

Research Letter

Usefulness of interphase FISH on uncultured amniocytes for rapid confirmation of low-level trisomy 7 mosaicism in a pregnancy with fetal intrauterine growth restriction and microcephaly

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Genetic counseling for trisomy 7 mosaicism at amniocentesis remains difficult because of the phenotypic variability [1]. Trisomy 7 mosaicism may present variable features ranging from normal to facial dysmorphisms, sparse hair, enamel dysplasia, pigmentary abnormalities, hypomelanosis of Ito, radial defects, Potter syndrome, Blaschkolinear malformation syndrome, and Goldenhar syndrome [2]. Trisomy 7 mosaicism may also be associated with maternal uniparental disomy for chromosome 7 (UPD 7) and Silver–Russell syndrome (SRS) [3–6]. Here, we report prenatal diagnosis of low-level trisomy 7 mosaicism by amniocentesis using cultured and uncultured amniocytes in the second trimester in a pregnancy with fetal intrauterine growth restriction (IUGR) and microcephaly. In this presentation, we demonstrate the application of molecular cytogenetic technologies in uncultured amniocytes for rapid confirmation of low-level trisomy 7 mosaicism and exclusion of UPD 7.

A 30-year-old primigravid woman underwent amniocentesis at 24 weeks of gestation because of microcephaly and IUGR. Prenatal ultrasound revealed a singleton fetus with a biparietal diameter (BPD) of 4.3 cm (17 weeks), an abdominal circumference (AC) of 16.2 cm (20 weeks) and

a femur length (FL) of 3.5 cm (21 weeks). Array comparative genomic hybridization (aCGH) investigation using CytoChip Oligo Array (BlueGnome, Cambridge, UK) on uncultured amniocytes revealed no gene dosage variation in all chromosomes (Fig. 1). In three out of 25 separated colonies of cultured amniocytes, an abnormal karyotype of 47,XX,+7 was noted, while the other 22 colonies had a karyotype of 46,XX (Fig. 2). The cytogenetic result of amniocentesis was 47,XX,+7[3]/46,XX[22]. The parental karyotypes were normal.

She underwent repeated amniocentesis at 26 weeks of gestation. Prenatal ultrasound at 26 weeks of gestation revealed a BPD of 5.35 cm (22 weeks), an AC of 17.71 cm (22 weeks) and an FL of 4.27 cm (24 weeks). Interphase fluorescence *in situ* hybridization (FISH), quantitative fluorescent polymerase chain reaction (QF-PCR) and a *PEG1/MEST* methylation-sensitive high-resolution melting PCR assay were applied to the uncultured amniocytes. Interphase FISH analysis on uncultured amniocytes using a 7q11.1-specific probe (RP11-432A1) showed three 7q-specific signals in 6/30 (20%) and two signals in 24/30 (80%) of uncultured amniocytes (Fig. 3). Polymorphic DNA marker analysis of uncultured amniocytes using QF-PCR and microsatellite markers specific for chromosome 7 revealed a biparental diallelic pattern with equal biparental inheritance of chromosome 7 and equal dosage in the biparental alleles (Fig. 4). The methylation-specific PCR assay identified the differential methylation of

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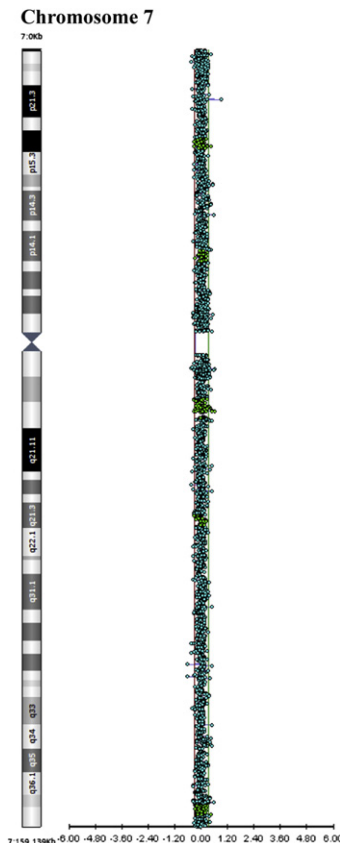


Fig. 1. Oligonucleotide-based array comparative genomic hybridization analysis using CytoChip Oligo array on uncultured amniocytes shows no genomic imbalance in chromosome 7.

the imprinted *PEG1/MEST* locus on 7q32 and revealed biparental inheritance of chromosome 7 in uncultured amniocytes (Fig. 5). The cytogenetic result of the repeated amniocentesis was 46,XX (39/39 colonies). The aCGH investigation using CytoChip Oligo Array (BlueGnome, Cambridge, UK) on uncultured amniocytes did not detect genomic imbalance in chromosome 7.

Prenatal ultrasound at 30 weeks of gestation revealed a BPD of 5.67 cm (24 weeks), an AC of 19.56 cm (24 weeks), an FL of 4.8 cm (26 weeks), ventriculomegaly, microcephaly, micrognathia, a narrow chest, and oligohydramnios. The fetus had intrauterine fetal death at 31 weeks. A 936-g malformed fetus was delivered with microcephaly, hypertelorism, micrognathia, a depressed nasal bridge, and low-set ears (Fig. 6). Cytogenetic analysis of the amniotic membrane, placenta, umbilical cord, and skin revealed a karyotype of 46,XX in 40 cells in all samples. The cultures of the blood and the tissues from lungs and liver were not successful.

The present case provides evidence for the usefulness of application of interphase FISH on uncultured amniocytes for rapid confirmation of low-level trisomy 7 mosaicism at amniocentesis. Trisomy 7 mosaicism at amniocentesis has been associated with pseudomosaicism [7], and discrepancy between the cytogenetic results of cultured amniocytes and the molecular results of uncultured amniocytes [1]. In the present case, the first amniocentesis revealed 12% (3/25) mosaicism for trisomy 7 in cultured amniocytes, and the second amniocentesis revealed a normal karyotype in all 39 colonies of cultured amniocytes. In the second amniocentesis, the interphase FISH on uncultured amniocytes revealed 20% (6/30) mosaicism for trisomy 7, but QF-PCR and aCGH failed to detect trisomy 7 mosaicism on uncultured amniocytes. This is because QF-PCR and aCGH have difficulty in detecting low-level mosaicism of trisomies [8,9]. We previously reported the successful detection of trisomy 7 mosaicism by interphase FISH, QF-PCR, and aCGH on uncultured amniocytes in a case with a higher level of 26% (13/50) mosaicism for trisomy 7 [2]. In the present case, however, only interphase FISH could detect a lower level of 20% mosaicism for trisomy 7. Therefore, we suggest that interphase FISH should be the molecular cytogenetic method of choice for rapid confirmation of low-level mosaicism at amniocentesis. Our case also provides evidence for cytogenetic discrepancy on cultured amniocytes in low-level trisomy 7 mosaicism between the first and repeated amniocenteses. We think that the abnormal cell line

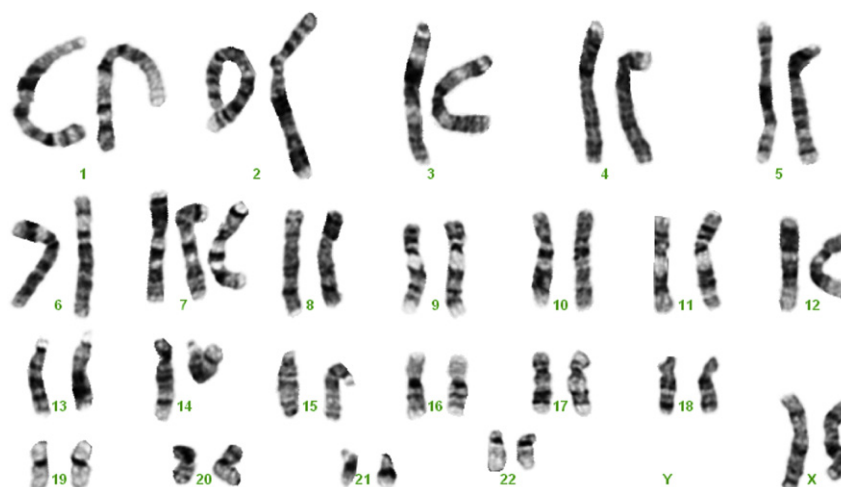


Fig. 2. A karyotype of 47,XX,+7.

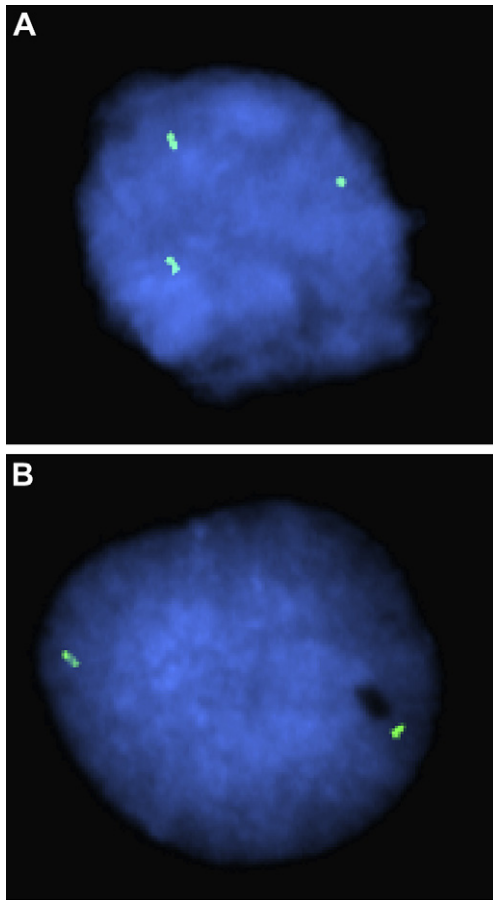


Fig. 3. Interphase fluorescence *in situ* hybridization analysis on uncultured amniocytes using a bacterial artificial chromosome probe RP11-432A1 (7q11.1; spectrum green) shows (A) three green signals in an abnormal cell with trisomy 7 and (B) two green signals in a normal cell with disomy 7.

might have disappeared after cell culture in the repeated amniocentesis.

In this study, cytogenetic analyses of the fetal skin and extraembryonic tissues such as membrane, placenta, and cord

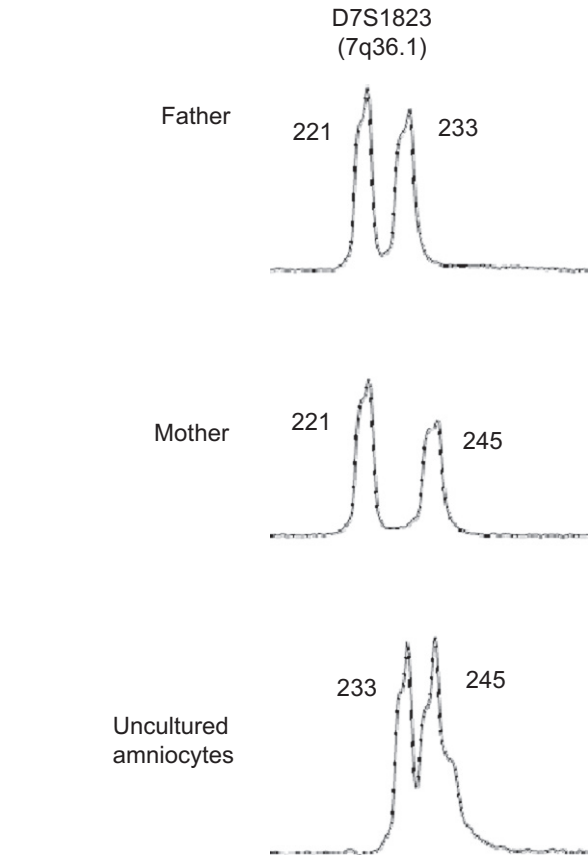


Fig. 4. Representative electrophoretogram of quantitative fluorescent PCR assay at a polymorphic DNA marker specific for chromosome 7 in the amniotic fluid sample with uncultured amniocytes shows a biparental diallelic pattern with equal biparental inheritance of chromosome 7 and equal dosage in the biparental alleles.

failed to detect trisomy 7 mosaicism. We think that incomplete tissue samplings and fetal death might explain this result, although the possibility of an origin of the abnormal cell line in the amnion could not be completely excluded [10,11].

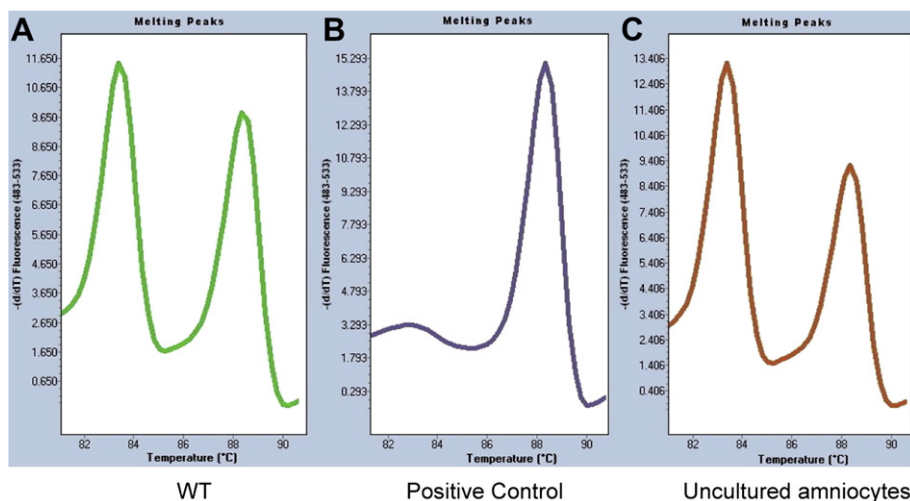


Fig. 5. *PEG1/MEST* methylation-sensitive high-resolution melting PCR assays show (A) a normal wild type (WT) with an unmethylated allele and a methylated allele, (B) a positive control of maternal uniparental disomy for chromosome 7 with only the methylated maternal allele, and (C) the uncultured amniocytes with an unmethylated allele and a methylated allele.

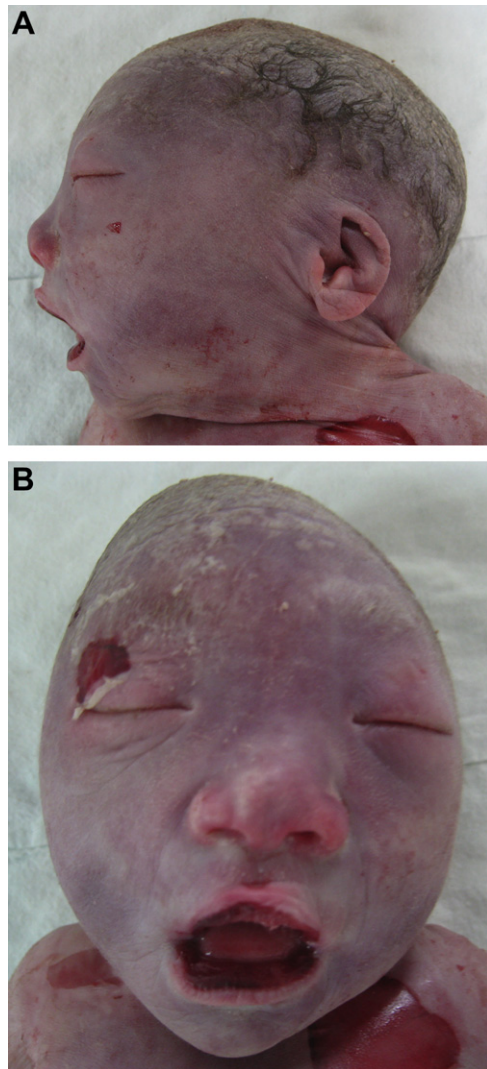


Fig. 6. The fetus at birth with microcephaly, hypertelorism, micrognathia, a depressed nasal bridge, and low-set ears.

Prenatal diagnosis of trisomy 7 mosaicism should raise a suspicion of UPD 7 and SRS [3–6]. Trisomy 7 mosaicism has variable and nonspecific clinical features. Our case represents a very unusual case with low-level trisomy 7 mosaicism at amniocentesis and an abnormal phenotype of IUGR, microcephaly, and facial dysmorphism but without UPD 7.

Acknowledgments

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