A Retrospective Study of Noninvasive Prenatal Testing for Chromosome Aneuploidies and Sub-Chromosomal Copy Number Variations in 24359 Single Pregnancies

Yuefang Liu  
huai'an maternal and child hospital

Longfei Cheng  
huai'an maternal and child hospital

Yuan Peng  
huai'an maternal and child hospital

Zhe Liang  
huai'an maternal and child hospital

Pan Qiong (✉ 18252586797@163.com)  
huai'an maternal and child health care hospital  https://orcid.org/0000-0001-8151-0923

Research

Keywords: Noninvasive prenatal testing (NIPT), Sub-chromosomal copy number variations (CNVs), Positive predictive value (PPV)

DOI: https://doi.org/10.21203/rs.3.rs-104459/v1

License: ©  This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
Abstract

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

**Methods:** A total of 24359 single pregnancies with different clinical indications were retrospectively analyzed. The positive predictive value (PPV) of chromosome aneuploidies and subchromosomal CNVs were analyzed. Pathogenicity of abnormal NIPT results were assessed according to American College of Medical Genetics and Genomics (ACMG).

**Results:** A total of 442 pregnancies (442/24359, 1.9%) were with abnormal NIPT results. 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 sub-chromosomal CNVs was 59.0% (46/78). The PPV for CNVs ≤ 5 Mb was 68.9% (31/45), for CNVs within 5-10 Mb was 83.3% (5/6) and for CNVs ≥ 10 Mb was 37.1% (10/27) respectively. The clinical information, prenatal diagnosis results and follow-up results of 46 true positive cases, 6 cases with sub-chromosomal CNVs inconsistent with NIPT and 1 false negative case were also described in detail.

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

Introduction

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 [2–3]. 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% [4–5].

Genomic structural changes, such as copy number variations (CNVs), are also always associated with human disease. Recently, further development and expansion use of NIPT has focused on microduplication/microdeletion syndromes (MMSs)[6]. The most common microdeletion is at 22q11.2, and recent reports indicate that the clinical incidence rate may exceed 1/1000 in the prenatal population[7]. 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[8]. In addition to 22q11.2 microduplication/microdeletion syndromes, other newly described CNVs like the distal 1q21.1 microdeletions/microduplications, 15q11.2 deletion, 16p13.11 deletion and 16p11.2 deletion/duplication are also identified as disease-causing CNVs[9–12]. Therefore, it is very important to evaluate the accuracy of NIPT for CNVs, which could help identify high-risk pregnancies and offer the possibility of a confirmatory invasive diagnostic test. However, there are many challenges for NIPT in clinical practice especially low positive predictive values (PPV). In the present study, we retrospectively analyzed 24359 single pregnancies including 125 cases of sub-chromosomal CNVs to assess the efficiency of NIPT technology on the detection of sub-chromosomal 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. 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 7906 (16.3%). The gestational age ranged from 9 to 34 weeks, and 56.9% had a gestational age from 17 to 20 weeks (see Table 1). The positive rate from younger group to older group was 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) | Number | Percent (100%) |
|---------------------------------|--------|----------------|
| ≤ 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  | /              |

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.

There were 442 pregnant 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 were with advanced maternal age more than 35 years (included), and 26 (5.8%, 26/442) pregnant cases had ultrasound abnormalities. 123 (27.8%, 123/442) cases were with high risk of 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) | Number | Percent (100%) |
|---------------------------------|--------|----------------|
| ≤ 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| /              |
Table 3

Reasons of 442 pregnant cases with chromosome aneuploidies and sub-chromosomal 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%          |

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

In 442 pregnancies with abnormal NIPT, there were 12 cases of T13, 35 cases of T18, 90 cases of T21, and 106 cases of sex chromosome abnormalities (SCAs) (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 of T21, 1 case of T13, 13 cases of T18, and 32 cases of SCAs (Table 4).

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% |
| SCAs                          | 106   | 27                           | 79            | 32             | 47  | 40.5% |
| Total                         | 243   | 65                           | 178           | 102            | 76  | 57.3% |

Nipt Results For Other Chromosome Aneuploidie

In 442 pregnancies with abnormal NIPT results, there were 74 cases of other chromosome aneuploidie including 39 cases of T7, 9 cases of T20, 12 case of T16, 3 cases of T10, 6 cases of T8, 3 cases of T9 and 2 cases of T14. Only 43 cases chosen amniocentesis for further diagnosis and result showed that there was only one true positive cases in T20. Therefore, the PPV for other chromosome aneuploidie in our study was 1.3%.

Table 5

Different PPVs according to pregnancies characteristics

| Indications                              | PPV of T21 | PPV of T18 | PPV of T13 | PPV of SCAs | PPV of CNVs |
|------------------------------------------|------------|------------|------------|-------------|-------------|
| Fetal structural abnormalities by ultrasound | 0          | 100%(1/1)  | 0          | 0           | 0           |
| Abnormal soft index of ultrasound        | 50.0%(1/2) | 100%(1/1)  | 0          | 0           | 0           |
| Abnormal serological screening           | 75.0%(24/32)| 55.6%(5/9) | 20.0%(1/5) | 36.7%(11/30)| 62.1%(18/29)|
| Advanced maternal age (≥ 35 years)      | 80.0%(24/30)| 60.0%(6/10)| 0%(0/2)    | 50.0%(11/22)| 46.2%(6/13) |
| Other                                    | 0          | 0          | 0          | 25.0%(1/4)  | 71.4%(5/7)  |
| No clinical indications                  | 25.0%(1/4) | 33.3%(1/3) | 0%(0/2)    | 37.5%(6/16) | 53.6%(15/28)|

Nipt Results For Cnvs

In addition, NIPT could also identify positive cases of sub-chromosomal CNVs. In 442 pregnancies with abnormal NIPT, there were 125 (28.3%,125/442) cases of sub-chromosomal CNVs. After genetic counseling about the clinical significance of these sub-chromosomal CNVs, 78 cases, including 27 cases with sub-chromosomal CNV ≥ 10M, 45 cases with CNV ≤ 5 M and 6 cases with CNV within 5–10 Mb, chosen to perform amniotic fluid puncture for further prenatal diagnosis. The PPV for sub-chromosomal CNVs screened by NIPT were 59% (46/78). The PPV for CNVs ≤ 5 Mb was 68.9% (31/45), for CNVs within 5–10 Mb was 83.3%(5/6) and for CNVs ≥ 10 Mb was 37.1% (10/27) respectively. Remaining 32 false positive cases included 6 cases with inconsistent CNV and 26 cases with no abnormality. 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). 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 6
The clinical information, prenatal diagnosis results and follow-up results of 15 cases with CNVs ≥ 10M.

| No | age | Preg-week | Indication                  | NIPT          | Karyotype                                      | Chip                                      | significance       | origin | Fr |
|----|-----|-----------|-----------------------------|---------------|-----------------------------------------------|-------------------------------------------|-------------------|--------|----|
| 1  | 38  | 26+0      | advanced age                | dup of 3q24- q29(146M-194M) | 46,XX,der(8)t(3;8) (q24:p23) | 3q24q29(144,804,358 – 197,851,444)x3,8p23.3p23.1(158,048 – 6,982,257)x1 | Pathogenic      |        | Tr |
| 2  | 26  | 19        | voluntary                  | dup of 13q14.11- q21.32 (46-65M) | 46,XN,dup(13) (q14q21) | 13q14.11q21.32(43,450,607 – 66,726,903)x3 | Pathogenic      | farther | Tr |
| 3  | 23  | 17+2      | voluntary                  | dup of 18p11.32- p11.21 (1M-11M), | normal | 15q11.2(22,754,322 – 23,222,284)x1 | Uncertain significance | unknown | ur |
| 4  | 24  | 17+2      | voluntary                  | dup of 4q24.3(19M-144M) | normal | 4p16.3p16.1(71,566-9,371,116)x1 | Pathogenic      | De novo | Tr |
| 5  | 30  | 20+6      | voluntary                  | del of Xq23-q28 (110M-153M) | 46,X,del(X)(q23) | Xp22.31(6,386,248-8,141,017)x3Xq23q28(110,798,069 – 152,651,757)x1 | Uncertain significance | unknown | Ne |
| 6  | 31  | 17+6      | voluntary                  | dup of 19q13.2-q13.43 (42M-57M) | normal | 11p14.1p13(27,473,981 – 33,896,715)x2 hmz | Uncertain significance | unknown | ur |
| 7  | 30  | 17+6      | High risk of serum screening | del of 5p15.33-p13.3 (1M-29M) | 46,XN,del(5) (p14.2p15.3) | 5p15.33p14.2(38,139 – 23,389,253)x1 | Pathogenic      | unknown | al |
| 8  | 33  | 18        | High risk of serum screening | del of 5p15.33-p15.2 (15M) | 46,XN,del(5)(p15.1) | 5p15.33p15.1(113,576 – 16,275,896)x1 | Pathogenic(cris-du-chat syndrome) | De novo | Tr |
| 9  | 22  | 19+4      | High risk of serum screening | del of 5q14.3 (48M ) | none | 5q14.3q23.2(82,812,442 – 121,787,549)x1 | Pathogenic      | unknown | Tr |
| 10 | 24  | 22        | voluntary                  | dup of 2q33.1-q37.3 (199M-241M) | 46,XN,dup(2) (q33.1q37.1) | none | Pathogenic      | unknown | Tr |
| 11 | 25  | 13+4      | voluntary                  | del of 11q12.1-q13.2(56M-67M) | normal | 16q23.1(77,909,692 – 78,568,430)x3 | Pathogenic      | unknown | nc |
| 12 | 38  | 16        | voluntary                  | dup of 3q27.1-q29 (183-196M) | none | 4.8M del of 5q35.2-5q35.3, 13.1M dup of 3q27.2-3q29 | 5q35 del syndrome | De novo | Cs |
| 13 | 24  | 19        | High risk of serum screening | dup of 16q13.3-13.11 (1-26M), dup of 16q12.2-24.3 (49-88M) | normal | 16p12.2(21,966,869 – 22,662,193)x1 | Uncertain significance | mother | cm |
| 14 | 27  | 19+4      | High risk of serum screening | dup of 10p15.3-p12.2 (1M-23M), del of 12q13.33-q13.32 (1M-5M) | 46,XN,der(12)t(10;12) (p12.31;p13.31) | 12p13.33p13.31(173,786-6,437,099)x1,10p15.3p12.31(100,047 – 20,255,943)x3 | Pathogenic      | unknown | Tr |
| No | age | Preg-week | Indication | NIPT | Karyotype | Chip | significance | origin | FI |
|----|-----|-----------|------------|------|-----------|------|--------------|--------|----|
| 15 | 24  | 18+2      | High risk of serum screening | del of 8p23.2-23.3 (1M-4M), dup of 21q21.3-22.3 (28M-46M) | 46,XN,der(8)t(8;21) (p23.2;q21.3) | 8p23.3p23.2(158,048−4,896,398)x1,21q21.3q22.3(27,985,829−48,093,361)x3 | Pathogenic | unknown | T |
Table 7
The clinical information, prenatal diagnosis results and follow-up results of 37 cases with CNV<10M and 1 false negative

| No | age | Preg week | Indication | NIPT | Karyotype | Chip | significance | origin |
|----|-----|-----------|------------|------|-----------|------|--------------|--------|
| 16 | 29  | 18+5      | Abnormal ultrasound | del of 5p14.1-p13.3(26M-29M) | normal | 5p14.1(26,067,126 - 28,837,434)x1,17p13.3(716,837-1,201,192)x3 | Uncertain significance | unknown |
| 17 | 24  | 16+1      | voluntary | dup of 16p12.2 (22M-23M) | normal | 16p12.2(21,841,353 - 22,431,031)x3 | Uncertain significance | unknown |
| 18 | 27  | 17+0      | voluntary | del of 15q13.1-q14(30M-34M) | normal | 15q13.2q13.3(30,955,149 - 32,513,176)x1 | 15q13.3 del syndrome | mother |
| 19 | 28  | 17+2      | voluntary | dup of Xq21.33-q22.3 (98M-107M) | normal | Xq21.33q22.3(97,776,700 - 107,811,504)x3 | Pathogenic Pelizaeus-Merzbacher disease | mother |
| 20 | 29  | 17+2      | voluntary | del of 17p13.3-p13.2 (0.1M-6M) | normal | 17p13.3p13.2(1,330,366 - 3,059,811)x1;17q12(34,824,845 - 36,339,294)x3 | Miller-Diekerlissencephaly syndrome | unknown |
| 21 | 29  | 14+6      | voluntary | dup of 8p23.3-p23.2 (1M-5M) | normal | 8p23.2(3,687,399-5,950,104)x4 | Uncertain significance | unknown |
| 22 | 31  | 19+4      | voluntary | del of 17p13.3 (0.1M-3M) | normal | 17p13.3(1,330,366-3,059,811)x1;17q12(34,824,845 - 36,339,294)x3 | Miller-Diekerlissencephaly syndrome | unknown |
| 23 | 34  | 21+0      | voluntary | del of 16p13.12-p12.3 (15M-17M) | normal | 16p13.12p13.11(14,780,640 - 16,458,424)x1 | 16p13.11 recurrent region (includes MYH11) | unknown |
| 24 | 28  | 16+6      | High risk of serum screening | dup of 22q11.21 (18.9M-22M) | normal | 3p26.3(1,536,945-2,579,649)x3;22q11.21(18,844,632-21,462,353)x3 | 22q11.2 dup syndrome | unknown |
| 25 | 33  | 19+3      | High risk of serum screening | del of Xp11.23-p11.22 (48-53M) | normal | Xp11.23p11.22(48,735,882 - 53,521,570)x1 | Uncertain significance | unknown |
| 26 | 24  | 18+6      | High risk of serum screening | dup of 21q21.1 (17M-18M) | normal | 21q21.1(17,775,056 - 19,154,417)x3 | Uncertain significance | unknown |
| 27 | 32  | 17+0      | Mild risk of serum screening | dup of 15q11.2-q13.1 (23M-28M) | normal | 15q11.2q13.1(22,764,491 - 29,071,810)x3 | 15q11-q13 dup syndrome | unknown |
| 28 | 25  | 17+0      | Mild risk of serum screening | del of 4q34.1-q34.3 (182M-183M) | normal | 4q34.3q35.1(182,542,070 - 183,305,274)x1 | Uncertain significance | unknown |
| 29 | 25  | 17+0      | Mild risk of serum screening | dup of 22q11.21(19M-20M) | normal | 22q11.21(18,648,855 - 21,800,471)x3 | 22q11.2 dup syndrome | unknown |
| 30 | 31  | 20+0      | Mild risk of serum screening | dup of 22q11.21-q11.23 (22M-24M) | normal | 22q11.21q11.23(21,059,669 - 24,629,406)x3 | 22q11.2 recurrent region (distal type I, D/E/F) | unknown |
| 31 | 22  | 17+5      | Mild risk of serum screening | dup of 17q12 (35M-37M) | normal | 17q12(34,822,465 - 36,243,365)x3 | 17q12 dup syndrome | mother |
| 32 | 27  | 24+5      | Widening of lateral ventricle | dup of 13q12.11-q12.12 (22M-24M) | none | 13q12.12(23,554,650 - 24,826,638)x3 | Uncertain significance | unknown |
| No | age | Preg week | Indication | NIPT | Karyotype | Chip | significance | origin |
|----|-----|-----------|------------|------|-----------|------|--------------|--------|
| 33 | 25 | 22 | voluntary | del of Xq27.1q27.3 (137M-143M) | none | Xq27.1q27.3(138,661,694 - 143,597,022)x1 | uncertain significance | unknown |
| 34 | 31 | 19 | Maternal mental retardation | dup of 22q12.1q12.2 (23M-24M) | none | 22q12.1q12.2(28,317,927 - 30,826,799)x2 hmz | uncertain significance | Mother-22q11.23(23,137,086-25,086,816)x1 |
| 35 | 22 | 26+2 | Maternal cleft lip and palate | del of 22q11.21 (18M-21M) | none | 22q11.21(18,648,855 - 21,800,471)x1 | DiGeorge syndrome | unknown |
| 36 | 29 | 23+5 | voluntary | del of 5p15.33-p15.2 (0M-3M) | none | 5p15.33(38,139-2,436,105)x1 | uncertain significance | mother |
| 37 | 32 | 17+2 | voluntary | dup of 22q11.21-q11.22 (21M-23M) | none | 22q11.21(81,674,866 - 88,970,446)x3 | uncertain significance | unknown |
| 38 | 27 | 16+5 | mild risk of serum screening | del of 5q12.1 (59M-61M) | none | 5q12.1(59,052,591 - 60,864,744)x1 | uncertain significance | mother |
| 39 | 27 | 17 | high risk of serum screening | dup of 10q23.1-23.2 (82-89M) | none | 10q22.3-23.2(81,674,866 - 88,970,446)x3 | likely pathogenic | De novo |
| 40 | 27 | 25+4 | Increased bowel echo and widened lateral ventricle | del of 15q11.2-q12 (24-27M) | normal | 15q11.2q13.1(23,300,172 - 28,536,634)x1 | uncertain significance | De novo |
| 41 | 27 | 21 | voluntary | dup of 3q12.3-q13.11 (101-104M) | normal | 3q12.3q13.11(101,694,516 - 104,402,138)x3 | uncertain significance | mother |
| 42 | 24 | 23+3 | mild risk of serum screening | dup of 17p12-p11.2 (14-16M) | none | 17p12(14,099,504 - 15,424,086)x3 | uncertain significance | De novo |
| 43 | 23 | 23 | Maternal mental retardation | del of 17q12 (34-36M) | normal | 17q12(34,822,465 - 36,410,720)x1 | uncertain significance | Mother(mental retardation) |
| 44 | 39 | 15+55 | History of adverse pregnancy | del of 4q12-q13.1 (53-62M) | none | 4q12(52,920,475 - 59,495,539)x1 | uncertain significance | De novo |
| 45 | 35 | 13+3 | voluntary | dup of 17p12 (14M-16M) | normal | 3q28(189,409,398 - 189,571,893)x1, 17p12(14,087,918 - 15,413,862)x3 | uncertain significance | De novo |
| 46 | 33 | 18 | high risk of serum screening | del of Xp22.32-p22.31(6M-8M) | none | Xp22.31(6,455,151-8,141,076)x1 | uncertain significance | mother |
| 47 | 25 | 20 | mild risk of serum screening | dup of 1p13.2-p12 (116-119M) | none | 1p13.2p12(115,582,990 - 120,527,348)x3, 15q11.2(22,770,421 - 23,276,605)x1 | uncertain significance | 15q11.2-motl1p13.2p12-de |
| 48 | 29 | 20 | Maternal mental retardation | del of Xq27.2-q27.3 (143M-147M) | none | Xq27.3(142,954,184 - 147,171,818)x0 | uncertain significance | unknown |
| 49 | 35 | 15+6 | Advanced age | del of 9p21.3-p21.1 (25M-30M) | none | 9p21.3p21.1(24,796,507 - 30,882,656)x1 | uncertain significance | mother |
| 50 | 28 | 22+5 | high risk of serum screening | dup of 7q32.3 (132M-135M) | none | 4q33q34(170,186,543 - 181,620,422)x2 hmz | uncertain significance | unknown |
Table 8

| No | age | Preg week | 22q Dup/del | bp start; stop (NCBI37/hg19) | significance | origin | Follow-up |
|----|-----|-----------|-------------|-----------------------------|--------------|--------|----------|
| 24 | 28  | 16 + 6    | duplication  | 3p26.31(1,536,945-2,579,649)x3;22q11.21(18,844,632-21,462,353)x3 | 22q11.2 dup syndrome | unknown | normal   |
| 29 | 25  | 17 + 3    | duplication  | 22q11.21(18,648,855-21,800,471)x3 | 22q11.2 dup syndrome | unknown | normal   |
| 30 | 31  | 20        | duplication  | 22q11.21q11.23(21,059,669-24,629,406)x3 | 22q11.2 recurrent region (distal type I, D-E/F) | unknown | congenital heart malformation, ventricular septal defect, aortic abnormalities |
| 34 | 31  | loss of heterozygosity | 22q12.1q12.2(28,317,927-30,826,759)x2 hmq | uncertain significance | mother with 22q11.23(23,700,639-25,088,816)x3 | unknown |
| 35 | 22  | 26 + 2    | deletion     | 22q11.21(18,648,855-21,800,471)x1 | DiGeorge syndrome | unknown | hoarseness, congenital heart disease |
| 37 | 32  | 17 + 2    | duplication  | 22q11.21q11.23(21,464,120-23,650,987)x3 | 22q11.2 dup syndrome | unknown | normal   |

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. 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 and this 4.2 Mb microdeletion was de novo. These 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, case 53 was tested again by improved the experimental method with better cffDNA enrichment. The results showed increased cffDNA fraction from 6.5–15.1% and a 34Mbp deletion in 4p16.3-p15.1 region, which is co-related with Wolf-Hirschhorn syndrome (WHS).

Discussion

In this study, we are the first to review the efficiency of NIPT for screening common chromosome aneuploidies as well as sub-chromosomal CNV within a cohort of 24359 single pregnancies in Huaiian area. This NIPT technology uses a semiconductor sequencing platform with high enrichment of cffDNA (20%-40%) to reliably detect subchromosomal deletions/duplications. The PPV for T21, T18, T13 and SCAs was 84.8%, 54.2%, 11.1% an 40.5% respectively.

In several recent studies, the PPV of T21 was 65–94%, the PPV of T18 was 47–85%, and the PPV of T13 was 12–62%[13–15]. Our results are consistent with previous studies. Interestingly, the PPV for CNVs was 59.0%, which is obviously higher than previous studies with 9–36%[16–18]. The reason for higher PPV of CNVs in our study may be related with our new enrichment strategy. The PPV for CNVs ≤ 5 Mb was 68.9% (31/45), for CNVs within 5–10 Mb was 83.3% (5/6) and for CNVs ≥ 10 Mb was 37.1% (10/27) respectively. However, previous reports demonstrated that PPV for CNVs

≥ 10 Mb was significantly higher than CNVs < 10 Mb [16–18]. Further analysis showed that there were only 27 cases with big CNVs (≥ 10 Mb) but 51 cases with small CNVs (< 10 Mb) suggesting that small CNVs occurred more frequently than big CNVs. Therefore, we speculate that frequent occurrence of small CNVs may be the potential cause of higher PPV of small CNVs.

Further analysis about the different PPV of NIPT according to pregnancies indications was performed. The results showed that the PPV of NIPT was the highest for T21 and was much lower for other aneuploidies. PPV of CNVs was close to T18 and much higher than T13. Advanced maternal age is a high risk factor for T21 so PPV of T21 in advanced maternal age is the highest. PPV of CNVs in advanced maternal age group was lower suggesting that advanced maternal age was not significantly related with CNVs. PPVs of CNVs in pregnancies with cleft lip, mental retardation or history of bad pregnancy was the highest suggesting high risk factors for CNVs.

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 parent, 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 was the most frequent in our research. The phenotype of the five patients with 22q11.2 microduplications were diverse, with symptoms ranging from being normal to mental retardation and congenital heart malformation. 22q11.2 microduplication is the second most common chromosomal abnormality secondary to Down syndrome [19]. However, the occurrence of 22q11.2 microduplications was more frequent than 22q11.2 microdeletion in our study, which was 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[20].

Studies have demonstrated that there is a small chance of a false negative result for NIPT[21]. In our study, there was 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[22–23]. In our research, extraction method for cffDNA 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 higher 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[24]. The karyotypes of cytotrophoblast and fetus may be different due to fetus are derived from the inner cell mass (ICM) of the blastocyst[21]. Other reasons for false negative results may be CPM and maternal mosaicism[25–27].

Conclusions

This study demonstrated that the PPV for T21, T18, T13 and SCAs was 85%, 54%, 11% and 41% respectively. The PPV for CNVs ≤ 5 Mb was 68.9% (31/45), for CNVs within 5–10 Mb was 83.3%(5/6) and for CNVs ≥ 10 Mb was 37.1% (10/27) 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, 24,359 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.

**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**

**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.

**Availability of data and materials**

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

**Competing interests**

The authors declare that they have no competing interests.

**Funding**

Supported by the Maternal and Child Health project of Jiangsu Province (No. F201670/F201714) (Pan Qiong); the “333 Project” Foundation of Jiangsu Province (No. BRA2017250) (Pan Qiong).

**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.

**Acknowledgements**

We would like express our gratitude for financial support from Maternal and Child Health project of Jiangsu Province (No. F201670/F201714) and the "333 Project" Foundation of Jiangsu Province (No. BRA2017250).

**References**

1. Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, et al. Presence of fetal DNA in maternal plasma and serum. Lancet. 1997;350(9076):485–7.
2. Filoche S, Lawton B, Beard A, Dowell A, Stone P. New screen on the block: non-invasive prenatal testing for fetal chromosomal abnormalities. J Prim Health Care. 2017;9(4):248–53.
3. McCullough RM, Almasri EA, Guan X, Geis JA, Hicks SC, Mazloom AR, et al. Non-invasive prenatal chromosomal aneuploidy testing—clinical experience: 100,000 clinical samples. PLoS One. 2014;9(10):e109173.

4. Hu H, Liu H, Peng C, Deng T, Fu X, Chung C, et al. Clinical Experience of Non-Invasive Prenatal Chromosomal Aneuploidy Testing in 190,277 Patient Samples. Curr Mol Med. 2016;16(8):759–66.

5. Liang D, Lin Y, Qiao F, Li H, Wang Y, Zhang J, et al. Perinatal outcomes following cell-free DNA screening in > 32,000 women: Clinical follow-up data from a single tertiary center. Prenat Diagn. 2018;38(10):755–64.

6. Oskarsdottir S, Vujic M, Fasth A. Incidence and prevalence of the 22q11 deletion syndrome: a population-based study in Western Sweden. Arch Dis Child. 2004;89(2):148–51.

7. Grati FR, Molina GD, Ferreira JC, Dupont C, Alesi V, Gouas L, et al. Prevalence of recurrent pathogenic microdeletions and microduplications in over 9500 pregnancies. 2015; 35(8):801–809.

8. McDonald-McGinn DM, Sullivan KE, Marino B, Philip N, Swillen A, Vorstman JA, et al. 22q11.2 deletion syndrome. Nat Rev Dis Primers. 2015.

9. Yaron Y, Jani J, Schmid M, Oepkes D. Current Status of Testing for Microdeletion Syndromes and Rare Autosomal Trisomies Using Cell-Free DNA Technology. Obstet Gynecol. 2015;126(5):1095–9.

10. Sentilhes L, Salomon LJ, Vayssiere C. Cell-free DNA Analysis for Noninvasive Examination of Trisomy. N Engl J Med. 2015;373(26):2581–2.

11. McDonald-McGinn DM, Tonnesen MK, Laufer-Cahana A, Finucane B, Emanuel BS, et al. Phenotype of the 22q11.2 deletion in individuals identified through an affected relative: cast a wide FISHing net! Genet Med. 2001;3(1):23–9.

12. Wincent J, Bruno DL, van Bon BW, Bremer A, Stewart H, Bongers EM, et al. Sixteen New Cases Contributing to the Characterization of Patients with Distal 22q11.2 Microduplications. Mol Syndromol. 2010;1(5):246–54.

13. Bianchi DW, Wilkins-Haug L. Integration of noninvasive DNA testing for aneuploidy into prenatal care: what has happened since the rubber met the road? Clin Chem. 2014;60(1):78–87.

14. Faas BH, de Ligt J, Janssen I, Eggink AJ, Wijnberger LD, van Vugt JM, et al. Non-invasive prenatal diagnosis of fetal aneuploidies using massively parallel sequencing-by-ligation and evidence that cell-free fetal DNA in the maternal plasma originates from cytotrophoblastic cells. Expert Opin Biol Ther. 2012;12(Suppl 1):19–26.

15. Jiang F, Ren J, Chen F, Zhou Y, Xie J, Dan S, et al. Noninvasive Fetal Trisomy (NIFTY) test: an advanced noninvasive prenatal diagnosis methodology for fetal autosomal and sex chromosomal aneuploidies. BMC Med Genomics. 2012;5(S7).

16. Kalousek DK. Pathogenesis of chromosomal mosaicism and its effect on early human development. Am J Med Genet. 2000;91(1):39–45.