The Value of Non-Invasive Prenatal Testing in the Diagnosis of Birth Defects: Insights from a Large Multicentre Study in Southern China

Meiying Cai

Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University

Na Lin

Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University

Xuemei Chen

Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University

Ying Li

Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University

Min Lin

Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University

Xianguo Fu

Ningde Municipal Hospital, Ningde Normal University

Shuqiong He

Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University

Liangpu Xu

Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University

Hailong Huang (✉ huanghailong@fjmu.edu.cn)

Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University

Research Article

Keywords: Non-invasive prenatal testing (NIPT), positive predictive value (PPV), chromosome microarray analysis (CMA), sex chromosomal aneuploidies (SCA), copy number variations (CNV)

Posted Date: August 6th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-778395/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Non-invasive prenatal testing (NIPT) is a fast, safe, and non-disruptive diagnostic method. At present, few studies have evaluated the screening efficiency of NIPT positive predictive value (PPV) in study subjects. Here, the results of NIPT in pregnant women were retrospectively analysed, and the detection rate, PPV and follow-up data were evaluated to determine its clinical value. A large multicentre study was conducted involving 52,855 pregnant women who received NIPT. Based on gestational age, amniotic fluid or umbilical cord blood were extracted for simultaneous karyotype and chromosome microarray analysis (CMA) in NIPT-positive patients. Among the 52,855 cases, 754 were NIPT-positive, with a positivity rate of 1.4%. Karyotype analysis and/or CMA confirmed 323 cases of chromosomal abnormalities, with a PPV of 45.1%. PPV of Trisomy 21 (T21), Trisomy 18 (T18), Trisomy 13 (T13), sex chromosomal aneuploidies (SCA) and copy number variations (CNV) were 78.9%, 35.3%, 22.2%, 36.9% and 32.9%, respectively. The PPV of T21, T18, and T13 increased with age whereas, the PPV of SCA and CNVs had little correlation with age. The PPV was significantly high in patients with advanced age along with an abnormal ultrasound.

NIPT had a high PPV for T21, and a low PPV for T13 and T18, while screening for SCA and CNVs showed clinical significance. However, in case of NIPT screening for SCA and CNVs, simultaneous karyotype and CMA should be performed to increase the detection rates. Interventional prenatal diagnosis is still required in NIPT-positive cases to avoid false positives or unnecessary termination of pregnancy.

Introduction

Chromosomal abnormalities are one of the most common causes of birth defects;^{1–4} currently there is no effective treatment. However, prenatal screening and diagnosis can prevent the birth of such children. Therefore, it is important to have a safe, simple, economical, and practical prenatal screening method to detect birth defects. Since Lo et al^{5,6} discovered cell-free foetal DNA (cffDNA) in a pregnant women's plasma for the first time in 1997, non-invasive prenatal testing (NIPT) to detect foetal chromosomal abnormalities has rapidly gained importance.^{7–10} NIPT uses high-throughput sequencing technology and bioinformatics tools to analyse and process data to determine the rate of risk of foetal chromosomal aneuploidy.⁸

Compared to traditional serological screening, the sensitivity and specificity of NIPT improved considerably for screening Trisomy 21 (T21), Trisomy 18 (T18), and Trisomy 13 (T13) syndromes.^{11,12} In addition, NIPT can also detect sex chromosomal aneuploidies (SCA) and copy number variations (CNV).^{13–15} This method has been widely applied and promoted as an accurate, safe, and fast method to detect foetal chromosomal aneuploidy. Moreover, NIPT avoids the risks of abortion, infection, and injury generally associated with the conventional interventional prenatal testing for pregnant women.¹⁶ Thus, NIPT can significantly reduce the rate of birth defects.

NIPT does have certain limitations. The positive predictive value (PPV) refers to the proportion of patients with actual disease in the screening positive population, which can reflect the diagnostic benefit of screening, thus having more reference value for clinical consultation and follow-up treatment. The PPV of NIPT is affected by population; data for the screening efficiency of NIPT PPV from different populations is limited. In this study, the results of NIPT in 52,855 pregnant women in a large multicentre study in Southern China were analysed retrospectively. The positivity rate and PPV of different populations along with follow-up were analysed to evaluate its significance in clinical applications.

Materials And Methods

Study subjects. A large multicentre study was conducted in Southern China from January 2014 to February 2021 wherein 52,855 pregnant women who underwent NIPT were enrolled as subjects. The age of the pregnant women ranged from 15 to 49 years, with an average age of 29.5 ± 4.7 years. The gestational age ranged from 11 to 36 weeks, with an average of 22.2 ± 2.4 weeks. Inclusion criteria for NIPT were as follows: single pregnancy, advanced age (expected age ≥ 35 years), serologically screened as high-risk group (risk value of $T21 \geq 1/270$ or $T18 \geq 1/350$), abnormality based on ultrasonography, previous miscarriages, and no indication (Fig. 1). Exclusion criteria for NIPT were as follows: pregnant women with malignant tumours or chromosomal abnormalities and those who have received blood transfusion or stem cell therapy or undergone transplantation surgery, within one year.

Laboratory methods for NIPT. The cell-free DNA blood collection tube or Ethylenediaminetetraacetic acid anticoagulant blood collection tubes (Streck, USA) were used to extract 10 mL of venous blood from the subjects, and plasma was separated. CffDNA was extracted from the peripheral blood of the subjects using the QIAamp DNA Microkit (Qiagen Limited, Germany). A library was constructed using the kits produced by Hangzhou Berui Hekang Gene Co., Ltd. Using the NextSeq CN500 sequencer, the proportion of each chromosome reads (% ChrN) and Z value of each chromosome was calculated. (cut-off: $|Z| = 3$).

Chromosome karyotype analysis. Depending on the gestational age of the pregnant woman, transabdominal amniocentesis was performed, and 20–30 ml of amniotic fluid or 1.0–2.5 ml of umbilical cord blood was extracted under ultrasonic guidance. The collected samples were cultured, treated with colchicine, and stained with Giemsa stain according to the laboratory standard operating procedures for G-banding karyotype analysis. Karyotypes were labelled according to the International System for Human Cytogenetic Nomenclature 2016; 40 karyotypes were counted in each case, and five karyotypes were analysed.

Chromosomal microarray analysis (CMA). The experiment was conducted in accordance with the standard operating procedures provided by Affymetrix for the following: DNA extraction and preparation, DNA digestion, ligation, amplification, purification, fragmentation, labelling, hybridization, washing, staining and scanning. Data analysis was done using the Chas 2.0 software. The CMA structure was analysed in combination with the relevant database to determine the nature of CNV. According to the American College of Medical Genetics and Genomics (ACMG) guidelines,¹⁷ CNV are divided into five categories: pathogenic, benign, and variation of uncertain clinical significance (VUS). For VUS, it is recommended that the pregnant woman and her spouse undergo parental testing for verification. According to the ACMG guidelines, if the CNV was inherited from a parent with normal phenotype, VUS might be benign; if it is a new mutation, VUS is likely to be pathogenic.

Follow-up of foetal pregnancy outcome and postnatal follow-up. Pregnant women who were NIPT-positive and confirmed by amniocentesis were followed up on call. Pregnancy outcome and the growth and development of the foetus after birth were recorded.

Statistical analysis. Microsoft Office Excel was used to input data, and SPSS 21.0 statistical software package was used to analyse the data. The results were compared by the chi-square test or Fisher's exact probability method. All statistical tests were conducted by bilateral test. The level of significance was set at $\alpha = 0.05$, and $P < 0.05$ was considered to be statistically significant.

Ethics declaration. All the pregnant women who underwent NIPT (age ranged from 15 to 49 years) gave the informed consent, and the study was approved by the Ethics Committee of Fujian Maternal and Child Health Hospital (2014-042). All experiments were performed in accordance with the relevant guidelines and regulations. Informed consent was obtained from all subjects and their legal guardian (s) wherever applicable.

Results

NIPT results statistics. Among 52,855 pregnant women, 754 were NIPT-positive, accounting for 1.4% (754/52855) of the total sample number. The 754 NIPT-positive patients included 171 cases of T21, 89 cases of T18, 48 cases of T13, 372 cases of SCA, and 74 cases of CNV.

Results of karyotype analysis and/or CMA. Among the 754 NIPT-positive pregnant women, 716 were tested for foetal chromosome karyotype analysis and 312 were tested for CMA simultaneously. By karyotype analysis and/or CMA, 323 cases of foetal chromosome abnormality were confirmed. The total PPV was 45.1% (323/716). The confirmed 312 chromosome abnormalities included T21 (131 cases), T18 (30 cases), T13 (10 cases), SCA (129 cases), and CNV (23 cases). PPV of T21, T18, T13, SCA, and CNV were 78.9%, 35.3%, 22.2%, 36.9%, and 32.9%, respectively (Fig. 1).

Karyotype analysis and CMA were performed simultaneously in 312 NIPT-positive cases. Results of the two methods were completely consistent in 292 cases, and the complete coincidence rate of test results was 93.6% (292/312). There were 20 cases of inconsistencies, including 14 cases of microduplication or microdeletion, two cases of balance translocation, and four cases of low proportion chimerism of sex chromosomes. Balanced translocations and low proportion chimerism of the sex chromosomes were normal with CMA. Chromosome microduplication or microdeletions were normal by karyotype analysis. (Table 1)

Table 1
Inconsistent results between Karyotype analysis and CMA

Case	NIPT+	Karyotype analysis	CMA
1	SCA	46,X,i(X)(q10)	N
2	SCA	46,X,i(X)(q10)	N
2	SCA	45,X[7]/46,XX[99]	N
4	SCA	45,X[6]/46,XX[72]	N
5	SCA	45,X[7]/46,XX[73]	N
6	SCA	45,X[41]/47,XXY[30]/46,XY[2]	N
7	SCA	N	arr[hg19] Yq11·223q11·23(25,863,808 – 27,609,692)×0
8	SCA	N	arr[hg19] Xq24q25(118,395,148 – 125,416,121)×1
9	SCA	N	arr[hg19] Xp22·33q28(168,551 – 155,233,098)×1-2
10	CNV	N	arr[hg19]22q11·21(18,648,855 – 21,800,471) ×1
11	CNV	N	arr[hg19] 17p11·2(16,600,022 – 18,746,988)×3
12	CNV	N	arr[hg19] 2p22·3(34,049,512 – 35,045,602)×3
13	CNV	N	arr[hg19]10q26·13q26·3(125,262,198 – 135,426,386)×1
14	CNV	N	arr[hg19]11q22·3(104,181,493 – 106,629,690)×1
15	CNV	N	arr[hg19] 16p12·2(21,740,199 – 22,718,351)×1
16	CNV	N	arr[hg19] 13q31·3q34(94,929,201 – 115,107,733)×1
17	CNV	N	arr[hg19]8q24·22q24·3(134,400,222 – 146,295,771)×3, 21q22·12q22·3(36,746,514 – 48,093,361)×1
18	CNV	N	arr[hg19] 5q23·1(115,690,982 – 116,314,598)×3
19	CNV	N	arr[hg19] 4q22·2q35·2(94,884,399 – 190,957,460)×3
20	T13	N	arr[hg19] 13q31·1q34(83,191,742 – 115,107,733)×3
NIPT: non-invasive prenatal testing; CMA: chromosomal microarray analysis; T13: trisomy 13 syndrome; SCA: sex chromosomal aneuploidies; CNV: copy number variations; N: normal.			

The distribution of chromosomal abnormalities, NIPT-positive and PPV in different age groups. According to age, 52,855 pregnant women were divided into two groups, the younger group (≤ 34 years old) (37,316 cases) and the older group (≥ 35 years old) (15,539 cases). According to the type of chromosome abnormality, they were divided into five groups, namely T21, T18, T13, SCA, and CNV. The ratios of NIPT positivity and PPV for different types of chromosomal abnormalities in the two age groups (young and old) are shown in Table 2. The PPV of T21, T18, and T13 increased with age and showed statistically significant differences between the elderly and young groups (the *P* values of T21, T18, and T13 between the two groups were 0.011, 0.009, and 0.007, respectively), while the difference in SCA and CNV between the elderly and young groups was not statistically significant (the *P* values of SCA and CNV between the two groups were 0.508 and 0.956, respectively) (Fig. 2).

Table 2
The distribution of chromosomal abnormalities, NIPT-positive and PPV in different age groups

Group	NIPT	T21		T18		T13		SCA		CNV	
		NIPT+	TP(PPV%)	NIPT+	TP(PPV%)	NIPT+	TP(PPV%)	NIPT+	TP(PPV%)	NIPT+	TP(PPV%)
≤34	37316	92	66(71.7)	50	12(24.0)	33	4(12.1)	256	97(37.9)	49	16(32.7)
≥35	15539	74	65(87.8)	35	18(51.4)	12	6(50.0)	94	32(34.0)	21	7(33.3)
Total	52855	166	131(78.9)	85	30(35.3)	45	10(22.2)	350	129(36.9)	70	23(32.9)
NIPT +: non-invasive prenatal testing positive; T21: trisomy 21 syndrome; T18: trisomy 18 syndrome; T13: trisomy 13 syndrome; SCA: sex chromosomal aneuploidies; CNV: copy number variations; TP: true positive; PPV: positive predictive value.											

Grouping of the indications of NIPT-positive and comparison with PPV. According to the indications of NIPT-positive, 716 NIPT-positive pregnant women were divided into five groups: among 370 cases in no indication group, 145 cases were diagnosed with foetal chromosomal abnormality and PPV was 39.2%; among 51 cases in serological screening high-risk group, 19 cases of foetal chromosomal abnormality were confirmed, and PPV was 37.3%; among 213 cases in the advanced age group, 107 cases were diagnosed with foetal chromosomal abnormalities and PPV was 50.2%; In the 58 abnormal ultrasound cases, 36 cases of foetal chromosomal abnormality were confirmed and PPV was 62.1%; there were 24 cases in the group containing 2 or more of the above indicators, and 16 cases were diagnosed with foetal chromosomal abnormality and PPV was 66.7% (Table 3). Statistical analysis indicated that PPV difference of various NIPT-positive indications was statistically significant ($P < 0.05$) (Fig. 3).

Table 3
The number of NIPT-positive cases and PPV in each indication group

Indication	NIPT+	TP	FP	PPV(%)
No indication	370	145	225	39.2
Serological screening high-risk	51	19	32	37.3
Advanced age	213	107	106	50.2
Ultrasonic abnormality	58	36	22	62.1
Two or more kinds of indicators	24	16	8	66.7
Total	716	323	393	45.1
NIPT +: non-invasive prenatal testing positive; TP: true positive; FP: false positive; PPV: positive predictive value.				

Obstetric follow-up of NIPT-positive pregnant women. Among 716 NIPT-positive cases, the follow-up rate was 94.7%, where 678 cases were followed-up successfully and 38 cases were lost to follow-up. Among the 678 cases, 374 were term delivery, with a rate of 52.2%; pregnancy was terminated in 289 cases, terminated pregnancy rate 40.4%; 14 cases resulted in spontaneous abortions or stillbirth, accounting for 2.0% (Table 4). Of the confirmed cases with SCA, in 15 cases, women delivered normally after genetic counselling (including nine cases with 47,XXX, four cases with low-proportion chimerism, and two cases with balanced chromosomal translocation). Of the confirmed cases with CNV, in 18 cases, women terminated the pregnancy.

Table 4
Obstetric follow-up of NIPT positive pregnant women

Chromosomal abnormal type	Karyotype analyses and (or) CMA	TD	TP	Abortion or stillbirth	Loss to follow-up	Total
T21	A	0	127	4	0	131
	N	24	0	1	10	35
T18	A	0	28	2	0	30
	N	50	0	2	3	55
T13	A	0	8	1	1	10
	N	34	0	0	1	35
SCA	A	15	108	2	4	129
	N	202	0	3	16	221
CNV	A	5	18	0	0	23
	N	44	0	0	3	47
Total		374	289	15	38	716
TP: terminated pregnancy; TD: term delivery; CMA: chromosomal microarray analysis; T21: trisomy 21 syndrome; T18: trisomy 18 syndrome; T13: trisomy 13 syndrome; SCA: sex chromosomal aneuploidies; CNV: copy number variations; A: abnormal; N: normal.						

Discussion

The accuracy rate of traditional serological screening is 66–80%, the rate of missed detection is 20–34%; the false positive rates and missed diagnosis rate are both relatively high.¹⁸ Although invasive prenatal diagnosis is the gold standard, there are risks of miscarriage and infection, which significantly reduces the compliance of pregnant women to invasive prenatal diagnosis. NIPT has high sensitivity and specificity for T21, T18, and T13, and its screening accuracy is significantly higher than that of traditional serological screening methods, and it can reduce the number of invasive prenatal diagnosis.¹⁹ Therefore, the development and application of NIPT plays an important role in the diagnosis of foetal chromosomal abnormalities. In this study, 754 of 52,855 cases were NIPT-positive, accounting for 1.4% of the total sample number, which was similar to the results of Sago et al.²⁰

In this study, the PPV of T21, T18, and T13 was lower than that of reported references,^{21–24} specifically PPV of T13 was significantly low. This could be because in the process of meiosis, if T13 abnormal fertilization occurs, the trisomy self-rescue mechanism is likely to occur, thus leading to a normal foetal karyotype. The placenta is composed of normal and abnormal chromosomal chimeras, namely, confined placental mosaicism (CPM).^{25,26} In addition, cfDNA detected by NIPT is mostly from placental trophoblast cells, hence, false negative results of T13 are easily detected during NIPT.²⁷ Therefore, strategies to improve the accuracy of T13 detection is to be further studied.

In this study, the PPV of T21, T18, and T13 in the advanced age group (≥ 35 years old) was significantly higher than that in the younger group (< 34 years old). The reason for this could be that women's ovarian function is more prone to decline with age, resulting in the formation of aging ovum. This leads to an increase in the probability of chromosomal variation in the resulting embryo. Therefore, gestation at an advanced age is an independent risk factor for foetal chromosomal aneuploidy.^{28,29} However, there was no difference between the PPV of SCA and CNV in the advanced and young age groups. This indicates that SCA and CNV have little correlation with age. In this study, we found that among the indications of NIPT-positive, the PPV of the group containing 2 or more kinds of indications, the group with abnormal ultrasound, and the advanced age group were the highest, which were 66.7%, 62.1%, and 50.2%, respectively. Abnormal foetal ultrasound is closely related to chromosomal aneuploidy.³⁰ Thus, patients with an abnormal foetal ultrasound should be prioritised. For cases with foetal ultrasound abnormalities and advanced age, direct extraction of amniotic fluid during the second trimester is now recommended for prenatal diagnosis. We also found that PPV was 39.2% in the group with no indication to voluntary requirements and 37.3% in the group at high risk of serological screening. Prior to the introduction of NIPT, serological screening for high-risk pregnant women was generally recommended for invasive prenatal diagnosis to determine the foetal chromosomal status. As the

most sensitive screening method, NIPT can effectively reduce the puncture rate in these pregnant women.^{31,32} As a result, many pregnant women with abnormal serological screening are choosing NIPT to avoid unnecessary invasive prenatal diagnoses.³³

Karyotype analysis can detect all chromosomal aneuploidy abnormalities and obvious chromosomal structural abnormalities that can be seen under the microscope. CMA is a molecular genetics technique developed in recent years.³⁴ CMA has significant advantages because it can detect chromosome microdeletions or microduplications that cannot be detected by conventional karyotype analysis at the genome level.^{35–37} However, CMA cannot detect balanced structural translocation and low proportion chimerism. For such abnormalities, karyotype analysis can be used. Balanced translocations and low-proportion chimerism of the sex chromosomes were normal with CMA. Chromosome microduplication or microdeletions were normal by karyotype analysis. Thus, in order to confirm PPV of SCA and CNV, the two methods should be used simultaneously to provide scientific and accurate molecular genetic diagnostic basis for targeted pre-pregnancy eugenics in the next pregnancy.

In this study, based on a large sample of NIPT test population, reliable PPV of T21, T18, T13, SCA, and CNV were obtained, which provided an important reference for clinical genetic counselling and management. However, the limitation of this study is the failure to complete the follow-up of all the cases after birth and the failure to conduct placenta test for all the NIPT false-positive cases, especially the NIPT false positive cases indicating SCA false positive cases. Hence, the presence of CPM or the influence of maternal DNA cannot be excluded.^{38–40} At the same time, no NIPT-negative cases were followed up in this study; hence, the sensitivity and false negative rate of NIPT could not be accurately calculated. Therefore, for cases where NIPT is normal or invasive prenatal diagnosis is refused, long-term extensive studies and follow-up are required.

Conclusion

In summary, NIPT does not cause any damage to the pregnant woman or the foetus. NIPT only has a high PPV for T21, and a low PPV for T13 and T18. However, the screening of CNV and SCA was found to be significant, which is more acceptable to pregnant women clinically. NIPT is used in pregnant women at an advanced age and those with an abnormal ultrasound with better PPV, but NIPT-positive cases still require invasive prenatal diagnosis to avoid false positives or unnecessary termination of pregnancy. For pregnant women with NIPT-positive SCA and CNV, it is recommended that both karyotype analysis and CMA be performed, to improve the detection rate of chromosomal abnormalities. This approach would be more conclusive in genetic counselling and guidance in planning subsequent pregnancies.

Data availability All data generated during and/or analyzed during the current study are available upon request by contact the corresponding author.

Declarations

Data availability

All data generated during and/or analyzed during the current study are available upon request by contact the corresponding author.

Acknowledgements

We thank the patients that participated in this study. This work was supported by the Fujian Provincial Natural Science Foundation (2020J01339) and the training project of young and middle-aged talents in health system of Fujian Province (2020GGA020).

Author contributions

M.Y.C. collected data and wrote the manuscript. N.L. designed study and searched literature. X.M.C. analysed data. Y.L. interpreted data. M.L. and S.Q.H. designed study. X.G.F. researched data and managed study. L.P.X. designed study and interpreted data. H.L.H. designed study and revised the manuscript.

Conflict of interest

The authors declare that they have no competing interest.

References

1. Franssen, M., Korevaar, J. C., Tjoa, W. M., Leschot, N. J. & Goddijn, M. Inherited unbalanced structural chromosome abnormalities at prenatal chromosome analysis are rarely ascertained through recurrent miscarriage. *Prenatal diagnosis*, **28**, 408–411 (2010).
2. Bianchi, D. W., Oepkes, D. & Ghidini, A. Current controversies in prenatal diagnosis 1: should noninvasive DNA testing be the standard screening test for Down syndrome in all pregnant women? *Prenatal diagnosis* **34**(2014).
3. Lewis, C., Silcock, C. & Chitty, L. S. Non-Invasive Prenatal Testing for Down's Syndrome: Pregnant Women's Views and Likely Uptake. *Public Health Genomics*, **16**, 223–232 (2013).
4. H. et al. Karyotype analysis with amniotic fluid in 12365 pregnant women with indications for genetic amniocentesis and strategies of prenatal diagnosis. *Journal of Obstetrics and Gynaecology* **36**, 293–296 (2015).
5. Lo, Y., Corbetta, N., Chamberlain, P. F., Rai, V. & Wainscoat, J. S. Presence of fetal DNA in maternal plasma and serum., **350**, 485–487 (1997).
6. Lo, Y. et al. Rapid clearance of fetal DNA from maternal plasma. *The American Journal of Human Genetics*, **64**, 218–224 (1999).
7. Non-invasive prenatal assessment of trisomy. 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. *BMJ: British Medical Journal (Overseas & Retired Doctors Edition)*, **342**, 217–217 (2011).
8. Chiu, R., Chan, K., Gao, Y., Lau, V. & Lo, Y. M. D. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. *Proceedings of the National Academy of Sciences* **105**, 20458–20463 (2009).
9. Zhang, J. et al. Non-invasive Prenatal Sequencing for Multiple Mendelian Monogenic Disorders Using Circulating Cell-free Fetal DNA. *Obstetrical & Gynecological Survey* **74** (2019).
10. Noninvasive prenatal diagnosis of cobalamin C (cblC) deficiency through target region sequencing of cell-free DNA in maternal plasma. *Prenatal diagnosis* **40** (2020).
11. Zimmermann, B. et al. Noninvasive prenatal aneuploidy testing of chromosomes 13, 18, 21, X, and Y, using targeted sequencing of polymorphic loci. *Prenatal diagnosis*, **98**, S28–S29 (2012).
12. Nicolaides, K. H., Syngelaki, A., Ashoor, G., Birdir, C. & Touzet, G. Noninvasive Prenatal Testing for Fetal Trisomies in a Routinely Screened First-Trimester Population EDITOR COMMENT. *American journal of obstetrics and gynecology*, **207**, 374371–374376 (2012).
13. Srinivasan, A., Bianchi, D., Huang, H., Sehnert, A. & Rava, R. Noninvasive detection of fetal subchromosome abnormalities via deep sequencing of maternal plasma. *American journal of human genetics*, **92**, 167–176 (2013).
14. Hume, J. H., Wardrop, J., Boomer, T., Almasri, E. & McCullough, R. M. Clinical outcome of subchromosomal events detected by whole-genome noninvasive prenatal testing. *Prenatal diagnosis*, **35**, 999–1004 (2015).
15. Liang, D. et al. Clinical utility of noninvasive prenatal screening for expanded chromosome disease syndromes. *Genetics in Medicine* (2019).
16. Amant, F., Verheecke, M., Wlodarska, I., Dehaspe, L. & Vermeesch, J. R. Presymptomatic Identification of Cancers in Pregnant Women During Noninvasive Prenatal Testing. *Jama Oncol*, **1**, 814–819 (2015).
17. Riggs, E. R. et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). **22**, 245–257 (2020).
18. Jean, G. et al. Non-invasive prenatal testing for fetal chromosome abnormalities: review of clinical and ethical issues. *The application of clinical genetics*, **9**, 15–26 (2016).
19. An, N., Li, L. L., Wang, R. X., Yue, J. M. & Liu, R. Z. Clinical and cytogenetic results of a series of amniocentesis cases from Northeast China: a report of 2500 cases. *Genetics & Molecular Research*, **14**, 15660–15667 (2015).
20. Sago, H. & Sekizawa, A. Nationwide demonstration project of next-generation sequencing of cell-free DNA in maternal plasma in Japan: 1-year experience. *Prenatal diagnosis* **35** (2015).
21. Liao, C., Yin, A. H., Peng, C. F., Fang, F. & Kang, Z. Noninvasive prenatal diagnosis of common aneuploidies by semiconductor sequencing. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 7415 (2014).
22. E. et al. Noninvasive detection of fetal trisomy 21: systematic review and report of quality and outcomes of diagnostic accuracy studies performed between 1997 and 2012. *Human reproduction update* **19**, 318–329 (2013).

23. Teoh *et al.* Clinical implementation of cell-free DNA-based aneuploidy screening: perspectives from a national audit. *Ultrasound in obstetrics & gynecology: the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*(2015).
24. Palomaki, G. E. *et al.* DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. *Genetics in Medicine*, **14**, 296–305 (2012).
25. Benn, P., Cuckle, H. & Pergament, E. Non-invasive prenatal testing for aneuploidy: current status and future prospects. *Ultrasound in obstetrics & gynecology: the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*, **42**, 15–33 (2013).
26. Wolstenholme, J. Confined placental mosaicism for trisomies 2, 3, 7, 8, 9, 16, and 22: their incidence, likely origins, and mechanisms for cell lineage compartmentalization. *Prenatal diagnosis*, **16**, 511–524 (2015).
27. Wang, Y. *et al.* Maternal mosaicism is a significant contributor to discordant sex chromosomal aneuploidies associated with noninvasive prenatal testing. *Clinical chemistry*, **60**, 251–259 <https://doi.org/10.1373/clinchem.2013.215145> (2014).
28. Hayata, K., Hiramatsu, Y., Masuyama, H., Eto, E. & Tamada, S. Discrepancy between Non-invasive Prenatal Genetic Testing (NIPT) and Amniotic Chromosomal Test due to Placental Mosaicism: A Case Report and Literature Review. *Acta Med. Okayama*, **71**, 181–185 (2017).
29. Nury, S., Jacques, C., Herrera, R. J., Mireia, S. & Brenner, C. A. Association between spindle assembly checkpoint expression and maternal age in human oocytes. *Molecular Human Reproduction*, S51-S51(2001).
30. Norton, M. E., Biggio, J. R., Kuller, J. A. & Blackwell, S. C. The role of ultrasound in women who undergo cell-free DNA screening. *American Journal of Obstetrics & Gynecology*, **216**, B2–B7 (2017).
31. Gil, M. M., Revello, R., Poon, L. C., Akolekar, R. & Nicolaides, K. H. Clinical Implementation of Routine Screening for Fetal Trisomies in the UK NHS: Cell-Free DNA Test Contingent on Results From First-Trimester Combined Test. *Obstetrical & Gynecological Survey***71** (2016).
32. Li, S. W., Barrett, A. N., Gole, L., Wei, C. T. & Choolani, M. The assessment of combined first trimester screening in women of advanced maternal age in an Asian cohort. *Singapore medical journal*, **56**, 47–52 (2015).
33. Vanstone, M., Yacoub, K., Giacomini, M., Hulan, D. & McDonald, S. Women's Experiences of Publicly Funded Non-Invasive Prenatal Testing in Ontario, Canada. *Qualitative Health Research***25** (2015).
34. Zhang, Y., Zhong, M. & Zheng, D. Chromosomal mosaicism detected by karyotyping and chromosomal microarray analysis in prenatal diagnosis. *Journal of cellular and molecular medicine*(2020).
35. Wapner, R. J. *et al.* Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis. *New England Journal of Medicine*(2012).
36. Brady, P. D. & Vermeesch, J. R. Genomic microarrays: a technology overview. *Prenatal diagnosis***32** (2012).
37. Karen *et al.* Chromosomal Microarrays for the Prenatal Detection of Microdeletions and Microduplications. *Clinics in Laboratory Medicine*(2016).
38. Wang, Y. *et al.* Two cases of placental T21 mosaicism: challenging the detection limits of non-invasive prenatal testing. *Prenatal diagnosis*, **33**, 1207–1210 (2013).
39. Snyder, M. W. *et al.* Copy-number variation and false positive prenatal aneuploidy screening results. *N Engl J Med*, **372**, 1639–1645 <https://doi.org/10.1056/NEJMoa1408408> (2015).
40. Mao, J. *et al.* Confined placental origin of the circulating cell free fetal DNA revealed by a discordant non-invasive prenatal test result in a trisomy 18 pregnancy. *Clin. Chim. Acta*, **433**, 190–193 (2014).

Figures

Fig. 1 Flowchart of the number of patients at different stages of NIPT screening for foetal chromosomal aneuploidy.

NIPT: non-invasive prenatal testing; CMA: chromosomal microarray analysis; T21: trisomy 21 syndrome; T18: trisomy 18 syndrome; T13: trisomy 13 syndrome; SCA: sex chromosomal aneuploidies; CNV: copy number variations; TP: true positive.

Figure 1

Exclusion criteria for NIPT were as follows: pregnant women with malignant tumours or chromosomal abnormalities and those who have received blood transfusion or stem cell therapy or undergone transplantation surgery, within one year.

Fig. 2 Comparison of PPV of different types of chromosomal abnormalities in different ages groups.

T21: trisomy 21 syndrome; T18: trisomy 18 syndrome; T13: trisomy 13 syndrome; SCA: sex chromosomal aneuploidies;

CNV: copy number variations; PPV: positive predictive value.

Figure 2

The PPV of T21, T18, and T13 increased with age and showed statistically significant differences between the elderly and young groups (the P values of T21, T18, and T13 between the two groups were 0.011, 0.009, and 0.007, respectively), while the difference in SCA and CNV between the elderly and young groups was not statistically significant (the P values of SCA and CNV between the two groups were 0.508 and 0.956, respectively)

Fig. 3 The NIPT-positive, TP and PPV in various indications.

①: No indication; ②: Serological screening high-risk; ③: Advanced age; ④: Ultrasonic abnormality; ⑤: Two or more kinds of indicators; NIPT +: non-invasive prenatal testing positive; TP: true positive; PPV: positive predictive value.

Figure 3

In the 58 abnormal ultrasound cases, 36 cases of foetal chromosomal abnormality were confirmed and PPV was 62.1%; there were 24 cases in the group containing 2 or more of the above indicators, and 16 cases were diagnosed with foetal chromosomal abnormality and PPV was 66.7% (Table 3). Statistical analysis indicated that PPV difference of various NIPT-positive indications was statistically significant ($P < 0.05$)