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CASE REPORT

Cell-free DNA testing in a trisomy 21 pregnancy with confined placental mosaicism for a cell line with trisomy for both chromosomes 18 and 21

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Key Clinical Message

NIPT (noninvasive prenatal testing) detected trisomy for two chromosomes. One trisomy reflected the fetal karyotype, and the other resulted from CPM (confined placental mosaicism). This case illustrates that extensive cytogenetic analysis can be required to identify CPM, and that patients should be counseled regarding the possibility of discordant NIPT results.

Keywords

Aneuploidy, cell-free fetal DNA, confined placental mosaicism, noninvasive prenatal testing.

Introduction

NIPT (noninvasive prenatal testing) technologies rely on massively parallel sequencing or single nucleotide polymorphism-based analysis of cell-free DNA (cfDNA) isolated from maternal plasma. The fetal fraction (cell-free fetal DNA, cffDNA), which represents approximately 10% of the cfDNA [1], has been found to be derived primarily from placental trophoblastic cells, rather than from the fetus [2]. Multiple reports have demonstrated that discrepancies between fetal genotype and NIPT results can occur as a consequence of CPM (confined placental mosaicism) for the detected aneuploidy [3–7].

Each commercial laboratory has independently defined the statistical measures by which it classifies patient results. In this case, the maternal sample was sent to Verinata (now Illumina) for the Verifi® prenatal aneuploidy test. Verinata employs a dual threshold method for test result classification. Values below the first cutoff are reported as "aneuploidy not detected," while values above the second cutoff are reported as "aneuploidy detected." Values between the two cutoffs are reported as "aneuploidy suspected." Sensitivity and specificity for this test are reportedly high: >99.9% and >99.8% for chromosome 21, and >97.4 and >99.6% for chromosome 18, respectively (Verinata). As a matter of company policy, Verinata reports only the qualitative result and does not release quantitative information about the thresholds for its tests or the patient's test values relative to the thresholds.

We present a case of trisomy 21 with CPM for a cell line with both trisomy 18 and trisomy 21, in which NIPT detected both abnormalities.

Case

A 32-year-old primigravida was referred by her local obstetrician at 17 weeks' gestation regarding positive second trimester quad screen results, in which the risk of trisomy 21 in the fetus was estimated to be 1/130. The results indicated no increased risk for either trisomy 18 or an open neural tube defect. Ultrasound examination identified a single intracardiac echogenic focus, increasing

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the risk [8] of trisomy 21 to 1/49. The patient was counseled regarding prenatal genetic testing options and declined both amniocentesis and NIPT. The patient returned to her local obstetrician for pregnancy management.

At 34 weeks, the patient was referred for further evaluation after a third trimester ultrasound revealed a fetal growth lag. After additional counseling, the patient elected to have the Verifi® NIPT. A result of “aneuploidy detected” was returned for chromosomes 18 and 21 and interpreted as consistent with trisomy for both chromosomes. The result for chromosome 13 was “aneuploidy not detected.” The patient was counseled regarding diagnostic testing options. Amniocentesis was declined.

At 38 weeks, oligohydramnios was identified by ultrasound, and labor was induced. A male infant was delivered with Apgars of 7/7 and weighing 2450 g. The newborn’s facial features, including upslanting palpebral fissures, epicanthal folds, and flat nasal bridge, were consistent with trisomy 21. There were no features concerning for trisomy 18 by physical examination. At 7 months of age, the infant was healthy and developing as expected for a child with trisomy 21.

A cord blood sample and eight placental biopsies (four from the fetal side, four from the maternal side) were taken for routine cytogenetics and FISH (fluorescence in situ hybridization) analyses. An extensive workup was performed to screen for evidence of fetal or placental mosaicism (Table 1). The cord blood sample was positive for trisomy 21 in all 50 cells analyzed by routine cytogenetics. FISH analysis was performed using probes for chromosomes 21 and 18 at the RUNX1 and BCL2 loci, respectively, in conjunction with a chromosome 12 probe (ETV6) to control for the ploidy level (Abbott Molecular, Abbott Park, Illinois, USA; Fig. 1). A total of 500 interphase cells from the cord blood sample was examined, and a fluorescence pattern consistent with trisomy 21 was observed in all. No evidence of trisomy 18 was observed.

Independent direct cytotrophoblast preparations were established from the eight placental biopsies. From each biopsy, 100 interphase cells were examined by FISH for evidence of aneuploidy for chromosomes 18 and 21. A fluorescence pattern consistent with trisomy 21 was observed in all eight specimens. A fluorescence pattern consistent with double trisomy for chromosomes 18 and 21 (48,XY,+18,+21) was observed in 49/800 cells distributed among only three of the eight biopsies. The majority (46/49) were found in one biopsy from the fetal side of the placenta. Two cells with double trisomy were identified in an additional biopsy from the fetal side, and only one cell with double trisomy was identified among the 400 cells examined from the four independent biopsy preparations from the maternal side of the placenta. There was no evidence of aneuploidy for chromosome 18 in the remaining five specimens. All 140 metaphase cells examined by routine cytogenetics from the eight biopsy sites had a trisomy 21 karyotype.

Table 1. Cell counts from interphase FISH and karyotype analysis of cord blood and placental biopsies.

Sample	Interphase FISH +21	Interphase FISH +18,+21	Cytogenetics 47,XY,+21
Cord blood	500	0	50
Placenta[1]			
Maternal side			
1	100	0	10
2	100	0	10
3	99	1	30
4	100	0	10
Fetal side			
5	54	46	30
6	100	0	10
7	98	2	30
8	100	0	10

[1]Interphase FISH performed on direct cytotrophoblast preparations; karyotyping performed on cultured whole villi.

Comment

Here, we report a pregnancy with two trisomies identified by NIPT, which were subsequently found to be due to true fetal trisomy 21 and CPM for trisomy 18. This case represents a uniquely complicated scenario for diagnosis and counseling. Nonmosaic double trisomy for chromosomes 18 and 21 has been reported in cases of early spontaneous abortion [9], but to our knowledge never in a third trimester pregnancy as in this case. The clinical

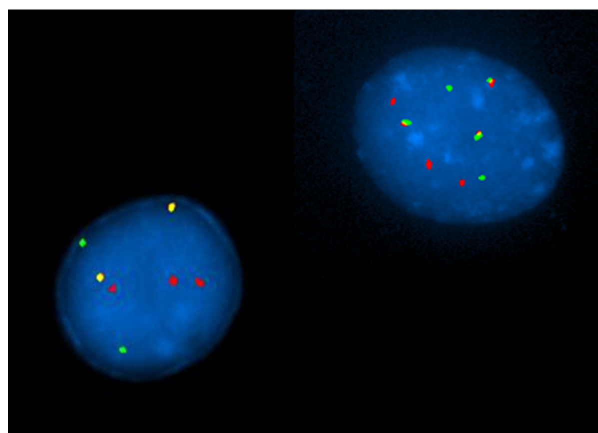

Figure 1. FISH (fluorescence in situ hybridization) probes for chromosomes 21 (red signal), 18 (yellow or fused red/green signal), and 12 (green signal) were used to identify cells with trisomy 21 (left) and both trisomy 18 and trisomy 21 (right).

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information available suggested trisomy 21 was more likely to be confirmed than trisomy 18. However, in the absence of prenatal diagnostic testing, it remained possible that either trisomy, both trisomies, or neither would be present in the fetus. Potential outcomes also included true fetal mosaicism and CPM involving one or both of the trisomies. Each of these possibilities and their likely effects on the health and development of the fetus were discussed with the patient.

This report adds to the growing body of literature documenting CPM as a common cause of discordant NIPT test results. In most previously reported cases, NIPT detected aneuploidy for a single chromosome, while the fetus was found to have either a normal chromosome complement [3, 4], or an entirely different aneuploidy [5, 7]. In the present case, NIPT results for chromosomes 18 and 21 were both reported as "aneuploidy detected." Given the average proportion of cells with evidence for trisomy 18 in the eight placental biopsies was quite low (49/800 cells, or 6.1%), but all eight samples were overwhelmingly positive for trisomy 21, it is of interest that the NIPT report did not distinguish the true trisomy 21 from the low-level CPM for trisomy 18. Assuming the relative excess cffDNA derived from chromosomes 21 and 18 is in proportion to the respective levels of trisomy identified in the placenta, the identical results for the two chromosomes may be due at least in part to the dual threshold method Verinata uses to detect aneuploidy. That is, even if the score for chromosome 18 were only marginally greater than the abnormal threshold and the score for chromosome 21 well above it, by definition both chromosomes would be reported positive for trisomy.

Alternatively, recent data suggest that cffDNA may not represent the average chromosome complement across all trophoblastic cells, but instead derives preferentially from trophoblastic cells in discrete regions of the placenta. Mao et al. [7] reported a case of fetoplacental mosaicism in which the fetus was nonmosaic for trisomy 18, and the placenta contained three cell lines: trisomy 18, trisomy 21, and euploid. NIPT was positive for trisomy 21, but failed to detect trisomy 18. Analysis of multiple placental biopsies showed highly variable proportions of the three cell lines, and the authors concluded that the cffDNA may have arisen primarily from regions of the placenta with a relative excess of trisomy 21 cells in the trophoblast. The current case is similar in that there was a highly uneven distribution of placental cells with double trisomy (ranging from 0% in five biopsies to a maximum of 46% in a single biopsy). Thus, it seems plausible that our patient's NIPT results reflect a disproportionate contribution of cffDNA from placental regions with a relatively high fraction of double trisomy cells.

Although it has been demonstrated that CPM is a relatively common contributor to discordant NIPT results, it is difficult to establish on a case-by-case basis. In the present case, trisomy 21 was present in all of the cells examined from cord blood and eight placental biopsy sites, but dual trisomy, 18 and 21, was observed in only 49 of the 940 cells examined from the eight biopsy sites and in none of the 550 cells examined from cord blood. Furthermore, the percentage of cells demonstrating dual trisomy was variable and ranged from 1% to 46% within the three positive placental biopsy sites. While it is typically not possible to assay multiple placental sites for an ongoing pregnancy, and it would be cost prohibitive to do so as extensively as done here after each birth, a thorough clinical evaluation and follow-up karyotyping and/or FISH studies should be attempted.

In summary, this report illustrates the necessity of considering the possibility of CPM following any positive NIPT result, but especially in rare cases of highly unusual findings, such as the double trisomy described here. The complicated nature of this case underscores the importance of discussing with patients the possibility of both atypical and discordant results during pretest counseling and consent.

Conflict of Interest

The authors have no conflicts of interest to disclose.

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