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Research Letter

Prenatal diagnosis of mosaic trisomy 2: Discrepancy between molecular cytogenetic analyses of uncultured amniocytes and karyotyping of cultured amniocytes in a pregnancy with severe fetal intrauterine growth restriction

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A healthy 40-year-old, gravida 2, para 0 woman underwent amniocentesis at 18 weeks of gestation because of her advanced maternal age. Cytogenetic analysis revealed a karyotype of 46,XX in 25 colonies of cultured amniocytes. She underwent repeated amniocentesis for investigation of genomic imbalance in the fetus at 23 weeks of gestation because of intrauterine growth restriction (IUGR). The biparietal diameter was 4.9 cm (<3rd centile), the abdominal circumference was 14 cm (<3rd centile), and the femur length was 2.6 cm (<3rd centile). Oligonucleotide-based array comparative genomic hybridization (aCGH) analysis using CytoChip Oligo (BlueGnome, Cambridge, UK) array showed that uncultured amniocytes had a small genomic gain in chromosome 2, consistent with the diagnosis of trisomy 2 mosaicism, whereas cultured amniocytes did not manifest any genomic imbalance in chromosome 2 (Fig. 1). Cytogenetic analysis of the cultured amniocytes revealed a karyotype of 46,XX in all 19 observed colonies. Interphase fluorescence *in situ* hybridization study of uncultured amniocytes showed three signals of chromosome 2q11.1-specific probe (RP11-468G5) (spectrum green) in 6 of 50 cells and two signals in the remaining 44 cells, indicating 12% (6 of 50) mosaicism for trisomy 2 in uncultured amniocytes (Fig. 2). Level II ultrasound of the fetus at 24 weeks of gestation revealed severe IUGR, decreased amount of amniotic fluid, and clinodactyly of the hands. The biparietal diameter was 5.1 cm (<3rd centile), the femur length was 2.9 cm (<3rd centile), the abdominal circumference was 15.2 cm (<3rd centile), and the amniotic fluid index was 9.4 cm. At 26 weeks of gestation, a 448-g growth-restricted fetus was delivered with low-set ears, macroglossia, clenched hands, and neonatal death. Conventional cytogenetic analysis revealed karyotypes of 47,XX,+2 [15]/46,XX[35] in amnion (Fig. 3); 46,XX (60 cells) in umbilical cord; and 46,XX (100 cells) in blood. The cultures of placenta and skin were not successful. aCGH analysis showed that the skin and blood had no genomic imbalance in chromosome 2; amnion had a small genomic gain in chromosome 2, consistent with the diagnosis of mosaic trisomy 2; and placenta had a complete genomic gain in chromosome 2, consistent with the diagnosis of trisomy 2 (Fig. 1). Quantitative fluorescent polymerase chain reaction analysis of different sampling tissues using polymorphic DNA markers revealed a diallelic pattern with unequal biparental inheritance of chromosome 2 in amnion and placenta and a diallelic pattern with equal biparental inheritance of chromosome 2 in skin and umbilical cord. The tissue of placenta had a dosage ratio of 2:1 (maternal allele:-paternal allele), consistent with the diagnosis of trisomy 2, and the tissue of amnion had a dosage ratio of 1.5:1 (maternal allele:paternal allele), consistent with the diagnosis of mosaic

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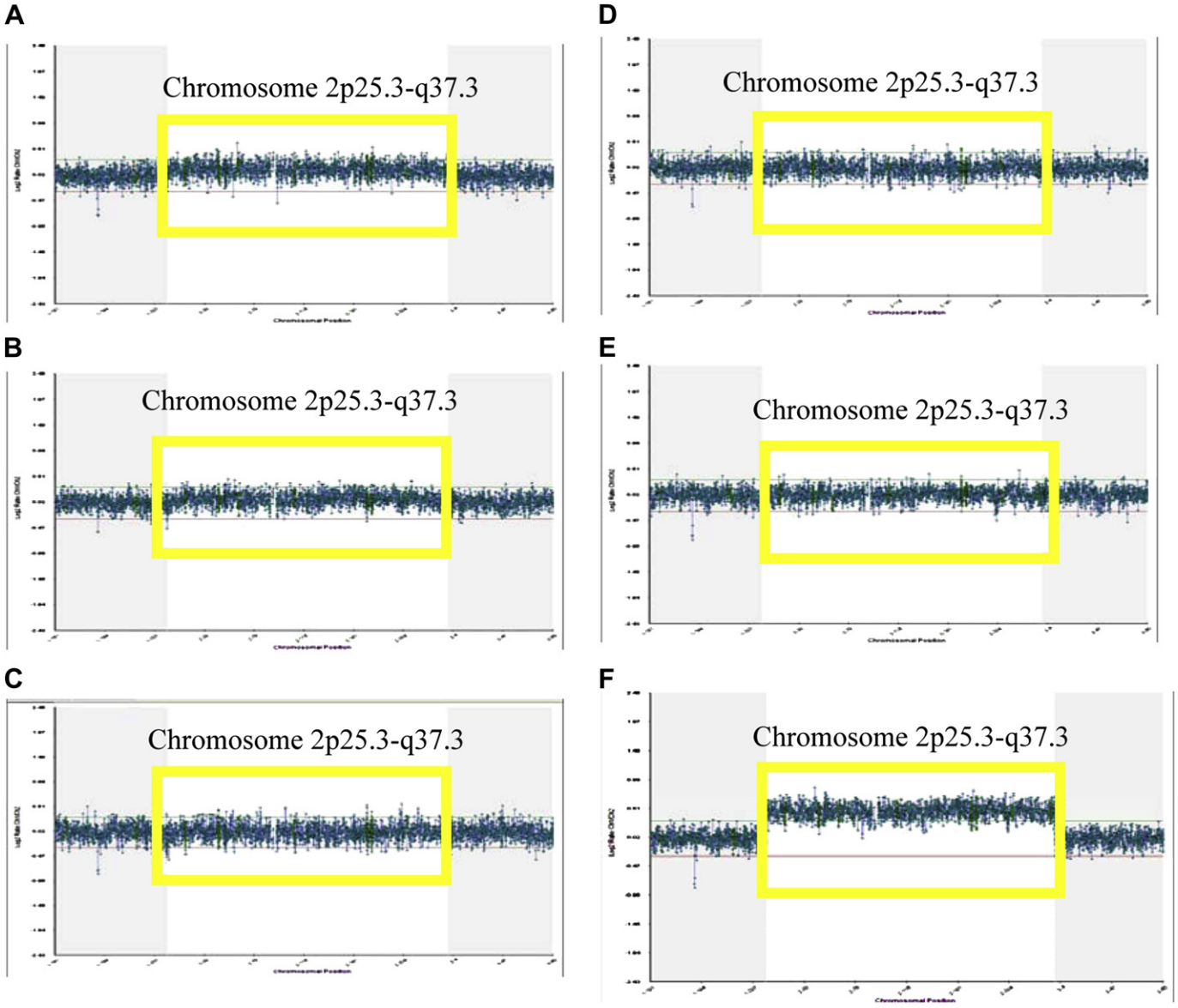

Fig. 1. Oligonucleotide-based array comparative genomic hybridization demonstrates that uncultured amniocytes (A) and amnion (B) had a small genomic gain in chromosome 2; cultured amniocytes (C), blood (D), and skin (E) do not have any genomic gain in chromosome 2; and placenta (F) had a complete gain in chromosome 2.

trisomy 2 (Fig. 4). The maternal allele dosage was greater than the paternal allele dosage, indicating a maternal origin of the trisomy 2 cell line. The results of quantitative fluorescent polymerase chain reaction excluded uniparental disomy (UPD) 2 in the fetus.

The present case provides evidence for discrepant cytogenetic findings of mosaic trisomy 2 between uncultured and cultured amniocytes, with uncultured amniocytes being positive for trisomy 2 by aCGH and interphase fluorescence *in situ* hybridization, and cultured amniocytes being negative for trisomy 2 by aCGH and conventional karyotyping. In the present case, molecular cytogenetic analyses showed that the tissues of placenta had trisomy 2, the tissues of amnion had mosaic trisomy 2, the tissues of skin and blood of the fetus had biparental disomy 2, the uncultured amniocytes had mosaic trisomy 2, and the cultured amniocytes had disomy 2. It is likely that the fetus had biparental disomy 2; the amnion had mosaic trisomy 2; the placenta had trisomy 2; and the uncultured amniocytes contained amnion epithelium-origin trisomy 2 amniocytes, which disappeared after long-term culture. Our observation was in accordance with the hypothesis of origin of amnion in mosaicism raised by Robinson et al [1]. A trisomic rescue occurs relatively late in the development, which causes a few cells of the inner cell mass to be diploid, resulting in cytogenetic discrepancies among placenta, amnion, and the fetus, and an efficient selection against the trisomic cells when biparental disomy is present.

The present case had complete trisomy 2 in placenta, mosaic trisomy 2 in amnion, and disomy 2 in the fetus, and was associated with severe IUGR and relative oligohydramnios. High

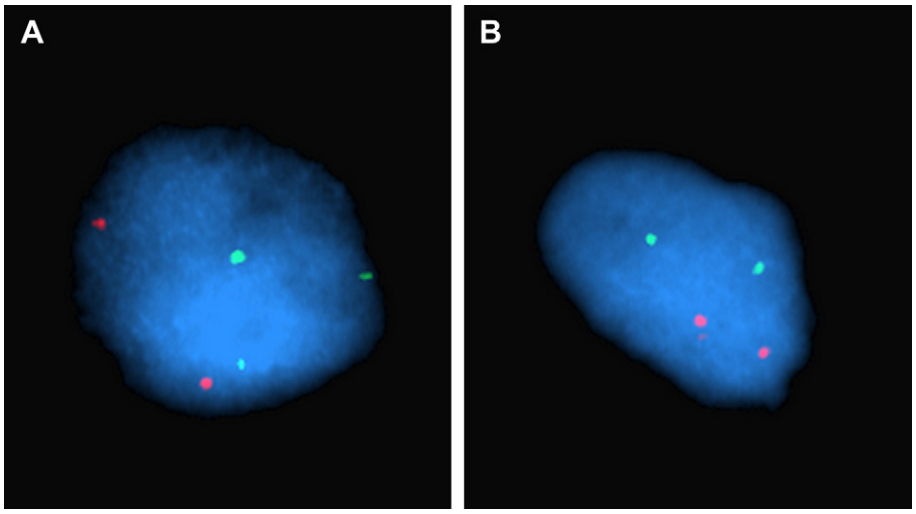

Fig. 2. Fluorescence *in situ* hybridization analysis of uncultured interphase amniocytes using bacterial artificial chromosome probes RP11-468G5 (2q11.1) (spectrum green) and RP11-299N13 (8q11.1) (spectrum red) (internal control) shows (A) three green signals in a cell with trisomy 2 and (B) two green signals in a cell with disomy 2. The result is consistent with the diagnosis of mosaic trisomy 2.

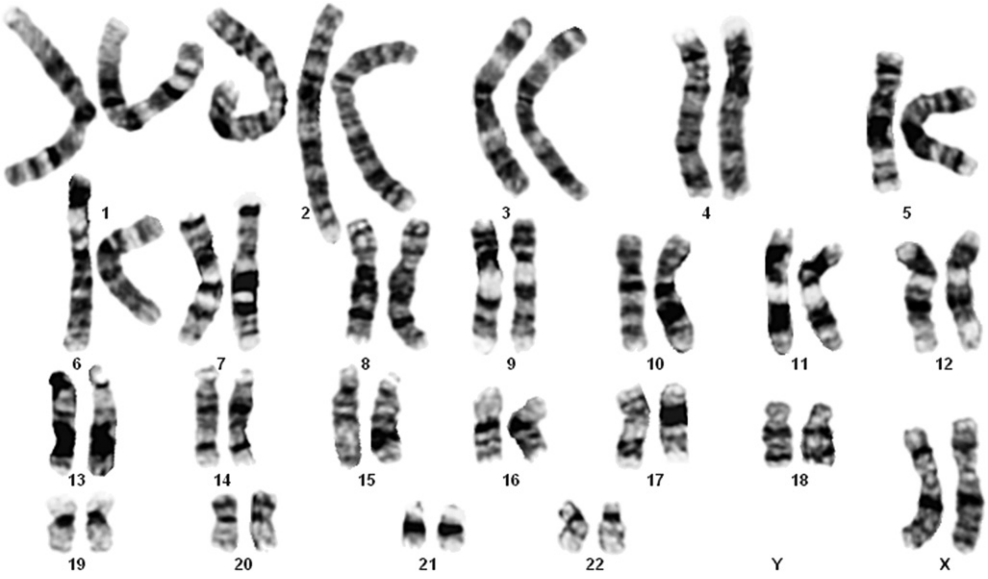

Fig. 3. A karyotype of 47,XX,+2.

levels of trisomy 2 and complete trisomy 2 in placenta have been well known to be associated with IUGR, oligohydramnios, and adverse fetal outcome. For instance, Hansen et al [2] reported maternal UPD in a diploid fetus and confined high levels of mosaic trisomy 2 in placenta in a pregnancy with IUGR, oligohydramnios, and fetal hypospadias. Roberts et al [3] reported trisomy 2 at chorionic villus sampling (CVS), mosaic trisomy 2 (8 of 90 cells) at amniocentesis, biparental disomy 2 in amniotic fluid and the fetus in a pregnancy with IUGR and neonatal death. Sifakis et al [4] reported complete trisomy 2 at CVS and mosaic trisomy 2 (1 of 53 cells) at amniocentesis in a pregnancy with severe IUGR, oligohydramnios, and absent end-diastolic flow in the fetus. IUGR may occur in high levels of trisomy 2 or complete trisomy 2 in placenta with the presence [2,5,6] or absence of fetal UPD 2 [3,7–10]. Robinson et al [1] additionally suggested that the presence of trisomy 2 in amnion correlates with poor pregnancy outcome even when the fetus carries diploid cells.

Sifakis et al [4] previously suggested that the presence of trisomy 2 in CVS cultures may not require further investigation by amniocentesis. However, our presentation shows that, in instances of trisomy 2 diagnosed by CVS, further investigation by amniocentesis using molecular cytogenetic technologies on both uncultured and cultured amniocytes is useful for our understanding of possible fetal and amniotic involvement.

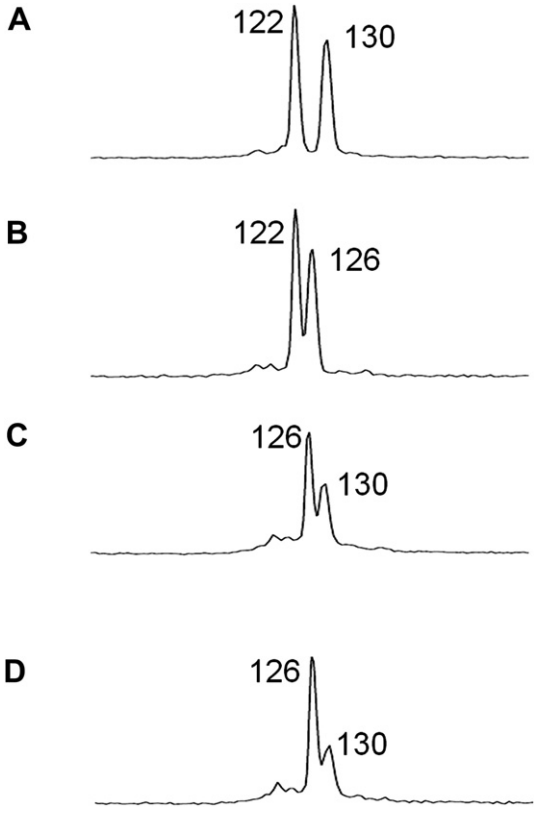

Fig. 4. Representative electrophoretograms of quantitative fluorescent polymerase chain reaction assays. The marker D2S1364 shows two peaks (126 bp:130 bp; maternal and paternal, respectively) of unequal fluorescent activity from two different parental alleles in the tissues of amnion with a dosage ratio of 1.5:1 (maternal:paternal) and in the tissues of placenta with a dosage ratio of 2:1 (maternal:paternal). (A) Father; (B) mother; (C) amnion; and (D) placenta.

Acknowledgments

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