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Research Letter

Interphase FISH on uncultured amniocytes at repeat amniocentesis for rapid confirmation of low-level mosaicism for tetrasomy 18p

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A 39-year-old, gravida 2, para 1, woman underwent amniocentesis at 18 weeks of gestation because of advanced maternal age. Cytogenetic analysis of cultured amniocytes revealed mosaic supernumerary isochromosome 18p. In four of 33 separated colonies of cultured amniocytes, an abnormal karyotype of 47,XY,+i(18)(p10) was noted, while the other 29 colonies had a karyotype of 46,XY. The karyotype of cultured amniocytes at first amniocentesis was 47,XY,+i(18)(p10)[4]/46,XY[29]. Level II ultrasound findings were unremarkable. She underwent repeat amniocentesis at 24 weeks of gestation, and cord blood sampling at 26 weeks of gestation. Cytogenetic analysis of cultured amniocytes revealed a karyotype of 47,XY,+i(18)(p10)[8]/46,XY[38] at the second amniocentesis. In eight of 46 separated colonies of cultured amniocytes, a karyotype of 47,XY,+i(18)(p10) was noted, while the other 38 colonies had a karyotype of 46,XY. Array comparative genomic hybridization (aCGH) on amniotic fluid using oligonucleotide array revealed a result of arr 18p11.3p11.1 (0–13,884,871) × 2~3. Cytogenetic analysis of cord blood lymphocytes revealed a karyotype of 46,XY in 100/100 cells. The woman underwent a third amniocentesis at 27 weeks of gestation. Interphase fluorescence *in situ* hybridization (FISH) on uncultured amniocytes, using an 18p11.32-specific probe (RP11-324G2) (178,384-350,551, dye: fluorescein isothiocyanate (FITC), green) and a control 18q23-specific probe (RP11-154H12) (77,437,746–77,600,269, dye: Texas Red), showed four green signals and two red signals in 7.1% (6/84 cells) of uncultured amniocytes, and two green signals and two red in 82.9% (78/84 cells) of uncultured amniocytes (Fig. 1). Whole-genome aCGH on the DNA extracted from the uncultured amniocytes obtained from 10 mL of amniotic fluid was performed using NimbleGen ISCA Plus Cytogenetic Array (Roche NimbleGen, Madison, WI, USA). aCGH detected a 15.31-Mb gene dosage increase at 18p11.32–18p11.21, or arr [hg 19] 18p11.32p11.21 (0–15,310,000) × 2.13 with a $\log_2$ ratio of 0.091 (Fig. 2). Cytogenetic analysis of cultured amniocytes revealed a karyotype of 47,XY,+i(18)(p10)[9]/46,XY[18] at the third amniocentesis. In nine of 27 separated colonies of cultured amniocytes, a karyotype of 47,XY,+i(18)(p10) was noted (Fig. 3), while the other 18 colonies had a karyotype of 46,XY. The woman decided to continue the pregnancy. A healthy 3450 g male baby was delivered uneventfully at term. Interphase FISH on uncultured urinary cells, using an 18p11.32-specific probe (RP11-324G2) (spectrum green) and an 18q23-specific probe (RP11-154H12) (spectrum red), showed four green signals and two red signals in 5.2% (5/97 cells) of uncultured urinary cells and two green signals and two red signals in 94.8% (92/97 cells) of uncultured urinary cells (Fig. 4). When examined at 1 month of age, the infant was apparently normal in growth and development without any phenotypic abnormalities.

Tetrasomy 18p (OMIM 614290) or isochromosome 18p syndrome is characterized by facial dysmorphism of low-set malformed ears, small pinched nose, small mouth, high-arched palate, micrognathia and prognathism, developmental delay, and cognitive impairment in most cases; neonatal feeding problems, growth

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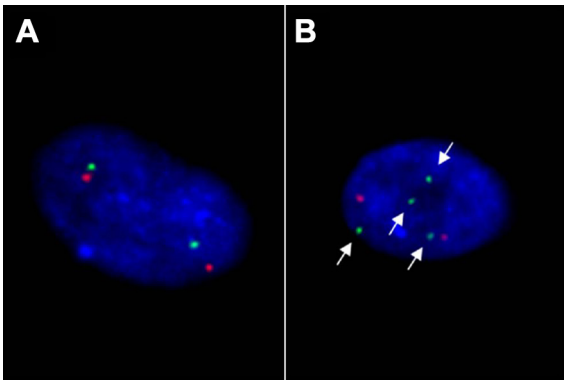

Fig. 1. Interphase fluorescence *in situ* hybridization (FISH) analysis of uncultured amniocytes using an 18p11.32-specific probe RP11-324G2 (spectrum green) and an 18q23-specific probe RP11-154H12 (spectrum red) shows: (A) two green signals and two red signals in an amniocyte with disomy 18; and (B) four green signals (arrows) and two red signals in an amniocyte with tetrasomy 18p.

retardation, microcephaly, strabismus, abnormal muscle tone, scoliosis/kyphosis, and variants on brain magnetic resonance imaging in >25% of the cases; the common findings of neonatal jaundice and respiratory distress, recurrent otitis media, hearing loss, seizures, refractive errors, constipation, gastroesophageal reflux, cryptorchidism, congenital heart defects, and foot anomalies; and occasional findings of hernias, myelomeningocele, kidney defects, short stature, and failure to respond to growth hormone stimulation [1,2]. However, patients with mosaic tetrasomy 18p have been reported to present variable phenotypic features ranging from an apparently normal phenotype to multiple abnormalities [3–8]. Prenatal diagnosis of mosaic tetrasomy 18p is uncommon. To our knowledge, only eight cases of prenatally detected mosaic tetrasomy 18p have been reported [3–9]. Diagnosis of low-level mosaicism for tetrasomy 18p in the patients should include

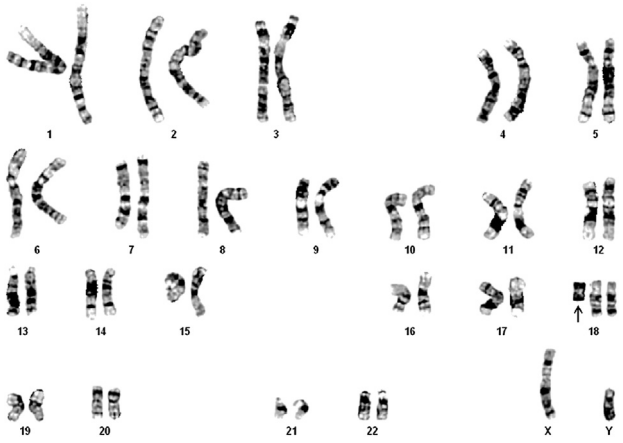

Fig. 3. A karyotype of 47,XY,+i(18)(p10). The arrow indicates a supernumerary isochromosome 18p.

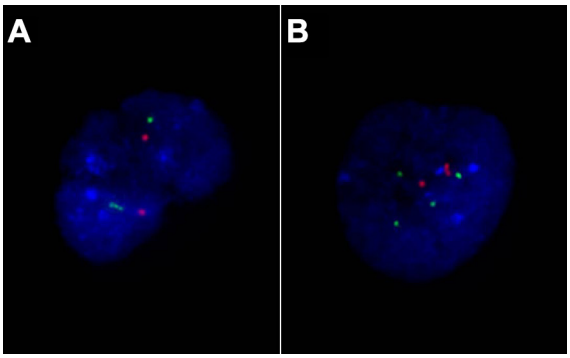

Fig. 4. Interphase fluorescence *in situ* hybridization (FISH) analysis on uncultured urinary cells using an 18p11.32-specific probe RP11-324G2 (spectrum green) and an 18q23-specific probe RP11-154H12 (spectrum red) shows: (A) two red signals and two green signals in a urinary cell with disomy 18; and (B) four green signals and two red signals in a urinary cell with tetrasomy 18p.

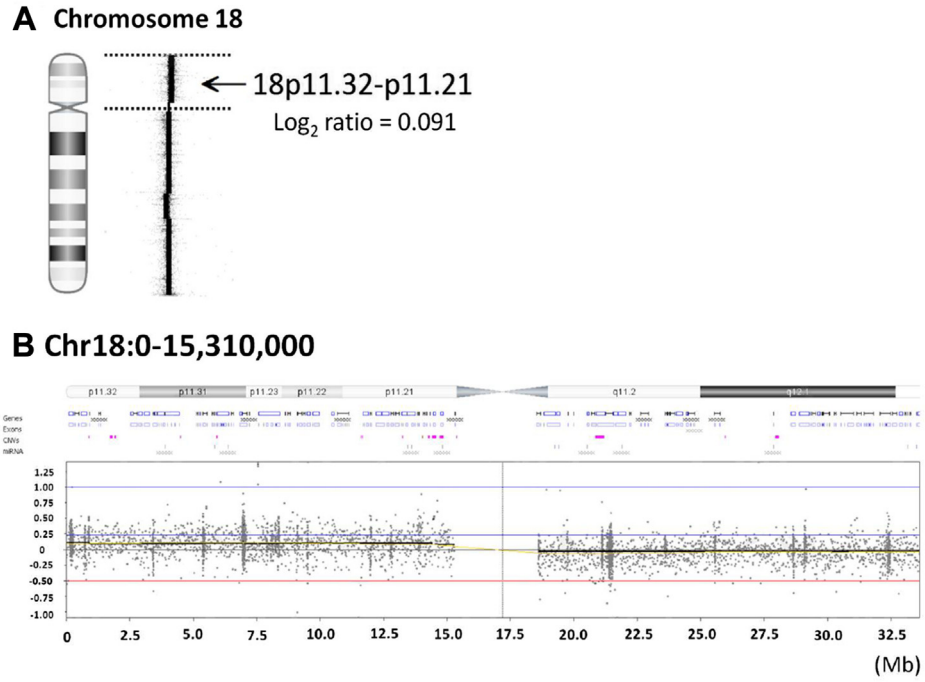

Fig. 2. (A) and (B) Whole-genome array comparative genomic hybridization analysis (aCGH) of the DNA extracted from uncultured amniocytes shows a gene dosage increase at 18p, or arr [hg19] 18p11.32p11.21 (0-15,310,000) × 2.13. The $\log_2$ ratio is 0.091.

genetic counseling of tetrasomy 18p in their offspring. Abeliovich et al [10] reported isochromosome 18p in a mother and her child. The woman had slight dysmorphism but normal development and 3% mosaicism for tetrasomy 18p, but her child had full isochromosome 18p syndrome and a karyotype of 47,XX,+i(18p). The present case, along with our previous observation [9], adds to the literature of low-level mosaicism for tetrasomy 18p detected prenatally by amniocentesis, with an apparently normal phenotype.

In the present case, the cord blood sampling revealed a normal karyotype. Conventional cytogenetic analysis on cultured amniocytes at three amniocenteses revealed mosaic tetrasomy 18p levels of 12.5% (4/32 colonies), 17.4% (8/46 colonies), and 33.3% (9/27 colonies), respectively, interphase FISH on uncultured amniocytes revealed a mosaic tetrasomy 18p levels of 7.1% (6/84 cells), and interphase FISH on uncultured urinary cells at birth revealed a mosaic tetrasomy 18p levels of in 5.2% (5/97 cells). The present case shows cytogenetic discrepancy between amniocytes and blood lymphocytes, indicating a limited value of cord blood sampling for confirmation of mosaic tetrasomy 18p at amniocentesis. The present case also shows significant variation of the mosaic level for tetrasomy 18p among different amniocenteses. Therefore, interphase FISH on uncultured amniocytes at repeat amniocentesis is very useful for rapid confirmation of the mosaic level in cases of mosaicism for tetrasomy 18p detected in the cultured amniocytes by amniocentesis.

Acknowledgments

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