RESEARCH ARTICLE

Detection of copy number variants using chromosomal microarray analysis for the prenatal diagnosis of congenital heart defects with normal karyotype

Tingting Song | Shanning Wan | Yu Li | Ying Xu | Yinghui Dang | Yunyun Zheng |
Chunyan Li | Jiao Zheng | Biliang Chen | Jianfang Zhang

Department of Obstetrics and Gynecology, the first affiliated hospital of Air Force Medical University, Xi'an, China

Correspondence: Jianfang Zhang, MD, and Biliang Chen, MS, Department of Obstetrics and Gynecology, the first affiliated hospital of Air Force Medical University, Xi'an 710032, China (zhangjf@fmmu.edu.cn; cblxjh@fmmu.edu.cn).

Background: With the increasing availability of chromosomal microarray analysis (CMA) for congenital heart defect (CHD), genetic testing now faces new challenges due to results with uncertain clinical impact. Studies are needed to better define the penetrance of genetic variants. The aim of the study was to examine the association between CMA and CHDs in fetuses with normal karyotype.

Methods: This was a retrospective study of 190 fetuses with normal karyotype that underwent CMA after a diagnosis of CHD by fetal ultrasound. Invasive prenatal diagnosis was performed between January 2015 and December 2016 at the first affiliated hospital of Air Force Medical University.

Results: Chromosomal microarray analysis detected pathogenic copy number variants (pCNVs) in 13/190 (6.84%) fetuses, likely pCNVs in 5/190 (2.63%), and variants of unknown significance (VOUS) in 14/190 (7.37%). Among those with pCNVs, none (0%) yielded a normal live birth. Among those with likely pCNVs, 2/5 (40.0%) yielded a live birth. Among the fetuses with VOUS, 10/14 (71.5%) yielded a live birth.

Conclusion: These results highlight the usefulness of CMA for prenatal genetic diagnosis of fetuses with CHDs and normal karyotype. In fetuses with CHD, the application of CMA could increase the detection rate of pCNVs causing CHDs. In this study, some VOUS were likely pathogenic, but additional studies are necessary to confirm these findings.

KEYWORDS

chromosomal microarray analysis, congenital heart defects, prenatal diagnosis, variants of unknown significance

1 | INTRODUCTION

Chromosomal microarray analysis (CMA) has been found to be useful to identify prenatal clinically relevant copy number variations (CNVs).¹ Compared with karyotype, CMA can achieve a better resolution for chromosomal imbalances and submicroscopic genomic alterations in the context of whole genome screening, both prenatal and postnatal.²⁻⁴ Therefore, it will soon be considered standard to

use CMA for the evaluation of pregnancies with anomalies.¹ This method should provide better decision guidance for the parents and physicians, but the complex ethics have to be weighed carefully.^{5,6}

Indeed, variants of unknown significance (VOUS) pose a challenge for proper prenatal genetic counseling⁷ because of their unknown penetrance and clinical impact. In addition, VOUS may be present in a substantial proportion of tested patients, for example, as high as in 15% in studies of hypertrophic cardiomyopathy,^{8,9} but

this proportion may vary depending on the number and nature of the genes being tested. Most importantly, despite the fact that their biological impact is unknown, their psychological impact on the parents and their family is undeniable as VOUS lead to stress in the face of uncertainty and because of the decision of continuing pregnancy or not.¹⁰ It has been shown that exploring uncertainty during the communication process is important for the patient and family experience in the presence of a VOUS.¹¹ Communication among the family members and the physicians is paramount.¹⁰ Furthermore, a VOUS may be in fact pathogenic, but has been classified as VOUS simply because of insufficient data to determine its real pathogenic potential. Therefore, a better understanding of the VOUS is necessary in order to provide adequate prenatal genetic counseling. Nevertheless, uncertainty will be an inherent part of genetic counseling for some time to come.¹²

Congenital heart defects (CHDs) represent the most frequent type of fetal anomalies in China, with a prevalence of eight per 1000 live births.¹³ CHDs are often found on ultrasound during prenatal diagnosis, and etiology is unknown in most cases,¹⁴ but recent findings highlighted molecular pathways in heart development. Chromosomal aberrations and single-gene defects are considered to be the main potential etiology of CHDs.^{15,16} The exact genetic causes of CHDs remain largely unknown, but CHDs have been associated with chromosomal abnormalities, chromosome 22q11.2 deletion,¹⁷ and rare CNVs.^{1,18} Studies showed that there are pathogenic CNVs associated with the tetralogy of Fallot, thoracic aortic aneurysms, and congenital left-sided heart disease, among others.^{18,19} Nevertheless, studies are still necessary to improve the definition of the role of CMA in CHD diagnosis due to the gap between studies focusing on prenatal and postnatal phenotypes. In addition, because of the increasing availability of CMA and of its sensitivity to any genetic change,^{20,21} VOUS found by CMA represent a growing problem.

Therefore, the aim of this study was to use CMA to analyze 190 fetuses with CHDs and normal karyotype, to follow the pregnancy, to examine the CHD status of the born infants, and to evaluate the potential value of CMA for the prenatal diagnosis of CHD. This study could also allow the estimation of the pathogenic potential of some VOUS, therefore improving genetic counseling.

2 | MATERIALS AND METHODS

2.1 | Case selection

A total of 207 fetuses diagnosed with CHD by fetal ultrasound and that underwent CMA were retrospectively reviewed. Invasive prenatal diagnosis was performed between January 2015 and December 2016 at the first affiliated hospital of Air Force Medical University (Shanxi Province, Northwest China). All parents had received prenatal counseling from a clinical geneticist regarding the risks related to invasive prenatal diagnosis, the benefits and limitations of CMA, and the risks of VOUS and

incidental findings. The inclusion criteria were as follows: (a) diagnosis of CHD by ultrasound, with or without other anomalies; (b) normal karyotype; and (c) CMA was performed. A total of 17 CHD cases with abnormal karyotype were excluded from the present analysis.

The institutional review board approved this study. Written informed consent was routinely obtained from the parents. The gestational age was calculated according to the early pregnancy ultrasound results and last menstrual period. The pregnancy outcomes were obtained by telephone follow-up.

2.2 | Karyotype analysis

Amniotic fluid (30 mL) or fetal blood (1 mL) was sampled at 18-32 gestational weeks by amniocentesis or cordocentesis after obtaining informed consent from the parents. Standard cytogenetic protocols were applied to the samples.²² The Giemsa-banding technique (450-550-band resolution) was used to analyze the cultured amniocytes or lymphocytes.

2.3 | DNA preparation

A QIAamp DNA Blood Mini Kit (Qiagen, Venlo, the Netherlands) was used to extract genomic DNA from amniotic fluid (10 mL) or umbilical cord blood (2 mL). The concentration and quality of genomic DNA were analyzed using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA).

2.4 | CMA

An Affymetrix Cytoscan 750k array (Affymetrix, Santa Clara, CA, USA) was used according to the manufacturer's protocol. The Cytoscan 750k array includes >750 000 markers spanning the entire human genome, including probes for single nucleotide polymorphisms (SNPs; $n = 200\,000$) and probes with a mean resolution of 100 kb for copy number variations (CNVs; $n = 550\,000$). The threshold of the CNV results was 100 kb (marker count ≥ 50). The DNA (250 ng) was amplified, labeled, and hybridized using the CytoScan 750k array platform (Affymetrix, Santa Clara, CA, USA), according to the manufacturer's protocol. After hybridization, a laser scanner was used to scan the arrays. CEL files were obtained. The Chromosome Analysis Suite v3.1 software (r8004) was used to analyze the CEL files, based on data from the genome version GRCh37 (hg19). Public databases such as DGV (<http://www.ncbi.nlm.nih.gov/dbvar/>), OMIM (<http://www.ncbi.nlm.nih.gov/omim/>), DECIPHER (<http://decipher.sanger.ac.uk/>), ISCA (<https://www.iscaconsortium.org/>), UCSC (<http://genome.ucsc.edu/>), and PubMed (<http://www.ncbi.nlm.nih.gov/pubmed/>) were used for the interpretation of the results and to analyze genotype-phenotype correlations. According to the American College of Medical Genetics (ACMG) guidelines,²³ the CNVs were classified as benign, likely benign, VOUS, likely pathogenic, or pathogenic. The parents were tested in the presence of a pathogenic CNV in the fetus. Only some patients were tested in the

FIGURE 1 Karyotyping and chromosomal microarray analysis results of congenital heart defect cases detected by ultrasound

TABLE 1 Types of CHDs and frequencies of fetuses with CNVs

CHD classification	Number of fetuses, n	Total number of fetus with CNVs, n	Number of fetuses with pathogenic CNVs, n	Number of fetuses with likely pathogenic CNV, n	Number of fetuses with VOUS, n
VSD	82	13	6	2	5
VSD, aortic abnormality	8	4	3		1
VSD, pulmonary artery abnormality	5	1	1		
VSD, DORV	1	1	1		
VSD, VR	1	1			1
VSD, PTA, PA	1	1			1
TOF	10	1	1		
SV	5	2	1	1	
VR	21	2		1	1
Aortic abnormality (AS, IAA)	5	2			2
Other CHD with CNVs observed ^a	30	4		1	3
Other CHD with no CNVs observed ^b	21	0		0	0

AS, aortic stenosis; CHD, congenital heart defects; CNV, copy number variation; DORV, double outlet right ventricle; IAA, interrupted aortic arch; PA, pulmonary atresia; PTA, persistent truncus arteriosus; SV, single ventricle; TOF, tetralogy of fallot; VR, vascular ring; VSD, ventricular septal defect.

^aIncluded mitral/tricuspid regurgitation, disproportionally large right ventricle and atrium, small inner aortic diameter, etc.

^bIncluded ventricle dysplasia, atrial septal defect, pulmonary artery abnormalities (PA, pulmonary stenosis, absent pulmonary valve), situs inversus viscerum, etc.

presence of a VOUS CNV in the fetus. Benign and likely benign CNVs were not considered for this study.

2.5 | FISH study of chromosome 22q11.2

Chromosome 22q11.2 deletion was analyzed by FISH on interphase nuclei using a commercial probe, according to standard protocols.²⁴

3 | RESULTS

3.1 | Characteristics of the cases

During the study period, 207 pregnancies with CHD detected ultrasonographically underwent both karyotype and CMA analysis. A total of 17 chromosomal aberrations were identified by karyotype

TABLE 2 Characteristics of congenital heart defect cases with pathogenic CNVs and normal karyotype

Case	Cardiac defect	Extra cardiac defect	Microarray Results	Size	Inheritance	Critical genes/region	Known syndrome/disease	Pregnancy outcome
1	VSD		arr[GRCh37] Xp22.31(6,455,151_8,135,568)x1	1.68 Mb	Maternal	HDHD1, STS		Born, dead
2	VSD		arr[GRCh37] 22q12.3(33,854,040_35,669,208)x1	1.82 Mb	De novo	LARGE	Walker-Warburg	TOP
3	SV, TA		arr[GRCh37] 11q24 .2q25(124,589,562_134,937,416)x1	10.3 Mb	De novo	JAM3, ETS1		TOP
4	VSD, PLSVC		arr[GRCh37] 17p13.3p13.2(204,999_3,784,378)x3	3.58 Mb	De novo	YWHAE, PFAFH1B1	17p13.3 duplication syndrome	TOP
5	APV, TR		arr[GRCh37] 3q29(195,678,474_197,353,975)x1	1.68 Mb	De novo		3q29 microdeletion syndrome	TOP
6	VSD, Small inner aortic diameter, disproportionately large RA & RV		arr[GRCh37] 1p36.33(849,466_2,121,139)x1 arr[GRCh37] Xp22.31(6,440,776_8,132,800)x3	1.27 Mb, 1.69 Mb	De novo		1p36 microdeletion syndrome	TOP
7	VSD		arr[GRCh37] 16p13.3(85,880_1,129,062)x1	1.04 Mb	De novo	HBA2, HBA1, SOX8	α -thalassaemia mental retardation	TOP
8	Tof		arr[GRCh37] 22q11.21(18,916,842_21,800,471)x1	2.89 Mb	De novo	TBX1	DiGeorge syndrome	TOP
9	VSD, PA, RAA		arr[hg19] Xq28(152,970,883-154,896,094)x2 arr[hg19] 6p25.3p24.3(381,117-7,790,535)x1	3.16 Mb	De novo	TBX1	DiGeorge syndrome	TOP
10	VSD		arr[GRCh37] 22q11.21(18,648,855_21,459,713)x1	2.81 Mb	De novo	TBX1	DiGeorge syndrome	TOP
11	VSD, AS, AAH		arr[GRCh37] 22q11.21(18,631,364_21,800,471)x1	3.17 Mb	De novo	TBX1	DiGeorge syndrome	TOP
12	VSD, CoAA		arr[GRCh37] 22q11.21(18,648,855_21,800,471)x1	3.2 Mb	De novo	TBX1	DiGeorge syndrome	TOP
13	Tof	cheilopalatognathus, posterior cranial fossa with cystic echo	arr[hg19] Xq28(152,970,883-154,896,094)x2 arr[hg19] 6p25.3p24.3(381,117-7,790,535)x1	1.93 Mb, 7.41 Mb	De novo	MECP2, DUSP22, FOXC1	chromosome 6pter-p24 deletion syndrome	TOP

Note. AS: aortic stenosis; APV: absent pulmonary valve; CoAA: coarctation of the aortic arch; PA: pulmonary atresia; PLSVC: persistent left superior vena cava; RAA: right-sided aortic arch; SV: single ventricle; TA: truncus arteriosus; Tof: tetralogy of fallot; TOP: termination of pregnancy; TR: tricuspid regurgitation; VSD: ventricular septal defect.

TABLE 3 Characteristics of congenital heart defect cases with likely pathogenic CNVs and normal karyotype

Cases	Cardiac defect	Microarray results	Size	Inheritance	Critical genes/ region	Pregnancy outcome
14	VSD, AS	arr[hg19] 15q11.2(22,770,421-23,277,436)x1	0.5 Mb	De novo	TUBGCP5, CYFIP1, NIPA2, NIPA1	Born, normal
15	VSD, DORV, PS	arr[hg19] 13q33.1q34(103,971,638-115,107,733)x2-3 arr[hg19] 4q32.3q35.2(166,546,468-190,957,460)x2-3	11.1 Mb, 24.4 Mb	De novo	Hand2	TOP
16	SV	arr[hg19] Xp22.31(6,455,151-8,132,677)x4	1.68 Mb	De novo	HDHD1, STS	TOP
17	VSD	arr[hg19] 8q21.3q22.1(89,634,344-93,391,982)x1	3.76 Mb	De novo	NBN, DECR1	Born, normal
18	RAA, VR, double arterial canal	arr[hg19] 15q11.2(22,770,421-23,625,785)x1	0.86 Mb	Maternal	TUBGCP5, CYFIP1, NIPA2, NIPA1	Born, normal

analysis, all double validated by CMA analysis. Among the 190 cases with normal karyotype, pathogenic CNVs (pCNVs) were detected in 6.8% (13/190) of the fetuses, likely pCNVs in 2.6% (5/190), and VOUS in 7.4% (14/190) (Figure 1). The mothers were 27 years of age, on average (range, 19-39 years). The mean gestational age was 28 ± 3 weeks (range of 18-36 weeks) at invasive prenatal diagnostic testing. CMA detected 32 (16.8%, 32/190) chromosomal aberrations among the 190 fetuses with ultrasonographically detected CHD and a normal karyotype (Table 1). Ultrasound features were highly variable among the fetuses, but were all determined as being abnormal.

3.2 | Detection rates of CNVs with normal karyotype by CMA

Chromosomal microarray analysis detected pCNVs in 13 (6.8%, 13/190) fetuses (Table 2), including five typical cases of DiGeorge syndrome, five cases of deletion, one case of duplication, and two cases of deletion plus duplication. The size of the deletion or duplication segment was between 1.68 and 10.3 Mb (Table 2). Five (5/190, 3.7%) fetuses were considered to be likely pCNVs, including 15q11.2 deletion in fetus 14, 15q11.2 deletion in fetus 18, Xp22.31 triplication in fetus 16, 8q21.3q22.1 deletion in fetus 17, and mosaic duplication of chromosome 13q33.1q34 and 4q32.3q35.2 in fetus 15 (Table 3). The size of the deletion or duplication segment was between 0.5 and 11.1 Mb. Fourteen (14/190, 7.40%) fetuses were considered to be with VOUS (Table 4). The size of the deletion or duplication segment was between 0.4 and 1.3 Mb. Among the fetuses with likely pCNVs, none (0%) yielded a normal live birth. Among the fetuses with pCNVs, two (40.0%) yielded a live birth. Among the fetuses with VOUS, 10 (71.5%) yielded a live birth, with the remaining four (28.5%) being terminated. No apparent abnormality was observed for all born-alive fetuses at 3 months.

4 | DISCUSSION

The ethics involved in prenatal genetic counseling and diagnosis are complex.^{5,6} Indeed, VOUS pose a challenge for proper prenatal genetic counseling.⁷ Therefore, a better understanding of the clinically significant genetic variants is necessary. Most fetal cases of CHD will be identified by conventional karyotyping, but this method is limited and most of the findings are aneuploidies and triploidies. The increasing use of CMA revealed a large number of CNVs in a number of disorders.²⁵ Recently, studies underlined the important role of CNVs in many developmental conditions such as CHD.²⁶⁻³⁰ CMA allows the detection of aneuploidies and large structural rearrangements (ie, microscopic alterations), as well as microdeletions, uniparental disomy (UPD), and duplications (ie, submicroscopic alterations). Using an array-based comparative genomic hybridization (aCGH) approach, Yan et al³¹ reported that the rate of pathogenic CNVs in 76 fetuses with CHD (no karyotype abnormalities and without 22q11.2 abnormalities) was 6.6%, and the rate of VOUS was 5.3%. The rate of VOUS was almost equal to that of pathogenic CNVs, posing a problem for adequate genetic counseling. Similarly, in the present cohort study, CMA detected pathogenic CNVs in 6.8% (13/190) of the cases; among them, there was one case of each of the following variants: 15q11.2 deletion, Xp22.31 triplication, 8q21.3q22.1 deletion, and mosaic duplication of chromosome 13q33.1q34 and 4q32.3q35.2 (each 1/190, 0.5%). The present study and the study by Yan et al³¹ suggest that different genomic approaches can yield similar results. Nevertheless, additional studies are necessary to determine whether these approaches could be complementary and could detect more cases. In addition, besides identifying additional VOUS, the use of different techniques does not provide additional data on the VOUS and association studies are necessary to determine their exact pathogenic potentials.

In the present study, all cases of pathogenic CNV either underwent termination of pregnancy ($n = 12$) or were stillborn ($n = 1$). Among the

TABLE 4 Characterizations of congenital heart defect cases with VOUS CNVs and normal karyotype

Cases	Cardiac defect	Extra cardiac defect	Microarray results	Size (Mb)	Pregnancy outcome
19	CoA		arr[GRCh37] 4p16.1(9,734,060_10,453,310)x3	0.7 Mb	Born, normal
20	VSD, Interrupted AA		arr[GRCh37] 17p13.1(9,980,134_10,439,179)x3	0.5 Mb	Born, VSD
21	Disproportionally large RA & RV, RV hypertrophy, small inner aortic diameter		arr[GRCh37] 3q26.32(176,541,218_177,004,937)x3	0.5 Mb	Born, disproportionally large RA & RV
22	VSD, VR		arr[GRCh37] 5q21.3(106,426,492_107,145,885)x3	0.7 Mb	Born, VSD
23	VSD, PA		arr[GRCh37] 12q24.33(133,216,695_133,616,108)x3, arr[GRCh37] Xq21.31(90,733,230_91,477,880)x3	0.4 Mb, 0.7 Mb	TOP
24	VSD		arr[GRCh37] 1p36. 33p36.32(2,194,420_2,865,958)x3, arr[GRCh37] 16p13.3(608,263_1,252,385)x3	0.7 Mb, 0.6 Mb	Born, VSD
25	RAA, ALSA, VR		arr[GRCh37] 7q11.21(63,317,724_63,958,602)x3, arr[GRCh37] 3p22.1p21.1 (42,160,760_52,459,515) hmz	0.6 Mb	TOP
26	VSD		arr[GRCh37] 1p36. 33p36.32(2,226,156_2,812,958)x3, arr[GRCh37] 10q21.3(68,157,444_68,551,407)x1	0.6 Mb, 0.4 Mb	Born, normal
27	TAPVC		arr[GRCh37] 15q25.3(87,560,764_88,338,733)x3	0.8 Mb	TOP
28	VSD		arr[GRCh37] 15q26.3(101,698,746_102,085,600)x1	0.4 Mb	TOP
29	VSD		arr[GRCh37] 7q31.1(110,444,128_111,757,838)x3	1.3 Mb	Born, normal
30	VSD, dispropotionally large LV & LA		arr[GRCh37] 3p26.3(915,730_1,407,687)x3	0.5 Mb	Born, normal
31	AS, AAH		arr[GRCh37] 4q32.3(166,148,331_167,125,325)x3	1.0 Mb	Born, normal
32	Hyperechogenic loci in LV, pulmonary valves unequal in size, enlarged inner diameter of distal main pulmonary artery	Sinus venosus (umbilical vein)	arr[hg19] 4q12(57,340,665-58,100,402)x3	0.76 Mb	Born, normal

five cases of likely pathogenic CNV, two underwent termination of pregnancy and three were born apparently normal. Among the 14 cases of VOUS, four underwent termination of pregnancy, four were born with CHD, and six were born apparently normal. Unfortunately, for now, our follow-up data end when the children were released from the hospital. Future studies will examine whether those children will develop cardiac symptoms. The reasons for the differences among the living children can be due to a number of reasons, including CNV penetrance and interactions with other genetic variants. Additional studies are necessary to better understand those CNVs.

It has been shown that the 22q11.2 deletion is related to cardiac anomalies.³² Indeed, *TBX1* is crucial to normal heart development. The prevalence of CNVs in 22q11.2 among fetuses with CHD is 1.6%-11.5%.^{29,32} Mercer-Rosa et al³³ reported that the 22q11.2 deletion was associated with the tetralogy of Fallot. In the present study, one case with the tetralogy of Fallot had the 22q11.2 deletion. The other fetuses with the 22q11.2 deletion had ventricular septal defect.

In addition to the 22q11.2 deletion, CMA detected other deletions or duplications that were associated with CHD in the present

cohort study. The deletion of a 11q24.2q25 region that encompasses the *JAM3* and *ETS1* genes had previously been associated with CHD.³⁴ *JAM3* and *ETS1* are strong candidate genetic modulators for cardiac phenotypes.³⁴ In the present study, a 10.3-Mb deletion at the 11q24.2q25 locus was observed in one case of single ventricle and truncus arteriosus.

CMA can be easily included in prenatal diagnostic panels after a positive finding of fetal ultrasound anomaly. Definitive pCNVs are of use for the clinician, but the VOUSs continue to be a challenging issue prenatally. The current rate of discovery of VOUS in prenatal diagnosis is estimated to be 0.3%-4.7%.^{31,35} In the present study, 14 fetuses were detected with VOUS, representing 7.4% of the fetuses or 43.8% of the fetuses with CNVs. This proportion is quite high, and additional studies are necessary to rule out the pathogenic or benign character of these VOUS. Nevertheless, some study reported that the 15q11.2 deletion was associated with neurological diseases (including schizophrenia, idiopathic generalized epilepsies, and abnormal behavior), but the 15q11.2 deletion was also observed in healthy controls.^{16,36,37} The knowledge about the penetrance of the 15q11.2 deletion on CHD is clearly incomplete. In the present study, the fetus with the 15q11.2 VOUS was born in December 2015 and displays no abnormality so far. Because potential benefits have to be weighed against risks, it is currently recommended that only pCNVs and likely pCNVs are to be disclosed to the parents²³ as VOUS can be associated with significant stress and anxiety.¹⁰ Even in the presence of pCNVs, the benefits and risks have to be carefully weighed. Nevertheless, the present study provides additional data about the pathogenic potential of some VOUS because of their association with ultrasound-detected CHD.

Microarrays tests for SNPs not only provide data about CNVs but can also identify mosaics. In the present cohort study, CMA revealed an 11-M mosaic partial trisomy of chromosome 13q and a 24.4-M mosaic partial trisomy of chromosome 4q in one fetus. The 4q region includes the *HAND2* gene, which encodes transcription factors that play essential roles in cardiac morphogenesis.³⁸ Previous studies showed that overexpression of *HAND2* affects heart development; indeed, *HAND2*-KO mice have right ventricle developmental defects and abnormal aortic arch arteries.³⁹ Tamura et al⁴⁰ showed that overexpression of *Hand2* causes heart defects in fetuses with trisomy distal 4q. In the present study, the case with mosaic partial trisomy of chromosome 4q have the cardiac phenotypes of double outlet right ventricle, ventricular septal defect, persistent left superior vena cava, and pulmonary stenosis is consistent with precious report.⁴¹

The present study is not without limitations. The sample size was relatively small. The follow-up mainly covered the pregnancy until termination or delivery, and the follow-up of the born children is short. CMA itself is a good and promising technique, but has some limitations such as its inability to detect balanced chromosomal rearrangements.²⁵

Genomic uncertainty is a challenge for genetic medicine.¹² Nevertheless, the present study underlines the usefulness of CMA

in the prenatal genetic diagnosis of fetuses with CHDs and normal karyotype. In fetuses with CHD, the application of CMA could increase the detection rate of pCNVs causing CHDs. In this study, VOUS were identified as being potentially pathogenic, providing new data for adequate genetic counseling, but additional studies are needed to confirm these findings.

ORCID

Jianfang Zhang <http://orcid.org/0000-0001-5074-4647>

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