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Analysis of the Detection Status of Non-Invasive Prenatal Testing in Sex Chromosome Abnormalities

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Abstract: *Objective:* To analyze the detection efficiency of non-invasive prenatal tests (NIPT) in single fetus sex chromosome abnormalities and explore the application value of NIPT in sex chromosome diseases. *Method:* A total of 47,770 singleton pregnant women received free NIPT at Dongguan Maternity and Infant Clinic. Pregnant women with NIPT results indicating sex chromosome abnormalities provided informed consent for amniocentesis and subsequent karyotype analysis and/or multiplex ligation probe amplification (MLPA) technology. The study also involved telephone follow-ups on test results and pregnancy outcomes, along with a retrospective analysis of the positive detection rate of sex chromosome abnormalities by NIPT. *Results:* Among the 47,770 pregnant women, NIPT identified sex chromosome abnormalities in 158 cases, resulting in a detection rate of 0.33%. Of these cases, 113 pregnant women opted for amniocentesis, while 36 declined. One newborn, whose parents refused puncture, was later diagnosed with cryptorchidism. 8 cases failed to follow up. Among the 113 cases undergoing amniocentesis, 55 were diagnosed with sex chromosome abnormalities. These included 7 cases of X monosomy, 24 cases of sex chromosome trisomy (14 cases of Klinefelter syndrome; 5 cases of Jacobs syndrome; 5 cases of trisomy X), and 24 cases with sex chromosome microdeletions and microduplications. Meanwhile, 58 cases had no abnormalities. The overall positive predictive value (PPV) of NIPT testing for sex chromosome abnormalities was 48.67%, with specific PPVs for monosomy X, Klinefelter syndrome, Jacobs syndrome, trisomy X, and sex chromosome microdeletions and microduplications being 6.19%, 12.39%, 4.42%, 4.42%, and 21.24% respectively. *Conclusion:* NIPT demonstrates high detection efficiency in sex chromosome diseases. However, the efficiency varies significantly across different chromosomal abnormalities. Pregnant women with NIPT results indicating sex chromosome abnormalities should undergo amniocentesis for karyotype analysis and MLPA.

Keywords: Non-invasive prenatal testing; Sex chromosome abnormalities; Karyotype analysis; Multiplex ligation probe amplification (MLPA)

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1. Introduction

Sex chromosome diseases can lead to risks such as underdevelopment or imperfect development of secondary gender characteristics, short stature, average intelligence, language learning disabilities and psychosocial adjustment disorders, infertility, and chromosomal abnormalities in offspring [1]. Because the structural abnormalities of fetal sex chromosome diseases are not obvious in ultrasound imaging, conventional serological screening is unsuitable for detecting these conditions. Interventional prenatal diagnosis by amniocentesis carries the risk of infection and miscarriage, preventing many pregnant women from undergoing the procedure. Consequently, numerous fetuses with sex chromosome diseases remain undetected and untreated until after puberty. In some cases, diagnosis occurs when individuals seek medical attention due to infertility during the reproductive period, causing significant harm to both society and families.

Currently, non-invasive prenatal testing (NIPT) demonstrates high accuracy in detecting trisomy 21, 18, and 13 [2]. With the continuous development of NIPT technology, it can also screen for sex chromosome abnormalities. However, the positive predictive value for sex chromosomes is reported differently in various pieces of literature [3-5]. This article analyzes the detection results of NIPT in fetal sex chromosomes to explore its clinical value in identifying sex chromosome abnormalities.

2. Materials and methods

2.1. General information

A retrospective analysis was employed to select 47,770 pregnant women who underwent free NIPT at Dongguan Maternal and Child Health Hospital from January 2016 to December 2017. The gestational age was more than 12 weeks, and there was no history of transplantation, blood transfusion, or tumor history within the past year, as well as no history of cellular immunity within the last three months. All research subjects were utilized for non-profit scientific research with informed consent and privacy protection as the underlying premise. The Ethics Committee of Dongguan Maternal and Child Health Hospital reviewed and approved the research.

2.2. Methods

2.2.1. NIPT detection

Upon signing the informed consent form, confirmation of a live fetus through B-ultrasound preceded the collection of 5 mL of maternal peripheral blood after 12 weeks of pregnancy to extract fetal cell-free DNA. DNA samples passing the test underwent construction, quantification, and sequencing template preparation. Subsequently, the samples were tested using the “Initialization” and “Ion P1 Hi-Q 200 V3 Kit” in the BioelectronSeq 4000 sequencer. The “Non-Invasive Prenatal Data Analysis and Management Software” was then utilized to analyze the data, applying the general function to calculate the Z value. The normal reference value range was -3 to +3.

2.2.2. Interventional prenatal diagnosis: amniotic fluid karyotype analysis and/or MLPA after amniocentesis

After fully informing the patient about the risks of amniocentesis and obtaining informed consent, the placental location was determined under ultrasound guidance. Following routine disinfection and draping, 40 mL of amniotic fluid was extracted for karyotype analysis and/or MLPA interventional prenatal diagnosis.

2.2.3. Follow-up outcomes

All pregnant women undergoing NIPT received telephone follow-ups to monitor subsequent examination results, pregnancy outcomes, and the fetal condition one year after birth.

3. Results

3.1. Analysis of NIPT results suggesting sex chromosome abnormalities

In this study, 158 cases were suggested to have sex chromosome abnormalities, yielding an abnormality detection rate of 0.33%. Among them, 113 cases underwent amniocentesis for prenatal diagnosis. Of these, 58 cases exhibited no abnormalities, 7 were diagnosed with monosomy X, 24 were diagnosed with sex chromosomes trisomy (14 cases of Klinefelter syndrome; 5 cases of Jacobs syndrome; 5 cases of trisomy X), and 24 cases were diagnosed with sex chromosome microdeletions and microduplications. The total positive predictive value (PPV) of NIPT for sex chromosome abnormalities is 48.67%, with specific PPVs for monosomy X (45,X; including mosaicism), Klinefelter syndrome (47,XXY), Jacobs syndrome (47,XYY), and trisomy X (47,XXX) being 6.19%, 12.39%, 4.42%, and 4.42%, respectively. The total PPV of NIPT in sex chromosome aneuploidies is 27.43%, and the PPV in sex chromosome microdeletions and microduplications is 21.24%. See **Table 1** for details.

Table 1. Analysis of amniocentesis results of NIPT suggesting chromosomal abnormalities

Amniocentesis results		Quantity (example)	Positive predictive value
No abnormalities		58	51.3%
Monosomy X (45,X)		7	6.2%
Microdeletion microduplication		24	21.2%
Sex chromosome trisomy	Klinefelter syndrome (47,XXY)	14	12.4%
	Jacobs syndrome (47,XYY)	5	4.4%
	Trisomy X (47,XXX)	5	4.4%
	Subtotal	24	21.2%
Total		113	100.0%

3.2. Analysis of interventional prenatal diagnosis results

Among the 113 pregnant women who underwent amniocentesis, 102 underwent karyotype analysis and prenatal diagnosis of MLPA, while 11 underwent only MLPA. Karyotype analysis was consistent with MLPA in 92 cases and inconsistent in 10 cases, resulting in a concordance rate of 90.2%. See **Table 2** for details.

Table 2. Analysis of interventional prenatal diagnosis results

Prenatal diagnosis	47,XXX	47,XXY	47,XYY	45,X	No abnormalities	Microdeletion microduplication	Total cases
MLPA	5	14	5	7	58	24	113
Karyotype analysis	5	14	4	6	58	15	102
Consistent	5	13	4	6	58	6	92
Consistency rate (%)	84.31%					6%	90.20%

Ten cases exhibited inconsistencies, as detailed in **Table 3**. For Cases 1, 2, 7, and 10, MLPA revealed structural abnormalities of sex chromosomes, while karyotype analysis showed no abnormalities, and no abnormalities were observed during childbirth. In the remaining cases, there were discrepancies in results between MLPA and karyotype analysis. Induction of labor during childbirth was conducted for Cases 3, 4, 5, and 6, whereas Cases 8 and 9 had no abnormal pregnancy outcomes.

Table 3. Cases with inconsistent results between MLPA and karyotype analysis

Case	NIPT results	MLPA	Karyotype analysis	Pregnancy outcome
Case 1	Sex chromosome abnormalities	46, XN,15pstk+	No abnormalities	No abnormalities
Case 2	Sex chromosome abnormalities	Microdeletion microduplication	No abnormalities	No abnormalities
Case 3	Sex chromosome abnormalities	Microdeletion microduplication	46,x,del(x)(q22)	Inducing labor
Case 4	Sex chromosome abnormalities	The probe signal in the short arm region of chromosome x is weakened, XP is missing, and the size is 52 Mb	Monomer	Inducing labor
Case 5	Sex chromosome abnormalities	X sex chromosome signal is weakened, which does not rule out the possibility of structural abnormalities or mosaicism in the sex chromosomes	45.X[11]/46, XX[24]	Inducing labor
Case 6	Sex chromosome abnormalities	There is a deletion at xp22.33p22.31.xq22.3928 and a mosaic deletion at xp22.31q22.3	45,xo[24]/46,x, +mar[16]	Inducing labor
Case 7	Sex chromosome abnormalities	There is a copy number duplication in the chromosome 16q13.1 region, with a size of 2.11 Mb	No abnormalities	No abnormalities
Case 8	Sex chromosome abnormalities	arr[hg19]16p13.11(15,499,445-16,289,059)X3, the fragment size is about 790kb and contains 5 OMIM genes	46,XN	No abnormalities
Case 9	Sex chromosome abnormalities	Copy number duplication occurs in the 15q11.2q13.3 region, with a duplication size of 10.1 Mb	47,XXY	No abnormalities
Case 10	Sex chromosome abnormalities	A copy number deletion occurred in the chromosome 15q11.2 region, with a deletion size of 506 kb	No abnormalities	No abnormalities

3.3. Telephone follow-up results

All fetuses that underwent NIPT were followed up one year after birth by telephone. Normal neonates identified through NIPT exhibited no obvious growth and development abnormalities after birth. Of the 158 cases suggesting sex chromosome abnormalities, telephone follow-up of the fetuses one year after birth revealed that 58 cases with prenatal diagnosis showed no abnormality and exhibited normal fetal growth and development. For the 7 cases of monosomy X, induction was carried out. For the 4 cases of trisomy X, induction was carried out, and no obvious abnormalities were found. Of the 5 Klinefelter syndrome cases and 14 Jacobs syndrome cases, all had induced labor. Of the 24 cases of microdeletion and microduplication, 13 cases underwent induced labor and termination of pregnancy, while 11 cases had full-term deliveries with no obvious abnormalities one year later. Among the 37 cases that refused puncture, 1 newborn was diagnosed with cryptorchidism after birth, 16 cases showed no obvious abnormalities during follow-up one year after birth, and 20 cases of pregnant women refused to follow up on pregnancy outcomes. Additionally, 8 cases experienced follow-up failure due to empty telephone numbers.

4. Discussion

Currently, NIPT is widely utilized in clinical routine screening for trisomy 21, trisomy 18, and trisomy 13 [6,7], demonstrating high accuracy and specificity. However, there is a lack of consensus on the screening conclusions for sex chromosome abnormalities, leading to controversy over whether NIPT should be extended to routine screening for such abnormalities.

4.1. Detection status of NIPT in sex chromosome diseases

NIPT is not extensively employed for sex chromosome abnormality screening, primarily due to its relatively low PPV for these conditions. The study by Xiaoli Huang *et al*. reported a total PPV of NIPT for sex chromosome abnormalities and aneuploidies were 45.68% and 44.29%, while PPVs in monosomy X, Jacobs syndrome, Klinefelter syndrome, trisomy X, and microdeletion microduplication, were 30.95%, 75.00%, 80.00%, 50.00%, and 54.55%, respectively [3]. Xinran Lu *et al*. reported a PPV of 12.5% in monosomy X, while the PPVs in Jacobs syndrome, Klinefelter syndrome, and trisomy X were 83.33%, 66.67%, and 51.72%, respectively [8]. Lingfang Tang *et al*. reported PPVs in monosomy X, Jacobs syndrome, Klinefelter syndrome, and trisomy X were 19.6%, 50.00%, 58.3%, and 47.8%, respectively [9]. This study revealed a total PPV of NIPT for sex chromosome abnormalities was 48.67%, with specific PPVs for monosomy X, Jacobs syndrome, Klinefelter syndrome, and trisomy X being 6.19%, 4.42%, 12.39%, and 4.42%, respectively. The total PPV of NIPT in sex chromosome aneuploidies is 27.43%, and the PPV in sex chromosome microdeletions and microduplications is 21.24 %, which all results appeared to be notably lower than the aforementioned studies. Despite discrepancies in results, the consensus remains that NIPT has a lower PPV in monosomy X. Discrepancies in PPV have been observed across different studies, reflecting variations in equipment, test strips, analysis platforms, DNA concentrations, and other factors. Factors influencing these variations also include maternal factors such as chromosome mosaicism and tumors, as well as placental and fetal factors such as mosaicism and twin pregnancies. Additionally, sample size significantly impacts research results, necessitating verification with a large number of clinical samples for reliable NIPT screening of sex chromosome abnormalities.

4.2. Diagnostic significance of different prenatal diagnosis methods for sex chromosome abnormalities

Chromosome karyotype analysis, while the primary method for detecting chromosomal aneuploidy, is complex, time-consuming (detection cycle of 3–4 weeks), and requires high technical skills [10]. MLPA technology, on the other hand, offers advantages such as being economical, fast, easy to operate, and highly sensitive, particularly for cases involving chromosomal microdeletions and microduplications in normal karyotypes.

In this study, karyotype analysis showed 92 cases consistent with MLPA and 10 cases inconsistent, resulting in a high consistency rate of 90.2%. Specifically, the consistency rate for sex chromosome aneuploidies was 84.31%, but notably lower at 6% for sex chromosome microdeletions and microduplications, which highlights the need for careful consideration in interpretation. The detection rate of sex chromosome aneuploidies between MLPA and karyotype analysis showed little difference, but MLPA demonstrated higher efficiency in detecting sex chromosome microdeletions and microduplications. This finding aligns with the detection of chromosomal abnormalities beyond sex chromosome abnormalities using MLPA and karyotype analysis [11,12].

Despite the advantages of MLPA, its inherent limitations may affect results, particularly when amniotic fluid cells are limited or specimens are contaminated with maternal blood [13]. Cases in this study (**Table 3**) revealed inconsistencies between karyotype analysis and MLPA, emphasizing the need for increased cell

analysis for accurate karyotype results. MLPA alone showed low accuracy for sex chromosome abnormalities, necessitating its combination with karyotype analysis for prenatal diagnosis to guide clinical work.

In conclusion, NIPT holds clinical value in screening for fetal sex chromosome abnormalities, offering the advantage of reducing unnecessary interventional prenatal diagnostic procedures and enabling early identification and intervention for these diseases. However, the complexity of clinical consultation and the rate of interventional prenatal diagnosis may increase when NIPT suggests sex chromosome abnormalities. The routine extension of NIPT to screen for sex chromosome abnormalities remains controversial. This report, by providing a detailed analysis of the sex chromosome abnormalities and their outcomes suggested by NIPT, contributes valuable clinical applications for NIPT in screening sex chromosome diseases. Nevertheless, due to the subtle clinical manifestations of sex chromosome diseases in early childhood, the report followed newborns until one year after birth. Therefore, the possibility of false-negative NIPT results cannot be completely ruled out. Further studies with larger sample sizes and longer follow-ups are essential.

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Disclosure statement

The authors declare no conflict of interest.

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