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Prenatal diagnosis of 913 fetuses samples using copy number variation sequencing

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Abstract

Background: The present study aimed to explore the etiological relationship between fetal abnormalities and copy number variations (CNVs) with the aim of intervening and preventing the birth of children with birth defects in time.

Methods: Samples of 913 fetuses with puncture indications were collected from January 2017 to December 2019. Karyotype analysis and CNV sequencing (CNV-seq) testing was performed for fetuses with ultrasonic abnormalities, a high risk of Down's syndrome and an adverse birth history. All cases were followed up.

Results: In total, 123 cases (13.47%) had abnormal karyotypes, including 109 cases with chromosome number abnormalities and 14 cases of chromosomal structural abnormalities. Thirty-seven (4.05%) cases with pathogenic CNVs were detected. The detection rate of pathogenicity CNVs was 12.82% for mixed indications, followed by 7.5% for an adverse birth history, 5.88% at high risk of non-invasive prenatal testing, 5.00% with an abnormal ultrasonic marker, 1.89% at high risk of screening for Down's syndrome and 1.45% with advanced maternal age. There were 12 (1.31%) cases with microduplications and 25 (2.74%) cases with microdeletions. Trisomy 21 (39.02%), trisomy 18 (13.82%) and Turner syndrome (9.76%) were the top three chromosome abnormalities. There were 104, 746 and 63 cases in the 11–13 weeks, 14–27 weeks 28–38 weeks gestational ages, respectively. The abnormal rates of fetal chromosome aneuploidy and the rate of pathogenic CNVs were decreased and increased with the increase of gestational age ($p < 0.05$), respectively.

Conclusions: Compared with karyotype analysis, CNV-seq can improve the detection rate of chromosomal abnormalities. CNV-seq combined karyotype analysis should be performed simultaneously in fetuses with puncture indications.

KEYWORDS
copy number variation sequencing, karyotype analysis, microdeletion, microduplication

1 | INTRODUCTION

Currently, there is no effective treatment for chromosomal disorders and prenatal diagnosis is an important means for avoiding the birth of children with chromosomal abnormalities. Prenatal diagnosis mainly involves the genetic testing of fetal cells such as villi, amniotic fluid and umbilical cord blood obtained by means of intervention. Karyotype analysis is still the gold criteria for the diagnosis of chromosomal diseases. It has been widely used in prenatal diagnosis. However, there are limitations to karyotype analysis. First, it will take a relatively long time (1–2 weeks) because of the cell culture. Second, the karyotype analysis technique can only diagnose a fetal chromosome with deletion and duplication of more than 5–10 Mb, and even minor structural changes may be missed.[1,2]

With the widespread use of high-resolution chromosomal analysis techniques in prenatal diagnosis, there is increasing evidence that pathogenic copy number variations (pCNVs) account for a certain percentage of the fetuses, such as for pregnant women with advanced age, ultrasound abnormalities or an adverse pregnancy history. Up to now, there have been over 300 types of chromosomal microdeletion/microduplication syndrome caused by pCNVs,[3,4] with a comprehensive incidence of almost 1 in 600,[3] accounting for half of the birth defects caused by chromosomal aberrations.[5] Studies have shown that 6–7% of fetuses with no abnormalities in karyotype analysis but with ultrasonic indications of structural abnormalities have definite or possible pathogenic CNVs.[6,7] In addition, 1.0–1.7% of fetuses with no abnormalities of karyotype analysis and ultrasound have definite or possible pathogenic CNVs.[6,8] A growing number of researchers and clinicians suggest that all pregnant women should be informed about the risk of the fetus developing pathogenic chromosomal abnormalities, not just the common aneuploidy.

Chromosomal microarray analysis (CMA) can be used to detect various microdeletion and microduplication syndromes caused by chromosomal microdeletion or microduplication.[9] In recent years, CMA has become a mature clinical high-resolution chromosome analysis technique. However, the high cost and low throughput of CMA limit its large-scale application in prenatal diagnosis. In addition, as a result of the limited coverage of chip probe used by CMA, some CNVs may not be detected. Currently, it is mainly used in prenatal diagnosis for fetuses with an abnormal chromosomal structure.[10] With the development of next generation sequencing (NGS) technology, the NGS-based copy number variation sequencing (CNV-seq) technology has gradually developed to become a detection method with high-throughput, higher accuracy and sensitivity, and lower costs.[11,12] CNV-seq based on NGS technology conducts sequencing analysis on samples, compares the sequencing results with the human reference genome and identifies CNVs through biological information analysis. CNV-seq can detect chromosomal aneuploidy, chromosomal CNVs over 100 kb and polyploidy.[13–15] In the present study, samples of fetuses with puncture indications were analyzed with CNV-seq, the sequencing results were compared with the sequence of the human reference genome and CNVs in the tested samples were found through bioinformation analysis.

2 | MATERIALS AND METHODS

2.1 | Participants

In total, 913 fetuses with indications of prenatal diagnosis receiving invasive prenatal diagnosis were collected from January 2017 to December 2019 at the Prenatal Diagnosis Center of Meizhou People's Hospital, Guangdong Province, China. Fetuses were recruited with respect to pregnant women with advanced age (age $\geq$ 35 years), fetuses with a high risk of screening for Down's syndrome, for those at high risk of non-invasive prenatal testing (NIPT) using cell-free fetal DNA from peripheral maternal blood, and for a fetal abnormality revealed by ultrasound. These samples were collected from fetuses during 11–36 weeks gestational age, including villi samples, amniotic fluid samples and umbilical blood samples. Both parents were informed about the advantages and limitations of karyotype analysis and CNV-seq and consented to test during genetic counseling. Parents' peripheral blood samples of each fetal sample were obtained to facilitate the identification of maternal contamination of fetal samples and to help identify the nature of CNVs when necessary.

2.2 | Fetal samples collection and pretreatment

Fetal sampling was performed in three ways: (i) chorionic villi sampling was performed under ultrasound guided at 1–14 weeks of gestation, and villus tissue was collected and sent for examination; (ii) amniocentesis was performed under ultrasound guided amniocentesis at 16–24 weeks gestation age, and 30 ml of amniotic fluid was taken and sent for examination; and (iii) umbilical cord puncture was performed under ultrasound guided at 24–32 weeks gestational age, and 2 ml of cord blood was collected and sent for examination.

2.3 | Chromosome karyotype analysis

Villi, amniotic fluid or umbilical cord blood were collected for cell inoculation, and samples from each pregnant woman were cultured for two lines. The cell state was observed under a microscope after 7 days, and the culture was continued after changing the liquid. Karyotyping was performed on G-band metaphases prepared from cultured cells of specimens obtained from chorionic villus sampling, amniocentesis and umbilical cord blood, in accordance with the laboratory's standardized procedures. The detected chromosomes were named according to the International System for Human Cytogenetic Nomenclature.

2.4 | Short tandem repeats (STR) analysis

Fetal samples may be confused by maternal cells and so STR analysis was conducted before carrying out detection in the fetal samples. Genomic DNAs were extracted from the peripheral blood of parents, as

well as villus, amniotic fluid or cord blood of the fetuses, using a DNA extraction kit (Tiangen Biotech Co., Ltd, Beijing, China). STR analysis was performed with the markers including D19S433, D5S818, D21S11, D18S51, D6S1043, AMEL, D3S1358,D13S317, D7S820, D16S539, CSF1PO, Penta D, D2S441, vWA, D8S1179, TPOX, Penta E, TH01, D12S391, D2S1338 and FGA (Microread Genetics Technology Co., Ltd, Beijing, China) using an ABI 3500xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). When all the polymorphic alleles of the mother were absent from the fetal sample, the fetal sample was considered free from maternal contamination.

2.5 | CNV-seq

The extraction of genomic DNA was performed using DNAeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA), and 50 ng of genomic DNA was used as the template to construct a sequencing library. Finally, sequencing was performed on BioelectronSeq 4,000 Platform (Thermo Fisher, Waltham, MA, USA). Wheeler Aligner, version 0.7.7 (https://acronyms.thefreedictionary.com/Burrows-Wheeler), was used to compare and analyze the sequence reading information with the human reference genome (GRCh37, UCSC Release HGL9) to obtain the bioinformatics results, determine the existence of chromosomal aneuploidy variation and CNVs, and evaluate the pathogenicity of a CNV detected by **ISCA** (https://isca.org.sg/), **Decipher** (https://decipher.sanger.ac.uk/), Clinvar (https://www.ncbi.nlm.nih.gov/clinvar/) and other databases. In the interpretation of CNVs, including five grades according to the American College of Medical Genetics and Genomics guidelines: (1) pathogenic CNVs (pathological CNVs, pCNVs), (2) likely pathogenic CNVs; (3) CNVs with unknown clinical significance; (4) likely benign CNVs; and (5) benign CNVs. CNV-seq testing and bioinformatics analysis were completed by CapitalBio Genomics Company (Dongguan, Guangdong Province, China).

2.6 | Follow-up and statistical analysis

All pregnant women were followed up by telephone to track pregnancy outcome and newborn health status. SPSS, version 21.0 (IBM Corp., Armonk, NY, USA) was used for data analysis. Data are reported with the descriptive statistics method and measurement data are expressed as the mean ± SD. A chi-squared test was used to analyze differences among the two groups. $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Characteristics of subjects and detection rate of karyotype and CNV abnormalities

The mean ± SD age of the pregnant women was 29.84 ± 5.82 years with a gestational duration of 19.85 ± 5.16 weeks. The reasons why 913 pregnant women underwent fetal sampling were divided into six categories: 43.81% (400/913) had a fetal abnormality revealed by ultrasound; 28.91% (264/913) had a high risk of screening for Down's syndrome; 7.56% (69/913) were pregnant women with advanced age; 5.59% (51/913) had undergone NIPT suggesting the existence of partial chromosome duplication and deletion; 5.48% (50/913) had both parents have the same type of thalassemia; and 4.38% (40/913) had previously given birth to abnormal children (Table 1).

The chromosome karyotype analysis of 913 fetus samples revealed that 123 cases (13.47%) had abnormal karyotypes, including 109 cases with chromosome number abnormalities and 14 cases of chromosomal structural abnormalities. Thirty-seven (4.05%; 37/913) cases with pathogenic CNVs were detected. According to the reasons for classification, the detection rate of pathogenicity CNVs was 12.82% for mixed indications, followed by 7.5% for an adverse birth history, 5.88% for a high risk of NIPT, 5.00% for an abnormal ultrasonic marker, 1.89% for a high risk of screening for Down's syndrome and 1.45% for advanced maternal age (Table 1).

3.2 | Detection results of fetuses with pathogenic CNVs

Thirty-seven (4.05%) cases with pathogenic CNVs were detected. The fragment size of the detected chromosomal pathogenic CNVs was

TABLE 1 The detection rate of karyotype and CNV abnormalities in various prenatal diagnostic indications

Indications	Number of cases	Abnormal karyotype (*n*)	Detectable rate (%)	Pathogenic CNVs (*n*)	Detectable rate (%)
Abnormal ultrasonic marker	400	36	9.00	20	5.00
High risk of screening for Down's syndrome	264	14	5.30	5	1.89
Advanced maternal age	69	3	4.35	1	1.45
High risk of NIPT	51	32	62.75	3	5.88
Both parents have the same type of thalassemia	50	0	0	0	0
Adverse birth history	40	2	5.00	3	7.50
Mixed indications	39	36	92.31	5	12.82
Total	913	123	13.47	37	4.05

TABLE 2 Detection results and outcomes of 37 fetuses with pathogenic CNVs detected

Num.	Specimen type	Maternal age (years)	Weeks of gestation	CNV result	• Syndrome/genes involved	Clinical feature	Karyotype	Outcome
1	AF	20	31	Chr2:111360000–113,080,000; 1.72 Mb del	• 2q13 deficiency syndrome	• Fetal right renal hydronephrosis, cyst	Normal	TOP
2	AF	28	28	Chr16:15500000–16,280,000; 0.78 Mb dup	NDE1,MYH11, ABCC6	• Fetal umbilical cord root cyst	Normal	IUD
3	AF	26	26	Chr1:146500000–147,840,000; 1.34 Mb dup	• 1q21.1 recurrent repetition syndrome	• Fetal ultrasound was abnormal, ventricular septal defect, tricuspid regurgitation, nasal bone loss	Normal	TOP
4	AF	33	21	Chr4:86440000–93,600,000; 7.16 Mb del	PKD2	Adverse birth history	Normal	TOP
5	AF	29	23	Chr18:120000–7,320,000; 7.20 Mb dup	None	Non-invasive trisomy 18 has a high risk;fetal heart proportion increases, right subclavian artery vagus	46,Xn,inv (18) (p11.32q11.2)	IUD
6	AF	28	18	Chr16:21960000–22,440,000; 0.48 Mb del	• Recurrent 16P12.1 microdeletion syndrome	The fetus has a large bladder with two renal pelvis and calyces separated and right locked Subosseous artery disorientation, bipedal varus	Normal	TOP
7	AF	43	17	Chr7:72720000–74,120,000; 1.40 Mb dup	• 7q11.23 repetitive syndrome	• Advanced age, high risk for T21	Normal	TOP
8	AF	29	16	ChrX:6460000–8,140,000; 1.68 Mb del	• X chain ichthyosis	• Single umbilical	Normal	TOP
9	AF	21	18	Chr16:14800000–16,840,000; 2.04 Mb del	MYH11, ABCC6	• Tang sieving 18 trisomy high risk	Normal	TOP
10	CV	23	12	Chr15:22760000–23,100,000; 0.34 Mb del	• 15q11.2 deficiency syndrome	• NT thickening = 3.4 mm	Normal	TOP
11	AF	28	28	Chr16:48540000–54,220,000; 5.68 Mb del	NOD2, SALL1	• Fetus has a shorter mandible	Normal	TOP
12	AF	33	19	Chr5:20000–23,640,000; 23.62 Mb del	Cri du chat syndrome	Non-invasive screening suggested 16.9Mdel on chromosome 5 and 17Mdup on chromosome 20, fetal ultrasound abnormalities, dilatation of the lateral and third ventricles, broadening of the posterior keyhole cistern, and vagus of the right subclavian artery	46,Xn,del (5) (p15.1 → pter)	TOP
13	AF	34	20	Chr16:21940000–22,420,000; 0.48 Mb del	• Recurrent 16P12.1 microdeletion syndrome	• Tang sieving 21 critical risk 1/316	Normal	IUD

TABLE 2 (Continued)

Num.	Specimen type	Maternal age (years)	Weeks of gestation	CNV result	• Syndrome/genes involved	Clinical feature	Karyotype	Outcome
14	CV	27	13	Chr22:18960000–21,460,000; 2.5 Mb del	• 22q11.2 microdeletion syndrome	• NT thickening = 4.0 mm	Normal	TOP
15	AF	29	25	Chr16:15480000–18,160,000; 2.68 Mb del	• 16P13.11 microdeletion syndrome	• Small ventricular septal defect	Normal	TOP
16	AF	33	17	Chr22:214600000–23,640,000; 2.18 Mb del	• 22q11.2 distal deletion syndrome	• NT thickening = 5.0 mm	Normal	TOP
17	AF	28	24	Chr9:200000–8,260,000; 8.06 Mb del	SMARCA2,JAK2	• Bilateral cleft lip and alveolar cleft, hard palate and soft palate	Normal	TOP
18	AF	21	17	Chr15:22760000–23,100,000; 0.34 Mb del	• 15q11.2 deficiency syndrome	• NT thickening = 3.6 mm	Normal	TOP
19	AF	20	24	Chr22:18920000–21,460,000; 2.54 Mb del	• 22q11.2 microdeletion syndrome	• Fetal spina bifida, cardiovascular dysplasia	Normal	TOP
20	AF	20	26	Chr11:121320000–134,800,000;13.48 Mb del	Jacobsen syndrome	The fetus has bilateral paracentricular cysts with cystic hyperplasia large, ventricular septal defect (contrapuntal type), subclavian artery vagus	Normal	IUD
21	AF	22	18	Chr2:111420000–113,100,000; 1.68 Mb dup	MERTK, TMEM86B	• Fetus lacks the second knuckle of both hands	Normal	TOP
22	CB	24	36	Chr5:100000–8,860,000; 8.76 Mb del	Cri du chat syndrome	• Duodenal obstruction, ventricular septal defect	Normal	TOP
23	AF	39	25	Chr22:17100000–18,560,000; 1.46 Mb dup	IL17RA, CECR1, ATP6V1E1	Fetal intrauterine growth restriction	Normal	TOP
24	AF	32	26	Chr17:14060000–15,700,000; 1.64 Mb del	• Hereditary stress susceptibility neurosis	• Single umbilical artery, right supraventricular vagus	Normal	TOP
25	AF	30	18	Chr7:143940000–159,120,000; 15.18 Mb del	CNTNAP2	Microcephaly	Normal	IUD
26	AF	36	18	Chr18:68100000–78,000,000; 9.9 Mb del	CYB5A, TSHZ1, CTDP1, TXNL4A	• Non-invasive suggestion: There is an 8M deletion on chromosome 18	Normal	TOP
27	AF	34	18	Chr16:15140000–16,280,000; 1.14 Mb dup	NDE1, NYH11, ABCC6	• Tang sieving 21trisomy high risk	Normal	TOP
28	AF	17	20	Chr1:146500000–147,800,000; 1.3 Mb dup	None	Lateral ventricle widened	Normal	TOP
29	AF	27	22	Chr16:28820000–29,040,000; 0.22 Mb dup	TUFM, ATP2A1, CD19, LAT	• NT thickening = 3.3 mm	Normal	TOP

(Continues)

TABLE 2 (Continued)

Num.	Specimen type	Maternal age (years)	Weeks of gestation	CNV result	• Syndrome/genes involved	Clinical feature	Karyotype	Outcome
30	AF	28	26	Chr13:48300000–58,340,000; 10.04 Mb del	SUCLA2, NUDT15, ITM2B, RB1, LPAR6, RCBTB1, RNASEH2B, ATP7B、ALG11	• Ependymal cyst, giant skull	Normal	TOP
31	AF	27	18	Chr16:15500000–18,180,000; 2.68 Mb del	• 16P13.11 microdeletion syndrome	Strong echo in right lower abdomen, hyperamniotic fluid	Normal	TOP
32	CB	29	30	Chr17:1700000–3,520,000; 1.82 Mb del	Miller– Dieker syndrome	Lateral ventricle widened	Normal	TOP
33	AF	31	26	Chr4: 80000–14,280,000; 14.2 Mb del	Wolf–Hirschhorn syndrome	Ventricular absence, vermiform hypoplasia of the cerebellum, small kidney size, gallbladder, spine, sacrococcygeal shape is incomplete, spine, low conical position, deformity of right hand	46,Xn,del (4) (pter→p15.2:)	IUD
34	AF	22	30	Chr2:20000–42,300,000; 42.28 Mb dup	• 2P distal trisomy syndrome	Left lateral ventricle widened	47,XN,der(2;21)(p21; p11.2)	TOP
35	AF	39	20	Chr4:168780000–190,780,000; 22 Mb dup	• Trisomy 4q distal trisomy syndrome	• Advanced age, high risk for T21	46,Xn,dup (4) (q32.2 → qter)	TOP
36	AF	31	26	Chr15:51100000–60,460,000; 9.36 Mb del	TCF12	Fetal lung cystadenoma, gallbladder undetected, hilar Small cyst	Normal	TOP
37	AF	35	22	Chr4:178120000–190,780,000; 12.66 Mb del	• Deletion syndrome of long arm end of chromosome 4	• Non-invasive indication of fetal chromosome 4 abnormality	46,Xn,ins(4)t(4;11) (q35;q14.2 → qter)	TOP

TOP, termination of pregnancy; IUD, intrauterine death; LB, live birth; CV, chorionic villi; AF, amniotic fluid; CB, cord blood.

TABLE 3 Results of chromosomal karyotype abnormalities and corresponding CNV results

Cases (*n*, %)	Karyotype result	CNV result	Consistency of the two results	Pregnancy outcome
I (48, 39.02%)	47,Xn,+21	47,Xn,+21	Conforming	TOP
II (17, 13.82%)	47,Xn,+18	47,Xn,+18	Conforming	TOP
III (12, 9.76%)	45,X0	45,X0	Conforming	TOP
IV (11, 8.94%)	47,XXY	47,XXY	Conforming	TOP
V (5, 4.07%)	47,Xn,+13	47,Xn,+13	Conforming	TOP
VI (4, 3.25%)	47,XYY	47,XYY	Conforming	TOP
VII (2, 1.63%)	47,xxx	47,xxx	Conforming	TOP
VIII (1, 0.81%)	47,Xn,+9	47,Xn,+9	Conforming	TOP
IX (1, 0.81%)	48,XXXY	48,XXXY	Conforming	TOP
X (1, 0.81%)	46,Xn,del (4)(pter→p15.2:)	Seq[hg19] 4pterp15.33(0.08 Mb–14.28 Mb) × 1	Conforming	TOP
XI (1, 0.81%)	46,Xn,ins(4)t(4;11)(q35; q14.2 → qter)	Seq[hg19] 4q34.3qter(178.12 Mb–190.78 Mb) × 1	Conforming	TOP
XII (1, 0.81%)	46,Xn,dup (4)(q32.2 → qter)	Seq[hg19] 4q32.3qter(168.78 Mb–190.78 Mb) × 3	Conforming	TOP
XIII (1, 0.81%)	47,XN,der(2;21)(p21;p11.2)	Seq[hg19] 2pterp21(0.02 Mb–42.3 Mb) × 3	Conforming	TOP
XIV (1, 0.81%)	46,Xn,del (5)(p15.1 → pter)	Seq[hg19] 5pterp14.2(0.1 Mb–23.88 Mb) × 1	Conforming	TOP
XV (1, 0.81%)	46,Xn,inv (18)(p11.32q11.2)	18q11.2q12.1(0.12 Mb–7.32 Mb) × 3	Conforming	TOP
XVI (10, 8.13%)	46,Xn,inv (9)(p13q13)	Nomal	Nonconforming	LB
XVII (1, 0.81%)	46,Xn,t(1;14)(q42;q13)	Nomal	Nonconforming	LB
XVIII (1, 0.81%)	46,Xn,inv (8)(p23.1q13)	Nomal	Nonconforming	LB
XIX (1, 0.81%)	46,Xn,t(3;13)(p11;q32)	Nomal	Nonconforming	LB
XX (1, 0.81%)	46,Xn,inv (7)(q22q32)	Nomal	Nonconforming	LB
XXI (1, 0.81%)	69, XXX	8q23.1q23.2(109.46 Mb–110.66 Mb)*3	Nonconforming	TOP
XXII (1, 0.81%)	92, XXX	17p11.2(17.1 Mb–20.22 Mb)*1	Nonconforming	TOP

TOP, termination of pregnancy; IUD, intrauterine death; LB, live birth.

220 kb to 42.28 Mb. There were 12 (1.31%; 12/913) with microduplications and 25 (2.74%; 25/913) with microdeletions. There are 18 known syndromes: 2q13 microdeletion syndrome, 1q21.1 microduplication syndrome, 16P12.1 microdeletion syndrome, 7q11.23 microduplication syndrome, X-linked ichthyosis, 15q11.2 microdeletion syndrome, Cri du chat syndrome, 22q11.2 microduplication syndrome, 16p13.11 microdeletion syndrome, 22q11.2 distal deletion syndrome, 22q11.2 microdeletion syndrome, Jacobsen syndrome, hereditary stress susceptibility neurosis, Miller–Dieker syndrome, Wolf–Hirschhorn syndrome, 2P distal trisomy syndrome, trisomy 4q distal trisomy syndrome and deletion syndrome of long arm end of chromosome 4. Among the fetuses with pathogenic CNVs, there were six intrauterine deaths (IUD) and 31 terminations of pregnancy (TOP) (Table 2).

3.3 | Comparison of karyotype analysis and CNV-seq results

The karyotype analysis was consistent with the CNVs detection results for chromosome aneuploidy abnormalities. Trisomy 21 (39.02%), trisomy 18 (13.82%) and Turner syndrome (9.76%) were the top three chromosome abnormalities. Fourteen cases with chromosomal structural abnormalities (12 cases of inversion and 2 cases of translocation) were not detected by high-throughput sequencing (Table 3).

3.4 | Comparison of CNV results among fetuses of different gestational ages

There were 104, 746 and 63 cases in the 11–13 weeks, 14–27 weeks and 28–38 weeks gestational ages, respectively. The abnormal rates of fetal chromosome aneuploidy were 25.10% (26/104), 11.00% (82/746) and 1.60% (1/63) in these groups, respectively, which decreased with the increase of gestational age ($\chi^2 = 24.287$, $p < 0.001$). The rates of variants of unknown significance (VOUS) were 23.10% (24/104), 31.50% (235/746) and 44.40% (28/63), respectively, which increased with the increase of gestational age ($\chi^2 = 10.041$, $p = 0.007$). The abnormal rate of pCNV was 1.92% (2/104), 3.89% (29/746) and 9.52% (6/63), respectively, and the difference was statistically significant ($\chi^2 = 6.866$, $p = 0.032$) (Table 4).

4 | DISCUSSION

Chromosome abnormalities include chromosome number abnormalities and chromosome structure abnormalities. Patients with chromosomal abnormalities are usually characterized by congenital mental retardation, delayed development, multiple malformations, sexual hypoplasia, repeated abortions and infertility. CNVs is a type of genetic structure variation that widely exists in the human genome. In recent years, it was confirmed to be related to many complex mental diseases in human beings, and it was noted that the information contained within it will be much larger than that for single nucleotide polymorphisms. Chromosome microdeletions and microduplications can lead to some complex clinical phenotypes (such as abnormal growth and development, mental retardation, deformity of the internal organs, endocrine abnormalities, etc.) of the syndrome, comprising common types of chromosome disease. More than 67 common chromosome microdeletion and microduplication syndromes have been found, with an incidence of approximately one-quarter of one in 0.25 in 4000 to 1 in 50,000.[16]

CNV-seq comprises a genomic copy number variation detection technology based on low-depth whole-genome sequencing technology. CNV-seq can detect CNVs of different sizes by adjusting the sequencing depth and changing the resolution. The resolution of the method used in the present study is 100 kb, which can make up for the deficiency of the low resolution of karyotype analysis. CNV-seq has the advantages of a wide detection range, high throughput, high resolution, simple operation and low DNA sample size, and many studies have evaluated the applicability of the method. Wang *et al.*[17] reported that the detection rate increased from 1.8% to 2.8% compared to the technology of karyotype analysis with respect to being pathogenic or possibly pathogenic, showing good reliability and accuracy. In the present study, 109 cases (11.94%) were detected with pathogenic variants by CNV-seq and karyotype analysis simultaneously, 101 cases were detected with pathogenic chromosome aneuploidy abnormality, including 48 cases with trisomy 21, 17 cases were detected with trisomy 18, five cases were detected with trisomy 13, one case was detected with trisomy 9 and 30 cases were detected with sex chromosome aneuploidy abnormality, whereas 37 cases were detected with pathogenic chromosome microdeletion (30 cases with definite pathogenic chromosome microdeletion and seven cases with possible pathogenesis of chromosome microdeletion). Both CNV-seq and karyotype analysis detected abnormal aneuploidy of pathogenic chromosomes, although 31 cases of pathogenic chromosome microdeletion were not detected in karyotype analysis. The results show that it is very necessary to use CNV-seq test in the prenatal diagnosis, which can significantly increase the detection rate of pathogenic chromosomal microdeletions and microduplications.

A number of syndromes were also identified in the present study, such as X-linked ichthyosis (sample 8), for which the main clinical characteristics are dark brown polygonal scales widely distributed in the neck, limbs, trunk and buttocks, which may be accompanied by corneal opacity that does not affect vision, as well as an increased incidence of cryptorchidism and testicular cancer. There was one patient with 15q11.2 deficiency syndrome (sample 10), for which the clinical symptoms included delayed movement, intellectual disabilities, autistic behavior, overall developmental delay, severe motor retardation, epilepsy, flexion contracture, epilepsy, spasm and short stature. There was one patient with Cri du Chat syndrome (46,Xn,del (5)

TABLE 4 Comparison of CNV results among pregnant women of different gestational ages

	Gestational weeks				
CNV result	**11–13 (*n*, %)**	**14–27 (*n*, %)**	**28–38 (*n*, %)**	**χ^2**	**p**
Number	104	746	63		
Chromosome aneuploidy	26 (25.10)	82 (11.00)	1 (1.60)	24.287	< 0.001
VOUS	24 (23.10)	235 (31.50)	28 (44.40)	10.041	0.007
pCNV	2 (1.92)	29 (3.89)	6 (9.52)	6.866	0.032

VOUS, variants of unknown significance.

(p15.1 → pter), sample 12), for which the clinical symptoms included a weak, sad, cat-like cry in infancy that improves with age, eyes at a widened distance, flat nose, low ear position, small jaw, growth retardation, severe mental retardation, heavier than normal weight. There was one patient with 22q11.2 microdeletion syndrome (sample 14), for which the clinical symptoms included high body size, prominent forehead, abnormal behavior, intellectual disability, horseshoe pronation, lower limb muscle atrophy, facial abnormalities, hypotonia, premature delivery, hypoplasia of the left heart, cleft palate, intrauterine growth retardation. There was one patient with Jacobsen syndrome (11q23 deletion syndrome, sample 20), for which the main clinical symptoms included skull deformity, eyes at a widened distance, ptosis, eye defects, lower oblique palpebral fissure, inner canthus, wide bridge of the nose, short nose, V-shaped mouth and small posterior rotating ear. Other clinical symptoms included eye, hearing, immune and hormonal abnormalities. There was one patient with Miller–Dieker syndrome (sample 21), a syndrome characterized by cardiac abnormalities, a prominent forehead, anencephaly, microcephaly and midface retraction, in which brain abnormalities usually cause severe mental retardation, developmental delay, seizures, low muscular tone and feeding difficulties. Our study confirmed that CNV-seq is an effective method for detecting these chromosomal variations. Therefore, the combined application of karyotype analysis and CNV-seq in the prenatal diagnosis of pregnant women with antenatal indications is of great clinical significance.

In total, 913 prenatal samples were collected in the present study. According to the reasons for the visit, they were mainly classified into an adverse birth history, a high risk of screening for Down's syndrome, both parents with the same type of thalassemia, advanced maternal age, a high risk of NIPT and abnormal ultrasonic marker. The overall detection rate of pCNVs was 4.05% (37/913). In the positive samples, there were 20 cases with an abnormal ultrasonic marker, five cases with a high risk of screening for Down's syndrome, three cases with a high risk of NIPT, three cases with an adverse birth history, one case with advanced maternal age and five cases with mixed indications. In terms of the detection rate of different types of CNVs, the detection rate of mixed indications was the highest, followed by an adverse birth history. However, the proportion of deviation caused by the small sample size of the first two samples in the present study cannot be excluded. The 22q11.2 microdeletion detection rate was highest, with the incidence of the disease in newborns being one in 4000, comprising the highest rate of microdeletion syndrome, which prompts genetic counseling for cardiac malformations, especially complex cardiac anomalies associated with deformity of other organs, for which it is suggested that chromosome karyotype analysis and CNV-seq detection be conducted at the same time, aiming to avoid the birth of children with birth defects.

In the present study, it was also found that the detection rate of chromosome aneuploidy abnormality decreases with an increase of gestational age, whereas the detection rate of VOUS increases. The detection rate of pCNV did not change with a change in gestational age. The reason why CNV-seq is not currently widely available in prenatal diagnosis is largely because of the VOUS result. It has been reported that the rate of VOUS is 0–12.3% for prenatal diagnosis sample detection.[2,14] The average detection rate of VOUS is approximately 1.7% in fetal samples with ultrasonic structural abnormalities and a normal chromosomal karyotype.[18] In the present study, 31.43% (287/913) cases have VOUS. Indeed, most VOUS cases were benign, being inherited from the parents, as confirmed from the parents' samples. The remaining 4.05% (37/913) cases of VOUS is true.

CNV-seq has its own limitations for the detection of chromosomal abnormalities. It cannot detect balanced structural abnormalities and chromosomal mosaicism. In the present study, 14 cases with chromosomal structural abnormalities (12 cases of inversion and two cases of translocation) were not detected by high-throughput sequencing. Therefore, it is not sufficient to rely solely on CNV-seq in prenatal diagnosis. CNV-seq and karyotype analysis should be combined to improve the detection rate of chromosomal abnormalities.

5 | CONCLUSIONS

In conclusion, CNV-seq can be used as an effective method for the prenatal genetic diagnosis of abnormal fetuses. Compared with traditional karyotype analysis, CNV-seq can improve the detection rate of chromosomal abnormalities, as well as identify chromosomal abnormalities that cannot be detected by karyotype analysis, such as CNVs and a chromosomal imbalance rearrangement with small segments. However, CNV-seq technology is unable to detect chromosomal structure rearrangements such as cross-translocation, inversion and loss of heterozygosity. With the continuous development of the next-generation sequencing technology and the improvement of CNV gene mapping, more pathogenic CNVs will be recognized, and CNV-seq technology will be widely used in prenatal diagnosis. We suggest that CNV-seq combined karyotype analysis should be performed simultaneously in fetuses with puncture indications.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

LL and ZZ conceived and designed the experiments. LS, BZ and YH recruited subjects and collected clinical data. ZZ conducted the laboratory testing. LL and ZZ analyzed the data. LL and ZZ prepared the manuscript. ZZ reviewed the manuscript.

ETHICAL APPROVAL

This study was conducted on the basis of the Declaration of Helsinki, and was supported by the Ethics Committee of the Meizhou People's Hospital.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. D'Amours G, Kibar Z, Mathonnet G, et al. Whole-genome array CGH identifies pathogenic copy number variations in fetuses with major malformations and a normal karyotype. *Clin Genet*. 2012;81:128-141.
2. Le Caignec C, Boceno M, Saugier-Veber P, et al. Detection of genomic imbalances by array based comparative genomic hybridisation in fetuses with multiple malformations. *J Med Genet*. 2005;42:121-128.
3. Nevado J, Mergener R, Palomares-Bralo M, et al. New microdeletion and microduplication syndromes: a comprehensive review. *Genet Mol Biol*. 2014;37(1 Suppl):210-219.
4. Vissers LE, Stankiewicz P. Microdeletion and microduplication syndromes. *Methods Mol Biol*. 2012;838:29-75.
5. Evans MI, Wapner RJ, Berkowitz RL. Noninvasive prenatal screening or advanced diagnostic testing: caveat emptor. *Am J Obstet Gynecol*. 2016;215:298-305.
6. Wapner RJ, Martin CL, Levy B, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. *N Engl J Med*. 2012;367:2175-2184.
7. Hillman SC, McMullan DJ, Hall G, et al. Use of prenatal chromosomal microarray: prospective cohort study and systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2013;41:610-620.
8. Callaway JL, Shaffer LG, Chitty LS, Rosenfeld JA, Crolla JA. The clinical utility of microarray technologies applied to prenatal cytogenetics in the presence of a normal conventional karyotype: a review of the literature. *Prenat Diagn*. 2013;33:1119-1123.
9. Vissers LE, Veltman JA, van Kessel AG, Brunner HG. Identification of disease genes by whole genome CGH arrays. *Hum Mol Genet*. 2005;14(Spec No. 2):R215-R223.
10. American College of Obstetricians and Gynecologists Committee on Genetics. Committee opinion no. 581: the use of chromosomal microarray analysis in prenatal diagnosis. *Obstet Gynecol*. 2013;122:1374-1377.
11. Hurd PJ, Nelson CJ. Advantages of next-generation sequencing versus the microarray in epigenetic research. *Brief Funct Genomic Proteomic*. 2009;8:174-183.
12. Su Z, Li Z, Chen T, et al. Comparing next-generation sequencing and microarray technologies in a toxicological study of the effects of aristolochic acid on rat kidneys. *Chem Res Toxicol*. 2011;24:1486-1493.
13. Liang D, Peng Y, Lv W, et al. Copy number variation sequencing for comprehensive diagnosis of chromosome disease syndromes. *J Mol Diagn*. 2014;16:519-526.
14. Dong Z, Zhang J, Hu P, et al. Low-pass whole-genome sequencing in clinical cytogenetics: a validated approach. *Genet Med*. 2016;18:940-948.
15. Xie C, Tammi MT. CNV-seq, a new method to detect copy number variation using high-throughput sequencing. *BMC Bioinformatics*. 2009;10:80-88.
16. Watson CT, Marques-Bonet T, Sharp AJ, Mefford HC. The genetics of microdeletion and microduplication syndromes: an update. *Annu Rev Genomics Hum Genet*. 2014;15:215-244.
17. Wang J, Chen L, Zhou C, et al. Prospective chromosome analysis of 3429 amniocentesis samples in China using copy number variation sequencing. *Am J Obstet Gynecol*. 2018;219:287.e281-287.e218.
18. Sahoo T, Cheung SW, Ward P, et al. Prenatal diagnosis of chromosomal abnormalities using array-based comparative genomic hybridization. *Genet Med*. 2006;8:719-727.

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