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Efficiency of Non-Invasive Prenatal Testing in Detecting Fetal Copy Number Variation: A Retrospective Cohort Study

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Purpose: Screening of pathological copy number variations (CNVs) is important for early-diagnosis of hereditary disease. This study was designed to investigate the efficiency of non-invasive prenatal testing (NIPT) in detecting fetal CNVs.

Methods: This retrospective analysis included fetuses with CNVs between January 2018 and December 2020. Karyotype analysis and CNV sequencing (CNV-seq) were performed. We then analyzed the positive predictive values of the subchromosomal microdeletions and microduplications.

Results: Fifty-eight subjects with aberrant CNVs were screened after NIPT, among which 44 finally underwent amniocentesis. CNV-seq confirmed the presence of CNVs in 24 cases. This indicated that false positivity rate of NIPT was 45.5%. Among 24 cases with CNVs after CNV-seq, only 4 showed consistent findings with karyotype analysis, which showed that karyotyping analysis yielded a missed diagnosis rate of 83.3% for the genome CNV. Positive predictive value (PPV) was 50.0% for CNVs with a length of <5 Mb after NIPT screening. PPV for CNVs with a length of 5 Mb-10 Mb was 33.3%, while that for CNVs with a length of ≥10Mb was 60%. For CNVs duplication after NIPT, the PPV was 65.2%, while that for deletion was 36.4%.

Conclusion: For CNVs detected after NIPT, it should be combined with ultrasonographic findings, karyotype analysis, CNV-seq or CMA to determine the pregnancy outcome. Expanding NIPT may increase the risk of unnecessary invasive surgery and unintended selective termination of pregnancy.

Keywords: non-invasive prenatal screening, genome copy number variation, next-generation sequencing, prenatal diagnosis

Introduction

Chromosomal abnormalities are gradually becoming the key cause for mortality and morbidity among fetuses,[1,2] including chromosome numerical abnormality, deletion or duplication, as well as pathogenic copy number variants (pCNVs). To date, prenatal screening, preimplantation genetic testing and prenatal diagnosis are effective for prevention of these conditions among fetuses.[3,4]

Noninvasive prenatal testing (NIPT) is based on the analysis of fetal cell-free DNA (cfDNA) in maternal blood for the detection of fetal chromosome abnormalities in high-risk pregnancies. NIPT for Trisomies 21, 18, and 13 has been common in clinical practice with a sensitivity of over 99% worldwide.[5] Since 2015, NIPT has been recommended as the first-line method for prenatal screening by the International Society for Prenatal Diagnosis (ISPD).[6] Nowadays, it has been utilized in the prenatal diagnosis of the aneuploidy of the whole chromosome.[7] Theoretically, NIPT could also detect the chromosomal microdeletions, microduplications, or CNVs.[8] Unlike common trisomies, the incidence of CNVs is independent of maternal age.[9] Due to a lack of effective screening methods for fetal chromosome CNVs, pregnant women may benefit from prenatal NIPT testing by providing reference for subsequent invasive prenatal diagnosis. This is beneficial for early clinical diagnosis and intervention, and more efficient prevention of birth defects. However, the

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widespread implementation of CNV detection in standard prenatal screening is limited, as the sensitivity in reflecting the genome anomaly is still under investigation. In addition, there is still a lack of studies with large sample sizes focusing on the clinical utility of NIPT in CNVs screening.

Next-generation sequencing (NGS)-based CNV-sequencing (CNV-seq) contributes to the screening of CNVs in the human genome, which is featured by high throughput, high resolution and no need for cell culture.[10,11] Therefore, it has been commonly utilized in the prenatal diagnosis. CNV-seq could precisely localize the fracture site for the micro-structural aberration and confirm the size of the chromosomal aberration.[12] In addition, it could precisely analyze the effects of chromosomal structural aberration on the clinical phenotypes. Therefore, in this study, CNV-seq was utilized to validate the aberrant signals of chromosomal microdeletion and micro-duplication screened by NIPT. This study was designed to analyze the accuracy of NIPT in the prenatal screening, along with the detection of pathogenic CNVs.

Materials and Methods

Subjects

In this retrospective analysis, pregnant women who underwent amniocentesis for prenatal diagnosis at The First Affiliated Hospital of Xinjiang Medical University from September 2017 and December 2020 due to high risk of NIPT screening for CNV results were selected as the subjects. Inclusion criteria were as follows: prenatal diagnostic indications, with NIPT indicating a high risk of chromosomal aneuploidy and a high risk of chromosomal loss/duplication. Exclusion criteria were as follows: threatened miscarriage, an axillary temperature of >37.2°C twice before surgery, a high tendency of bleeding, and signs of pelvic or intrauterine infection.

Fifty-eight subjects with aberrant CNVs after NIPT were eligible in this study, among which 14 refused further genetic test. After signing the informed consent, 44 patients underwent amniocentesis in order to obtain amniotic fluid, and then fetal chromosomal karyotyping analysis was performed together with CNV-seq analysis. After validation of the aberrant CNVs in fetus following invasive prenatal diagnosis, peripheral blood samples were collected and then parental CNVs analysis was performed in order to confirm the genetic origin of CNVs. Meanwhile, genetic consulting was recommended.

Ethical Approval

Written informed consent was obtained from all individuals included in this study. This study has complied with all the relevant national regulations, institutional policies and in accordance with the tenets of the Helsinki Declaration. The study protocols were approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (approval No.: K202201-13).

Sample Collection

Under the ultrasonic guidance, amniotic fluid (20 mL) was obtained under sterilized conditions. The samples were then transferred to Eppendorf tubes. Some samples were cultured in the AmnioMax-II medium, while the other samples were subject to CNV-seq analysis.

NIPT

Venous blood (5 mL) was collected from each subject using potassium–ethylenediaminetetraacetic acid tubes and centrifuged for 10 min at 1600 × g at 4◦C within 8h after blood collection. The plasma was then centrifuged at 4◦C and 16,000 × g for 10 min to obtain cell-free plasma. Plasma circulating cell-free DNA (cfDNA) was extracted from maternal plasma using the Circulating Nucleic Acid kit (Berry Genomics, Beijing, China). DNA library was constructed as previously described using enzymatic reactions, molecular labeling and PCR.[13] DNA fragments were subjected to end repair and linker ligation. After PCR amplification and pooling, single-strand cyclization and DNA nanosphere preparation were carried out to construct a library for sequencing. Each sample was sequenced using the BGISEQ-500 platform and a combinatorial probe-anchored polymer sequencing method, and bioinformatics analysis was performed using BGI Halos software (Shenzhen, China). First, the reference genome window division was used for sequence alignment

correction, so as to reduce the sequencing depth and determine the CNV breakpoint position more accurately. Then, the normal control reference data were used to correct the GC content between batches and within batches of samples, and conduct regional correction for the regions with uneven sequencing data on the genome to effectively improve the detection accuracy. Finally, the position and size of CNVs were determined by binary segmentation and Z-test. On average, cell-free fetal DNA represented only 10% of cell-free DNA in maternal plasma (fetal fraction), while the remaining 90% was of maternal origin. The most critical parameters for CNV detection in the NIPT environment included fetal fraction, size of the aberration, sequencing depth, and the biological variability of the region. A sufficient fetal fraction, which comprised the proportion of fetal DNA against the predominant background of maternal DNA, is vital in the detection of an aberration inversely proportional to its size.

Chromosomal Karyotyping Analysis Based on G Banding

Cells were harvested after a culture of amniotic fluid for 9–10 days, and then karyotyping analysis was carried out. For each sample, 4 karyotypes were analyzed. In the case of mosaicism, 100 mitosis phases were counted. The chromosomal images were analyzed using Leica system. MetaSystems was used for the chromosome analysis (Zeiss, Germany). The denomination of the karyotype was carried out using the International System for Cytogenetic Nomenclature (ISCN, 2013 version).

CNV-Seq and Bioinformatic Analysis

Amniotic fluid and peripheral blood samples were sent to Anoroad Biotech (Beijing, China) for CNV-seq analysis and bioinformatic analysis. The sequencing results were aligned with the reference sequences in the database, to identify aneuploidies and CNVs. The variations were then analyzed using the guidelines proposed by the American College of Medical Genetics.[14] The analysis merely focused on the CNVs with a length of >100 kb. The clinical significance of CNVs was in line with the proposals and guidelines of the American College of Medical Genetics and Genomics (ACMGG).[15] Interpretation of results was conducted based on DECIPHER (https://decipher.Sanger.ac.Uk/), DGV (http://dgv.tcag.Ca/), OMIM (http://omim.Org), after taking the parental phenotypes and CNV-seq alignment results into consideration. Finally, the CNVs were divided into benign CNVs (bCNVs), variants of uncertain significance CNVs (VOUS CNVs), and pathogenic CNVs (pCNVs), respectively.

Results

Population Characteristics

Fifty-eight subjects with aberrant CNVs after NIPT were eligible in this study, among which 14 refused further genetic test. After signing the informed consent, 44 patients (75.8%) underwent amniocentesis in order to obtain amniotic fluid, and then fetal chromosomal karyotyping analysis was performed together with CNV-seq analysis. The median age was 31 yrs (range 25–39), 8 cases (13.6%) aged ≥35 yrs were high risk pregnant women for amniocentesis. The concentration of fetal-free DNA was 7.25% (range 5.98–10.22%). After validation of the aberrant CNVs in fetus following invasive prenatal diagnosis, peripheral blood samples were collected, and then parental CNVs analysis was performed in order to confirm the genetic origin of CNVs. Meanwhile, genetic consulting was recommended.

NIPT Findings

Among the CNVs detected by NIPT, all were single duplication or deletion. Chromosome 22 showed the most CNVs in amount (13.6%), followed by chromosome 5 (11.4%). The proportion of duplication and deletion was 47.8% and 52.3%, respectively.

Capacity of NIPT Screening for CNV, Deletion and Duplication

After the CNV-seq validation, the positive predictive value (PPV) was 50.0% for CNVs with a length of <5 Mb on NIPT. The PPV for CNVs with a length of 5 Mb-10 Mb was 33.3%, while that for CNVs with a length of ≥10Mb was 60%. For the CNVs duplication after NIPT, the PPV was 65.2%, while that for the deletion was 36.4% (Table 1).

Table 1 Prediction of Deletion/Duplication for the Various CNV Fragments Using NIPT

Fragment	Micro-Duplication, NIPT+/CNV-seq+	Micro-Deletion, NIPT+/CNV-seq+	Total
<5Mb	10/16 (62.5%)	5/14 (35.7%)	15/30(50%)
5Mb-10Mb	2/4 (50.0%)	0/2 (0%)	2/6(33.33%)
≥10Mb	3/4 (75.0%)	3/6 (50.0%)	6/10(60%)
Total	15/24 (62.5%)	8/22 (36.4%)	23/46(50%)

Comparison of NIPT, Chromosomal Karyotyping Analysis and CNV-Seq results

Forty-four cases with CNVs after NIPT screening underwent invasive prenatal screening, together with fetal chromosomal karyotyping analysis and CNV-seq. For the 44 with CNVs after NIPT, CNV-seq confirmed the presence of CNVs in 24 cases (54.5%). Among the 24 cases with CNVs after CNV-seq, 10 showed positive findings in karyotyping analysis, including 3 with polymorphism, 1 with balanced translocation, 4 with consistent findings with CNV-seq, as well as 2 with chromosomal aberrations that could not be detected by CNV-seq. Therefore, karyotyping analysis yielded a missed diagnosis rate of 83.3% (20/24) for the genome CNV. In 9 cases (37.5%), the CNVs locations revealed by NIPT was consistent with those obtained after CNV-seq (Table 2).

Follow-Up Data

In total, 26 types of CNVs were identified in 24 cases after the CNV-seq test, including 10 with pCNVs (8 cases), 2 with likely CNVs, and 14 with CNVs of unknown significance. In order to validate the source of CNV, we further analyzed the CNVs in the peripheral blood in parents. Eight cases were confirmed with pCNVs, including two with novel variants that were finally Termination of Pregnancy (TOP), one with pCNVs from the mother (finally TOP), one with pCNVs from father (the fetus was finally born with no anomaly), as well as four cases did not undergo comparison with the parents (2 TOP, and 2 with no anomaly in the post-birth follow-up). For the 2 with likely pathogenic CNVs, the families decided to choose abortion after genetic consultation. Fourteen cases showed unknown clinical significance, among which eight did not perform parental comparison, five were from the mother, and one from father. Only one case (1/14) was willing to decide on an abortion; however, no significant malformation was noticed in the fetal phenotype after TOP. The others were healthy in the post-birth follow-up.

Discussion

Karyotyping analysis is the gold standard for cytogenetic test.[16] However, there are some disadvantages to it, including a limited resolution (only 5–10 MB), time-consuming, as well as a high risk for test failure.[17] Unlike common primary trisomic disease, maternal age showed no relationship with the probability of CNVs.[9] CNVs have been reported to induce some microdeletion/microduplication syndromes (MMS) such as Williams-Beuren Syndrome (WBS), Angelman/Prader Willi Syndrome (PWS), and Charcot Marie Tooth Syndrome (CMTS). Up to June 2016, there were over 70 mmS involving 50,000 cases among explicitly marked CNV diseases recorded in DECIPHER. The incidence of pCNV was nearly 1/600,[18] which occupied half of the birth defects caused by chromosomal aberration. There might be possibilities of pCNVs (1.0–1.7%) in those with no aberrant changes in karyotyping and ultrasonic findings.[19] Therefore, prenatal screening contributed to the reduction of severe birth defects. The efficiency of FISH technology is hindered by the location and quantity of probes, which cannot evaluate unknown DNA fragments.[20] The coverage of chip probes used in chromosome microarray analysis (CMA) is limited, and some pCNVs may not be detected.[21] With the development of high throughput sequencing technology, CNV-seq technique based on NGS technology serves as a new method for prenatal diagnosis with high resolution, high throughput and low cost.[22] Using the NIPT technique, the aneuploidy of chromosome 13, 18, 21 can be clearly identified.[23]

The validity of this part of the NIPT remains to be proven. The American College of Obstetrics and Gynecology (ACOG) recommends that "routine cell-free DNA screening for microdeletion syndromes should not be performed."[24]

Table 2 Comparison of NIPT, Karyotype Analysis and CNV-Seq Results

Case No.	Age, yrs	Gestational Age	NIPT	Karyotype Analysis	CNV-Seq	Chromosome Syndrome	Source	Fetal out-Come
1	37	22	Duplication, in 16q11.2-q23.3, 37.6MB	46, XN[100]	16p13.3q24.3(85,880–90,155,062)x2-3	47,XN,+16[27%]/46,XN[73%] on chromosome 16	Novel	TOP
2	26	19+1	Duplication (8M in length) on chromosome 22	46,X.i(X)(910)[32]/46,X,del(X)(p10)[6]	Dup(22)(q11.21) CN:3.1Mb	22q11.2 duplication	Novel	TOP
5	32	20+2	Duplication (3.85 Mb) on 5q34-5q34	46,XN,?15p+	Dup(5)(q34q34)CN: 3.45Mb	With unknown significance	No comparison	Normal delivery
7	31	18+6	Duplication (5M) on 13q31.1–13q31.2	46,XN	Dup(13)(q31.1q31.2)CN:3.8Mb	With unknown significance	No comparison	Normal delivery
8	27	21+3	Duplication (9.35 Mb) on the 1q4.3–1q4.4	46,XN	(1–22)x2,(XN)x1	None		Normal delivery
9	31	22	Deletion on the long arm of chromosome 3	46,XN?t(3;20)	Del(3)(q26.31q26.32)CN:3.6Mb	With unknown significance	No comparison	Normal delivery
12	29	22	Deletion in sexual chromosome	46,X.i(X)(910)[32]/46,X,del(X)(p10)[6]	46,XN,del(X)(p22.33p11.21)CN:53.45Mb/46,XN,del(X)(p22.33p11.21)CN:53.45Mb,dup(X)(p11.1q28)CN:99.05Mb	Xp monosomy syndrome, Xq26.3 duplication syndrome, Xq27.3-q28 duplication syndrome	No comparison	TOP
13	30	20+3	Chromosome 7 duplication syndrome	46,XN,der(13)?t(7;13)	Dup(7)(q33q36.3)CN:24.175Mb,del(13)(q34q34)CN:2.95Mb。	7q partial monosomy syndrome; 13q distal deletion syndrome	No comparison	TOP
14	29	22	Duplication on chromosome 9	46,XN,del (9)(p23)	Del(9)(p24.3p22.3) CN:14.9Mb	9p distal deletion syndrome	No comparison	Normal delivery, normal fetus
15	34	22+1	Duplication (4.35 Mb) on chromosome 4	46,XN	Dup(4)(p15.1p14).CN:4.15Mb	With unknown significance	Father	Normal delivery, normal fetus
16	33	20+3	Duplication (5.85 Mb) on the long arm of chromosome 21	46,XN	Dup(21)(q21.1q21.1)。CN:2.1Mb	With unknown significance	Mother	Normal delivery, normal fetus
19	27	22+6	2.15Mb duplication(xq28-28.3) on chromosome X	46,XN	Dup(X)(q28q28)CN:3.65Mb	MECP2; anomalies in boy, and no anomalies in girl; duplication syndrome	Mother	TOP
21	32	20+3	Deletion and duplication on chromosome 16	46,XN	Dup(16)(p12.3p12.)CN:1.4Mb	With unknown significance	Mother	Normal delivery, normal fetus
23	28	19+3	Deletion of 3.35 Mb on the long arm of chromosome 10	46,XN,14pstk+[mat]	Del(10)(q21.1-q21.1)CN:13.1Mb	With unknown significance	No comparison	Normal delivery, normal fetus
24	26	17	Deletion (1.68 Mb) on Xp22.13	46,XN	Del(X)(p22.31p22.31)CN:1.75Mb	Recessive X-linked ichthyosis	No comparison	Normal delivery
25	39	19+5	Deletion (4.6 Mb) on the 4q34.3–4q34.3	46,XN	Del(4)(q34.3-q34.3)CN:4.5Mb	With unknown significance	No comparison	Normal delivery, normal fetus
28	35	18+6	Duplication on the chromosome X	46,XN	46,XN[71]/46,XN,dup(X)(p11.1q12)CN:7.35M[29]	With unknown significance	Mother	Normal delivery, normal fetus

(*Continued*)

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Table 2 (Continued).

Case No.	Age, yrs	Gestational Age	NIPT	Karyotype Analysis	CNV-Seq	Chromosome Syndrome	Source	Fetal out-Come
29	33	22+2	Duplication (4.55 Mb) on the long arm of 10q11.22–10q11.23	46,XN	Dup(10)(q11.22-q11.23)CN:3.65Mb	With unknown significance	No comparison	Normal delivery, normal fetus
30	28	22	Deletion (1.5 Mb) on chromosome 17	46,XN	Del(17)(q12q12)CN:1.45Mb	17q12 deletion syndrome	Mother	Normal delivery, normal fetus
31	32	18	Duplication on 1q23-q41	46,XN,dup(1)(q22.3q24.2)	Dup(1)(q23.3q24.2) CN:7.8Mb	Likely pathogenic CNVs	Novel	TOP
35	39	20+1	Duplication on chromosome 9	47,XN,+mar[47]/46,XN[53]	46,XN[42]/46,XN,dup(9)(p21.2P13.1)CN:12.9Mb[58]	Likely pathogenic CNVs	Novel	TOP
36	27	21+4	Duplication (3.35 Mb) on chromosome 17	46,XN,9qh+	Dup(17)(p12p12)CN:1.3M	Associated with the Charcot-Marie-Tooth disease	No comparison	Normal delivery, normal fetus
38	28	20+2	Deletion in the short arm of chromosome 4	46,XN	Del(4)(p16.3p16.1)CN:2.8Mb	With unknown significance	No comparison	TOP
39	31	19+5	Duplication (3. 6Mb) on 22q11.1-q11.21	46,XN	Dup(22)(q11.1q11.21)CN:3.6Mb	22q11.2 duplication syndrome	Father	Normal delivery, normal fetus
41	27	18	Deletion (4.10 Mb) on 3q25.33–26.1	46,XN	Del(3q25.33–26.1)CN:4.05M	With unknown significance	Mother	Normal delivery

Abbreviation: TOP, Termination of Pregnancy.

The American Society of Human Genetics (ASHG) and the European Society of Human Genetics (ESHG) also agree that "routine cell-free DNA screening for microdeletion syndromes is not recommended at this time" because extensive screening for NIP including sub-chromosomal aneuploidy may result in reduced diagnostic specificity.[25] In future, more studies involving a large sample size are required. Fetal-free DNA concentration directly affects the detection efficiency of NIPT.[26] To avoid false negative results, the fetal-free DNA concentration was ranged between 5.98% and 10.22% with an average of 7.25% in our study. We screened 44 cases with CNVs after NIPT, among which 24 pregnant women presented CNVs after invasive prenatal screening. This indicated that the PPV of NIPT screening for CNVs was 54.5% and the false positivity rate of NIPT was 45.5%, which was similar to that of a previous study of 55%.[27] Indeed, there are other studies that only evaluate MMS. Wapner et al developed a targeted single-nucleotide polymorphism-based sequencing approach to detect large deletions associated with five microdeletion syndromes with detection rates of >97%.[9] Ten cases showed positive karyotyping findings. Three were polymorphism, including 1 (case 5) with 46, XN, 15p+, 1 (case 23) with 46, XN, 14pstk+, 1 (case 36) with 46, XN, 9qh+. One (Case 9) showed balanced translocation with a karyotype of 46, XN,?t(3;20). This validated the fact that CNV-seq cannot detect polymorphism and balanced translocation. Four cases showed consistent findings with CNV-seq; however, we could not identify the location of CNVs fragments. Two cases showed aberrant chromosome findings not in terms of CNV-seq, including case 2 with dup(22)(q11.21) CN:3.1Mb. Its Karyotype was 46,X.i(X)(910)[32]/46,X,del(X)(p10) as the karyotype can find mosaicism rather than chromosome ultrastructural anomaly. One case (case 35) showed 46,XN[42]/46,XN,dup(9)(p21.2P13.1) CN:12.9Mb [58], with the Karyotype of 47,XN,+mar[47]/46,XN[53]. All the fetuses were aborted. Therefore, the missed diagnosis rate for CNVs based on the karyotype analysis was 83.3%, but it could identify additional polymorphism, balanced translocation, and chimera that would not have been detected by any other method as well. In 9 cases (37.5%), the CNVs position after NIPT was consistent with CNV-seq results; however, the majority only demonstrated duplication or deletion, which could not display the position and size of CNVs. Besides, the PPV for CNVs of various sizes was different for NIPT. Our data showed that NIPT showed that PPV were higher for the CNVs in a range of <5 Mb or ≥10 Mb, while its prediction capacity was lower in those with a length of 5–10Mb. Theoretically, the detection power of NIPT reduces rapidly with the decreasing size of the deletion/duplication.[28] Our data may be related to the sequencing depth and data volume of NIPT. These data may be unstable on some occasions. For instance, in a previous study, the detection rate was only 14.3% (1/7) for the seven samples with CNVs <5 Mb and >1 Mb by NIPT.[29]

Fetus with ultrastructural anomaly is mainly featured by development delay, mental retardation, and autism, which is a great burden to the public health.[30] Nevertheless, partial CNVs may present differences in the penetrance and phenotype. In this study, follow-up was conducted to the CNVs samples. CNVs comparison and analysis were conducted in 12 cases to the peripheral blood obtained from parents. Four showed novel variants and were finally aborted. Six were maternally inherited and two from father, which were born finally. Twelve cases denied peripheral blood CNVs comparison, including 2 with pCNVs that were finally aborted and 1 with unknown clinical significance that was finally aborted. The others were normally delivered, and were normal in the follow-up. In this study, 14 novel CNV polymorphisms were identified based on NIPT and CNV-seq, which provided solid evidence for the human genome research. Prenatal invasive test indicated presence of CNVs, and then further examinations on the parents were required to confirm the source of CNVs and evaluate the pathogenicity. This contributed to the prediction of risks for the next pregnancy.

False positives may be due to placental chimerism or maternal abnormalities. According to reports, chromosomal abnormalities that occur only in the placenta rather than the fetus are known as localized placental chimeras, with an incidence rate of approximately 1–2%. With the widespread application of NIPT in clinical practice and its shift towards CNV screening, it is expected that there will be more false positives caused by abnormal maternal/placental origin. NIPT is a screening method that requires prenatal invasive testing to confirm the presence of CNVs in the fetus. It is recommended to further examine the parents of the fetus to determine the source of CNVs and comprehensively evaluate their pathogenicity. Therefore, the NIPT positive report of CNV must be interpreted with caution.

Nowadays, NIPT has been considered to be superior in the prenatal screening. In this study, our data showed that the CNVs screened by NIPT combined with CNV-seq and karyotype analysis contributed to the screening of pCNVs in clinical practice. Nevertheless, there are some limitations for the NIPT, and thus, it is only utilized as a screening method.

In cases of any abnormalities after NIPT, prenatal diagnosis should be given for the confirmation. Besides, as there are gene polymorphisms in the CNVs, it is necessary to inform the patients and the family members about the CNVs. Occasionally, parental analysis is required to confirm the source of CNVs, in order to evaluate the pathogenicity of CNVs. In future, prospective clinical studies with a large sample size are required to further confirm the clinical data.

Conclusion

In summary, for the CNVs detected after NIPT, it should be combined with the ultrasonographic findings, karyotype analysis, CNV-seq, or CMA in order to determine the pregnancy outcome. For those with negative findings, ultrasonic follow-up was recommended. The predictive ability of NIPT for CNVs is limited, and most have good pregnancy outcomes. Expanding NIPT may increase the risk of unnecessary invasive surgery and unintended selective termination of pregnancy. Genetic counseling and fertility management are crucial.

Data Sharing Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report there are no competing interests to declare.

References

1. Wang Q, Zeng X, Yu L, Zhao S. Research progress of non-invasive prenatal detection for fetal chromosomal microdeletion/microreplication. *J PractGynecol Endocrinol*. 2019;v.6(13):22–24.
2. Evans MI, Wapner RJ, Berkowitz RL. Noninvasive prenatal screening or advanced diagnostic testing: caveat emptor. *Am J Obstet Gynecol*. 2016;215(3):298–305. doi:10.1016/j.ajog.2016.04.029
3. Kimelman D, Pavone ME. Non-invasive prenatal testing in the context of IVF and PGT-A. *Best Pract Res Clin Obstet Gynaecol*. 2021;70:51–62. doi:10.1016/j.bpobgyn.2020.07.004
4. Shaw J, Scotchman E, Chandler N, Chitty LS. PREIMPLANTATION GENETIC TESTING: non-invasive prenatal testing for aneuploidy, copy-number variants and single-gene disorders. *Reproduction*. 2020;160(5):A1–a11. doi:10.1530/rep-19-0591
5. Gil MM, Quezada MS, Revello R, Akolekar R, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. *Ultrasound Obstet Gynecol*. 2015;45(3):249–266. doi:10.1002/uog.14791
6. Benn P, Borrell A, Chiu RW, et al. Position statement from the Chromosome Abnormality Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. *Prenat Diagn*. 2015;35(8):725–734. doi:10.1002/pd.4608
7. Taylor-Phillips S, Freeman K, Geppert J, et al. Accuracy of non-invasive prenatal testing using cell-free DNA for detection of Down, Edwards and Patau syndromes: a systematic review and meta-analysis. *BMJ Open*. 2016;6(1):e010002. doi:10.1136/bmjopen-2015-010002
8. Srinivasan A, Bianchi DW, Huang H, Sehnert AJ, Rava RP. Noninvasive detection of fetal subchromosome abnormalities via deep sequencing of maternal plasma. *Am J Hum Genet*. 2013;92(2):167–176. doi:10.1016/j.ajhg.2012.12.006
9. Wapner RJ, Babiarz JE, Levy B, et al. Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes. *Am J Obstet Gynecol*. 2015;212(3):332.e1–9. doi:10.1016/j.ajog.2014.11.041
10. Gu W, Deng X, Lee M, et al. Rapid pathogen detection by metagenomic next-generation sequencing of infected body fluids. *Nat Med*. 2021;27(1):115–124. doi:10.1038/s41591-020-1105-z
11. Lan L, Wu H, She L, et al. Analysis of copy number variation by sequencing in fetuses with nuchal translucency thickening. *J Clin Lab Anal*. 2020;34(8):e23347. doi:10.1002/jcla.23347

12. Lan L, She L, Zhang B, He Y, Zheng Z. Prenatal diagnosis of 913 fetuses samples using copy number variation sequencing. *J Gene Med*. 2021;23(5):e3324. doi:10.1002/jgm.3324
13. Qi Y, Yang J, Hou Y, et al. The significance of trisomy 7 mosaicism in noninvasive prenatal screening. *Hum Genomics*. 2019;13(1):18. doi:10.1186/s40246-019-0201-y
14. 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. doi:10.1097/GIM.0b013e3182217a3a
15. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405–424. doi:10.1038/gim.2015.30
16. Tamura Y, Santo M, Araki Y, et al. Chromosomal copy number analysis of products of conception by conventional karyotyping and next-generation sequencing. *Reprod Med Biol*. 2021;20(1):71–75. doi:10.1002/rmb2.12351
17. Ishida C, Gupta V. Genetics, Molecular Testing. In: *StatPearls*. StatPearls Publishing; 2021.
18. Nevado J, Mergener R, Palomares-Bralo M, et al. New microdeletion and microduplication syndromes: a comprehensive review. *Genet Mol Biol*. 2014;37(1 Suppl):210–219. doi:10.1590/s1415-47572014000200007
19. Chen Y, Yu Q, Mao X, Lei W, He M, Lu W. Noninvasive prenatal testing for chromosome aneuploidies and subchromosomal microdeletions/microduplications in a cohort of 42,910 single pregnancies with different clinical features. *Hum Genomics*. 2019;13(1):60. doi:10.1186/s40246-019-0250-2
20. Liu H, Liu J, Wu L. Expert consensus on the application of low-depth whole genome sequencing technology in prenatal diagnosis. *Chin J Med Genet*. 2019;36(4):293–296. doi:10.3760/cma.j.issn.1003-9406.2019.04.001
21. Liu H, Liu J, Wu L, Ye L. Expert consensus on the application of low-depth whole genome sequencing in prenatal diagnosis. *Zhonghua Yi Xue Yi Chuan Xue Za Zh*. 2019;36(4):293–296.
22. Hu T, Liu S, Liu S. [Guidelines for the application of copy number variation testing in prenatal diagnosis]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*. 2020;37(9):909–917. Polish
23. Riopelle RJ, Gawel MJ, Libman I, et al. A double-blind study of bromocriptine and L-dopa in de novo Parkinson's disease. Short-term results. *Eur Neurol*. 1988;28(1):11–14.
24. Committee Opinion No. 640: cell-free DNA screening for fetal aneuploidy. *Obstet Gynecol*. 2015;126(3):e31–e37. doi:10.1097/aog.0000000000001051
25. Dondorp W, de Wert G, Bombard Y, et al. Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening. *Eur J Hum Genet*. 2015;23(11):1438–1450. doi:10.1038/ejhg.2015.57
26. Benn P. Non-invasive prenatal testing using cell free DNA in maternal plasma: recent developments and future prospects. *J Clin Med*. 2014;3(2):537–565. doi:10.3390/jcm3020537
27. Lo KK, Karampetsou E, Boustred C, et al. Limited clinical utility of non-invasive prenatal testing for subchromosomal abnormalities. *Am J Hum Genet*. 2016;98(1):34–44. doi:10.1016/j.ajhg.2015.11.016
28. Chen S, Lau TK, Zhang C, et al. A method for noninvasive detection of fetal large deletions/duplications by low coverage massively parallel sequencing. *Prenat Diagn*. 2013;33(6):584–590. doi:10.1002/pd.4110
29. Li R, Wan J, Zhang Y, et al. Detection of fetal copy number variants by non-invasive prenatal testing for common aneuploidies. *Ultrasound Obstet Gynecol*. 2016;47(1):53–57. doi:10.1002/uog.14911
30. Gorkin DU, Barozzi I, Zhao Y, et al. An atlas of dynamic chromatin landscapes in mouse fetal development. *Nature*. 2020;583:7818):744–751. doi:10.1038/s41586-020-2093-3