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The Application of SNP Microarray Technique in Fetal Central Nervous System Abnormality

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Abstract: Objective: To explore the application significance of SNP microarray technique in fetal central nervous system deformity and the relationship between chromosome abnormality and fetal nervous system abnormality. **Methods**: Collection of 40 abnormal amniotic fluid and abortion casese of fetal nervous system abnormalities sereened by Ultrasonic testing and Nuclear magnetic resonance (NMR) were tested by SNP microarray technology, of the 40 samples, 32 samples of amniotic fluid were additionally analyzed with traditional karyotype. **Results:** The success rate of fetal nervous system anomaly detection was 100%. At the same time, in 32 cases of amniotic fluid analysis, 31 cases were successfully cultured, and the success rate was 96.9%. There were 7 cases of chromosome abnormality (17.5%), 2 cases with abnormal number(5%), 5 cases of structural abnormalities（12.5%）.32 samples of amniotic fluid were tested both by traditional karyotype analysis and SNP microarray technique, the traditional karyotype analysis only found 2 cases of numerical chromosomal abnormalities. SNP chromosome microarray technology also found 2 cases structura1 chromosomal abnormalities in the sample of lateral ventricle and hydrocephalus. Additonally detected out 3 cases of structura1 chromosomal abnormalities among 8 cases of abortion samples with fetal nervous system abnormality. **Conclusion**: SNP microarray can not only detect the numerical abnormalities of chromosome and large fragments of structural abnormalities, but also detect the microdeletion and microduplation of chromosomes, so as to help fully understanding the status of the chromosomal abnormalities of fetal nervous system abnormality, Particularly, gene copy number variation (CNV) is closely related to fetal central nervous system abnormality.

Keywords: Fetal Central Nervous Malformation, SNP Microarray, Chromosome Number/Structure Abnormality, Gene Copy Number Variation

1 Introduction

The development of the central nervous system is carried out strictly in accordance with procedures and is one of the most complex systems in the human body. Nervous system malformation is the most common fetal malformation [1], which reflects the termination of the development of the nervous system at a certain stage. Although the etiology of central nervous system abnormalities is highly heterogeneous, genetic factor is considered to be the major reason [2-3], especially trisomy 13 and trisomy 18. However, with the rapid development of molecular genetics, it has been found that between the nervous system malformation and deletion/duplication of chromosome fragments also have a certain relationship [4].

At present, most hospitals use the G-banding karyotype analysis technique and fluorescence in situ hybridization (FISH) technique for chromosome inspection. G-banding karyotyping is consistently considered as the golden standard for checking chromosomes. However, there are many deficiencies in this technique, which are mainly manifested in the in vitro culture of fetal exfoliated cells. The failure rate is about 10% to 40%; The culture period is long, it takes about 4 to 6 weeks; It is easily contaminated by the mother cells and leads to false inlay[5]. Although FISH technology does not need cell culture, it can only detect specific known regions and the technical operation is difficult, time-consuming and low-flux [6]. Chromosomal microarray technology can perform

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high-resolution detection within the whole genome with sensitivity that is more than 100 times higher than that of karyotype analysis. It can accurately detect chromosome microdeletions, microreplication syndromes [7], uniparental disomy, loss of Heterozygosity and so on, the result is more accurate and reliable.

2 Materials and Methods

2.1 Samples collection

During the period from May 2016 to January 2018, 40 cases of fetal nervous system abnormalities were detected by ultrasound or MRI at the Linyi Women and Children Hospital. These included 22 structural abnormalities including the incomplete development of the corpus callosum, disappearance of the transparent compartment, loss of the cerebellar mound, and expansion of the ventricles. There were 18 cases of flexible target abnormalities such as lateral ventricle widening, choroid plexus cyst, and polyhydramnios. Thirty-two of them accepted amniocentesis and amniotic fluid exfoliated cells tested by SNP chromosome microarray analysis and additionally by conventional cell culture. The other 8 cases of artificial abortion, the SNP chromosome microarray analysis was agreed to analyze the skin or muscle tissue of the aborted embryos.

2.2 SNP Microarray Detection

The whole genome DNA is detected strictly in accordance with Affymetrix CytoScan 750K Array operating procedures (Hangzhou Bo Sheng Company). Amniotic fluid exfoliating cells, flow products extracting DNA, fragmentation, amplification, purification, quantification and hybridization after labeling, the test results were searched online for specific structures and functions of the target gene, such as DGV-human normal copy number variation database, DECIPHER-known syndrome and case database, OMIM Genes-human Mendelian (disease) gene database, and Genes-NCBI database. Deletion, duplication fragments were classified as pathogenic, ambiguous and polymorphic.

2.3 Karyotype analysis

According to the amniotic mid-term karyotype harvest operation procedure, the results were analyzed after inoculation, liquid exchange, harvesting, droping, and staining.

2.4 Statistical analysis

The direct counting method was used to count the frequency of chromosomal abnormalities and the frequency of calculation.

3 Results

In 40 fetal abnormality samples detected by fetal ultrasound or nuclear magnetic resonance, there were 7 chromosomal abnormalities, the abnormal rate is 17.5%, 2 number abnormalities (1 trisomy 18 and 1 trisomy 13), the abnormal rate is 5%, 5 structural abnormalities, the abnormal rate was 12.5%. In the 32 amniotic fluid samples detected by traditional karyotype analysis and SNP chromosomal microarray, only two abnormalities were detected out with traditional karyotype analysis. But the SNP chromosome microarray technique not only detected two abnormalities, but also detected two cases of chromosome abnormalities in lateral ventricle broadening and hydrocephalus. In addition, 3 chromosome abnormalities were detected with SNP chromosome microarray technique in 8 cases of abortive chromosomes, and all 5 structural abnormalities were pathogenic. Specific pathogenic sites and gene functions are shown in Table 1.

Table 1 5 cases of chromosomal abnormalities in the central nervous system

Cases	Gestational age	Prenatal diagnosis indications	Abnormal type and the length of CNV	Pathogenic conditions of the related gene
1	21▼	lateral ventriculomegaly	A 616Kb microduplication in	The region contains gene IL1RAPL1 IL1RAPL1 is associated with neurodevelopment. Some essays say it may also lead to mental retardation.
2	28▼	Lateral ventricles is slightly wider Transparent insulation cavity is disappeared	A 18.2Mb duplication in 11q23.3q25	This area contains 123 genes such as APOA4, APOC3and APOA1 .
			A 3.4Mb duplication in 22q11.1-q11.21	This area contains 37 genes such as XKR3, IL17RA, and CECR1 two variant fragments can lead to Emanuelsyndrome. This syndrome is mainly brain corpus callosum dysplasia, facial deformity, head malformation, mental retardation, stunting, congenital, heart disease and other clinical manifestations.

3	29w	Fetal cerebellar vermis developmental abnormalities Third ventricle is dilated	A 23.8 Mb deletion in 4p16.3 -p15.2	This segment lacks 93 genes including ZNF141, PDE6B and ATP5I. This area contains wolf - hirschhorn syndrome (WHS), the mutant fragment can lead to growth retardation, corpus callosum hypoplasia, mental defects and other clinical manifestations.
4	22w	Hydrocephalus	A 1.93 Mb deletion in the 1q21.1-q21.2	This fragment contains 13 genes including NBPF10, HYDIN2 and NBPF12., the deletion of the fragment can cause the development of nervous system diseases. Mainly include clinical symptoms such as systemic growth retardation, small head deformity mental retardation and short stature.
5	26+3w	third and fourth Ventricular is dilated	A 1.6Mb deletion in 16p13.11	This fragment contains 10 genes such as NDE1, MYH11, and ABCC1. this variant contains a region of 16p13.11 microdeletion syndrome, which can lead to mental retardation and multiple congenital malformations

4 Discussion

The incidence of central nervous system abnormalities is 0.14%-0.16% in the live birth, and the rate of stillbirth is as high as 3%-6% [8]. Fetal nervous system malformations include anencephaly, malformation of the corpus callosum, hydrocephalus, Dandy-Walker syndrome, microcephaly, subarachnoid cyst, widened posterior fossa, and poor cerebellar dysplasia. The development process of the nervous system is complex, and the etiology and pathogenesis of its distortion are still unclear. In addition to teratogenicity caused by disturbances in the development of neural tube and brain bubble caused by teratogenic factors in the environment, many reports have been reported in relation to chromosome number, especially trisomy 13 and trisomy 18 [4]. This study also detected one case of trisomy 13 and one case of trisomy 18 in 40 cases of fetal neurological abnormalities. Studies have shown that 39% of trisomy 13 and 25% of trisomy 18 have a nervous system deformity detected by ultrasonography. Also in this study, trisomy 13 and trisomy 18 were detected in the case of ultrasound-induced microcephaly and polyhydramnios. In recent years, with the rapid development of molecular genetics, there has been a certain relationship between neurological malformations and deletion/duplication of chromosome fragments. In this case, 5 cases of chromosome abnormalities were detected, and the abnormal rate was much higher than the number of abnormalities. This is due to the high resolution of the SNP chromosome microarray. Traditional karyotype analysis did not detect microreplicates of 616 kb in the Xp21.3 region in case 1 and micduplication of 1.6 Mb in 16p13.11 in case 5 in amniotic fluid culture samples. Therefore, the SNP chromosomal microarray can detect more chromosomal abnormalities than the traditional karyotype.

These five chromosome abnormalities involved Emanuelsyndrome (ES), Wolf-Hirschhorn syndrome (WHS) and 16p13.11 microdeletion syndrome. These syndromes are related to neurocognition, growth and development. Case 1 contains only one gene, IL1RAPL1, which codes for high levels of brain neuronal proteins and regulates neurite outgrowth and extracellular secretion. The studies of Franek and other researchers showed that the function of IL1RAPL1 protein is related to mental retardation. In addition to mental retardation, there are other features including low muscle tone, funnel-shaped chest, prominent chin, and one eyebrowetc[9]. In the late follow-up, this pregnant women have induced labor. Case 2 is related with Emanuel syndrome. The syndrome (ES) is a rare chromosomal disorder characterized by multiple congenital malformations and developmental disorders. The reported malformations are growth retardation, central nervous system abnormalities (most common are microcephaly), heart defects, reproductive bowel, musculoskeletal abnormalities, and kidney damage.

It is generally the descendant of a carrier who have the balanced translocation of chromosome 11and 22. Carriers of this balanced transposition are usually not clinically symptomatic and are usually identified in cases of unbalanced translocations of offspring after birth, derived (22)t(11,22) syndrome, and Emanuel Syndrome is also known as Derived 22 Syndrome, Derived 11; 22 Syndrome, Partial trisomy 11; 22, or Derived (22) t(11,22) Syndrome, and this case is on chromosome der(11:22). There are repetitions that should be part of the trisomy of chromosome11and22. Therefore, ES is an unbalanced translocation syndrome, and its production is mainly caused by a chromosome segregation anomaly in the 3:1 segregation of parental balanced translocation carriers during the first meiotic division[10].In this case, because parents rejected the

chromosomal comparison, it is impossible to know the origin and characteristics of the chromosome. Follow-up fetuses were aborted. In case 3, Wolf-Hirschhorn syndrome (WHS) is caused by the partial deletion of the short arm of chromosome 4. Mainly caused by partial deletion of the short arm of chromosome 4, it is characterized by severe growth restriction and mental defects, microcephaly, "Greek helmet" phase and midline fusion defects. The key area for the development of the disease lies distal to the Huntington's disease-associated G8 (D4S10). Studies have reported prenatal diagnosis of intrauterine growth restriction, low fat, foot malformations, etc. WHS should be suspected and prenatal diagnosis [11]. In case 5, the 16p13.11 locus is a genomic hotspot, especially a locus of low-copy repeats. The absence of 16p13.11 is associated with a wide range of mental disorders including schizophrenia, autism, mental retardation, mental retardation, epilepsy and minor microcephaly. The NDE1 gene contained in this gene is the most important gene for the neurodevelopmental phenotype associated with the 16p13.11 microdeletions. The gene NDE1 distributes e-homolog 1 protein, which is located in the centrosome and mitotic spindles and interacts with cytosolic dyne and liss brain neuron-1 (LIS1).This protein plays a crucial role in microtubule organization, mitosis, and neuronal migration, which is critical for the growth of mammalian brain and human cerebral cortex [12-13]. Follow-up pregnant women decided to induce labor.

Among the 18 flexible target abnormalities, only 2 cases of chromosomal abnormalities were detected, 1 case was detected in the Fetal cerebral ventriculomegaly, 1 case was detected in the polyhydramnios, and the remaining 16 cases had normal karyotypes. This brings a dilemma for prenatal diagnosis, that is, there are both normal and abnormal karyotypes in abnormal fetal ultrasound, which brings great pressure and anxiety to guide women to determine the fetal stay or not. Follow-up of 3 cases of Fetal cerebral ventriculomegaly, 5 cases of choroid plexus cysts choose abortion, and the remaining 8 cases choose to continue pregnancy.

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