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Contribution of single-gene defects to congenital cardiac left-sided lesions in the prenatal setting

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KEYWORDS: congenital heart defects; *KMT2D*; prenatal diagnosis; single-gene defects; whole-exome sequencing

CONTRIBUTION

What are the novel findings of this work?
We revealed that single-gene defects contributed substantially (13/66 (19.7%)) to the genetic cause of congenital cardiac left-sided lesions (LSLs) in a prenatal cohort without aneuploidy or pathogenic copy-number variants. Furthermore, *KMT2D* mutations occurred in 10.6% (7/66) of LSL cases in this cohort.

What are the clinical implications of this work?
Our findings suggest that prenatal whole-exome sequencing should be performed to identify potential diagnostic genetic variants and facilitate perinatal decision-making and management in cases in which conventional tests (karyotyping and microarray) are not diagnostic.

ABSTRACT

Objectives *To explore the contribution of single-gene defects to the genetic cause of cardiac left-sided lesions (LSLs), and to evaluate the incremental diagnostic yield of whole-exome sequencing (WES) for single-gene defects in fetuses with LSLs without aneuploidy or a pathogenic copy-number variant (pCNV).*

Methods *Between 10 April 2015 and 30 October 2018, we recruited 80 pregnant women diagnosed with a LSL who had termination of pregnancy and genetic testing. Eligible LSLs were aortic valve atresia or stenosis, coarctation of the aorta, mitral atresia or stenosis and hypoplastic left heart syndrome (HLHS). CNV sequencing (CNV-seq) and WES were performed sequentially on specimens from these fetuses and their parents. CNV-seq was used to identify aneuploidies and pCNVs, while WES was used to identify diagnostic genetic variants in cases without aneuploidy or pCNV.*

Results *Of 80 pregnancies included in the study, 27 (33.8%) had a genetic diagnosis. CNV-seq analysis identified six (7.5%) fetuses with aneuploidy and eight (10.0%) with pCNVs. WES analysis of the remaining 66 cases revealed diagnostic genetic variants in 13 (19.7%) cases, indicating that the diagnostic yield of WES for the entire cohort was 16.3% (13/80).* KMT2D *was the most frequently mutated gene (7/66 (10.6%)) in fetuses with LSL without aneuploidy or pCNVs, followed by* NOTCH1 *(4/66 (6.1%)). HLHS was the most prevalent cardiac phenotype (4/7) in cases with a* KMT2D *mutation in this cohort. An additional six (9.1%) cases were found to have potentially deleterious variants in candidate genes.*

Conclusions *Single-gene defects contribute substantially to the genetic etiology of fetal LSLs.* KMT2D *mutations accounted for approximately 10% of LSLs in our fetal cohort. WES has the potential to provide genetic diagnoses in fetuses with LSLs without aneuploidy or pCNVs.*

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INTRODUCTION

Congenital cardiac left-sided lesions (LSLs) constitute roughly 10% of all neonatal congenital heart defects (CHDs) and 20% of all prenatal CHDs[1], and have a disproportionately high contribution to the costs of long-term disability and mortality arising from CHDs[2]. When associated with genetic abnormalities, such as aneuploidy, pathogenic copy-number variants (pCNVs) and single-gene defects (SGDs), the prognosis of LSLs is much worse owing to the presence of neurodevelopmental disorders and/or extracardiac malformations; therefore, accurate genetic diagnosis of LSLs as early as possible is an important area of study.

Prenatal genetic diagnosis can provide valuable information for guiding pregnancy and perinatal decision-making and management[3]. In clinical practice, genetic testing, such as karyotyping and chromosomal microarray analysis, are recommended for identifying chromosomal abnormalities in fetuses with CHDs. Overall, approximately 18–22% of fetal CHDs are associated with clinically relevant chromosomal abnormalities[4], which means that more than 70% of these pregnancies do not receive a definitive genetic diagnosis to guide future care and genetic counseling.

Several whole-exome sequencing (WES) studies have shown that SGDs are important genetic contributors to CHD in children and adults[5–8]. In general, SGDs are found in 3–5% of all CHDs[9]. However, these results are from the postnatal setting, thus they cannot inform prenatal counseling. To date, only two case series of unselected structural anomalies involving 77 and 34 fetuses, respectively, with CHD have evaluated the feasibility and value of WES in prenatal diagnostic testing, and the detection rates of WES for diagnostic genetic variants (DGVs) of CHD were 5% and 20.6%, respectively[10,11]. No papers have been published on the role of SGDs in pregnancies with LSLs. The primary aim of this study was to explore the contribution of SGDs to the genetic cause of LSLs in the prenatal setting. We also aimed at determining the genetic diagnostic capacity of WES in fetal CHD.

METHODS

This study was approved by the institutional review board of the Medical Ethics Committee of Beijing Anzhen Hospital, which is a regional and national referral center. Signed informed consent was obtained from all parents who agreed to participate in the study. Between 10 April 2015 and 30 October 2018, all pregnancies with a LSL leading to termination of pregnancy in our center were screened for eligibility for inclusion in this study. All pregnancies underwent a routine fetal anatomy ultrasound scan. If CHD was suspected, echocardiography was subsequently performed in Beijing Anzhen Hospital. Cases of CHD were classified according to the modified etiological classification method for CHDs proposed by Botto *et al.*[12]. Eligible LSLs were aortic valve atresia or stenosis, coarctation of the aorta (CoA), mitral atresia or stenosis and hypoplastic left heart syndrome (HLHS). Parents of fetuses with eligible LSLs who opted for termination of pregnancy and genetic testing were offered participation in the study. Father–mother–fetus trio samples or fetal-proband-only samples were collected for sequencing. CNV sequencing (CNV-seq) was performed to identify aneuploidies and pCNVs, and WES was used to identify DGVs in cases without aneuploidy or pCNVs. A history of familial occurrence of cardiac defects was investigated by telephone follow-up and self-reports by the parents of the proband.

Fetal ultrasound and echocardiography examination

All ultrasound examinations were performed by experienced operators using a GE Voluson E8 ultrasound system (GE Healthcare, Milwaukee, WI, USA) with a transabdominal 2–4-MHz curvilinear transducer, or an Aloka SSD ultrasound system (Aloka, Tokyo, Japan) with a transabdominal 3–6-MHz curvilinear transducer. A complete fetal echocardiographic examination, including two-dimensional, M-mode and color and pulsed Doppler echocardiography, was performed according to the American Society of Echocardiography guidelines and standards for performance of the fetal echocardiogram to ascertain the presence of LSLs[13].

Copy-number variation sequencing

Both CNV-seq and WES were provided as part of a purely research-based protocol. CNV-seq was performed as described previously[14,15]. Briefly, RNA-free high-molecular-weight genomic DNA was isolated from the umbilical cord using the DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. The quality and concentration of genomic DNA in the samples were assessed using the Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Approximately 5 million sequencing reads per sample were mapped to the NCBI human reference genome (hg19/GRCh37) using the Burrows–Wheeler Aligner (BWA) tool and then allocated to 20-kb sequencing bins with 5-kb sliding to achieve a higher resolution for CNV detection. CNV-seq profiles of each chromosome were represented as log2 of the mean CNV of each sequencing bin along the length of the chromosome.

Detected CNVs were evaluated based on a scientific literature review and the following public databases: DECIPHER (https://decipher.sanger.ac.uk/), DGV (http://dgv.tcag.ca/), the 1000 Genomes Project (http://www.internationalgenome.org/), OMIM (http://omim.org/), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar), ClinGen (https://www.clinicalgenome.org/) and ISCA CNV (https://www.iscaconsortium.org). CNVs were classified into three categories according to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines for the interpretation of CNVs: benign, of uncertain clinical significance and

pathogenic[16]. In this study, we reported only pathogenic CNVs (pCNVs).

Whole-exome sequencing

Genomic DNA was extracted from the umbilical cord and parental blood using a Qiagen DNA Blood Midi/Mini kit (Qiagen GmbH). DNA libraries were prepared using an Agilent liquid capture system (Agilent SureSelect Human All Exon V6; Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's protocol. The size distribution and concentration of the libraries were determined using Agilent 2100 Bioanalyzer (Agilent Technologies) and quantified using real-time polymerase chain reaction. The DNA library was sequenced using Illumina HiSeq 4000 or Illumina NovaSeq (Illumina, Inc., San Diego, CA, USA) for paired-end 150-bp reads according to the manufacturer's protocol.

Raw image files were processed using Bcl To Fastq (Illumina Inc.) for base calling and generating raw data. Low-quality sequencing reads were filtered out using a quality score of ≥ 20. The reads were aligned to the NCBI human reference genome (hg19/GRCh37) using the BWA tool. BAM files were subjected to single-nucleotide polymorphism (SNP) analysis, duplication marking, indel realignment and recalibration using GATK and Picard tools.

After variant detection, ANNOVAR was used for annotation (http://wannovar.wglab.org/). We determined the frequency of each variant in the dbSNP147 (https://www.ncbi.nlm.nih.gov/snp/), 1000 Genomes Project, Exome Variant Server (http://evs.gs.washington.edu/EVS), Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org/), gnomAD (http://gnomad-old.broadinstitute.org/) and an in-house database to remove common SNPs (minor allele frequency $> 0.1\%$). Then, non-synonymous, splicing, frameshift and non-frameshift variants, as well as variants located in splice sites within 10 base pairs of an exon, were prioritized for evaluation. SIFT (http://sift.jcvi.org), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2), MutationTaster (http://www.mutationtaster.org) and CADD (http://cadd.gs.washington.edu) were used to predict the pathogenicity of missense variants, while Human Splicing Finder (http://www.umd.be/HSF.html) and MaxEntScan (http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html) were used to evaluate the effects on splicing. Furthermore, databases such as OMIM, ClinVar and Human Gene Mutation Database (http://www.hgmd.org) were used to determine variant harmfulness and pathogenicity where appropriate.

All reported variants were classified according to the ACMG recommendations[17,18]. The presence of secondary findings was not considered in the overall classification of a case. The overall case was classified into one of the following four categories: (1) positive result: when a DGV, including pathogenic and probably pathogenic variants, was identified in a known disease gene associated with the reported phenotype; (2) candidate gene: when a variant predicted to be deleterious was identified in a novel candidate gene that has not been previously implicated in CHD or for which the published data to support CHD association are not yet definitive. Supporting data could be based on model organism data, tolerance of the gene to sequence variation or data about tissue or developmental timing of expression. Further research is required to evaluate any of the suggested candidate genes; (3) uncertain result: when a variant of uncertain significance was identified in a known disease gene and the fetal phenotype was consistent with the reported disease spectrum (e.g., uncertainty is limited to the pathogenicity of the variant owing to a lack of parental samples to assess for *de-novo* occurrence and determine the phase of variants in recessive disorders); (4) negative result: when no variant was detected in the genes associated with the reported phenotype. Sanger sequencing confirmation was performed for all DGVs in cases with a positive result.

RESULTS

Between 10 April 2015 and 30 October 2018, 388 sequentially identified pregnant women with a fetus diagnosed with LSL were screened for eligibility for inclusion in our study. Of these, 90 (23.2%) chose to continue the pregnancy and eventually gave birth to a live neonate without undergoing genetic testing, and 218 (56.2%) chose to terminate their pregnancy and declined genetic testing. Thus, 80 (20.6%) pregnancies were eligible for inclusion in the study and underwent CNV-seq and WES sequentially. Figure 1 presents the progression and findings of genetic analysis in the study cohort.

Demographics, cardiac and extracardiac characteristics and sequencing information of the study cohort are summarized in Table 1 and detailed in Table S1. All parents, except one mother, were healthy and non-consanguineous according to self-reporting. The median maternal age was 29 (range, 21–40) years, and the fetuses were assessed at a median gestational age of 24 (range, 16–39) weeks. In total, 18 (22.5%) fetuses had extracardiac malformations detected on ultrasound and 62 (77.5%) had isolated CHD. HLHS was the most common LSL type ($n = 48$ (60.0%)), followed by CoA ($n = 22$ (27.5%)). There was a greater proportion of males than females in this cohort, which is consistent with the known epidemiology of LSLs[19].

Diagnostic yield of CNV-seq

Of the 80 pregnancies that underwent CNV-seq analysis, 14 (17.5%) had a chromosomal abnormality, comprising six (7.5%) cases with aneuploidy and eight (10.0%) with pCNVs (Figure 1, Tables 2 and 3). These 14 cases were prospectively excluded from WES analysis.

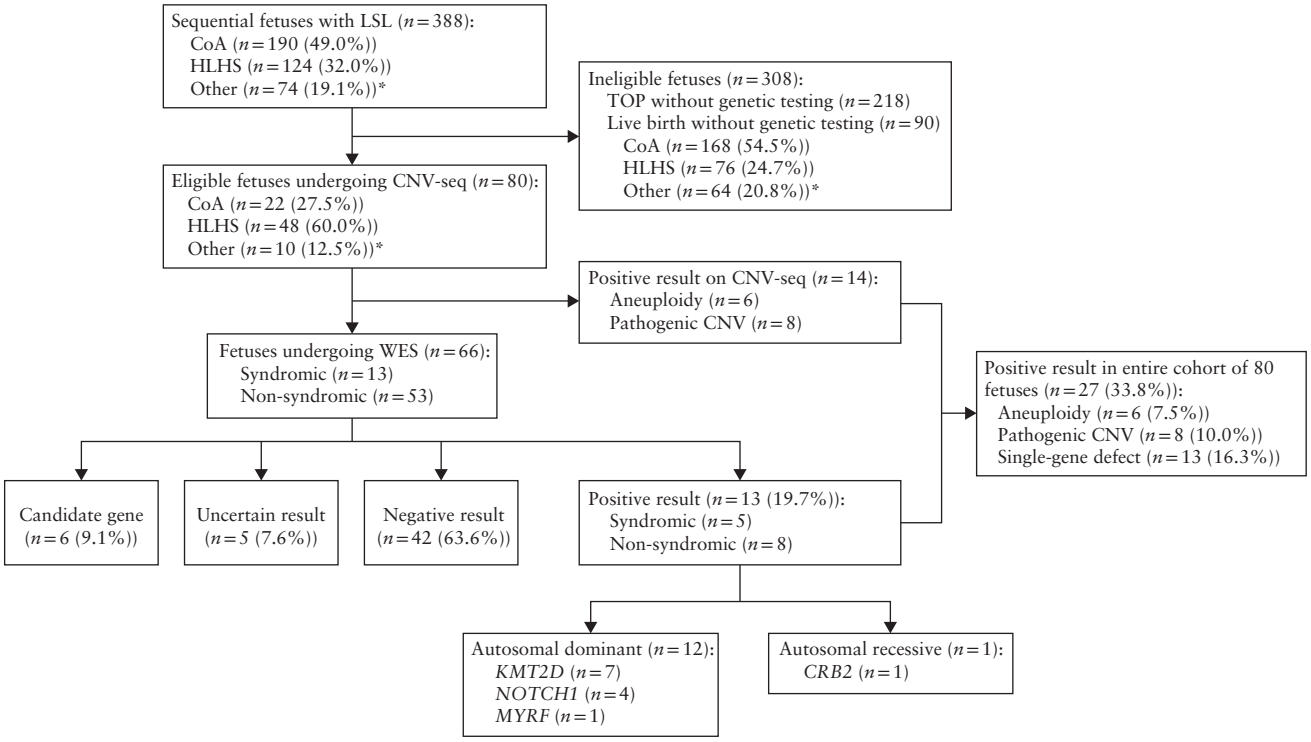

Figure 1 Flowchart showing inclusion in study of pregnancies with fetal congenital cardiac left-sided lesions (LSL) that opted for termination of pregnancy (TOP) and genetic testing, and findings of next-generation sequencing analysis. In this cohort, 'syndromic' and 'non-syndromic' refer to presence and absence, respectively, of fetal structural abnormalities detected on ultrasound. *Other includes: aortic valve atresia, aortic valve stenosis, mitral atresia and mitral stenosis. CNV, copy-number variant; CNV-seq, CNV sequencing; CoA, coarctation of the aorta; HLHS, hypoplastic left heart syndrome; WES, whole-exome sequencing.

Table 1 Demographic and clinical characteristics and sequencing information in 80 pregnant women diagnosed with fetal cardiac left-sided lesion (LSL)

Variable	*Value*
Maternal age (years)	29 (21–40)
GA at diagnosis (weeks)	24 (16–39)
LSL type	
HLHS	48 (60.0)
CoA	22 (27.5)
AS	6 (7.5)
AS and CoA	1 (1.3)
Aortic valve atresia	1 (1.3)
AS and MS	1 (1.3)
Mitral atresia	1 (1.3)
Extracardiac malformation	
Yes	18 (22.5)
No	62 (77.5)
Fetal gender	
Male/female ratio	1.6:1
Male	48 (60.0)
Female	30 (37.5)
45,X	2 (2.5)
Genetic sequencing	
CNV-seq	80 (100.0)
WES*	66 (82.5)
Father–mother–fetus trio	52/66 (78.8)
Fetal proband only	14/66 (21.2)

Data are given as median (range), *n* (%) or *n*/*N* (%). *Fourteen cases with aneuploidy or pathogenic copy-number variant were prospectively excluded from whole-exome sequencing (WES) analysis. AS, aortic valve stenosis; CNV-seq, copy-number variation sequencing; CoA, coarctation of the aorta; GA, gestational age; HLHS, hypoplastic left heart syndrome; MS, mitral stenosis.

Table 2 Aneuploidies identified by copy-number variation sequencing in study cohort

ID	*Aneuploidy*	*LSL type*	*Extracardiac malformation*
10	Trisomy 18	HLHS	Congenital diaphragmatic hernia
36	Trisomy 18	HLHS	None
44	Trisomy 18	HLHS	None
101	Trisomy 18	CoA	Polydactyly; overlapping finger; micrognathia; low-set ears; rocker-bottom foot; horseshoe kidney
12	Turner syndrome (45,X)	HLHS	None
56	Turner syndrome (45,X)	HLHS	Fetal cystic hygroma

CoA, coarctation of the aorta; HLHS, hypoplastic left heart syndrome; LSL, cardiac left-sided lesion.

Contribution of single-gene defects

Of the 66 pregnancies that underwent WES analysis, 13 (19.7%) had a positive result, indicating that the diagnostic yield of WES for the entire cohort was 16.3% (13/80) (Figure 1). The overall diagnostic yield of CNV-seq and WES was 33.8% for the entire cohort. Information on the genotype and phenotype of the 13 cases with a positive WES result are shown in Table 4. Of the remaining cases, variants in candidate genes were identified in six (9.1%), five (7.6%) were classified as uncertain result and 42 (63.6%) had a negative result (Table S2). In

Table 3 Pathogenic copy-number variants identified by copy-number variation sequencing in study cohort

ID	*LSL type*	*Extracardiac malformation*	*Copy-number variant*	*Size (Mb)*	*Associated syndrome*
21	HLHS	None	seq[hg19]dup(7)(p22.3) chr7:g.10000_2464000dup	2.454	None
			seq[hg19]del(11)(q24.2q25) chr11:g.126778000_134946000del	8.168	Jacobsen syndrome
55	HLHS	None	seq[hg19]del(11)(q24.2q24.3) chr11:g.123400000_134945000del	11.545	Jacobsen syndrome
95	AS + CoA	None	seq[hg19]del(11)(q23.3q25) chr11:g.121197000_134946000del	13.749	Jacobsen syndrome
			seq[hg19]dup(19)(q13.43) chr19:g.56341000_59106000dup	2.765	None
22	CoA	None	seq[hg19]del(1)(p36.33p36.32) chr1:g.572000_3371000del	2.799	1p36 deletion syndrome
29	HLHS	Unilateral polycystic kidney dysplasia; congenital diaphragmatic hernia	seq[hg19]del(15)(q26.2q26.3) chr15:g.96205000_102399000del	6.194	None
32	CoA	Intrauterine growth restriction	seq[hg19]del(4)(p16.3p16.24) chr4:g.65000_18004000del	17.930	Wolf–Hirschhorn syndrome
48	HLHS	None	seq[hg19]dup(12)(q13.3q14.1) chr12:g.52895000_58205000dup	5.310	None
			seq[hg19]dup(12)(q24.33) chr12:g.132185000_133815000dup	1.630	None
49	CoA	None	seq[hg19]del(7)(q11.23) chr7:g.72727000_74340000del	1.613	Williams–Beuren syndrome

AS, aortic valve stenosis; CoA, coarctation of the aorta; del, deletion; dup, duplication; HLHS, hypoplastic left heart syndrome; LSL, cardiac left-sided lesion; Mb, megabases.

the cohort of pregnancies that underwent WES analysis, one (1.5%) case with CoA had an ACMG secondary finding in *PKP2*[18], which is associated with arrhythmogenic right ventricular dysplasia 9 (OMIM: 609040).

KMT2D mutations

Unexpectedly, we identified seven cases with DGVs in *KMT2D* (six *de-novo* loss-of-function (LOF) variants and one in-frame deletion variant of unknown origin) (Table 4), accounting for 53.8% (7/13) of the positive results, 8.8% (7/80) of the entire cohort and 10.6% (7/66) of cases without aneuploidy or pCNVs. More than half ($n = 4$) of the seven cases with DGVs in *KMT2D* had HLHS. Two of the other three cases had ultrasound-detected extracardiac malformations.

We also identified four cases with DGVs in *NOTCH1*, comprising two *de-novo* variants, one of unknown origin and one inherited from the asymptomatic mother (Table 4). We did not perform echocardiography in the mother who transmitted the *NOTCH1* mutation to the affected fetus (Case 53), leaving open the possibility of incomplete penetrance or variable expressivity of the phenotype, such as bicuspid aortic valve (BAV) or other asymptomatic cardiac anomaly. All four cases with DGVs in *NOTCH1* had isolated CHD, without obvious extracardiac pathology on ultrasound.

Additionally, we identified two cases with DGVs in two genes (*MYRF* and *CRB2*) that are not yet well established as human CHD genes.

DGVs were identified more than twice as frequently in fetuses with extracardiac malformations (5/13 (38.5%)) than in those without extracardiac malformations (8/53 (15.1%)); however, the difference was not statistically significant (correct chi-square test, $P = 0.13$). When considering the known inheritance patterns of DGVs in the 13 cases with a positive result, 12 (92.3%) were dominant (with variants in *KMT2D*, *NOTCH1* and *MYRF*) and one (7.7%) was recessive (with variants in *CRB2*).

DISCUSSION

This is the first study to assess the contribution of SGDs to LSLs in a fetal population. Our findings show that WES analysis can yield incremental DGVs in 19.7% of cases with LSL without aneuploidy or pCNVs. *KMT2D* mutations accounted for a substantial fraction (10.6%) of LSLs in this cohort. Despite focusing primarily on SGDs, we also found 7.5% cases with aneuploidy and 10.0% cases with pCNVs in the entire cohort. Our findings suggest that, first, SGDs are probably as important to the pathogenesis of LSLs as are chromosomal abnormalities, and, second, prenatal WES should be performed to identify potential DGVs and facilitate perinatal decision-making and management in cases in which conventional tests (karyotyping and microarray) are not diagnostic.

Although the concept of using WES in fetuses with structural anomalies (including CHD) is not novel, the strength of this study is that we selected only fetuses with a specific CHD, namely LSL, rather than including cases with any structural anomaly[10,11]. Our approach, while narrow in its focus, led to a greater diagnostic yield with

Table 4 Clinical phenotype and pathogenic/probably pathogenic variants identified in 13 cases with a positive result on whole-exome sequencing

ID	*LSL type*	*Extracardiac malformation*	*Gene*	*Variant*	*Zygosity*	*Disease, inheritance model*	*Origin*	*Reference (PMID)*	*Classification*
74	CoA	Multiple*	*KMT2D*	NM_003482.3 c.8430dupA p.Gln2811Thrfs*34	Het	Kabuki syndrome 1, AD	*De novo*	Novel	Pathogenic
83	HLHS	Abnormal lung lobation; anal atresia	*KMT2D*	NM_003482.3 c.15920_15921 +2delCAGT p.Leu5308Alafs*36	Het	Kabuki syndrome 1, AD	*De novo*	Novel	Pathogenic
51	AA	None	*KMT2D*	NM_003482.3 c.8074_8075delCG p.Arg2692Alafs*31	Het	Kabuki syndrome 1, AD	*De novo*	Novel	Pathogenic
28	HLHS	None	*KMT2D*	NM_003482.3 c.1845_1846del p.Leu617Phefs*5	Het	Kabuki syndrome 1, AD	*De novo*	Novel	Pathogenic
33	MA	Situs inversus; cleft palate	*KMT2D*	NM_003482.3 c.6595delT p.Tyr2199Ilefs*65	Het	Kabuki syndrome 1, AD	*De novo*	27302555, 28256057, 25972376	Pathogenic
84	HLHS	None	*KMT2D*	NM_003482.3 c.8159G>A p.Trp2720*	Het	Kabuki syndrome 1, AD	*De novo*	Novel	Pathogenic
85	HLHS	None	*KMT2D*	NM_003482.3 c.16489_16491del p.Ile5497del	Het	Kabuki syndrome 1, AD	Unknown	27302555	Probably pathogenic
31	CoA	None	*NOTCH1*	NM_017617.5 c.3643+1G>A	Het	Aortic valve disease 1, AD; Adams–Oliver syndrome 5, AD	*De novo*	Novel	Pathogenic
53	HLHS	None	*NOTCH1*	NM_017617.5 c.4015-2A>G	Het	Aortic valve disease 1, AD; Adams–Oliver syndrome 5, AD	Maternal	Novel	Probably pathogenic
23	AS	None	*NOTCH1*	NM_017617.5 c.4837C>T p.Gln1613*	Het	Aortic valve disease 1, AD; Adams–Oliver syndrome 5, AD	Unknown	Novel	Probably pathogenic
86	HLHS	None	*NOTCH1*	NM_017617.5 c.2452dupC p.Leu818Profs*10	Het	Aortic valve disease 1, AD; Adams–Oliver syndrome 5, AD	*De novo*	Novel	Pathogenic
43	CoA	Pulmonary sequestration	*MYRF*	NM_001127392.2 c.789delC p.Ser264Alafs*8	Het	Cardiac–urogenital syndrome, AD	*De novo*	Novel	Pathogenic
38	HLHS	Pulmonary hypoplasia	*CRB2*	(1) NM_173689.6 c.2029C>T p.Arg677Cys	C-Het	FSGS9, AR; VMCKD, AR	Paternal	Novel	Probably pathogenic
				(2) NM_173689.6 c.3076_3077insTGGCG CGGCCCCGGCCCG GCGCGGCCCC p.Arg1038Alafs*45			Maternal	Novel	Probably pathogenic

*Unilateral microtia, unilateral polycystic kidney dysplasia, unilateral renal agenesis, absent gallbladder, cleft palate, unilateral atresia of the external auditory canal, lung segmentation defects. AA, aortic valve atresia; AD, autosomal dominant; AR, autosomal recessive; AS, aortic valve stenosis; C-Het, compound heterozygous; CoA, coarctation of the aorta; FSGS9, focal segmental glomerulosclerosis 9; Het, heterozygous; HLHS, hypoplastic left heart syndrome; LSL, cardiac left-sided lesion; MA, mitral atresia; VMCKD, ventriculomegaly with cystic kidney disease.

a higher prevalence of DGVs, compared with the 5% reported previously[10].

KMT2D is the causative gene of Kabuki syndrome 1 (OMIM:147920), which is an autosomal dominant disorder characterized by developmental delay and/or intellectual disability, infantile hypotonia and typical facial features. Other findings may include CHD, growth defect with feeding difficulties, anorectal anomalies, skeletal anomalies, renal malformations and persistence of fetal fingertip pads.

We found that *KMT2D* was the most frequently mutated gene in fetuses with LSL without aneuploidy or pCNVs, with a mutation rate of 10.6% in this cohort. However, in the largest postnatal genetic study of CHD to date, 10 cases with LOF variants in *KMT2D* were identified, accounting for only 1.3% of all LSLs[5]. This difference

may be due to bias in our study owing to its small sample size and the inclusion of only pregnancies that were terminated, which could represent a more severe end of the LSL spectrum in comparison with LSL in patients encountered postnatally. Patients encountered prenatally may be more likely to have extracardiac pathology that could affect their survival, leading to under-representation in postnatal series. In contrast, previous postnatal series have included BAV, which would not have been included in a fetal series owing to the inability to resolve such a subtle pathology prenatally in the vast majority of patients[5].

We also found that HLHS was the most prevalent type of LSL in fetuses with *KMT2D* mutation, accounting for four (66.7%) of the six cases with *KMT2D* LOF variants. Consistent with our results, HLHS accounted for 80% (8/10) of LSL cases with *KMT2D* LOF variants in the largest genetic study of CHD[5]. By contrast, a previous postnatal study found that CoA was the most prevalent LSL in cases with Kabuki syndrome, and HLHS represented only 10.5% of LSLs[20]. This difference could be related again to the more severe spectrum of LSLs encountered prenatally and the fact that the study of Digilio *et al.*[20] focused only on clinically diagnosed Kabuki syndrome, which potentially resulted in the omission of genetically abnormal cases with more subtle clinical features.

A benefit of performing WES, particularly trio-WES, is emphasized in the identification of candidate genes or genes of unknown significance that have as yet not been associated with CHD. This was illustrated by Case 43 in our cohort, with an ultrasound diagnosis of CoA and pulmonary sequestration, in which *MYRF* was considered a candidate gene with a *de-novo* LOF variant. Recent studies have implicated *MYRF* in LSLs and congenital malformations of the lung[21,22]. The variant was subsequently classified as pathogenic on reassessment by the multidisciplinary team.

On the other hand, genes and variants of unknown significance can also be problematic for prenatal counseling, especially if the fetal phenotype is non-specific, as they can lead to uncertainty and dilemmas with regard to clinical management and cause significant parental anxiety and make decision-making challenging. Given that the application of WES for investigating fetal structural anomalies will inevitably increase, there is an urgent unmet need for a formal curation procedure and for shared, publicly available, large-scale, carefully curated clinical and genomic datasets for prenatal diagnostics[3,10,23,24].

This study has some limitations that should be acknowledged. The sample size was relatively small, and only a very small number of cases underwent autopsy (data not shown). We did not undertake systematic cardiovascular assessment of parents recruited into the study, and therefore we were unable to distinguish between parents without CHDs and those with asymptomatic CHDs. In addition, subtle dysmorphic fetal features cannot be determined using fetal ultrasound, thus some phenotypes, in particular neurodevelopmental disorders, are impossible to determine in the prenatal setting. Furthermore, we did not enroll all pregnant women with fetal LSLs but only those who opted for termination, which introduced a selection bias, potentially towards more serious disease. Finally, 14 fetal samples were analyzed as singletons, so information regarding the presence or absence of identified variants in the parents is lacking, leading to a high rate of uncertain results in these cases.

In conclusion, our study shows that SGDs contribute substantially to the genetic cause of LSLs in the prenatal setting. We found that *KMT2D* mutations accounted for as many as 10.6% of LSLs, with HLHS being the most prevalent cardiac phenotype in these fetuses. The role of *KMT2D* mutations in fetal LSLs should be evaluated more comprehensively in larger robust studies.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Table S1 Characteristics and phenotype of 80 fetuses diagnosed with cardiac left-sided lesion (LSL)

Table S2 Variants identified by whole-exome sequencing in cases with candidate genes and uncertain results in study cohort

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Contribución de los defectos unigénicos a las lesiones cardíacas congénitas del lado izquierdo en el ámbito prenatal

RESUMEN

Objetivos Estudiar la contribución de los defectos unigénicos a la causa genética de las lesiones cardíacas del lado izquierdo (LCLI) y evaluar el desempeño del diagnóstico incremental de la secuenciación hologenómica (SHG) para los defectos unigénicos en los fetos con LCLI sin aneuploidía o sin variación patógena en el número de copias (pCNV, por sus siglas en inglés).

Métodos Entre el 10 de abril de 2015 y el 30 de octubre de 2018 se reclutaron 80 mujeres embarazadas diagnosticadas con LCLI, las cuales se sometieron a una interrupción del embarazo y a pruebas genéticas. Las LCLI elegibles eran la atresia o estenosis de la válvula aórtica, la coartación de la aorta, la atresia o estenosis mitral y el síndrome del hemicardio izquierdo hipoplásico (SHIH). La secuenciación CNV (CNV-seq) y la SHG se realizaron de forma secuencial en muestras de estos fetos y de sus padres. La CNV-seq se utilizó para identificar las aneuploidías y las pCNV, mientras que la SHG se utilizó para identificar las variantes genéticas de diagnóstico en los casos sin aneuploidías o pCNV.

Resultados De 80 embarazos incluidos en el estudio, 27 (33,8%) tuvieron un diagnóstico genético. El análisis de la CNV-seq identificó seis (7,5%) fetos con aneuploidía y ocho (10,0%) con pCNV. El análisis de la SHG de los 66 casos restantes manifestó variantes genéticas de diagnóstico en 13 (19,7%) casos, lo que indica que el comportamiento del diagnóstico del SHG para toda la cohorte fue del 16,3% (13/80). El KMT2D fue el gen que mutó más frecuentemente (7/66 (10,6%)) en los fetos con LCLI sin aneuploidía o pCNV, seguido de NOTCH1 (4/66 (6,1%)). El SHIH fue el fenotipo cardíaco más prevalente (4/7) en los casos con mutación de KMT2D en esta cohorte. En seis casos (9,1%) adicionales se encontraron variantes potencialmente perjudiciales en los genes con riesgo.

Conclusiones Los defectos unigénicos contribuyen sustancialmente a la etiología genética de las LCLI fetales. Las mutaciones de KMT2D representaron aproximadamente el 10% de las LCLI en esta cohorte fetal. La SHG tiene el potencial de proporcionar diagnósticos genéticos en fetos con LCLI sin aneuploidía o sin pCNV.

产前单基因缺陷对先天性心脏左侧病变的促成

摘要

目标 探究单基因缺陷对先天性心脏左侧病变（LSL）遗传病因的促成，并评估全外显子组测序（WES）对LSL胎儿（无异倍体或致病性拷贝数变异）单基因缺陷的递增诊断率。

方法 在2015年4月10日至2018年10月30日之间，我们招募了80名被诊断患有LSL的孕妇，她们都已终止妊娠并进行了基因检测。符合本研究条件的LSL包括主动脉瓣闭锁或狭窄、主动脉缩窄、二尖瓣闭锁或狭窄和左心发育不全综合征（HLHS）。对来自胎儿及其父母的样本依次进行了CNV测序（CNV-seq）和WES。CNV-seq用于识别异倍体和致病性拷贝数变异（pCNVs），而WES则用于在无异倍体或pCNVs的病例中识别诊断性遗传变异体。

结果 在本研究的80例受试怀孕中，有27例（33.8%）得到了基因诊断。CNV-seq分析识别出六个（7.5%）有异倍体的胎儿和八个（10.0%）有pCNVs的胎儿。对剩下的66例采用WES分析，揭示出13个（19.7%）病例中有基因变异，表明WES对所有受试者的诊断率为16.3%（13/80）。KMT2D是LSL胎儿中（无异倍体或pCNVs）最频繁的突变基因（7/66（10.6%）），其次是NOTCH1（4/66（6.1%））。在所有受试者中有KMT2D突变的病例中，HLHS是最常见的心脏表型（4/7）。我们在另外六例（9.1%）中发现了含潜在有害变异的候选基因。

结论 单基因缺陷极大地促成了胎儿LSL的遗传病因。在我们的所有受试胎儿中，KMT2D变异约占LSL的10%。WES具有为LSL胎儿（无异倍体或pCNVs）提供基因诊断的潜力。