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Original Article

The influence of fetal gender and maternal characteristics on fetal cell-free DNA in maternal plasma

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ABSTRACT

Objective: To examine the possible effects of fetal gender and maternal characteristics on concentration of fetal cell-free DNA (cfDNA).
Methods: Maternal plasma that collected from 2638 singleton pregnancies women were analyzed using non-invasive prenatal testing for aneuploidy by next generation sequence technology. The effects of fetal gender and maternal BMI on fetal cfDNA was measured by Pearson correlation analysis.
Results: The proportion of fetal cfDNA was positively correlated with gestational age (regression equation: $Y = 16.2483 + 6.8856X$, $r = 0.1660$, $p < 0.0001$); and negatively correlated with BMI (Body Mass Index) (regression equation: $Y = 25.6342 - 19.0065X$, $r = -0.2146$, $p < 0.0001$); Concentration of female fetal cfDNA (mean fetal cfDNA is 13.07%, $p < 0.0001$) is higher than male fetal cfDNA (mean fetal cfDNA is 8.37%, $p < 0.0001$).
Conclusions: Fetal cfDNA increases stably between 12 and 20 weeks of gestation, and increases in a higher rate after 20 weeks. The maternal BMI is an important factor affecting fetal cfDNA, should be paid enough attention in clinical application.

Introduction

In 1997, Lo et al. [1] found the existence of fetal cell-free DNA (cfDNA) in the maternal plasma. They used real-time quantitative PCR to successfully amplify the Y-chromosome-specific sequence (SRY gene sequence) of male fetus from the total cfDNA of maternal plasma. It was first confirmed that cfDNA can spread to the maternal peripheral blood circulation. Although the existence of cfDNA has been confirmed by many researches, but their release mechanisms have not been completely clarified. In general, three release mechanisms are considered [2]: First, fetal nucleated cells (lymphocytes, granulocytes and nucleated erythrocytes etc.) undergo apoptosis and release cfDNA after entering maternal peripheral blood. Second, cfDNA enters maternal blood through the maternal-fetal interface. The concentration of cfDNA in amniotic fluid is almost 200 times higher than maternal plasma, and it is speculated that the concentration gradient may lead to direct transmission of DNA molecules. Third, cfDNA is released after apoptosis of placental trophoblast cells. This statement has received more experimental support. Go et al. [3] discovered the existence of chromosome 21-specific mRNA fragment LOC90625 in maternal plasma, which provides a possibility to detect fetal aneuploidy disease using high-throughput sequencing technology.

About 5 million defective children are born every year in the world, about one million new birth defects are added every year in China [4]. 70% of major birth defects are caused by genetic factors. The main causes of these hereditary diseases are chromosomal abnormalities and genetic mutations [5]. Aneuploidy abnormalities account for 95% of chromosomal abnormalities in live infants. There is no effective treatment, even the cause of the disease is unknown. Prenatal diagnosis is one of the main means to avoid birth defects [6,7]. Traditional prenatal diagnostic techniques rely on invasive procedures to obtain fetal genetic material, which have potential risks to both pregnant women and fetuses. With the development of medical genetics and molecular biology technologies, prenatal diagnostic technologies continue to develop in the directions of rapid, early and noninvasive. In recent years, non-invasive prenatal testing (NIPT) has been alternative choice for prenatal diagnosis.

There are many factors affect the accuracy of NIPT detection, mainly the concentration of fetal cfDNA. Low fetal cfDNA proportion resulting in a higher false negative rate and failure result [8]. To ensure the accuracy of the test results, it is very important to study the influencing factors of fetal cfDNA concentration.

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Methods

This study has been approved by the Ethics Committee of Jiayin International Hospital, and all participated pregnant women signed informed consent and voluntarily participated in this study.

Subjects: From 1 January 2017 to 30 June 2018, 2638 maternal plasma samples were collected for NIPT testing. Recruitment criteria are as follows: (1) at least 12 weeks gestation. (2) There were no clear chromosomal abnormalities in both spouses. (3) Pregnant women did not receive allogeneic blood transfusion, transplantation, cell therapy or immunotherapy in last one year. (4) After exclusion of abortion and loss of contact, the rest of 2202 cases are confirmed by amniocentesis and followed-up after delivery.

Preparation of samples: Fetal DNA was extracted with DNA Extraction Kit (Guangzhou Darui Co., Ltd.) from maternal plasma which was isolated via centrifugation. Sequencing library was constructed using Ion Xpress Plus Fragment Library Kit (Life Technologies), each library has a unique tag that distinguish from other samples. The constructed library was quantified using Qubit-iT dsDNA HS Assay Kit. The library samples were quantitatively mixed into a chip for sequencing on the Ion Proton instrumentation according to the manufacturer's instructions. The data from each sample is required to be more than 3 M, which ensures that the average sequencing depth is 0.1X.

data analysis: All sequencing data were compared to the human genome reference sequence (NCBI Build37/hg19) by TMAP software, and the results performed deduplication analysis using BamDuplicates command from the DA8600 instrument. Mapping Quality Scores (MAPQ) is greater than 10 and sequence length is greater than 35 bp as the unique matching sequence, and the obtained matching sequence is subjected to GC correction using local weighted regression method, finally calculated the percentage of Y chromosomes. The ratio of fetal cfDNA is approximately equal to the percentage of Y chromosome. $p < 0.05$ was statistically significant.

Results

We obtained 2202 plasma samples from singleton pregnancy patients. Maternal and fetal characteristics are shown in Table 1. The mean fetal fraction in the total population was 10.26% (range: 1.95–25.87%), while mean gestational age 16.95 (range: 12–26), mean maternal age 31.1 (range: 17–55), mean BMI 23.7 (range: 15.6–39.7). The statistical analysis demonstrated that proportion of fetal cfDNA was positively associated with gestational age (regression equation: Y = 16.2483 + 6.8856X, r = 0.1660, $p < 0.0001$) (Table 2).

Samples grouped by the World Health Organization (WHO) standard for Asian BMI. The data analysis shown that significant negative correlation between fetal cfDNA and maternal BMI (regression equation: Y = 25.6342–19.0065X, r = −0.2146, $p < 0.0001$) (Table 3). Approximately 1.6% samples of this study failed in detection because of fetal fraction less than 4%. The patients who had a failed test have common features—younger gestational age or higher BMI. About 3.9 weeks after the first blood draw, 35 patients underwent a second blood draw resulted in greater than 4% fetal percent on the second blood draw. One woman underwent a third blood draw at 24 weeks 6 day, who caring a male fetus and with BMI 39, and obtained sufficient fetal cfDNA. It suggests that low cfDNA concentration due to higher maternal weight can be overcome by waiting for a later gestational age.

After confirmation of NIPT results by follow-up and karyotype analysis, we examined statistical relations between fetal gender and fetal cfDNA. As shown in Fig. 1, the average cfDNA of female fetuses is relatively higher than male fetuses in the same gestational weeks. The mean fetal cfDNA was 12.15% ($p = 0.0322$) in female fetuses and 7.53% ($p = 0.024$) in male fetuses at 12 weeks, 13.04% ($p = 0.0171$) and 8.32% ($p = 0.0318$) at 20 weeks respectively. The increment of cfDNA was shown stable at this gestational period, 0.08% in female fetuses per week and 0.11% in male fetuses. In this study we also found that cfDNA of male fetuses (0.78% per week, $p < 0.0001$) increased with greater trend than female fetuses (0.53% per week, $p = 0.0094$) after 20 weeks.

Table 1
Characteristics of the study samples.

Maternal Characteristics	Mean	Median (Range)
Maternal Age (Years)	31.1 ± 5.1	30 (17–55)
Maternal Weight (kg)	62.7 ± 10.1	61.5 (40–110)
Maternal Height (m)	1.625 ± 0.05	1.63 (1.45–1.85)
Maternal BMI	23.7 ± 3.6	23.1 (15.6–39.7)
Fetal Fraction (%)	10.26 ± 3.58	9.95 (1.95–25.87)
Gestational Age (weeks)	16.95 ± 2.89	16.57 (12–26)

Table 2
Distribution of fetal cell-free DNA of different gestational weeks with ≥4% fetal cell-free DNA.

Gestational weeks	n	cfDNA ($\bar{x} \pm s$,%)	cfDNA≥4% [n (%)]
≥12~<13	178	10.01±3.37	172 (96.62)
≥13~<14	144	10.44±3.74	139 (96.53)
≥14~<15	157	10.45±3.55	152 (96.81)
≥15~<16	224	9.75±3.32	220 (98.21)
≥16~<17	513	9.98±3.43	505 (98.44)
≥17~<18	303	10.38±3.80	299 (98.67)
≥18~<19	217	10.31±3.64	214 (98.62)
≥19~<20	145	10.52±3.54	144 (99.31)
≥20~<21	104	10.69±3.82	103 (99.04)
≥21~<22	50	11.95±3.46	50 (100)
≥22~<23	75	10.75±3.04	75 (100)
≥23~<24	46	11.02±3.25	46 (100)
≥24~<25	19	11.14±4.29	19 (100)
≥25~<26	16	14.00±6.01	16 (100)
≥26~<27	11	14.43±4.20	11 (100)

Table 3
Fetal cell-free DNA and proportion of pregnancies with ≥4% fetal cell-free DNA.

Body type	Maternal BMI	n	cfDNA ($\bar{x} \pm s$,%)	cfDNA≥4% [n (%)]
Thin	≥15.5~<17	14	12.49±3.98	14(100)
	≥17~<18.5	91	11.17±3.95	90(98.90)
Normal	≥18.5~<21.25	464	10.96±3.56	462(99.56)
	≥21.25~<24	737	10.51±3.47	729(98.91)
Slightly fat	≥24~<25.5	312	10.26±3.46	308(98.72)
	≥25.5~<27	218	9.38±3.48	215(98.62)
Moderate obesity	≥27~<28.5	157	9.03±3.34	150(95.54)
	≥28.5~<30	88	9.28±3.48	83(94.32)
Severe obesity	≥30~<35	106	9.01±3.62	100(94.33)
Extreme obesity	≥35~<40	15	10.17±5.13	14(93.33)

Discussion

Three possible pathways for fetal cfDNA have been demonstrated: apoptosis in placental trophoblast cells, fetal hematopoietic nucleated cells and direct transfer through maternal-fetal

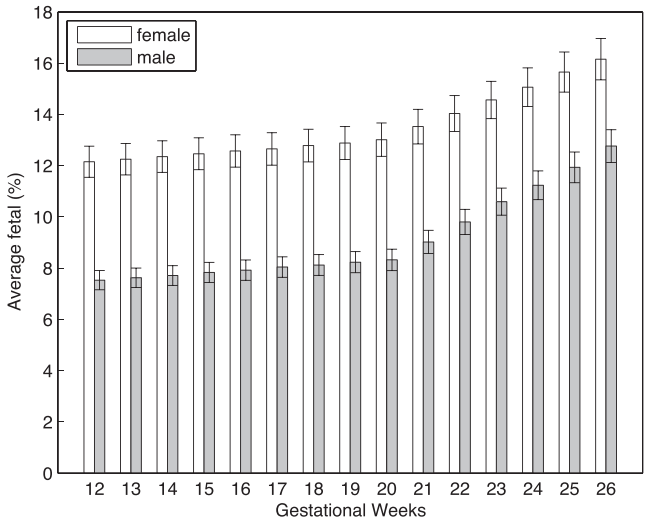

Fig. 1. Average fetal fraction of male and female fetuses.

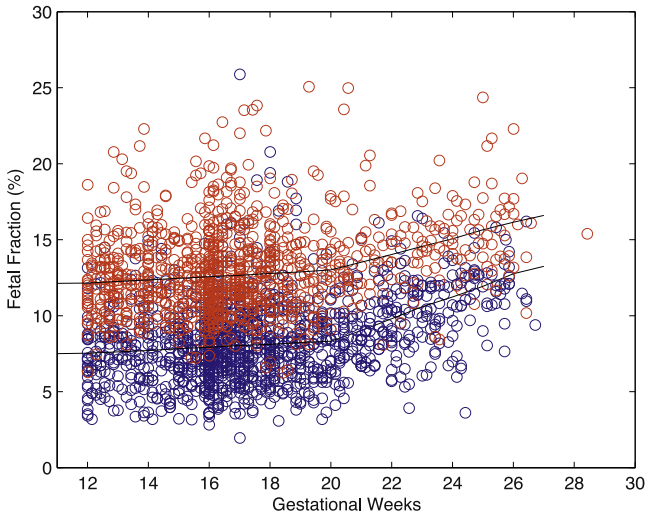

Fig. 2. Contribution of male and female fetal cfDNA. The cfDNA of female fetuses (red open circles) is relatively higher than male fetuses (blue open circles) in the same gestational weeks. From 12 weeks to 20 gestation weeks, the fetal cfDNA increased incrementally. Starting at 20 weeks of gestation, a greater weekly increase in fetal percent can be expected, especially male fetuses (0.78% per week, below line) increased with greater trend than female fetuses (0.53% per week, upper line).

interface [2]. Fetal cfDNA used in detection of fetal trisomy 2118 and 13 via NIPT [9]. Low fetal concentration lead to false positive rates or failure test [10]. Previous study reported fetal fraction in maternal plasma is associate with gestational age, maternal BMI and pregnancy-associated plasma protein A [11]. other researchers have found that significantly higher fetal fraction can be detected from pregnant women with high preeclampsia [12]. Our research result further validates these concepts.

In this study we tested 2202 single-pregnant women's plasma who is undergoing fetal aneuploidy screening for routine prenatal care. The percentage of fetal cfDNA has a strong negative correlation with maternal BMI. Despite BMI does not directly reflect body type, we also consider that increased adipocytes and blood volume dilute fetal cfDNA resulting in a decreased proportion of cfDNA in maternal peripheral blood. In clinical application, pregnant women with large BMI values may delay the blood collection during pregnancy, avoiding the fetal cfDNA concentration lower than theoretical value of high-throughput sequencing, thus requiring secondary blood draw.

Our study also examined that the fetal cfDNA have been in an uptrend but relatively stable before 20 weeks, probably due to the relatively small changes in fetal development rate and placental volume. The average cfDNA of female fetuses was higher than male fetuses at the same gestational age (Fig. 2). Probably on account of the Y chromosome is only one-third of the X chromosome. The DA8600-based high-throughput sequencing technology replicates DNA fragments by emulsion PCR, and this tiny numerical difference is infinitely magnified, corresponding proportion of male fetuses cfDNA less than female fetuses. After 20 weeks, fetal cfDNA increased rapidly, and the increment rate of male fetal cfDNA was more severe than female fetuses. There are many differences in human growth due to gender divisions. It was reported early as 1952 that, boys weighed about 100 g more than girls in natural pregnancies born [13]. Affected by genes and hormones, this difference appears in 20 weeks, and the BPD value of male fetuses was 1.7% higher per week than female fetuses ($p < 0.0001$) [14]. This distinction maybe causes the proportional difference of fetal cfDNA between male and female fetuses. This conclusion is for comparison of a large amount of data, the fetal gender cannot be judged by single sample's cfDNA. Our data provide a theoretical basis for relevant detection techniques based on fetal cfDNA. The limitation of our study is small quantity of clinical data, this conclusion requires more data to support.

Because of its high sensitivity, specificity and non-invasiveness, NIPT is gradually being accepted by more and more people. NIPT improves the detection rate of chromosome aneuploidy diseases and makes up for the deficiency of invasive prenatal diagnosis, has good clinical application value. However, NIPT also has several limitations, low fetal cfDNA concentration can cause high failure test rate and false positive/negative results. Therefore, effects of fetal DNA concentration and different maternal conditions should be considered when analyze NIPT result and make corresponding strategy adjustments and result corrections.

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References

[1] Dennis Lo YM, Corbetta N, Chamberlain PF, Vik Rai MRCOG, Sargent IL, Redman CWG, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485–7.
[2] Bianchi DW. Circulating fetal DNA: its origin and diagnostic potential—a review. Placenta 2004;25:S93–S101.
[3] Go ATJI, Visser A, Mulders MAM, Westerman BA, Blankenstein MA, Oudejans CBM, et al. First-trimester fetal MRNA of placental origin in maternal plasma. Am J Obstet Gynecol 2003;189: S111-S111.
[4] Driscoll DA, Susan G. Clinical practice. Prenatal screening for aneuploidy. N Engl J Med 2009;360:2556.
[5] Nicopoullos JDM, Gilling-Smith C, Almeida PA, Homa S, Nice L, Tempest H, et al. The role of sperm aneuploidy as a predictor of the success of intracytoplasmic sperm injection? Hum Reprod 2008;23:240.
[6] Bryndorf T, Lundsteen C, Lamb A, Christensen B, Philip J. Rapid prenatal diagnosis of chromosome aneuploidies by interphase fluorescence in situ hybridization: a one-year clinical experience with high-risk and urgent fetal and postnatal samples. Acta Obstet Gynecol Scand 2015;79:8–14.
[7] Witters I, Devriendt K, Legius E, Matthijs G, Van Schoubroeck D, Van Assche FA, et al. Rapid prenatal diagnosis of trisomy 21 in 5049 consecutive uncultured amniotic fluid samples by fluorescence in situ hybridisation (FISH). Prenat Diagn 2002;22:29–33.
[8] Sikora A, Zimmermann BG, Rusterholz C, Birri D, Kolla V, Lapaire O, et al. Detection of increased amounts of cell-free fetal DNA with short PCR amplicons. Clin Chem 2010;56:136–8.

[9] Sparks AB, Struble CA, Wang ET, Song K, Oliphant A. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. Am J Obstet Gynecol 2012;206:319 e311-319.e319.
[10] Benn P, Borell A, Chiu R, Cuckle H, Dugoff L, Faas B, et al. Position statement from the Aneuploidy Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. Prenat Diagn 2013;33:622–9.
[11] Ashoor G, Syngelaki A, Poon LC, Rezende JC, Nicolaides KH. Fetal fraction in maternal plasma cell-free DNA at 11-13 weeks' gestation: relation to maternal and fetal characteristics. Fetal Diagn Ther 2013;31:237–43.
[12] England LJ, Schisterman EF. Two-stage elevation of cell-free fetal DNA in maternal sera before onset of preeclampsia. Am J Obstet Gynecol 2004;190:707–13.
[13] Karn MN, Penrose LS. Birth weight and gestation time in relation to maternal age, parity and infant survival. Ann Hum Genet 2012;16:147–64.
[14] Persson PH, Grennert L, Gennser G. Impact of fetal and maternal factors on the normal growth of the biparietal diameter. Acta Obstet Gynecol Scand 2011;57:21–7.