Research Letter

Interphase FISH on uncultured amniocytes at repeat amniocentesis for rapid diagnosis of true mosaicism in a case of level II mosaicism involving trisomy 21 in a single colony from an in situ culture of amniocytes

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A R T I C L E   I N F O

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A 25-year-old, gravida 2, para 1, woman underwent amniocentesis at 16 weeks of gestation because of abnormal maternal serum screening and a Down syndrome risk of 1/81. Cytogenetic analysis of cultured amniocytes revealed level II mosaicism involving trisomy 21 and a karyotype of 47XX,þ21[1]/46XX[16]. Of 17 colonies of cultured amniocytes, only one colony had the karyotype of 47XX,þ21, whereas the other 16 colonies had the karyotype of 46XX. The single colony with trisomy 21 had four metaphase cells, and all the four cells had the karyotype of 47XX,þ21 (Fig. 1). The father had a karyotype of 46XY, and the mother had a karyotype of 46XX. Prenatal ultrasound findings were unremarkable. At 19 weeks of gestation, the woman underwent repeat amniocentesis. Interphase fluorescence in situ hybridization (FISH) and array comparative genomic hybridization (aCGH) were performed on uncultured amniocytes, and conventional cytogenetic analysis was performed on cultured amniocytes. aCGH on uncultured amniocytes using NimbleGen ISCA Plus Cytogenetic Array (Roche NimbleGen, Madison, WI, USA) showed no genomic imbalance. Interphase FISH on uncultured amniocytes using a 21q11.2-specific probe (RP11-138015, dye FITC, 15,627,586-15,772,076) [hg 19] showed three green signals in 10.5% (10 of 95 cells) of uncultured amniocytes and two green signals in 89.5% (85/95 cells) of uncultured amniocytes. The result was nuc ish(RP11-138015×3)[10/95] (Fig. 2). Cytogenetic analysis of cultured amniocytes revealed a karyotype of 46XX in 25 of 25 colonies. The parents decided to continue the pregnancy. A healthy 2958-g female baby was delivered uneventfully at 39 weeks of gestation with no phenotypic features of Down syndrome. Cytogenetic analysis of cord blood lymphocytes revealed a karyotype of 46XX in 40 of 40 cultured lymphocytes. Interphase FISH on uncultured urinary cells using the 21q11.2-specific probe of RP11-138015 showed the result of nuc ish(RP11-138015×3)[2/54] (Fig. 3). In 54 uncultured urinary cells, 3.7% (2/54 cells) showed three green signals, whereas 96.3% (52 of 54 cells) showed two green signals. The neonate had normal physical and psychomotor development at 1 month of age at follow-up.

We previously observed low-level true mosaicism confirmed by interphase FISH on uncultured amniocytes at repeat amniocentesis following prenatal diagnosis of suspected mosaicism in a single colony of cultured amniocytes involving trisomy 2 [1,2] or trisomy 8 [3]. In this presentation, we report an additional case involving trisomy 21 with a similar condition. The current case, along with our previous observations, suggests that level II mosaicism detected in cultured amniocytes under the circumstance of two or more
cells with the same chromosome aberration in a single colony from an in situ culture of amniocytes should be watched closely for the possibility of low-level true mosaicism.

The current case provides evidence that interphase FISH on uncultured amniocytes is very useful for confirmation of true mosaicism at repeat amniocentesis. The current case had trisomy 21 in 4/4 metaphase amniocytes in a single colony of cultured amniocytes. Hsu and Benn [4], in the revised guidelines for the diagnosis of mosaicism in amniocytes, suggested that in case of prenatal diagnosis of autosomal trisomy involving chromosome 2, 5, 8, 9, 12, 13, 14, 15, 16, 18, 20, 21, or 22 in a single colony in an in situ culture of amniocytes, an extensive workup involving the examination of 24 colonies from two further separate cultures is required. The current case had 5.9% (1 of 17 colonies) mosaicism for trisomy 21 in cultured amniocytes at first amniocentesis, but no (0 of 25 colonies) mosaicism for trisomy 21 in cultured amniocytes at repeat amniocentesis. However, there were 10.5% (10 of 95 cells) mosaicism for trisomy 21 in uncultured amniocytes at repeat amniocentesis, and 3.7% (2 of 54 cells) mosaicism for trisomy 21 in uncultured urinary cells. The Association for Clinical Cytogenetics [5] suggested that in case of level II mosaicism in a single colony from an in situ culture of amniocytes, interphase FISH may be used to investigate the mosaicism. We emphasize that interphase FISH on uncultured amniocytes at repeat amniocentesis is practical for differential diagnosis of true mosaicism from pseudomosaicism under such circumstances.

In the current case, both cultured amniocytes at repeat amniocentesis and cultured cord blood showed no mosaicism. However, interphase FISH on uncultured amniocytes at repeat amniocentesis and uncultured urinary cells obtained after birth

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**Fig. 1.** A karyotype of 47,XX,+21.

**Fig. 2.** Interphase fluorescence in situ hybridization analysis of uncultured amniocytes using the bacterial artificial chromosome (BAC) probe RP11-138015 (21q11.2; spectrum green, dye FITC) at repeat amniocentesis shows (A) two green signals in a disomy 21 amniocyte and (B) three green signals in a trisomy 21 amniocyte.
showed low-level mosaicism. We previously found that uncultured amniocytes have a higher frequency of trisomy 21 cells than lymphocytes in fetuses with mosaic trisomy 21 and suggested that interphase FISH on uncultured amniocytes will provide more accurate information on the trisomy 21 mosaic percentage than that acquired by cultured blood lymphocytes [6]. In patients with mosaic trisomy 21, there is a positive correlation between the mosaic percentage and the severity of the phenotype, and an inverse correlation between the overall survival and the mosaic percentage [7–9]. This presentation stresses the importance of undertaking interphase FISH on uncultured amniocytes for rapid differential diagnosis of true mosaicism from pseudomosaicism in case of level II mosaicism involving trisomy 21 in a single colony from an in situ culture of amniocytes.

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