1	601920	Alagille syndrome; deafness congenital heart defects and posterior embryotoxon; tetralogy of fallot	Heart septal defect
KANSL1	612452	Koolen de vries syndrome	Heart septal defect
KAT6B	605880	Noonan syndrome; young simpson syndrome; blepharophimosis intellectual deficit syndrome sbbys type; genitopatellar syndrome; noonan syndrome	Heart septal defect; atrioventricular septal defect
KDM6A	300128	Kabuki make up syndrome	Heart septal defect
KIAA0196	610657	3c syndrome; dandy walker like malformation	Heart septal defect; heart atrial septal defect
KIF7	611254	Acrocallosal syndrome; joubert syndrome 18	Heart septal defect
KMT2D	602113	Kabuki make up syndrome	Heart septal defect
KRAS	190070	Cardiofaciocutaneous syndrome; noonan syndrome; costello syndrome	Heart septal defect
L1CAM	308840	Hirschsprung disease	Heart ventricular septal defect
LAT2	605719	Williams syndrome	Heart septal defect
LBR	600024	Pelger huet anomaly	Heart septal defect
LETM1	604407	Wolf hirschhorn syndrome	Heart septal defect
LIMK1	601329	Williams syndrome	Heart septal defect
LMNA	150330	Heart hand syndrome slovenian type; left ventricular noncompaction; restrictive dermopathy lethal	Heart septal defect
LONP1	605490	Codas syndrome	Heart septal defect
LRP2	600073	Donnai barrow syndrome	Heart septal defect
LRP5	603506	Osteoporosis pseudoglioma syndrome	Heart septal defect
LTBP2	602091	Weill marchesani syndrome	Heart septal defect
LTBP4	604710	Cutis laxa	Heart septal defect
MAGT1	300715	Congenital disorder of glycosylation	Heart septal defect
MAP2K1	176872	Cardiofaciocutaneous syndrome; noonan syndrome	Heart septal defect
MAP2K2	601263	Cardiofaciocutaneous syndrome	Heart septal defect
MED12	300188	Lujan fryns syndrome; x linked intellectual deficit; x linked mental retardation	Heart septal defect
MEGF8	604267	Carpenter syndrome	Heart septal defect
MEOX1	600147	Isolated klippel feil syndrome	Heart septal defect
MGAT2	602616	Congenital disorder of glycosylation type iia	Heart septal defect
MGP	154870	Keutel syndrome	Heart septal defect
MID1	300552	Opitz frias syndrome	Heart septal defect; atrioventricular septal defect
MKKS	604896	Mckusick kaufman syndrome	Heart septal defect
MKS1	609883	Meckel syndrome type 1	Heart septal defect; atrioventricular septal defect
MLXIPL	605678	Williams syndrome	Heart septal defect
MMP14	600754	Torg winchester syndrome	Heart septal defect
MMP2	120360	Torg winchester syndrome	Heart septal defect
MOGS	601336	Congenital disorder of glycosylation	Heart septal defect
MPDU1	604041	Congenital disorder of glycosylation	Heart septal defect
MPI	154550	Congenital disorder of glycosylation	Heart septal defect
MSX1	142983	Wolf hirschhorn syndrome	Heart septal defect

MX1	147150	Fanconi anemia	Heart septal defect
MYH6	160710	Atrial septal defect 3; dilated cardiomyopathy 1ee; hypertrophic cardiomyopathy 14	Heart septal defect; heart atrial septal defect
MYL2	160781	Cardiomyopathy familial hypertrophic 10	Heart septal defect
NAA10	300013	N-terminal acetyltransferase deficiency; ogden syndrome	Heart septal defect
NEK8	609799	Renal hepatic pancreatic dysplasia	Heart septal defect
NEK9	609798	Arthrogryposis perthes disease and upward gaze palsy	Heart septal defect
NELFA	606026	Wolf hirschhorn syndrome	Heart septal defect
NFIX	164005	Marshall smith syndrome; sotos syndrome	Heart septal defect
NIPBL	608667	Cornelia de lange syndrome	Heart septal defect
NKX2-5	600584	Atrial septal defect 7; conotruncal heart malformations; tetralogy of fallot; ventricular septal defect 3	Heart atrial septal defect; heart septal defect
NKX2-6	611770	Conotruncal heart malformations; persistent truncus arteriosus	Heart septal defect
NODAL	601265	Heterotaxy visceral 5 autosomal	Heart septal defect
NOS3	163729	Fabry disease	Heart septal defect
NOTCH2	600275	Alagille syndrome; hajdu cheney syndrome	Heart septal defect
NOTCH3	600276	Lateral meningocele syndrome	Heart septal defect
NPHP1	607100	Acrocallosal syndrome; joubert syndrome	Heart septal defect
NPHP3	608002	Renal hepatic pancreatic dysplasia	Heart septal defect
NRAS	164790	Noonan syndrome	Heart septal defect
NRG1	142445	Hirschsprung disease	Heart septal defect; heart ventricular septal defect
NRTN	602018	Hirschsprung disease	Heart septal defect; heart ventricular septal defect
NSD1	606681	Sotos syndrome	Heart septal defect
NSDHL	300275	Child syndrome	Heart septal defect
OFD1	300170	Primary ciliary dyskinesia; acrocallosal syndrome; joubert syndrome 18	Heart septal defect
OTX2	600037	Microphthalmia syndromic 3	Heart septal defect
PALB2	610355	Fanconi anemia	Heart septal defect
PAX2	167409	Microphthalmia syndromic 3	Heart septal defect
PAX3	606597	Waardenburg syndrome type 3	Heart septal defect
PCNT	605925	Microcephalic osteodysplastic primordial dwarfism type II	Heart septal defect
PCSK5	600488	Heart septal defects; heart ventricular septal defects	Heart septal defects; heart ventricular septal defects
PEX1	602136	Zellweger syndrome	Heart septal defect
PEX10	602859	Zellweger syndrome	Heart septal defect
PEX11B	603867	Zellweger syndrome	Heart septal defect
PEX12	601758	Zellweger syndrome	Heart septal defect
PEX13	601789	Zellweger syndrome	Heart septal defect
PEX14	601791	Zellweger syndrome	Heart septal defect
PEX16	603360	Zellweger syndrome	Heart septal defect
PEX19	600279	Zellweger syndrome	Heart septal defect
PEX2	170993	Zellweger syndrome	Heart septal defect
PEX26	608666	Zellweger syndrome	Heart septal defect
PEX3	603164	Zellweger syndrome	Heart septal defect
PEX5	600414	Zellweger syndrome	Heart septal defect
PEX6	601498	Zellweger syndrome	Heart septal defect
PGM1	171900	Congenital disorder of glycosylation	Heart septal defect
PHOX2B	603851	Hirschsprung disease	Heart septal defects; heart ventricular septal defects
PIEZO2	613629	Marden walker syndrome	Heart septal defect
PIGA	311770	Multiple congenital anomalies hypotonia seizures syndrome 2	Heart septal defect
PIGL	605947	Chime syndrome; zunich neuroectodermal syndrome	Heart septal defect
PIGN	606097	Multiple congenital anomalies hypotonia seizures syndrome 1	Heart septal defect
PIK3CA	171834	Megalencephaly capillary malformation polymicrogyria syndrome; megalencephaly cutis marmorata telangiectatica congenita	Heart septal defect
PIK3R2	603157	Megalencephaly polymicrogyria polydactyly hydrocephalus syndrome	Heart septal defect

PITX2	601542	Microphthalmia syndromic 3	Heart septal defect
PITX3	602669	Anterior segment mesenchymal dysgenesis; microphthalmia syndromic 3	Heart septal defect
PMM2	601785	Congenital disorder of glycosylation	Heart septal defect
PORCN	300651	Focal dermal hypoplasia	Heart septal defect
PQBP1	300463	Hamel cerebro palato cardiac syndrome; renpenning syndrome 1	Heart septal defect
PTEN	601728	Leopard syndrome	Heart septal defects; atrioventricular canal defect
PTPN11	176876	Leopard syndrome; noonan syndrome; tetralogy of fallot	Heart septal defect; atrioventricular septal defect
PUF60	604819	Verheij syndrome	Heart septal defect
PYCR1	179035	Cutis laxa	Heart septal defect
RAB23	606144	Carpenter syndrome	Heart septal defect
RAD21	606462	Cornelia de lange syndrome	Heart septal defect
RAD51C	602774	Fanconi anemia	Heart septal defect
RAF1	164760	Leopard syndrome; noonan syndrome; dilated cardiomyopathy 1nn	Heart septal defect; atrioventricular septal defect
RAI1	607642	Potocki lupski syndrome	Heart septal defect
RARB	180220	Microphthalmia syndromic 12	Heart septal defect
RASGEF1A	614531	Hirschsprung disease	Heart septal defect; heart ventricular septal defect
RAX	601881	Microphthalmia syndromic 3	Heart septal defect
RBM10	300080	Tarp syndrome	Heart septal defect
RBM8A	605313	Thrombocytopenia absent radius syndrome	Heart septal defect
RBPJ	147183	Adams oliver syndrome	Heart septal defect
RECQL4	603780	Rapadilino syndrome; baller gerold syndrome	Heart septal defect; heart atrial septal defect
RET	164761	Hirschsprung disease	Heart septal defect; heart ventricular septal defect
RFC2	600404	Williams syndrome	Heart septal defect
RFT1	611908	Congenital disorder of glycosylation	Heart septal defect
RIT1	609591	Noonan syndrome	Heart septal defect
RMRP	157660	Cartilage hair hypoplasia	Heart septal defect
RNU4ATAC	601428	Microcephalic osteodysplastic primordial dwarfism type I	Heart septal defect
ROR2	602337	Brachydactyly type b; robinow syndrome autosomal recessive	Heart septal defect
RPGRIP1L	610937	Acrocallosal syndrome; joubert syndrome	Heart septal defect
RPL11	604175	Diamond blackfan anemia	Heart septal defect
RPL15	604174	Diamond blackfan anemia	Heart septal defect
RPL26	603704	Diamond blackfan anemia	Heart septal defect
RPL35A	180468	Diamond blackfan anemia	Heart septal defect
RPL5	603634	Aase syndrome; diamond blackfan anemia	Heart septal defect
RPS10	603632	Diamond blackfan anemia	Heart septal defect
RPS17	180472	Diamond blackfan anemia	Heart septal defect
RPS19	603474	Diamond blackfan anemia	Heart septal defect
RPS24	602412	Diamond blackfan anemia	Heart septal defect
RPS26	603701	Diamond blackfan anemia	Heart septal defect
RPS28	603685	Diamond blackfan anemia	Heart septal defect
RPS7	603658	Diamond blackfan anemia	Heart septal defect
SALL1	602218	Townes brocks syndrome	Heart septal defect
SALL4	607343	Duane radial ray syndrome	Heart septal defect
SEMA3C	602645	Truncus arteriosus persistent	Heart septal defect
SEMA3E	608166	Charge syndrome	Heart septal defect
SETBP1	611060	Schinzel giedion midface retraction syndrome	Heart septal defect
SH2B1	608937	Proximal 16p11 2 microdeletion syndrome	Heart septal defect
SHANK3	606230	Phelan mcdermid syndrome	Heart septal defect
SHH	600725	Single upper central incisor	Heart septal defect; atrioventricular septal defect
SHOC2	602775	Noonan syndrome	Heart septal defect
SIX3	603714	Microphthalmia syndromic 3	Heart septal defect
SIX6	606326	Microphthalmia syndromic 3	Heart septal defect
SLC19A2	603941	Thiamine responsive megaloblastic anemia syndrome	Heart septal defect

SLC29A3	612373	Dysosteosclerosis; faisalabad histiocytosis; h syndrome; histiocytosis lymphadenopathy plus syndrome	Heart septal defect
SLC35A1	605634	Congenital disorder of glycosylation	Heart septal defect
SLC35C1	605881	Congenital disorder of glycosylation	Heart septal defect
SLX4	613278	Fanconi anemia	Heart septal defect
SMAD4	600993	Myhre syndrome	Heart septal defect
SMARCA2	600014	Coffin siris syndrome	Heart septal defect
SMARCA4	603254	Coffin siris syndrome	Heart septal defect
SMARCB1	601607	Coffin siris syndrome	Heart septal defect
SMARCE1	603111	Coffin siris syndrome	Heart septal defect
SMC1A	300040	Cornelia de lange syndrome	Heart septal defect
SMC3	606062	Cornelia de lange syndrome	Heart septal defect
SNRPB	182282	Cerebrocostomandibular syndrome	Heart septal defect
SNX3	605930	Microphthalmia syndromic 8	Heart septal defect
SOS1	182530	Noonan syndrome	Heart septal defect
SOX2	184429	Microphthalmia syndromic 3	Heart septal defect
SPECC1L	614140	Opitz gbbb syndrome type ii	Heart septal defect
SRCAP	611421	Floating harbor syndrome	Heart septal defect; heart ventricular septal defect
SRD5A3	611715	Congenital disorder of glycosylation	Heart septal defect
STAMBP	606247	Microcephaly capillary malformation syndrome	Heart septal defect
STRA6	610745	Microphthalmia syndromic 9	Heart septal defect
STRADA	608626	Polyhydramnios megalencephaly and symptomatic epilepsy	Heart septal defect
SYNE1	608441	Emery dreifuss muscular dystrophy 4 autosomal dominant	Heart septal defect
TBL2	605842	Williams syndrome	Heart septal defect
TBX1	602054	22q11.2 deletion syndrome; conotruncal anomaly face syndrome; conotruncal heart malformations; digeorge syndrome; shprintzen syndrome; tetralogy of fallot; velocardiofacial syndrome	Heart septal defect
TBX20	606061	Atrial septal defect 4	Heart septal defect; heart atrial septal defect
TBX3	601621	Ulnar mammary syndrome	Heart septal defect
TBX5	601620	Holt oram syndrome	Heart septal defect; heart atrial septal defect; atrioventricular septal defect
TCF4	602272	Pallister hall syndrome	Heart septal defect
TCTN1	609863	Acrocallosal syndrome; joubert syndrome	Heart septal defect
TCTN2	613846	Acrocallosal syndrome; joubert syndrome	Heart septal defect
TCTN3	613847	Acrocallosal syndrome; joubert syndrome 18	Heart septal defect
TDGF1	187395	Holoprosencephaly	Heart septal defect
TFAP2B	601601	Char syndrome; patent ductus arteriosus 2	Heart septal defect
TGDS	616146	Catel manzke syndrome	Heart septal defect
TGFBR1	190181	loeys dietz syndrome type 1a	Heart septal defect
TGFBR2	190182	loeys dietz syndrome type 1b	Heart septal defect
TGIF1	602630	Holoprosencephaly	Heart septal defect
TLL1	606742	Atrial septal defect 6	Heart septal defect; heart atrial septal defect
TMEM138	614459	Acrocallosal syndrome; joubert syndrome	Heart septal defect
TMEM165	614726	Congenital disorder of glycosylation	Heart septal defect
TMEM216	613277	Acrocallosal syndrome; joubert syndrome	Heart septal defect
TMEM231	614949	Acrocallosal syndrome; joubert syndrome	Heart septal defect
TMEM237	614423	Acrocallosal syndrome; joubert syndrome	Heart septal defect
TMEM67	609884	Acrocallosal syndrome; joubert syndrome	Heart septal defect
TNF	191160	Fanconi anemia	Heart septal defect
TP63	603273	Ankyloblepharon ectodermal defects cleft lip palate; hay wells syndrome	Heart septal defect
TSFM	604723	Combined oxidative phosphorylation deficiency 3	Heart septal defect
TTC21B	612014	Acrocallosal syndrome; joubert syndrome	Heart septal defect
TTC37	614589	Trichohepatoenteric syndrome 1	Heart septal defect
TUSC3	601385	Congenital disorder of glycosylation	Heart septal defect
TWSG1	605049	Holoprosencephaly	Heart septal defect
TXNL4A	611595	Burn mckeown syndrome	Heart septal defect

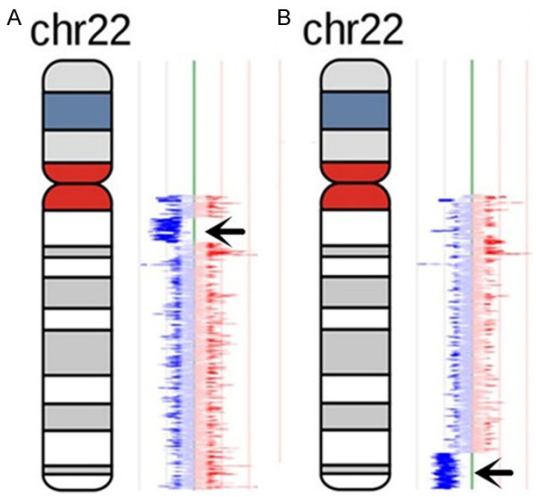
UBR1	605981	Johanson blizzard syndrome	Heart septal defect
UFD1L	601754	22q11.2 deletion syndrome	Heart septal defect
UMPS	613891	Orotic aciduria	Heart septal defect
VAX1	604294	Microphthalmia syndromic 3	Heart septal defect
VEGFA	192240	Heart septal defects ventricular	Heart septal defect; heart ventricular septal defects
VIPAS39	613401	Arthrogryposis renal dysfunction and cholestasis 2	Heart septal defect
VPS13B	607817	Cohen syndrome	Heart septal defect
VPS33B	608552	Arthrogryposis renal dysfunction and cholestasis 1	Heart septal defect
VSX2	142993	Microphthalmia syndromic 3	Heart septal defect
WBSCR22	615733	Williams syndrome	Heart septal defect
WBSCR27	612546	Williams syndrome	Heart septal defect
WDPCP	613580	Congenital heart defects hamartomas of tongue and polysyndactyly; orstavik lindemann solberg syndrome	Heart septal defect; atrioventricular septal defect
WHSC1	602952	Wolf hirschhorn syndrome	Heart septal defect
WT1	607102	Meacham syndrome	Heart septal defect
YY1AP1	607860	Grange syndrome	Heart septal defect
ZEB2	605802	Mowat wilson syndrome	Heart septal defect
ZIC3	300265	Double outlet right ventricle; heterotaxy visceral x linked; situs ambiguus; vacterl association; vacterl association x linked	Heart septal defect
ZMPSTE24	606480	Restrictive dermopathy lethal	Heart septal defect

Abbreviations: AVSD, atrioventricular septal defect; CHD, congenital heart disease.

Supplementary Table 2. AVSD-associated gene list from related published literatures

Gene	OMIM ID	CHD-associated syndromes or diseases	CHD Phenotypes	Genetic variation in human AVSD	Play a role in AVSD formation in animal models
COL1A2	120160	Ehlers danlos syndrome autosomal recessive cardiac valvular form; osteogenesis imperfecta	Heart septal defect; atrioventricular septal defect	No	Yes
COL6A1	120220	-	Heart septal defect; atrioventricular septal defect	Yes	Yes
COL6A2	120240	-	Heart septal defect; atrioventricular septal defect	Yes	Yes
NR2F2	107773	Congenital heart defects multiple types, 4	Heart septal defect; atrioventricular septal defect	Yes	Yes
DSCAM	602523	Down's syndrome	Heart septal defect; atrioventricular septal defect	Yes	No
DNAHC11	603339	Ciliary dyskinesia, primary, 7, with or without situs inversus	Heart septal defect; atrioventricular septal defect	No	Yes
FOXP1	605515	Mental retardation with language impairment and with or without autistic features	Heart septal defect; atrioventricular septal defect	Yes	Yes
ACVR1 (*ALK2*)	102576	Cardiac death sudden	Heart septal defect; atrioventricular septal defect	Yes	Yes
BMP5	112265	-	Heart septal defect; atrioventricular septal defect	No	Yes
COL18A1	120328	Heart valve diseases	Heart septal defect; atrioventricular septal defect	No	Yes
CYR61 (*CCN1*)	602369	-	Heart septal defect; atrioventricular septal defect	No	Yes
FBLN2	135821	-	Heart septal defect; atrioventricular septal defect	Yes	Yes
FGF2	134920	Cardiomegaly; myocardial ischemia	Heart septal defect; atrioventricular septal defect	No	Yes
FRZB	605083	-	Heart septal defect; atrioventricular septal defect	Yes	Yes
GATA5	611496	Familial atrial fibrillation	Heart septal defect; atrioventricular septal defect	Yes	Yes
HEY2	604674	Brugada syndrome; cardiomyopathy hypertrophic	Heart septal defect; atrioventricular septal defect	Yes	Yes
ROCK1	601702	-	Heart septal defect; atrioventricular septal defect	No	Yes
WNT9A	602863	-	Heart septal defect; atrioventricular septal defect	No	Yes
ALDH1A2	603687	-	Tetralogy of Fallot	No	Yes
HAND1	602406	-	Atrial Septal Defect, Hypoplastic Left Heart	No	Yes
SMAD6	602931	-	Aortic Valve Disease	No	Yes

Abbreviations: AVSD, atrioventricular septal defect; CHD, congenital heart disease.



Supplementary Figure 1. Two CNVs larger than 1 Mb were detected by low-pass WGS. They were heterozygous deletions at chromosome 22q11.21 (A) and chromosome 22q13.31q13.33 (B), the length of them were 2.78 Mb and 4.29 Mb, respectively.

Supplementary Table 3. Primers Design for AVSD-associated genes contained in CNVs

Gene	Forward Primer	Reverse Primer	Product Length
NOTCH2	AGGCACCTGTATTGACCTTG	TCCAATCCTATCCATGCACTG	142 Bp
COL11A1	CAGGTGGAACTTTCCCAGAA	GCAGGTTTTCCAGTGTGGTC	169 Bp
NIPBL	GCTGGCACCTGAACTAAGTAC	GTAAAGGAGATGGAAGAGGCAG	150 Bp
EHMT1	GCCAGTAAAGATCCCAGAGAAG	GTAGCACTGGTTCTGAGGTAG	150 Bp
NR2F2	TCAAAGTGGGCATGAGACG	CGCAACAGCAGGGAAATATATC	142 Bp
COL6A1	CGAATGCGAGATTTTGGACATC	ACGAAGTCCTTGGCAATCTC	138 Bp
COL6A2	CAGCCCTCAAGTTTGCCTAC	TCACTCTCGTGCTTCTCGTG	196 Bp
SMC1A	GGTAGAGGATGAGGTGTTTGAAG	ACTGAATGCCCAAGCGAG	149 Bp

Supplementary Table 4. CNVs containing the AVSD-associated genes in DECIPHER

Candidate gene	Patients, CNVs and phenotypes
NOTCH2	Patient 250,335 with 14.55 Mb deletion at 1p12p21.1 has ASD, VSD
	Patient 317,280 with 4.20 Mb deletion at 1p12p13.2 has VSD
COL11A1	None
NIPBL	Patient 4,651 with 177.78 Kb deletion at 5p13.2 with VSD
	Patient 285,915 with 22.17 Mb duplication at 5p13.2q11.2 has ASD
	Patient 341,218 with 422.15 Kb duplication at 5p13.2 has ASD
	Patient 350,097 with a heterozygous and definitely pathogenic frameshift variant (Val2227PhefsTer25) has complete AVSD (SNV)
EHMT1	Patient 771 with 3.03 Mb deletion at 9q34.3 has VSD
	Patient 1,003 with 2.22 Mb deletion at 9q34.3 has ASD, VSD
	Patient 250,053 with 192.76 Kb deletion at 9q34.3 has ASD
	Patient 251,553 with 561.12 Kb deletion at 9q34.3 has ASD
	Patient 269,405 with 293.64 Kb deletion at 9q34.3 has ASD
	Patient 285,975 with 589.35 Kb definitely pathogenic deletion at 9q34.3 has ASD, VSD
NR2F2	Patient 2,219 with 8.54 Mb duplication at 15q26.1q26.3 has AVSD
	Patient 251,099 with 6.60 Mb deletion at 15q26.2q26.3 has VSD
	Patient 256,144 with 10.65 Mb duplication at 15q26.1q26.3 has ASD

	Patient 259,934 with 7.10 Mb deletion at 15q26.2q26.3 has VSD
	Patient 277,356 with 3.56 Mb deletion at 15q26.2q26.3 has VSD
	Patient 286,739 with 3.71 Mb likely pathogenic deletion at 15q26.2q26.3 has VSD
	Patient 259,383 with a likely pathogenic SNV has complete AVSD
COL6A1	None
COL6A2	None
TBX1	Patient 256,300 with 2.40 Mb deletion at 22q11.21 has AVSD
	Patient 286,085 with 2.49 Mb definitely pathogenic deletion at 22q11.1q11.21 has ASD, VSD
	Patient 300,420 with 2.42 Mb definitely pathogenic duplication at 22q11.21 has ASD, VSD
SHANK3	Patient 253,900 with 86.55 Kb duplication at 22q13.33 has AVSD
	Patient 353,765 with 55.33 Kb duplication at 22q13.33 has VSD
SMC1A	Patient 256,035 with 1.32 Mb duplication at Xp11.22 has VSD

Abbreviations: CNV, copy number variation; AVSD, atrioventricular septal defect; ASD, atrial septal defect; VSD, ventricular septal defect; SNV, single nucleotide variants.

Supplementary Table 5. Primers Design for Sanger sequencing

Gene	Position	Nucleotide changes	Forward Primer	Reverse Primer	Product Length
COL11A1	1:103496805	652-5->TT	TTTCCTGAGCCAGAAGATAACA	CAAAAACTGCACTGCGATGT	427 Bp
	1:103412451	3266C>T	ACATGCCAGACACATATGCAG	TGGATTCAACTGTTTCTCTTTGG	388 Bp
COL6A2	21:47532276	499G>A	ATCCACGTGTACTTCGTGCTG	TCACCATGACCTTGATGATGC	586 Bp
	21:47532456	679G>A	CTGGCCAACATGACGGAG	GGTAAAGTGAGGCCCGGAG	384 Bp
	21:47552204	2798G>A	ACGACGACCCTCTCAACG	AGGAGCTGGAGAGGTGCAG	588 Bp
C5ORF42	5:37170162	6443A>G	CACCCGGCTGACTTTTGTAT	CTGTGCATTTAGGGGAAAGC	355 Bp
	5:37125396	8746G>A	TGCCAAATTACAAATGTATCCAA	AGGTAACAAATTGGAGTGAGTTGAC	434 Bp
	5:37243184	608A>G	AAGGCAGGAGGACTGCTTG	CTGCCTCTGGCTCAGAAAAA	504 Bp
GLI3	7:42188023	169G>A	ATAAAGCGCGCACACACAC	GCTCTCAAAGTTGCTGTGAATG	481 Bp
	7:42188028	164G>A	ATAAAGCGCGCACACACAC	GCTCTCAAAGTTGCTGTGAATG	481 Bp
LRP2	2:170038738	9937G>A	TTACATGAACAGCCTTCTCGG	TAGCTTGGGTAGGAAACTGGG	315 Bp
	2:170038761	9914G>A	TTACATGAACAGCCTTCTCGG	TAGCTTGGGTAGGAAACTGGG	315 Bp
GATA6	18:19751148	43G>C	CTTGTTAACCCGTCGATCTCC	TCAGTGAACAGCAGCAAGTCC	439 Bp
	18:19751656	551G>A	CTGCTGTTCACTGACCTCGAC	GTATGGAGGGCTGTCGGC	369 Bp
HSPG2	1:22161303	10589G>A	TGTCCCAAGTGAACAGAAAGG	TTGGGCAGTCTATGGCCTC	454 Bp
	1:22206994	2057T>C	GACAAGCCAGAATAGCCAATG	TAGGGCTGGGAGCAAAGG	408 Bp
	1:22217079	353C>T	TCAAGTACTCCGACTCCAGCTG	TATTTCCGAGCCCTGGTGA	226 Bp