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RESEARCH ARTICLE

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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

Tingting Song and Ying Xu are contributed equally to this work.

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 are 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 ≥ 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

TABLE 1 Characterizations of CNS abnormalities cases with pathogenic CNVs and normal karyotype

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_106,093,135	17.2	*ZIC2*, *VGCNL1*, *ZIC5*	TOP
5	lateral ventriculomegaly	Single umbilical artery	Loss	2p11.2	83,592,209_89,128,106	5.54	*FOXI3*, *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	130,333,948_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 *SKI*	Born, death
9	lisencephaly	Nasal bone dysplasia	Loss	6p21.1	44,032,138_45,486,795	1.45	*RUNX2*	TOP
10	Cerebellar vermis missing		Loss	6p25.3p25.2	384,096_3,827,197	3.4	*FOXQ1*, *FOXF2*, *FOXC1*	TOP
11	Arachnoid cyst	CHD, ectopical kidney	Loss	4q31.3q32.1	153,328,608_158,214,998	4.9	Uncertain	TOP
12	Danker_walker		Loss Loss	5q13.3q14.1 5q14.3q15	75,642,770_79,936,342 84,428,488_97,070,754	4.29 12.6	5q14.3 Deletion Neurocutaneous Syndrome *MEF2C*	TOP
13	Hydrocephalus, Spinal bifda		Loss Gain	1q21.1q21.2 Xp22.31	145,895,746_147,830,830 6,449,752_8,134,765	1.94 1.69	1q21.1 deletion syndrome	TOP
14	lateral ventriculomegaly		Loss Gain	21q22.13q22.3 6p25.3p22.3	39,373,647_48,093,361 156,974_25,066,393	8.72 24.9	*FOXQ1*, *FOXF2*, *FOXC1*	TOP
15	lateral ventriculomegaly, Posterior Cranial Fossa		Loss Gain	17p13.3 15q24.1q26.3	525_2,158,383 73,768,298_102,429,040	2.15 28.7	Miller–Dieker Syndrome	TOP
16	Posterior Cranial Fossa	TOF, Cleft lip and palate	Loss Gain	6p25.3p24.3 Xq28	381,117_7,790,535 152,970,883_154,896,094	7.41 1.93	*FOXQ1*, *FOXF2*, *FOXC1*	TOP
17	Posterior Cranial Fossa		Loss Gain	3p21.31p21.2 3p22.1	47,409,497_52,148,326 39,620,069_41,796,286	4.7 2.2	*PLXNB1*, *CELSR3*, *DOCK3*	TOP
18	Blake's Pouch Cyst		Loss Gain	9p24.3p24.2 9p24.2p22.2	208,454_2,715,213 2,716,920_17,186,374	2.5 14.5	*KANK1*, *DOCK8*	TOP
19	lateral ventriculomegaly		Loss Gain	6q27 6q25.3q27	168,168,883_170,914,297 156,197,501_168,167,204	2.7 11.9	6q terminal deletion syndrome, *C6orf70*	TOP
20	Meningoceles, lateral ventriculomegaly	Single umbilical artery, oligohydramnios	Loss Gain	1q43q44 6p25.3p22.3	238,536,090_249,224,684 330,740_19,488,333	10.7 19.2	*FOXQ1*, *FOXF2*, *FOXC1* 1q44 deletion syndrome	TOP

(Continues)

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 Gain	1q43q44 7q36.1q36.3	242,702,622_249,224,684 150,301,319_159,119,707	6.5 8.8	1q44 deletion syndrome *SHH*	TOP
22	Choroid plexus cyst		Loss	17q12	34,822,465_36,243,365	1.42	17q12 deletion syndrome	TOP
23	Absent cavum septum pellucidum,	VSD	Loss Gain Gain	4q35.1q35.2 4p12q13.2 4q13.2q21.23	185,081,688_190,957,460 47,632,643_69,435,889 69,541,893_86,815,623	5.87 21.8 17.3	4q deletion Syndrome	TOP
24	lateral ventriculomegaly		Gain	2p16.1p14	61,123,434_66,911,895	5.79	uncertain	Born, development delay
25	lateral ventriculomegaly	Polyhydramnios	Gain	7q11.23	72,701,098_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 Gain	22q11.1q11.21 11q23.3q25	16,888,899_20,312,661 116,683,754_134,937,416	3.4 18.2	*GRIK4*	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.

size. Likely, pathogenic CNVs types were summarized in Table 2. In addition, VOUS CNVs or LOHs were detected in 3.27% (11/336), including microdeletions or microduplications varying from 129 kb to 1.39 Mb in size, and over 10 Mb LOH. The VOUS results were summarized in Table 3.

3.2 | The Types of Fetal CNS abnormalities and various CNVs incidence

In our present study, the incidence of CNVs was different in the different types of CNS abnormalities. 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 (18.2%, 2/11), blake's pouch cyst (16.7%, 1/6), cerebellar vermis missing (33.3%, 1/3) and agenesis of the corpus callosum (100%, 2/2) was relatively higher than other single CNS abnormalities. The detection rate of pCNVs in two or more CNS abnormalities or plus other ultrasound abnormalities was 12.3% (14/114), higher than the fetuses with single CNS abnormalities (5.86%, 13/222). The difference was statistically significant ($P < .05$). The occurrence of fetuses with pathogenic CNVs, likely pathogenic CNVs and VOUS in different types of CNS abnormalities were summarized in Table 4.

3.3 | Clinical follow-up

In the present study, the mean duration of telephone follow-up among those cases was 6 months, range from 1 month to 2.5 years. All cases with pCNVs either underwent termination of pregnancy (n = 23) or were liveborn (n = 4). Among the 4 cases of pathogenic CNV, the fetus 1 with 10q11.22q11.23 deletion and fetus 26 with 16p11.2 duplication was born, no obvious abnormal was observed at 6 months, but the postnatal follow-up was short and the information was not comprehensive. The fetus 8 with a 1p36.33p36.31 deletion delivered by cesarean, agenesis of the corpus callosum, patent of ductus atriosus and patent foramen ovale, hypotonia, dysmorphic features included large anterior fontanel, high 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 24 with 2p16.1p14 duplication delivered by cesarean, mild hypospadias, atrial septal defect, development delay, speech delay were observed after birth, he still cannot walk alone at 2 years 3 months. Among the 8 cases of likely pathogenic CNV, 4 underwent termination of pregnancy, 3 were born apparently normal and 1 lost to follow-up. Among the 11 cases of VOUS, 4 underwent termination of pregnancy, 6 were born with apparently normal and 1 was died after birth. Among the 290 fetuses of normal CMA results, 228 were born with apparently normal, 2 were died after birth, 36 underwent termination of pregnancy and 24 lost to follow-up. The detail clinical follow-up assessments

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 al[7] showed that the detected rate of pathogenic CNVs in 46 fetuses with CNS was 10.9%. A meta-analysis by De Wit MC et al[12] 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 relation 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, 21q22.13q22.3, Xq28, 3p21.31p21.2, 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 al[16] 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	other	Copy number	Cytoband	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.

TABLE 3 Characterizations of CNS abnormalities cases with VOUS CNVs and normal karyotype

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 Gain	15q13.3 17p13.3	32,003,537_32,444,042 2,339,684_2,825,460	0.44 0.49	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 14.7	106,514,772-121,272,606	14.7	Born, normal
43	Hydrocephalus	Vascular circle	LOH LOH	1p36.11p34.3 16q21q23.1	24,349,271-34,868,452 61,161,679-75,377,750	10.5 41.2	TOP
44	Lateral ventriculomegaly		LOH	1p33p31.3	47,948,617-62,446,802	14.5	Born, normal
45	Blake's Pouch Cyst		LOH	7q32.1q35	128,770,822-144,281,590	15.5	TOP
46	Choroid plexus cyst		LOH LOH	2p24.2p16.1 14q21.2q24.1	16,822,735-56,261,491 47,164,539-69,843,549	39.4 29.7	Born, development delay

Abbreviations: CNS, Central nervous system; CNVs, copy number variants; LOH, Loss of heterozygosity; TOP Termination of pregnancy; VOUS, variants of unknown significance.

TABLE 4 Types of CNS abnormalities and frequencies of fetuses with CNVs

CNS abnormalities classification	Number of fetuses	pCNVs	lpCNVs	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/meningoceles	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; lpCNVs, 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 lpCNVs	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; lpCNVs, likely pathogenic copy number variants; pCNVs, pathogenic copy number variants; 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[16] 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. The detection rate of likely pathogenic CNVs was 2.38%, but 50% of them with the deletion or duplication of 15q11.2 BP1-BP2 region involving *TUBGCP5*, *CYFIP1*, *NIPA2*, and *NIPA1* genes. 15q11.2 BP1-BP2 deletion or duplication had been reported over 200

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.[17,18] 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.[17] 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,[19] 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,[20,21] but the clinical phenotypes of duplication carriers are milder than deletion carriers.[20] 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.

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CONFLICT OF INTERESTS

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

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REFERENCES

1. Onkar D, Onkar P, Mitra K. Evaluation of fetal central nervous system anomalies by ultrasound and. its anatomical co-relation. *J Clin Diagn Res*. 2014;8(6):AC05-07.
2. Almli LM, Alter CC, Russell RB, et al. Association between infant mortality attributable to birth defects and payment source for delivery – United States, 2011–2013. *MMWR Morb Mortal Wkly Rep*. 2017;66(3):84-87.
3. Van den Veyver IB. Prenatally diagnosed developmental abnormalities of the central nervous system and genetic syndromes: a practical review. *Prenat Diagn*. 2019;39(9):666-678.
4. Coe BP, Stessman HAF, Sulovari A, et al. Neurodevelopmental disease genes implicated by de novo mutation and copy number variation morbidity. *Nat Genet*. 2019;51(1):106-116.
5. Lei T, Feng J-L, Xie Y-J, et al. Chromosomal aneuploidies and copy number variations in posterior fossa abnormalities diagnosed by prenatal ultrasonography. *Prenat Diagn*. 2017;37(11):1160-1168.
6. Krutzke SK, Engels H, Hofmann A, et al. Array-based molecular karyotyping in fetal brain malformations: Identification of novel candidate genes and chromosomal regions. *Birth Defects Res A Clin Mol Teratol*. 2016;106(1):16-26.
7. Sun L, Wu Q, Jiang S-W, et al. Prenatal diagnosis of central nervous system anomalies by high-resolution chromosomal microarray analysis. *Biomed Res Int*. 2015;2015: 1-9.
8. Kearney HM, Thorland EC, Brown KK, Quintero-Rivera F, South ST. American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. *Genet Med*. 2011;13(7):680-685.
9. Song T, Wan S, Li YU, et al. Detection of copy number variants using chromosomal microarray analysis for the prenatal diagnosis of congenital heart defects with normal karyotype. *J Clin Lab Anal*. 2019;33(1):e22630.
10. Fu F, Chen F, Li RU, et al. Prenatal diagnosis of fetal multicystic dysplastic kidney via high-resolution whole-genome array. *Nephrol Dial Transplant*. 2016;31(10):1693-1698.
11. Su L, Huang H, An G, et al. Clinical application of chromosomal microarray analysis in fetuses with increased nuchal translucency and normal karyotype. *Mol Genet Genomic Med*. 2019;7(8):e811.
12. de Wit MC, Srebniak MI, Govaerts LCP, et al. Additional value of prenatal genomic array testing in fetuses with isolated structural ultrasound abnormalities and a normal karyotype: a systematic review of the literature. *Ultrasound Obstet Gynecol*. 2014;43(2):139-146.
13. Delahaye A, Khung-Savatovsky S, Aboura A, et al. Pre- and postnatal phenotype of 6p25 deletions involving the FOXC1 gene. *Am J Med Genet A*. 2012;158A(10):2430-2438.
14. de Vos IJ, Stegmann AP, Webers CA, Stumpel CT. The 6p25 deletion syndrome: an update on a rare neurocristopathy. *Ophthalmic Genet*. 2017;38(2):101-107.

15. Ovaert C, Busa T, Faure E, et al. FOXC1 haploinsufficiency due to 6p25 deletion in a patient with rapidly progressing aortic valve disease. *Am J Med Genet A*. 2017;173(9):2489-2493.
16. Zou Z, Huang L, Lin S, et al. Prenatal diagnosis of posterior fossa anomalies: Additional value of chromosomal microarray analysis in fetuses with cerebellar hypoplasia. *Prenat Diagn*. 2018;38(2):91-98.
17. Mohan KN, Cao YE, Pham J, et al. Phenotypic association of 15q11.2 CNVs of the region of breakpoints 1–2 (BP1-BP2) in a large cohort of samples referred for genetic diagnosis. *J Hum Genet*. 2019;64(3):253-255.
18. Picinelli C, Lintas C, Piras IS, et al. Recurrent 15q11.2 BP1-BP2 microdeletions and microduplications in the etiology of neurodevelopmental disorders. *Am J Med Genet B Neuropsychiatr Genet*. 2016;171(8):1088-1098.
19. Kang DS, Shin E, Yu J. 1p36 deletion syndrome confirmed by fluorescence in situ hybridization and array-comparative genomic hybridization analysis. *Korean J Pediatr*. 2016;59(Suppl 1):S14-S18.
20. Chen C-P, Chern S-R, Wu P-S, et al. Prenatal diagnosis of a 3.2-Mb 2p16.1-p15 duplication associated with familial intellectual disability. *Taiwan J Obstet Gynecol*. 2018;57(4):578-582.
21. Hancarova M, Vejvalkova S, Trkova M, et al. Identification of a patient with intellectual disability and de novo 3.7 Mb deletion supports the existence of a novel microdeletion syndrome in 2p14-p15. *Gene*. 2013;516(1):158-161.

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