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Overall evaluation of the clinical value of prenatal screening for fetal-free DNA in maternal blood

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Abstract

Objective: To explore the clinical value of prenatal screening for fetal-free DNA in maternal blood.

Methods: A total of 10,275 maternal blood samples were collected from October 2012 to May 2016 at the prenatal diagnosis center of Changzhou Woman and Children Health Hospital.

Results: Among 10,275 pregnant women accepted noninvasive prenatal testing (NIPT), 9 cases could not get the results after collected the blood second times. The rate of NIPT failure was 0.09%. Seventy-two cases got the NIPT positive results of trisomy 21/trisomy 18/trisomy 13, and the detection rate, specificity, positive predictive value (PPV), and false positive rate were 98.59%, 99.99%, 97.22%, and 0.02%. The top-3 indications of the study were advanced age women (34.90%), high risk (25.22%), and intermediate risk (19.56%). They all had the satisfactory results of NIPT. Fifty-seven pregnant women had the high risk of fetal sex chromosomal aneuploidies (SCA). After informed consent, 33 cases accepted prenatal diagnosis. Eighteen cases were confirmed as sex chromosome aneuploidies. The PPV was 54.54%. Compared with other SCA, the PPV of Turner syndrome was lower. One case was false negative after followed up.

Conclusions: NIPT showed a broad application prospects for prenatal screening and diagnosis of fetal chromosomal diseases. We should deepen mining and analyzing the clinical data, and explore the use of NIPT more reasonably from the perspective of evidence-based medicine.

Abbreviations: DR = detection rate, FPR = false positive rate, NIPT = noninvasive prenatal testing, PPV = positive predictive value, SCA = sex chromosomal aneuploidies, T13 = trisomy 13, T18 = trisomy 18, T21 = trisomy 21.

Keywords: cffDNA, high-throughput sequencing, maternal, noninvasive prenatal testing, prenatal screening

1. Introduction

Recently, noninvasive prenatal testing (NIPT) for common fetal aneuploidies was proved to be a better prenatal screening program, which was detected cell-free DNA obtained from maternal plasma by massively parallel sequencing (MPS). Nowadays, NIPT was widely used to prenatal screen the trisomy 21 (T21), trisomy 18 (T18), trisomy 13 (T13), and presented good accuracy. Some studies reported that it has the detection rate (DR) of 99.2% with a false positive rate (FPR) of 0.09% for trisomy 21; 96.3% and 0.13% for trisomy 18; and 91.0% and 0.13% for trisomy 13; respectively.[1] Some professional membership associations have issued the committee opinions or guidelines about the clinical application of NIPT, such as the American College of Obstetricians and Gynecologists (ACOG), International Society for Prenatal Diagnosis, the American College of Medical Genetics, and so on. They all thought NIPT is one better technology in screening for the common autosomal trisomies, especially T13, T18, and T21.

Changzhou Woman and Children Health Hospital affiliated to Nanjing Medical University is the only prenatal diagnosis center in the city, and applied the NIPT from 2012. More than 10,000 pregnant women have accepted the NIPT detection. In present study, we mined and analyzed the clinical data, and explored the use of NIPT more reasonably from the perspective of evidence-based medicine.

2. Materials and methods

2.1. Patients and design

The pregnant women who accepted prenatal screening and diagnosis in Changzhou Woman and Children Health Hospital affiliated to Nanjing Medical University from October 2012 to

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BY and B-YL have contributed equally to this work.

BY and H-YW carried out the assays and participated in designing the study. BZ, B-YL, QZ, Y-PC, and X-QZ carried out clinical consultation. BZ, BY, and H-YW carried out laboratory tests and performed the statistical analysis. BY and JJ conceived the study, participated in its design and coordination, and helped draft the manuscript.

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Table 1

Indications of 10,275 prenatal women accepted NIPT.

Indications	n	Constituent ratio, %
Advanced age women	3585	34.90
High risk of prenatal screening	2591	25.22
Intermediate risk of prenatal screening	2010	19.56
Voluntary demand	1517	14.76
Ultrasonic structural abnormality	182	1.77
Assisted reproductive conception	194*	1.93
Twins	119*	1.16
Others	136	1.32
Total	10,275	100

NIPT = noninvasive prenatal testing.
* Fifty-nine cases with twins were assisted reproductive conception.

May 2016 were recruited for this study. After prenatal screening in second trimester, a total of 10,275 prenatal women accepted NIPT. They were 18 to 49 years old and their gestational weeks were 13 to 27^{+5} w. The indications of the cases were high risk of prenatal screening, intermediate risk, women of advanced maternal age (AMA), and so on. Table 1 showed the baseline characteristics of the pregnant women.

The study design and protocol were reviewed and approved by the ethics committee of Changzhou Woman and Children Health Hospital affiliated to Nanjing Medical University.

2.2. Prenatal screening in second trimester

The concentrations of AFP, free βHCG, and free E3 were detected by time-resolved immunofluorescence assay. Combined with maternal age, gestational age, body weight, and diabetes, the risk values were calculated by Lifecycle software (4.0), including the risk value of neural tube defects (NTD), T21 and T18; high risk: T21 > 1/300, T18 > 1/350; intermediate risk: T21 1/300 to 1/1000, T18 1/350 to 1/1000; and AMA: maternal age ≥ 35.

2.3. Laboratory methodology

Five milliliters blood of all the cases was collected by simple needle aspiration. Within 48 h of collection, the maternal blood samples were centrifuged at 1600g for 10 min at 4°C.

The plasma was then transferred to microcentrifuge tubes and centrifuged at 1600g for 10 min at 4°C. The plasma DNA was extracted from 1 mL plasma of each sample using QIAamp Circulating Nucleic Acid Kit from Qiagen (Hilden, Germany). The resulting plasma DNA was used to make libraries for sequencing using the modified ChIP Seq protocol, as described previously.[2] DNA libraries from 12 plasma samples were indexed using 6nt barcodes and quantified with KAPA SYBR fast qPCR kit (Woburn, MA). These libraries were then pooled and loaded. One lane of an Illumina Next CN 500 v2 flow cell was used to perform the sequencing using a single-ended 43-bp sequencing protocol following the manufacturer's instructions.

The sequences from each library were split according to their unique indexes. The split sequences were then mapped to the unmasked human genome sequence (hg19). SOAP2 mapping algorithm was used to obtain the results as previously described.[3] The sequences of each sample that were mapped to each chromosome were counted, and the GC content was calculated. Normalized chromosome representation and CG correction were used to generate a Z-score as previously described.[3] Each pair of chromosomes was defined as increased if its Z-score > 3 and decreased if its Z-score is <−3.

2.4. Statistical analysis

The data were analyzed using EmpowerStats x64 software. $P < .05$ was chosen to be statistically significant. We calculated the DR, specificity, positive predictive value (PPV), and FPR in different groups. Chi-squared test was employed to compare differences for continuous variables between 2 groups.

3. Results

3.1. NIPT failure

Among 10,275 pregnant women accepted NIPT, 33 cases (0.32%) could not get the result and they all collected the blood second times. Twenty-four cases (72.7%, 24/33) succeed, while 9 cases still had no effective results. The rate of NIPT failure was 0.09% (9/10,275). Among 9 pregnant women with NIPT failure, 5 cases (5/9) were due to the high level of total maternal-free DNA, 4 cases (4/9) with low level of fetal-free DNA (<4%). Among the 24 cases who got effective results after collected the blood second times, NIPT suggested that 1 case with the high risk of T21 and it was confirmed as 46, XN, rob(21; 21) by the prenatal diagnosis. Among 9 cases who had no NIPT results, 1 case normal delivered. There were 3 cases induced labor in late pregnancy due to the maternal factors or the fetal factors. Others were still in pregnancy.

3.2. Efficiency of NIPT for T21/T18/T13

Among 10,266 prenatal women who got the effective results of NIPT, 72 cases got the NIPT positive results of T21/T18/T13, including 57 cases of T21, 14 cases of T18, and 1 of T13. After informed consent, they all accepted prenatal diagnosis by amniotic fluid cell analysis. Table 2 showed their prenatal diagnosis results. The DR, specificity, and PPV were 98.59%, 99.99%, and 97.22%, respectively. After following up, we found that 1 case was NIPT false negative result. However, it was detected by prenatal ultrasonic check with absence of nasal bone, and was prenatal diagnosed by umbilical cord blood. Meanwhile,

Table 2

Prenatal diagnosis results of 72 cases with NIPT positive results of T21/T18/T13.

	NIPT+	TP	FP	FN	DR	Specificity, %	PPV
T21	57	56	1	1	98.25%	99.99	98.25%
T18	14	13	1	0	100%	100	91.67%
T13	1	1	0	0	1/1	100	1/1
Total	72	70	2	1	98.59%	99.99	97.22%

DR = detection rate, FN = false negative, FP = false positive, NIPT = noninvasive prenatal testing, NIPT+ = NIPT positive result, PPV = positive predictive value, TP = true positive.

Table 3

Comparison of the NIPT results between different indications.

	n	NIPT+	TP	FP	FN	DR, %	PPV, %	Abnormal rate, %
Advanced age women	3585	19	19	0	0	100	100	0.53
High risk	2591	41	39	2	1	97.5	95.12	1.51
Intermediate risk	2010	10	9	1	0	100	90.0	0.45

DR = detection rate, FN = false negative, FP = false positive, NIPT = noninvasive prenatal testing, NIPT+ = NIPT positive result, PPV = positive predictive value, TP = true positive.

2 cases were NIPT false positive, FPR was 0.02%. AMA (34.90%), high risk (25.22%), and intermediate risk (19.56%) were the top-3 indications of NIPT in present study. They all had the satisfactory results of NIPT, as shown in Table 3.

3.3. Efficiency of NIPT in AMA women

In present study, the women whose age >35 years old were the largest subjects. Among 3585 advanced maternal age women, there were 19 cases with the NIPT positive results of T21/T18/T13, including 16 cases of T21 and 3 of T18. All cases accepted prenatal diagnosis via amniocentesis, and got the consistent results. We did not found the false negative case after followed up. So the efficiency of NIPT could was satisfactory, and the application of NIPT significantly reduce the rate of invasive prenatal diagnosis which was only 0.6% (19/3585). Because the AMA women should directly choose prenatal diagnosis rather than prenatal screening in China.

3.4. Efficiency of NIPT for SCA

Meanwhile, NIPT results also suggested that 57 cases might exist abnormalities of fetal sex chromosome. After informed consent, 33 women accepted prenatal diagnosis via amniocentesis. As shown as Table 4, 18 cases were confirmed as true positive results while 15 women were proved carried the normal babies. The PPV of NIPT for fetal sex chromosomal aneuploidies (SCA) was 54.54%. Among the 57 cases, 27 cases were suggested as Turner syndrome (45, X), 12 cases as Klinefelter syndrome (47, XXY), 8 cases as XXX syndrome (47, XXX), 3 cases as XYY syndrome (47, XYY), and 7 cases might exist X chromosome microdeletions. Table 4 showed the PPV of NIPT in different types of SCA disease. The PPV in Turner syndrome was the lowest (29.41%).

3.5. Distribution situation of the indications of T21

We diagnosed a total of 57 Down syndrome cases in present study including 56 cases found by NIPT and 1 case by prenatal ultrasonic check. Among 57 pregnant women, 31 cases (55%) were high risk of prenatal screening. The ages of 16 women (29%) were higher than 35 years old. The prenatal screening risk of 15.8% (9/57) women was between the value of high risk and 1/1000. The NIPT false negative case just came from the intermediate risk.

Table 4

PPV of NIPT in different SCA disease.

	NIPT+	TP	FP	No diagnosis	PPV %
Turner syndrome	27	5	12	10	29.41 (5/17)
Klinefelter syndrome	12	7*	2	3	77.78 (7/9)
XXX syndrome	8	5	0	3	100 (5/5)
XYY syndrome	3	1	0	2	100 (1/1)
ChrX-(Y)	7	0	1	6	—
Total	57	18	15	24	54.54

FP = false positive, NIPT = noninvasive prenatal testing, NIPT+ = NIPT positive result, PPV = positive predictive value, SCA = sex chromosomal aneuploidies, TP = true positive.

* Two cases were detected chromosome X partial duplication (0.4 and 0.8 Mb) by Chromosomal Microarray Analysis in amniotic fluid cells, while not clear cause disease.

4. Discussion

In the past few years, NIPT has been widely used to screen for T21, T18, and T13. According our 10,275 clinical data, we also confirmed that the DR, specificity, and PPV were 98.59%, 99.99%, and 97.22%, respectively, which was similar to many other researches.[4,5] So it is well known that NIPT was a very efficient method for prenatal screening and diagnosis, and it is helpful for the early detection of birth defects.

We first discussed the problem of NIPT detection failure. Some studies reported the failure rate of NIPT was 0.12% to 8.09%.[6] We found that the NIPT failure rate of single blood sampling was 0.58% and there was still 0.09% after collected blood second times. The rate was similar to Taneja's report,[7] but was lower than many other reports. It might be due to the platform which we used. Massive parallel sequencing (MPS) is considered to have a low failure rate compared with other platform, such as chromosome-specific sequencing and single-nucleotide polymorphism analysis.[6] The reason of NIPT failure mainly included too low value of fetal-free DNA or too high value of total maternal-free DNA. Meanwhile, we found that 72.7% pregnant women could get effective results after sampling second times and most women had normal pregnant outcome. Therefore, it should not be simply identified as high risk of fetal chromosomal aneuploidies because single NIPT failure. However, it was an noteworthy problem that the pregnancy outcomes of NIPT failure after collected blood again. Although we just collected a few data, we still found that 3 mothers induced abortion in the late pregnancy Gil[1] also found that the incidence rate of fetal chromosomal disease was significant higher in failure detection group. So we should pay more attention to the pregnant women with NIPT failure, and provide enhanced genetic counseling, prenatal diagnosis, ultrasound imaging assessment, and so on. This point was consistent with the update Committee Opinion of the American College of Women and women's college of physicians (ACOG)[8] in 2015.

Our study confirmed that NIPT for T21/T18/T13 had a good efficiency with high accuracy, specificity, PPV, and low FPR. However, we also found that there were still some false negative and false positive results. If the result of NIPT was positive, the women must be further confirmed by prenatal diagnosis, and it was necessary to follow-up the pregnant outcome. Recently, the problem of prenatal screening and diagnosis for AMA caught our attention. In our study, the group of AMA was the biggest one, occupying 34.9%. Some prospective studies showed that the program of prenatal screening in second trimester was also due to

the detection of Down syndrome, and could reduce the rate of amniocentesis. However, it did not advocate pregnant women more than 35 years to prenatal screening routinely in China. After fully informed consent, only a few of elderly pregnant women were willing to accept the interventional prenatal diagnosis. It caused the occurrence of missed cases. According the results of our study, NIPT had a very good screening effect in advanced reproductive age women, and easy to be accepted. It could significantly reduce the rate of invasive prenatal diagnosis which may be unnecessary. On the other hand, the results also suggested that the application of NIPT had a good screening effect for the women of intermediate risk. Some researchers reported the rate of fetal chromosomal abnormalities was 3.9% in this group. Among 56 Down syndrome fetuses, 14% cases come from the intermediate-risk mothers. According to the traditional program of prenatal screening and diagnosis, they will be missed diagnosis. Therefore, we think that the management of intermediate-risk population will be one of the most effective ways to reduce missed diagnosis od birth defects.

Apart from the common fetal aneuploidy as mentioned above, some studies showed NIPT also contributed to screen the fetal sex chromosome aneuploidy. However, the DR from different studies had obvious differences. The range of DR was from 66.7% to 100%.[1] In present study, we found that the PPV of NIPT for fetal SCA was 54.54%, which was similar to the reports of Porreco et al[9] and Yao et al.[10] Although NIPT had any effect in the prenatal screening of fetal sex chromosome abnormalities, but the FPR was higher. The NIPT positive result of SCA could not be used as the direct indication of induced labor. Among the different type of SCA, the PPV of Turner syndrome was the lowest (only 29.41%), and was relatively satisfactory in other SCAs, such as Klinefelter syndrome, XXX syndrome, and XYY syndrome. When the clinicians met NIPT positive results of SCA, they should carefully consult based on the type of diseases. At the same time, the traditional way of follow-up could not get the accurate results of sex chromosome aneuploidy disease. Because the patients with sex chromosome aneuploidy are usually mild in the neonatal period, without any physical or intellectual disability. It was also very difficult to diagnose the SCA disease by cell karyotype for every newborn baby. So we only observed one index (PPV) in this study, without analyzing the DR, specificity, and so on.

In conclusion, NIPT showed a broad application prospects for prenatal screening and diagnosis of fetal chromosomal diseases. We should deepen mining and analyze the clinical data, and explore the use of NIPT more reasonably from the perspective of evidence-based medicine.

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References

[1] 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 2015;45:249–66.
[2] Chiu RW, Chan KC, Gao Y, et al. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. Proc Natl Acad Sci USA 2008;105:20458–63.
[3] Song Y, Huang S, Zhou X, et al. Noninvasive prenatal testing for fetal aneuploidies in the first trimester of pregnancy. Ultrasound Obstet Gynecol 2014;45:55–60.
[4] Norton ME, Jacobsson B, Swamy GK, et al. Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med 2015;372:1589–97.
[5] 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;45:530–8.
[6] Yaron Y. The implications of non-invasive prenatal testing failures: a review of an under-discussed phenomenon. Prenat Diagn 2016;36: 391–6.
[7] PA, Snyder HL, de Feo E, Kruglyak KM, et al. Noninvasive prenatal testing in the general obstetric population: clinical performance and counseling considerations in over 85000 cases. Prenat Diagn 2016;36: 237–43.
[8] Committee opinion no 640: cell-free DNA screening for fetal aneuploidy. Obstet Gynecol 2015;126:e31–7.
[9] Porreco RP, Garite TJ, Maurel K, et al. Noninvasive prenatal screening for fetal trisomies 21, 18, 13 and the common sex chromosome aneuploidies from maternal blood using massively parallel genomic sequencing of DNA. Am J Obstet Gynecol 2014;211:365.e1–2.
[10] Yao H, Jiang F, Hu H, et al. Detection of fetal sex chromosome aneuploidy by massively parallel sequencing of maternal plasma DNA: initial experience in a Chinese hospital. Ultrasound Obstet Gynecol 2014;44:17–24.