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ORIGINAL RESEARCH

Application of Single Nucleotide Polymorphism Microarray in Prenatal Diagnosis of Fetuses with Central Nervous System Abnormalities

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Background: The current gold standard of karyotype analysis for prenatal diagnosis of fetuses with central nervous system (CNS) abnormalities has some limitations. Here, we assessed the value of single nucleotide polymorphism (SNP) arrays as a diagnostic tool.

Methods: The results of prenatal diagnosis of 344 fetuses with CNS abnormalities as determined by ultrasonographic screening were retrospectively analyzed. All fetuses underwent chromosomal karyotype analysis and genome-wide SNP array analysis simultaneously. The resultant rates and frequencies of genomic abnormalities were compared.

Results: Karyotype analysis found 45 (13.2%) abnormal CNS cases, while SNP array found 60 (17.4%) cases. SNP array detected 23 (6.7%) cases of submicroscopic abnormalities that karyotype analysis did not find. The detection rate of karyotype analysis was 8.1% in the group with isolated CNS anomalies, but 16.5% in the group with CNS abnormalities plus extra ultrasound anomalies. Detection rates of SNP array were 12.4% and 20.8% in these two groups, respectively. Statistical analysis showed that the detection rates of both methods were significantly higher in the group with CNS malformations and other ultrasound anomalies than in the group with isolated CNS anomalies. Abnormal chromosomes were detected most frequently in fetuses with holoprosencephaly.

Conclusion: Genome-wide SNP array technology can significantly improve the positive detection rate of fetuses with CNS abnormalities. Combining karyotype analysis and SNP array technology is recommended for detecting the development of fetuses with abnormal CNS.

Keywords: central nervous system abnormalities, chromosome, karyotype analysis, single nucleotide polymorphism microarray, prenatal diagnosis

Introduction

Fetal central nervous system (CNS) defects are caused by abnormal CNS development during the embryonic period. Common CNS defects include anencephaly, neural tube defects, choroid plexus cyst, ventriculomegaly, hydrocephalus, abnormalities of the corpus callosum and cavum septum pellucidum, holoprosencephaly, lissencephaly, cerebellar and posterior fossa abnormalities, as well as some neurological syndromes, such as Dandy-Walker malformation and Joubert syndrome. Collectively, fetal CNS malformations are the most common and serious congenital malformations.[1] The prevalence of CNS abnormalities in live births is 0.14%–0.16%, reaching as high as 3%–6% in stillbirths.[2–4] Depending on the mechanism of formation, fetal CNS defects may be the intracranial manifestation of

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malformations in other systems, or they may indicate a fetal chromosomal abnormality, which is usually considered the primary cause.[4,5]

Although karyotype analysis is considered the gold standard for prenatal cytogenetic detection,[6] it has some limitations. The technique cannot consistently detect microdeletions and microduplications less than 5 Mb in size. In addition, karyotype analysis requires cell culture, has a long reporting period, and has a higher risk of sample contamination that leads to experiment failure.[7] Duplication/deletion of chromosomal segments less than 5–10 Mb can cause microdeletion and microduplication syndromes that play an important role in multiple malformations and mental retardation.[8–10] Indeed, about 6%–15% of genetic diseases are associated with microduplication/microdeletion of genomic segments (also known as copy number variations, CNVs).[11,12] Conventional karyotype analysis cannot detect CNVs, which are only detectable through high-resolution techniques.

Chromosomal microarray analysis (CMA) has become a first-line technique for prenatal diagnosis of fetuses with structural abnormalities identified through ultrasound.[13–15] CMA can be separated into comparative genomic hybridization (CGH) chips and single nucleotide polymorphism (SNP) chips. Both techniques can detect microdeletions and microduplications across the whole genome, as small as 50–100 kb. The SNP array can detect regions of homozygosity, triploidy, and maternal cell contamination, which the CGH array cannot. In addition, since CMA does not require cell culture, the results can be reported within 3–4 days; therefore, it is increasingly widely used in clinical diagnosis.

In this study, chromosome karyotype and SNP array analyses were performed on 344 fetuses with CNS abnormalities. The results were compared to evaluate the application value of SNP arrays in prenatal diagnosis, which should provide guidance for genetic counseling.

Methods

Study Population

This retrospective study included 344 pregnant women admitted to Fujian Provincial Maternity and Children's Hospital, China, from January 2016 to July 2020, and excluded those with twin pregnancies and those who did not undergo both chromosome karyotype and SNP array analyses. All 344 fetuses were diagnosed with CNS abnormalities via ultrasonographic screening. Written informed consent for participation was received for all patients. The study was approved by the Protection of Human Ethics Committee of Fujian Maternity and Child Health Hospital and complied with the Declaration of Helsinki.

Interventional Surgery

Interventional surgeries were performed by obstetricians using standard clinical procedures under ultrasound guidance.[16,17] Chorionic villi (~10 mg) were extracted using choriocentesis at 9–13 weeks, amniotic fluid (~20–40 mL) was extracted via amniocentesis at 18–24 weeks, and cord blood (~4 mL) was extracted via umbilical vein puncture after 24 weeks.

Chromosome Karyotype Analysis

Fetal chromosome karyotype analysis was performed in accordance with our laboratory's routine operation at the 320–400 band level resolution. Giemsa banding (G-banding) karyotypes were analyzed and diagnosed according to the International System for Human Cytogenomic Nomenclature (ISCN2016).[18]

SNP Array and Data Interpretation

Fetal DNA was extracted using the QIAGEN DNA Mini Kit (Qiagen, Valencia, CA, USA) and analyzed using the CytoScan 750K gene chip detection platform (Affymetrix Inc., Santa Clara, CA, USA). CNV thresholds were set to report deletions >200 kb or duplications >500 kb. Data were analyzed using the Chromosome Analysis Suite (ChAS; Affymetrix). SNP array results were assessed with reference to the following databases: Database of Genomic Variants (DGV, http://projects.tcag.ca/variation), Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (DECIPHER, https://decinher.sanger.ac.uk/), International Standards for Cytogenomic Arrays (ISCA, https://www.iscaconsortium.org/) Consortium, and Online Mendelian Inheritance in Man (OMIM, http://www.omim.org). Peripheral blood from parents was extracted for SNP array detection when appropriate. These databases, scientific literature, and ultrasonography findings were used to systematically evaluate the clinical significance of CNVs, which were then categorized as pathogenic, variants of unknown significance (VUSs), or benign, following the American College of Medical Genetics and Genomics standards and guidelines for interpretation and reporting of postnatal constitutional CNVs.[19] VUSs were further subdivided into likely pathogenic, VUSs with no subclassification, and likely benign.

Statistical Analyses

Data are expressed as frequencies and rates. The detection rate was compared in groups using a chi-square test, with a P value < 0.05 indicative of statistical significance.

Results

Patient Clinical Characteristics

The average age of the 344 pregnant women was 29.9 years (range: 18–45); gestational age ranged from 12–37 weeks. Two cases were diagnosed with choriocentesis, 178 cases with amniocentesis, and 164 cases with umbilical vein puncture. Among the 344 cases, 137 (39.8%) had simple nervous system malformations and 207 (60.2%) had combined extrinsic nervous system malformations.

Karyotype Analysis

Fetal amniotic fluid cell cultures failed in 2 out of 344 cases; the overall success rate was 99.4%. Of the 342 successful cultures, 297 (86.8%) had a normal karyotype (including two chromosome polymorphisms). Of the 45 (13.2%) cases with an abnormal karyotype, 28 had abnormal chromosomal numbers, including 6 cases of trisomy 21, 11 cases of trisomy 18, 5 cases of trisomy 13, and 6 cases of sexual and other chromosome abnormalities (including three chimeras). Among the 17 chromosomal structural abnormalities detected, 7 cases had balanced chromosomal structural abnormalities, and the remaining 10 had large-fragment duplication/deletion (Table 1).

Table 1 Karyotype Analysis Results Showing Chromosomal Abnormalities

Type of Chromosomal Abnormalities	Description of Chromosome Abnormalities	Number of Cases
Numerical abnormalities		28
	Trisomy 21	6
	Trisomy 18	11
	Trisomy 13	5
	Sexual chromosome and other numerical abnormalities	6
Structural abnormalities		17
	Balanced chromosomal structural abnormalities	7
	Imbalanced chromosomal structural abnormalities	10

SNP Array

The SNP array revealed 284 cases with normal results and 60 cases with abnormal results, with an abnormality rate of 17.4% (60/344). The detection rate appeared higher than traditional chromosome karyotype analysis, but this difference was not statistically significant ($\chi^2 = 2.43$, $P > 0.05$). Among the 60 abnormal SNP array results, 25 cases had numerical abnormalities (including one chimera), and 12 cases had structural abnormalities in large segments. In addition, the SNP array detected 23 (6.7%) cases of submicroscopic abnormalities that karyotype analysis did not find. In one case of structural abnormalities in large segments, SNP array identified two additional microdeletions. After searching the database and literature, we concluded that 4 of the 24 (one case had two microdeletions) submicroscopic abnormalities were clearly pathogenic mutations, 5 were likely pathogenic, 8 were mutations with unknown clinical significance, and 7 were likely benign mutations. Table 2 presents details of the 23 cases with submicroscopic abnormalities.

Detection Rates of Anomalies in Fetuses with Isolated CNS Anomalies and Fetuses with CNS Anomalies Plus Extra Ultrasound Anomalies

The detection rates of karyotype analysis were 8.1% (11/136) in the group with isolated CNS anomalies and 16.5% (34/206) in the group with CNS anomalies plus extra ultrasound anomalies; the respective detection rates of SNP array were 12.4% (17/137) and 20.8% (43/207) (Table 3). For both analyses, detection rates were significantly higher in the group with CNS malformations and other ultrasound anomalies than in the group with isolated CNS anomalies (karyotype: $\chi^2 = 5.08$, $P < 0.05$; SNP array: $\chi^2 = 4.01$, $P < 0.05$).

Incidence of Chromosomal Abnormalities in Different Types of Fetal CNS Anomalies

Both karyotype and SNP array analyses detected abnormal chromosomes most frequently in fetuses with holoprosencephaly. Table 4 depicts the relationship between different types of CNS anomalies and the incidence of chromosomal abnormalities.

Discussion

The CNS is one of the most complex systems in the human body, and CNS malformations are the most common of all fetal

Table 2 Copy Number Variations Detected in Fetuses with CNS Anomalies Using SNP Array

Cases	SNP Result (hg19)	Size	Genes Involved	Sonographic Findings	Inheritance Pattern[†]	Clinical Significance	Pregnancy Outcomes
1	2q36.1q36.2 (224,459,152–225,330,583)x3	871 kb	SCG2, MRPL44, SERPINE2	Posterior cranial fossa widened	Unknown	VUS	Live birth
2	3p22.1(42,875,130–43,309,436)x1	434 kb	CYP8B1, POMGNT2	Ventriculomegaly	Unknown	VUS	Live birth
3	3q29(195,678,474–197,340,833)x1	1.6 Mb	CEP19, DLG1, FBXO45, PAK2, SENP5	Choroid plexus cysts, The fourth ventricle communicates with the posterior cranial fossa, Increased NT	De novo	Pathogenic	TOP
4	5q33.2q33.3 (154,435,034–156,727,811)x3	2.29Mb	HAVCR1, HAVCR2, ITK, SGCD	Ventriculomegaly, Intestinal hyperechogenicity	Paternal	Likely benign	Live birth
5	5q35.3(179,194,643–179,767,135)x3	572 kb	LTC4S, MAML1, SQSTM1	Ventriculomegaly, Absence of corpus callosum	Maternal	Likely benign	Live birth
6	7p22.3p14.3(43,376–31,039,092)x3, 14q32.33(105,090,669–106,257,269)x1, 14q32.33(106,705,895–107,284,437)x1	30.9Mb, 1.1Mb, 579 kb	AHR, ETV, HDAC9, IGF2BP3, ACTB; JAG2, MTA, AKT1; no OMIM gene	Choroid plexus cysts	Maternal[‡]	Pathogenic VUS Likely benign	TOP
7	7q36.3(155,347,675–156,348,660)x3	1.0 Mb	SHH, RBM33, CNPY1	Ventriculomegaly, Hydrocephaly	De novo	VUS	Live birth
8	8p23.2(3,703,883–5,940,433)x3	2.2Mb	CSMD1	Choroid plexus cysts	Unknown	Likely benign	Live birth
9	14q21.2q21.3 (46,782,405–49,288,860)x1	2.5Mb	LINC00871, RPL10L, MDGA2, MIR548Y, LINC00648	Ventriculomegaly, Hydrocephaly	Unknown	VUS	Live birth
10	15q11.2(22,770,421–23,277,436)x1	507 kb	CYFIP1, NIPA1, NIPA2	Dandy-Walker syndrome, VSD	Paternal	Likely pathogenic	Lost to follow-up
11	15q13.3(31,999,631–32,444,043)x3	444 kb	OTUD7A, CHRNA7	Severe cerebral edema with interstitial edema	Unknown	VUS	Lost to follow-up
12	15q13.3(32,021,609–32,439,524)x3	418 kb	OTUD7A, CHRNA7	Narrow lateral ventricle, Intestinal hyperechogenicity, Left ventricular echogenic foci	Maternal	Likely benign	Live birth
13	15q13.3(32,011,458–32,444,043)x3	433 kb	OTUD7A, CHRNA7	Choroid plexus cysts	Unknown	VUS	Live birth

(*Continued*)

Table 2 (Continued).

Cases	SNP Result (hg19)	Size	Genes Involved	Sonographic Findings	Inheritance Pattern†	Clinical Significance	Pregnancy Outcomes
14	16p11.2(28,810,324–29,032,280)x1	222 kb	SH2B1, SPNS1, RABEP2, ATXN2L, NFATC2IP, LAT, ATP2A1, TUFM, CD19	Ventriculomegaly, Left ventricular echogenic foci, Intestinal hyperechogenicity	Unknown	Likely pathogenic	Live birth
15	16p11.2(29,591,326–30,176,508)x1	585 kb	ALDOA, CDIPT, MAZ, TAOK2	Ventriculomegaly, Hydrocephalus, Posterior cranial fossa widened, FGR	Unknown	Likely pathogenic	Lost to follow-up
16	16p13.11(15,422,960–16,508,123) x1	1.0 Mb	MARF1, MYH11, NDE1	Ventriculomegaly, Intestinal hyperechogenicity	De novo	Likely pathogenic	Live birth
17	16p13.11(15,058,820–16,309,046)x3	1.25Mb	MARF1, MYH11, NDE1, NTAN1	Ventriculomegaly	Paternal	Likely pathogenic	Live birth
18	17p12(14,099,504–15,491,533)x1	1.3Mb	CDRT1, COX10, PMP22, HS3ST3B1	Corpus callosum agenesis, Small CSP, Renal sinus separation, Oligohydramnion	Unknown	Pathogenic	Live birth
19	17p13.3p13.2(525–5,204,373)x1	5.2Mb	ASPA, MNT, CRK, GP1BA	Ventriculomegaly, Dysplasia of cerebellar vermis, Polyhydramnios	Unknown	Pathogenic	TOP
20	17q12(34,822,465–36,307,773)x1	1.4Mb	HNF1B	Choroid plexus cysts, Left ventricular echogenic foci, Mild tricuspid regurgitation, Renal cortical hyperechogenicity, Collecting system dissociate	Unknown	Pathogenic	TOP
21	18q11.2(19,620,590–21,572,153)x3	1.9Mb	GATA6, RBBP8	Ventriculomegaly, Mild tricuspid regurgitation	Paternal	Likely benign	Live birth
22	18q21.33(59,581,098–59,784,858)x1	204 kb	PIGN	CSP was not evident, Ventriculomegaly, ACC, Intestinal hyperechogenicity, Ventricular echogenic foci	Unknown	Non-pathogenic recessive genetic disease carrier	Live birth
23	20q13.2(53,545,723–54,866,110)x3	1.3Mb	CBLN4, MC3R	Choroid plexus cysts	Unknown	VUS	Live birth

Notes: † Unknown: Patient refused to undergo pedigree verification. ‡ The pregnant woman was a carrier of a balanced translocation, karyotype: 46, XX, t(7;14)(p15;p32.3).

Abbreviations: VSD, ventricular septal defect; FGR, fetal growth retardation; CSP, cavity of septum pellucidum; ACC, agenesis of the corpus callosum; TOP, termination of pregnancy; VUS, variants of unknown significance.

Table 3 Detection Rates of Abnormalities in Fetuses with CNS Anomalies Linked to Different Ultrasound Findings

Ultrasound Findings	Number of Cases	Number of Abnormal Cases		Detection Rate (%)	
		Karyotype	SNP Array	Karyotype	SNP Array
Isolated CNS anomaly	137	11	17	8.1%	12.4%
CNS anomalies combined with extra ultrasound anomalies	207	34	43	16.5%	20.8%
Cardiovascular system	68	4	5		
Digestive system	17	2	5		
Urinary system	15	1	1		
Increased NT or NF	7	1	2		
Abnormal growth indicators	21	1	2		
Others	11	4	4		
Multisystem anomaly	68	21	24		
Total	344	45	60	13.2%	17.4%

Table 4 Types of Fetal CNS Anomalies and Abnormal Chromosome Incidence

CNS Anomalies Classification	Number of Cases[†]	Number of Abnormal Cases	
		Karyotype	SNP Array
Ventriculomegaly and holoprosencephaly	170	16(9.5%)	26(15.3%)
Choroid plexus cysts	92	17(18.5%)	21(22.8%)
Posterior cranial fossa widened	43	5(11.6%)	6(14.0%)
Abnormalities of the corpus callosum	16	1(6.3%)	4(25.0%)
Abnormalities of septum pellucidum or CSP	20	0	2(10.0%)
Cerebellar hypoplasia	16	3(18.8%)	3(18.8%)
Arachnoid cyst	6	0	0
Subependymal cyst	8	1(12.5%)	1(12.5%)
Blake's porch cyst	6	2(33.3%)	0
Holoprosencephaly	6	4(66.7%)	4(66.7%)
Other CNS abnormalities[‡]	16	2(12.5%)	4(26.7%)

Notes: [†]If there were two or more simultaneous CNS abnormalities, the case was counted in each group.[‡]This category includes narrowed ventricular, cerebral white matter lesions, cortical dysplasia, and encephalocele.

defects.[20] Common teratogenic factors include chromosomal abnormalities, intrauterine infections, and the use of certain drugs. Understanding the etiology of CNS anomalies is very important for evaluating fetal prognosis and recurrence risk. Numerous studies have shown that fetal CNS anomalies and numerical chromosomal abnormalities are closely linked.[21–23] Advancements in molecular biology have gradually revealed genomic mutations that cause microdeletion or microduplication syndromes associated with fetal CNS malformations.[24] In recent years, CMA has become increasingly popular for prenatal diagnosis because of its short detection cycle, high throughput, and high resolution. In our study, karyotype analysis and SNP array analysis detected 45 (13.2%) and 60 (17.4%) abnormal cases, respectively; the different detection rates were not statistically significant. Nevertheless, SNP array identified an additional 23 cases (6.7%) carrying submicroscopic abnormalities that escaped karyotype analysis, and 39.1% (9/23) of these cases were pathogenic or likely pathogenic, consistent with previous studies.[25,26]

Moreover, some CNVs were clearly related to CNS abnormalities. For example, duplication of the 7q36 region in case 3 (Table 2) involves the SHH gene, which is linked to holoprosencephaly.[27] Likewise, in case 9, the microdeletion in the 14q21.2q21.3 is related to the MDHA2 gene, which is involved in the development of the nervous system.[28] The microdeletion of 16p11.2 in cases 14 and 15 is an important genetic risk factor for neurodevelopmental disorders, and it also affects the development of brain structure.[29–31]

These findings indicate that chromosome microarray is necessary when an ultrasound suggests fetal CNS abnormalities but the karyotype analysis suggests the opposite. An

SNP array provides more information for assessing fetal prognosis. The clinical significance of genetic information is conducive to genetic counseling and assessment of recurrence risk. The detection rate of karyotype analysis was 8.1% in fetuses with isolated CNS anomalies and 16.5% in fetuses with CNS anomalies plus extra ultrasound anomalies, while detection rates of SNP array were 12.4% and 20.8% in these two groups, respectively. Detection rates were significantly higher in the group with CNS malformations plus other ultrasound anomalies than in the group with isolated CNS anomalies. Thus, fetuses with CNS anomalies (especially if combined with extra system malformations) likely have more chromosomal abnormalities. These findings are similar to previous reports, although some of the studies only showed a higher detection rate of non-isolated CNS abnormalities and no significant difference between the two groups.[3,32]

In this study, detection of holoprosencephaly (66.7%, 4/6) had the highest positive rate among the different types of CNS abnormalities. All abnormal cases were trisomy 18 syndromes. Studies show that 39% of reported trisomy 13 cases in fetuses had neurological malformations, such as holoprosencephaly and dilatation of the cisterna magna.

Despite several advantages, SNP array cannot completely replace karyotype analysis because it cannot detect balanced chromosomal structural abnormalities, such as balanced translocations and inversions. Although most of these anomalies are inherited from parents and do not always affect fetal phenotypes, they can cause spontaneous abortion during reproduction or generate unbalanced chromosomal structures in the fetus. In addition, SNP arrays may ignore a low proportion of mosaic chromosomal abnormalities. Therefore, combining SNP arrays with karyotype analysis will provide more genetic information for clinical guidance and the two methods cannot be replaced by each other.

This study had some possible limitations. First, the study focused on fetuses with CNS abnormalities, whether structural malformations or soft index abnormalities, and we did not analyze the correlation between the two and chromosomal abnormalities. Second, the two methods used in this study cannot identify pathogenic mutations at the gene level, which can be addressed in future research by the application of Next Generation Sequencing (NGS) technology.

Conclusion

In summary, fetal CNS abnormalities, which are related to chromosomal aneuploidy and CNVs, are important indicators for prenatal diagnosis. Chromosomal abnormality rate increases significantly when malformations occur across different systems. SNP array can improve the detection rate of genetic causes in fetuses with CNS abnormalities during prenatal diagnosis. Therefore, when such fetuses are identified through ultrasound, a prenatal diagnosis that includes both karyotype analysis and SNP array should be recommended. The integrated application of karyotype analysis and SNP array can provide us with more genetic information to avoid a missed diagnosis, provide more basis for genetic counseling, and better understand the genetic etiology of CNS abnormalities.

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Disclosure

The authors report no conflicts of interest in this work.

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