

Short Communication

Rapid positive confirmation of mosaicism for a small supernumerary marker chromosome as r(8) by interphase fluorescence *in situ* hybridization, quantitative fluorescent polymerase chain reaction, and array comparative genomic hybridization on uncultured amniocytes in a pregnancy with fetal pyelectasis

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Abstract

Objective: This study aimed at presenting prenatal diagnosis and molecular cytogenetic characterization of a small supernumerary marker chromosome (sSMC) derived from chromosome 8 by fluorescence *in situ* hybridization (FISH), quantitative fluorescent polymerase chain reaction (QF-PCR), and array comparative genomic hybridization (aCGH) on uncultured amniocytes.

Materials, Methods, and Results: A 32-year-old woman underwent amniocentesis at 19 weeks of gestation because of fetal pyelectasis. Amniocentesis revealed a *de novo* ring-shaped sSMC in two of 21 colonies of cultured amniocytes. Repeated amniocentesis at 22 weeks of gestation revealed a karyotype of 47,XY,+mar[8]/46,XY[32] in cultured amniocytes. Spectral karyotyping and FISH confirmed that the sSMC was derived from chromosome 8. She underwent a third amniocentesis at 26 weeks of gestation. Oligonucleotide-based aCGH analysis on uncultured amniocytes demonstrated a 43 Mb genomic gain in chromosome 8 encompassing 8p22→q12.1. Polymorphic DNA marker analysis of the uncultured amniocytes revealed a maternal origin of the sSMC and excluded uniparental disomy 8. Interphase FISH analysis showed three D8Z2 signals in 8/40 (20%) of uncultured amniocytes. The cultured amniocytes had a karyotype of 47,XY,+r(8)(p22q12.1)[3]/46,XY[37]. The pregnancy was carried to term, and an apparently normal baby, weighing 3300 g, was delivered with mild hydronephrosis but no other phenotypic abnormalities. The cord blood was found to have a karyotype of 47,XY,+r(8)(p22q12.1)[2]/46,XY[38].

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Conclusion: Prenatal diagnosis of fetal pyelectasis should alert obstetricians of chromosome aberration. Interphase FISH, QF-PCR, and aCGH analyses on uncultured amniocytes are helpful in rapid positive confirmation of an sSMC detected at amniocentesis.

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Keywords: array comparative genomic hybridization; interphase fluorescence *in situ* hybridization; prenatal diagnosis; quantitative fluorescent polymerase chain reaction; small supernumerary marker chromosome; supernumerary ring chromosome 8

Introduction

Chromosome 8-derived small supernumerary marker chromosomes (sSMCs) have been reported in at least 32 cases [1–3]. However, very few cases have been investigated by comprehensive molecular characterization and identification of the genetic component of such supernumerary marker chromosomes. Herein, we present our experience with mosaic chromosome 8-derived sSMC associated with fetal pyelectasis where additional molecular workup was performed on uncultured amniocytes, including interphase fluorescence *in situ* hybridization (FISH), quantitative fluorescent polymerase chain reaction (QF-PCR), and array comparative genomic hybridization (aCGH).

Materials, methods, and results

A 32-year-old, gravida 3, para 1, woman underwent amniocentesis at 19 weeks of gestation because of fetal pyelectasis. Amniocentesis revealed a ring-shaped sSMC in two

of 21 colonies of cultured amniocytes. The karyotype was 47,XY,+mar[2]/46,XY[19]. The parental karyotypes were normal. Repeated amniocentesis at 22 weeks of gestation found an increase of marker percentage with a karyotype of 47,XY,+mar[8]/46,XY[32] in cultured amniocytes. The sSMC was characterized by spectral karyotyping (SKY) using 24-color SKY probes (Applied Spectral Imaging, Carlsbad, CA, USA) (Fig. 1) and the whole chromosome painting probe 8 (WCP8) (Cytocell, Adderbury, Oxfordshire, UK) (Fig. 2). The sSMC was derived from chromosome 8. At 26 weeks of gestation, level II ultrasound revealed right hydronephrosis in the fetus. The fetal biometry was appropriate for gestational age, and other internal organs were unremarkable. The patient and her family still felt worried and fearful, so they consulted another medical center and underwent a third amniocentesis for further evaluation of the mosaic sSMC situation. Molecular cytogenetic techniques using aCGH, interphase FISH, and QF-PCR were applied to the uncultured amniocytes. The aCGH investigation using CytoChip Oligo array (BlueGnome, Cambridge, UK) on uncultured amniocytes manifested a 43

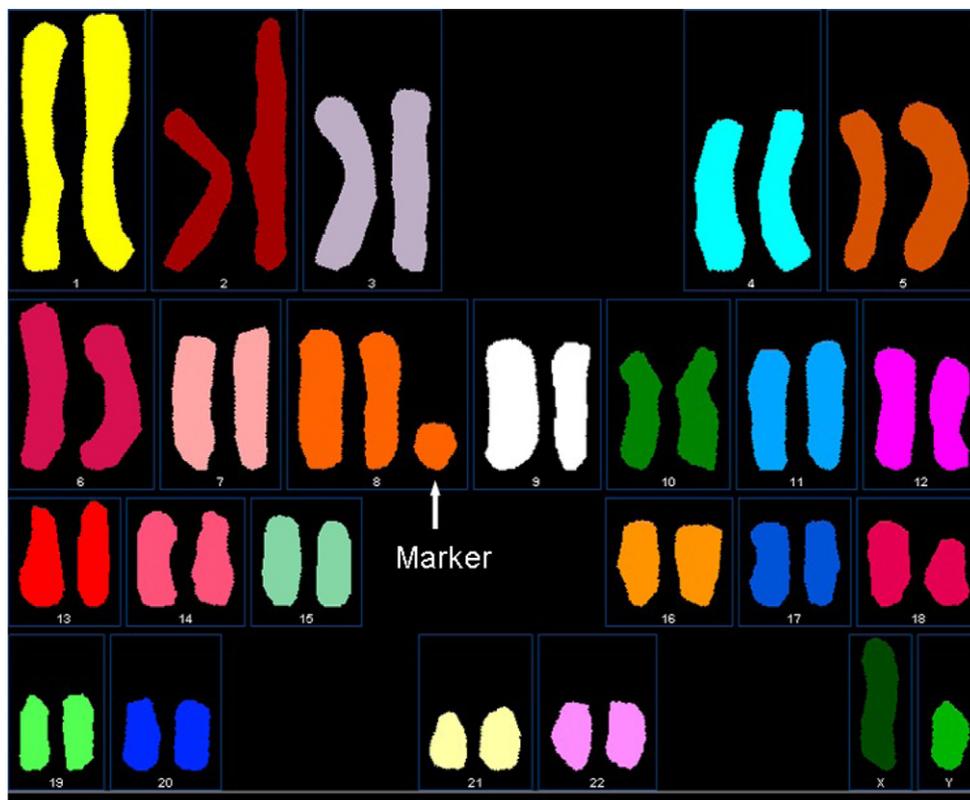


Fig. 1. SKY analysis of cultured amniocytes using 24-color SKY probes shows a small supernumerary marker chromosome derived from chromosome 8 (arrow). SKY = spectral karyotyping.

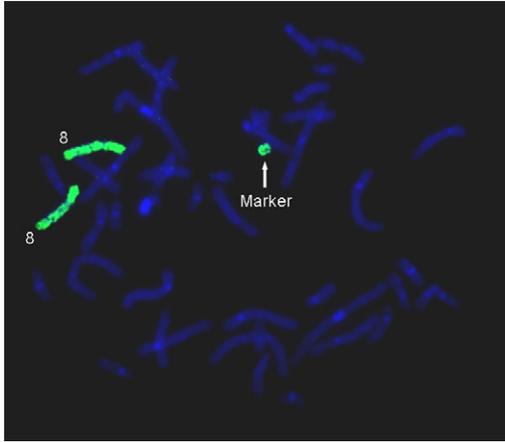


Fig. 2. Fluorescence *in situ* hybridization analysis of cultured amniocytes using a chromosome 8 whole chromosome painting probe (spectrum green) shows a chromosome 8-derived small supernumerary marker chromosome (arrow).

Mb genomic gain in chromosome 8 encompassing 8p22→q12.1 (18,100,180–61,104,884 bp) (UCSC hg18, NCBI Build 36, March 2006) (Fig. 3). Polymorphic DNA marker analysis of the uncultured amniocytes using chromosome 8-specific microsatellite markers revealed a biparental diallelic pattern for chromosome 8 (Fig. 4, Table 1). The microsatellite markers specific for the region between 8p22 and q12.1, such as D8S322, D8S1133, D8S2332, and D8S1102, revealed an increase of gene dosage in the maternal allele (Fig. 4, Table 1). Interphase FISH analysis on uncultured amniocytes using an 8p11.1–q11.2-specific probe (Vysis CEP8, D8Z2) (Abbott Laboratories, Abbott Park, IL, USA) showed three D8Z2 signals in eight (20%) and two signals in 32 (80%) of 40 uncultured amniocytes (Fig. 5). Cytogenetic analysis of the cultured amniocytes revealed a karyotype of 47,XY,+r(8)(p221q12.1)[3]/46,XY[37] (Fig. 6). The parents decided to continue with the pregnancy. At 38 weeks of gestation, a male baby with a body weight of 3300 g was delivered. He was apparently normal except for mild bilateral hydronephrosis. Postnatal cytogenetic analysis of the cord

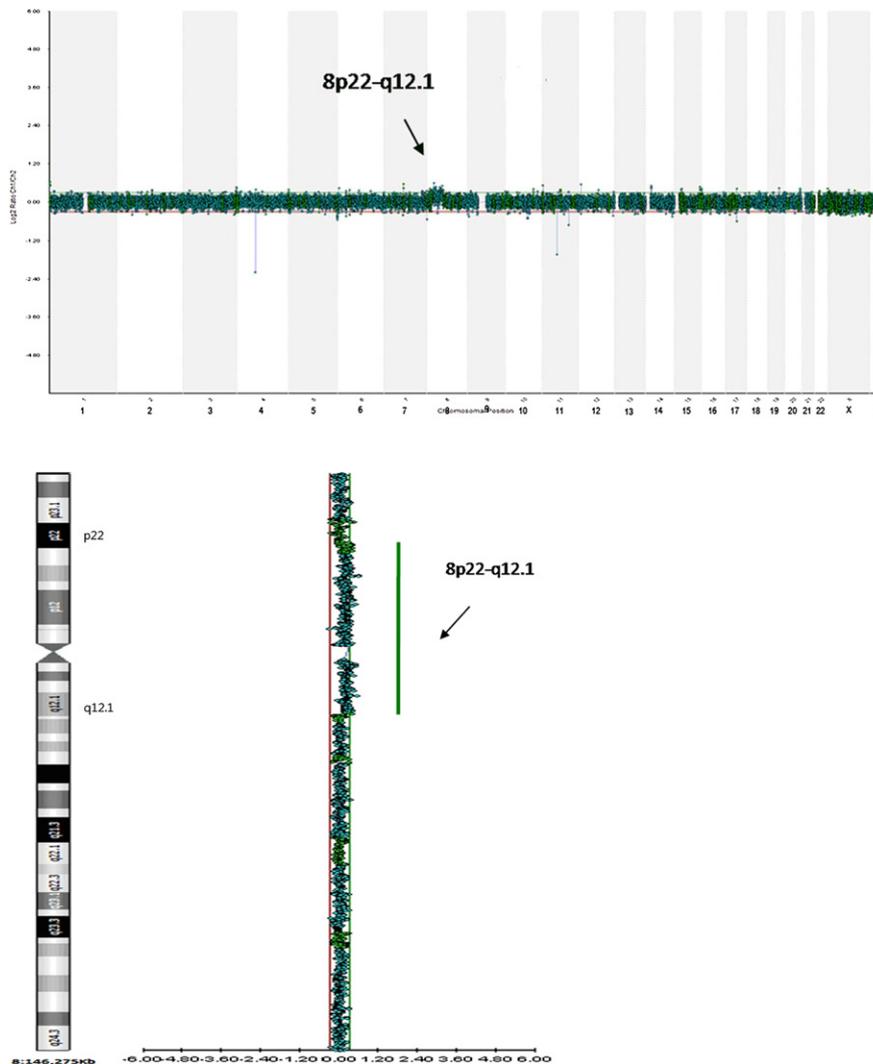


Fig. 3. Oligonucleotide-based array comparative genomic hybridization analysis using CytoChip Oligo array on uncultured amniocytes shows a 43 Mb genomic gain in chromosome 8 encompassing the region of 8p22→q12.1.

blood, umbilical cord, and placenta revealed the karyotype of 47,XY,+r(8)(p22q12.1)[2]/46,XY[38], 47,XY,+r(8)(p22q12.1)[2]/46,XY[18], and 47,XY,+r(8)(p22q12.1)[4]/46,XY[36], respectively.

Discussion

Application of molecular cytogenetic techniques on uncultured amniocytes has been well described in rapid positive confirmation of mosaicism for trisomies such as mosaic trisomy 2, mosaic trisomy 7, mosaic trisomy 8, and mosaic trisomy 9 [4–8]. In this study, we additionally showed the usefulness of interphase FISH, QF-PCR, and aCGH on uncultured amniocytes in rapid positive confirmation of mosaicism for an sSMC at amniocentesis. We found variations of the level of mosaicism between different amniocentesis. In this study, the first, second, and third amniocentesis revealed a mosaic level of 9.5%, 20%, and 7.5%, respectively, in cultured amniocytes, and the interphase FISH analysis on uncultured amniocytes revealed a mosaic level of 20%. Therefore, interphase FISH analysis on uncultured amniocytes may serve as a more truthful reflection of the mosaic level in their original, uncultured state against the results from different amniocentesis karyotyping where analysis was done on cultured cells. The present study also showed a discrepancy of mosaic level between the uncultured amniocytes and the cultured lymphocytes (20% vs. 5%). It is evident that interphase FISH on uncultured amniocytes provides more accurate information on mosaicism than blood lymphocytes.

QF-PCR and aCGH may have difficulty in rapid positive confirmation of mosaicism detected at amniocentesis in cases with low-level mosaicism. QF-PCR assay has been reported to detect mosaicism as low as 15% of the whole sample [9]. The detection rate for mosaicism using aCGH on uncultured amniocytes varies according to the different products of array chips [4–8]. Previously, we successfully detected mosaic

Table 1

Genotypic information of the father, mother, and uncultured amniotic fluid cells at short tandem repeat markers specific for chromosome 8 obtained by quantitative fluorescent polymerase chain reaction assays.^a

Markers	Locus	Father	Mother	Uncultured amniocytes
D8S1042	8p23.2	189, 189	189, 189	189, 189
D8S376	8p23.1	127, 127	127,127	127,127
D8S322	8p21.3	234, 250	234, 242	242, ^b 250
D8S1133	8q11.21	188, 188	200, 200	188, 200 ^b
D8S2332	8q12.1	174, 174	166, 178	166, ^b 174
D8S1102	8q12.1	192, 204	192, 200	192, 200 ^b
D8S1987	8q13.1	233, 233	221, 233	221, 233
D8S569	8q21.13	196, 204	196, 204	196, 204
D8S385	8q22.3	156, 168	152, 184	152, 156

^a Alleles (base pair sizes) are listed below each individual.

^b With increased dosage.

trisomy by aCGH using CytoChip Oligo array on uncultured amniocytes in a case of mosaic trisomy 9 with 48% (12/25) mosaicism, a case of mosaic trisomy 2 with 12% (6/50) mosaicism, and a case of mosaic trisomy 7 with 26% (13/50) mosaicism [6–8]. In this case of mosaic supernumerary r(8), both aCGH using CytoChip Oligo array and QF-PCR were able to detect a mosaic level of 20% (8/40) in uncultured amniocytes.

The peculiar aspect of the present case is the association of an sSMC 8 with fetal pyelectasis. In the present case, prenatal diagnosis of a mosaic supernumerary r(8)(p22q12.1) was achieved by amniocentesis in a 32-year-old woman because of the ultrasound findings of fetal pyelectasis in the second trimester. Renal abnormalities such as hydronephrosis and megacystitis have been well known to be associated with mosaic trisomy 8 [5,10,11]. Renal abnormalities have also been observed in patients with a supernumerary ring/marker chromosome 8. For instances, Butler et al [12] reported right hydronephrosis with bilateral vesicoureteral reflux in a patient with 45% mosaicism for a supernumerary r(8) in fibroblasts. Spinner et al [13] reported mild hydronephrosis and kidney malrotation in a patient with 56% mosaicism for a supernumerary marker 8p11q11 in blood cells. Starke et al [14] reported prenatally detected slight bilateral pyelectasis in a fetus with 54% mosaicism for a supernumerary marker 8p11q11 in amniocytes. Loeffler et al [15] reported left-sided renal hypoplasia and an enlarged right kidney with a duplicated collecting system in a patient with 70% mosaicism for a supernumerary r(8)(p12q12) in blood cells. Filges et al [16] reported a left pelvic kidney in a patient with 60% mosaicism for a supernumerary r(8)(p11.21q21.2) in blood cells. Chen et al [17] reported left multicystic kidney in a fetus with 93% mosaicism for a supernumerary r(8)(p11.21q11.1) in amniocytes. The present case further adds to the preexisting evidence that gene dosage increase in 8p11.21→q21.2 can result in fetal pyelectasis.

In conclusion, a low-level mosaicism for a supernumerary r(8)(p22q12.1) can present fetal pyelectasis, and prenatal diagnosis of fetal pyelectasis should raise suspicion of chromosome aberration. Molecular cytogenetic analyses on

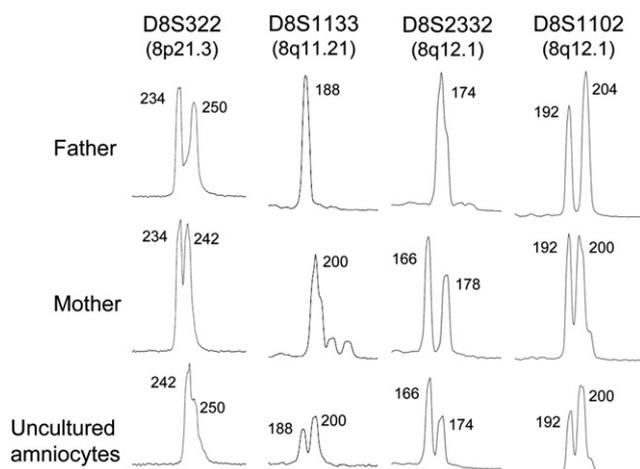


Fig. 4. Representative electrophoretograms of quantitative fluorescent polymerase chain reaction. The markers D8S322, D8S1133, D8S2332, and D8S1102 show two peaks of unequal fluorescent activity from two different parental alleles in uncultured amniocytes with a dosage increase in the maternal allele and a dosage ratio of 1:1.2 (paternal: maternal).

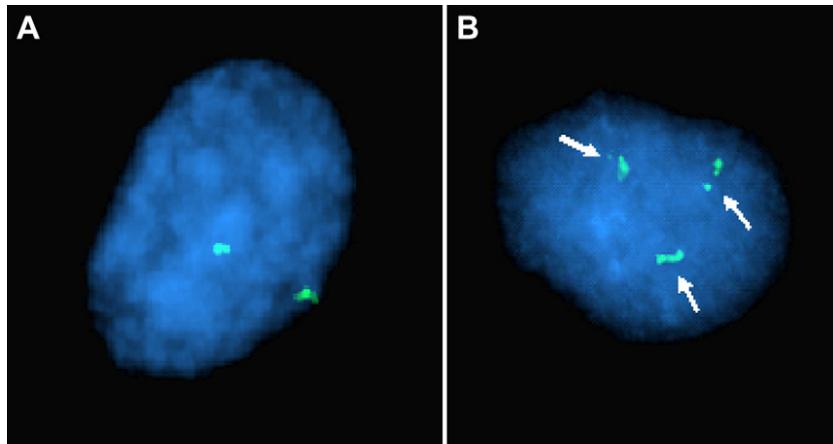


Fig. 5. Interphase fluorescence *in situ* hybridization analysis on uncultured amniocytes using an 8p11.1–q11.2-specific probe (Vysis CEP8, D8Z2; spectrum green) shows (A) two green signals in a cell with disomy 8 and (B) three green signals (arrows) in a cell with a supernumerary r(8).

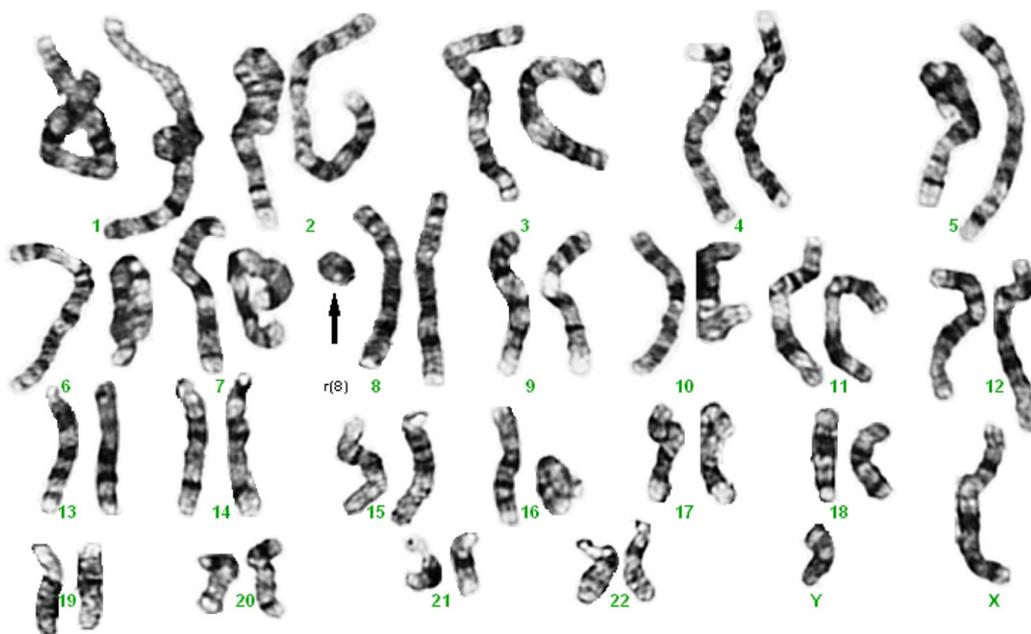


Fig. 6. The G-banded karyotype of 47,XY,+r(8)(p22q12.1). The arrow indicates a supernumerary ring chromosome derived from chromosome 8.

uncultured amniocytes have the advantage of rapid positive confirmation of a supernumerary ring chromosome detected at amniocentesis.

Acknowledgments

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