Reproductive Sciences (2024) 31:1089–1107
https://doi.org/10.1007/s43032-023-01399-2

GENETICS: ORIGINAL ARTICLE

Clinical Experience of Prenatal Chromosomal Microarray Analysis in 6159 Ultrasonically Abnormal Fetuses

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Received: 13 September 2023 / Accepted: 27 October 2023 / Published online: 27 November 2023

Abstract

A single-center retrospective study of G-band karyotyping and chromosomal microarray analysis (CMA) for the invasive prenatal diagnosis of 6159 fetuses with ultrasound abnormalities was conducted. This study aimed to investigate the incidence rates of chromosomal abnormalities and pregnancy outcomes and postpartum clinical manifestations by long-term follow-up and to explore the correlation between different types of prenatal ultrasound abnormalities and pathogenic chromosomal abnormalities. The overall incidence of pathogenic chromosomal aberrations in fetuses with ultrasound abnormalities was 7.58% (467/6159), which comprised 41.7% (195/467) with chromosome number abnormalities, 57.6% (269/467) with pathogenic copy-number variations (pCNVs), and 0.64% (3/467) with uniparental disomy (UPD). In addition, 1.72% (106/6159) with likely pathogenic copy-number variations (lpCNVs) and 3.04% (187/6159) with variants of unknown significance (VOUS) were detected by CMA. Ultrasound abnormalities were categorized into structural anomalies and soft marker anomalies. The incidence rate of pathogenic and likely pathogenic chromosomal abnormalities was significantly higher among fetuses with structural anomalies than soft markers (11.13% vs 7.59%, $p < 0.01$). We retrospectively analyzed the prenatal genetic outcomes for a large cohort of fetuses with different types of ultrasound abnormalities. The present study showed that the chromosomal abnormality rate and clinical outcomes of fetuses with different types of ultrasound abnormalities varied greatly. Our data have important implications for prenatal genetic counseling for fetuses with different types of ultrasound abnormalities.

Keywords Prenatal diagnosis · Ultrasound abnormalities · Copy number variations · Chromosomal microarray analysis · Uniparental disomy

Introduction

Ultrasonic examination plays an important role in the discovery and diagnosis of fetal abnormalities, including soft markers and structural anomalies. Fetal structural abnormalities are found in up to 3% of all pregnancies [1], and these fetuses are at increased risk of chromosomal abnormalities. In addition, with the increasing capabilities and experience of technicians, an increasing number of ultrasound abnormalities are being detected, especially the abnormalities in various soft markers. Soft marker abnormalities can be easily detected by ultrasound examination during the second trimester. Previous studies have shown that soft marker abnormalities have a minor impact on fetal development and usually resolve in the third trimester, but they are considered a potential risk factor for chromosomal abnormalities [2, 3]. The incidence of chromosomal abnormalities varies among fetuses with different types of ultrasound abnormalities. It is particularly important to use suitable methods for genetic prenatal diagnosis of ultrasound abnormalities in fetuses.

Assessment of genome-wide copy-number variations (CNVs) is recommended as the first level of testing for the cytogenetic assessment of these fetuses with ultrasound abnormalities [4, 5]. Chromosomal microarray analysis (CMA) is already widely utilized in invasive prenatal diagnostics for fetuses with ultrasound abnormalities, advanced

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maternal age, aberrant first trimester screening, and other situations [6]. .CMA has been found to be useful to identify prenatal clinically relevant CNVs. In addition to chromosomal aneuploidy and CNVs, CMA with single nucleotide polymorphism (SNP) probes is also effective in detecting uniparental disomy (UPD), loss of heterozygosity (LOH), triploidy, and chimerism [7, 8]. Several previous studies showed that the application of CMA is valuable for fetuses with ultrasound abnormalities [9, 10], but focused on specific cases of pregnancy outcomes, and the sample sizes were limited. The large-scale studies on the association between chromosomal abnormality rates and ultrasound abnormalities in different groups are still lacking. Therefore, when encountering a certain type of ultrasound abnormality, clinicians may experience difficulties in choosing the most appropriate test. So further large sample studies are still necessary to clarify the correlation between different types of ultrasound abnormalities and chromosomal abnormalities, and provide more data for clinicians.

The present study retrospectively investigated the clinical ultrasound manifestations and outcomes of 6159 fetuses with ultrasound abnormalities by CMA and karyotyping with long-term follow-up. The present study further evaluated the clinical application of CMA in the prenatal diagnosis of CNVs. In particular, the potential diagnostic rates of CMA for different subgroups of ultrasound abnormalities were also evaluated. In addition, subgroup analyses were performed to better understand the genetic causes of ultrasound abnormalities and to make recommendations for prenatal genetic testing for each type of ultrasound abnormality.

Materials and Methods

Subjects

A total of 6159 fetuses with ultrasound abnormalities detected by fetal ultrasound or echocardiography and that underwent CMA and karyotyping were retrospectively reviewed. Invasive prenatal diagnosis was performed between January 2015 and December 2021 at the First Affiliated Hospital of the Fourth Military Medical University (Shanxi Province, Northwest China). All parents received prenatal counseling from a clinical geneticist about the risks associated with an invasive prenatal diagnosis, the advantages and limitations of CMA, and the risks of variants of unknown significance (VOUS) and incidental findings. The CMA results and following outcomes were analyzed: incidence rates of chromosomal anomalies in different group ultrasound abnormalities and the prognosis of fetuses. All pregnant women routinely provide written informed consents. In the present study, the amniotic fluid samples of fetuses were collected at 18 to 35 weeks of gestation, chorionic villus samples were collected from 26 fetuses at 11 to 13 weeks of gestation, and umbilical cord blood samples were collected from 22 fetuses at 24 to 28 weeks due to oligohydramnios. The pregnancy outcomes were obtained by telephone follow-up.

Karyotype Analysis

The amniotic fluid, chorionic villus, and umbilical cord blood samples were cultured and karyotyped according to standard cytogenetic protocols. The Giemsa-banding technique (450-550-band resolution) was used to analyze the cultured amniocytes or lymphocytes.

Chromosomal Microarray Analysis (CMA)

A QIAamp DNA Blood Mini Kit (Qiagen, Venlo, the Netherlands) was used to extract genomic DNA from amniotic fluid, chorionic villus, and umbilical cord blood samples. An Affymetrix CytoScan 750K array (Affymetrix, Santa Clara, CA, USA) was used and the procedure was performed according to the standard manufacturer's protocol as described in our previous publication [11]. The Chromosome Analysis Suite v4.2 software was used to analyze the CEL files, based on data from the genome version GRCh37 (hg19). CNVs larger than 100 kb or those that affected more than 50 contiguous probes were considered, and regions of homozygosity larger than 10 Mb were analyzed.

Public databases such as DGV (http://www.ncbi.nlm.nih.gov/dbvar/), ClinGen(https://search.clinicalgenome.org/kb/gene-dosage), 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 [12], the CNVs were classified into five categories: pathogenic copy-number variations (pCNVs), likely pathogenic copy-number variations (lpCNVs), benign, likely benign, and variants of unknown significance (VOUS). In the present study, to determine whether the pCNVs, lpCNVs and VOUS detected by CMA are de novo or inherited, some parents were tested, but benign and likely benign CNVs were not considered for the present study.

Clinical Follow-up Assessments and Statistical Analysis

Clinical follow-up assessments about the pregnancy outcomes, the detail data on postnatal conditions, and prenatal and postnatal development were performed regularly by telephone from 6 months to 3 years. SPSS 24.0 statistical

software was used for statistical analysis of the data. Comparisons between groups were conducted using the chi-square test or the Fisher exact test. A p-value < 0.05 was considered statistically significant in the tests.

Results

Study Subjects

CMA detection was performed on 9141 pregnancies during the 7-year study period in our center. Among them, 6159 pregnant women underwent genetic CMA testing due to ultrasound abnormalities. The detection rates of pCNVs, lpCNVs, VOUS, and other findings in fetuses with different subgroups of ultrasonographic structural anomalies and soft markers are summarized in Tables 1 and 2.

Prevalence of Chromosomal Abnormalities

The overall incidence rate of pathogenic chromosomal abnormalities in fetuses with ultrasound abnormalities was 7.58% (467/6159). Among these cases, 57.6% (269/467) were with pCNVs, 41.7% (195/467) with numerical chromosomal abnormalities, and 0.64% (3/467) with UPD.

The 195 fetuses with chromosomal number abnormalities were comprised of 92 trisomy 21, forty-four trisomy 18, five trisomy 13, 26 monosomy X, five XXX, seven XXY, five XYY, ten mosaicisms (including one mosaic trisomy 8, one mosaic trisomy 18, one mosaic trisomy 22, one mosaic trisomy 16, one mosaic trisomy 21, and five mosaic sex chromosome), and one triploid (69,XXX).

Two hundred sixty-nine (4.37%, 269/6159) cases with pCNVs were detected. The fragment size of chromosomal pCNVs detected ranged from 73 to 80.1 Mb. There were 190 (3.08%; 190/6159) with microdeletions, 46 (0.75%; 46/6159) with microduplications, and 33 (0.54%; 33/6159) cases with both deletions and duplications. A total of 35 microdeletion or microduplication syndromes were found in 187 cases, including 22q11.2 microdeletion syndrome, 22q11.2 microduplication syndrome, 1p36 microdeletion syndrome, 15q11.2 microdeletion syndrome, 15q13.3 microdeletion syndrome, 16p11.2 microdeletion syndrome, 16p11.2 microduplication syndrome, 16p13.11 microdeletion syndrome, 17q12 microdeletion syndrome, 17q12 microduplication syndrome, 1q21.1 microdeletion syndrome, 1q21.1 microduplication syndrome, 1q44 deletion syndrome, 2q13 microdeletion syndrome, 3q29 microdeletion syndrome, 6q25.1 microdeletion syndrome, 7q11.23 microduplication syndrome, 8p23.1 deletion syndrome, 8p23.1 microduplication syndrome, alpha-thalassemia/mental retardation syndrome, type 1, cri du chat syndrome, hereditary stress susceptibility neurosis, Jacobsen syndrome, KBG syndrome, Miller-Dieker syndrome, Pallister-Killian syndrome, Phelan–McDermid syndrome, Smith-Magenis syndrome, tetrasomy 18p

Table 1 Summary of chromosomal aberrations among the 2982 fetuses with ultrasonographic structural anomalies

Category of anomaly		Microarray results (*n* (%))					
		Total	Aneuploid	pCNVs	Others	lpCNVs	VOUS
Cardiovascular system	Tetralogy of Fallot, interrupted aortic arch, transposition of the great arteries, pulmonary artery sling	1795	38 (2.12%)	96 (5.35%)	0	29 (1.62%)	52 (2.9%)
Central nervous system	Agenesis of corpus callosum, Dandy–Walker malformation, pachygyria, cerebral dysplasia, hydrocephalus	173	0	19 (10.98%)	0	4 (2.31%)	10 (5.78%)
Genitourinary system	Enlarged polycystic and echogenic kidneys, hypospadias, common cloacal deformity	273	4 (1.47%)	8 (2.93%)	0	9 (3.29%)	12 (4.39%)
Skeletal system	Shortened and bowing bone, dysplasia of thoracic vertebra and ribs, hemivertebra, scoliosis, syndactyly	232	2 (0.86%)	12 (5.17%)	0	3 (1.29%)	3 (1.29%)
Gastrointestinal system	Esophago-tracheal fistula, mesenteric cyst, situs in vs	84	1 (1.19%)	3 (3.57%)	0	4 (4.76%)	3 (3.75%)
Respiratory system	Dysplasia of right lung	86	1 (1.16%)	2 (2.33%)	0	0	1 (1.16%)
Facial	Micrognathia, bilateral anophthalmia, unilateral microphthalmia, depressed nasal bridge	79	1 (1.27%)	1 (1.27%)	0	0	5 (6.33%)
Multiple	Two or more ultrasonographic structural anomalies	260	51(19.62%)	36 (13.85%)	3 (1.15%)[a]	5 (1.92%)	9 (3.46%)
Total		2982	98 (3.39%)	177 (5.94%)	3	54 (1.81%)	95 (3.42%)

pCNVs pathogenic copy-number variants, *lpCNVs* likely pathogenic copy-number variants, *VOUS* variant of uncertain significance

[a]Two fetuses with uniparental disomy and one with triploid

Table 2 Summary of chromosomal aberrations among the 3177 fetuses with ultrasonographic soft markers

Category of anomaly	Microarray results (*n* (%))					
	Total	Aneuploid	pCNVs	Others	lpCNVs	VOUS
NT/NF	275	39 (14.18%)	6 (2.18%)	0	4 (1.45%)	11 (4%)
Absent/hypoplastic nasal bone	182	7 (3.85%)	4 (2.2%)	0	3 (1.65%)	2 (1.1%)
Mild ventriculomegaly	258	3(1.16%)	15(5.81%)	0	7 (2.71%)	8 (3.1%)
CPCs	266	7 (2.63%)	4 (1.5%)	0	3 (1.13%)	9 (3.38%)
Posterior cranial fossa	15	0	0	0	1 (6.67%)	0
EICF	495	5 (1.01%)	7 (1.41%)	1 (0.2%)[a]	8 (1.61%)	19 (3.84%)
Echogenic bowel	104	2 (1.92%)	1 (0.96%)		1 (0.96%)	1 (0.96%)
SUA	91	1 (1.1%)	8 (8.79%)	0	2 (2.2%)	3 (3.3%)
Mild pyelectasis	31	0	0	0	1 (3.23%)	1 (3.23%)
Polyhydramnios/oligohydramnios	148	1 (0.68%)	6 (4.05%)	0	2 (1.35%)	7 (4.73%)
Subclavian artery vagus	107	0	1 (0.99%)	0	2 (1.87%)	0
Cardiac soft markers	281	4 (1.42%)	9 (3.2%)	0	2 (0.71%)	11 (3.91%)
FGR	51	0	2 (3.92%)	0	0	5 (9.8%)
Multiple soft markers	615	24 (3.9%)	20 (3.25%)	0	10 (1.63%)	19 (3.09%)
Others	258	3 (1.16%)	9 (3.49%)	0	6 (2.33%)	2 (0.78%)
Total	3177	96 (3.02%)	92 (2.9%)	1	52 (1.64%)	98 (3.08%)

pCNVs pathogenic copy-number variants, *lpCNVs* likely pathogenic copy-number variants, *VOUS* variant of uncertain significance, *NT/NF* nuchal translucency/nuchal fold, *CPCs* choroid plexus cysts, *EICF* echogenic intracardiac focus, *SUA* single umbilical artery, *FGR* fetal growth retardation

[a]One fetus with uniparental disomy

syndrome, Williams-Beuren syndrome, Wolf–Hirschhorn syndrome, MECP2 duplication syndrome, and X-linked ichthyosis. In the remaining cases, some rare CNVs such as 21q22.12q22.3 microdeletion, 6q27 1 Mb microdeletion, and 2p16.1p14 microduplication were included; the details are summarized in Table 3.

In addition, 1.72% (106/6159) with lpCNVs and 3.04% (187/6159) with VOUS were detected by CMA; the details of lpCNVs are summarized in Table 4 and VOUS are summarized in Supplementary table 1.

Subgroup Analysis of the Different Types of Ultrasound Abnormalities

There were 2982 fetuses with ultrasonographic structural anomalies, 9.32% (278/2982) with pathogenic chromosomal abnormalities including pCNVs, aneuploid, and UPD. 1.81% (54/2982) with lpCNVs and 3.42% (95/2982) with VOUS were detected by CMA. Congenital heart diseases (CHDs) were the most common ultrasound abnormalities presented in 1795 fetuses; the detection rate of pathogenic and likely pathogenic chromosomal abnormalities for fetuses with CHDs was 7.47% (134/1795) and 1.62% (29/1795) respectively. The incidence of pathogenic chromosomal abnormalities for fetuses with skeletal and central nervous system abnormalities was respectively 6.03% and 10.98%. Three thousand one hundred seventy-seven fetuses with ultrasonographic soft markers, 5.95% (189/3171) with pathogenic anomaly. 1.64% (52/3177) with lpCNVs, and 3.08% (98/3177) with VOUS were detected by CMA. The detection rate of pathogenic and likely pathogenic chromosomal abnormalities in fetuses with ultrasonographic structural anomalies (332/2982, 11.13%) was significantly higher than that in fetuses with ultrasonographic soft markers (241/3177, 7.59%) ($p < 0.001$).

Clinical Follow-up Assessments

In the current study, the average telephone follow-up time for these fetuses was 1 year, ranging from 3 months to 3 years. Among 194 aneuploid fetuses, 183 underwent termination of pregnancy, 2 were lost to follow-up, 9 were born without obvious clinical defects including six with XXY, one with XXX, and two with 45,X. Among 269 fetuses with pCNVs, 205 underwent termination of pregnancy, 24 were lost to follow-up, and 35 were born. However, there were 5 fetuses who had postnatal death and 8 fetuses showed developmental delay, hypotonia, and feeding difficulties after birth, others without obvious clinical defects at follow-up. Among the 106 fetuses of lpCNV, 35 underwent termination of pregnancy, 12 were lost to follow-up, 53 were born apparently normal, 1 had postnatal death, and 5 showed developmental delay after birth. Among 193 cases of VOUS, 33 underwent termination of pregnancy due to the chromosomal abnormalities, 40 were lost to follow-up, 111 were born

Table 3 Characteristics of rare pCNVs among the 6159 fetuses with ultrasonically abnormal

Cases	Ultrasound findings	pCNVs (GRCh37)	Size of CNVs (Mb)	Copy number	Karyotyping results	Outcomes
1	DORV	arr[GRCh37] 4q27q35.2(121974109_190957460)x3 arr[GRCh37] 8p23.3p23.1(158049_10783584)x1	68.98 10.63	Gain Loss	46,XY,der(8)t(4;8)(q27p23.1)	Top
2	VSD	arr[GRCh37] 6q24.3q25.1(148689991_149893356) x1	1.2	Loss	46,XY	Top
3	Lateral ventriculomegaly, porencephalia	arr[GRCh37] 13q33 .1q34(104,703,176_115,107,733)x1	10.4	Loss	46,XY	Top
4	EICF	arr[GRCh37] 15q26 .1q26.3(91,665,684_102,429,040)x3	10.8	Gain	46,XX	Top
5	VSD	arr[GRCh37] 18q22.2q23(67923602_78013728)x1	10.1	Loss	46,XY	Born, development delay
6	PLSVC	arr[GRCh37]14q32 .2q32.33(101013829_106071820)x1	5.06	Loss	46,XX	Top
7	VSD, aortic straddle	arr[GRCh37] 18q12.1q23(29271053_78013728)x3 arr[GRCh37] 3p26.3p26.1(61892_7439472)x1	48.7 7.38	Gain Loss	46,XY	Top
8	VSD	arr[GRCh37] 8q22 .3q24.3(103,697,953_146,295,771)x3 arr[GRCh37] 13q34(111,384,129_115,107,733)x1	42.6 3.72	Gain Loss	46,XX	Top
9	Single ventricle and atrium	arr[GRCh37] 21q22.12q22.3(36800863_48093361) x1	11.3	Loss	46,XY, 14pstk+, r(21) (p11q22)	Top
10	VSD	arr[GRCh37] 10q26. 12q26.3(121,951,796_135,426,386)x3 arr[GRCh37] 2p25.3(12,770_3,262,730) x1	13.5 3.25	Gain Loss	46,XX	Top
11	VSD	arr[GRCh37] 18q11 .2q23(23,295,110_78,013,728)x3 arr[GRCh37] 18p11.32(136,227_1,426,361)x1	54.7 1.3	Gain Loss	46,XX	Top
12	VSD, lateral ventriculomegaly	arr[GRCh37] 7q31.1q36.2 (112,635,571_154,503,381)x3 arr[GRCh37] 7q36 .2q36.3(154,509,025_159,119,707)x1	48.1 4.6	Gain Loss	46,XX	Born, death
13	EICF	arr[GRCh37] 4q34 .3q35.2(179,190,469_190,957,460)x1	11.8	Loss	46,XY,del(4)(q34)	Top

Table 3 (continued)

Cases	Ultrasound findings	pCNVs (GRCh37)	Size of CNVs (Mb)	Copy number	Karyotyping results	Outcomes
14	CoA	arr[GRCh37] 2q14 .1q14.3(114,486,367_126,517,961)x1	12	Loss	46,XY	Top
15	VSD, lateral ventriculomegaly	arr[GRCh37] 5q14.3q15(86,733,471_92,377,914)x1	5.64	Loss	46,XX	Top
16	TOF	arr[GRCh37] 19q13. 32q13.33(47,186,440_48,139,059)x1	0.95	Loss	46,XY	Born, development delay
17	VSD	arr[GRCh37] 17p13.3p13.2(204,999_3,784,378)x3	3.58	Gain	46,XX	Top
18	VSD, EICF	arr[GRCh37] 22q12.3(33,854,040_35,669,208)x1	1.82	Loss	46,XY	Top
19	VSD, IAA	arr[GRCh37] 5p13.3(29,117,172_31,284,397)x3 arr[GRCh37] 5p12q11.1(45,675,290_49,588,319)x3	2.17 3.91	Gain Gain	46,XY	Top
20	VSD	arr[GRCh37] 6q14 .1q16.1(82,660,373_95,092,084)x1	12.4	Loss	46,XX	Top
21	Hydronephrosis	arr[GRCh37] 9p24.3p23(208,454_13,683,509)x1	13.5	Loss	46,XX	Loss to follow-up
22	Lateral ventriculomegaly	arr[GRCh37] 17q22q25.3(56,944,915_81,041,823)x3 arr[GRCh37] Xq21. 31q28(91,495,299_155,233,098)x1	24.1 63.7	Gain Loss	46,X, der(X)t(X;17) (q21.3;q22)	Top
23	Lateral ventriculomegaly	arr[GRCh37] 17p13.3(2,549,665_3,159,264)x1	0.61	Loss	46,XX	Top
24	Lateral ventriculomegaly	arr[GRCh37] 6q27(169,907,267_170,914,297)x1	1	Loss	46,XY	Loss to follow-up
25	Lateral ventriculomegaly	arr[GRCh37] 6q25 .3q27(156,197,501_168,167,204)x3 arr[GRCh37] 6q27(168,168,883_170,914,297)x1	11.9 2.7	Gain Loss	46,XX	Top
26	NT	arr[GRCh37] 18p11.32p11.21(136227_15136153)x1	15	Loss	45,XY, der(18)t(18;21) (q11;p11.1),-18	Top
27	Lateral ventriculomegaly	arr[GRCh37] 6p25.3p22.3(156,974_25,066,393)x3 arr[GRCh37] 21q22. 13q22.3(39,373,647_48,093,361)x1	24.9 8.72	Gain Loss	46,XX,-21, +der(21) t(6;21) (p22.3;q22.1)	Top
28	Lateral ventriculomegaly	arr[GRCh37] 2p16.1p14(61,123,434_66,911,895)x3	5.79	Gain Loss	46,XY	Born, development delay
29	Choroid plexus cyst	arr[GRCh37] 12q21 .2q21.31(78,090,627_83,477,230)x1	5.39	Loss	46,XY	Born, normal

Table 3 (continued)

Cases	Ultrasound findings	pCNVs (GRCh37)	Size of CNVs (Mb)	Copy number	Karyotyping results	Outcomes
30	NT	arr[GRCh37] Yq11.2 23q11.23(24741035_28420380)x0	3.7	Loss	46,XY	Born, normal
31	NT	arr[GRCh37] 1p36.22(10445922_11939442)x1	1.49	Loss	46,XX	Top
32	NT	arr[GRCh38] Yp11.2q11.222(2650425_19556683) x2 arr[GRCh37] Yq11.222q12(20618888_28799654)x0	16.9 8.2	Gain Loss	46,X [24]/46,X,der(Y)dup(Y) (pterq11.2)del(Y)(q11.2)[36]	Top
33	NT, lateral ventriculomegaly	arr[GRCh37] Yq11.21q11.23(13800956_28799654) x1	15	Loss	——	Top
34	SUA	arr[GRCh37] 2q37.1q37.3(232990480_242782258) x1	9.79	Loss	46,XX	Loss to follow-up
35	SUA	arr[GRCh37] 12q13. 12q13.13(49393538_54406950)x3	5.01	Gain	46,XX	Top
36	SUA	arr[GRCh37] 18p11. 32p11.31(136,227_3,483,199)x1 arr[GRCh37] 18q21. 33q23(61,103,653_78,013,728)x1	3.3 16.9	Loss Loss	46,XY, r(18)(p11.3q21.3)	Top
37	DORV, absent pulmonary valve	arr[GRCh37] 2q31 .1q32.1(170,548,729_187,302,116)x1	16.8	Loss	46,XX,del(2)(q31.1→q32.2)	Top
38	Posterior cranial fossa	arr[GRCh37] 3p21. 31p21.2(47,409,497_52,148,326)x1	4.7	Loss	46,XY	Loss to follow-up
39	Posterior cranial fossa, ARSA	arr[GRCh37] 10q26 .2q26.3(130,333,948_135,426,386)x1	5.1	Loss	46,XY	Top
40	Blake's pouch cyst	arr[GRCh37] 9p24 .2p22.2(2,716,920_17,186,374)x3 arr[GRCh37] 9p24.3p24.2(208,454_2,715,213)x1	14.5 2.5	Gain Loss	46,XX	Top
41	Lateral ventriculomegaly	arr[GRCh37] 13q31 .2q33.2(88,867,776_106,093,135)x1	17.2	Loss	46,XX, del(13)(q31.2q33.2)	Top
42	Lateral ventriculomegaly, single umbilical artery	arr[GRCh37] 2p11.2(83,592,209_89,128,106)x1	5.54	Loss	46,XY	Top
43	Dandy–Walker, EICF, polyhydramnios	arr[GRCh37] 5q13 .3q14.1(75,642,770_79,936,342)x1 arr[GRCh37]5q14 .3q15(84,428,488_97,070,754)x1	4.29 12.6	Loss Loss	46,XY,-4,-5,+der(4)t(4;5) (q21;q15),+der(5)t(4;5) (q21;q14),del(5) (q14q15)	Top
44	Cerebellar vermis missing	arr[GRCh37]6p25 .3p25.2(384,096_3,827,197)x1	3.4	Loss	46,XX	Loss to follow-up

Table 3 (continued)

Cases	Ultrasound findings	pCNVs (GRCh37)	Size of CNVs (Mb)	Copy number	Karyotyping results	Outcomes
45	VSD, aortic straddle, FGR, Hypoplastic nasal bone	arr[GRCh37] Xp22.33p22.31(168,551_9,418,597)x2 arr[GRCh37] 4p16.3p16.1(68,345_10,091,098)x1	9.25 10.02	Gain Loss	46,XY	Top
46	SUA, NT	arr[GRCh37] 10p14p12.1(8,218,666_25,853,592)x1	17.6	Loss	46,XX,del(10)(p12.1)	Top
47	SUA, absent nasal bone, absence of right kidney	arr[GRCh37] 13q13 .3q34(38,052,485_112,312,366)x3 arr[GRCh37] 13q34(112,318,044_115,107,733)x1	74.3 2.79	Gain Loss	46,XY	Top
48	VSD, oligohydramnios	arr[GRCh37] 12q24. 21q24.33(115,688,366_133,687,046) x3	18	Gain	46,XX	Top
49	VSD, absent cavum septum pellucidum, FGR	arr[GRCh37] 4p12q13.2(47,632,643_69,435,889)x3 arr[GRCh37] 4q13 .2q21.23(69,541,893_86,815,623)x3 arr[GRCh37] 4q35 .1q35.2(185,081,688_190,957,460)x1	21.8 17.3 5.87	Gain Gain Loss	——	Top
50	Lateral ventriculomegaly, polyhydramnios, VSD, Short femur length	arr[GRCh37] 7q32.3q36.3(130536339_159119707) x3 arr[GRCh37] 10q26. 13q26.3(126924247_135426386)x1	28.6 8.5	Gain Loss	46,XY	Top
51	SUA, EICF polyhydramnios	arr[GRCh37] 8p12p11.21(32959945_42054971)x4	9.09	Triplication	46,XX,qdp(8)(p12p11.21)	Top
52	Polyhydramnios, VSD	arr[GRCh37] 6p22.3p22.2(21363210_25320148)x1	3.96	Loss	46,XX	Top
53	EICF	arr[GRCh37] 21q11 .2q22.11(15,016,486_34,251,578)x1	19.2	Loss	46,XY,del(21)(q11.2q22.1)	Top
54	Strephenopodia	arr[GRCh37] 1p36.33p36.32(849467_2879469)x3	20.3	Gain	46,XY	Born, development delay
55	VSD, aortic straddle	arr[GRCh37] 2q31.2q33.1(179223124_199580726) x3	20.4	Gain	46,XY,dup(2)(q31.2q33.1)	Top
56	PLSVC, short femur length	arr[GRCh37] 14q23.1(59,548,268_61,453,634)x1	1.9	Loss	46,XX	Top
57	FGR, VSD	arr[GRCh37] 4p16.3p15.2(68,345_24,886,024)x3 arr[GRCh37] 4q34 .3q35.2(183,030,135_190,957,460)x1	24.8 7.9	Gain Loss	46,XY	Top

Table 3 (continued)

Cases	Ultrasound findings	pCNVs (GRCh37)	Size of CNVs (Mb)	Copy number	Karyotyping results	Outcomes
58	Short femur length, VSD	arr[GRCh37] 4q13.1q22.1(65509730_88308111)x1	22.8	Loss	46,XY,del(4)(q13.1;q13.2)	Top
59	Orofacial clefts, TOF, posterior fossa cyst	arr[GRCh37] Xq28(152,970,883_154,896,094)x2 arr[GRCh37] 6p25.3p24.3(381,117_7,790,535)x1	1.93 7.41	Gain Loss	46,XY	Top
60	VSD, SUA	arr[GRCh37]8q24. 13q24.3(124,437,925_146,295,771)x3 arr[GRCh37] 7p22.3p22.1(43,376_4,896,209)x1	21.9 4.9	Gain Loss	46,XX,-7,+der(7)t(7;8) (p22.3;q24)	Top
61	Posterior fossa cyst, diaphragmatic hernia	arr[GRCh37] 5q32q35.3(148089501_180715096)x3 arr[GRCh37] 20p13(61663_918358)x1	32.6 0.86	Gain Loss	46,XY	Top
62	Absent nasal bone	arr[GRCh37] 6p21.1(44,032,138_45,486,795)x1	1.45	Loss	46,XX	Top
63	Echogenic bowel, renal cyst	arr[GRCh37] 2p25.3p23.3(12,770_26,779,066)x3	26.8	Gain	46,XX,der(7)t(2;7)(p23.3;p22)	Top
64	SUA, diaphragmatic hernia	arr[GRCh37] 5q23 .2q33.1(122,987,868_150,316,262)x3	27.3	Gain	46,XY	Top
65	Orofacial clefts	arr[GRCh37] Xq25(122,971,404_123,987,126)x2	1.02	Gain	46,XY	Loss to follow-up
66	Absent nasal bone	arr[GRCh37] 4q25q31.21(112,633,330_ 143,808,984)x1	31.2	Loss	46,XY,del(4)(q33)	Loss to follow-up
67	Mild hydronephrosis, EICF	arr[GRCh37] 7p22.1(4869176_5986394) x3 arr[GRCh37] 7p22.1(6263894_6868186) x3	1.12 0.6	Gain Gain	46,XY	Top
68	Polyhydramnios, short femur length	arr[GRCh37] 12p13.33p11.21(173787_31611816)x3 arr[GRCh37] 4q34.3q35.2(181344971_190957460) x1	31.4 9.6	Gain Loss	46,XX	Top
69	Oligohydramnios, renal abnormality	arr[GRCh37] 8q13 .1q13.2(66,739,561_69,208,366)x1 arr[GRCh37] 8q21.11(75,289,991_78,283,479)x1	2.47 2.99	Loss Loss	46,XY	Top
70	Polyhydramnios, short femur length	arr[GRCh37] 15q11 .2q13.3(22,770,421_32,444,043)x4	9.7	Triplication	46,XX	Top
71	Oligohydramnios	arr[GRCh37] Xq25q26.3(127758808_135081344)x2	7.32	Gain	46,XY	Top

Table 3 (continued)

Cases	Ultrasound findings	pCNVs (GRCh37)	Size of CNVs (Mb)		Copy number	Karyotyping results	Outcomes
72	Polyhydramnios	arr[GRCh37] 11q22.1q22.2(98697497_102751491) x1 arr[GRCh37] 11q14.1q21(83001205_97058917)x1	4.05 14.1		Loss Loss	46,XY	Top
73	Polyhydramnios	arr[GRCh37] 22q12.1q12.2(27146527_29832257)x1	2.69		Loss	46,XX	Top
74	Short femur length	arr[GRCh37] 8q12 .1q12.2(60,092,529_62,013,253)x3	1.92		Gain	46,XY	Top
75	Polyhydramnios, polydactyly, EICF	arr[GRCh37] 4q12(55511100_56758837)x1 arr[GRCh37] 7p14.1p12.1(41538488_52380146)x1	1.25 10.8		Loss Loss	46,XX, der(4;9;10)t(4;9;10) (q12.1;q34.3;p15.2)del(7) (p12.3p12.1)	Top
76	Echogenic bowel	arr[GRCh37] 2q37.1q37.3(233686273_242782258) x3	9.1		Gain	46,XY	Top
77	Syndactylus, VSD	arr[GRCh37] 15q11 .2q22.2(22,770,421_62,967,090)x3	40.2		Gain	46,XY	Top
78	Spine abnormality	arr[GRCh37] 18q12.1q23(31430547_78013728)x3 arr[GRCh37] 15q26.3(99664502_102429040)x1	46.7 2.76		Gain Loss	46,XX	Top
79	Posterior cranial fossa, clinodactyly	arr[GRCh37] 12q13. 13q24.33(53642243_133777562)x2_3	80.1		Mosaic gain	46,XY	Top
80	Absent cavum septum pellucidum	arr[GRCh37] 4q21.1q32.1(77011568_159682971)x3	82.7		Gain	46,XX,dup(4)(q21.1q32.1)	Top
81	FGR	arr[GRCh37] 14q32. 13q32.33(95,859,273_107,284,437)x3 arr[GRCh37] 3p26.3p26.1(61,891_6,969,298)x1	11.4	6.9	Gain	46,XX	Loss to follow-up
82	Spine abnormality, ureterectasia	arr[GRCh37] 7q36 .1q36.3(149,481,498_159,119,707)x1	9.6		Loss	46,XY	Top

pCNVs pathogenic copy-number variants, *Top* termination of pregnancy, *DORV* double-outlet right ventricle, *VSD* ventricular septal defect, *EICF* echogenic intracardiac focus, *COA* coarctation of aorta, *TOF* tetralogy of Fallot, *IAA* interruption of aortic arch, *NT* nuchal translucency, *ARSA* aberrant right subclavian artery, *FGR* fetal growth restriction, *SUA* single umbilical artery, *PLSVC* persistent left superior vena cava

——, there is no result of karyotyping

Table 4 Characteristics of lpCNVs among the 6159 fetuses with ultrasonically abnormal

Number	Ultrasound findings	pCNVs (GRCh37)	Size of CNVs	Copy number	Karyotyping results	Outcomes
1	VR	arr[GRCh37] 2q11.1q11.2(96069545_97678612)x3	1.61 Mb	Gain	46,XY	TOP
2	VSD	arr[GRCh37] 17q11.2(28997894_30425992)x3	1.43 Mb	Gain	46,XY	TOP
3	CoA	arr[GRCh37] 2p25.1p24.2(9059161_18673642)x3	9.6 Mb	Gain	46,XX	TOP
4	VSD	UPD(8)	UPD(8)	UPD	46,XX	Born, normal
5	VR	arr[GRCh37] 2q13q14.1(111371702_113142794)x3	1.8 Mb	Gain	46,XY	TOP
6	VR	arr[GRCh37] 7q35(146050361_146492864)x1	442 kb	Loss	46,XY	Born, normal
7	VSD	arr[GRCh37] 15q11.2(22813068_23290813)x3	478 kb	Gain	46,XX	Born, normal
8	VSD	arr[GRCh37] 15q11.2(22813068_23290813)x3	478 kb	Gain	46,XX	Born, normal
9	ARSA	arr[GRCh37] 1q43(237540615_240488993)x1	2.95 Mb	Loss	46,XY	TOP
10	VSD	arr[GRCh37] 22q11.23(23652587_25002659)x3	1.35 Mb	Gain	46,XX	Born, normal
11	VSD	arr[GRCh37] 1p36. 33p36.32(2,208,464_3,641,595)x3	1.43 Mb	Gain	46,XX	Loss to follow-up
12	VSD	arr[GRCh37] 16p11.2(28,708,186_29,051,191)x1	343 kb	Loss	46,XY	Born, development delay
13	EICF	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XY	Born, normal
14	VSD	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XX	Born, normal
15	VSD	arr[GRCh37] 3p26.3(688,613_1,144,815)x1	456 kb	Loss	46,XX	Born, normal
16	VSD, APVC, hooked hand	arr[GRCh37] 12q21.32(88,131,931_88,960,660)x1	829 kb	Loss	46,XY	TOP
17	SV, AA	arr[GRCh37] 1q21 .1q21.2(146,569,489_147,398,268)x3	828 kb	Gain	46,XY	TOP
18	VSD	arr[GRCh37] 5q11 .2q12.1(55,074,278_59,670,602)x1	4.6 Mb	Loss	46,XX	TOP
19	VR	arr[GRCh37] 7p14.3(32,947,709_34,917,263)x1	1.97 Mb	Loss	46,XY	Born, normal
20	The inner diameter of the main pulmonary artery widened	arr[GRCh37] 22q11. 22q11.23(22,724,607_24,014,514)x1	1.29 Mb	Loss	46,XX	TOP
21	VSD	arr[GRCh37] 1q21 .1q44(143932350_249224684)×2-3	105 Mb	Mosaic loss	46,XY	Born, normal
22	VSD	arr[GRCh37] 19p13.3(364,936_1,922,093)x3	1.56 Mb	Gain	46,XX	TOP
23	ARSA	arr[GRCh37] 16q21(57,666,355_64,669,301)x1	7 Mb	Loss	46,XY	TOP
24	VR	arr[GRCh37] 22q11. 22q11.23(22,997,928_25,041,592)x3	2 Mb	Gain	46,XY	TOP
25	PAS	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XY	Born, normal
26	Hydropericardium, tricuspid regurgitation	arr[GRCh37] 16p13.11(15,338,152_16,282,869)x3	945 kb	Gain	46,XX	Born, normal

Table 4 (continued)

Number	Ultrasound findings	pCNVs (GRCh37)	Size of CNVs	Copy number	Karyotyping results	Outcomes
27	VSD, DORV, pulmonary valve stenosis	arr[GRCh37] 13q33 .1q34(103,971,638_115,107,733)x2_3 arr[GRCh37] 4q32 .3q35.2(166,546,468_190,957,460) x2_3	11.1 Mb 24.4 Mb	Mosaic gain	46,XY	TOP
28	VSD	arr[GRCh37] 8p23.1(8102818_11822454)x3	3.72 Mb	Gain	46,XY	TOP
29	Pulmonary valve enlargement	arr[GRCh37] 17p13.3(526_1311263)x1	1.31 Mb	Loss	46,XX	Born, normal
30	Lateral ventriculomegaly	arr[GRCh37] 16p12.2(21405328_21931248)x1	526 kb	Loss	46,XX	Born, development delay
31	Lateral ventriculomegaly	arr[GRCh37] 15q11.2(22813068_23290813)x3	478 kb	Gain	46,XX	Born, normal
32	Lateral ventriculomegaly	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XY	Born, normal
33	Lateral ventriculomegaly	arr[GRCh37] 22q11. 22q11.23(22,997,928_25,041,592)x3	2.04 Mb	Gain	46,XY	Born, normal
34	Lateral ventriculomegaly	arr[GRCh37] 7p22.1(5,367,121_5,764,090)x3	396 kb	Gain	46,XX	Born, normal
35	Lateral ventriculomegaly	arr[GRCh37] 1q25.1(174,394,236_174,657,340)x1	263 kb	Loss	46,XY	Born, normal
36	Lateral ventriculomegaly	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XY	Born, normal
37	Choroid plexus cyst	arr[GRCh38] 15q11.2(22582283_23380638)x3	798 kb	Gain	46,XY	TOP
38	Choroid plexus cyst	arr[GRCh37] 2p16.1(59,564,391_60,738,227)x3	1.17 Mb	Gain	46,XY	Born, normal
39	Choroid plexus cyst	arr[GRCh37] 7p22.1(5,212,615_6,332,465)x3	1.12 Mb	Gain	46,XY	Born, development delay
40	NT	arr[GRCh37] 2q13q14.1(111388620_113111856)x3	1.72 Mb	Gain	46,XX	Born, normal
41	NF	arr[GRCh37] 22q11.21(20312661_21464764)x1	785 kb	Loss	46,XY	Born, normal
42	NT	arr[GRCh38] 15q11.2(22582283_23380638)x3	798kb	Gain	46,XY	Born, normal
43	NT	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XX	Born, death
44	EICF	arr[GRCh37] 8p23.2(3688710_4041995)x1	353 kb	Loss	46,XY	Loss to follow-up
45	EICF	arr[GRCh37] 15q11.2(22770422_23288350)x3	518 kb	Gain	46,XY	Loss to follow-up
46	EICF	arr[GRCh37] 2p16.3(51039020_51574265)x1	535 kb	Loss	46,XY	Born, normal
47	EICF	arr[GRCh37] 22q11.21(21058888_21464764)x1	406 kb	Loss	46,XX	Loss to follow-up
48	EICF	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XY	Born, normal
49	EICF	arr[GRCh37] 16p13.11(15,481,747_16,282,869)x4	801 kb	Gain	46,XY	Born, normal
50	EICF	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XY	TOP
51	EICF	arr[GRCh37] 15q11.2(22,770,421_23,290,788)x3	520 kb	Gain	46,XX	Born, normal

Table 4 (continued)

Number	Ultrasound findings	pCNVs (GRCh37)	Size of CNVs	Copy number	Karyotyping results	Outcomes
52	Echogenic bowel	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XX	Born, normal
53	Nasal bone dysplasia	arr[GRCh37] 16p13.11(15058823_16538596)x3	1.48 Mb	Gain	46,XX	Born, normal
54	Nasal bone dysplasia	arr[GRCh37] 9p24.2p24.1(4022149_4903904)x3	882 kb	Gain	46,XY	Born, normal
55	Nasal bone dysplasia	arr[GRCh37] 8q21.11q21.12(76,270,643_78,666,602)x1	2.4 Mb	Loss	46,XY	TOP
56	SUA	arr[GRCh37] 8q11.23q12.1(53696145_59562664)x1	5.87 Mb	Loss	46,XY	Born, normal
57	SUA	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XX	Born, normal
58	Hydrocephalus	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XX	TOP
59	Cisterna magna widen	arr[GRCh37] 17q11.2(29,379,983_30,352,918)x3	972 kb	Gain	46,XY	TOP
60	Absent cavum septum pellucidum	arr[GRCh37] 2p16.3(50730534_50943528)x1	213 kb	Loss	46,XY	Loss to follow-up
61	Cerebellar hemisphere dysplasia, renal dysplasia	arr[GRCh38] 15q11.2(22582283_23375045)x3	793 kb	Gain	46,XY	TOP
62	Hemivertebra	arr[GRCh38] 10q11.22q11.23(45721102_50114612)x1	4.39 Mb	Loss	46,XX	TOP
63	TOF, lateral ventriculomegaly, Orofacial clefts	arr[GRCh37] 12q13.12(49,244,956_49,887,003)x4	642 kb	Gain	46,XX	TOP
64	VR, nasal bone dysplasia	arr[GRCh37] 2p16.1p15(59457176_62680102)x3	3.22 Mb	Gain	46,XX	TOP
65	ARSA, SUA, VSD	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XX	Born, normal
66	SUA, PLSVC	arr[GRCh37] 5q35.3(178,493,921_180,715,096)x1	2.2 Mb	Loss	46,XX	Born, development delay
67	EICF, ectopic kidney, arachnoid cyst	arr[GRCh37] 4q31.3q32.1(153,328,608_158,214,998)x1	4.9 Mb	Loss	46,XX	Loss to follow-up
68	The left kidney cyst	arr[GRCh37] 16p13.3(6665697_7073999)x1	408 kb	Loss	46,XX	Born, normal
69	EICF, hydronephrosis	arr[GRCh37] 17q21.31(42127391_43586368)x3	1.46 Mb	Gain	46,XY	TOP
70	Dysplasia of the left kidney	arr[GRCh37] 22q11.21(20312661_21464764)x1	1.15 Mb	Loss	46,XY	Born, normal
71	Multicystic dysplastic kidneys	arr[GRCh37] 16p12.2(21931248_22442007)x1	511 kb	Loss	46,XX	TOP
72	Multicystic dysplastic kidneys; polydactyly	arr[GRCh37] 6p24.3p24.2(8,105,101_10,992,444)x1	2.89 Mb	Loss	46,XX	TOP
73	Multicystic dysplastic kidneys	arr[GRCh37] 8q13.3(72195441_72527179)x4	332 kb	Gain	46,XX	Loss to follow-up
74	Pyelectasis	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XY	Loss to follow-up

Table 4 (continued)

Number	Ultrasound findings	pCNVs (GRCh37)	Size of CNVs	Copy number	Karyotyping results	Outcomes
75	Multicystic dysplastic kidneys	arr[GRCh37] 1q21.2(148,585,323_149,792,200)x1	1.2 Mb	Loss	46,XX	TOP
76	Lung cystic adenoma	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XX	Born, normal
77	Pyelectasis	arr[GRCh37] 16p11.2(29,591,326_30,191,848)x3	600 kb	Gain	46,XY	Born, normal
78	Lateral ventriculomegaly, EICF	arr[GRCh37] 15q11.2(22770422_23625785)x3	855 kb	Gain	46,XY	Born, normal
79	SUA, polyhydramnios	arr[GRCh37] 1q43q44(243135526_249224684)x3	6.09 Mb	Gain	46,XY	TOP
80	Nasal bone dysplasia, EICF	arr[GRCh38] 1q21.1(143966472_145470346)x3 arr[GRCh38] 15q25.2q25.3(84242064_85247430)x1	1.5 Mb 1 Mb	Gain Loss	46,XX	Born, normal
81	Choroid plexus cyst ,EICF	arr[GRCh37] 1p33p32.2(47948618_58918842)x2 hmz arr[GRCh37] 9q21.33q31.3(89673026_112008165)x2 hmz arr[GRCh37] 14q31 .3q32.33(88388547_105382003)x2 hmz arr[GRCh37] 16p13.3p12.1(6479830_25784920)x2 hmz	10.9 Mb 22.3 Mb 16.9 Mb 19.3 Mb	LOH	46,XY	TOP
82	EICF, echogenic bowel	arr[GRCh37] 2q13(110,876,775_111,400,720)x1	524 kb	Loss	46,XX	Born, normal
83	Absent nasal bone, EICF	arr[GRCh37] Yq11.2 23q11.23(24,741,034_28,420,380)x0	3.68 Mb	Loss	46,XY	TOP
84	EICF, SUA	arr[GRCh37] 2p16.1p15(60,021,142_62,492,163)x3	2.47 Mb	Gain	46,XX	Loss to follow-up
85	SUA, ectopic kidney	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XY	Born, normal
86	EICF, nasal bone dysplasia	arr[GRCh37] 22q11.23(23652518_25002659)x3	1.35 Mb	Gain	46,XY	Born, normal
87	Polyhydramnios, Nasal bone dysplasia	arr[GRCh37] Yq11.2 23q11.23(24,741,034_28,420,380)x0	3.7 Mb	Loss	46,XY	Born, normal
88	Polyhydramnios	arr[GRCh37] 15q11.2(22813068_23290813)x3	478 kb	Gain	46,XY	Born, normal
89	Polyhydramnios	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XX	Born, development delay
90	Bone bending	arr[GRCh37] 15q11.2(22770422_23288350)x3	518 kb	Gain	46,XX	TOP
91	ARSA	arr[GRCh37] 16q13q21(56219551_62178737)x1	5.96 Mb	Loss	46,XX	TOP
92	ARSA	arr[GRCh37] 15q11.2(22813068_23290813)x3	478 kb	Gain	46,XX	Born, normal
93	The arch of the aorta has a small diameter	arr[GRCh37] 16p13.11(14897402_16538596)x3	1.64 Mb	Gain	46,XY	Loss to follow-up
94	Ductus venosus absent	arr[GRCh37] 15q11.2(22813068_23290813)x3	478 kb	Gain	46,XX	Born, normal

Table 4 (continued)

Number	Ultrasound findings	pCNVs (GRCh37)	Size of CNVs	Copy number	Karyotyping results	Outcomes
95	DA	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x3	518 kb	Gain	46,XX	Loss to follow-up
96	DA	arr[GRCh37] 19p13.3(4,332,379_5,176,361)x3	844 kb	Gain	46,XX	Loss to follow-up
97	Posterior Cranial Fossa	arr[GRCh37]10q11. 22q11.23(46,293,590_51,903,756)x1	5.6 Mb	Loss	46,XY	Born, normal
98	Choledochus cyst	arr[GRCh37] 16p13.3(3,922,380_4,999,985)x3	1.08 Mb	Gain	46,XX	TOP
99	Dysplasia of rib	arr[GRCh38] 1q21.1(145002039_146057702)x3	1.06 Mb	Gain	46,XY	Born, normal
100	Diaphragmatic hernia	arr[GRCh37] 15q11.2(22813068_23290813)x3	478 kb	Gain	46,XX	TOP
101	EICF	arr[GRCh37]9q33 .3q34.11(128746809_133782954) x1_2	5.04 Mb	Mosaic loss	46,XY	TOP
102	Enhanced echo in both kidneys, PRUV, Auricle abnormal	arr[GRCh37] 15q11.2(22813068_23290813)x3	478 kb	Gain	46,XY	Born, normal
103	Strephenopodia, EICF	arr[GRCh37] 15q11.2(22,770,421_23,288,350)x4	518 kb	Gain	46,XY	Born, normal
104	Seroperitoneum	arr[GRCh37] 16p13.11(15,058,820_16,538,596)x3	1.48 Mb	Gain	46,XY	Born, normal
105	Battledore placenta	arr[GRCh37] 16p13.3(85,880_364,183) x1	278 kb	Loss	46,XX	Born, normal
106	Cyst of cord, EICF	arr[GRCh37] 3q29(195,739,427_197,386,180)x3	1.65 Mb	Gain	46,XX	Born, normal

pCNVs pathogenic copy-number variants, *lpCNVs* likely pathogenic copy-number variants, *CNVs* copy-number variants, *TOP* termination of pregnancy, *VR* vascular ring, *VSD* ventricular septal defect, *CoA* coarctation of the aorta, *ARSA* aberrant right subclavian artery, *APVC* anomalous pulmonary venous drainage, *SV* single ventricle, *AA* aortic atresia, *PAS* pulmonary artery stenosis, *DORV* double-outlet right ventricle, *NT* nuchal translucency, *NF* nuchal fold, *EICF* echogenic intracardiac focus, *SUA* single umbilical artery, *TOF* tetralogy of Fallot, *PLSVC* persistent left superior vena cava, *DA* duodenal atresia, *PRUV* persistent right umbilical vein

apparently normal, 5 showed developmental delay after birth, and 4 had postnatal death. In the 5393 fetuses with normal results, 41 died after birth, 651 underwent termination of pregnancy, 5352 fetuses were apparently normal at birth, and 565 were lost to follow-up. In the present study, the detail clinical follow-up evaluation for different types of CMA results after prenatal diagnosis are summarized in Table 5.

Table 5 Clinical follow-up assessment of fetuses with different types of CMA results after prenatal diagnosis

Different types of CMA results	Total numbers	Born	TOP	Lost to follow-up
Fetuses with aneuploid	194	9 (4.6%)	183 (94.3%)	2 (1.03%)
Fetuses with pCNVs	269	40 (14.9%)	205 (76.2%)	24 (8.92%)
UPD/Triploid	4	0	4 (100%)	0
Fetuses with lpCNVs	106	59 (55.7%)	35 (33%)	12 (11.3%)
VOUS	193	120 (62.2%)	33 (17.1%)	40 (20.7%)
Normal CMA results	5393	4177 (77.5%)	651 (12.1%)	565 (10.5%)
Total	6159	4405 (71.5%)	1111 (18%)	643 (10.4%)

Abbreviations: *CMA* chromosomal microarray analysis, *TOP* termination of pregnancy, *pCNVs* pathogenic copy-number variants, *lpCNVs* likely pathogenic copy-number variants, *VOUS* variants of unknown significance, *UPD* uniparental disomy

Discussion

Most of the existing studies have focused on soft markers or structural anomalies, but large-scale studies are lacking. Currently, limited data on the clinical outcomes of pregnancies with specific types of ultrasound abnormalities are available. Previous studies have shown that CMA detects 6 to 18.7% of chromosomal abnormalities in fetuses with ultrasound abnormalities [9, 13] and may identify 1.5 to 7.4% of pathogenic CNVs in fetuses with ultrasound abnormalities and normal karyotypes [10, 14]. Our study indicated that the overall frequency of pathogenic chromosomal abnormalities including aneuploidy and CNVs was 7.58% (467/6159); the rate of CNVs with normal karyotype detected by CMA was 3.75% (231/6159), which was in accord with some previous studies [15, 16]. The fetuses with ultrasonographic structural anomalies (9.32%, 278/2982) were significantly higher than fetuses with soft markers (5.95%, 189/3177). We report for the first time that the incidence of chromosomal abnormalities in fetuses with ultrasound structural abnormalities was 9.32% (278/2982) and ranged from 2.53 to 34.62% in groups with different structural anomalies. The incidence of pathogenic chromosomal abnormalities was highest among fetuses with two or more ultrasound structural abnormalities (34.62%, 90/260). lpCNVs were 1.62% (29/1795). The incidence of pathogenic chromosomal abnormalities among fetuses with different structural anomalies is as follows: central nervous system malformations were 10.98% (19/173), congenital heart defects were 7.47% (134/1795), skeletal system malformations were 6.03% (14/232), gastrointestinal system malformations were 4.76% (4/84), and genitourinary system malformations were 4.39% (12/273). Our study findings are slightly different from those of previous studies, in which cardiovascular system, central nervous system, and musculoskeletal system malformations were mostly associated with pathogenic CNVs, but the incidence of chromosomal abnormalities was not completely uniform among fetuses with the same type of ultrasound abnormalities in different studies [17–19]; this may be caused by selection bias, different populational factors, and different sample sizes. However, the incidence rate of pathogenic chromosomal abnormalities among fetuses with respiratory system and facial malformations was relatively low, with rates of 3.49% (3/86) and 2.53% (2/79) respectively. Among the fetuses with soft markers, the overall frequency of pathogenic chromosomal abnormalities including aneuploidy and CNVs was 5.95% (189/3177); aneuploidy was 3.02% (96/3177), which accounts for 51.06%; pCNVs was 2.9% (92/3177), which accounts for 48.94%. The incidence of chromosomal aneuploidy in fetuses with ultrasonographic soft markers is high. The incidence of pathogenic chromosomal abnormalities was highest in fetuses with thickened nuchal fold (16.36%, 45/275), aneuploidy accounts for 86.7% (39/45), and pCNVs account for 13.3% (6/45). Hu et al.'s [2] previous study also showed that the incidence of aneuploidy was highest in thickened nuchal fold fetuses compared with other soft markers. The incidence of pathogenic chromosomal abnormalities for fetuses with thickened nuchal translucency in our study was higher than that in Hu et al.'s report, but the sample size is larger and more representative. Karyotype analysis can detect most abnormalities in fetuses with nuchal translucency (NT) abnormalities, but there was still 2.18% fetuses with pCNVs; CMA is more meaningful. Our study indicated that the use of CMA in prenatal diagnosis is necessary and can significantly improve the detection rate of pathogenic CNVs. In addition, for fetuses with an absent/hypoplastic nasal bone, the incidence rate of aneuploidies and pCNVs was 3.85% (7/182) and 2.2% (4/182) respectively, so the incidence of aneuploidies especially trisomy 21 was also higher in this group. Huang et al. [20] also showed a strong correlation between chromosomal abnormalities and fetal nasal bone hypoplasia. Excluding fetuses with NT thickening and absent/hypoplastic nasal bones, the incidence of pCNVs was high among fetuses with other ultrasonographic soft marker abnormalities. For fetuses with mild ventriculomegaly, the incidence rate of chromosome aneuploidies and pCNVs was 1.16% (3/158) and 5.81% (15/158) respectively, and the incidence of pCNVs was significantly higher than that of aneuploidies. Previous studies have reported that the incidence of chromosomal abnormalities ranges from 5.7 to 12.1% in different cohorts [21–24]. The overall incidence of pathogenic chromosomal abnormalities was 6.98%, and this difference may be attributed to selection bias. CMA is a promising prenatal diagnosis tool that can provide valuable data for accurately assessing fetal prognosis and deciding whether to continue pregnancy during prenatal clinical consultation.

In the present study, a total of 35 types of microdeletion/microduplication syndromes were detected in 187 fetuses, such as 22q11.2 microdeletion syndrome, 1p36 microdeletion syndrome, 15q11.2 microdeletion syndrome, 3q29 microdeletion syndrome, and Williams-Beuren syndrome. The 22q11.2 microdeletion syndrome was the most common chromosomal microdeletion syndrome, showing a wide phenotypic spectrum include congenital heart disease, gastrointestinal symptoms, and psychiatric illnesses and has an estimated incidence of 1/4000–6000 livebirths [25–27]. 22q11.2 microdeletion

syndrome is closely related to congenital heart diseases (CHDs), which prompts genetic counseling, especially for complex heart abnormalities associated with other malformations, for which it is suggested that CMA detection be conducted to prevent the birth of children with birth defects. High-resolution CMA allowing for the detection of submicroscopic imbalances, except for the usual microdeletion syndromes, was also helpful to detect single-gene diseases caused by deletions. In the present study, 7 male fetuses with *DMD* gene deletion without any family history of dystrophinopathy were incidentally detected using CMA, and karyotyping of the fetuses showed normal 46,XY. The deletion was further verified by denaturing high-performance liquid chromatography, and parental study revealed maternal inheritance or de novo inheritance. The deletion or disruption of the *DMD* gene may result in Duchenne muscular dystrophy (DMD) or Becker muscular dystrophy (BMD). DMD seriously affects the quality of life and survival of the patients and currently, there is no effective treatment. Prenatal diagnosis is necessary to provide accurate prognostic information for genetic counseling and potential options for the family regarding clinical management.

CMA exhibits a high efficiency for the diagnosis of fetal chromosomal abnormalities and unavoidable and multiple VOUS with unclear relevance to the detected clinical phenotypes [14]. The identification of VOUS during prenatal diagnosis continues to be a challenging issue prenatally, which may lead to difficulty in clinical genetic counseling and stress for pregnant women and their families and even result in excessive induction of labor. VOUS has been identified in less than 5% of all prenatal samples [28, 29]. In the current study, a 1.72% prevalence rate of lpCNVs and a 3.04% prevalence rate of VOUS were detected in the 6159 fetuses, which was consistent with previous reports [9, 30], but higher than those of a previous study [10]. These differences may be caused by different interpretation biases and reporting standards. 15q11.2 duplication, including the BP1-BP2 region, encompasses four highly conserved genes: *TUBGCP5*, *NIPA1*, *NIPA2*, and *CYFIP1*, which are the most common lpCNVs (31.1%, 33/106). CNVs involving this region present a major challenge in prenatal testing because they have been reported in affected individuals with healthy family members of affected probands. Most CNVs in this area are inherited without significant clinical manifestations from parents. The phenotypes associated with CNVs are known for their variability, incomplete penetrance, and wide phenotypical spectrum, even among members of the same family [31]. The microduplication of 15q11.2 had a low penetrance, but increased the risk of developmental delays and mental retardation [32, 33]. CMA with SNP probes can also detect loss of heterozygosity (LOH) and uniparental disomy (UPD). Excluding clearly imprinted genes, the clinical significance of LOH and UPD is unclear, and the recessive disease-causing genes contained in the regions increase the risk of hereditary diseases, making genetic counseling difficult. Therefore, further studies are needed to accurately assess fetuses with VOUS.

Conclusions

Fetuses with ultrasound abnormalities are at increased risk of chromosomal abnormalities including CNVs and aneuploidy. Our present study aimed to investigate the incidence rates of chromosomal abnormalities and pregnancy outcome and postpartum clinical manifestations by long-term follow-up. Detection by CMA with SNP probes can be used as an effective method for the prenatal genetic diagnosis of fetal ultrasound abnormalities and can enhance the detection rate of chromosomal abnormalities. Prenatal CMA should be recommended for fetuses with ultrasound abnormalities. The present study also provides important data including the prevalence and distribution of chromosomal abnormalities among fetuses with different types of ultrasound aberrations and pregnancy outcomes that may assist physicians and geneticists in proper genetic counseling.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s43032-023-01399-2.

Acknowledgements We thank all the family members for participating in the research and the support of financial fundings.

Author Contributions JZ, LJ, and HY conceived and designed the study. TS drafted the manuscript. YX revised the manuscript. YL and JZ performed the statistical analysis and participated in its design. FG revised the grammar of the manuscript. All authors read and approved the final manuscript submitted for publication.

Funding This work was supported by the Xi'an City Innovation Ability strong base plan-medical research project under Grant 21YXYJ0106 and the National Natural Science Foundation of China under Grant 82172993.

Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing Interests The authors declare no competing interests.

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