

Research Letter

Rapid positive confirmation of trisomy 21 mosaicism at amniocentesis by interphase FISH, QF-PCR and aCGH on uncultured amniocytes

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A 32-year-old, gravida 3, para 2, woman electively underwent amniocentesis at 16 weeks of gestation. Cytogenetic analysis of cultured amniocytes revealed a karyotype of 47,XX,+21[6]/46,XX[14]. In 20 colonies of cultured amniocytes, six colonies had a karyotype of 47,XX,+21, whereas the other 14 colonies had a karyotype of 46,XX. Repeat amniocentesis was performed at 19 weeks of gestation. Interphase fluorescence *in situ* hybridization (FISH) analysis on uncultured amniocytes using the bacterial artificial chromosome (BAC) clone probe RP11-91N21 (21q11.2) showed three 21q-sepcific signals in 48.1% (25/52) of the amniocytes and two 21q-sepcific signals in 51.9% (27/52) of the amniocytes (Fig. 1), indicating 48.1% mosaicism for trisomy 21 in uncultured amniocytes. The cultured amniocytes had a karyotype of 47,XX,+21[13]/46,XX[18] in the repeat amniocentesis. The pregnancy was terminated at 21 weeks of gestation, and a 420-g fetus was delivered with bilateral low-set ears. Cytogenetic analyses revealed a karyotype of 47,XX,+21[4]/46,XX[36] in cord blood and a karyotype of 47,XX,+21[20]/46,XX[30] in umbilical cord. Quantitative fluorescent polymerase chain reaction (QF-PCR) analysis on the umbilical

cord showed unequal fluorescent activity from two alleles with a dosage ratio of 1:1.22 (Fig. 2).

A 40-year-old, gravida 3, 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,XY,+21[13]/46,XY[12]. Repeat amniocentesis was performed at 19 weeks of gestation. Interphase FISH analysis on uncultured amniocytes using the BAC clone probe RP11-91N21 (21q11.2) showed three 21q-sepcific signals in 54% (27/50) of the amniocytes and two 21q-sepcific signals in 46% (23/50) of the amniocytes, indicating 54% mosaicism for trisomy 21 in uncultured amniocytes. QF-PCR analysis on uncultured amniocytes showed unequal fluorescent activity from two alleles with a dosage ratio of 2:1 (Fig. 3). The cultured amniocytes had a karyotype of 47,XY,+21[7]/46,XY[17] in the repeat amniocentesis. The pregnancy was terminated at 22 weeks of gestation, and a 524-g fetus was delivered with hypertelorism, a depressed nasal bridge, low-set ears and malposition of the toes but without dysplasia of mid phalanx of the fifth fingers (Fig. 4). Cytogenetic analyses revealed a karyotype of 47,XY,+21[13]/46,XY[27] in cord blood, a karyotype of 47,XY,+21[5]/46,XY[35] in umbilical cord and a karyotype of 47,XY,+21 in placenta (40/40 cells).

A 39-year-old, primigravid woman underwent amniocentesis at 19 weeks of gestation because of advanced maternal

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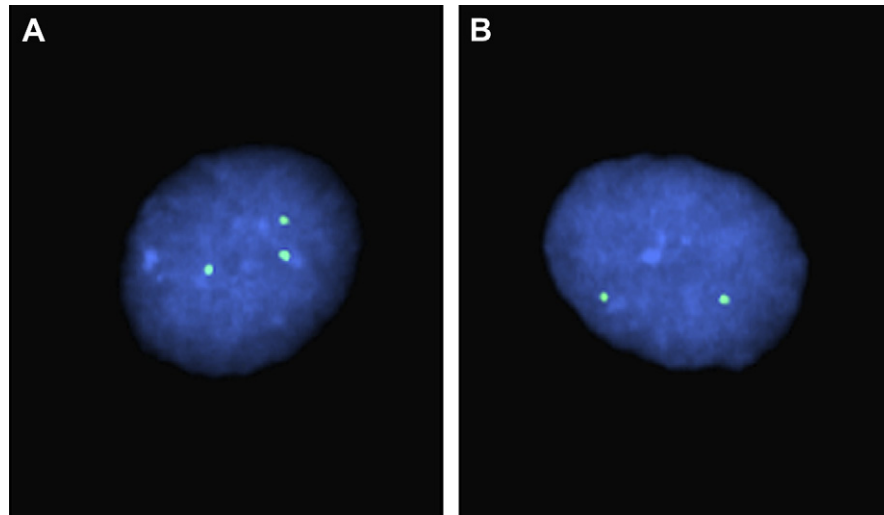


Fig. 1. Interphase fluorescence *in situ* hybridization analysis on uncultured amniocytes using a bacterial artificial chromosome probe RP11-91N21 (21q11.2; spectrum green) shows (A) three green signals in an abnormal cell with trisomy 21; and (B) two green signals in a normal cell with disomy 21.

age. Cytogenetic analysis of cultured amniocytes revealed a karyotype of 47,XY,+21[5]/46,XY[14]. The parental karyotypes were normal. Repeat amniocentesis was performed at 22 weeks of gestation. Interphase FISH analysis on uncultured amniocytes using the BAC clone probe RP11-91N21 (21q11.2) showed three 21q-sepcific signals in 23.1% (12/52) of the amniocytes and two 21q-sepcific signals in 76.9% (40/52) of the amniocytes, indicating 23.1% mosaicism for trisomy 21 in uncultured amniocytes. QF-PCR analysis on uncultured amniocytes showed unequal fluorescent activity from two alleles with a paternal: maternal dosage ratio of 1:1.3 (Fig. 5). Oligonucleotide-based array comparative genomic hybridization (aCGH) analysis on uncultured amniocytes

using CytoChip Oligo array (BlueGnome, Cambridge, UK) showed a partial genomic gain in chromosome 21 (Fig. 6). The molecular results were consistent with the diagnosis of mosaic trisomy 21. The cultured amniocytes had a karyotype of 47,XY,+21[4]/46,XY[17] in the repeat amniocentesis. The parents elected to terminate the pregnancy at 24 weeks of gestation, and a 704-g fetus was delivered without facial dysmorphisms or dysplasia of mid phalanx of the fifth fingers (Figs. 7 and 8). Cytogenetic analyses revealed a karyotype of 47,XY,+21[2]/46,XY[8] in cord blood, 47,XY,+21[1]/46,XY[39] in umbilical cord.

We previously reported application of molecular cytogenetic techniques in uncultured amniocytes in prenatal diagnosis of mosaicism for trisomy 8, trisomy 9 and trisomy 2 [1–4]. In this report, we additionally provide evidence for the usefulness of interphase FISH, QF-PCR and aCGH on uncultured amniocytes in rapid positive confirmation of

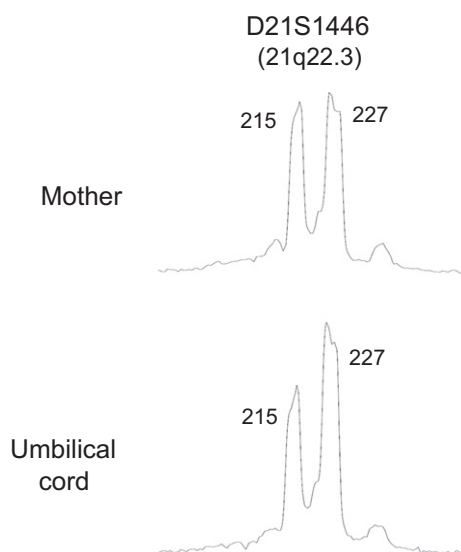


Fig. 2. Representative electrophoretograms of quantitative fluorescent polymerase chain reaction (QF-PCR) assays at short tandem repeat (STR) markers specific for chromosome 21 in the umbilical cord sample of Case 1. The marker D21S1446 (21q22.3) shows two different alleles with unequal fluorescent activity in umbilical cord.

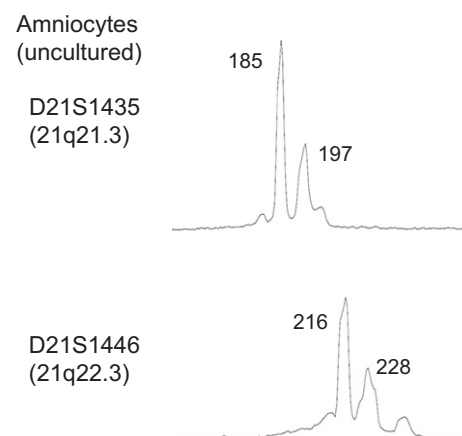


Fig. 3. Representative electrophoretograms of quantitative fluorescent polymerase chain reaction (QF-PCR) assays at short tandem repeat (STR) markers of D21S1435 (21q21.3) and D21S1446 (21q22.3) in the amniotic fluid samples with uncultured amniocytes of Case 2. The markers D21S1435 and D21S1446 show two different alleles with unequal fluorescent activity in uncultured amniocytes.



Fig. 4. The craniofacial appearance of the fetus in Case 2.

trisomy 21 mosaicism. Wallerstein *et al* [5] suggested that repeat amniocentesis may be considered when there is insufficient number of cells or cultures to establish a diagnosis of mosaicism, and the risk of abnormal outcome in pregnancies with less than 50% cultured trisomic 21 cells and greater than 50% cultured trisomic 21 cells was 45% (27/60) versus 59.5% (22/37). In this presentation, variation of the level of mosaicism between different cultured amniocytes was observed in Cases 1 and 2. In Case 1, the first amniocentesis revealed 30% cultured trisomic cells and the second amniocentesis revealed 42% cultured trisomic cells, and interphase FISH on uncultured amniocytes revealed 48.1% trisomic cells. In Case 2, the first amniocentesis revealed 52% cultured trisomic cells and the second amniocentesis revealed 29.2% cultured trisomic

cells, and interphase FISH on uncultured amniocytes revealed 54% trisomic cells. In Case 3, the first amniocentesis revealed 26.3% cultured trisomic cells and the second amniocentesis revealed 19% cultured trisomic cells, and interphase FISH on uncultured amniocytes revealed 23.1% trisomic cells. In this regard, interphase FISH analysis on uncultured amniocytes is helpful for determining the probable level of mosaicism in trisomy 21 mosaicism at amniocentesis.

In this study, QF-PCR was able to detect mosaicism for trisomy 21 in uncultured amniocytes in Cases 2 and 3 with the mosaic level of 54% (27/50) and 23.1% (12/52), respectively, and aCGH using CytoChip Oligo array was able to detect mosaicism for trisomy 21 in uncultured amniocytes in Case 3 with a mosaic level of 23.1%. QF-PCR assay has been reported to detect mosaicism as low as 15% of the whole sample [6], and aCGH has been reported to detect mosaicism as low as 20% in the peripheral blood samples [7,8] or even the level limit of 10% at prenatal diagnosis [9]. We previously successfully detected mosaic trisomy by aCGH using CytoChip Oligo array in two independent cases on uncultured amniocytes with 48% (12/25) mosaicism for trisomy 9 and 12% (6/50) mosaicism for trisomy 2, respectively [3,4]. However, the detection rate of mosaic trisomy using aCGH on uncultured amniocytes is variable according to different products of array chips. For instance, we previously failed to detect mosaic trisomy by aCGH using CMDX array chips on uncultured amniocytes in two separate cases with 25% (5/20) mosaicism for trisomy 8 and 18% (9/50) mosaicism for trisomy 9, respectively [1,2]. Lau *et al* [10] failed to detect mosaic trisomy by QF-PCR on uncultured amniotic fluid samples in two independent cases with 16.7% (5/30) and 2.9% (1/35) mosaicism for trisomy 21, respectively. Therefore, QF-PCR and aCGH may have difficulty in rapid positive confirmation of mosaic trisomy detected at amniocentesis in cases with low-level mosaicism.

Wallerstein *et al* [5] suggested that fetal blood sampling has a role in mosaic trisomy 21 as the risk for abnormal outcome increases with positive confirmation with 1/4 (25%) positive confirmation in phenotypically normal cases versus 3/6 (50%)

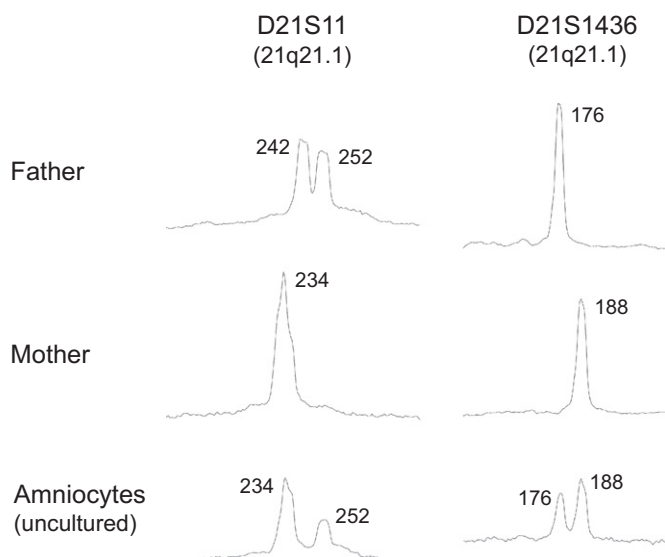


Fig. 5. Representative electrophoretograms of quantitative fluorescent polymerase chain reaction (QF-PCR) assays at short tandem repeat (STR) markers of D21S11 (21q21.1) and D21S1436 (21q21.1) in the parents and uncultured amniocytes of Case 3. The markers D21S11 and D21S1436 show two different parental alleles with unequal fluorescent activity and a dosage increase in the maternal allele in uncultured amniocytes.

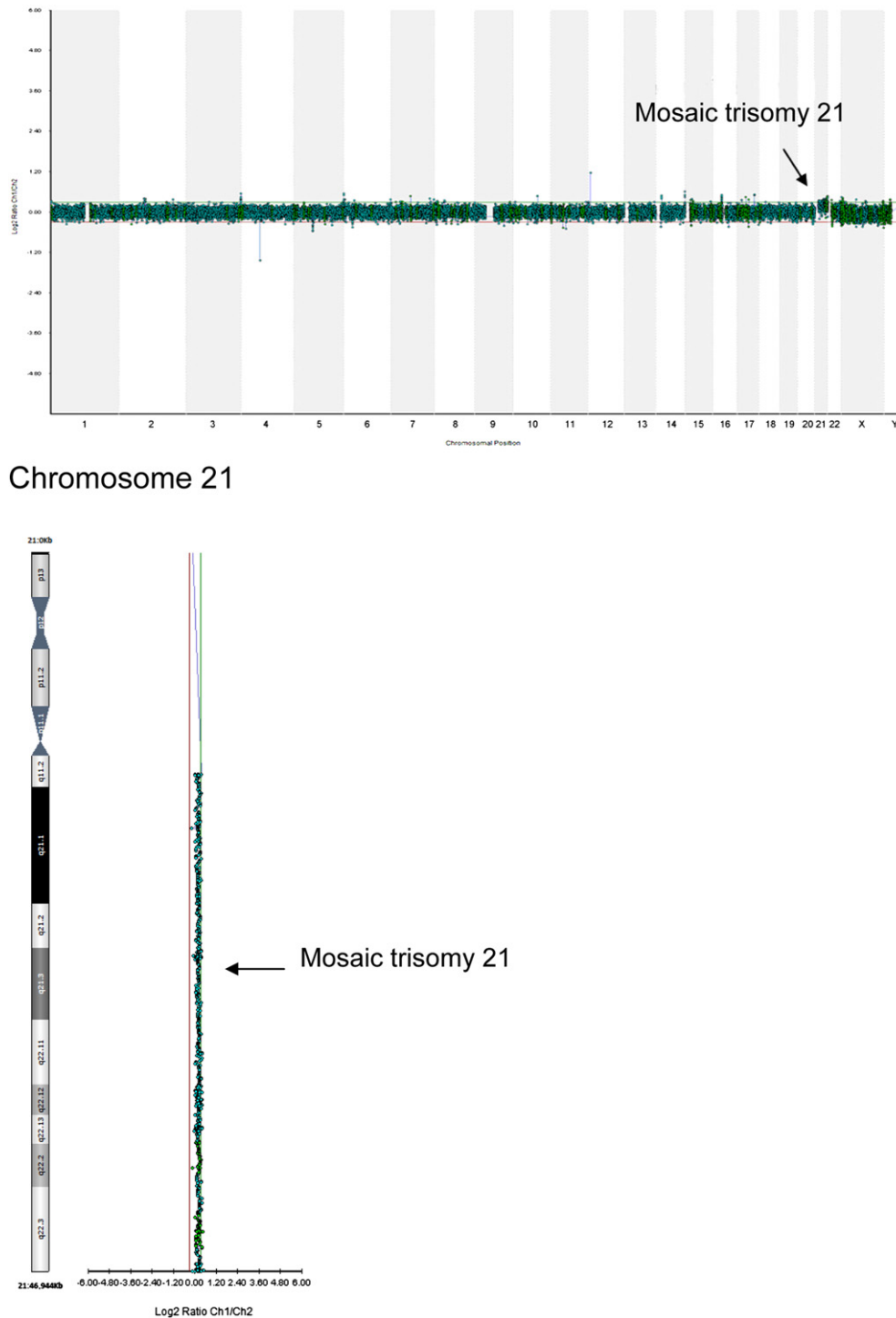


Fig. 6. Oligonucleotide-based array comparative genomic hybridization analysis using CytoChip Oligo array (BlueGnome, Cambridge, UK) on uncultured amniocytes in Case 3 shows a partial genomic gain in chromosome 21.

positive confirmation in phenotypically abnormal cases. However, the percentage of trisomy 21 in lymphocytes is usually lower than other tissues in individuals with mosaic trisomy 21, and fetal blood sampling may lead to a false negative result. For instance, in a study of 107 individuals with mosaic trisomy 21, Papavassiliou *et al* [11] observed that buccal cells showed a significantly higher frequency of

trisomy 21 than lymphocytes ($48.06\% \pm 3.44\%$ vs. $30.13\% \pm 3.99\%$; mean \pm standard error). In this study, the percentage of trisomy 21 cells in uncultured amniocytes of Cases 1–3 was 48.1%, 54% and 23.1%, respectively, and the percentage of trisomy 21 cells in cord blood of Cases 1–3 was 10%, 32.5% and 20%, respectively. It is evident that uncultured amniocytes have higher frequency of trisomy 21 cells



Fig. 7. The craniofacial appearance of the fetus in Case 3.

than lymphocytes in fetuses with mosaic trisomy 21, and accordingly interphase FISH on uncultured amniocytes will provide more accurate information of the percentage of trisomic cells than blood lymphocytes acquired by fetal blood sampling.

It has been suggested that there is a significant positive correlation between the percentage of mosaicism and the severity of the phenotype, and an inverse correlation between

overall survival and the percentage of mosaicism in patients with mosaic trisomy 21 [11–13]. This presentation stresses the importance of undertaking molecular cytogenetic analyses on uncultured amniocytes in order to maximize the accuracy of rapid positive confirmation of trisomy 21 mosaicism at amniocentesis.

Acknowledgments

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Fig. 8. The hand of Case 3 shows no dysplasia of midphalanx of the fifth finger.

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