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Understanding the Limitations of Circulating Cell Free Fetal DNA: An Example of Two Unique Cases

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Abstract

Circulating cell free fetal DNA (cffDNA) is an effective screening modality for fetal aneuploidy. We report two cases of false positive results. The first case involves a female, with self-reported Down syndrome. CffDNA returned positive for trisomy 18 leading to a maternal diagnosis of mosaicism chromosome 18 with normal fetal karyotype. The second case involves a patient with an anomalous fetal ultrasound and cffDNA positive for trisomy 13. Amniocentesis demonstrated a chromosome 8p duplication/deletion. False positive cffDNA may arise in clinical scenarios where diagnostic testing is clearly indicated. Practitioners should recognize the limitations of cffDNA.

Keywords

Prenatal diagnosis; Cell free fetal DNA; Fetal anomalies

Introduction

Since Lo et al first published their work regarding the ability of massively parallel sequencing (MPS) of circulating cell free fetal DNA (cffDNA) to detect autosomal trisomies, multiple publications have confirmed the high sensitivity and specificity of this technique. Traditional prenatal screening methods provide detection rates of fetal aneuploidy between 85% and 90% with screen positive rates of up to 5% [1]. With a false positive rate of less than 1%, integrating cffDNA into current screening algorithms has the potential to decrease the amount of invasive procedures resulting from false positives associated with serum and ultrasound screening [2].

The technique extracts cffDNA from the maternal plasma. Z-scores are then calculated measuring the relative amount of chromosome fragments from the sample in comparison to expected values for non-aneuploidy pregnancies. For this principle to hold true, the maternal karyotype must be normal, since MPS does not distinguish between fetal and maternal DNA fragments. Therefore, in the presence of a maternal trisomy, one would expect to see an

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increased proportion of fragmented DNA for the affected chromosome even with a normal fetus.

Additionally, when anomalies are encountered on ultrasound, the standard of care is to offer diagnostic testing consisting of chorionic villus sampling or amniocentesis. The use of cffDNA in either instance can lead to both false positive or false negative results.

Case Report

We present two cases of false positive cffDNA testing. The first case is a patient in whom a false positive MPS result of a fetal trisomy 18 led to the diagnosis of a maternal mosaic with ring chromosome. The second case is a fetus with complex abnormalities on anatomy ultrasound, with a false positive MPS result of a fetal trisomy 13 leading to the diagnosis of a terminal deletion and interstitial duplication of chromosome 8.

Case 1

A 23-year-old African American, primiparous female was referred at 16 weeks EGA for consultation with maternal fetal medicine because of a personal history of mental retardation. The patient stated that she herself had been diagnosed with Down syndrome on day 4 of life. On physical exam, the patient lacked the classic stigmata of trisomy 21, such as simian creases, a flat nasal bridge, or epicanthal folds [3].

The patient returned for a sonographic anatomic survey and genetic counseling at 19 weeks. With the exception of bilateral renal pylectasis, the fetal anatomic survey was within normal limits. The patient provided a copy of her karyotype, completed 23 years ago, which reported trisomy 21. After review of the karyotype, an amniocentesis for definitive diagnosis was offered and declined. Following extensive counseling, the patient opted to use cffDNA as a screening tool for her fetus; she had been given a potential 50% risk for fetal Down syndrome based on her own karyotype. The cffDNA screened positive for trisomy 18, an unexpected result in the presence of an essentially normal anatomy scan and a presumed maternal karyotype of trisomy 21. Given the significance of trisomy 18, the patient was reoffered an amniocentesis for definitive diagnosis, which was accepted. The results of the fetal karyotype via FISH and microarray were normal.

The patient was offered a repeat personal karyotype utilizing advances in technology made over the past 20 years. The patient's karyotype, utilizing a combination of cytogenetics and single nucleotide polymorphisms microarray demonstrated 35% mosaicism of a supernumerary ring derived from chromosome 18, thus explaining the initial cffDNA result of trisomy 18.

Case 2

A 26-year-old African American gravida four, para three was found to have multiple fetal anomalies on her 20-week anatomy ultrasound, including a inferior vermian hypoplasia, two vessel umbilical cord and a ventricular septal defect. The patient's past medical history and prenatal exposures were unremarkable. After genetic counseling, the patient opted for noninvasive fetal testing utilizing cffDNA, which returned positive for trisomy 13.

The patient underwent fetal MRI, which demonstrated an inferior vermian hypoplasia, a thin appearing corpus callosum, small cerebellum, two vessel cord, polyhydramnios (AFI 30) and mild pyelectasis. After identification of the additional findings, the patient agreed to amniocentesis with reflex to microarray. The initial karyotype demonstrated an apparent duplication of the short arm of chromosome 8. The microarray demonstrated an 8.10 MB terminal deletion of 8pTER to 8p23.1 and a 31.8 MB interstitial duplication of 8p23.1 to 8p11.1. Previously reported cases in this region include anomalies of the central nervous system, renal and cardiac systems [4].Patients with this duplication also present with severe intellectual disability and multiple minor abnormalities [5]. The patient declined further genetic testing for herself and partner and elected to continue the pregnancy.

Discussion

The above cases highlight several important elements for consideration when utilizing advanced screening methods for prenatal diagnosis. In case 1, although the patient's original diagnosis proved to be incorrect, the offering of cffDNA in the presence of a known maternal kartyotype was erroneous. Though the result of trisomy 18 generated the recommendation for amniocentesis, in the setting of an abnormal maternal karyotype, chorionic villus sampling or amniocenterisis is the appropriate test to provide a definitive diagnosis or absence of fetal anueploidy. In subsequent pregnancies, appropriate screening for fetal aneuploidy consists of first or second trimester screening, followed by chorionic villus sampling or amniocentesis should screening via ultrasound or analyte testing suggest aneuploidy.

Though the above case illustrates the incorrect application of cffDNA, in the presence of a normal ultrasound and a test result suspicious for trisomy 13 or 18, a false positive result is part of the differential diagnosis. While up to 75% of fetuses affected with trisomy 21 may lack major congenital abnormalities on, almost all pregnancies affected by trisomy 13 or 18 have demonstrated anomalies via ultrasound [6]. If a normal fetal karytope and microarray are determined via amniocentesis or chorionic villous sampling, maternal karyotype with reflex microarray may be warranted, particularly given the possibility of maternal chromosomal mosaicism. Mosacism can result in a wide range of phenotypic expression of various disorders, ranging from the complete absence of phenotypic abnormalities to severely affected individuals. This information could be of reproductive value to the patient, along with alerting the patient and her clinician that she will need definitive prenatal diagnosis in subsequent pregnancies because of the substantial risk for fetal aneuploidy.

Diagnostic testing is also indicated in the presence of fetal anomalies, especially if the anomalies do not correspond with the suspected trisomy by cffDNA. As case 2 demonstrates, fetal anomalies can result from a number of chromosomal rearrangements, duplications and deletion syndromes. There are reports of individuals with small amounts of extra 8p material that are healthy, develop normally and have healthy children [4]. The abnormalities appear to include developmental delay, learning difficulties, hypotonia, heart defect and agenesis of the corpus callosum. The effects of 8p duplications depend mostly on the genetic material repeated and the exact breakpoints [7]. Published case reports involving chromosome 8p23.1 range from large terminal deletions that are easily detectable by routine

chromosome analysis to small interstitial deletions which are best identified using molecular techniques such as array comparative genomic hybridization [8].

Currently, in the United States, cffDNA testing is validated for trisomy 21, 13, 18, 22q11.2 deletion syndrome, triploidy, sex chromosome aneuploidies and certain micro-deletions. Until the technology progresses to detect duplication/deletion syndromes or gross unbalanced chromosomal rearrangements, invasive testing with cytogenetic analysis with reflex to microarray is indicated initially. CffDNA is not an optimal substitute, particularly in the face of subtle anomalies or anomalies atypical for standard chromosome aneuploidies. If a deletion or duplication is detected, parental karyotype, possibly with microarray is also indicated to further characterize their carrier status.

While the introduction of cffDNA has dramatically reduced the number of invasive procedures, understanding the indications and limitations of noninvasive prenatal testing is central to its application and performance.

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