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Relationship Between Gene-Phenotype and Clinical Manifestations of Chromosomal Copy Number Variations Indicated by Non-Invasive Prenatal Testing

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Abstract: *Objective*: To analyze the clinical value of non-invasive prenatal testing (NIPT) in detecting chromosomal copy number variations (CNVs) and to explore the relationship between gene expression and clinical manifestations of chromosomal copy number variations. *Methods:* 3551 naturally conceived singleton pregnant women who underwent NIPT were included in this study. The NIPT revealed abnormalities other than sex chromosome abnormalities and trisomy 13, 18, and 21. Pregnant women with chromosome copy number variations underwent genetic counseling and prenatal ultrasound examination. Interventional prenatal diagnosis and chromosome microarray analysis (CMA) were performed. The clinical phenotypes and pregnancy outcomes of different prenatal diagnoses were analyzed. Additionally, a follow-up was conducted by telephone to track fetal development after birth, at six months, and one year post-birth. *Results:* A total of 53 cases among 3551 cases showed chromosomal copy number variation. Interventional prenatal diagnosis was performed in 36 cases: 27 cases were negative and 8 were consistent with the NIPT test results. This indicates that NIPT's positive predictive value (PPV) in CNVs is 22.22%. *Conclusion:* NIPT has certain clinical significance in screening chromosome copy number variations and is expected to become a routine screening for chromosomal microdeletions and microduplications. However, further interventional prenatal diagnosis is still needed to identify fetal CNVs.

Keywords: Non-invasive prenatal testing; Chromosomal copy number variation; Chromosomes 1 and 3; Chromosome 4; Chromosome 7; Chromosome 15; Prenatal diagnosis

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1. Introduction

Chromosome copy number variations (CNVs) refer to the duplication or deletion of chromosome segments in the genome of more than 1 kb. They are widely distributed in the human genome and are an important genetic basis

that leads to various genetic diseases, disease susceptibility, and individual clinical phenotype differences. Studies have shown that chromosomal microdeletion/microduplication syndrome accounts for 1–2% of all neonatal congenital anomalies [1]. However, the pathogenicity of the chromosomal copy number variation depends on the size and location of the abnormal copy number fragment and the involvement of key genes. In-depth studies are still needed to determine whether a CNV is pathogenic. According to relevant literature [2], about 2.5% of those with normal karyotype have pathogenic chromosomal microdeletions/microduplication syndromes related to clinical phenotypes; about 6% of those with abnormal fetal ultrasound structures have pathogenic chromosomal microdeletions. Besides, 1.7% of fetuses with normal ultrasound structures have pathogenic chromosomal microdeletions/microduplications. Currently, more than 300 chromosomal microdeletion/microduplication syndromes (MMS) caused by pathogenic copy number abnormalities have been discovered [3]. Unfortunately, there is currently no clear and effective treatment for chromosomal diseases. These diseases can only be prevented through prenatal screening and diagnosis. NIPT is a highly precise screening method for fetal trisomy 13, trisomy 18, and trisomy 21 [4]. With the continuous development of technology, although non-invasive prenatal testing (NIPT) can screen for many chromosome number abnormalities [5], the incomplete understanding of the genetic types of human chromosomal microdeletion/microduplication syndromes and the variability in their clinical phenotypes leads to the need for more clinical samples for a more comprehensive an accurate screening. This article will focus on genes with abnormal chromosome copy numbers. The phenotype, fetal ultrasound structure, and pregnancy outcomes of the patients are described and discussed in detail to provide clinical reference and basis for pathogenic microdeletions/microduplications of chromosomal CNVs.

2. Objects and methods

2.1. Research objects

3551 naturally conceived singleton pregnant women who received free NIPT at Dongguan Maternal and Child Health Hospital from September 2016 to May 2017 were selected. The NT-corrected gestational age ranged from 12 weeks to 24 weeks, and they had no history of transplantation, no history of tumors within one year, no history of blood transfusion, and no history of cellular immunity within three months. Chromosome microarray analysis (CMA) was performed on pregnant women whose NIPT indicated chromosomal microdeletions and microduplications with their consent. The research subjects gave informed consent and were informed of privacy protection. This study is a non-profit study and was reviewed and approved by the Ethics Committee of Dongguan Maternal and Child Health Hospital.

2.2. Research methods

2.2.1. NIPT detection method

5 mL of peripheral blood was collected and the maternal plasma was separated by centrifugation, followed by an extraction of fetal DNA. After passing the library construction, quantification, and sequencing templates, the qualified DNA samples were sequenced using the “Initialization” and “Ion P1 Hi-Q 200 V3 Kit” in the sequencer (Model: BioelectronSeq 4000). At the same time, the “Non-invasive Prenatal Data Analysis and Management Software” was used to analyze the sequencing data and calculate the Z-score of the sample chromosomes, with the normal reference value being -3 to +3.

2.2.2. CMA detection method

The patients with NIPT indicating microdeletions/microduplications of chromosomes (except chromosomes 13, 18, and 21) were informed of the risks of amniocentesis. The patients signed an informed consent and

routine disinfection was performed while avoiding the position of the fetal limbs and placenta under ultrasound guidance. After draping, 40 m: of amniotic fluid was extracted to extract fetal DNA for CMA. At the same time, the peripheral blood of both spouses was also collected for CMA.

2.2.3. Follow-up outcomes

The patients were followed up by telephone to inquire about fetal color ultrasound results during pregnancy, the performance and outcomes of any interventional prenatal diagnoses, and the growth and development of the fetus after birth, at six months, and one year post-birth.

3. Results

3.1. NIPT test results

A total of 53 cases among the 3551 cases (except for chromosomes 13, 18, 21, and sex chromosomes) showed chromosome copy number abnormalities, 36 cases underwent interventional prenatal diagnosis and 17 cases did not undergo prenatal diagnosis. Among them, 6 cases failed to be followed up, and 8 were born. No abnormalities were found after birth. One case was born with a low birth weight (2.25 kg), but no abnormalities were found during follow-up one year after birth. There was one case of intrauterine fetal death at 37+ weeks of gestation, and 1 case required induction of labor due to an unplanned pregnancy. 227 cases of prenatal diagnosis were negative, 8 were consistent with the NIPT test results, and 1 was inconsistent with the NIPT test results. The PPV of NIPT in chromosome copy number variation was 22.22%. Telephone follow-up showed that 27 cases were negative, 26 cases showed no abnormalities in fetal growth and development from birth to one year post-birth, and 1 case underwent induction of labor due to chorioamnionitis at 20+ weeks of pregnancy. The details are shown in **Table 1**.

Table 1. NIPT in copy number abnormalities of chromosomes (except chromosomes 13, 18, and 21)

NIPT exception categories	Abnormal NIPT results	CMA results			NIPT chromosome copy number abnormality PPV
		Consistent with NIPT	Inconsistent with NIPT	Negative	
Microdeletion	13	3	0	19	23.08
Microduplication	38	4	1	8	10.53%
Both	2	1	0	0	50%

3.2. Prenatal diagnostic test results and clinical manifestations

A total of 9 cases underwent CMA testing. The CMA results of the parents and fetus, the fetal ultrasound structure, and the pregnancy outcomes of each case are detailed in **Table 2**. Among them, the prenatal diagnosis of Case 2 showed that there was a copy number deletion in the 1q21.1q21.2 region, with a size of 4.188 Mb; a copy number deletion in the chromosome 3p26.3p26.1 region, with a size of 7.354 mb; and its three-dimensional color ultrasound showed complete transposition of the major arteries and ventricular septal defect in the fetal heart. The prenatal diagnosis of Case 4 showed that loss of heterozygosity (LOH) occurred in the chromosome 4q28.3q32.3 region, with a size of 29.96 mb (**Figure 1**). Besides, there was a mutation of unknown significance and ultrasound examination showed no abnormalities. The prenatal diagnosis of Case 7 showed that copy number duplication occurred in the 15q11.2q13.3 region, with a size of 10.1 Mb, and the fetal CMA was 47, XN, +mar. arr{hg19}15q11.2q13.3 (22,700,421-32,915,732)×4 (**Figure 1**). In this case, the fetus was stillborn in utero at 4+ months of gestation, and the autopsy showed structural abnormalities of the fetal heart.

Table 2. CMA test results, clinical phenotypes, and pregnancy outcomes

Number of cases	Age	NT (mm)	Three-dimensional B-ultrasound	NIPT results	CMA test results			Pregnancy outcome
					Fetus	Father	Mother	
Case 1	44	1.6	-	Microduplication on chromosome 18, microdeletion on chromosome 22	(1) Duplication on chromosome 18 (2) Deletion on chromosome 22	-	-	-
Case 2	30	1.3	Fetal heart complete transposition of the great arteries, ventricular septal defect	Microdeletion on the short arm of chromosome 3	Copy number deletion of chromosome bands 1q21.1 to q21.2, with a size of 4.188 Mb; a copy number deletion occurred in the 3p26.3p26.1 region, with a size of 7.354 Mb	-	-	Induced labor
Case 3	22	1.2	-	Z-score of chromosome 5 = 2.289, del: 1M-20M	5p microdeletion syndrome	-	-	Induced labor
Case 4	34	1.5	-	Chromosome 4 may be overrepresented.	Loss of heterozygosity (LOH) occurred in the 4q28.3q32.3 region, with a size of 29.96 mb.	-	-	-
Case 5	37	1.1	-	Z-score of chromosome 7 = 14.430, dup: 63M-157M, dup: 1M-55M.	46,XN,ins(7;11)(p13;q22.3q14)	-	-	-
Case 6	41	1.2	-	There may be an increase in chromosome 15	9.5b microduplication (hg19) in the 15q11.2q13.2 region, and log2 > 1.	-	-	Induced labor
Case 7	41	1.5	4+ months pregnant, fetal death in utero	The Z value of chromosome 15 is 2.874, dup: 24M-28M	CNVs occurred in the 15q11.2q13.3 region. CMA: 47, XN,+mar. arr{hg19}15q11.2q13.3(22，700,421-32,915,732)×4.	-	-	Intrauterine fetal death at 4+ months of pregnancy
Case 8	37	1.3	20+ weeks of pregnancy (1) Biparietal diameter: 45 mm, < -4SD (2) Head circumference: 183 mm, < -2SD (3) Abdominal circumference: 150 mm, < close to -3SD (4) Femur length 33 mm, < close to -2SD (5) Humerus length 31 mm, < -2SD	There may be an increase in chromosome 7	46,XN,t(7;16)(q21;q21)[4]/46,XN[36]	-	-	Inducing labor
Case 9	26	2.6	-	Microdeletion on chromosome 10	Copy number deletion on chromosome 10, with a size of 3.833 Mb	-	Similar missing fragments were identified in both the mother and the fetus	-

Note: "-" Indicates that the result is normal

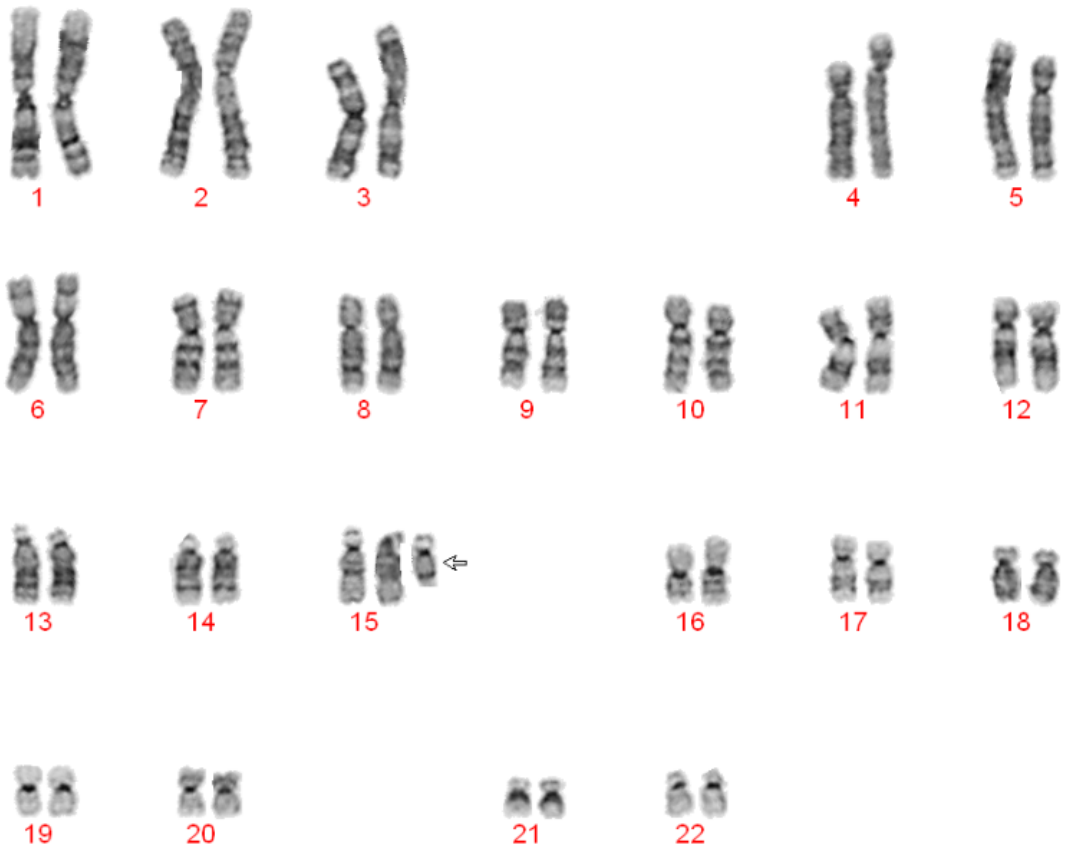

Figure 1. 47,XN,+mar.arr[hg19]15q11.2q13.3(22,700,421-32,915,723)×4

4. Discussion

4.1. The application value of NIPT in chromosome copy number variation

With the advancement of NIPT, there have been more and more reports of NIPT being used for copy number abnormalities outside chromosomes 13, 18, and 21. Tan *et al.* [6] reported that the PPV of NIPT in CNVs was 32.46%; Hong *et al.* [7] reported that the PPV of NIPT in CNVs was 30.95%; Pang *et al.* [8] and Yang *et al.* [9] also reported the PPV of NIPT in CNVs was 32.14% and 30.96%, respectively. The detection of CNVs is related to factors such as fetal cell-free DNA concentration, chromosomal microdeletion micro repeat fragment size, sequencing data volume, and sequencing depth [10]. The total PPV of NIPT in CNVs based on the results of this study was 22.22%, which was lower than the percentage reported in other studies. The reason for the smaller percentage may be attributed to the smaller sample size used in this study. Because prenatal diagnostic testing is an invasive procedure, there might be risks such as miscarriage, needle stick injury, and infection. In this study, 17 pregnant women who underwent NIPT but did not undergo prenatal diagnosis showed CNVs, accounting for 32.08% of the 53 cases with chromosome copy number abnormalities. Some CNVs show no obvious signs at the infant stage and early childhood. Therefore, it is unknown whether CNVs were present in the 17 cases. In addition, in this study, the PPV of NIPT in microdeletions was 23.08%, the PPV in microduplications was 10.53%, and the PPV in both microdeletions and microduplications was 50%. The substantial differences observed among these values may be attributed to variations in sample sizes. Further verification with a substantial amount of clinical data will be necessary to draw more robust conclusions.

4.2. The relationship between gene expression and clinical manifestations of CNVs

More than 300 pathogenic chromosomal microdeletions and microduplications have been identified over the years, and their clinical manifestations vary. However, a large number of clinical samples will be needed to deduce the relationship between the CNVs and certain clinical manifestations.

The 1q21.1 region is complex, with multiple low-copy repeats. These low-copy repeats gather together to form four segmental repeat regions, with sizes ranging from 270 kb to 2.2 mb, making this region prone to repeated deletions and duplications. This chromosomal subregion is a research hotspot for non-allelic homologous recombination. In this study, the CMA test in Case 2 showed that a copy number deletion occurred in the 1q21.1q21.2 region, with a size of 4.188 Mb; a copy number deletion occurred in the 3p26.3p26.1 region, with a size of 7.354 Mb; three-dimensional color ultrasound revealed that the fetus had complete transposition of the major arteries and ventricular septal defect, so labor was induced. Some studies have shown that 1q21.1 microdeletion can cause fetal growth retardation, microcephaly and craniofacial malformations, abnormal cardiac structural development, hydrocephalus, agenesis of the corpus callosum, and genitourinary system abnormalities [11-13]. However, Fu et al. [14] reported that microduplications/microdeletions 1q21.1 can still result in a successful pregnancy and delivery. These reports illustrate the variability of 1q21.1 chromosome CNV and the complexity of the pathogenic mechanism. In addition, there was a copy number deletion in the 3p26.3p26.1 region in Case 2. The copy number deletion in this region can lead to 3p deletion syndrome, which is a rare genomic disease. This disease is characterized by low birth weight, mental retardation and poor physical development, low muscle tone, microcephaly, and micrognathia. There also might be other manifestations such as long philtrum, polydactyly, kidneys, stomach and intestinal abnormalities, low-set ears, congenital heart disease, etc. In Case 2, the ultrasound showed cardiac structural abnormalities, which may be caused by 1q21.1 microdeletion, but it is more likely to be caused by 3p26.3p26.1 copy number deletion. Currently, 3p deletion syndrome has been verified as the main lesion area. The pathogenic mechanism of the 1q21.1 microdeletion is more complex and requires continuous verification with a large number of clinical samples.

In addition, the CMA test of Case 3 in this study revealed that 5p15 deletion can cause Cri-du-chat syndrome [15], which can manifest as cat-like meowing in the neonatal period, also known as cat-meowing syndrome. Its clinical manifestations include severe growth and development retardation, intellectual disability, language impairment, moon face, small head, narrow eye openings, wide spacing between the eyes, and a small mandible, and other abnormal facial features. Some groups also present with congenital heart malformations, abaxial palmar three-rays, and transverse to the palm. Nevertheless, the mortality rate is low, and most children can survive to adulthood. Cases 6 and 7 in this study demonstrated 15q11.2q13.3 microduplications. Microdeletions/microduplications in this part of the chromosome can lead to Prader-Willi syndrome or Angelman syndrome [15]. If it is of paternal origin, the clinical manifestations can include moderate mental retardation, hyposexuality, cryptorchidism, hypoplasia of external genitalia, short body, small hands and feet, almond-shaped eyes, strabismus, and narrow forehead. This condition is known as the Prader-Willi syndrome. If the genetic abnormality originates from the maternal side, symptoms may manifest as severe mental retardation, frequent smiling, limited language abilities, and hyperactivity. Other indicators include a flat back of the head, cerebellar ataxia, and episodes of unconscious epilepsy, which are characteristic features of Angelman syndrome.

In this study, the clinical significance of genetic abnormalities in Cases 4, 5, and 8 is currently unclear although microdeletions/microduplications on chromosomes 4 and 7 have been associated with severe teratogenic and fatal diseases [16]. The types and regions of chromosomal structural variations in the study differ from those reported. Case 8's prenatal B-ultrasound examination showed that the fetal head circumference

and biparietal diameter were both less than two standard deviations for the gestational age. The parents' chromosome test showed no abnormality. There are many reasons for the clinical phenotype of the fetus in cerebellar infants. However, there is currently no information about chromosome 7 at home and abroad. It has been reported that the translocation of the chromosome and the long arm two region 1 of chromosome 16 can lead to cerebellar infants. In this case, it is recommended that the pregnancy be terminated. In Cases 4 and 5, after the mother and her family were fully informed of the situation, they requested to continue the pregnancy. The fetus was followed up by phone after birth and from half a year old to one year old. No obvious abnormalities were found during that period.

5. Conclusion

In summary, chromosomal CNV has diverse clinical manifestations and there is currently no effective treatment for this condition. However, it is crucial that chromosomal structural abnormalities with serious pathogenicity be detected through simple and effective methods before birth as it can greatly improve the quality of the population. Through this clinical sample analysis, NIPT has certain feasibility in detecting chromosomal CNVs. It is unclear whether NIPT can be extended to detect chromosomal CNVs or temporarily extended to pathogenic chromosomal microdeletions /microduplications with clear clinical significance. The detection provides a clinical reference, but many samples are still needed for verification due to the variability of clinical manifestations of chromosomal CNVs.

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Disclosure statement

The authors declare no conflict of interest.

References

[1] Wang Z, Guo Q, Zhou Y, 2016, Current Status and Prospects of Genetic Testing for Chromosomal Microdeletion and Microduplication Syndrome. Chinese Journal of Laboratory Medicine, 39(06): 407–409.

[2] Wapner RJ, Martin CL, Levy B, et al., 2012, Chromosomal Microarray Versus Karyotyping for Prenatal Diagnosis. New Engl J Med, 367(23): 2175–2184.

[3] Liang D, Cram DS, Tan H, et al., 2019, Clinical Utility of Noninvasive Prenatal Screening for Expanded Chromosome Disease Syndromes. Genet Med, 21(9): 1998–2006.

[4] Norton ME, Jacobsson B, Swamy GK, et al., 2015, Cell-Free DNA Analysis for Noninvasive Examination of Trisomy. N Engl J Med, 372(17): 1589–1597.

[5] Malcher C, Yamamotog L, Burnham P, et al., 2018, Development of a Comprehensive Noninvasive Prenatal Test. GenetMol Biol, 41(3): 545–554.

[6] Tan L, Huang T, Li Q, et al., 2021, Application of Non-Invasive Prenatal Testing Technology in Fetal Chromosome Copy Number Variation. Journal of Practical Medicine, 37(22): 2934–2938.

[7] Hong S, Tan M, Wei H, et al., 2023, Analysis of the Positive Predictive Value of NIPT in Copy Number Variation Screening. Chinese Journal of Eugenics and Genetics, 31(04): 804–810.

[8] Pang Y, Wang C, Tang J, et al., 2021, Clinical Application of Noninvasive Prenatal Testing in Detecting Fetal Chromosomal Diseases. Mol Cytogenet, 14(1): 31.

[9] Yang J, Wu J, Peng H, et al., 2021, Performances of NIPT for Copy Number Variations at Different Sequencing Depths Using the Semiconductor Sequencing Platform. Hum Genomics, 15: 41.

[10] Zhao C, Tynan J, Ehrich M, et al., 2015, Detection of Fetal Subchromosomal Abnormalities by Sequencing Circulating Cell-Free DNA from Maternal Plasma. Clin Chem, 61: 608–616.

[11] Haldeman-Englert CR, Jewett T, 2015, 1q21.1 Recurrent Microdeletion. GeneReviews, 8: 93–97.

[12] Mefford H, Hulten M, 2014, 1q21.1 Microdeletions. Unique, 16: 359.

[13] Riddell JVB, Houle A, Franc-Guimond J, et al., 2015, Prenatal Vesico-Allantoic Cyst Outcome — A Spectrum from Patent Urachus to Bladder Exstrophy. Prenat. Diagn., 35: 1342– 1346. https://www.doi.org/10.1002/pd.4702

[14] Fu W, Zhuang J, Wang Y, et al., 2022, Clinical Phenotype and Genetic Analysis of 1q21.1 Microdeletion/Microduplication Fetuses. Progress in Modern Obstetrics and Gynecology, 31(12): 929–933. https://www.doi.org/10.13283/j.cnki.xdfckjz.2022.12.008

[15] Lu G, (ed) 2002 Prenatal Genetic Disease Diagnosis (1st Edition), Guangdong Science and Technology Press, Guangzhou.

[16] Wang W, Hu C, Bi X, et al., 2018, Analysis of 10 Patients with Duplications of 15q11q13 Region and Autism Features. Zhonghua Yi Xue Yi Chuan Xue Za Zhi, 35(1): 23–28.