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

Yuefang Liu
huaian maternal and child hospital
Longfei Cheng
huaian maternal and child hospital
Yuan Peng
huaian maternal and child hospital
Zhe Liang
huaian maternal and child hospital
Pan Qiong (18252586797@163.com)
huaian maternal and child health care hospital https://orcid.org/0000-0001-8151-0923

Research

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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% an 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 3966(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 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	/

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)		
≤ 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 histroy	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)	0(0/1)	0	16.7%(1/6)	60.0%(3/5)
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	siginificance	origin	Fo
1	38	26 + 0	advanced age	dup of3q24-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		Te
2	26	19	voluntary	dup of13q14.11-q21.32 (46-65M)	46,XN,dup(13)(q14q21)	13q14.11q21.32(43,450,607 – 66,726,903)x3	Pathogenic	farther	Te
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	un
4	24	17 + 2	voluntary	dup of4q24.3(1(19M-144M)	normal	4p16.3p16.1(71,566-9,371,116) × 1	Pathogenic	De novo	Te
5	30	20 + 6	voluntary	del ofXq23-q28 (110M-153M)	46,X,del(X)(q23)	Xp22.31(6,386,248-8,141,017)x3;Xq23q28(110,798,069–152,651,757)x1	Uncertain significance	unknown	No fe
6	31	17 + 6	voluntary	dup of19q13.2-q13.43 (42M-57M)	normal	11p14.1p13(27,473,981 – 33,896,715)x2 hmz	Uncertain significance	unknown	un
7	30	17 + 6	High risk of serum screening	del of5p15.33-p13.3 (1M-29M)	46,XN,del(5)(p14.2p15.3)	5p15.33p14.2(38,139 – 23,389,253)x1	Pathogenic	unknown	ab
8	33	18	High risk of serum screening	del of5p15.33-p15.2 (15M)	46,XN,del(5)(p15.1)	5p15.33p15.1(113,576 – 16,275,896)x1	Pathogenic(cri-du-chat syndrome)	De novo	Te
9	22	19 + 4	High risk of serum screening	del of5q14.3 (48M)	none	5q14.3q23.2(82,812,442 – 121,787,549) × 1	Pathogenic	unknown	Te
10	24	22	voluntary	dup of2q33.1-q37.3 (199M-241M)	46,XN,dup(2)(q33.1q37.1)	none	Pathogenic	unknown	Te
11	25	13 + 4	voluntary	del of11q12.1-q13.2(56M-67M)	normal	16q23.1(77,909,692 – 78,568,430)x3	Pathogenic	unknown	no
12	38	16	voluntary	dup of3q27.1-q29 (183-196M)	none	4.8M del of5q35.2-5q35.3, 13.1M dup of3q27.2-3q29	5q35 del syndrome	De novo	Fe co m
13	24	19	High risk of serum screening	dup of16q13.3-13.11 (1-26M), dup of16q12.2-24.3 (49-88M)	normal	16p12.2(21,966,869 – 22,662,193)x1	Uncertain significance	mother	no
14	27	19 + 4	High risk of serum screening	dup of10p15.3-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	Te

No	age	Preg-week	Indication	NIPT	Karyotype	Chip	siginificance	origin	Fe
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	Te

Table 7

The clinical information, prenatal diagnosis results and follow-up results of 37 cases with CNV < 10M and 1 false negative c

No	age	Preg week	Indication	NIPT	Karyotype	Chip	siginificance	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	PathogenicPelizaeus-Merzbacher disease	mother
20	29	17 + 2	voluntary	del of17p13.3-p13.2 (0.1M-6M)	normal	17p13.3p13.2(4,888-4,818,558)x1	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,)	46,XN,inv(9)p11q13	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 4 q34.1-q34.3 (182M-183M)	normal	4q34.3q35.1(182,542,070– 183,305,274)x1	uncertain significance	unknown
29	25	17 + 3	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 of17q12 (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	siginificance	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 – 30,826,759)x2 hmz	uncertain significance	Mother-22q11.23(23,7 – 25,086,816)x3
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) × 1	CRI-DU-CHAT syndrome	De novo
37	32	17 + 2	voluntary	dup of 22q11.21-q11.22 (21M-23M)	none	22q11.21q11.23(21,464,120 – 23,650,987)x3	22q11.2 dup syndrome	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	angelman syndrome prader-Willi syndrome	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	17p12 recurrent (HNPP/CMT1A) region (includes PMP22)	mother
43	23	23	Maternal mental retardation	del of 17q12 (34-36M)	normal	17q12(34,822,465 – 36,410,720)x1	17q12 recurrent del syndrome	Mother(menta 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	likely pathogenic	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	17p12 recurrent (HNPP/CMT1A)region	unknown
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	pathogenic	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- motl 1p13.2p12-de
48	29	20	Maternal mental retardation	del of Xq27.2-q27.3(143M-147M)	none	Xq27.3q28(142,954,184 – 147,171,818)x0	likely pathogenic	unknown
49	35	15 + 6	Advanced age	del of 9p21.3-p21.1(25M-30M)	none	9p21.3p21.1(24,796,507 – 30,288,265)x1	uncertain significance	mother
50	28	22 + 5	high risk of serum screening	dup of 7q32.3 (132M-135M)	none	4q33q34.3(170,186,543 – 181,620,422)x2 hmz	uncertain significance	unknown

No	age	Preg week	Indication	NIPT	Karyotype	Chip	siginificance	origin
51	35	22	voluntary	del of Xq24q25 (117-122M)	none	Xq24q25(117,865,893 – 122,724,000)x1	uncertain significance	unknown
52	30	12	voluntary	dup of 8p23.2 (3-5M)	none	8p23.2(3,687,399-5,950,104)x4	uncertain significance	unknown
53	28	20 + 3	High risk of serum screening	low risk	46,XX,del(4)(p14)	none	pathogenic	De novo

Figure 2 Fetal free DNA fraction of NIPT between true positive group (X ± S = 17.3 ± 5.7, n = 46) and false positive group(X ± S = 14.0 ± 7.6, n = 32)(p = 0.0185).

Different Ppv According To Pregnancies Characteristics

We also analyzed different PPV according to pregnancies characteristics, Different PPVs of NIPT according to pregnancies indications are shown in Table 5. The total PPV of T21 was 84.8%, the PPV of T21 fetuses in women of advanced maternal age was 80.0% and in abnormal serological screening group was 75.0%. Similarly, the PPV of T18 and SCAs in advanced maternal age group were also the highest. It is worth noting that PPVs of CNVs in pregnancies with cleft lip, mental retardation or history of bad pregnancy was the highest.

Five Cases With Distal 22q11.2 Microdeletions And Microduplications

In our study, the PPV of 22q11.2 microdeletions and microduplications is 100% (5/5). There were four fetuses with 22q11.2 duplication syndrome and one with 22q11.2 deletion syndrome (Table 8). Case 24, 29 and 37 were all clinically healthy after birth but case 30 was with ventricular septal defect, aortic abnormalities and 1 bright spot in the left ventricle subsequently detected by ultrasound. Case 35 was confirmed to have a 22q11.21 deletion syndrome with hoarseness, atrial septal defect, patent ductus arteriosus and myocardial injury after birth. However, it was not clear that whether these mutations were de novo or inherited from parents. Case 34 was confirmed to have the loss of heterozygosity in 22q12.1q12.2 whose clinical phenotypes were unavailable. Fetal mother were also advised to have a array comparative genomic hybridization because of her mental retardation. CMA analysis showed that 22q11.23 microduplication may contribute to her mental retardation.

Table 8
The clinical information, prenatal diagnosis results and follow-up results of six cases distal 22q11.2 microdeletions and microduplications.

No	age	Pregnant week	22q Dup/del	bp start; stop (NCBI37/hg19)	significance	origin	Follow-up
24	28	16 + 6	duplication	3p26.3(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 hmz	uncertain significance	mother with 22q11.23(23,700,639 – 25,086,816)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 reviewed the efficiency of NIPT for screening common chromosome aneuploidies as well as sub-chromosomal CNV within a cohort of 24359 single pregnancies in Huaian 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 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 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 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 microdeletions 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 was 59.0%. 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, 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 CytoScanTM 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

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.

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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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Figures

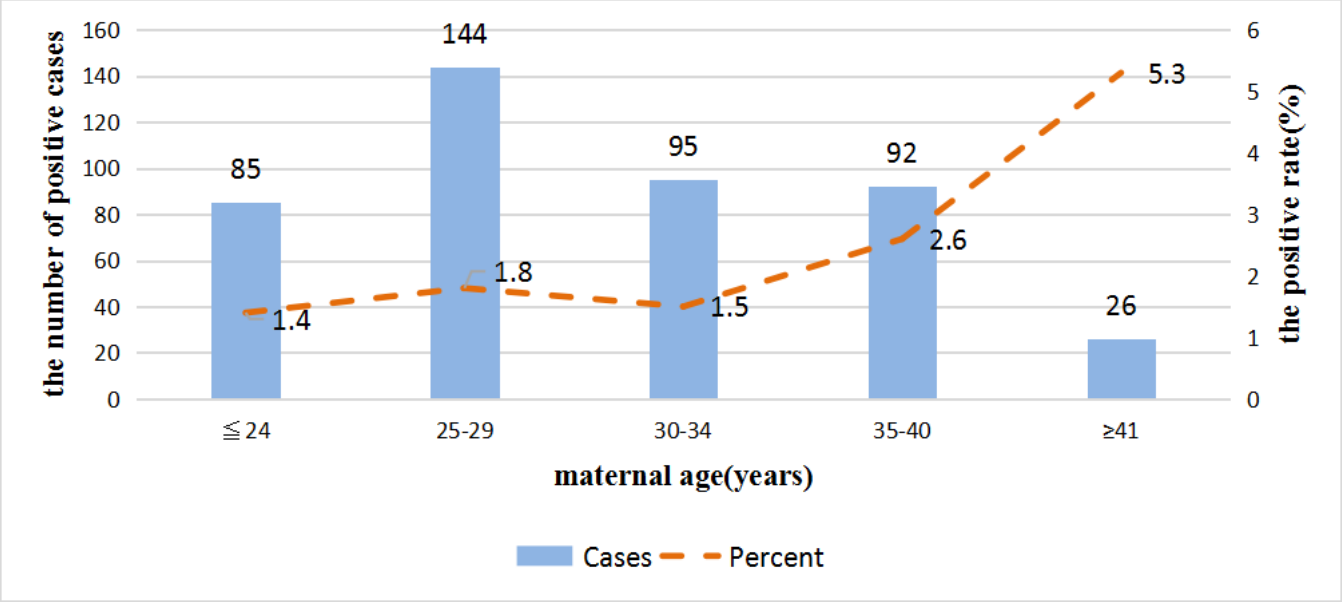

Figure 1

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.

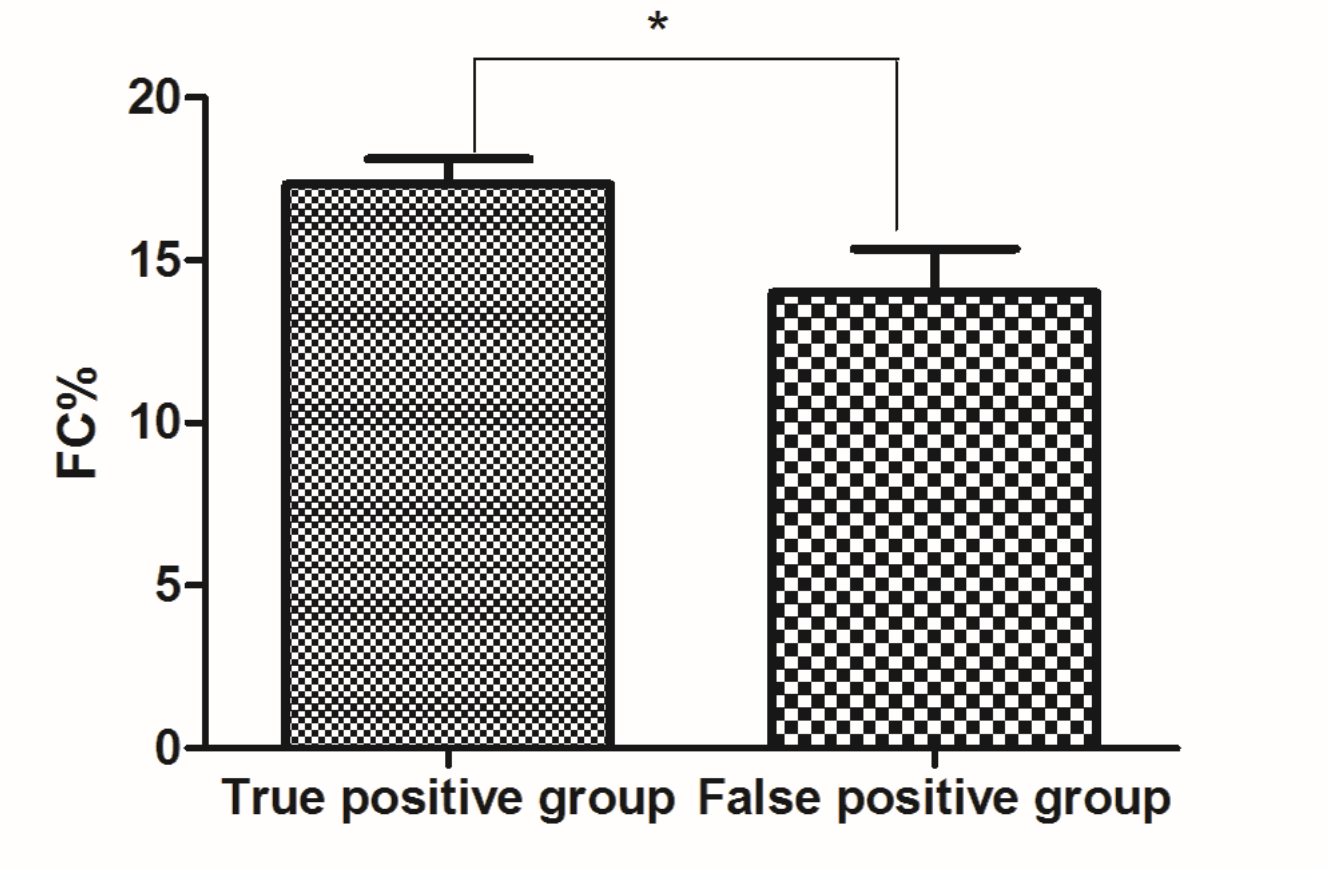

Figure 2

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