Detection of trisomies 13, 18 and 21 using non-invasive prenatal testing

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Abstract. The clinical performance of non-invasive prenatal testing (NIPT) in the Down's syndrome screening based on 1,901 pregnant women in a Chinese hospital was investigated. This was a retrospective analysis of NIPT study in singleton pregnancy (n=1,901). The NIPT test is offered routinely as a prenatal screening test for common fetal aneuploidies, including trisomy 13 (T13), T18 and T21 to pregnant women with risk factors of one or more anomalies. Maternal peripheral blood (5 ml) was collected in an ethylenediaminetetraacetic acid (EDTA) tube at a gestational age of 12+0 to 32+6 weeks. The samples were delivered at -80˚C to the certified Shenzhen BGI Clinical Laboratory Center. Sequencing data were analyzed using a proprietary algorithm. Women with positive NIPT results were recommended to receive karyotype analysis and amniotic fluid puncture for further validation. The cases were followed up for 56 days after delivery. All the patients underwent ultrasound examination, and the majority of patients (91.16%) showed normal findings. In contrast, 136 (7.15%) showed ultrasound anomalies. The most common anomaly was echogenic heart focus (n=80), accounting for 4.21% of the patients. Twenty-two cases were classified by the NIPT to be positive for the T21 (n=15), T18 (n=5) and T13 (n=2), respectively, while the others (n=1,879) were classified to be NIPT negative cases. Among these cases, the fetal outcome data were obtained in 1,483 cases, while 396 were lost to follow-up. The majority of cases (75.47%) were normal at birth. Neonatal death was observed in 1 case. Five pregnant women decided termination of pregnancy despite the presence of NIPT negativity. In conclusion, NIPT technique is feasible for the prenatal screening of T18 and T21 with higher sensitivity and specificity compared with conventional methods.

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Introduction

Down's syndrome screening is offered to all pregnant women in many counties (1). Conventionally, the combined screening test involving fetal ultrasound examination and maternal serum biomarkers determination (2) is used to generate a risk assessment. Generally, such a procedure is performed at a gestational age of 10 weeks and 14+1 weeks with a rate of detection ~85% with a rate of false positive of 2.5% (3).

To the best of our knowledge, the majority of pregnant women wanted to be informed if Down's syndrome was suspected. On clinical suspicion, most of them would prefer to undergo an invasive diagnostic procedure, which is currently the main method in clinical practice. Accordingly, invasive tests may carry a risk of miscarriage of ~0.5% (4). Extensive studies have been focused on developing an alternative method with highflyer or equal accuracy and non-invasive features (5,6). Non-invasive prenatal testing (NIPT) for fetal aneuploidies is now available and is generally suggested as a screening test in clinical practice (7). Up to now, NIPT has been considered as committee opinions or guidelines for the clinical screening for the more common autosomal trisomies, such as trisomy 13 (T13), T18 and T21 (8,9). However, only few studies have reported the use of NIPT for the prenatal screening of Down's syndrome due to small sample size.

In the present study, we report a performance evaluation of NIPT-based Down's syndrome screening in our hospital with a large patient cohort of 1,901 singleton pregnancies. Our study indicated that NIPT is feasible for the prenatal screening of T18 and T21 with higher sensitivity and specificity for the Down's syndrome screening, compared with conventional methods.

Patients and methods

Patients. The study protocols were approved by the Ethics Committee of Maternal and Children's Hospital of Shaanxi Province (Xi'an, China). Written informed consent was obtained from each patient. The study was a retrospective analysis of NIPT in singleton pregnancy over the period from March 2012 to March 2015 at the Maternal and Children's

Table I. Basic characteristics of the 1,901 pregnant women of NIPT.

Characteristics	No. (%)
Maternal age (years)	
≤20	0.21% (4/1,901)
20-24	8.63% (164/1,901)
25-29	40.50% (770/1,901)
30-34	25.25% (480/1,901)
35-39	21.25% (404/1,901)
40-44	4.00% (76/1,901)
≥45	0.16% (3/1,901)
Mean	30.57
Range	19-45
Gestation at NIPT test (weeks)	
12+0 to 12+6	1.00% (19/1,901)
13+0 to 13+6	1.63% (31/1,901)
14+0 to 14+6	1.68% (32/1,901)
15+0 to 15+6	3.26% (62/1,901)
16+0 to 16+6	7.26% (138/1,901)
17+0 to 17+6	17.31% (329/1,901)
18+0 to 18+6	21.25% (404/1,901)
19+0 to 19+6	16.10% (306/1,901)
20+0 to 20+6	10.89% (207/1,901)
21+0 to 21+6	8.15% (155/1,901)
22+0 to 22+6	4.73% (90/1,901)
23+0 to 23+6	3.16% (60/1,901)
24+0 to 24+6	1.53% (29/1,901)
25+0 to 25+6	0.68% (13/1,901)
26+0 to 26+6	0.58% (11/1,901)
27+0 to 27+6	0.11% (2/1,901)
28+0 to 28+6	0.16% (3/1,901)
29+0 to 29+6	0.00% (0/1,901)
30+0 to 30+6	0.11% (2/1,901)
31+0 to 31+6	0.11% (2/1,901)
32+0 to 32+6	0.05% (1/1,901)
Unknown	0.26% (5/1,901)
Ultrasound findings	
Normal	91.16% (1733/1,901)
Unknown	1.63% (31/1,901)
Abnormal ultrasonographic soft markers or ultrasound anomalies	7.15% (136/1,901)
Thickened NT/NF	0.16% (3/1,901)
Echogenic heart focus	4.21% (80/1,901)
Renal pelvic dilatation	0.11% (2/1,901)
Echogenic heart focus + renal pelvic dilatation	0.16% (3/1,901)
Echogenic heart focus + renal pelvic dilatation+ choroid plexus cysts	0.05% (1/1,901)
Renal pelvic dilatation + widened ventricle	0.05% (1/1,901)
Echogenic heart focus + choroid plexus cysts	0.16% (3/1,901)
Choroid plexus cysts	0.42% (8/1,901)
Widened ventricle	0.26% (5/1,901)
Echogenic heart focus + widened ventricle	0.21% (4/1,901)

Table I. Continued.

Characteristics	No. (%)
Echogenic heart focus + widened ventricle +small nasal bone	0.05% (1/1,901)
Echogenic heart focus + widened ventricle + thickened NF	0.05% (1/1,901)
Echogenic bowel	0.16% (3/1,901)
Echogenic heart focus + echogenic bowel	0.05% (1/1,901)
Absent nasal bone	0.05% (1/1,901)
Echogenic heart focus + small nasal bone	0.05% (1/1,901)
Tricuspid regurgitation	0.05% (1/1,901)
Others	0.89% (17/1,901)
Prior screening test	
High risk (T21)	49.55% (942/1,901)
Critical high risk (T21)	14.94% (284/1,901)
High risk (T18)	1.16% (22/1,901)
Critical high risk (T18)	0.16% (3/1,901)
High risk (T21+T18)	0.11% (2/1,901)
Critical high risk (T21+T18)	0.11% (2/1,901)
Low risk	3.58% (68/1,901)
None	28.83% (548/1,901)
Unknown	1.58% (30/1,901)
Previous trisomy 21 pregnancies	0.32% (6/1,901)
Pregnancy by assisted reproductive techniques	1.53% (29/1,901)

NIPT, non-invasive prenatal testing.

Hospital of Shaanxi Province. The prenatal screening test findings for common fetal aneuploidies, including T13, T18 and T21, were set as previously described (10). The NIPT test is offered routinely as a prenatal screening test for common fetal aneuploidies, including T13, T18 and T21 to pregnant women with risk factors of one or more anomaly. In total, 1,901 pregnant women (19-45 years, median 30.57 years) received the NIPT test, and all received prior prenatal screening (Down's syndrome screening).

Sequencing. Maternal peripheral blood (5 ml) was collected in an ethylenediaminetetraacetic acid (EDTA) tube at a gestational age of 12+0 to 35+6 weeks. The blood sample was stored at 4˚C immediately after collection. Plasma was isolated within 8 h with a two-step centrifugation protocol according to the previous description (11). Subsequently, samples were delivered at -80˚C to the ISO/IEC 17025 (International Organization for Standardization/International Electro technical Commission)-certified at Shenzhen BGI Clinical Laboratory Center (Shenzhen, China). The cell-free DNA extraction, library construction, sequencing, and bioinformatics analysis were performed according to the previous study (11).

Bioinformatics analysis. Sequencing data were analyzed using a proprietary algorithm. The binary hypothesis T-score of particular chromosomes in each sample was determined, as reported previously (11). Briefly, to assess the fetal risk of T21, T18 and T13, the sample with a T-score ≥3.0 for these chromosomes was classified as positive, whereas a T-score <3.0 was classified as negative for the indicated trisomy.

Karyotype analysis and amniotic fluid puncture. Women with positive NIPT results were recommended to receive karyotype analysis and amniotic fluid puncture for further validation. The amniotic fluid puncture was performed as routinely described. The karyotype analysis was performed according to the International System for Human Cytogenetic Nomenclature guidelines (12).

Follow-up. Follow-up was performed to NIPT negativity cases via telecommunication. The follow-up duration was 56 days after delivery. A total of 396 cases were lost to follow-up.

Results

Patient characteristics. A total of 1,901 pregnant women of Han Chinese background, with a mean maternal age of 30.57 years (19-45 years), were included in this study. The gestational age was from 12 weeks to 32+6 weeks at blood sample collection. The patient characteristics were listed in Table I. All these patients received prior screening tests using conventional methods previously, and most of them were classified as risks for T21 and/or T18. However, among these patients, 22 cases were classified by the NIPT to be positive for the T21 (n=15), T18 (n=5) and T13 (n=2), respectively.

Table II. Details of the 22 non-invasive prenatal testing (NIPT) positive cases.

No.	Sample ID	Maternal age, year	Ultrasound findings	Prior screening test results	Gestation, week	NIPT		Outcome
						High risk for	Fetal karyotpe	
1	13B0148333	31	Normal	1:45 (T21)	21+5	T21	47,XN,+21	Termination of pregnancy
2	13B0148379R	37	Normal	-	22+5	T18	46,XN	Normal
3	14B1017282	38	Normal	Unknown	19	T21	47,XN,+21	Termination of pregnancy
4	14B1017311	32	Normal	Unknown	14+5	T21	47,XN,+21	Termination of pregnancy
5	14B1017323	24	Normal	1:26 (T21)	17+2	T21	47,XN,+21	Termination of pregnancy
6	15B1103208D	29	A vanishing twin	-	16+2	T18		Stillbirth
7	PDB12AC00025	37	Normal	1:33 (T21)	17+2	T21	47,XN,+21	Termination of pregnancy
8	PDB12AC00114	34	Normal	1:55 (T21)	18+4	T21	-	Intrauterine fetal death
9	PDB12AC00146	27	Echogenic heart focus	1:25 (T21)	20+2	T21	47,XN,+21	Termination of pregnancy
10	PDB13AC00011	31	Normal	1:166 (T21)	18+2	T21	47,XN,+21	Termination of pregnancy
11	PDB13AC00027R	33	Normal	1:144 (T21)	21	T13	Declined	Termination of pregnancy
12	PDB13AC00166	30	Normal	1:140 (T21)	18+3	T21	47,XN,+21	Termination of pregnancy
13	PDB13AC00213R	33	Normal	1:100 (T21)	22	T18	Declined	Termination of pregnancy
14	PDB13AC00484R	28	Normal	1:20 (T21)	18+2	T21	-	Intrauterine fetal death
15	PDB13AC00485	33	Normal	1:37 (T21)	16+5	T21	Declined	Loss to follow up
16	PDB13AC00614	29	Echogenic heart focus	1:280 (T21)	21+5	T21	47,XN,+21	Termination of pregnancy
17	PDB13AC00729	31	Norma	1:270 (T21)	18+6	T21	47,XN,+21	Termination of pregnancy
18	PDB13AC00834	30	Normal	1:260 (T21)	15+5	T13	46,XN	Normal
19	PDB13AC00952	40	Normal	-	12+5	T21	47,XN,+21	Termination of pregnancy
20	PDB13AC01023	24	Normal	1:270 (T21)	19+5	T18	47,XY, +18(70%) 48,XYY, +18(30%)	Termination of pregnancy
21	PDB13AC01225	22	Normal	1:177 (T21)	19+2	T21	47,XN,+21	Termination of pregnancy
22	PDB13AC01245R	27	Normal	1:270 (T21)	21	T18	46,XN	Normal

While the others (n=1,879) were classified to be NIPT negative cases.

Karyotype confirmation. Among the 22 pregnant women confirmed with positivity of NIPT tests, 15 showed high risks for T21, 5 showed high risks for T18 and 2 showed high risks for T13, respectively (Table II). Finally, 16 underwent amniotic fluid puncture for further validation. Among the 15 cases with high risks of T21, the karyotype results were confirmed with T21 in 12 cases, while the other 3 refused the amniotic fluid puncture. Three underwent amniotic fluid puncture among the 5 cases with high risk of T18. Karyotype results indicated normal karyotype in 2 patients, while the other one showed karyotype of 47, XY,+18 (70%) and 48, XYY,+18 (30%). Among the 2 cases with high risks of T13, 1 declined to receive the amniotic fluid puncture, while the other 1 showed a normal karyotype.

Pregnancy outcome. The fetal outcome data for the 6 pregnant women with NIPT-positivity but declined to undergo amniotic fluid puncture were obtained after the expected

Table III. The pregnancy outcome of 1,879 NIPT negative cases.

Follow-up	No. of the cases (%)
Fetal outcome available	1,483 (78.92)
Confirmed normal at birth	1,418 (75.47)
Still birth	59 (3.14)
Neonatal death	1 (0.05)
Termination of pregnancy	5 (0.27)
Abnormal ultrasound findings	2 (0.11)
Abnormal ultrasound findings-chromosomal abnormalities	1 (0.05)
Personal reason	2 (0.11)
Loss to follow-up	396 (21.08)
Failed to contact	390 (20.76)
Refused to provide information	6 (0.32)

NIPT, non-invasive prenatal testing.

Table IV. Comparison of specificity (Sp) and sensitivity (Se) of NIPT and prior screening test.

A, T21

	Prior screening test					NIPT					
Results	No.	Se	Sp	FPV	PPV	Positive	Se	Sp	FPV	PPV	Validation
High risk	752	88.89%	29.08%	70.92%	1.06%	10	100%	100%	0%	100%	8(47,XN,+21); 2(Intrauterine fetal death)
Critical high risk	234	88.89%	29.08%	70.92%	1.06%	1	100%	100%	0%	100%	1(47,XN,+21)
Low risk	72	88.89%	29.08%	70.92%	1.06%	0	100%	100%	0%	100%	-
None	377	-	-	-	-	1	100%	100%	0%	100%	1(47,XN,+21)
Unknown	9	-	-	-	-	2	100%	100%	0%	100%	2(47,XN,+21)
Total	1,444	-	-	-	-	14	100%	100%	0%	100%	12(47,XN,+21); 2(Intrauterine fetal death)

B, T18

	Prior screening test					NIPT					
Results	No.	Se	Sp	FPV	PPV	Positive	Se	Sp	FPV	PPV	Validation
High risk	19	0%	98.20%	1.80%	0/0+10	0	100%	99.86%	0.14%	33.33%	-
Critical high risk	4	0%	98.20%	1.80%	0/0+10	0	100%	99.86%	0.14%	33.33%	-
Low risk	1,035	0%	98.20%	1.80%	0/0+10	3	100%	99.86%	0.14%	33.33%	1[47,XY,+18(70%); 48,XYY,+18(30%)]; 1(Termination of pregnancy); 1(46,XN)
None	377	-	-	-	-	1	100%	99.86%	0.14%	33.33%	1(46,XN)
Unknown	9	-	-	-	-	0	100%	99.86%	0.14%	33.33%	-
Total	1,444	-	-	-	-	4	100%	99.86%	0.14%	33.33%	1[47,XY,+18(70%); 48,XYY,+18(30%)]; 1(Terminationof pregnancy); 2(46,XN)

C, T13

	Prior screening test					NIPT					
Results	No.	Se	Sp	FPV	PPV	Positive	Se	Sp	FPV	PPV	Validation
Total	-	-	-	-	-	2	0%	99.93%	0.07%	0%	1(Termination of pregnancy); 1(46, XN)

NIPT, non-invasive prenatal testing.

confinement date. All the 12 pregnant women validated with T21 through karyotype decided termination of pregnancy. For the 3 pregnant women with NIPT-positivity declining to receive amniotic fluid puncture, the fetal outcome in 2 cases were intrauterine fetal death while the other 1 was lost in the follow-up. One pregnant with NIPT positivity with high risks for T18 still decided delivery without undergoing amniotic fluid puncture. Another two pregnant women with NIPT positivity with high risks for T13 and T18 decided to determine the pregnancy. One pregnant woman decided to terminate the pregnancy after classified as NIPT positivity after karyotype analysis (Table II).

In the 1,879 cases with negative NIPT testing, the fetal outcome data were obtained in 1,483 cases, while 396 were in lost. The majority of cases (75.47%) were normal at birth. Neonatal death was observed in 1 case. Five pregnant women

decided termination of pregnancy despite the presence of NIPT negativity (Table III).

Evaluation of NIPT capacity. Among the 1,879 cases with NIPT negativity, follow-up was successfully performed in 1,504 cases including 21 with NIPT positivity and 1,483 with NIPT negativity. The stillbirth cases (n=60) were excluded from the NIPT performance evaluation. Thus, a total of 1,444 cases were used for the analysis finally. The sensitivity of the prior screening test for T21 was 88.89%, but the specificity was only 29.13% (Table IV). Among the 15 cases classified as NIPT positivity for T21, one patient was lost in the follow-up. Thus, 14 patients were included in the analysis, and the sensitivity and specificity were both at 100%. For the conventional screening test, the respective sensitivity and specificity for the T18, were 73.27 and 98.20% while the sensitivity and specificity for the NIPT were 100 and 99.86%, respectively (Table IV).

Discussion

NIPT has been widely used for the prenatal screening of T21, T18 and T13 in the last few years. Up to now, it is still lacking large scale clinical studies focused on the efficiency in the general population. Besides, there are really some concerns on the clinical performance. The present study including 1,901 cases was performed to investigate the clinical performance of NIPT on the sensitivity and specificity on the screening of T21, T18 and T13, respectively.

Conventional prenatal screening involves amniotic fluid puncture, chorionic villus sampling and umbilical vein puncture (13). However, these methods are invasive and cause high risks of infection, and even abortion. Recently, NIPT for common fetal aneuploidies by massively parallel sequencing of maternal plasma DNA has been considered as a screening method in clinical practices (14). Up to now, several studies have been carried out to focus on the application of such a technique on the screening for the T13, T18 and T21 (14-16). For example, Liang and collegues reported the efficiency of NIPT based on 412 samples with full karyotyping and sequencing results (11). These authors showed a detection sensitivity of 100% and a specificity of 99.71%, and indicated that the NIPT of fetal chromosome aneuploidies for all 24 chromosomes was feasible in a single sequencing event. In our study, a large sample size with a total of 1,901 cases was used. On this basis, we evaluated the efficiency and clinical performance of NIPT in the screening for the T13, T18 and T21, respectively. We found that, among the 22 NIPT-positive pregnant women, all chose to be informed of the potential results, suggesting that most of the pregnant women want to know the potential information as much as possible in order to make the final decision. Most of the patients (86.3%) decided to undergo amniotic fluid puncture. For the cases (n=1483) with negative NIPT findings, 1,418 (95.61%) were normal at birth, indicating the efficiency of NIPT screening was extremely feasible as previously described in other studies.

Serum screening technique has been commonly used in the clinical practice in the past decades. Giving an estimated detection sensitivity of 90% and a rate of false positivity of 2-5%; the most commonly used method for the screening of Down syndrome and T18 being based on the combination of maternal age, ultrasound examination findings of the fetus, as well as the concentration of related markers (17,18). Such a method is labor-consuming and involves extensive procedures. Invasive methods with amniocentesis as a representative method, is effective for the prescreening with definite results, but such a technique bears a miscarriage rate of 0.5% (10). Compared with these methods, the NIPT technique is non-invasive (with only a blood sample collection of <10 ml) and easy to perform. In 2012, the committee of Prenatal Diagnosis in China gave positive opinion on the NIPT technique (19). On this basis, it is reasonable to believe that the application of NIPT in clinical practice may be further extended.

Indeed, the NIPT is suitable to the screening of pregnant women at a gestational age of 12+0 to 26+6 weeks, which is more extensive than the serum screening (12+0 to 16+6 weeks) and amniotic fluid puncture (16+0 to 22+6 weeks), respectively (3). Another advantage of the NIPT is the satisfactory sensitivity and specificity (20). In our study, the sensitivity of the prior screening test for T21 was 88.89%, but the specificity was only 29.13% while the NIPT showed 100% for both sensitivity and specificity for T21 screening. For the T18, the sensitivity and specificity was, respectively, 73.27 and 98.20% for the conventional screening test relatively to 100 and 99.86% for NIPT. Compared with the conventional methods, the sensitivity and specificity of NIPT for the screening of T18 and T21 was remarkably higher. However, the sensitivity of detection of T13 was 99.93%, but the specificity was not satisfactory, suggesting that large samples are still needed to focus on the performance of NIPT for the screening of T13.

In conclusion, NIPT technique is feasible for the prenatal screening of T18 and T21 with higher sensitivity and specificity compared with conventional methods. We believe that the genome sequencing-based NIPT technique is applicable in clinical practice, and deserves extensive application in future.

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