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Research Letter

Application of interphase FISH to uncultured amniocytes for rapid confirmation of true trisomy 2 mosaicism in the case of suspected amniocyte mosaicism involving trisomy 2 in a single colony

Chih-Ping Chen [a,b,c,d,e,f,g,*], Fang-Yu Hung [h], Schu-Rern Chern [b], Peih-Shan Wu [i], Jun-Wei Su [a,j], Wayseen Wang [b,k]

[a] *Department of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei, Taiwan*
[b] *Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan*
[c] *Department of Medicine, Mackay Medical College, New Taipei City, Taiwan*
[d] *Department of Biotechnology, Asia University, Taichung, Taiwan*
[e] *School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan*
[f] *Institute of Clinical and Community Health Nursing, National Yang-Ming University, Taipei, Taiwan*
[g] *Department of Obstetrics and Gynecology, School of Medicine, National Yang-Ming University, Taipei, Taiwan*
[h] *Department of Obstetrics and Gynecology, Hsinchu Mackay Memorial Hospital, Hsinchu, Taiwan*
[i] *Gene Biodesign Co. Ltd, Taipei, Taiwan*
[j] *Department of Obstetrics and Gynecology, China Medical University Hospital, Taichung, Taiwan*
[k] *Department of Bioengineering, Tatung University, Taipei, Taiwan*

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We previously reported prenatal diagnosis of mosaic trisomy 2 and mosaic trisomy 8 on amniocentesis with the abnormal cell line limited to a single colony [1,2]. Here we present an additional case of prenatal diagnosis of trisomy 2 mosaicism with a similar condition.

A 35-year-old, gravida 2, para 1 woman underwent amniocentesis at 17 weeks of gestation because of advanced maternal age. Cytogenetic analysis of cultured amniocytes revealed a karyotype of 47,XX,+2[1]/46,XX[19]. Of 20 colonies of cultured amniocytes, only one colony had the karyotype of 47,XX,+2; the other 19 colonies had the karyotype of 46,XX. The single colony with trisomy 2 had four metaphase cells, each of which had a karyotype of 47,XX,+2 (Fig. 1). The parental karyotypes were normal, and fetal ultrasound findings were unremarkable. Repeat amniocentesis was performed at 19 weeks of gestation. Array comparative genomic hybridization (aCGH), interphase fluorescence *in situ* hybridization (FISH), and quantitative fluorescent polymerase chain reaction (QF-PCR) were carried out on uncultured amniocytes, and conventional cytogenetic analysis was performed on cultured amniocytes. aCGH analysis of uncultured amniocytes using NimbleGen ISCA Plus Cytogenetic Array (Roche NimbleGen, Madison, WI, USA) showed no genomic imbalance in chromosome 2. Interphase FISH analysis of uncultured amniocytes using a 2q11.1-specific probe (RP11-468G5, spectrum green; 95,903,645-96,106,005; NCBI build 37) showed three green signals in 3% (3/100 cells) and two green signals in 97% (97/100 cells) of uncultured amniocytes (Fig. 2). QF-PCR analysis excluded uniparental disomy (UPD) 2. Cytogenetic analysis of cultured amniocytes revealed a karyotype of 46,XX in 32/32 colonies. After genetic counseling, the woman decided to continue the pregnancy. A healthy 3,400-g female baby was delivered uneventfully at 40 weeks of gestation with no phenotypic abnormalities. Cytogenetic analysis of cord blood revealed a karyotype of 46,XX in 40/40 cultured blood lymphocytes. Interphase FISH analysis of uncultured urinary cells using the RP11-468G5 probe showed three green signals in 3.9% (4/103 cells) and two green signals in 96.1% (99/103 cells) of urinary cells (Fig. 3). The neonate showed normal growth and psychomotor development at 1 month of age.

The present case had trisomy 2 in 4/4 metaphase amniocytes in a single colony of cultured amniocytes. According to revised guidelines for the diagnosis of mosaicism in amniocytes, 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 requires extensive workup involving the examination of 24 colonies from two further separate cultures [3]. The present case had approximately 3% mosaicism for trisomy 2 in uncultured amniocytes and uncultured urinary

* Corresponding author. Department of Obstetrics and Gynecology, Mackay Memorial Hospital, 92, Section 2, Chung-Shan North Road, Taipei, Taiwan.
E-mail address: cpc_mmh@yahoo.com (C.-P. Chen).

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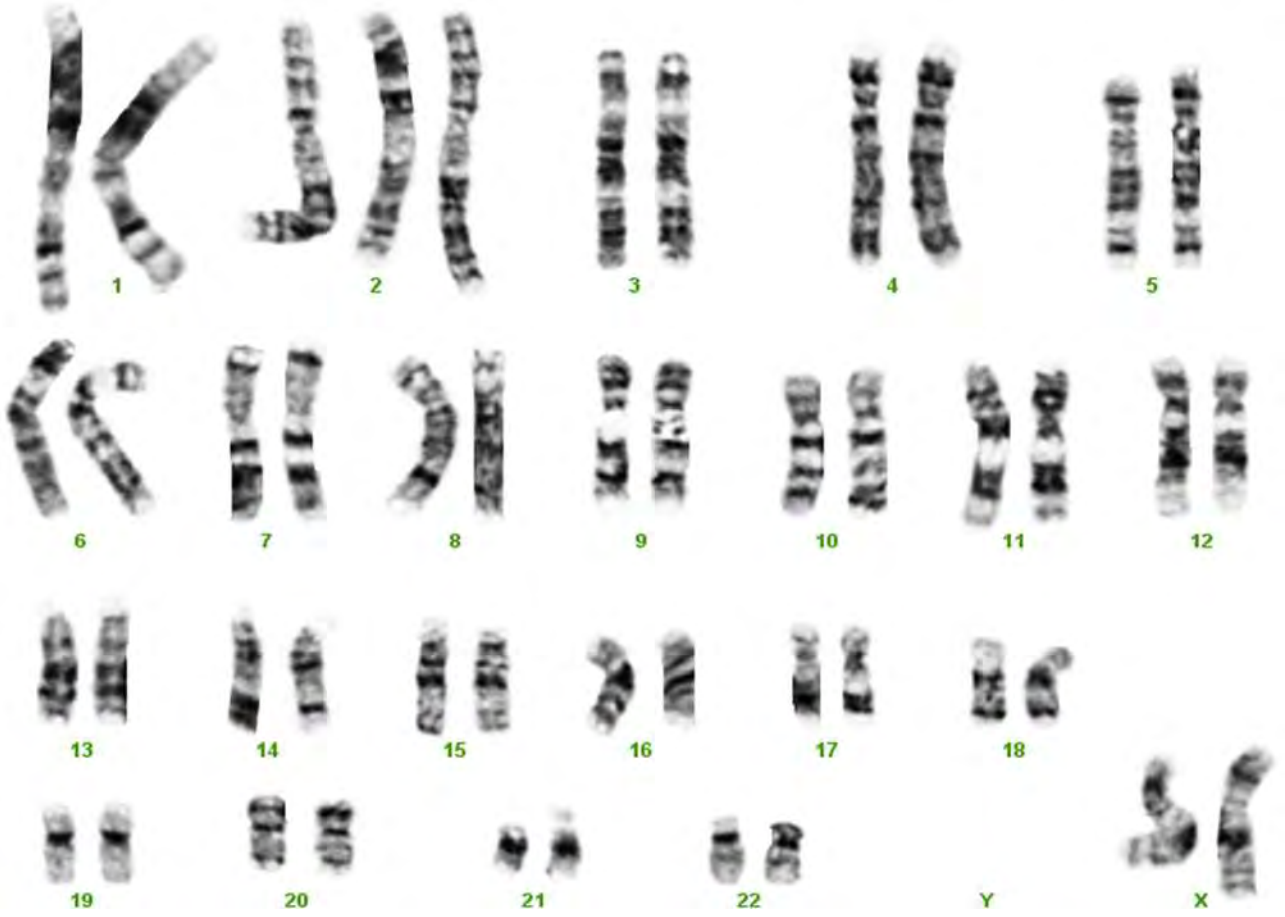

Fig. 1. A karyotype of 47,XX,+2.

cells, but examination of more than 24 colonies from two further separate cultures revealed no other colony with trisomy 2. The Association for Clinical Cytogenetics [4] suggests that level II mosaicism on amniocentesis, such as two or more cells with the same abnormality in a single abnormal colony from an *in situ* culture, should alert to the possibility of clinically significant aneuploidy, and interphase FISH may be used to investigate mosaicism. In the present case, the first amniocentesis revealed 5% (1/20 colonies) mosaicism for trisomy 2 in cultured amniocytes, but repeat amniocentesis

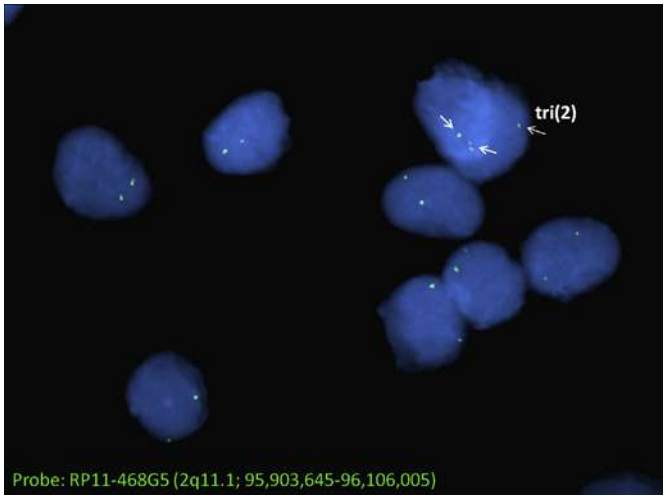

Fig. 2. Interphase fluorescence *in situ* hybridization (FISH) analysis of uncultured amniocytes using the bacterial artificial chromosome (BAC) probe RP11-468G5 (2q11.1; spectrum green) on repeat amniocentesis shows three green signals (arrows) in an amniocyte with trisomy 2 [tri(2)].

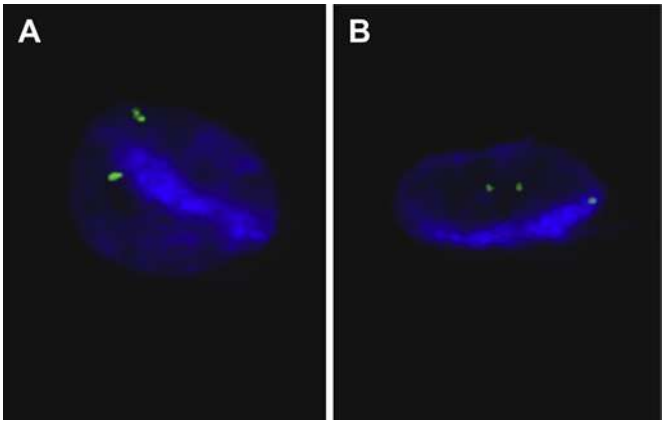

Fig. 3. Interphase FISH analysis of uncultured urinary cells using the BAC probe RP11-468G5 (2q11.1; spectrum green) after birth shows (A) two green signals in a normal urinary cell with two copies of chromosome 2 and (B) three green signals in an abnormal urinary cell with trisomy 2.

revealed no mosaicism (0/32 colonies) for trisomy 2 in cultured amniocytes. It is likely that the abnormal cell line with trisomy 2 in our case disappeared after long-term amniocyte culture. Cord blood analysis also showed no mosaicism (0/40 colonies) for trisomy 2 in cultured lymphocytes. In our case, interphase FISH for uncultured amniocytes on repeat amniocentesis revealed 3% (3/100 cells) mosaicism for trisomy 2, and interphase FISH for uncultured urinary cells confirmed 3.9% (4/103 cells) mosaicism for trisomy 2. We suggest that interphase FISH for uncultured amniocytes on repeat amniocentesis is very practical for differential diagnosis of true mosaicism from pseudomosaicism during prenatal diagnosis of suspected amniocytes in a single colony.

In conclusion, interphase FISH is useful for rapid confirmation of low-level trisomy 2 mosaicism in uncultured amniocytes on amniocentesis and of low-level trisomy 2 mosaicism in uncultured urinary cells at birth.

Acknowledgments

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