A retrospective study of noninvasive prenatal testing for chromosome aneuploidies and subchromosomal copy number variations in 24359 single pregnancies

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Abstract

Background: With the development of whole-genome sequencing, small subchromosomal deletions and duplications could be found by non-invasive prenatal testing(NIPT). Our study is aimed to review the efficacy of NIPT as a screening test for aneuploidies and subchromosomal copy number variations (CNVs) in 24359 single pregnancies.

Methods: A total of 24359 single pregnancies with different clinical features were retrospectively analyzed. Pathogenicity of abnormal NIPT results were assessed according to [American College of Medical Genetics and Genomics\(ACMG\)](#). Chromosome aneuploidies and subchromosomal CNVs were confirmed by karyotyping and chromosomal microarray analysis(CMA).

Results: A total of 442 pregnancies (442/24359,1.9%) were with abnormal NIPT results. The positive predictive value (PPV) for trisomy 21(T21), trisomy 18 (T18), trisomy 13 (T13), and sex chromosome aneuploidies (SCAs) was 84.8%, 54.2%, 11.1% and 40.5% respectively. The PPV for subchromosomal CNVs was 59.0% (46/78). The clinical information, prenatal diagnosis results and follow-up results of 46 true positive cases, 6 cases with subchromosomal CNVs inconsistent with NIPT and 1 case of false negative were also demonstrated in detail.

Conclusion: Our data have potential significance in demonstrating the significance of NIPT not only for common whole chromosome aneuploidies but also for subchromosomal CNV. Besides, the clinical information, prenatal diagnosis results and follow-up results of 52 cases with subchromosomal CNV and 1 case of false negative would provide important guidance for genetic counseling.

Background

The discovery of cell-free fetal DNA (cffDNA) in maternal plasma by Lo et al in 1997 has opened up new approaches for NIPT^[1]. At present, NIPT has been gradually applied as a first-tier aneuploidy screening strategy in clinical practice^[2–4]. Previous large-scale clinical studies have revealed high accuracy of NIPT in screening on trisomy 21, 18 and 13, with sensitivity and specificity higher than 95%^[5, 6], and the PPV range of trisomy 21 was 65–94%, trisomy 18 was 47–85%, and trisomy 13 was 12–62%^[4, 7, 8].

Genomic structural changes, such as CNVs, are also always associated with human disease. More recently, further development and expansion use of NIPT has focused on microduplication/microdeletion syndromes(MMSs)^[9]. The most common microdeletion is at 22q11.2, and recent reports indicate the clinical incidence rate may exceed 1/1000 in the prenatal population^[10]. The 22q11.2 microdeletion syndrome causing DiGeorge syndrome has a very broad clinical phenotype that can include congenital heart disease, palatal, gastrointestinal, genitourinary anomalies, immunodeficiency, endocrine disturbance, developmental delay, cognitive deficits and psychiatric illness^[11]. However, there are many challenges in clinical practice including false-positive rate and low accuracy. Therefore, it was very important to assess the efficacy of NIPT technology on the detection of subchromosomal CNVs.

In the present study, we retrospectively analyzed 24359 single pregnancies undergoing NIPT, including 125 cases of subchromosomal CNVs.

Results

An overview of clinical data

Among the 24367 cases undergoing NIPT, 8 cases were not eligible for the next analysis due to the low concentration of fetal DNA, so the remaining 24359 cases were under-analyzed in the present study. Based on these results, the maternal age for the 24359 pregnancies ranged from 16 to 51 years old. The group aged 25 to 29 years was the majority (8084, 33.2%). Pregnant women older than 35 years were 3966(16.3%). The gestational age ranged from 9 to 34 weeks, and 56.9% of the group had a gestational age from 17 to 20 weeks (see Table 1). The positive rate from younger group to older group were 1.4% (85/6097), 1.8%(144/8084), 1.5%(95/6212), 2.6%(92/3472) and 5.3%(26/494) respectively. (Fig. 1). Older group(≥ 41) has the highest positive rate.

Table 1
Maternal age and gestational age of 24359 blood sampling

Maternal age at NIPT (years)	Number	Percent (100%)
≤ 24	6097	25.0%
25–29	8084	33.2%
30–34	6212	25.5%
35–40	3472	14.3%
≥ 41	494	2.0%
Advanced maternal age (≥ 35 years old)	3966	16.3%
Gestational age at NIPT (weeks)		
≤ 8	0	0%
9–12	680	2.8%
13–16	6525	26.8%
17–20	13867	56.9%
21–24	2612	10.7%
25–28	639	2.6%
≥ 29	36	0.1%
Range (weeks)	12–34	/

There were 442 pregnancy cases with chromosome aneuploidies and submicroscopic anomalies, whose basic information and clinical reasons for NIPT were collected(Table 2 and Table 3). We found that 61(13.8%,61/442) cases had advanced maternal age more than 35 years (included), and 26(5.8%, 26/442)pregnancy cases had ultrasound abnormalities. 123(27.8%, 123/442) cases were with the results of positive serum biochemistry screening. The number of the voluntary group were 210(47.5%, 210/442). Other reasons included poor fertility history and maternal chromosomal abnormality or mental retardation.

Table 2
Clinical information of 442 pregnancy cases with chromosome aneuploidies and subchromosomal CNVs

Maternal age at NIPT (years)	Number	Percent (100%)
≤ 24	77	17.4%
25–29	148	33.9%
30–34	95	21.5%
35–40	97	21.9%
≥ 41	25	5.7%
Advanced maternal age (≥ 35 years old)	122	27.6%
Gestational age at NIPT (weeks)		
≤ 8	0	0%
9–12	13	2.9%
13–16	116	26.2%
17–20	252	57.0%
21–24	46	10.4%
25–28	13	2.9%
≥ 29	2	0.5%
Range (weeks)	12–30 ⁺¹	

NIPT results for T21, T18, T13 and sex chromosome aneuploidies

In 442 pregnancy with abnormal NIPT, there were 12 cases of T13, 35 cases of T18, 90 cases of T21, and 106 cases of sSCAs (Table 4). Among them, there were 102 true-positive cases, 76 false-positive cases, and 65 unverified cases that chose to continue gestation or to terminate the pregnancy. For the 102 true-positive cases, there were 56 cases for T21, 1 case for T13, 13 cases for T18, and 32 cases for sex chromosome abnormalities (Table 4).

For the 56 true-positive cases of T21, there were 23 cases with advanced maternal age(≥ 35) and 24 cases with positive serum biochemistry screening. For the 32 true-positive cases of sex chromosome abnormalities, there were 11 cases with advanced maternal age(≥ 35) and 10 cases with positive serum biochemistry screening.

Table 3
Reasons of 442 pregnancy cases with chromosome aneuploidies and subchromosomal CNVs

Reasons	Number	Percent (100%)
advanced age	61	13.8%
ultrasound abnormalities	26	5.9%
Abnormal serum biochemistry screening	123	27.8%
poor fertility history	2	0.5%
voluntary detection	210	47.5%

Table 4
PPV of common chromosome aneuploidie

Common chromosome aneuploidie	Cases	Unverified	prenatal diagnosis	True Positive	False positive	PPV
T21	90	24	66	56	10	84.8%
T18	35	11	24	13	11	54.2%
T13	12	3	9	1	8	11.1%
sex chromosome	106	27	79	32	47	40.5%
total	243	65	178	102	76	57.3%

NIPT Results For Other Chromosome Aneuploidie

In 442 pregnancy with abnormal NIPT, there were 74 cases of other chromosome aneuploidie including 38 cases of trisomy 7 (T7), 9 cases of trisomy 20 (T20), 12 case of trisomy 16(T16)and so on. One pregnancy with the result of T16 further chosen karyotyping and microarray for prenatal diagnosis. The result of karyotyping was normal but microarray showed that fetus was not only suspected to be a low proportion (< 20%) of trisomy 16 but also there was a loss of heterozygosity (LOH) about 15.3 Mb fragment in the 16q23.1q24.3 segment. LOH means that chromosomes of a homologous pair are inherited from the same parent, which increases the risk of imprinting disorder. Study has reported that LOH were often caused by trisomic rescue and one third of these trisomic rescues lead to a LOH^[12]. Currently, there is no evidence for imprinted genes encoded by 16q23.1q24.3.

The imprinted genes has already been proven for chromosomes 6, 7, 11, 14, 15 and 20, and LOH of these chromosomes contribute to the associated imprinting disorders. Thus, when NIPT results showed chromosome aneuploidie in chromosomes 6, 7, 11, 14, 15 and 20, both karyotyping and microarray should be considered to detect the mosaic trisomy and LOH.

Fetal Subchromosomal CNVs

In addition, NIPT could also identify positive cases of subchromosomal CNVs. In 442 pregnancy with abnormal NIPT, there were 125 (28.3%,125/442) cases of subchromosomal CNVs. Clinical significance of these CNVs included uncertain significance, possible pathogenicity and pathogenicity. After genetic counseling about the clinical significance of these subchromosomal CNVs, 78 cases, including 27 cases with subchromosomal CNV $\geq 10M$ and 51 cases with subchromosomal CNV $< 10M$, chosen to perform amniotic fluid puncture for further prenatal diagnosis. 51 cases of them chosen both karyotyping and microarray as prenatal diagnosis methods, while 3 of them only chose karyotyping and 24 of them only chose microarray. The ppv for subchromosomal CNVs screened by NIPT were 59.0% (46/78). Remaining 32 false positive cases included 6 cases with different CNV and 26 cases with no abnormality. The ppv for subchromosomal CNVs $\geq 10M$ was 37.1%(10/27) and for subchromosomal CNVs $< 10M$ was 70.6% (36/51).

We also analyzed indications of 78 prenatal diagnosis cases with subchromosomal CNVs, we found that 20 of 37 cases with high risks of serum screening were true positive. Interestingly, 20 of 35 cases without any indications were true positive (Table 5). Among 52 abnormal cases, 31 cases were correlated to microdeletion or microduplication syndromes suggesting that NIPT may be an important method to find potential birth defect. Moreover, we compared fetal free DNA concentration between 46 true positive cases and 32 false positive cases. The result showed that true positive cases had higher fetal fraction than false positive cases ($p = 0.013$) (Fig. 2). In addition to fetal concentration, abnormal maternal chromosome also increases the false positive rate of the fetal chromosome. The clinical information, prenatal diagnosis results and follow-up results of 52 abnormal cases are shown in the table 6 and table 7 (sorted by CNV size)

Table 5
Indications for 78 prenatal diagnosis cases

Indications	Prenatal diagnosis case	True positive case	PPV
Abnormal biochemistry screening	37	21	56.8%
ultrasound abnormalities	5	3	60.0%
advanced age	13	6	46.2%
voluntary detection	35	20	57.1%
total	78	46	59.0%

Six Cases Distal 22q11.2 Microdeletions And Microduplications

The chromosome region 22q11.2 has long been recognized as a hotspot for genomic rearrangement. This region contains several large segmental duplication/low copy repeats (LCRs) that function as mediators of non-allelic homologous recombination (NAHR) and predispose the genomic region to chromosomal rearrangements^[13]. 22q11.2 deletion syndrome and 22q11.2 duplication syndrome are caused by genomic rearrangement of 22q11.2. Some researchers believe that the incidence of 22q11.2 deletion syndrome is higher than 22q11.2 duplication syndrome, with an estimated frequency of 1 in 4,000–6,000 live births^[14]. However, retrospective analysis of our study suggested that 22q11.2 duplication syndrome occurred more frequently. The clinical manifestation of five fetuses with 22q11.2 duplication syndrome and one with 22q11.2 deletion syndrome were analyzed in detail followed (table 8).

Case 24 And 29

Both of these two cases were confirmed to have 22q11.21 microduplication and their parents did not accept CMA to verify the source of this mutation. Fortunately, these two cases were clinically healthy after birth. It has been reported that the duplication of the 22q11.2 can cause different degrees of mental retardation, learning disabilities and growth retardation. However, there are also cases without any clinical phenotype, which indicates that 22q11.21 microduplication may have incomplete manifestation or performance differences^[15]. Therefore, it is difficult to determine the pathogenicity of 22q11.21 microduplication.

Case 30

Case 30 was confirmed to have 22q11.21q11.23 microduplication by CMA. Ventricular septal defect, aortic abnormalities and 1 bright spot in the left ventricle were subsequently detected by ultrasound. Parents of the fetus decided to terminate pregnancy.

This fragment overlaps a recurrent 22q11.2 distal region (LCR22-D to LCR22-H). Studies have reported that this 22q11.21q11.23 microduplication had been found in patients with clinical phenotype such as mild developmental delay, facial abnormalities, and speech delay. Some patients' 22q11.21q11.23 microduplication were inherited from their parents with normal phenotypes^[16].

Case 37

Case 37 was confirmed to have 22q11.21q11.23 microduplication by CMA and also confirmed to be clinically healthy after birth.

Case 34

Case 34 was confirmed to have the loss of heterozygosity in 22q12.1q12.2. clinical phenotypes was unavalibale. Fetal mother were also advised to have a array comparative genomic hybridization because of her mental retardation. CMA analysis showed her with 22q11.23 microduplication.

Case 35

The 22q11.21 deletion syndrome(DiGeorge syndrome/velocardiofacial syndrome) is characterized by congenital heart defects, immune deficiency, transient neonatal hypocalcemia, velopharyngeal insufficiency and a distinctive facial appearance but also by learning disabilities, behavioral anomalies and hypotonia. The majority of the 22q11.2 deletions are de novo^[17]. Case 35 is confirmed to have a 22q11.21 deletion syndrome with hoarseness, atrial septal defect, patent ductus arteriosus and myocardial injury after birth. The origin of 22q11.21 deletion in case 35 was unknown.

One case of Xp22.31 microdeletion

Case 46 was detected to have a 1.6 Mb microdeletion in Xp22.31 by NIPT, which often causes ichthyosis (X-linked recessive genetic disease). Most female carriers of Xp22.31 microdeletion have a normal phenotype, a few female carriers may show abnormal symptoms due to inactivation of X chromosomes and all male carriers show ichthyosis^[18]. In our study, case 46 chosen to perform prenatal diagnosis for further confirmation and the result showed that the fetus was female and this mutation is inherited from her normal mother.

One case of 5.4 Mb microdeletion in 9p21.3-p21.1

Case 49 was detected to have a 5.4 Mb microdeletion in 9p21.3-p21.1(24,796,507 – 30,288,265) by NIPT, which contains 8 OMIM genes such as *TEK* and *C9orf72*. Heterozygous mutations of *TEK* gene often causes venous malformations, multiple cutaneous and mucosal(autosomal dominant genetic disease). *C9orf72* is related with Frontotemporal dementia and/or amyotrophic lateral sclerosis 1(autosomal dominant genetic disease). However, case 49 was healthy and this 5.4 Mb microdeletion was inherited from his/her healthy mother. Interestingly, our laboratory had previously detected a 4.2 Mb microdeletion in 9p21.2p21.1(26,210,360 – 30,492,812) by CMA from a fetus with NT = 1.3 cm. This 4.2 Mb microdeletion was de novo. Theses results suggested that 9p21.3-p21.1 microdeletion may have penetrance difference.

A false negative NIPT result for case 53 with 4p14 microdeletion

The NIPT result of case 53 with high risk of serum screening was normal. System structure screening (other hospital) in 24 week showed fetal growth retardation. The fetal was diagnosed as neonatal pneumonia, low weight, congenital heart disease and hyperbilirubinemia after birth. Karyotype detection of peripheral blood showed 46,XX,del(4)(p4). Thus, we improved the experimental method for better cffDNA enrichment and retrospectively tested case 53^[19]. The results showed increased cffDNA fraction

from 6.5–15.1% and a 34Mbp deletion in 4p16.3-p15.1 region, which is related with Wolf-Hirschhorn syndrome (WHS).

Discussion

In recent years, NIPT has been widely used in clinical practice for prenatal screening of T21, T18 and T13. While screening for the whole genome, NIPT also found some chromosomal deletions and duplications at the submicroscopic level. The researches about the feasibility of using NIPT for screening of chromosome CNVs have been performed. In 2011, Peters et al reported for the first time that whole-genome sequencing of fetal free DNA from plasma of pregnant women detected a deletion of approximately 4.2 Mb in the p11.22p12.1 region with a large amount of sequencing data (243M)^[20]. Since then, many scholars have detected chromosomal microdeletions and microduplications by NIPT. These researches all have high sequencing depth and the detection resolution can be as low as 300 kb^[21–22].

In this study, we reviewed the use of NIPT in the context of screening for common chromosome aneuploidies as well as subchromosomal CNV within a cohort of 23373 single pregnancies. The PPV for T21, T18, T13 and SCAs was 84.8%, 54.2%, 11.1% and 40.5% respectively. At the same time, we also found 125 positive cases with subchromosomal CNVs. 78 of 125 chosen for further detection, with 46 (59.0%, 46/78) true-positive cases and 32 false positive cases (41.0%, 32/78) validated by chromosome karyotyping and CMA. Previous researches have shown that the extent of chromosomal abnormalities presented in plasma of women with aneuploidy pregnancies is linearly correlated with the cfDNA fraction, thus the test accuracy of NIPT largely relies on the fetal fraction^[23]. In our study, the content of fetal free DNA fragments in the plasma of pregnant women varied greatly and can reach 35% and the cell-free fetal DNA between the true positive cases and false positive cases showed significant difference ($p < 0.05$). Possible reasons causing the false positives also include abnormal maternal chromosome and confined placental mosaicism (CPM) were also reported to cause the false positive cases.

chromosomal microdeletions and microduplications have become increasingly recognized as significant contributors to human diseases^[24], which are present in approximately 1.7% of structurally normal pregnancies^[25]. Other studies have suggested that PPV of microdeletions detected by NIPT have not demonstrated a sufficiently low false-positive rate to be deemed practical^[26]. However, for our studies, NIPT had higher PPV (59%) for microdeletions and microduplications. Among the 46 true positive cases and 7 abnormal false positive cases, 31 cases were correlated to microdeletion or microduplication syndromes with 6 cases inherited from parents, 3 cases de novo and other 22 cases unavailable. Early detection of pathogenic and potentially pathogenic CNVs by NIPT has good benefit in prenatal screening.

22q11.2 microduplication is the most frequent in our research. The phenotype of the five patients with 22q11.2 microduplications are diverse, with symptoms ranging from being normal to mental retardation and congenital heart malformation. 22q11.2 microdeletions is the second most common chromosomal abnormality secondary to Down syndrome^[27]. However, the occurrence of 22q11.2 microduplications is

more frequent than 22q11.2 microdeletion in our study, which is contrary to previous research conclusion. The rare occurrence of 22q11.2 microduplication cases may be explained by the absence of a defined phenotype and incomplete penetrance^[28].

Studies have demonstrated that there is a small chance of a false negative result for NIPT^[29]. In our study, there is a false negative case in 79 validated NIPT. The most common factor associated with these false negative results is the low fetal fraction, which are often affected by maternal weight, gestational age and extraction method^[30–31]. In our research, extraction method for cfDNA enrichment was the main reason for the false negative cases. Therefore, improved extraction method for elevating fetal fraction were immediately used in December 2018, which may be the potential reason for improved the overall performance of NIPT and high PPV in this research. Faas BH et al in 2012 clarified that cell free fetal DNA in the maternal plasma originates from cytotrophoblastic cells derived from trophoblast of the blastocyst^[32]. The karyotypes of cytotrophoblast and fetus may be different due to fetus are derived from the inner cell mass (ICM) of the blastocys^[29]. Other reasons for false negative results may be CPM and maternal mosaicism^[33–35].

Conclusions

This study demonstrates that the PPV for T21, T18, T13 and SCAs was 85%, 54%, 11% and 41% respectively. The PPV for CNVs was 59.0%. The PPV for CNVs < 10 Mb is 70.6% and for ≥ 10 Mb 37.1% respectively. Our data have potential significance in demonstrating the usefulness of NIPT not only for common whole chromosome aneuploidies but also for CNVs.

Materials And Methods

Patients

From 2015 to July 2019, 24359 pregnant women opted for NIPT to screen fetal chromosome aneuploidies. Informed written consent was obtained from all pregnant women who agreed to receive NIPT. Pregnancies with high risks were divided into advanced maternal age, ultrasound abnormalities, poor fertility history, positive serum screening, and other groups in this study.

NIPT sequencing

Maternal peripheral blood (5 ml) was collected in an ethylenediaminetetraacetic acid (EDTA) tube. The blood sample was stored at 4°C immediately after collection. Afterwards, cfDNA extraction, library construction, quality control, and pooling were performed according to the JingXin Fetal Chromosome Aneuploidy (T21, T18, T13) Testing Kits (CFDA registration permit No. 0153400300). Sequencing reads were filtered and aligned to the human reference genome (hg19). Combined GC correction and Z-score testing methods were used to identify fetal autosomal aneuploidies. A cut off value of Z-score > 3 was used to determine whether the ratio of the chromosomes was increased. Here, each chromosome with an

absolute value of the Z-score greater than 3 was marked with chromosome aneuploidies or microdeletions/ microduplications.

Chromosome karyotype analysis

Banding cytogenetics was performed on G-banded metaphase chromosomes of cultured peripheral blood lymphocytes using routine techniques. Karyotypes were interpreted according to the International System for Human Cytogenetic Nomenclature.

Chromosome karyotype analysis

The aCGH platform from CytoScan™ 750 k chip made by American Affymetrix was employed in this study. The standard operating procedures for genomic DNA digestion, ligation, amplification, purification, fragmentation, labeling, chip hybridization, washing and scanning, and data analysis were performed. Pathogenicity of genomic DNA fragments were determined with reference to the international public pathological CNVs database, international public pathological CNVs database, international public benign CNVs database and human genetics cytogenetics microarray database.

Abbreviations

NIPT: Noninvasive prenatal testing; cffDNA: Cell-free DNA; CMA: Chromosomal microarray analysis; CNVs: Copy number variants; MMS: Microdeletion/microduplication syndromes; PPV: Positive predictive value; LCRs: Low copy repeats; NAHR: Non-allelic homologous recombination; CPM: Confined placental mosaicism; ICM: Inner cell mass.

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Huaian Maternal and Child Health Care Hospital.

Consent for publication

The authors declare that they have no competing interests and the patients in this case report had provided their consent for publication.

Competing interests

The authors declare that they have no competing interests.

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Authors'contributions

All authors have materially participated in the study and manuscript preparation. YF Liu, LF Chen, Y Peng, Z Liang, X Jin and NN Yan collected all clinical data. YF Liu participated in the data analysis and drafted the manuscript. Q Pan designed the work and drafted and revised the manuscript. All authors have approved the final article.

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Tables 6-8

Due to technical limitations, Tables 6-8 are provided in the Supplementary Files section.

Figures

Figure 1

The positive rate of NIPT for aneuploidy and subchromosomal CNV increases with maternal age and the number of positive cases in the five age groups.

Figure 2

Fetal free DNA fraction of NIPT between true positive group($\bar{X} \pm S = 17.3 \pm 5.7$, $n=46$) and false positive group($\bar{X} \pm S = 14.0 \pm 7.6$, $n=32$)($p=0.0185$).

Supplementary Files

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