Application of fetal cell-free DNA enrichment in non-invasive prenatal screening: experience from a single center in Eastern China

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Sequencing cell-free DNA (cfDNA) from the peripheral blood of pregnant women has been successfully applied in prenatal screening for fetal chromosomal aneuploidies, known as non-invasive prenatal screening (NIPS).[1,2] With large-scale clinical practices, it is recognized that the accuracy and reliability of this method largely relies on the proportion of fetal cfDNA presenting in total cfDNA from maternal plasma, known as the fetal fraction.[3]

Previously, we reported a fetal cfDNA enrichment method to increase the fetal fraction for NIPS, reducing “no call” and false negative results.[4] However, the performance of this method has not been assessed in an actual prenatal screening setting. Additionally, it remains unclear whether fetal fraction-associated factors, such as maternal body mass index (BMI) and gestational age (GA), affect the efficacy of fetal cfDNA enrichment.

In this study, we compared the original NIPS method and NIPS with fetal cfDNA enrichment in parallel in a clinical setting. This study was approved by the Institutional Review Board of the Nanjing Maternity and Child Health Care Hospital (2018FYYL007). A total of 3599 singleton pregnancies, confirmed by ultrasound reports, having NIPS in the Medical Genetics Center in the Nanjing Maternity and Child Health Care Hospital were recruited from May 2018 to December 2018. The median maternal age and GA were 29 years and 17.6 weeks, respectively. The median maternal BMI was 21.8 kg/m². Informed written consent was obtained from all pregnant women before blood collection. All plasma samples were aliquoted. Both the original NIPS method and NIPS with fetal cfDNA enrichment were used in parallel to test each sample.

The procedures of NIPS and cfDNA enrichment were similar to those described in our previous report.[4] A “no call” result was defined as failure to generate a reportable result because of a low fetal fraction or a “grey zone” of z-score for a blood sample. All cases were followed-up based on prenatal diagnostic test or newborn physical test results, from either medical records or telephone interviews. Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR). Differences between the fetal fraction with and without enrichment were compared using the paired Student’s t-test and Chi-square test. Differences among the maternal BMI and GA groups were calculated using one-way analysis of variance (ANOVA). The associations between fold change of the fetal fraction and GA, and fold change of the fetal fraction and maternal BMI, were determined by linear regression analysis. All tests were two-tailed and a P value of 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 19.0 (IBM Corp., Armonk, NY, USA).

Seventy-five cases were excluded from calculation of testing parameters because they lacked follow-up information. The original NIPS method showed high risk results for trisomies and 17 “no call” results, with a sensitivity of 100.0% and specificity of 99.97% for common trisomies. The NIPS with fetal cfDNA enrichment returned 10 high risk for trisomies and 2 “no call” results, with a sensitivity of 100.0% and specificity of 99.95%. These results revealed a significantly reduced “no call” rate when using NIPS with fetal cfDNA enrichment compared with no enrichment (0.06% vs. 0.47%, P < 0.001).

To accurately determine the effect of fetal cfDNA enrichment by the size-selection method, we focused on 1800 pregnancies with male fetuses, of which the fetal fractions can be precisely calculated using the Y-chromosome cfDNA ratio. We found that the average fetal fraction was significantly increased by approximately...
1.8-fold using the enrichment procedure (10.18 ± 3.77% vs. 17.52 ± 5.85%, P < 0.001) [Supplementary Figure S1, http://links.lww.com/CM9/A339]. To further explore the relationship between the efficacy of cfDNA enrichment (fold change of the fetal fraction) and GA, we used linear regression analysis, which revealed a positive correlation between GA and the fetal fraction from either the original NIPS or NIPS with cfDNA enrichment results. However, there was no significant correlation between fold change of the fetal fraction and GA (P = 0.950) [Figure 1A].

Figure 1: Relationship between the increased level of fetal fraction after enrichment and fetal fraction associated factors. (A) There was no correlation between fold change of the fetal fraction after enrichment and gestational age (P = 0.950). (B) Fold change of the fetal fraction after enrichment in groups with different ranges of gestational age. The average fetal fraction increase in groups of gestational age at 12.0–14.0, 14.1–21.0 and 21.1–26.4 weeks were 1.70-, 1.76- and 1.68-fold, respectively. Fold change of the fetal fraction after enrichment was significantly higher in the 14.1–21 weeks group compared with the 12.0–14.0 weeks (P = 0.016) and 21.1–26.4 weeks (P < 0.001) groups. There was no significant difference (P = 0.766) between those of 12.0–14.0 weeks and 21.1–26.4 weeks groups. (C) There was a significant positive correlation between fold change of the fetal fraction after enrichment and maternal body mass index (BMI) (P < 0.0001). Fold change of the fetal fraction reduced by 0.017% for every BMI increase of per 1 kg/m². (D) Fold change of the fetal fraction after enrichment in groups with different ranges of maternal BMI. The average increases of the fetal fraction in the maternal BMI ≤ 18.4, 18.5–24.9, 25.0–29.9 and ≥30 kg/m² groups were 1.69-, 1.75-, 1.80- and 1.84-fold, respectively. There were significant differences in fold changes of the fetal fraction between the low weight and overweight groups (P < 0.001), the low weight and obese groups (P = 0.012), as well as the normal weight and overweight groups (P < 0.001). There were no significant differences between the low weight and normal weight groups (P = 0.066), normal weight and obese groups (P = 0.150), and overweight and obese groups (P = 0.900).
Interestingly, analysis of subgroups of cases with different ranges of GA revealed a significantly higher fold change in the fetal fraction of the 14.1 to 21.0 weeks GA group compared with the 12.0–14.0 (P = 0.016) and 21.1–26.4 (P < 0.001) weeks GA groups [Figure 1B].

Analysis of the effect of maternal BMI on the efficacy of cfDNA enrichment found that the fetal fraction was negatively correlated with maternal BMI, for both the original NIPS and NIPS with fetal cfDNA enrichment methods. Additionally, we found a positive correlation between the increase in the fetal fraction and maternal BMI (P < 0.001) [Figure 1C]. Analysis of subgroups of cases with different ranges of BMI revealed that fold change of the fetal fraction after fetal cfDNA enrichment was significantly lower in the low weight group compared with the overweight (P < 0.001) and obese (P = 0.012) groups, and was significantly lower in the normal weight group compared with the overweight group (P < 0.001) [Figure 1D].

Previous reports regarding the performance of NIPS with fetal cfDNA enrichment were mostly based on retrospective results.[5,6] Here, we used 3599 samples to compare the original NIPS and NIPS with fetal cfDNA enrichment methods in parallel in a clinical setting to assess the clinical performance of this new NIPS method. We found that the increased fetal fraction after cfDNA enrichment was positively correlated with maternal BMI. A possible explanation was that our size-selection method might provide a better enrichment effect when reducing the elevated maternal cfDNA found in obese women. More importantly, our results indicated that NIPS with fetal cfDNA enrichment may be preferable for testing pregnant women with a high BMI.

GA has been reported to have a positive correlation with the fetal fraction. Our results found that the fold change in fetal fraction did not exhibit a linear correlation with GA. Notably, the increase in the fetal fraction was significantly higher in the 14.1–21.0 weeks GA group compared with the 12.0–14.0 and 21.1–26.4 weeks GA groups. This relationship revealed that improved fetal cfDNA enrichment resulted from pregnancy at 14.0 to 21.0 weeks GA, suggesting this may represent an appropriate GA window for NIPS with fetal cfDNA enrichment, especially for those with an initial NIPS failure because of a low fetal fraction during early gestation.

In conclusion, this study demonstrated the feasibility of the new NIPS method in clinical practice. Additionally, our analyses revealed that the fold change of the fetal fraction after fetal cfDNA enrichment had a positive linear correlation with maternal BMI, but not GA. Furthermore, we demonstrated that a better efficacy of fetal cfDNA enrichment could be achieved with pregnant women with a higher BMI and an appropriate GA window.

**Funding**
The study was supported by grants from the National Key R&D Program of China (No. 2018YFC1002402), Nanjing Outstanding Youth Grant for Medical Science and Technology (No. JQX18008), and National Natural Science Foundation of China (No. 81770236).

**Conflicts of interest**
None.

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**How to cite this article:** Lin Y, Liang D, Hu P, Li H, Luo CY, Xu ZF. Application of fetal cell-free DNA enrichment in non-invasive prenatal screening: experience from a single center in Eastern China. Chin Med J 2021;134:104–106. doi: 10.1097/CM9.0000000000011112