

BRIEF REPORT

Detection of 21q11.2-q22.11 deletions in a fetus by NIPT

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Background: Non-invasive prenatal testing (NIPT) is extensively used in the detection of fetal trisomies 21, 18, and 13, which is promptly becoming a common clinical practice. Concerned about the clinical application of non-invasive detection of the fetal autosomal duplications or deletion.

Case Presentation: A 34-year-old, healthy pregnant woman was referred to the First Affiliated Hospital of the Air Force Medical University. The ultrasound examination indicates that low-lying placenta, the fetus has a left ventricular bright spot and small amount of pericardial effusion. NIPT was chosen to further screen for fetal chromosomal abnormalities. NIPT results indicated an approximately 18 Mb deletion, which was verified by prenatal diagnosis. The chromosome microarray analysis (CMA) result showed about 19.2 Mb deletions in 21q11.2-q22.11. The karyotype analysis result showed 46,XN,del(21)(q11.2q22.1). Prenatal diagnosis was consistent with NIPT results, and the paternal karyotype revealed no obvious abnormalities.

Conclusion: In this study, we successfully detected and diagnosed deletions of large fragments in chromosome 21 in a fetus using NIPT. This indicates that NIPT can provide effective genetic information for detecting fetal subchromosomal deletions/duplications.

KEYWORDS

chromosomal microarray-based analysis, karyotype analysis, non-invasive prenatal testing

1 | BACKGROUND

Non-invasive prenatal testing (NIPT) are widely used in common detection of fetal trisomies 21, 18, and 13, which is fast becoming a common clinical practice.¹ The test is relatively high specificity and sensitivity.² Recently, a growing body of attention has been used for non-invasive detection of clinical applications of fetal autosomal duplications and deletions.^{3,4} Here, we report a case with abnormal second trimester combined testing, NIPT results indicated a deletion on chromosome 21, which was also confirmed on multiple methods of prenatal diagnosis, the final cytogenetic characterization of trisomy 21q11.2-q22.11 deletion syndrome associated with chromosomal microarray-based analysis and chromosome

karyotype. This syndrome is early infantile epileptic encephalopathy. This case suggested that NIPT is a valuable screening method, about autosomal > 10M fragment genetic abnormalities in pregnancy screening.

1.1 | Case presentation

A 34-year-old, gravida 2, para 1, healthy pregnant woman was referred to the First Affiliated Hospital of the Air Force Medical University. The pregnant woman was 164 cm tall and weighed 71 kg with normal hallmark developmental milestones. The ultrasound examination at a gestational age of 26 weeks indicates that low-lying placenta, the fetus has a left ventricular bright spot and

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small amount of pericardial effusion. Pregnant woman did not have serological screening, an NIPT was choosed to further screen fetal chromosomal abnormalities. NIPT results indicated chromosome 21 was abnormal. Then, prenatal diagnosis was confirmed, including karyotyping and chromosome microarray analysis, the results were consistent with NIPT results.

2 | MATERIALS AND METHODS

2.1 | Non-invasive prenatal testing

Non-invasive prenatal testing methods follow standard procedures, including the isolation of cell-free DNA(two-step centrifugation), library construction(end repair, interface connection and gap repair, PCR), library quantify, sequencing, the sequencing was performed using an Ion Torrent (Life Technologies, California, USA) sequencing system, and bioinformatics analysis. Z-score and GC-correction testing methods were used to identify the fetus autosomal aneuploidy, as described in an article by Liao.⁵ Z scores ranged between - 3 to 3 were considered to indicate low risk for a trisomy chromosome.

2.2 | Chromosome karyotype analysis

Chromosome karyotype analyses with under sterile conditions, was performed for the foetus and parents, on cultured amniocytes according to standard protocols. The experimental process includes routine fixation, production and dyeing treatment, microscopic examination, and analysis of the karyotype (G-banding) of amniotic fluid.

2.3 | Chromosomal microarray-based analysis

The experimental steps including genomic DNA extraction, DNA digestion, ligation, PCR, PCR product check, PCR product purification, quantitation, fragmentation, fragmentation QC Gel, labeling, and hybridization with the arrays were performed according to the manufacturer's standard protocols (Affymetrix Inc, Santa Clara, CA, USA), finally washed, stained, and scanned. Chromosomal microarray-based analysis used a CytoScan 750K array (Affymetrix Inc).The results were analyzed using chromosome analysis suite software.to interpret the data and analyze the genotype-phenotype correlations by public databases (DECIPHER, OMIM, DGV, UCSC, NCBI).

3 | RESULTS

3.1 | Non-invasive prenatal testing

The NIPT results showed that the Z-score of chromosome 21 was -6.876, and demonstrated an approximately 18 Mb deletion, respectively, the scores suggested that deletions of fetal DNA fragments may occur in chromosome 21. Therefore, studies have shown

that the analysis of fetal copy number by maternal plasma sequencing has the potential to detect large fetal deletions/duplications (> 10 Mb) by Chen et al.⁶ The NIPT results are verified as shown in the figure. (Figure 1A,B,C,D).

3.2 | Chromosomal microarray-based analysis

Chromosomal microarray-based analysis results showed about 19.2 Mb deletions in 21q11.2-q22.11 (15,016,486-34,251,578)x1. The deletion region contained 21 OMIM genes. Decipher(patient: 291626, 285024, 285691, etc.), ISCA (patient: nssv577813, nssv577815,etc.), Clinvar,PubMed, and other databases showed that patients with deletion region have clinical manifestations such as stunting, poor speech expression, and cognitive impairment. The absence of this region has pathogenic significance.

3.3 | Chromosome karyotype analysis

Karyotype analysis of amniotic fluid showed chromosome structural abnormalities 46,XN,del(21)(q11.2q22.1). We also analyzed the chromosome karyotype of the parents, and the results of the parents showed no obvious abnormalities.

4 | DISCUSSION

In recent years, NIPT has become more and more accepted by people. This test is designed to screen fetus for chromosomal disorders, including Down syndrome, Edwards' syndrome, Patau syndrome.⁷ Meanwhile, more and more studies have shown that NIPT can effectively detect fetal subchromosomal abnormalities, and research in this direction has great potential. Researchers have done related research. Someone found about 16 Mb deletions in 21q11.1-q21.3 by NIPT, and the results were validated.⁸ There are other researchers who found there was an approximately 50.94 Mb duplication in q11.32-q21.2 of chromosome 18 and an approximately 58.46 Mb deletion in p22.33-p11.1 of chromosome X by NIPT, the result was also confirmed.⁹ In our study, we successfully detected deletions in chromosomes 21 in fetus using NIPT, and further located the specific deletion region precisely using CMA and karyotype analysis, the results of NIPT have been confirmed.

The deletion region (21q11.2-q22.11) contains some disease-causing genes (Table 1), including LIPI,LPDL, PRED5, PRSS7, ENTK, APP, AAA, CVAP, AD1, SOD1, ALS1, MRAP, FALP, C21orf61, GCCD2, FGD2, C21orf59, CILD26, SYNJ1, PARK20, EIEE53. The deletion of two or more genes in this region is critical, which is consistent with Lyle's hypothesis.¹⁰ Of these genes, the deletion of APP gene may be involved in Alzheimer disease-1 caused by mutation in the gene encoding the amyloid precursor protein on chromosome 21q, this is a genetically heterogeneous disorder and may contribute to intellectual disability.¹¹ C21orf59, CILD26 genes are an autosomal recessive disorders caused by defective ciliary movement. The human gene deletion has neonatal respiratory

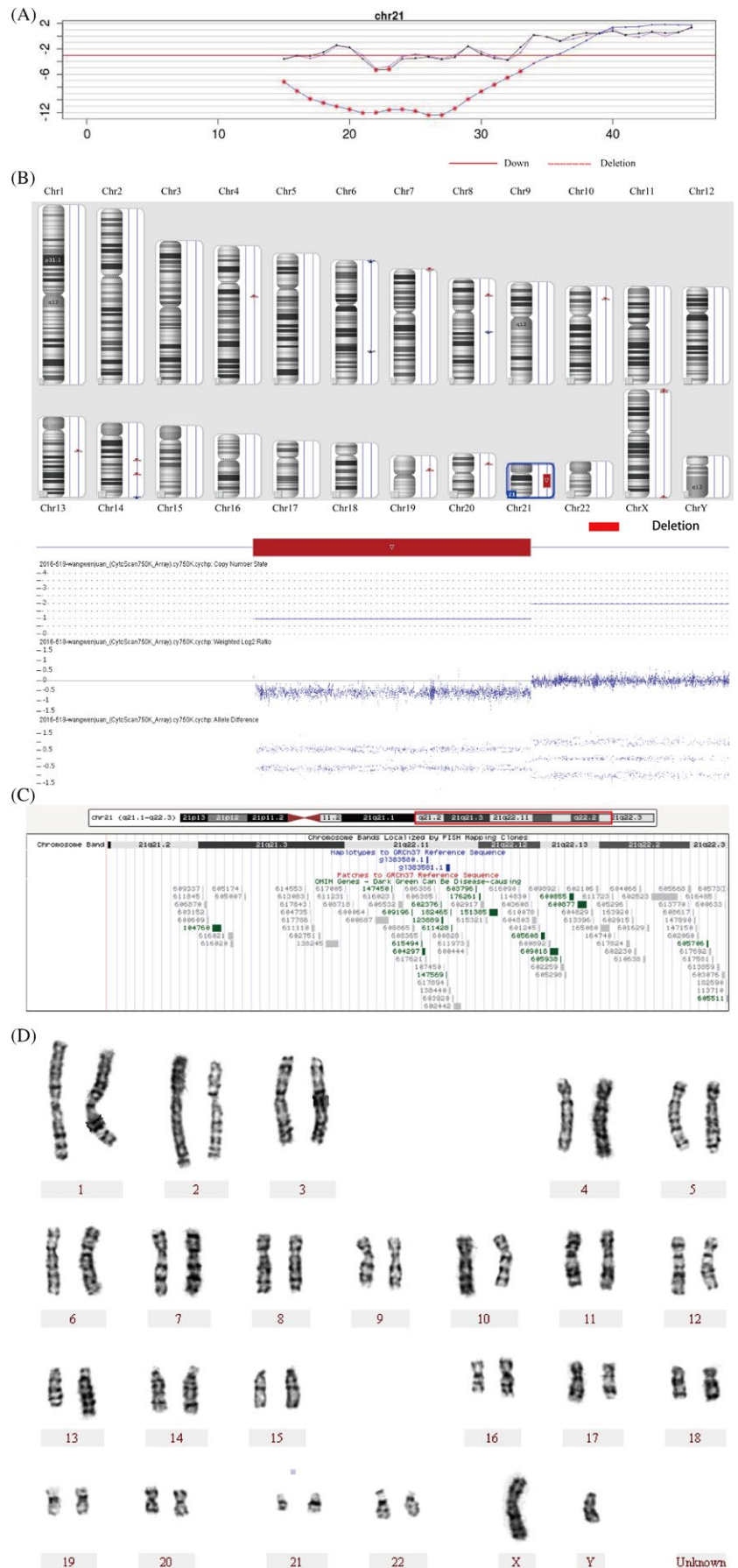


FIGURE 1 A, Analysis result of fetal chromosome 21 karyotype obtained by non-invasive prenatal testing. The red dot area refers to the deletion part chr21; B, Approximately, 19.2 Mb deletions in 21q11.2-q22.11 of chromosome 21 was validated by chromosome microarray-based analysis; C, Microarray profile of chromosome 21 showing the region of deletions and the corresponding OMIM genes; D, The fetal karyotype was 46,XN,del(21)(q11.2q22.1)

Gene	Phenotype
LIPI,LPDL,PRED5	{Hypertriglyceridemia, susceptibility to}
PRSS7,ENTK	Enterokinase deficiency.
SOD1,ALS1	Amyotrophic lateral sclerosis 1.
MRAP,FALP,C21orf61,GCCD2,FGD2	Glucocorticoid deficiency -2.
C21orf59, CILD26	Ciliary dyskinesia, primary, 26.
SYNJ1,PARK20,EIEE53	Epileptic encephalopathy, early infantile-53. Parkinson disease 20, early-onset.
APP,AAA, CVAP,AD1	Alzheimer disease-1, familial. Cerebral amyloid angiopathy, Dutch, Italian, Iowa, Flemish, Arctic variants.

TABLE 1 Genes in the region of 21q11.2-q22.11 and the diseases with which they are associated

distress, recurrent upper and lower airway disease, and bronchiectasis.¹² Early infantile epileptic encephalopathy-53 (EIEE53) is caused by homozygous or compound heterozygous mutation in the SYNJ1 gene on chromosome 21q22. A critical reduction of the dual phosphatase activity of SYNJ1 underlies a severe disorder with neonatal refractory epilepsy and a neurodegenerative disease course.¹³ Early infantile epileptic encephalopathy-53 is a severe neurodegenerative disorder characterized by onset of intractable seizures in infancy. Affected individuals show hypotonia and very poor or absent global development, resulting in severe intellectual disability and spastic quadriplegia.^{14,15} Some patients may die in childhood. In this case, we verified the parents of the fetus, and parental studies revealed that the deletion of this region of the fetal chromosome 21 was not inherited by the parents. For this condition, in our opinion, it will have birth defects if the fetus is born. Finally, the parents opted for termination of pregnancy.

5 | CONCLUSION

In conclusion, combining NIPT, chromosomal microarray-based analysis and karyotype analysis, we successfully detected a deletion on chromosomes 21 with a severe disorder neonatal refractory epilepsy and a neurodegenerative disease, neonatal respiratory distress, recurrent upper and lower airway disease, bronchiectasis and so on. This case shows that NIPT can detect fetal trisomies 21, 18, and 13, and can detect deletions or duplications of large fragments. NIPT can play a greater role in the detection of more complex diseases in the future. However, although NIPT is increasingly used in clinical practice. It is still a screening test that requires prenatal diagnosis to accurately confirm the presence of abnormal chromosome structures.

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