Placental mosaicism for Trisomy 13: a challenge in providing the cell-free fetal DNA testing

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Abstract

Purpose We investigated the disagreement between the positive cell-free fetal DNA test for trisomy 13 and the standard cytogenetic diagnosis of one case.

Methods Cell-free fetal DNA testing was performed by massively parallel sequencing. We used conventional cytogenetic analysis to confirm the commercial cell-free fetal DNA testing. Additionally, postnatal fluorescent in situ hybridization (FISH) testing was performed on placental tissues.

Results The cell-free fetal DNA testing result was positive for trisomy 13. G-banded analysis of amniotic fluid was normal, 46, XY. FISH testing of tissues from four quadrants of the placenta demonstrated mosaicism for trisomy 13.

Conclusions A positive cell-free fetal DNA testing result may not be representative of the fetal karyotype because of placental mosaicism. Cytogenetic analysis should be performed when abnormal cell-free fetal DNA test results are obtained.

Keywords Noninvasive prenatal diagnosis · Positive cell-free fetal DNA testing · Trisomy 13 · Placental mosaicism

Introduction

Trisomy 21, Down's syndrome, occurs in 1 in 800 live births. Trisomy 13 occurs in about 1 of every 10,000 newborns, and the incidence of trisomy 18 is estimated to be 1 in 6,000 live births [1]. The detection of such fetal chromosomal aberrations is an important indication for prenatal diagnosis.

Capsule Reported one challenge case to cell-free fetal DNA testing, positive for trisomy 13 but disagree with the standard cytogenetic diagnosis results because of placental mosaicism.

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In the past decades, prenatal screening identified more than 90 % of affected fetuses. However, there is still a need for invasive testing in 3–5 % of the population [2]. These invasive tests carry a risk of miscarriage of about 0.2–0.3 % [3–5], and classical cytogenetic analysis, which is highly accurate, has the limitation of a 7–10-day turn-around time because of the requirement for cell culture [6].

Two themes have dominated recent technological advances in prenatal diagnosis: interrogation of the fetal genome in increasingly high resolution and the development of non-invasive methods of fetal testing using cell-free DNA in maternal plasma. These two areas of advancement have converged with several recent reports of non-invasive assessment [7]. Chromosome-selective sequencing of loci from chromosomes 21 and 18 in maternal plasma cell-free fetal DNA (cffDNA) has been successfully applied in non-invasive prenatal testing (NIPT) for fetal trisomy 21 and 18 [8–10]. Additionally, cffDNA testing was used to detect aneuploidy of other chromosomes, including 13, 18, 21, X, and Y [11]. Lau et al. reported that NIPT can detect a wide range of fetal, placental, and maternal chromosomal abnormalities [12].

For several decades, screening for aneuploidy with maternal serum markers and fetal ultrasound scanning has focused on increasing sensitivity while minimizing the number of invasive diagnostic tests [13]. Now, the introduction of NIPT resulted in a significant decrease in invasive diagnostic testing [14]. The use of NIPT as a replacement for current prenatal diagnosis techniques remains controversial.

Sampling of cffDNA in maternal plasma is non-invasive and does not present risk to the fetus. Additionally, the technique has opened new possibilities for non-invasive prenatal diagnosis [15]. Knowledge has advanced extremely rapidly since its use was first reported in 1997. Of the total maternal plasma DNA during pregnancy, 3.4–6.2 % was of fetal origin [16]. cffDNA in pregnant women's plasma is useful for non-invasive prenatal diagnosis. The major source of circulating cffDNA is the trophoblast derived from the embryo [17, 18].

This technique requires millions of DNA molecules to be sequenced, counted, mapped to a reference human genome, and analyzed using sophisticated bioinformatics tools. The recent application of massively parallel sequencing technology has made NIPT feasible on a scale that is suitable for large clinical trials and commercial release [7]. Non-invasive assessment of the entire fetal genome has the potential to detect rare disorders, taking prenatal diagnosis to the next level [19].

Although the test methods demonstrate excellent discrimination between euploid pregnancies and those with trisomy 13, 18, or 21, the necessity of establishing a quantitative cut-off point inevitably will result in some false-positive and false-negative results [20]. Determining the reason for false-positive or false-negative results is significant to apply and extend the use of the noninvasive cfDNA test.

In this study, we report one case that had a positive cfDNA test result for trisomy 13 that was not confirmed by conventional cytogenetic analysis of amniotic fluid. The child was a boy with a normal karyotype. The discordance between the cfDNA test and the cytogenetic tests may reflect a limitation of the positive predictive value of the testing. We investigated the cause of the conflicting test results.

Materials and methods

A 32-year-old woman had second-trimester serum integrated screening with adjusted risks for Down's syndrome of 1:240 and trisomy 18 of 1:12,000, and open neural tube defect was low risk. The patient requested a cfDNA test at 16 weeks 6 days' gestation. The result was reported as negative for trisomy 21 and 18, but it showed an increased representation of chromosome 13 materials. A normal fetal anatomic survey was obtained, and the woman opted for amniocentesis. Chromosome analysis of cultured amniotic cells showed a normal male karyotype using 60 cells from 15 different colonies. Therefore, the mother chose to continue the pregnancy and delivered a healthy male infant. The infant had a normal clinical examination, and cord blood was not taken for karyotyping. Postnatal fluorescent in situ hybridization (FISH) was used to test chromosome 13 and 21 of the cytotrophoblast from the placenta.

Prenatal screening

Sample collection and processing

Three milliliters of fasting venous blood was taken from the pregnant woman at 16 weeks of gestation. After incubation for 30 min, the sample was centrifuged for 10 min at 3,000 revolutions/min. The course should be completed within 2 h and the hemolytic specimen should be removed.

Serum analysis and risk assessment

The serum samples were separated within 24 h of collection. Triple markers (α -fetoprotein, free β -human chorionic gonadotropin, and unconjugated estriol) were measured using an Auto-MDELFIA analyzer platform (Perkin Elmer Inc., Turku, Finland). In combination with gestational weeks, weight, and age, we evaluated the risk with Life Cycle software (Life Cycle™ Perkin Elmer Inc., Waltham, MA, USA). The screen positive cut-off value was set at 1/270 (T21) and 1/350 (T18) for the option of amniocentesis.

Non-invasive prenatal diagnosis by massively parallel sequencing

A maternal peripheral venous blood sample (5 mL) was collected into Streck cell-free DNA Blood Collection Tube, and the sample was not hemolyzed. After incubation for 30 min, the sample was sent to Beijing BerryGenomics Co., Ltd. (China), by overnight courier while being kept frozen on dry ice. We performed all subsequent analyses at the company.

The indications for testing that are provided by BerryGenomics Co., Ltd., are as follows.

Gestational age: 12–26 weeks. Testing is currently available for women with advanced maternal age, personal or family history of chromosome abnormality, fetal ultrasound with findings that are associated with an increased risk for aneuploidy, or positive screening test result (www.berrygenomics.com).

Cytogenetic analysis for detection of aneuploidy

Chromosomal analyses of amniocentesis samples were carried out using standard protocols. Amniotic fluid samples were cultured in the AmnioMAX-C100 culture medium (Invitrogen, Carlsbad, CA, USA). Metaphase chromosomes were stained using the Giemsa-trypsin-Giemsa (GTG) banding method. Twenty metaphases were analyzed, and karyotypes were described in accordance with International System for Human Cytogenetic Nomenclature 2009.

FISH detection of placental tissues

FISH analysis [21–23] was performed on placental tissues.

Preparation of placental tissues and probes

Tissues (0.5 g) were obtained from each of 4 quadrants of the placenta. Samples were minced and put into centrifuge tubes.

Commercially available FISH probes specific for aneuploidies of chromosomes 13 and 21 were used in this study. Probes for chromosomes 13 and 21 were procured from GP Medical Technologies (Beijing, China).

Quantitative analysis

On the basis of FISH scoring results, the samples were considered to be informative normal and abnormal. Informative normal samples were defined as samples in which ≥ 80 % of all nuclei spread from each autosomal hybridization demonstrated 2 signals. Informative mosaic samples were defined as those in which >20 % of the nuclei spreads had a variation in signal number from the majority.

Results

We used high throughput DNA sequencing of cfDNA as an NIPT to investigate aneuploidy of chromosomes 13, 18, and 21 (Table 1). The estimated value for chromosome 13 was outside the normal reference value, indicating that the fetal karyotype included trisomy 13.

Next, we used cytogenetic analysis of cultured amniocytes to verify the high throughput DNA sequencing results. In contrast to the sequencing method, this analysis revealed a karyotype of 46, XY (Fig. 1).

Finally, we used FISH analysis of tissue from quadrants of the placenta. Three chromosome 13 signals were evident in 2 of the quadrants, and 2 signals were evident in the other two quadrants of the placenta, and the percent normal and abnormal signals are 75 % and 25 %, respectively. This indicated mosaicism for trisomy 13 in 2 quadrants and a normal karyotype in the other two (Fig. 2).

Discussion

A reliable and convenient method for non-invasive prenatal diagnosis has long been sought to reduce the risk of miscarriage and allow earlier testing. Recently, strategies were developed for the non-invasive diagnosis of fetal aneuploidy (trisomies 13, 18, and 21) by fetal nucleic acid analysis in

maternal plasma [24, 25]. Validation studies in high-risk patients who have undergone non-invasive diagnostic testing showed a sensitivity of >98 % for the detection of trisomy 21 and a remarkably low false-positive rate (<0.5 %) [9, 26, 27].

We realize there are different methodologies for NIPT, including shotgun massively parallel sequencing, targeted massively parallel sequencing, single nucleotide polymorphism-based approaches, and methylated DNA-based approaches [28]. The most common technique that is currently used to detect and identify specific cfDNA sequences is polymerase chain reaction (PCR) and its variants (nested PCR, real-time PCR, and digital PCR) combined with next-generation DNA sequencing technology [2]. There is less confidence in NIPT as a screen for trisomy 13 because of technical issues and the infrequency of the condition [29].

There is report that non-invasive prenatal diagnosis of trisomy 13 by maternal plasma DNA sequencing is achievable with low false-positive rates [19]. Others reported that non-invasive diagnostic testing had a somewhat lower sensitivity for detecting trisomy 13 [27, 30], and achieving the level that was obtained for trisomy 21 is difficult [31].

In our study, the cfDNA test results indicated an increased representation of chromosome 13 material (Table 1), but these results conflicted with those of amniotic fluid cytogenetic analysis, revealing an interesting result. Post-natal FISH tests of tissues from quadrants of the placenta demonstrated mosaicism for trisomy 13 in two of the quadrants and a normal karyotype in the other two (Fig. 2). This result is not concordant with the standard prenatal cytogenetic diagnosis.

The observations in clinical practice of several cases of positive cfDNA tests for trisomy 13 that were not confirmed by cytogenetic testing of the pregnancy may reflect a limitation of the positive predictive value of this testing. Other mechanisms, such as a low percentage of mosaicism or confined placental mosaicism, might also lead to positive cfDNA testing results [20, 32]. Therefore, cfDNA testing can lead to incorrect results that are not representative of the fetus.

The positive predictive value (i.e., the proportion of positive test results that are true positives) has improved substantially, but it remains less than ideal. Now, the origin of the cfDNA remains uncertain. Evidence suggests that placental trophoblast cells are the principal source [33–37]. Previous studies reported that confined placental mosaicism is present in human conceptions [38, 39]. It occurs in approximately 1–3 % of viable pregnancies according to chorionic villus sampling. The great majority of pregnancies with confined placental mosaicism proceed uneventfully, resulting in normal liveborn infants [40–44].

Table 1 Non-invasive prenatal diagnosis of chromosome aneuploidy

Chromosome	Estimated value	Normal reference range ^a	Interpretation
13	3.45	−3.0–3.0	Trisomy
18	−1.33	−3.0–3.0	No obvious abnormality
21	1.19	−3.0–3.0	No obvious abnormality

^a Of the samples that have an abnormality, >99 % will have an estimated value that falls outside the normal reference range provided by Beijing BerryGenomics Co., Ltd. (www.berrygenomics.com)

Fig. 1 Cytogenetic analysis of cultured amniocytes with the G-banding karyotype 46, XY. This showed that the fetus had a normal karyotype

cffDNA testing is associated with some challenges, and the different types of confined placental mosaicism may have different impacts on the release of cffDNA [45]. The high sensitivity of cffDNA testing and the low false-positive rate has led some investigators to speculate about the possibility of cffDNA replacing diagnostic testing in the future [46, 47]. However, we know that the testing should solve the problem of false-positive results before it can be used in the general population of low-risk obstetric patients.

Conclusions

In conclusion, we demonstrated that a positive cffDNA test result does not ensure an affected pregnancy because of placental mosaicism. This experience reinforces the importance of offering invasive testing to confirm cffDNA results before parental decision making. Understanding the mechanism and frequency of false-positive and false-negative cases will be important before the cffDNA test is offered widely to the general population of low-risk obstetric patients.

Fig. 2 Fluorescent in situ hybridization (FISH) results of placental tissue (green, chromosome 13; red, chromosome 21). A: Normal karyotype with 2 chromosome 13 signals and 2 chromosome 21 signals; B: karyotype with trisomy 13

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