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Research Letter

Cytogenetic discrepancy between uncultured amniocytes and cultured amniocytes in mosaic isochromosome 20q detected at amniocentesis

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A 31-year-old, gravida 2, para 1, woman underwent amniocentesis at 16 weeks of gestation at a medical center because of an abnormal maternal serum screening result (Down syndrome risk = 1/52). In 4 of 17 separated colonies of the cultured amniocytes, an abnormal karyotype of 46,XY,i(20)(q10) was noted, whereas the other 13 colonies had a karyotype of 46,XY. The conventional cytogenetic result of the cultured amniocytes thus was 46,XY,i(20)(q10)[4]/46,XY[13]. The parental karyotypes were normal. The prenatal ultrasound findings were unremarkable. She was referred to the hospital for genetic counseling and repeated amniocentesis. Array comparative genomic hybridization (aCGH), fluorescence *in situ* hybridization (FISH), and quantitative fluorescent polymerase chain reaction (QF-PCR) were applied to both uncultured and cultured amniocytes after repeated amniocentesis. Molecular cytogenetic analyses of the uncultured amniocytes revealed no abnormal findings. aCGH of the uncultured amniocytes did not manifest any genomic imbalance in chromosome 20 (Fig. 1). Polymorphism DNA marker investigation of the uncultured amniocytes using microsatellite markers specific for chromosome 20q revealed a diallelic pattern and seemingly equal biparental inheritance of chromosome 20q (Fig. 2).

Interphase FISH study of the uncultured amniocytes using 20q13.33-specific probe (RP11-266K16) found two signals in all 50 uncultured amniocytes, indicating no evidence of isochromosome 20q (Fig. 3). On the other hand, molecular cytogenetic analyses of the cultured amniocytes revealed abnormal findings. aCGH of the cultured amniocytes manifested genomic imbalance in chromosome 20 with a deletion in chromosome 20p and a duplication in chromosome 20q (Fig. 1). QF-PCR analysis of the cultured amniocytes revealed a diallelic pattern with unequal biparental inheritance of chromosome 20q with a dosage ratio of 1:1.3 (maternal allele:paternal allele) (Fig. 2). Interphase FISH study of the cultured amniocytes found three 20q-specific signals in 7 of 25 (28%) cultured amniocytes, indicating the presence of mosaic isochromosome 20q in cultured amniocytes (Fig. 4). In 8 of 24 (33.3%) separated colonies of the cultured amniocytes, an abnormal karyotype of 46,XY,i(20)(q10) was noted, whereas 16 of 24 (66.7%) colonies had a karyotype of 46,XY. The conventional cytogenetic result of the cultured amniocytes in repeated amniocentesis was 46,XY,i(20)(q10)[8]/46,XY[16] (Fig. 5). In this case, cytogenetic discrepancy between uncultured and cultured amniocytes in mosaic isochromosome 20q was evident. The woman decided to continue the pregnancy. Level II obstetric ultrasonography revealed normal fetal growth biometry, a normal fetal craniofacial profile, and no structural abnormalities. At 40

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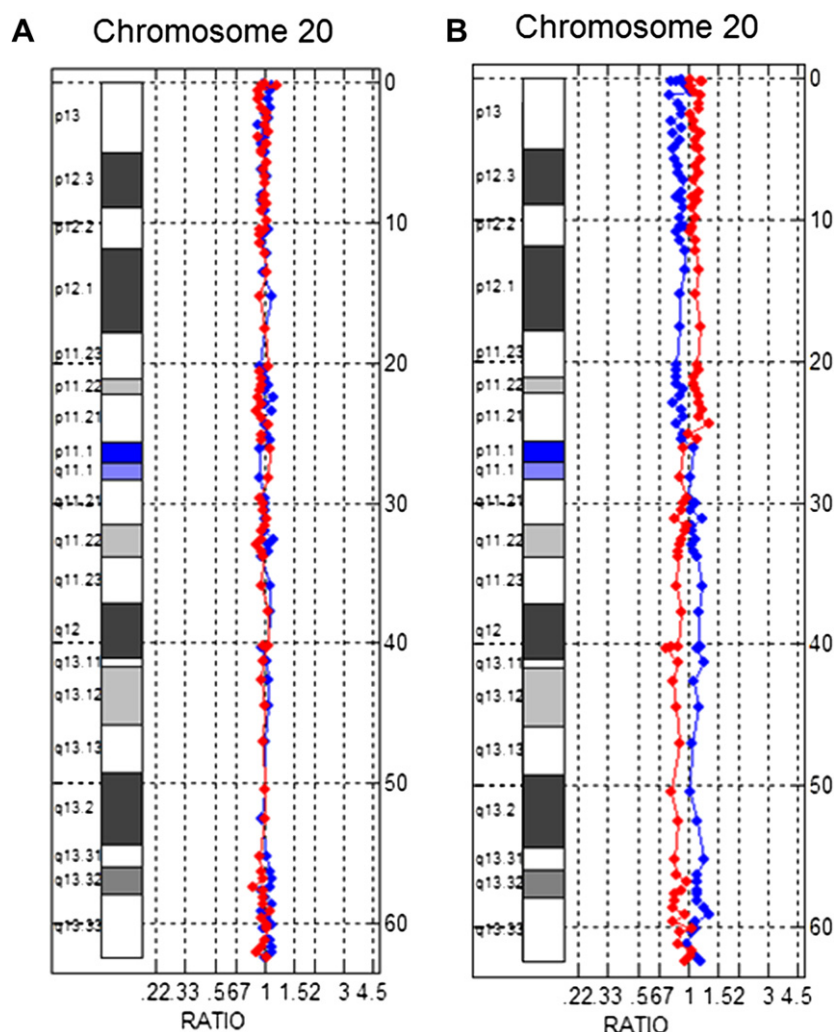


Fig. 1. BAC-based aCGH of (A) uncultured amniotic fluid cells and (B) cultured amniotic fluid cells using CMDX BAC-based aCGH CA3000 Chips (CMDX, Irvine, CA, USA) shows no genomic imbalance in chromosome 20 of uncultured amniocytes and genomic imbalance in chromosome 20 of cultured amniocytes with a deletion in chromosome 20p and a duplication in chromosome 20q. aCGH = array comparative genomic hybridization; BAC = bacterial artificial chromosome.

weeks of gestation, a 3,395-g healthy male baby was delivered uneventfully. Cytogenetic analysis of the blood lymphocytes (100 cells) of the baby revealed a karyotype of 46,XY. At a routine pediatric follow-up examination at 3 months of age, the infant showed normal growth and normal psychomotor development.

The present case provides evidence for cytogenetic discrepancy between uncultured and cultured amniocytes in mosaic isochromosome 20q detected at amniocentesis and shows that mosaic isochromosome 20q in cultured amniocytes can be a tissue-limited cell culture artifact. Our observation may explain the clinical findings that most reported cases with prenatally detected mosaicism for isochromosome 20q resulted in normal pregnancy outcomes [1–3]. Robinson et al [3] suggested that the isochromosome 20q presumably arises through a postzygotic error and can persist only in a few specific cell types.

Although there is a predominantly normal outcome of prenatally detected isochromosome 20q, genetic counseling for mosaic isochromosome 20q detected at amniocentesis still should be done cautiously. Detailed fetal ultrasound

evaluation and molecular cytogenetic investigation of uncultured amniocytes may be helpful for genetic counseling when parents decide to continue the pregnancy. To date, at least four reports with mosaic isochromosome 20q detected at amniocentesis have been described to be associated with phenotypic abnormalities [1,4–6]. Chevrons et al [4] reported prenatal diagnosis of mosaic isochromosome 20q with a mosaic chromosome constitution of 8 of 13 colonies of cultured amniocytes having the abnormal karyotype of 46,XX,i(20q). The fetus was associated with diaphragmatic hernia, ventricular dilation, a large calvarium, anophthalmia, craniofacial dysmorphism, and a single umbilical artery. Pfeiffer et al [5] reported prenatal diagnosis of mosaic isochromosome 20q with a mosaic chromosome constitution of 24 of 26 colonies of cultured amniocytes having the abnormal karyotype of 46,XY,i(20q). The blood, placenta, and skin had the karyotype of 46,XY. The umbilical cord had the karyotype of 46,XY,i(20q)[13]/46,XY[18]. FISH of interphase nuclei presented three signals of 20q13-specific probe in 6% of buccal epithelium and in 13.7% of urinary sediment. The infant

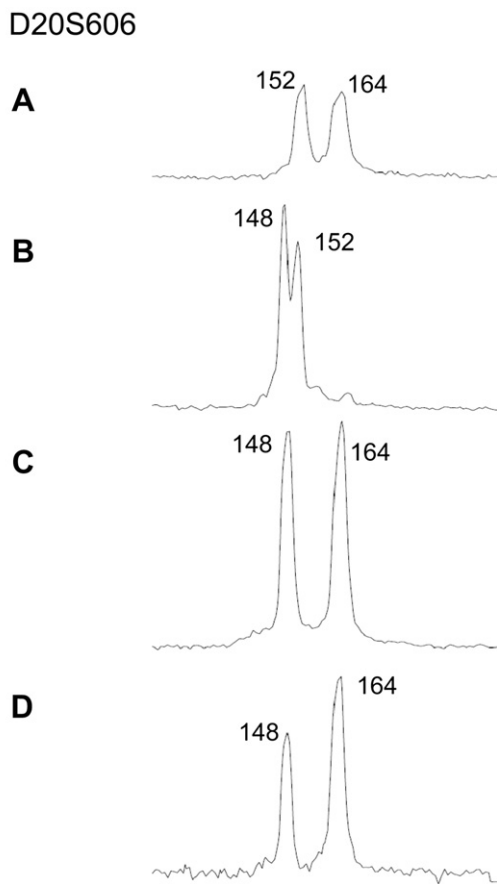


Fig. 2. Representative electrophoretograms of quantitative fluorescent polymerase chain reaction assays. The marker D20S606 (20q13.2) shows two peaks (maternal:paternal, 148 bp:164 bp) of equal fluorescent activity from two different parental alleles in uncultured amniocytes and two peaks of unequal fluorescent activity in cultured amniocytes with a dosage ratio of 1:1.3 (maternal:paternal). (A) Father; (B) mother; (C) amniotic fluid cells (uncultured); (D) amniotic fluid cells (cultured).

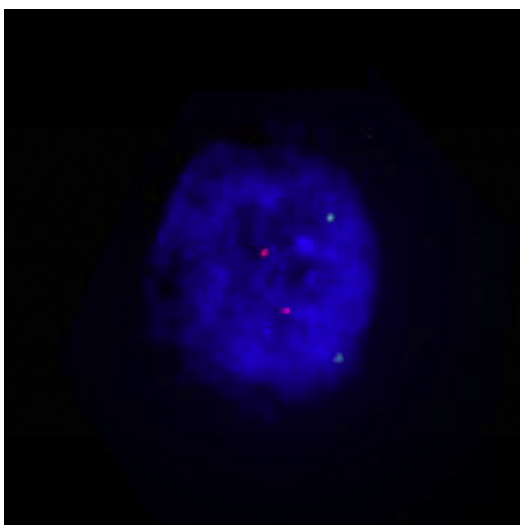


Fig. 3. Fluorescence *in situ* hybridization analysis of uncultured interphase amniocytes using a 20q-specific probe RP11-266K16 (20q13.33) (spectrum red) and a 6q-specific probe RP11-448N11 (6q11.1–q11.2) (spectrum green) as internal control shows two signals of the 20q-specific probe.

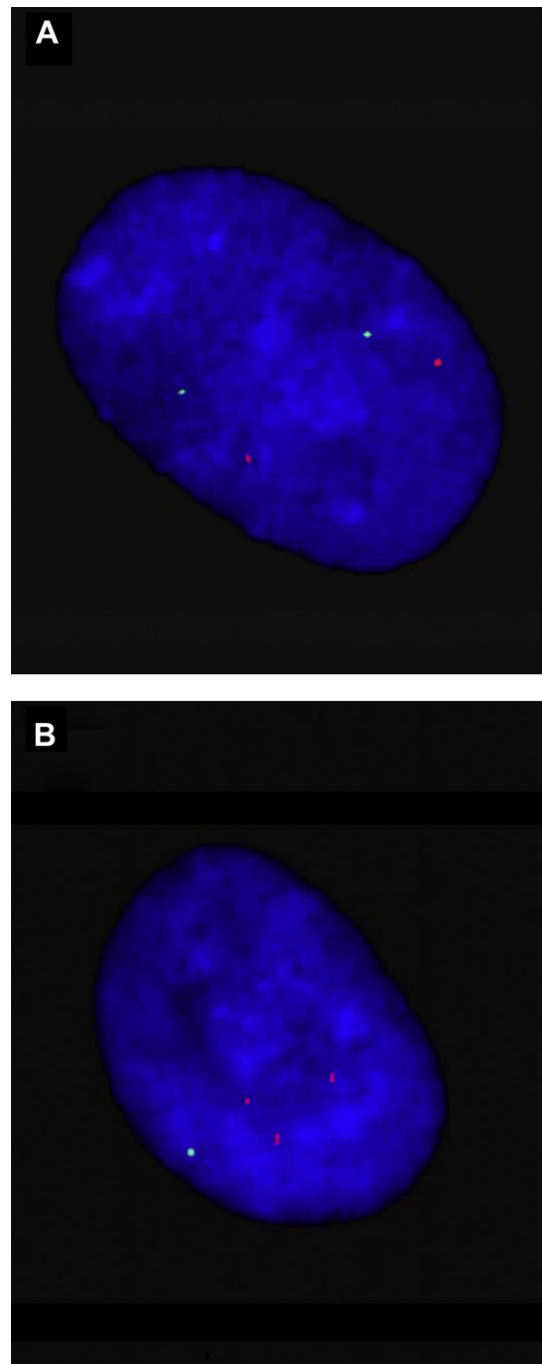


Fig. 4. Fluorescence *in situ* hybridization analysis of cultured interphase amniocytes using a 20q-specific probe RP11-266K16 (20q13.33) (spectrum red) and a 20p-specific probe RP11-530N10 (20p13) (spectrum green) shows (A) a normal cell with two normal chromosomes 20 (two red signals and two green signals) and (B) an abnormal cell with isochromosome 20q (three red signals and one green signal).

manifested with scars on the scalp, orbital cysts, coloboma of the papilla, and dyssegmentation of the thoracic spine. Chen [1] reported prenatal diagnosis of mosaic isochromosome 20q with a mosaic chromosome constitution of 14 of 28 colonies of cultured amniocytes having the abnormal karyotype of 46,XX,i(20q). The blood, placenta, skin, and liver had the karyotype of 46,XX. The infant manifested with

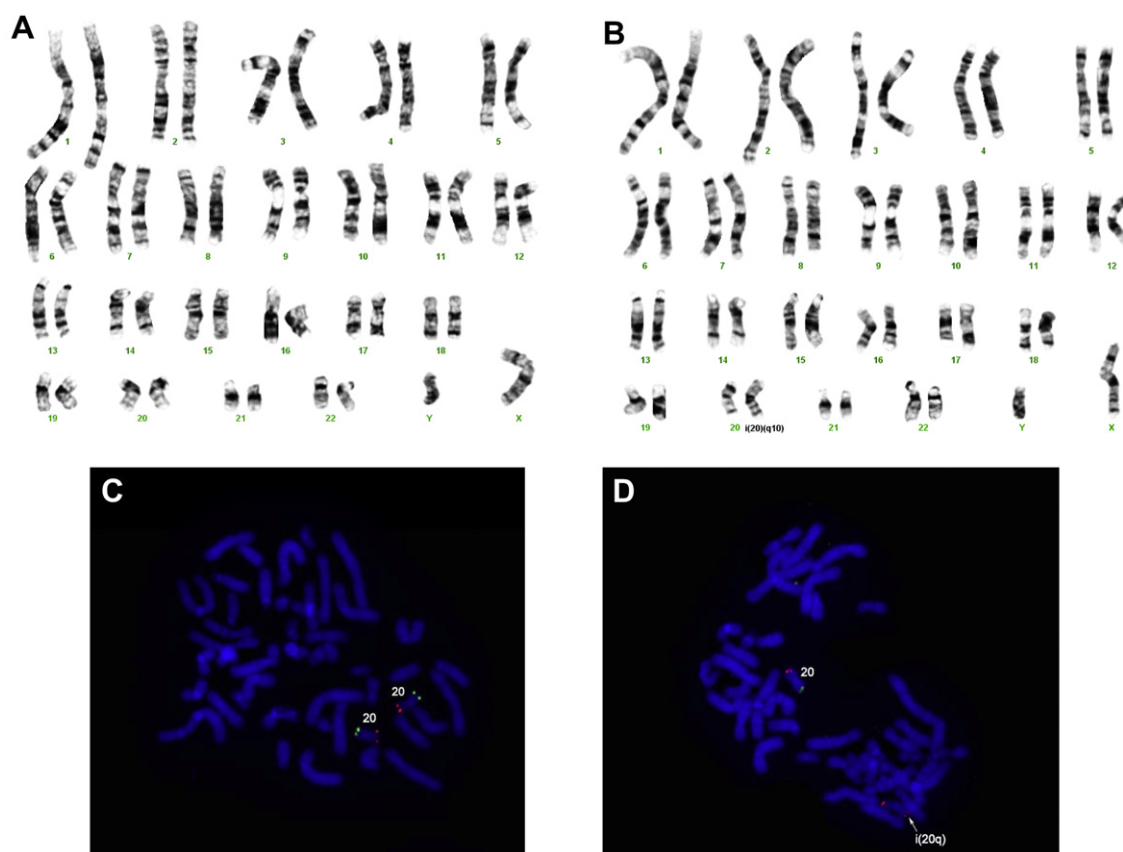


Fig. 5. (A) A karyotype of 46,XY. (B) A karyotype of 46,XY,i(20)(q10). (C) Corresponding metaphase FISH finding of a cultured amniocyte with 46,XY. (D) Corresponding metaphase FISH finding of a cultured amniocyte with 46,XY,i(20)(q10). Red signal: RP11-266K16 (20q13.33); green signal: RP11-530N10 (20p13). FISH = fluorescence *in situ* hybridization.

arthrogryposis multiplex congenita, amyoplasia, and a single umbilical artery, which were likely a casual relationship with the mosaic i(20q). Goumy et al [6] reported a prenatal diagnosis of mosaic isochromosome 20q with a mosaic chromosome constitution of 14 of 15 colonies of cultured amniocytes having the abnormal karyotype of 46,XY,i(20q). Interphase FISH study revealed two signals of 20q12-specific probe in all 20 uncultured amniocytes and three signals in 24% of 260 cultured amniocytes. aCGH study of cultured amniocytes revealed no genomic imbalance. The pregnancy was terminated, and the fetus manifested with hydrocephalus, spinal malformation, craniofacial dysmorphism, and clubfeet. However, there was no successful fetal tissue sampling for confirmation. The phenotype–genotype correlation in that case was inconclusive.

This is the first report of prenatal molecular cytogenetic investigation of mosaic isochromosome 20q using interphase FISH, aCGH, and QF-PCR on both uncultured and cultured amniocytes. This report confirms that cytogenetic discrepancy occurs between uncultured and cultured amniocytes in mosaic isochromosome 20q detected at amniocentesis and implies that mosaic isochromosome 20q detected at amniocentesis can be a cell culture artifact. We suggest that prenatal diagnosis of mosaic isochromosome 20q by amniocentesis without fetal anomalies and without mosaic i(20q) on uncultured amniocytes is likely to result in a normal fetal outcome.

Acknowledgments

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