The accuracy and feasibility of noninvasive prenatal testing in a consecutive series of 20,626 pregnancies with different clinical characteristics

Yunyun Zheng | Jia Li | Jianfang Zhang | Hong Yang

Department of Obstetrics and Gynecology, XJing Hospital of Air Force Medical University, Xi’an, China

Correspondence
Hong Yang, Department of Obstetrics and Gynecology, XJing Hospital of Air Force Medical University, No. 169 Changle West Road, XinCheng District, Xi’an 710032, Shaanxi, China.
Email: hongyang202203@163.com

Funding information
National Key Research & Development Program of China, Grant/Award Number: 2016YFC1000703 and 2016YFC1000700

Abstract
Background: To evaluate the accuracy and feasibility of noninvasive prenatal testing (NIPT) according to the results of NIPT and pregnancy outcomes with different indications.

Methods: Between October 2014 and December 2020, 20,626 pregnant women who received NIPT were included in this study. The positive predictive value (PPV) of trisomy 21, 18, and 13 (T21, T18, T13), sex chromosome abnormalities (SCAs), other chromosomal aneuploidies, and chromosomal microdeletion/microduplication were calculated. The positive results of NIPT were confirmed by amniocentesis, Karyotype analysis, and chromosome microarray analysis (CMA).

Results: In total, 263 positive cases (263/20,626, 1.28%) were detected by NIPT, of which T21, T18, and T13 were 69, 26, and 9 cases, respectively. Sex chromosome abnormalities (SCAs), other chromosomal aneuploidies, and copy number variants (CNVs) were 69, 12, and 38 cases, respectively. There were true positive in 49 of T21, 13 of T18, 1 of T13, 32 of SCAs, 1 of other chromosomal aneuploidies, and 15 of CNVs. The PPV was high in T21, moderate in T18 and SCAs, and low in T13 and other chromosomal abnormalities.

Conclusion: NIPT has high accuracy, specificity and can effectively avoid the occurrence of birth defects, but it cannot replace prenatal diagnosis. The accuracy, specificity, and sensitivity of NIPT in detecting sex chromosomes, chromosome microdeletion/microduplication, and other chromosomal abnormalities should be improved.

KEYWORDS
accuracy, chromosomal microdeletion/microduplication, chromosome aneuploidies, copy number variants, feasibility, noninvasive prenatal testing, sex chromosome abnormalities
1 | INTRODUCTION

The issue of birth defects has attracted more and more attention from scholars worldwide. Chromosomal abnormalities are main pathogenic factors for inducing birth defects.\(^1\) Chromosomal abnormalities include trisomy21 (T21), trisomy18 (T18), trisomy13 (T13), sex chromosome aneuploidies (SCAs), common microdeletions,\(^2\) rare autosomal aneuploidies, and partial deletions and duplications.\(^3\)–\(^6\) It is estimated that the incidences of T21, T18 and T13 are about 1/800, 1/6000 and 1/10,000, respectively.\(^7,8\) The incidence of SCAs is about 1/500.\(^9,10\) The incidence of chromosome abnormalities at birth is as high as 1/600.\(^2\) Prenatal screening is an effective intervention mean for fetal chromosomal diseases. The traditional detection methods mainly include prenatal serological screening and B-ultrasound screening for preliminary screening and prenatal diagnosis. Due to the low detection rate and high false-positive rate of maternal serological screening, prenatal screening technology is continuously improved and developed.\(^11\) Also, it is necessary to find an efficient, reliable, and economical method of prenatal pregnancy screening for use following assisted reproduction.\(^12\)

In 2011, the massively parallel sequencing method has been used to detect maternal plasma free DNA (cfDNA). In clinical applications in China, its detection rate is as high as 99.2% for screening T21, T18, and T13 syndromes.\(^5,13–15\) In recent years, genome-wide screening studies have also been performed to evaluate SCAs and copy number variants (CNVs).\(^16,17\) Clinically, noninvasive prenatal testing (NIPT) based on high-throughput sequencing is usually used to analyze cell-free DNA in maternal plasma to evaluate the risk for common fetal aneuploidies by quantifying the fetal chromosome complement. This valuable prenatal screening technology shows high sensitivity and specificity and is increasingly widely applied in pregnant women.\(^18\) In this study, we aimed to evaluate the accuracy and feasibility of NIPT according to the results of NIPT and pregnancy outcomes.

2 | SUBJECTS AND METHODS

2.1 | Study subjects

This study was approved by Xijing Hospital of the Air Force Medical University, China. A total of 20,626 pregnant women who voluntarily underwent NIPT from October 2014 to December 2020 were included in this study. They signed informed written consent before NIPT. Besides, they were at least 12 weeks pregnant (12–24 weeks). Information of pregnant women including age, height, weight, gestational age, pregnancy history, whether serological screening, ultrasound testing, and assisted pregnancy was recorded. Under the principle of informed consent and voluntariness, invasive prenatal diagnostic techniques were recommended for pregnant women who had poor NIPT (failed) results. Additionally, chromosomal abnormalities were analyzed. When an abnormality that matched a high-risk NIPT diagnosis was observed through invasive prenatal diagnosis or B-ultrasound, fetal aneuploidy with a positive NIPT result was considered. The pregnant women without abnormal results continued pregnancy. Two telephone follow-ups were conducted to register and sort out the pregnancy outcomes.

2.2 | cfDNA processing and sequencing

The peripheral blood (5 ml) was obtained from pregnant women and centrifuged twice within 8 h to separate the plasma. Protocols described in previous study were strictly followed during subsequent molecular detection and bioinformatics processes, including DNA extraction, library construction, and sequencing.\(^5,6,10\) In addition to T21, T18 and T13, we presented the abnormalities of other chromosomal screenings in additional information of the subjects’ reports (not shown). Figure 1 illustrated the entire testing process.
2.3 | Karyotype analysis

All chromosomes of the sample, T21, T18, T13, sex chromosomes, and chromosome microdeletion/microduplication were analyzed. Amniocentesis was recommended for pregnant women with positive NIPT results. Karyotype analysis was performed with amniotic fluid cell culture according to the standard techniques.\(^1\)

2.4 | Chromosome microarray analysis (CMA)

Chromosome microarray analysis (CMA) was carried out in strict accordance with the standard.\(^2\) The detection process included digestion, ligation, polymerase chain reaction (PCR) amplification, fragmentation, labeling, chip hybridization, dyeing, and scanning, with AffymetrixCytogenetic Cyto Scan 750K Array Gene Chip (Affymetrix Inc, Santa Clara, CA, USA). Databases, including DECIPHER, OMIM, DGV and UCSC, were used as references to evaluate the array data and analyze genotype–phenotype correlations.\(^3\)

2.5 | Follow-up

Follow-up of all study participants was completed from subsequent phone calls done by the Information Platform of Xijing Hospital, China. The follow-up information included data on delivery outcomes such as survival, induced labor, or abortion, neonatological care, and the presence or absence of birth defects.

2.6 | Statistical analysis

All statistical analyses were performed using Statistical Product and Service Solutions (SPSS) version 19.0 software (SPSS, Inc.). Data were expressed as means ± standard deviation (SD). Differences among groups were compared by analysis of variance (ANOVA). \(p < 0.05\) was considered statistically significant. We used formulas to calculate the sensitivity, specificity, false-negative rate, and predictive value as follows: sensitivity was calculated as true-positive number/(true-positive number + false-negative number) \(\times 100\%\); specificity was calculated as true-negative number/(true-negative number + false-positive number) \(\times 100\%\); the positive predictive value was calculated as true-positive number/(true-positive number + false-positive number).

3 | RESULTS

3.1 | Clinical characteristics

There were 20,626 blood samples being sequenced. There were 36 cases (1.7\%) failed to perform due to insufficient fetal scores (<4\%). Blood samples of 218 (1.06\%) cases were collected again because of borderline Z-score or low fetal fraction, with 166 (0.80\%) effective NIPT results. Finally, 20,538 subjects with the age of \(27 \pm 13\) years and gestational age of \(18 \pm 5\) weeks were included in this study. The age distribution and gestational age distribution are shown in Table 1.

3.2 | NIPT for T21, T18, T13, and SCAs

Of the 223 cases with prenatal diagnosis, there were 69 of T21, 26 of T18, 9 of T13, 69 of SCAs, 12 of other chromosome aneuploidy, and 38 of CNVs. A total of 49 of T21, 13 of T18, 1 of T13, 32 of SCAs, 1 of other chromosome aneuploidy, and 15 of CNV were confirmed to be true positive. The flowchart is shown in Figure 2. There were 263 abnormalities detected by NIPT, of which 223 were also detected by prenatal diagnostic testing. The cases with NIPT abnormalities were confirmed by Karyotype analysis and CMT, respectively (Table 3). There were 111 true positives and 112 false positives. The NIPT sensitivity of T21, T18, T13, SCAs, other chromosome aneuploidy, and CNVs was 100\%. The specialties of them were 99.90\%, 99.94\%, 99.96\%, 99.82\%, 99.95\%, 99.89\%, and the PPVs of them was 71.01\%, 50.00\%, 11.11\%, 46.38\%, 8.33\%, 39.47\%, respectively (Table 4).
3.3 | NIPT results for other chromosome aneuploidies and CNVs

Among the 223 pregnant women who had amniocentesis and confirmed NIPT positive results, 12 had other chromosomal abnormalities and 38 had CNV. The test results were basically consistent with the results of NIPT, and the diagnosis results were all pathogenic or possibly pathogenic. A total of 13 cases of chromosome microduplications were detected, including chromosomes 2, 3, 8, 9, 10, 13, 18, 20, and 22 (one case had both chromosome 10 duplication and chromosome 20 duplication). Among them, chromosome 22 had the highest incidence of microduplication. Three cases of chromosome microdeletion were detected, including chromosomes 10, 15, 21 (Table 5). CNVs were categorized into CNVs ≤5 Mb, 5–10 Mb, and >10 Mb groups according to the length, with 8, 3, and 6 cases, respectively.

### TABLE 2 Detection of fetal aneuploidies in different indications

| Clinical features                           | n = 20,626 | Rate (%) |
|---------------------------------------------|------------|----------|
| Abnormal fetal structure                    | 1468       | 7.12     |
| High risk of serological screening          | 4128       | 20.01    |
| Critical risk of serological screening      | 719        | 3.49     |
| Advanced age                                | 3786       | 18.36    |
| No clinical indications                     | 1277       | 6.19     |
| NT ≥ 3.0mm                                  | 360        | 1.75     |
| Trisomy 18 high-risk                        | 544        | 2.64     |
| No serology screening                       | 6695       | 32.46    |
| History of adverse pregnancy                | 1161       | 5.63     |
| Assisted reproduction                        | 224        | 1.09     |
| Twins                                       | 264        | 1.28     |

Note: Abnormal fetal structure: malformation; High risk of serological screening: the high risk of fetal neural tube malformation or chromosomal malformation.

### TABLE 3 Fetal karyotypes of NIPT positives

| NIPT (n) | Fetal karyotypes | Numbers |
|----------|------------------|---------|
| T21(69)  | 47,XN,+21        | 47      |
|          | 47,XN,+21/46,XN  | 2       |
|          | 46,XN            | 20      |
| T18(26)  | 47,XN,+18        | 13      |
|          | 46,XN            | 13      |
| T13(9)   | 47,XN,+13        | 1       |
|          | 46,XN            | 8       |
| SCAs(69) | 45,X            | 6       |
|          | 47,XXX          | 8       |
|          | 47,XXY          | 13      |
|          | 47,XYY          | 3       |
|          | 46,X,del(X)      | 1       |
|          | 45,X[20%]/46,XX[80%] | 1    |
|          | 46,XN           | 37      |

### FIGURE 2 Flowchart of NIPT results and follow-up

3.3 | NIPT results for other chromosome aneuploidies and CNVs

Among the 223 pregnant women who had amniocentesis and confirmed NIPT positive results, 12 had other chromosomal abnormalities and 38 had CNV. The test results were basically consistent with the results of NIPT, and the diagnosis results were all pathogenic or possibly pathogenic. A total of 13 cases of chromosome microduplications were detected, including chromosomes 2, 3, 8, 9, 10, 13, 18, 20, and 22 (one case had both chromosome 10 duplication and chromosome 20 duplication). Among them, chromosome 22 had the highest incidence of microduplication. Three cases of chromosome microdeletion were detected, including chromosomes 10, 15, 21 (Table 5). CNVs were categorized into CNVs ≤5 Mb, 5–10 Mb, and >10 Mb groups according to the length, with 8, 3, and 6 cases, respectively.
At present, newborn birth defect is a worldwide problem, and there is a high incidence in China. Traditional serological prenatal screening has a 5%–50% misdiagnosis rate, and it is insensitive for other fetal chromosomal abnormalities. 

In recent years, chromosomal microdeletion/microduplication, sex chromosome abnormalities may be the main causes of birth defects. With the discovery of cfDNA in pregnant women, NIPT has been widely used in the screening of prenatal genetic diseases, with high diagnostic accuracy for T21, T18, and T13, and the ability to screen transgenation. In this study, the accuracy and feasibility of NIPT in 20,626 pregnant women were analyzed for providing more data support for the application of NIPT.

In this study, 263 high-risk patients were identified by NIPT. The true-positive rates of T21, T18, T13, SCA, other chromosome abnormalities, and CNV were 49, 13, 1, 32, 1, and 15, respectively.

It was suggested that the sensitivity and specificity of NIPT for chromosome abnormalities are high. It has been reported that the PPV range of T21 is 65%–94%, T18 is 47%–85%, and T13 is 12%–62%. Norton et al. performed NIPT on more than 15,000 pregnant women from multiple centers. Statistical analysis showed that the sensitivity and specificity of NIPT for the detection of trisomy 21 fetuses were 100%, 99%, 90%, and 100% for trisomy 18 fetuses, and 100% for trisomy 13 fetuses. Wu et al. retrospectively analyzed the NIPT test results of 11,118 pregnant women and concluded that the positive predictive values of NIPT for trisomy 21 syndrome, trisomy 18 syndrome, trisomy 13 syndrome, and sex chromosome aneuploidy were 92.16%, 91.67%, 36.36%, and 59.26%, respectively. Recently, the detection range of NIPT has been expanding and has been extended to the screening of fetal chromosome microdeletion/microduplication syndrome (MMS), namely NIPT-plus. Our results are consistent with previous domestic studies. It is too early to judge the true

| No | Age | Weeks | NIPT indication | NIPT results | Karyotype results | CMA results |
|----|-----|-------|----------------|--------------|------------------|------------|
| 1  | 34  | 18+3  | High risk of trisomy 21 | Chr2: dup2 | 46,XN | Chr2:2p15 dup,2.0 Mb |
| 2  | 35  | 14    | Advanced age | Chr3: dup3 | 46,XN | Chr3:3q13.31 dup,2.0 Mb |
| 3  | 27  | 20+2  | High risk of trisomy 21 | Chr8: dup8 | 46,XN | Chr8:8q13.2: q13.3dup, 2.7 Mb |
| 4  | 35  | 14    | Advanced age | Chr8: dup8 | 46,XN | Chr8:3q13.31dup,2.0 Mb |
| 5  | 24  | 18+1  | High risk of trisomy 18 | Chr9: dup9 | 47,XY,+9 | 47,XY,+9 |
| 6  | 32  | 17    | No serology screening | Chr10: dup10 | 46,XN | Chr10:10p15.3-p12.1 dup, 25.5 M; Chr20:q13.13-q q13.33 dup, 14.2 M |
| 7  | 31  | 16+1  | History of adverse pregnancy | Chr13: dup13 | 46,XN | Chr13:13q21dup,16.0 Mb |
| 8  | 35  | 15+5  | Advanced age | Chr18: dup18 | 46,XN | Chr18:18p11.32-p11.21dup, 20.0 Mb |
| 9  | 33  | 19    | No serology screening | Chr22:dup22 | 46,XN | Chr22: 2q11.2dup,10.0 Mb |
| 10 | 34  | 19+2  | High risk of trisomy 21 | Chr22: dup22 | 46,XN | Chr22:2q11.23-q12.3dup,8.80 Mb |
| 11 | 22  | 16+5  | High risk of trisomy 21 | Chr22: dup22 | 46,XN | Chr22:2q11.21dup,3.80 Mb |
| 12 | 30  | 14    | Twins | Chr22: dup22 | 46,XN | Chr22:2q12.3-q13.1dup,6.60 Mb |
| 13 | 28  | 17+4  | High risk of trisomy 21 | Chr22: dup22 | 46,XN | Chr22:2q11.21del,2.40 Mb |
| 14 | 29  | 18+1  | History of adverse pregnancy | Chr10: del10 | 46,XN | Chr10:10p14 del,3.30 Mb |
| 15 | 34  | 19+2  | High risk of trisomy 21 | Chr15: del15 | 46,XN | Chr15:15q26.3del,2.90 Mb |
| 16 | 34  | 27+5  | Advanced age | Chr21: del21 | 46,XN | Chr21:21del(16-33Mb),18.0 Mb |

### Table 4 Performance of NIPT for detecting fetal chromosomal aneuploidies

| Chromosome abnormality | Positive | TP  | FP  | Sensitivity (%) | Specificity (%) | PPV (%) |
|------------------------|----------|-----|-----|-----------------|-----------------|---------|
| Trisomy 21             | 69       | 49  | 20  | 100             | 99.90           | 71.01   |
| Trisomy 18             | 26       | 13  | 13  | 100             | 99.94           | 50.00   |
| Trisomy 13             | 9        | 1   | 8   | 100             | 99.96           | 11.11   |
| SCA                    | 69       | 32  | 37  | 100             | 99.82           | 46.38   |
| Other chromosome aneuploidy | 12   | 2   | 10  | 100             | 99.95           | 8.33    |
| CNVs                   | 38       | 15  | 23  | 100             | 99.89           | 39.47   |

---

**DISCUSSION**

At present, newborn birth defect is a worldwide problem, and there is a high incidence in China. Traditional serological prenatal screening has a 5%–50% misdiagnosis rate, and it is insensitive for other fetal chromosomal abnormalities. In recent years, chromosomal microdeletion/microduplication, sex chromosome abnormalities may be the main causes of birth defects. With the discovery of cfDNA in pregnant women, NIPT has been widely used in the screening of prenatal genetic diseases, with high diagnostic accuracy for T21, T18, and T13, and the ability to screen transgenation. In this study, the accuracy and feasibility of NIPT in 20,626 pregnant women were analyzed for providing more data support for the application of NIPT.

In this study, 263 high-risk patients were identified by NIPT. The true-positive rates of T21, T18, T13, SCA, other chromosome abnormalities, and CNV were 49, 13, 1, 32, 1, and 15, respectively.
sensitivity and false-negative rate of SCAs in this study, because these clinical symptoms are not easily identified in neonatal examination and require long-term prospective follow-up. Some positive cases were proved to be false-positive, and the causes may include low fetal score, vanishing twin, abnormal maternal chromosome, localized placental chimaera, etc.

According to the sensitivity and specificity of NIPT to detect T21, NIPT is more accurate than serological screening. The American College of Medical Genetics and Genomics (ACMG) recommends NIPT for pregnant women to avoid the omission of more abnormalities. Pregnant women ≥35 years old are easily affected by environment, ovarian function degeneration, and egg aging. Pregnancy in the elderly is high-risk for chromosome aneuploidy. In our study, 3786 pregnant women ≥35 years old (18.36%) still selected NIPT, and they were high-risk with chromosome variation during the embryonic period.

NIPT is usually carried out to screen T21, T18, T13, and sex chromosomes, and it is also gradually applied to the study of other chromosomal abnormalities and chromosome CNVs. However, there are not enough clinical data to support NIPT in chromosomal abnormalities and chromosome CNVs. However, there are fewer cases of microdeletions in this study. In addition, there are indeed some problems with clinical manifestations. Therefore, we hope that the cases of this study can provide some data support.

NIPT was also used to analyze other chromosomal abnormalities and CNV screening studies. NIPT can detect microdeletion and microduplication syndromes associated with phenotypes screened, diagnosed, and studied. In clinic, microdeletions occur more frequently than microduplications. However, there are fewer cases of microdeletions in this study. In addition, there are indeed some problems with clinical manifestations. Therefore, we hope that the cases of this study can provide some data support.

NIPT was also used to analyze other chromosomal abnormalities and CNV screening studies. NIPT can detect microdeletion and microduplication of the fetal genome of more than 300 Kb. In this study, 12 cases of other chromosomal abnormalities and 38 cases of CNVs were detected using NIPT technology. After prenatal diagnosis, the results of 15 cases of CNVs and one case of chromosomal abnormality were basically consistent with the results of NIPT. Among them, eight cases had CNVs ≤5 Mb, three had CNVs between 5 and 10 Mb, and six had CNVs >10 Mb. Some subchromosomal microdeletions and microduplications have recurrent CNVs, such as 1p36, 3q, 11q23, 22q11.2 deletion syndromes. In our study, there are 3q in two cases and 22q11.2 deletion syndromes in one case. After verification, all 16 cases are pathogenic or possibly pathogenic, and further follow-up showed that the pregnancy outcomes were induced labor. It is worth noting that in our study, only 32% of deletions and duplications are related to known abnormalities. Many abnormalities may be normal genetic mutations and have no clinical significance. With the development of sequencing technology and the accumulation of clinical research, the expansion of the database may explain these unknown abnormalities. NIPT for subchromosomal microdeletion and microduplication is still in its infancy. So far, no technology has fully verified that its tests have reached a statistically significant level. Therefore, it is very important to carefully study NIPT data.

5 | CONCLUSION

In this study, NIPT has high accuracy, specificity, and acceptance of pregnant women and can effectively avoid the occurrence of birth defects. However, it is only a prenatal screening method and cannot replace prenatal diagnosis. Therefore, it is of great significance to increase its accuracy, specificity, and sensitivity in detecting sex chromosomes, chromosome microdeletion, microduplication, and other chromosomal abnormalities in clinical practice.

AUTHOR CONTRIBUTIONS

This work was funded by the National Key Research and Development Program of China. All the authors were involved in drafting and revising the work, obtaining, analyzing, and interpreting data. Ji Li and Hong Yang contributed to research design, clinical and data acquisition, analysis, and interpretation. All authors have provided final approval for submissions and agree to be responsible for all aspects of their work.

ACKNOWLEDGEMENT

None.

FUNDING INFORMATION

This study was supported by the National Key Research & Development Program of China (2016YFC1000700, 2016YFC1000703).

CONFLICT OF INTERESTS

All authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

The datasets are available from the corresponding author on reasonable request.

CONSENT FOR PUBLICATION

None.

ORCID

Jianfang Zhang https://orcid.org/0000-0001-5074-4647
Hong Yang https://orcid.org/0000-0003-4786-6402

REFERENCES

1. BlueCross BlueShield Association. Sequencing-based tests to determine fetal down syndrome (trisomy 21) from maternal plasma DNA. Technol Eval Cent Assess Program Exe Summ. 2013;27(10):1-6.
2. Wapner RJ, Babiarz JE, Levy B, et al. Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes. Am J Obstet Gynecol. 2015;212(3):332.e1–9.
3. Pertile MD, Halks-Miller M, Flowers N, et al. Rare autosomal trisomies, revealed by maternal plasma DNA sequencing, suggest increased risk of feto-placental disease. Sci Transl Med. 2017;9(395):eaan1240.
4. Fiorentino F, Bono S, Pizzuti F, et al. The clinical utility of genome-wide non invasive prenatal screening. Prenat Diagn. 2017;37(6):593-601.
5. Zheng Y, Chen B, Wan S, et al. Detection of 21q11.2-q22.11 deletions in a fetus by NIPT. J Clin Lab Anal. 2019;33(3):e22711.

6. Zheng Y, Wang G, Li J, et al. Non-invasive prenatal testing detects duplication abnormalities of fetal chromosome 12. Eur J Obstet Gynecol Reprod Biol. 2020;253:278-284.

7. Driscoll DA, Gross S. Clinical practice. Prenatal screening for aneuploidy. N Engl J Med. 2009;360(24):2556-2562.

8. Ben P, Cuckle H, Pergament E, et al. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. Obstet Gynecol. 2012;119(6):1270-1271.

9. Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn. 2011;31(1):7-15.

10. Zheng Y, Wan S, Dang Y, et al. Clinical experience regarding the clinical application of noninvasive prenatal testing for pregnant women with assisted reproductive pregnancy. Int J Womens Health. 2021;13:1167-1174.

11. Jin XX, Xu YF, Ying X, Qian YQ, Jin PZ, Dong MY. Clinical application of non-invasive prenatal screening for the detection of fetal mosaics and microduplications in autosomal chromosomes. Mol Genet Genomic Med. 2020;8(8):e1339.

12. Jin XX, Xu YF, Ying X, Qian YQ, Jin PZ, Dong MY. Clinical application of noninvasive prenatal testing for pregnant women with assisted reproductive pregnancy. Int J Womens Health. 2021;13:1167-1174.

13. Gil MM, Quezada MS, Revello R, et al. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol. 2017;50(3):302-314.

14. Gil MM, Quezada MS, Bregant B, Ferraro M, Nicolaides KH. Implementation of maternal blood cell-free DNA testing in early screening for aneuploidies. Ultrasound Obstet Gynecol. 2013;42(1):34-40.

15. Zhang H, Gao Y, Jiang F, et al. Non-invasive prenatal testing for trisomies 21, 18 and 13; clinical experience from 146,958 pregnancies. Ultrasound Obstet Gynecol. 2015;45(5):530-538.

16. Liang D, Cram DS, Tan H, et al. Clinical utility of noninvasive prenatal screening for expanded chromosome disease syndromes. Genet Med. 2019;21(9):1998-2006.

17. Porreco RP, Garite TJ, Maurel K, et al. Noninvasive prenatal screening for fetal trisomies 21, 18, and 13 in the common sex chromosome aneuploidies from maternal blood using massively parallel genomic screening of DNA. Am J Obstet Gynecol. 2014;211(4):365.e1-e12.

18. Yehui L, Hailiang L, He Y, et al. Clinical performance of non-invasive prenatal served as a first-tier screening test for trisomy 21, trisomy 18, trisomy 13, and sex chromosome aneuploidy in a pilot city in China. Hum Genomics. 2020;14(1):21.

19. McGowan-Jordan J, Simons A, Schmid M. An international system for human cytogenomic nomenclature. Reprint Cytogenet Genome Res. 2016;149:1-2.

20. Chiu RW, Akolekar R, Zheng YWL, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. BMJ. 2011;342:c7401.

21. Zheng J, Yang X, Haiyan L, et al. Prenatal diagnosis of sex chromosome mosaicism with two marker chromosomes in three cell lines and a review of the literature. Mol Med Rep. 2019;19(3):1791-1796.

22. Redon R, Ishikawa S, Fitch KR, et al. Global variation in copy number in the human genome. Nature. 2006;444(7118):444-454.

23. Koumbaris G, Kyri E, Tsangarakas K, et al. Cell-free DNA analysis of targeted genomic regions in maternal plasma for non-invasive prenatal testing of trisomy 21, trisomy 18, trisomy 13, and fetal sex. Clin Chem. 2016;62(6):848-855.

24. Tong H, Jin Y, Xu Y, et al. Prenatal diagnosis of trisomy 21, 18 and 13 by quantitative pyrosequencing of segmental duplications. Clin Genet. 2016;90(5):451-455.

25. Manotaya S, Xu H, Uerpairojkitt B, et al. Clinical experience from Thailand: noninvasive prenatal testing as screening tests for trisomies 21, 18 and 13 in 4736 pregnancies. Prenat Diagn. 2016;36(3):224-231.

26. Hu H, Wang L, Jiayan W, et al. Noninvasive prenatal testing for chromosome aneuploidies and subchromosomal microduplications in a cohort of 8141 single pregnancies. Hum Genomics. 2019;13(1):14.

27. Tian C, Deng T, Zhu X, et al. Evidence of compliance with and effectiveness of guidelines for noninvasive prenatal testing in China: a retrospective study of 189,809 cases. Sci China Life Sci. 2020;63(3):319-328.

28. 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):1095-1099.

29. Norton ME, Jacobsson B, Swamy GK, et al. Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med. 2015;372(17):1589-1597.

30. Norton ME, Wapner RJ. Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med. 2015;373(26):2582.

31. Wu QC, Sun L, Xu YS, et al. Retrospective analysis of 11118 cases of non-invasive prenatal genetic testing results in Xiamen. Chin J Eugen Genet. 2017;25(1):31-33.

32. Lu W, Huang T, Wang XR, et al. Next-generation sequencing: a follow-up of 36,913 singleton pregnancies with noninvasive prenatal testing in Central China. J Assist Reprod Genet. 2020;37(12):3143-3150.

33. Bevilacqua E, Ordóñez E, Hurtado I, et al. Screening for sex chromosome neuploidy by cell-free DNA testing: patient choice and performance. Fetal Diagn Ther. 2018;44(2):98-104.

34. Cheung SW, Patel A, Leung TY. Accurate description of DNA based noninvasive prenatal screening. N Engl J Med. 2015;372(17):1675-1677.

35. Anthony RG, Skotko BG, Benkendorf JL, et al. Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics. Genet Med. 2016;18(10):1056-1065.

36. April LH, Holli MD, Jennifer LV, et al. Positive cell-free fetal DNA testing for trisomy 13 reveals confined placental mosaicism. Genet Med. 2013;15(9):729-732.

37. Francesca RG, Jose F, Peter B, et al. Outcomes in pregnancies with a confined placental mosaicism and implications for prenatal screening using cell-free DNA. Genet Med. 2020;22(2):309-316.

38. Richardson MC, Guo M, Fauser BC, Macklon NS. Environmental and developmental origins of ovarian reserve. Hum Reprod Update. 2014;20(3):353-369.

39. Crawford NM, Steiner AZ. Age-related infertility. Obstet Gynecol Clin North Am. 2015;42(1):15-25.

40. Anja W, Kristin M, Elisabeth K, et al. Microdeletion and microduplication syndromes. J Histochem Cytochem. 2012;60(5):3463-3468.

41. Quezada MS, Gil MM, Francisco C, Orosz G, Nicolaides KH. Screening for trisomies 21, 18 and 13 by cell-free DNA analysis of maternal blood at 10-11 weeks' gestation and the combined test at 11-13 weeks. Ultrasound Obstet Gynecol. 2015;45(1):36-41.

42. Nancy CR, Peter B, Aubrey M. Current controversies in prenatal diagnosis 1: should NIPT routinely include microdeletions/microduplications? Prenat Diagn. 2016;36(1):10-14.

43. Mark IE, Ronald JW, Richard LB, et al. Noninvasive prenatal screening or advanced diagnostic testing: caveat emptor. Am J Obstet Gynecol. 2016;215(3):298-305.

44. Chamberlain SJ, Lalande M. Angelman syndrome, a genomic imprinting disorder of the brain. J Neurosci. 2010;30(30):9958-9963.

45. Cheon CK. Genetics of Prader-Willi syndrome and Prader-Willi-like syndrome. Ann Pediatr Endocrinol Metab. 2016;21(3):126-135.

46. Ichimiyi Y, Wada Y, Kunishima S, et al. 11q23 deletion syndrome (Jacobsen syndrome) with severe bleeding: a case report. J Med Case Reports. 2018;12(1):3.
47. Verheij E, Speleman L, Mink van der Molen AB, Thomeer H. Congenital respiratory tract disorders in 22q11.2 deletion syndrome. *Int J Pediatr Otorhinolaryngol*. 2018;104:1-4.

48. Zhang Z, Wang J, Li N, et al. Cutis laxa in a patient with 1p36 deletion syndrome. *J Dermatol*. 2018;45(7):871-873.

49. Ramieri V, Tarani L, Costantino F, et al. Microdeletion 3q syndrome. *J Craniofac Surg*. 2011;22(6):2124-2128.

How to cite this article: Zheng Y, Li J, Zhang J, Yang H. The accuracy and feasibility of noninvasive prenatal testing in a consecutive series of 20,626 pregnancies with different clinical characteristics. *J Clin Lab Anal*. 2022;36:e24660. doi: [10.1002/jcla.24660](https://doi.org/10.1002/jcla.24660)