Taiwanese Journal of Obstetrics & Gynecology 54 (2015) 58–61

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Short Communication

Detection of no isochromosome 20q by interphase fluorescent *in situ* hybridization on uncultured amniocytes in a pregnancy with mosaic isochromosome 20q in cultured amniocytes at amniocentesis

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ARTICLE INFO

Article history:
Accepted 11 December 2014

Keywords:
amniocentesis
amniocytes
isochromosome 20q
mosaicism
prenatal diagnosis

ABSTRACT

Objective: To present prenatal diagnosis and molecular cytogenetic characterization of mosaic isochromosome 20q at amniocentesis.
Materials and methods: A 36-year-old woman underwent amniocentesis at 17 weeks of gestation because of advanced maternal age, and conventional cytogenetic analysis revealed a karyotype of 46,XY,i(20)(q10)[12]/46,XY[7]. Repeated amniocentesis was performed at 20 weeks of gestation. During repeated amniocentesis, array comparative genomic hybridization (aCGH), interphase fluorescence *in situ* hybridization (FISH), and quantitative fluorescent polymerase chain reaction (QF-PCR) were performed on uncultured amniocytes, and conventional cytogenetic analysis and interphase FISH were performed on cultured amniocytes.
Results: Conventional cytogenetic analysis of cultured amniocytes revealed a karyotype of 46,XY,i(20)(q10)[4]/46,XY[16]. Interphase FISH analysis on 217 uncultured amniocytes did not detect isochromosome 20q, aCGH on the DNA extracted from uncultured amniocytes showed no genomic imbalance, and QF-PCR analysis on the DNA extracted from uncultured amniocytes excluded uniparental disomy 20 (UPD 20). Interphase FISH analysis on 115 cultured untouched amniocytes revealed 13% (15/115 cells) mosaicism for isochromosome 20q.
Conclusion: Mosaic isochromosome 20q detected at amniocentesis can be a cell culture artifact. Detailed ultrasound examination, performing interphase FISH and/or aCGH on uncultured amniocytes for confirmation of true mosaicism, and performing QF-PCR to exclude UPD 20 may be useful under such a circumstance.
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Introduction

Mosaic isochromosome 20q identified at amniocentesis has been shown to be a benign condition in most reported cases [1–6]. Robinson et al [3] suggested that the isochromosome 20q arises due to a postzygotic error, and its growth persists *in vitro* in a few specific cell types of amniocytes. We previously reported on the

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http://dx.doi.org/10.1016/j.tjog.2014.12.002
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cytogenetic discrepancy between uncultured amniocytes and cultured amniocytes, and suggested that the mosaic isochromosome 20q detected at amniocentesis is a cell culture artifact [4–6]. Here, we present an additional case in which no isochromosome 20q was detected by interphase fluorescence *in situ* hybridization (FISH) on uncultured amniocytes in a pregnancy with mosaic isochromosome 20q in cultured amniocytes at amniocentesis.

Materials and methods

Clinical description

A 36-year-old, primigravid woman underwent amniocentesis at 17 weeks of gestation because of advanced maternal age. Conventional cytogenetic analysis using cultured amniocytes revealed a karyotype of 46,XY,i(20)(q10)[12]/46,XY[7]. Among 19 colonies of cultured amniocytes, 12 colonies had a karyotype of 46,XY,i(20)(q10), whereas the other seven colonies had a karyotype of 46,XY. Prenatal ultrasound findings were unremarkable. The woman requested repeated amniocentesis at 20 weeks of gestation. During repeated amniocentesis, array comparative genomic hybridization (aCGH) was performed on the DNA extracted from uncultured amniocytes, interphase FISH was applied on uncultured amniocytes and cultured amniocytes, and quantitative fluorescent polymerase chain reaction (QF-PCR) analysis using informative polymorphic DNA markers was performed on the DNAs extracted from uncultured amniocytes and parental blood samples. Conventional cytogenetic analysis was performed on cultured amniocytes and parental blood samples.

Conventional cytogenetic analysis

Routine cytogenetic analysis by G-banding techniques at the 550 bands of resolution was performed. Samples of amniotic fluid and parental blood were collected, and they were subjected to cell culture according to the standard cytogenetic protocol.

Array comparative genomic hybridization

Whole-genome aCGH on the DNA extracted from uncultured amniocytes derived from 10 mL of amniotic fluid was performed using NimbleGen ISCA Plus Cytogenetic Array (Roche NimbleGen, Madison, WI, USA). The NimbleGen ISCA Plus Cytogenetic Array has 630,000 probes and a median resolution of 15–20 kb across the entire genome (according to the manufacturer). The DNA from uncultured amniocytes was extracted first. The extraction was carried out using the QIAamp DNA Mini kit (Qiagen, Inc., Valencia, CA, USA) by following the manufacturer's protocol. Then, 0.5 μg of the extracted DNA was labeled in Cy5 dye and compared with an equivalent amount of normal female genomic DNA (G1521, Promega, Madison, WI, USA) labeled in Cy3 dye to perform the aCGH experiment. The experiment was performed according to the procedures recommended in the Roche NimbleGen ISCA plus Cytogenetic Array's user manual. The data were finally represented using Nexus 6.1 (BioDiscovery, Hawthorne, CA, USA).

Fluorescence in situ hybridization

Interphase FISH analysis was performed on 100 uncultured amniocytes using a 20q13.33-specific bacterial artificial chromosome (BAC) probe (RP11-266K16) (62,706,872-62,959,918) [hg 19] [fluorescein isothiocyanate (FITC), green spectrum] according to the standard FISH protocol. Interphase FISH analysis was performed on another 117 uncultured amniocytes using a 20q11.21-specific BAC probe (RP11-702M8) (30,180,454-30,348,569) [hg 19] (FITC, green spectrum) according to the standard FISH protocol. For the RP11-702M8 probe, a control study was performed on 136 interphase amniocytes from a normal database containing untouched cultured amniocytes. Interphase FISH analysis was performed on 115 cultured untouched amniocytes of this case using a 20q13.3-specific BAC probe of RP11-266K16 (FITC, green spectrum) and a 20p12.2-specific BAC probe of RP11-2E8 (10,613,237-10,817,493) [hg 19] (red spectrum) according to the standard FISH protocol.

Quantitative fluorescent polymerase chain reaction

QF-PCR analysis was performed using genomic DNAs extracted from uncultured amniocytes and parental blood samples. Primers specifically flanking polymorphic markers on chromosome 20 such as D20S605 (20p12.1) and D20S1083 (20q13.2) were used to exclude uniparental disomy 20 (UPD 20).

Results

G-banding chromosome analysis of cultured amniocytes at repeated amniocentesis revealed a karyotype of 46,XY,i(20)(q10)[4]/46,XY[16]. Among 20 colonies of cultured amniocytes, four colonies had a karyotype of 46,XY,i(20)(q10) (Fig. 1), whereas the other 16 colonies had a karyotype of 46,XY. Whole-genome aCGH analysis on the DNA extracted from uncultured amniocytes showed no genomic imbalance. Interphase FISH analysis on 100 uncultured amniocytes using a 20q13.33-specific probe of RP11-266K16 (green spectrum) revealed two green signals in all 100 cells (Fig. 2). Interphase FISH analysis on another 117 uncultured amniocytes using a 20q11.21-specific probe of RP11-702M8 (green spectrum) revealed two green signals in all 117 cells (Fig. 2). In the 136 interphase-cultured amniocytes of normal control, 130 cells had two green signals, four cells had one green signal, and two cells had three signals. Interphase FISH analysis on 115 cultured untouched amniocytes of this case using a 20q13.3-specific probe of RP11-266K16 (green spectrum) and a 20p12.2-specific probe of RP11-2E8 (red spectrum) showed two green signals and two red signals in 100 cultured untouched amniocytes, and one red signal and three green signals in 15 cultured untouched amniocytes, indicating the presence of mosaic isochromosome 20q in cultured amniocytes (Fig. 3). QF-PCR analysis of uncultured amniocytes excluded UPD 20 (Fig. 4). The patient was advised to continue the pregnancy.

Discussion

Chen [1] previously reported on the prenatal diagnosis of 50% (14/28 colonies) mosaicism for isochromosome 20q at amniocentesis using cultured amniocytes, and postnatal diagnosis of a karyotype of 46,XX in the blood, placenta, skin, and liver in a fetus with arthrogryposis multiplex congenita (amyoplasia) and a single umbilical artery. Chen [2] additionally reported a case with 26.9% (7/26 colonies) mosaicism for isochromosome 20q at amniocentesis using cultured amniocytes, and a karyotype of 46,XY in the cord blood, amniotic membrane, placenta, umbilical cord, liver, lung, and skin in a fetus with no phenotypic abnormalities. Chen et al [4] later reported cytogenetic discrepancy between uncultured amniocytes and cultured amniocytes in mosaic isochromosome 20q detected at amniocentesis. In that case, there were 23.5% (4/17 colonies) mosaicism for isochromosome 20q at the first amniocentesis and 33.3% (8/24 colonies) mosaicism for isochromosome 20q at repeated amniocentesis using cultured amniocytes. Interphase FISH analysis on 50 uncultured amniocytes did not detect isochromosome 20q, and aCGH on the DNA extracted from uncultured amniocytes showed no genomic imbalance; however,

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