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CASE REPORT

Cell-free fetal DNA in the maternal circulation originates from the cytotrophoblast: proof from an unique case

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Key Clinical Message

Noninvasive prenatal testing (NIPT) and direct karyotyping of cytotrophoblast were normal for a male fetus, but cultured chorionic villus mesenchymal cells and umbilical cord fibroblasts showed nonmosaic trisomy 18. This observation provides direct evidence for the cytotrophoblastic origin of cell-free fetal DNA and yields a biological explanation for falsely reassuring NIPT results.

Keywords

Cell-free fetal DNA (cfDNA), cytotrophoblast, mesenchyme, noninvasive prenatal testing, placenta.

What is Already Known About This Topic?

Noninvasive prenatal testing (NIPT) has the same biological limitations as analysis of direct preparations of cytotrophoblast cells from chorionic villi. Published cases in which both NIPT and direct analysis of cytotrophoblast showed a 45,X karyotype in women carrying an euploid fetus proved the cytotrophoblastic origin of cfDNA, providing a biological explanation for abnormal results of NIPT not confirmed in the fetus.

What Does This Report Add?

Noninvasive prenatal testing and direct karyotyping of cytotrophoblast were normal for a male fetus, but cultured chorionic villus mesenchymal cells and umbilical cord fibroblasts showed nonmosaic trisomy 18. This observation provides direct evidence for the cytotrophoblastic origin of cfDNA and yields a biological explanation for falsely reassuring NIPT results.

Chorionic villus sampling (CVS) was performed in a 30-year-old primigravid woman at a gestational age of 13 weeks. She was referred because of an increased nuchal translucency (4.8 mm) and an omphalocele in the fetus. A direct analysis of cytotrophoblast cells of chorionic villi (short-term culture, STC) showed a 46,XY karyotype in six metaphases, whereas cultured mesenchymal core cells (long-term culture, LTC) revealed 47,XY,+18 in six metaphases. Fluorescence in situ hybridization (FISH) with the CEP 18 (locus D18Z1) probe, combined with CEP X (locus DXZ1) and CEP Y (locus DYZ3, all Abbott-Vysis probes), resulted in a 46,XY karyotype in all 73 additional metaphases from the STC and 47,XY,+18 in all 100 additional metaphases from the LTC.

Follow-up ultrasonographic imaging at 16 1/7 weeks' gestation demonstrated severe growth retardation, an omphalocele, an univentricular heart and clenched fists, all consistent with trisomy 18. The omphalocele was 33 mm in diameter and contained liver and intestines. On request of the parents the pregnancy was terminated at 17 weeks. Before induction and with the patient's

consent, 20-mL peripheral blood was drawn for NIPT and processed according to the standard protocols. Briefly, cell-free DNA was extracted from 1-mL plasma and sequenced (35 bp) on the Solid 5500xl Wildfire sequencing platform (Life Technologies, Foster City, CA, USA). Statistical analysis was performed based on the *z*-score algorithm [1] with additional GC-correction [2]. The threshold for trisomy was set at ≥3.00. A *z*-score of 2.42 was found for chromosome 18, indicating euploidy for this chromosome. The *z*-score for the Y-chromosome was 35.63, indicative of a male fetal karyotype and a relatively high fetal fraction in maternal plasma. This makes it highly unlikely that the false-negative, normal NIPT result is due to a low fetal fraction. The parents did not agree to take a fetal biopsy for cytogenetic studies but allowed investigation of umbilical cord and placenta.

In cultured umbilical cord fibroblasts, we found trisomy-18 in all 107 metaphases investigated. The placenta was biopsied at 10 evenly spaced positions at the fetal side. For each biopsy, cytotrophoblast and mesenchyme cells were separated [3] and investigated by FISH using CEP 18 combined with CEP 11 (locus D11Z2, as a control for diploidy, provided by Abbott-Vysis) and CEP Y (as a control for nonmaternal origin; only cells with a signal for the Y chromosome were scored). The results are shown in Table 1. In the cytotrophoblast, seven of 10 biopsies showed a low percentage trisomy 18 cells (average 3.6%, range 0–13%). In the other three nonadjacent biopsies, this varied from 40% to 80%. In the mesenchyme, all 10 biopsies predominantly contained trisomy 18 cells (average 74%, range 59–90%. These observations can reflect real mosaicism in both cytotrophoblast and mesenchyme (confined placental mosaicism type III), contamination with tissues of embryonic origin such as amnion (because biopsies were taken from the fetal side), or incomplete separation of cytotrophoblast and mesenchyme cells during preparation. We also performed FISH on sections (4 μm) from placental tissue, using the standard methods. FISH on formalin-fixed, paraffin-embedded tissue sections using CEP 18 showed two signals each in cytotrophoblast cells and three in about 30% of mesenchymal cells (Fig. 1). Taken together, these observations are consistent with placental mosaicism with trisomic rescue in the cytotrophoblast [4] in a trisomy 18 pregnancy.

It is generally assumed that apoptosis of villous cytotrophoblast cells, a process occurring in all pregnant

Table 1. Percentages of trisomy-18 cells in 10 placental biopsies.

	Cytotrophoblast		Mesenchyme	
Biopsy	%	*n*	%	*n*
1	0	23	59	100
2	1	100	75	100
3	2	52	88	100
4	80	60	88	100
5	44	150	86	100
6	40	154	90	90
7	13	100	69	120
8	0	100	59	100
9	1	101	65	100
10	6	52	64	100

n, number of cells investigated.

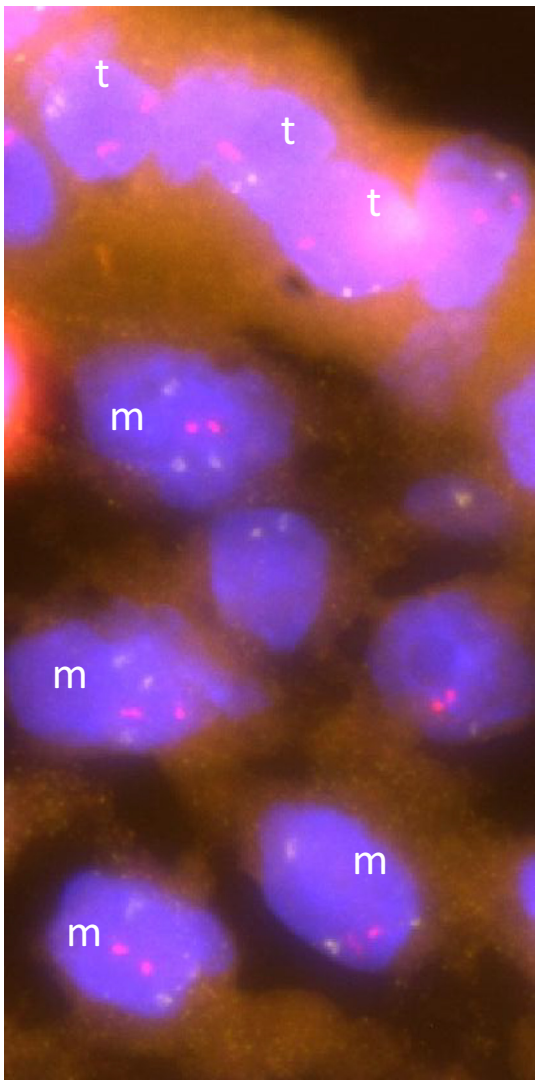

Figure 1. Formalin-fixed, paraffin-embedded tissue section of the placenta after fluorescence in situ hybridization with DNA-probes (Cytocell) specific for chromosome 21 (covering *DYRK1A*, *KCNJ6*, *DSCR4*, *DSCR8* genes, in red) and 18 (locus D18Z1, in blue) with DAPI as counterstain for DNA. Cytotrophoblastic cells (t) show two signals for chromosome 18 per nucleus, while mesenchymal cells (m) show three signals.

women, leads to the release of cfDNA into the circulation [5]. The concordance between NIPT and cytogenetic investigation of cytotrophoblast cells in our case directly demonstrates that the cfDNA in the maternal circulation is mainly, or perhaps exclusively, derived from the cytotrophoblast, in line with earlier case reports [6–8]. Similar cases of a false-negative NIPT result for trisomy 18 due to placental mosaicism were reported recently by others [9, 10]. Karyotyping of STC and LTC has enabled the accumulation of a huge body of cytogenetic knowledge on the biology of placental mosaicism [11], which will be extremely useful in the era of NIPT. It has been calculated from large series of consecutive patients undergoing CVS that false negative NIPT results involving trisomy 13, 18, or 21 are to be expected in 1/107 cases [4].

For clinical practice, our case implies that when NIPT results are normal but an ultrasonographic anomaly scan is not, karyotyping or array-based aneuploidy detection should always be offered. Vice versa, in the absence of ultrasonographic anomalies an abnormal NIPT result should always be confirmed before concluding that the fetus has an abnormal karyotype. Our case implies that NIPT using cfDNA has the same biological limitations as direct analysis of chorionic villi. Patients opting for NIPT should be made aware of this during pretest counseling. Delivering NIPT in clinical practice can benefit to a great extent from the existing knowledge on cell-line-specific mosaicism that sets limits to diagnostic accuracy.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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