Non-invasive prenatal testing (NIPT) detected chromosome aneuploidies and beyond in a clinical setting

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Abstract: The discovery of circulating cell-free fetal DNA (cffDNA) in maternal plasma opened up a new area in detecting fetal chromosome aneuploidies. Many studies have indicated that this technology had a high sensitivity and specificity. In this study, we have examined 4180 patients who underwent non-invasive prenatal testing (NIPT). Total of 36 cases of common trisomies (22 were trisomy 21, 8 were trisomy 18 and 6 were trisomy 13) were identified by NIPT; all were further confirmed by amniocentesis analysis except one case of trisomy 13. Moreover, four cases of subchromosome abnormalities also were identified by NIPT. Further investigations by array based comparative genomic hybridization (aCGH) revealed that the abnormality was fetal origin in two, and maternal origin in two. In addition, in one case of negative NIPT, based on ultrasound abnormality, aCGH showed a 2 M small deletion on chromosome 15. In conclusion, NIPT is a powerful tool in detecting fetal chromosome trisomy, even in subchromosome abnormalities.

Keywords: Non-invasive prenatal testing (NIPT), chromosome aneuploidies, subchromosome abnormalities, genetic counseling

Introduction

The circulating cell-free fetal DNA (cffDNA) in maternal plasma was first discovered more than one decade ago [1]. Since then, epigenetic differences between the placenta and maternal blood cells [2] and the ratio between alleles of a SNP in placental expressed PLAC4 mRNA in maternal plasma [3, 4] were gradually clarified, and therefore were employed for non-invasive prenatal diagnosis. In particular, with the progress of next generation sequencing, massively parallel genomic sequencing of maternal plasma DNA was successfully used to detect of fetal chromosome trisomy [5] as well as other hereditary disease [6]. And this method, namely, non-invasive prenatal testing (NIPT), has shown high sensitivity and specificity for patients of fetal aneuploidies in many studies [7-10]. However, concerns on NIPT, such as false positive and false negative reports have also been discussed in other studies [11-13].

4180 pregnant of singleton pregnancy that came to our center for NIPT were recruited in our research. 22 cases of trisomy 21, 8 cases of trisomy 18 and 6 cases of trisomy 13 were identified by NIPT. All cases of trisomy were further confirmed by amniocentesis analysis except one case of trisomy 13. Moreover, four cases of subchromosome abnormalities also were identified by NIPT. Furthermore, in one case of negative NIPT, based on ultrasound abnormality, aCGH showed a 2 M microdeletion on chromosome 15. So far, fetal outcomes were ascertained in 3510 cases.

Materials and methods

Ethics statement

Ethical approval was obtained from Ethics Committee of Henan Provincial People’s Hospital, China. And all participants involved in this research have agreed and signed the written consents.
NIPT application in a clinical setting

Study subjects

We consecutively recruited consenting pregnant women from Medical Genetic Institute of Henan Province, Henan Provincial People’s Hospital, from 24 April 2014 to 24 Dec 2015. All involved study subjects were singleton pregnancy.

Sample preparation

Maternal blood was collected at least at 12 weeks of gestation. Maternal plasma was separated by centrifuging the blood sample twice, and then plasma DNA was extracted using the protocol reported elsewhere [14]. 10 ml of amniotic fluid were collected from NIPT-positive pregnant, and then the standard karyotyping and aCGH were performed.

Plasma DNA sequencing, data align and analysis

DNA libraries were prepared by using DNA extracted from the collected plasma (TruSeq DNA Sample Prep Kit, Illumina, Inc). Unique DNA adapter sequences were added to each sample so that pooled sequencing runs. Total of 96 different adapters were used to distinguish the individual maternal plasma sample. From DNA extraction to sequencing, each step has a quality control. Total of 4 lanes on flow cells were sequenced with Next Seq CN500 sequencer (Illumina) by standard single-end 45 bp reads (include 8 bp index). Q20, the sequencing quality value, was over 90% for each base. The amount of each sequencing reads was around 3.5-5.5 million, and the GC content was around 39-42%. Sequences only mapped to one location in the repeat-masked reference human genome with no mismatch were analyzed by SOAP2 bioinformatic algorithm. The number of sequence reads that aligned to chromosome were counted, the z-score was further calculated with the formula described elsewhere [5].

Results and discussion

Total of 4180 pregnant women were recruited in this research. The average of maternal age (MA) was 32 (ranging, 20-44) years old. The mean of gestation age (GA) was 20.1 (ranging, 17-26) weeks. The largest indication for testing is positive serum screening at 48.0%. This is followed by advanced maternal age at 41.0%, personal or family history at 4.8%, and ultrasound findings at 3.9% not suitable for invasive tests at 2.3%, respectively (Table 1).

In this study, 22 cases of trisomy 21, 8 cases of trisomy 18 and 6 cases of trisomy 13 were identified by NIPT. The remains showed low-risk pregnancy (Table 2). Patients with a positive NIPT result were advised to undergo invasive testing for a prenatal karyotyping analysis. Karyotyping diagnostic tests confirmed 22 cases of trisomy 21, 8 of trisomy 18 and 5 of trisomy 13, while one case of trisomy 13 detected by NIPT showed negative karyotyping analysis. Further analysis revealed that this case of pregnancy had maternal chromosome duplication on chromosome 13. This result suggested that NIPT is a reliable approach in clinical practice under a strict protocol and quality control.

Beyond chromosome aneuploidy, NIPT also could detect genomic microdeletion or microduplication although sensitivity and specificity are still unclear. In this research, four cases of subchromosome abnormalities were detected by NIPT (Figure 1). Three cases were further confirmed by aCGH although cytogenetic analysis showed negative results. The fourth cases showed positive results both in cytogenetic analysis and aCGH analysis (Figure 2). Further aCGH analysis showed that two cases of subchromosome abnormality were from maternal and two cases were from fetal.

Table 1. Characteristic of the patients in our cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA (years) (mean, range)</td>
<td>32 (20-44)</td>
</tr>
<tr>
<td>GA (weeks) (mean, range)</td>
<td>20.1 (17-26)</td>
</tr>
<tr>
<td>Serum biochemical Screening (High risk)</td>
<td>2006 (48.0%)</td>
</tr>
<tr>
<td>Maternal age (≥35)</td>
<td>1714 (41.0%)</td>
</tr>
<tr>
<td>Personal/family reason</td>
<td>201 (4.8%)</td>
</tr>
<tr>
<td>Ultrasound findings</td>
<td>163 (3.9%)</td>
</tr>
<tr>
<td>Not suitable for invasive tests</td>
<td>96 (2.3%)</td>
</tr>
</tbody>
</table>

The average maternal age of the patient in the cohort is 32 (range from 20-44) years, and the average of gestation age is 20.1 (range from 17-26) weeks. The cohort can be sorted into positive serum biochemistry screening results, advanced maternal age (≥35 years of age at the due date in singleton pregnancies), personal/family reason, ultrasound findings, and not suitable for invasive tests at 48.0%, 41.0%, 4.8%, 3.9%, and 2.3% respectively.
NIPT application in a clinical setting

Table 2. Results of the cohorts in NIPT, karyotyping and aCGH

<table>
<thead>
<tr>
<th></th>
<th>NIPT</th>
<th>Karyotyping</th>
<th>aCGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 21</td>
<td>22</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>8</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>6</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>A case with ultrasound findings</td>
<td>Negative</td>
<td>Normal</td>
<td>2 M Del on Chr 15</td>
</tr>
<tr>
<td>Microduplication</td>
<td>5 M Dup on Chr 13</td>
<td>Normal</td>
<td>1.2 M Dup on Chr 13 of maternal origin</td>
</tr>
<tr>
<td>Microduplication</td>
<td>3 M Dup on Chr 4</td>
<td>Normal</td>
<td>0.5 M Dup on Chr 4 of fetal origin</td>
</tr>
<tr>
<td>Microduplication</td>
<td>10 M Dup on Chr 15</td>
<td>46XY, dup (15) (q11.2q14)</td>
<td>11.8 M Dup on Chr 15 of fetal origin</td>
</tr>
<tr>
<td>Microduplication</td>
<td>10 M Dup on Chr 16</td>
<td>Normal</td>
<td>1.5 M Dup on Chr 16 of maternal origin</td>
</tr>
</tbody>
</table>

Both NIPT and karyotyping could effectively detect trisomy 21, trisomy 18 and trisomy 13. In addition, aCGH detected a 2 M Del on Chr 15, while negative results were identified by NIPT and karyotyping analysis in this case. Four cases of microduplication were detected by NIPT, and aCGH showed similar results. The remaining samples were not detected any anomalies by any of these three tests. -, not performed. Chr, chromosome. Dup, duplication. Del, deletion.

Figure 1. Subchromosome abnormalities were detected by NIPT. A: Showed a 5 M duplication on chromosome 13. B: Showed a 3 M duplication on chromosome 4. C: Showed a 10 M duplication on chromosome 15. D: Showed a 10 M duplication on chromosome 16. Black arrow indicates subchromosome abnormalities on each chromosome detected by NIPT.
NIPT application in a clinical setting

In this study, there is a pregnant woman who received her negative NIPT report. With increasing gestation weeks, abnormal ultrasound of the fetus was found. Further invasive test was suggested to this patient. After the pregnant agreed to take invasive test, karyotyping analysis showed negative result, while aCGH analysis of amniotic fluid showed a 2 M small deletion on chromosome 15 (Table 2), which was likely related to the fetal phenotype. Therefore, NIPT is not the best option for subtle anomalies in current. And post-test genetic counseling in clinical is essential even when a negative NIPT result was obtained.

So far, fetal outcome of the NIPT-negative cases was ascertained in 3474 and NIPT-positive cases in 36, collectively accounting for 83.97% of the cohort. Overall, the positive rate was 0.53%, 0.19%, and 0.14% for trisomy 21, 18 and 13 respectively. Because one case of trisomy 13 was not confirmed, the false positive rate (FPR) was evaluated as 0.02% in detecting chromosome aneuploidy. The remains are still followed up in progress in current.

Taken together, our data suggested that NIPT is a powerful tool in chromosome trisomy and has much potential in subchromosome abnormalities. But it is still a screening tool and should not be used as a diagnostic method. To be noted, post-test genetic counseling in clinical utilization of NIPT must be provided to pregnant women.

Acknowledgements

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Disclosure of conflict of interest

None.

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References


Figure 2. Copy number variations were examined by aCGH. A: Confirmed a 1.2 M duplication on chromosome 13. B: Confirmed a 0.5 M duplication on chromosome 4. C: Confirmed a 1.8 M duplication on chromosome 15. D: Confirmed a 1.5 M duplication on chromosome 16. Red arrow indicates subchromosome abnormalities on each chromosome detected by aCGH.
NIPT application in a clinical setting


