Detection of submicroscopic chromosomal aberrations by chromosomal microarray analysis for the prenatal diagnosis of central nervous system abnormalities

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Abstract

Background: Central nervous system (CNS) abnormalities are a group of serious birth defects associated with high rates of stillbirths, infant death, or abnormal development, and various disease-causing copy number variations play a much more important role in the etiology of CNS abnormalities. This study intends to present a retrospective study of the prenatal diagnosis and the pregnancy outcome of fetuses diagnosed with CNS abnormalities, and evaluate the clinical value of chromosomal microarray analysis (CMA) in prenatal diagnosis of CNS abnormalities.

Methods: A total of 356 fetuses with CNS abnormalities with or without other ultrasound abnormalities subjected to invasive prenatal diagnosis at the first affiliated hospital of Air Force Medical University from January 2015 to August 2018. All cases have performed both karyotyping and CMA concurrently, but 20 fetuses with chromosome aneuploidy were excluded in the current study.

Results: The CMA identified pathogenic copy number variants (pCNVs) in 27/336 (8.03%) fetuses, likely pCNVs in 8/336 (2.38%) fetuses, and variants of unknown significance (VOUS) in 11/336 (3.27%) fetuses. A total of 222 cases had single CNS abnormalities and the pCNVs detection rate was 5.86% (13/222), the remaining 114 cases including CNS abnormalities plus other structural abnormalities, ultrasonographic soft markers and two or more CNS abnormalities, the pCNVs detection rate was 12.3% (14/114).

Conclusions: Fetuses with CNS abnormalities have a higher risk of chromosomal abnormalities, our study showed that CNVs play an important role in the etiology of CNS abnormalities. The application of CMA could increase the detection rate of pCNVs causing CNS abnormalities.

Keywords
central nervous system abnormalities, chromosomal microarray analysis, copy number variations, loss of heterozygous, prenatal diagnosis
1 | INTRODUCTION

The incidence of CNS abnormalities is 0.14%-0.16% of live births and as high as 3%-6% of stillbirths.\(^1\) CNS abnormalities are a group of severe birth defects associated with high rates of stillbirths, infant deaths, or abnormal development.\(^2\) There are many factors leading to CNS abnormalities, such as maternal infections, chromosomal abnormalities, and single gene disorders; however, the etiology of fetal CNS abnormalities is unknown in most cases.\(^3\)-\(^5\) Previous studies have shown that genetic factors are a main cause of CNS abnormalities, but disease-causing copy number variations have a much more important role in the etiology of CNS abnormalities.\(^6\),\(^7\) There are currently no effective treatments for chromosomal-related diseases, including aneuploidy, CNVs, and monogenic disorders, which result in enormous financial and mental burdens on family and society. Thus, prenatal diagnosis is necessary for CNS malformations to reduce birth defects and improve quality of life. The high-resolution genome coverage, CMA analysis, has been widely used in invasive prenatal diagnostics for the detection of submicroscopic genomic alterations, while the association between CMA results and ultrasound abnormalities is poorly defined. Several studies have indicated that the application of CMA is valuable for fetuses with CNS anomalies, but the number of cases is limited. Therefore, further large-scale sample studies are needed to clarify the application of CMA in the prenatal diagnosis of CNS abnormalities.

In the current study, we performed a systematic analysis of 336 fetuses with various types of CNS abnormalities using the CMA approach to search for potentially disease-causing candidate genes and CNVs for fetuses with different types of CNS abnormalities. In addition, we analyzed the impact of prenatal diagnosis on neonatal outcomes and pregnancy outcomes and provided additional information for prenatal genetic counseling of fetuses with CNS abnormalities.

2 | MATERIALS AND METHODS

2.1 | Case selection

This retrospective cohort study included 336 fetuses diagnosed with CNS abnormalities by fetal ultrasound with or without other ultrasound abnormalities underwent invasive prenatal diagnostic testing at the First Affiliated Hospital of the Air Force Military Medical University from January 2015 to August 2018. All pregnant couples had received prenatal genetic counseling from a clinical geneticist, including information regarding the risks of amniocentesis, the advantages and limitations of karyotype and CMA. Written informed consents for invasive prenatal diagnosis and CMA analysis were routinely obtained from the pregnant couples after genetic counseling.

2.2 | Chromosomal microarray analysis, CMA

Genomic DNA (gDNA) was extracted from uncultured amniocytes or umbilical cord blood using a QIAamp DNA Blood Mini Kit (Qiagen, Venlo, The Netherlands) according to the standard manufacturer's instructions. The concentration and quality of gDNA were measured by Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). An Thermo Fisher Cytoscan 750k array (Thermo Fisher Scientific, Santa Clara, CA, USA) was applied to detected CNVs and loss of heterozygous (LOH) according to the manufacturer’s instructions. 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 \(\geq 50\)). The results were analyzed by Chromosome Analysis Suite 3.30 software, and the annotations of genome version were GRCh37 (hg19).

2.3 | Data interpretation

Public databases including DGV (http://www.ncbi.nlm.nih.gov/dbvar/), ISCA (https://www.iscaconsortium.org/), UCSC (http://genome.ucsc.edu), OMIM (http://www.ncbi.nlm.nih.gov/omim), PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) DECIPHER (http://decipher.sanger.ac.uk/) and our in-house database were used to analyze the CMA results. The detected CNVs were classified as benign, likely benign, VOUS, likely pathogenic and pathogenic in accordance with the American College of Medical Genetics (ACMG) guidelines.\(^8\)

2.4 | Clinical follow-up assessment and statistical analysis

Clinical follow-up assessments about prenatal and postnatal development, pregnancy outcome were done regularly by telephone. Statistical analysis was performed using SPSS version 17.0. Data analysis was carried out using chi-square test.

3 | RESULTS

3.1 | Detection rates of CNVs with normal karyotype by CMA

In this cohort, the mean maternal age was 29 years (range from 20 to 46) and the mean gestational age at diagnosis was 26 ± 2 weeks (range from 18 to 35) of gestation. The total pathogenic CNVs (pCNVs) were detected in 8.03% (27/336) of the fetuses, comprising 15 duplications and 25 deletions in a total of 27 fetuses. There are 16 fetuses with a single change, 9 fetuses with two changes (deletion and duplication), 1 fetus with two deletions, 1 fetus with two duplications and one deletion. Pathogenic CNVs types were summarized in Table 1. Likely, pCNVs were detected in 2.38% (8/336), and CNVs were associated with deletion from 239 kb to 3.1 Mb in size and duplication ranging from 396 kb to 972 kb in
| Case | CNS abnormalities | Extra CNS abnormalities | CNV type | Cytoband | Chromosome physical location (hg19) | Size (Mb) | Critical genes/region | Pregnancy outcome |
|------|------------------|-------------------------|----------|----------|-----------------------------------|-----------|----------------------|------------------|
| 1    | Posterior Cranial Fossa | | Loss | 10q11.22q11.23 | 46,293,590_51,903,756 | 5.6 | WDFY4 | Born, normal |
| 2    | lateral ventriculomegaly | | Loss | 16p11.2 | 28,807,417_30,190,029 | 1.38 | SH2B1, TBX6 | TOP |
| 3    | lateral ventriculomegaly, porencephalia | | Loss | 13q33.1q34 | 104,703,176_115,107,733 | 10.4 | ARHGEF7, SOX1, UPF3B | TOP |
| 4    | lateral ventriculomegaly | | Loss | 13q31.2q33.2 | 88,867,776_106,093,756 | 17.2 | ZIC2, VGCNL1, ZIC5 | TOP |
| 5    | lateral ventriculomegaly | Single umbilical artery | Loss | 16p11.2 | 83,592,209_89,128,106 | 5.54 | FOX13, REEP1 | TOP |
| 6    | lateral ventriculomegaly | | Loss | 16p13.11 | 14,892,975_16,538,596 | 1.65 | 16p13.11 microdeletion syndrome, NDE1, NTAN1 | TOP |
| 7    | Posterior Cranial Fossa | vascular circle | Loss | 10q26.2q26.3 | 130,333,276_135,426,386 | 5.1 | CALY, INPP5A, DPYSL4 | TOP |
| 8    | Agenesis of the corpus callosum | | Loss | 1p36.33p36.31 | 1,028,553_5,851,366 | 4.8 | 1p36 deletion syndrome | TOP |
| 9    | Isencephaly | Nasal bone dysplasia | Loss | 5q13.3q14.1 | 2,028,653_35,613,366 | 1.45 | RUNX2 | Born, death |
| 10   | Cerebellar vermis missing | | Loss | 5q13.3q14.1 | 75,642,770_79,936,342 | 4.29 | 5q14.3 Deletion | Neurocutaneous Syndrome MEF2C | TOP |
| 11   | Arachnoid cyst | CHD, ectopical kidney | Loss | 5q14.3q15 | 84,428,488_97,070,750 | 12.6 | Uncertain | TOP |
| 12   | Danker_walker | | Loss | 1q21.1 | 44,032,138_45,486,795 | 1.45 | RUNX2 | TOP |
| 13   | Hydrocephalus, Spinal bifida | | Loss | 1q21.1q21.2 | 145,896,746_147,830,830 | 4.7 | 2.2 | 1q21.1 deletion syndrome | TOP |
| 14   | lateral ventriculomegaly | | Loss | 2q11.23q22.3 | 39,373,647_48,093,361 | 8.72 | FOXQ1, FOXF2, FOXC1 | TOP |
| 15   | lateral ventriculomegaly, Posterior Cranial Fossa | | Loss | 2q11.23q22.3 | 39,373,647_48,093,361 | 8.72 | FOXQ1, FOXF2, FOXC1 | TOP |
| 16   | Posterior Cranial Fossa | TOF, Cleft lip and palate | Loss | 6p25.3p24.3 | 381,117,770,535 | 7.41 | FOXQ1, FOXF2, FOXC1 | TOP |
| 17   | Posterior Cranial Fossa | | Loss | 3p21.32p22.1 | 47,409,497_52,148,326 | 4.7 | PLXNB1, CELSR3, DOCK3 | TOP |
| 18   | Blake's Pouch Cyst | | Loss | 9p24.3p22.2 | 208,454,275,123 | 2.5 | KANK1, DOCK8 | TOP |
| 19   | lateral ventriculomegaly | | Loss | 6q27 | 168,168,883_170,914,297 | 2.7 | 6q terminal deletion syndrome, G6orf70 | TOP |
| 20   | Meningocoele, lateral ventriculomegaly | Single umbilical artery, oligohydramnios | Loss | 1q43q44 | 238,536,090_249,224,684 | 10.7 | FOXQ1, FOXF2, FOXC1 | TOP |
|      |                  |                       | Gain    | 6p25.3p22.3 | 330,740_19,488,333 | 19.2 | 1q44 deletion syndrome | TOP |

(Continues)
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In our present study, the incidence of CNVs was different in the different types of CNS abnormalities and various CNVs incidence. There were 222 cases with single CNS abnormalities and 114 cases with two or more CNS abnormalities or plus other ultrasound abnormalities including ultrasonographic soft markers and structural abnormalities. The detection rate of pCNVs in fetuses with posterior cranial fossa blake's pouch cyst (16.7%), cerebellar vermis missing (13.3%, 1/7), and agenesis of the corpus callosum (10.0%, 2/2) was relatively higher than other single CNS abnormalities. The detection rate of pathogenic CNVs in two or more CNS abnormalities or plus other ultrasound abnormalities including ultrasonographic soft markers and structural abnormalities was 12.3% (4/336). In addition, VOUS CNVs or LOHS were detected in 3.7% (11/336).

### 3.3 Clinical follow-up

In the present study, the mean duration of telephone follow-up was 6 months, but the postnatal follow-up was short and the information was not comprehensive. The fetus 8 with a 1p36.33p36.31 deletion was born by cesarean, agenesis of the corpus callosum, patent of ductus atrioum and patent foramen ovale, hypotonia, dysmorphic features included large anterior fontanel, low forehead, small nose with a broad base and low-set ears were observed after birth. Unfortunately, the baby suffered from severe pneumonia and died two months after birth. The fetus 26 with 16p11.2 duplication was born by cesarean, agenesis of the corpus callosum, GRIK4, and heterotaxia. The fetus 27 with 22q11.1q11.2 deletion was born by cesarean and 2p16.1p14 duplication was born by cesarean, agenesis of the corpus callosum, TOP.

### Table 1 (Continued)

| Case | CNS abnormalities | Extra CNS abnormalities | CNV type | Cytoband | Chromosome physical location (hg19) | Size (Mb) | Critical genes/region | Pregnancy outcome |
|------|-------------------|-------------------------|----------|----------|------------------------------------|-----------|-----------------------|------------------|
| 21   | Agenesis of the corpus callosum | Loss | 1q43.4q44 | 242,702,622_249,224,684 | 6.5 | 1q44 deletion syndrome | TOP |
| 22   | Choroid plexus cyst | Loss | 1q21.23 | 34,822,465_36,243,365 | 1.42 | 17q12 deletion syndrome | TOP |
| 23   | Absent cavum septum pellucidum | Loss | 4q35.1q35.2 | 150,301,319_159,119,707 | 8.8 | 4q deletion Syndrome | TOP |
| 24   | lateral ventriculomegaly | Gain | 2p16.1p14 | 61,233,434_66,911,895 | 5.79 | uncertain | Born, development delay |
| 25   | lateral ventriculomegaly | Polyhydramnios | 7q11.23 | 72,701,908_74,133,586 | 1.43 | 7q11.23 duplication syndrome | TOP |
| 26   | Choroid plexus cyst | Gain | 16p11.2 | 29,591,326_30,243,606 | 0.65 | TBX6 | Born, normal |
| 27   | Cerebellar vermis missing, lateral ventriculomegaly | Gain | 22q11.1q11.21 | 16,888,899_20,312,661 | 3.4 | GRK4 | TOP |

Abbreviations: CHD, congenital heart disease; CNS, central nervous system; CNV, copy number variant; TOF, tetralogy of fallot; TOP, Termination of pregnancy; VSD, ventricular septal defect.
after prenatal diagnosis of the fetuses with CNS abnormalities in this study were summarized in Table 5.

4 | DISCUSSION

Although CMA was widely applied in prenatal diagnosis for fetuses with structural malformations or ultrasonographic soft markers such as congenital heart defects, renal abnormalities, CNS abnormalities, increased nuchal translucency and so on,7,9-11 there are not enough studies especially for fetuses with CNS abnormalities illuminate the relationship between CNVs and the abnormalities detected by prenatal ultrasound. In previous study, Lijuan Sun et al7 showed that the detected rate of pathogenic CNVs in 46 fetuses with CNS was 10.9%. A meta-analysis by De Wit MC et al12 published in 2014 found a pooled prevalence of pathogenic was 6.2% (35/563 cases) for CNS abnormalities. In addition, the sample size was relatively small in previous single study of CNS abnormalities,7 further studies in larger cohorts are necessary to validate the relationship between genotypes and phenotypes. In the current study, we report our experience with the use of CMA for analysis of 336 fetuses with CNS malformations with or without other structural abnormalities. In addition, we searched for causative mutations characterized by a loss or gain of genomic material and attempted to illustrate the relationship between CNVs and CNS malformations. Our data showed that the total pathogenic CNVs in 336 fetuses with CNS abnormalities was 8.03%, but the sample size of in the present cohort study was relatively large compared to previous studies, thus our study was valuable and more representative. It is noteworthy that fetuses with CNS abnormalities are at higher risk for CNVs, and the risk increases with abnormalities (the more abnormalities the higher the risk). The detection rates for pathogenic CNVs in fetuses with two or more CNS abnormalities (12.3%) or in addition to structural malformations were significantly higher than fetuses with isolated CNS abnormalities (5.86%); however, the detection rate of pathogenic CNVs in fetuses with posterior cranial fossae, Blake's pouch cysts, an absent cerebellar vermis, and agenesis of the corpus callosum were also high, but the sample sizes were relatively small, which could limit the clinical usefulness of our observations.

There are several CNVs which may be associated with CNS abnormalities. The total rate of pathogenic CNVs was 8.03% in the current study. We detected some microdeletion and microduplication syndromes associated with CNS abnormalities, including the 16p13.11 microdeletion syndrome, 1p36 deletion syndrome, 5q14.3 deletion neurocutaneous syndrome, 1q21.1 deletion syndrome, Miller–Dieker syndrome, 6q terminal deletion syndrome, 1q44 deletion syndrome, 17q12 deletion syndrome, 4q deletion syndrome, and 7q11.23 duplication syndrome in 11 fetuses. In addition, some rare disease-causing CNVs in 16 fetuses were detected. Our results further demonstrate that the chromosomal regions, including 10q11.22q11.23, 16p11.2, 13q33.1q34, 13q31.2q33.2, 2p11.2, 10q26.2q26.3, 6p21.1, 6p25.3p25.2, 4q31.3q32.1, 21q22.1q22.3, Xq28, 3p21.3p21.2, 3p22.1, 9p24.3p24.2, 9p24.2p22.2, 2p16.1p14, 22q11.1q11.21, and 11q23.3q25 may be related to CNS abnormalities. The deletion or duplication of 6p25.3 involving the FOXC1 gene was common in fetuses with CNS abnormalities. A previous study showed that the 6p25.3 deletion is a rare, but well-known entity. The major clinical manifestations include developmental delay, a special facial appearance, congenital heart disease, and CNS abnormalities.13-15 Aldinger et al16 reported that the FOXC1 gene is necessary for normal cerebellar development and is a main contributor to Dandy-Walker malformation.

**TABLE 2** Characterizations of CNS abnormalities cases with likely pathogenic CNVs and normal karyotype

| Cases | Clinical feature | Copy number | Chromosome physical location (hg19) | Size (kb) | Inheritance | Pregnancy Outcome |
|-------|-----------------|-------------|-----------------------------------|----------|-------------|------------------|
| 28    | Meningoceles    | Loss        | 2p15                              | 61,595,331-61,834,624 | 239         | De novo TOP      |
| 29    | Posterior Cranial Fossa | EICF | Loss               | 15q11.2           | 22,770,421-23,082,237 | 312       | Unknown Born, normal |
| 30    | Lateral ventriculomegaly | Loss | 15q11.2           | 22,770,421-23,277,436 | 507         | De novo Born, normal |
| 31    | Lateral ventriculomegaly, agenesis of the corpus callosum | Loss | Xq26.3q27.1      | 136,388,326-139,518,268 | 3100        | Mat TOP          |
| 32    | Lateral ventriculomegaly | Gain   | 7p22.1            | 5,367,121-5,764,090 | 396         | Unknown Born, normal |
| 33    | Lateral ventriculomegaly | Gain   | 15q11.2           | 22,770,421-23,288,350 | 518         | Unknown TOP      |
| 34    | Lateral ventriculomegaly | Gain   | 17q11.2           | 29,379,983-30,352,918 | 972         | De novo TOP      |
| 35    | Lateral ventriculomegaly | Gain   | 15q11.2           | 22,770,421-23,288,350 | 518         | Pat Lost to follow up |

Abbreviations: CNS, central nervous system; CNVs, copy number variants; EICF, echogenic intracardiac foci; Mat, maternal; Pat, paternal; TOP, termination of pregnancy.
| Cases | Clinical feature                                      | other         | CNV type | Cytoband   | Chromosome physical location (hg19) | Size (Mb) | Pregnancy Outcome       |
|-------|------------------------------------------------------|---------------|----------|------------|------------------------------------|-----------|--------------------------|
| 36    | Lateral ventriculomegaly                             | Loss          | 6p25.3   | 1,637,727-1,767,134 | 0.13 | Born, death              |
| 37    | Elargement of cerebellomedullary cistern             | Loss          | 18p11.31 | 4,471,611-5,675,587 | 1.2  | Born, normal             |
| 38    | Cerebellum abnormal                                  | Loss          | 3q11.2q12.1 | 97,623,364-99,013,835 | 1.39 | Born, normal             |
| 39    | Lateral ventriculomegaly                             | Gain          | 15q13.3  | 32,003,537-32,444,042 | 0.44 | TOP                      |
| 40    | Lateral ventriculomegaly                             | LOH           | 14q24.3q31.3 | 74,973,739-87,318,306 | 12.3 | Born, normal             |
| 41    | Lateral ventriculomegaly                             | LOH           | 14q32.13q32.33 | 95,377,700-107,279,475 | 11.9 | TOP                      |
| 42    | Arachnoid cyst                                       | LOH           | 11q22.3q24.1 | 106,514,772-121,272,606 | 14.7 | Born, normal             |
| 43    | Hydrocephalus                                        | Vascular circle | LOH 1p36.11p34.3 | 24,349,271-34,868,452 | 10.5 | TOP                      |
| 44    | Lateral ventriculomegaly                             | LOH           | 1p33p31.3 | 47,948,617-62,446,802 | 14.5 | Born, normal             |
| 45    | Blake's Pouch Cyst                                   | LOH           | 7q22.1q35 | 128,770,822-144,281,590 | 15.5 | TOP                      |
| 46    | Choroid plexus cyst                                  | LOH           | 2p24.2p16.1 | 16,822,735-56,261,491 | 39.4 | Born, development delay |
|       |                                                      | LOH           | 14q21.2q24.1 | 47,164,539-69,843,549 | 29.7 |                          |

Abbreviations: CNS, Central nervous system; CNVs, copy number variants; LOH, Loss of heterozygosity; TOP, Termination of pregnancy; VOUS, variants of unknown significance.
Four fetuses with a deletion or duplication of 6p25.3, including the FOXC1 gene, were detected in the present study, further supporting that the CNVs of 6p25.3p25.2 might contribute to CNS abnormalities.

CMA is a whole-genome high-resolution technique for discovering aneuploidies, polyploid, microdeletions, microduplications, and UPD, so a series of interpretation of variants of unknown significance (VOUS) were detected by CMA. Zhi et al. reported that the rate of VOUS in posterior fossa anomalies fetuses was 7.7%. The sample size in the current study was relatively large and some CNVs that inherited from parents VOUS were excluded, our data showed that the total VOUS in CNS fetuses was 5.65%. However, the VOUS remain posing a problem for adequate genetic counseling because the clinical phenotype information was limited, especially for fetuses with CNS abnormalities.

### Table 4: Types of CNS abnormalities and frequencies of fetuses with CNVs

| CNS abnormalities classification | Number of fetuses | pCNVs | IpCNVs | VOUS |
|---------------------------------|-------------------|-------|--------|------|
| Lateral ventriculomegaly        | 107               | 5 (4.67%) | 4 (3.74%) | 5 (4.67%) |
| Choroid plexus cyst            | 59                | 2 (3.39%) | 0      | 1 (1.69%) |
| Posterior Cranial Fossa        | 11                | 2 (18.2%) | 0      | 0     |
| Other CNS malformation         | 7                 | 0      | 0      | 1 (14.3%) |
| Cerebellomedullary cistern      | 7                 | 0      | 0      | 1 (14.3%) |
| Arachnoid cyst                  | 6                 | 0      | 0      | 1 (16.7%) |
| Blake's pouch cyst              | 6                 | 1 (16.7%) | 0      | 1 (16.7%) |
| Subependymal cyst               | 4                 | 0      | 0      | 0     |
| Cerebellar vermis missing       | 3                 | 1 (33.3%) | 0      | 0     |
| Exencephaly                     | 2                 | 0      | 0      | 0     |
| Agenesis of the corpus callosum | 2                 | 2 (100%) | 0      | 0     |
| Encephalocele/meningocele       | 2                 | 0      | 1 (50%) | 0     |
| Cavum septum pellucidum         | 2                 | 0      | 0      | 0     |
| Dandy-Walker syndrome           | 1                 | 0      | 0      | 0     |
| Holoprosencephaly               | 1                 | 0      | 0      | 0     |
| Cerebellar hypoplasia           | 1                 | 0      | 0      | 0     |
| Hematencephalon                 | 1                 | 0      | 0      | 0     |
| Plus ultrasonographic soft markers | 69             | 6 (8.7%) | 1 (1.45%) | 0     |
| Plus structural malformations   | 23                | 5 (21.7%) | 0      | 1 (4.35%) |
| Two or more CNS anomalies       | 22                | 3 (13.6%) | 2 (9.09%) | 0     |
| Total                           | 336               | 27 (8.03%) | 8 (2.38%) | 11 (3.27%) |

Abbreviations: CNS, central nervous system; CNVs, copy number variants; IpCNVs, likely pathogenic copy number variants; pCNVs, pathogenic copy number variants; VOUS, variants of unknown significance.

### 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 pCNVs             | 27            | 4(14.8%) | 23 (85.2%) | 0                 |
| Fetuses with IpCNVs            | 8             | 3 (37.5%) | 4 (50%) | 1 (12.5%)         |
| VOUS                            | 11            | 7 (63.6%) | 4 (36.4%) | 0                 |
| Normal CMA results              | 290           | 230 (79.3%) | 36 (12.4%) | 24 (8.28%)        |
| Total                           | 336           | 244 (72.6%) | 67 (19.9%) | 25 (7.4%)         |

Abbreviations: CMA, chromosomal microarray analysis; IpCNVs, likely pathogenic copy number variants; pCNVs, pathogenic copy number variants; TOP, Termination of pregnancy; VOUS, variants of unknown significance.
individuals in previous publications. The published literature showed that the phenotypic spectrum of the CNV carriers was wide, ranging from association with different phenotypes to being non-pathogenic, the mainly neurodevelopmental disorders, including developmental delay, dysmorphic features, epilepsy and autism group of disorders. However, not all individuals with the CNV share a clinical phenotype, in some cases the parent carrying deletion or duplication was even observed to be normal. So it is challenging for us to prenatal diagnosis and genetic counseling.

The clinical follow-up assessments were completed after prenatal diagnosis in our study. The results showed that most fetuses with pCNVs had labor induced after genetic counseling, but 4 fetuses with pCNVs were born alive. Fetus 8 had a 1p36.33p36.31 deletion, including 50 OMIM genes, that overlapped with the 1p36 deletion syndrome. The 1p36 deletion syndrome is characterized by facial dysmorphism, mental retardation, developmental delay, congenital heart defects, hypotonia, and seizures, but the mother selected to continue pregnancy after genetic counseling. Agenesis of the corpus callosum, a patent ductus arteriosus and foramen ovale, hypotonia, dysmorphic features (including a large anterior fontanel, high forehead, a small nose with a broad base, and low-set ears) were observed after birth. Unfortunately, the baby had severe pneumonia and died 2 months after birth. A 2p16.1p14 duplication involving 22 OMIM genes was detected in fetus 24. A deletion of the same region is a well-known neurodevelopmental syndrome characterized by intellectual disability, facial dysmorphism, delayed psychomotor development, autistic behavior, short stature, craniofacial dysmorphism of microcephaly, hypoplastic corpus callosum, and other brain malformations, but the clinical phenotypes of duplication carriers are milder than deletion carriers. Fetus 24 in our study was delivered by cesarean section and had mild hypospadias, an atrial septal defect, development delay, and speech delay, and he was unable to walk without assistance at 27 months of age. This finding provides a basis supporting duplication of 2p16.1p14 as a contributor to CNS abnormalities. Our study showed that the fetuses with pathogenic CNVs had a poor prognosis. Among the 290 fetuses with normal CMA results, 266 fetuses had follow-up evaluations. Specifically, 228 (85.7%) were born apparently normal. Our follow-up assessments showed that fetuses with normal CMA results had a good prognosis after birth.

In conclusion, the submicroscopic deletions and duplications identified in the present study will advance the molecular understanding of etiology in CNS abnormalities. The availability of the extra information provided by CMA in prenatal diagnosis for fetuses with CNS abnormalities was remarkable, and the rate of undiagnosed or underlying genomic disorders was decreased. Our study not only provides information for clinical consultation, but may also allow more accurate genetic diagnosis and a better understanding of the etiology and mechanisms involved in CNS abnormalities.

ACKNOWLEDGMENTS

We thank the Key Research and Development Program of Shaanxi Province (2019ZDLSF01-06), Scientific and Technological Projects for Social development of Shaanxi Province (2016SF-254) and Hospital discipline booster program of the First Affiliated Hospital of the Air Force Medical University (Grant No. XJZT18MJ53). We thank all the pregnant women and research workers for their participation.

CONFLICT OF INTERESTS

All authors declare that they have no any conflict of interests.

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**How to cite this article:** Song T, Xu Y, Li Y, et al. Detection of submicroscopic chromosomal aberrations by chromosomal microarray analysis for the prenatal diagnosis of central nervous system abnormalities. *J Clin Lab Anal*. 2020;34:e23434. [https://doi.org/10.1002/jcla.23434](https://doi.org/10.1002/jcla.23434)