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Short Communication

Chromosome 18p deletion syndrome presenting holoprosencephaly and premaxillary agenesis: Prenatal diagnosis and aCGH characterization using uncultured amniocytes

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ABSTRACT

We present prenatal diagnosis of a de novo distal 18p deletion involving 14.06 Mb at 18p11.32–p11.21 by aCGH using uncultured amniocytes in a pregnancy with fetal holoprosencephaly and premaxillary agenesis. QF-PCR analysis showed that distal 18p deletion was from maternal origin. Metaphase FISH analysis confirmed haploinsufficiency of *TGIF*. We discuss the functions of the genes that are deleted within this region. The present case shows the usefulness of applying aCGH on uncultured amniocytes for rapid aneuploidy diagnosis in cases with prenatally detected fetal structural abnormalities.

1. Introduction

Chromosome 18p deletion [del(18p)] syndrome (OMIM 146390), first described by de Grouchy et al. (1963) and de Grouchy (1969), is a contiguous gene deletion syndrome that is characterized by mental retardation, short stature, growth retardation, craniofacial dysmorphisms of round face, short protruding philtrum, palpebral ptosis, large dysplastic ears, wide mouth, dental abnormalities and abnormalities of the limbs, genitalia, eyes, heart and brain (Brenk et al., 2007; Turleau, 2008). In patients with del(18p) syndrome, about two-thirds of the cases have a de novo pure terminal deletion, while one-third of the cases are caused by a de novo unbalanced translocation, malsegregation of a parental translocation or inversion, and a ring chromosome or isochromosome 18q (Chen et al., 2001, 2008, 2010, 2011a; Turleau, 2008). Women with del(18p) syndrome may be fertile, and familial del(18p) syndrome has been reported (Maranda et al., 2006; Tsukahara et al., 2001).

Prenatal diagnosis of de novo del(18p) associated with HPE is very rare (Lim et al., 2008; Sepulveda, 2009). Rapid aneuploidy diagnosis of del(18p) by aCGH using uncultured amniocytes has not previously been described. Here, we report prenatal diagnosis and molecular cytogenetic characterization of a distal 18p deletion by aCGH and QF-PCR in a fetus with holoprosencephaly (HPE) and premaxillary agenesis (PMA).

2. Methods for detection

2.1. Array-CGH

Whole-genome aCGH on 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. The DNA

Abbreviations: aCGH, Array comparative genomic hybridization; HPE, Holoprosencephaly; del, Deletion; PMA, Premaxillary agenesis; OMIM, Online Mendelian Inheritance in Man; FISH, Fluorescence in situ hybridization; BAC, Bacterial artificial chromosome; QF-PCR, Quantitative fluorescent polymerase chain reaction; STRs, Short tandem repeats; CNS, Central nervous system.

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from the cells in amniotic fluid was extracted first. It was done by following the manufacturer's protocol of QIAamp DNA Mini kit (Qiagen, Inc., Valencia, CA, USA). Then, the 0.5 μg of the extracted DNA was labeled in Cy5 dye compared with equivalent amount of normal female gDNA (G1521, Promega) labeled in Cy3 dye to perform the aCGH experiment. The experiment was performed according to the procedures recommended from Roche NimbleGen ISCA plus Cytogenetic Array's user guide. The data were finally represented by using Nexus 6.1 (BioDiscovery, Hawthorne, CA, USA).

2.2. Conventional cytogenetic analysis

Routine cytogenetic analysis by G-banding techniques at the 550 bands of resolution was performed. About 16 mL of amniotic fluid was collected, and the sample was subjected to in situ amniocyte culture. Twenty colonies of cultured amniocytes were investigated in the in situ culture of amniocytes. Parental bloods were collected, and the samples were subjected to lymphocyte culture. Twenty cells of cultured lymphocytes were investigated in the culture of lymphocytes.

2.3. FISH

FISH analysis was performed on the cultured amniocytes using 18p11.31-specific BAC probe RP11-325E18 encompassing *TGIF* (spectrum red) (3,332,636–3,511,777) and 18q23-specific BAC probe RP11-154H12 (spectrum green) (77,421,494–77,584,017), according to the standard FISH protocol. Briefly, probes and the slide were prepared as follows. Three hundred nanogram of each labeled probe was mixed with 9 μg of human Cot-1 DNA (Invitrogen). The probe cocktail was purified by adding 13 μL of 10 M NH_4OAc and 130 μL of cold ethanol to carry out ethanol precipitation. After incubating at −80 °C for 30 minutes and centrifuging at 4 °C for 30 minutes, air-dried pellet was resuspended in 10 μL of hybridization buffer (10% dextran sulfate, 50% formamide/2× SSC, pH 7.0, custom-made). The well-mixed probe cocktail was denatured at 76 °C for 10 minutes, and then reannealed at 37 °C for 20 minutes.

Meanwhile, slide preparation was proceeded. The slide was treated with 0.005% pepsin in 0.01 N HCl at 37 °C for 2 minutes and washed with PBS briefly. The slide was dehydrated in an ethanol series (70%, 80% and 100% at room temperature, each for 2 minutes) and then air-dried. After that, the slide was denatured in the denature solution (70% formamide/2× SSC, pH 7.0) at 76 °C for 3 minutes, and the slide was dehydrated again with an ethanol series described above for 2 minutes each and air-dried. All of 10 μL probe cocktail was dropped onto the slide and covered with a 22 × 22 mm coverslip. After sealing the margin with rubber cement, the slide was placed in a humidified chamber and incubated at 37 °C overnight.

After removing the coverslip, the slide was washed in 3 serial washing solutions (50% formamide/2× SSC, pH 7.0) at 45 °C, each for 5 minutes, followed by once in 2× SSC, once in 1× SSC and once in 0.1× SSC at 45 °C for 5 minutes each. The slide was rinsed in ddH_2O and air-dried. Counterstain was done by dipping the slide in 4′,6-diamidino-2-phenylindole (20 ng/mL of DAPI solution) for 5 minutes, then rinsed the slide with ddH_2O and air-dried. The slide was mounted with anti-fade mounting medium (VECTOR Laboratories, Inc.) and covered with a 24 × 50 mm coverslip.

2.4. QF-PCR

QF-PCR analysis was performed by using genomic DNAs of uncultured fetal tissues and parental bloods as described elsewhere (Chen et al., 2000). Briefly, primers specifically flanking STRs markers on chromosome 18p region such as D18S818 (18p11.32), D18S976 (18p11.31) and D18S391 (18p11.31) were applied to undertake polymorphic marker analysis and parental origin determination of the deletion.

3. Results

3.1. Clinical description

A 36-year-old, gravida 2, para 1, woman was referred to the hospital for genetic counseling at 19 weeks of gestation because of advanced maternal age and sonographic findings of craniofacial abnormalities. She and her husband were not related and healthy. The husband was 34 years old. There was no family history of diabetes mellitus or congenital malformations. The woman was not expose to teratogenic agents, irradiation or infectious disease during this pregnancy. Maternal urine examination revealed no glycosuria. Level II ultrasound at 19 weeks of gestation showed HPE and median facial cleft consistent with the diagnosis of HPE-PMA (Fig. S1). Amniocentesis was performed at 19 weeks of gestation. Whole-genome aCGH analysis was performed on uncultured amniocytes to determine genomic imbalance. Conventional cytogenetic analysis was performed on cultured amniocytes to determine chromosome abnormalities. Metaphase FISH analysis was performed on cultured amniocytes to determine the deletion of *TGIF*. The parents elected to terminate the pregnancy. QF-PCR analysis was performed on fetal tissues and parental bloods to determine parental origin of the deletion.

3.2. Molecular and cytogenetic data and postnatal findings

Whole-genome aCGH analysis on uncultured amniocytes detected a 14.06-Mb deletion at 18p11.32–p11.21, or arr 18p11.32p11.21 (64,223–14,124,700) × 1 (NCBI build 37) (Fig. 1). The deleted region encompasses 140 genes including the 44 OMIM genes of *USP14*, *THOC1*, *COLEC12*, *CETN1*, *TYMS*, *ENOSF1*, *YES1*, *ADCYAP1*, *NDC80*, *EMILIN2*, *LPIN2*, *MYOM1*, *MYL12B*, *TGIF*, *DLGAP1*, *ZBTB14*, *EPB41L3*, *ARHGAP28*, *LAMA1*, *PTPRM*, *NDUFV2*, *ANKRD12*, *TWSG1*, *RALBP1*, *PPP4R1*, *RAB31*, *VAPA*, *APCDD1*, *NAPG*, *PIEZO2*, *GNAL*, *CHMP1B*, *MPPE1*, *IMPA2*, *CIDEA*, *AFG3L2*, *SPIRE1*, *PSMG2*, *PTPN2*, *SEH1L*, *LDLRAD4*, *RNMT*, *MC5R* and *MC2R*. The karyotype of 20 cultured lymphocytes of paternal blood was 46,XY. The karyotype of 20 cultured lymphocytes of maternal blood was 46, XX. The karyotype of 20 colonies of cultured amniocytes was 46,XX, del(18)(p11.21)dn (Fig. 2). Metaphase FISH analysis on 13 cultured amniocytes showed absence of 18p11.31-specific probe signal on the aberrant chromosome 18, indicating an 18p deletion encompassing *TGIF* (Fig. 3). QF-PCR assays showed only the paternal allele in the fetus on the informative markers of D18S818 (18p11.32), D18S976 (18p11.31) and D18S391 (18p11.31), indicating a distal 18p deletion from maternal origin (Fig. 4). A 280-g female fetus was delivered with hypertelorism, epicanthic folds, median cleft lip and palate, and large low-set ears (Fig. S2).

4. Discussion

The present case prenatally manifested HPE-PMA. In the present case, aCGH on uncultured amniocytes rapidly detected a distal 18p deletion. Rapid aneuploidy diagnosis using uncultured amniocytes by aCGH has the advantage of rapid prenatal diagnosis of chromosomal abnormalities in cases with fetal structural anomalies without the need of cell culture (Chen et al., 2011b, 2012).

The peculiar aspect of the present case is the association with a 14.06-Mb deletion at 18p11.21 → pter encompassing *TGIF* and *TWSG1* and the phenotype of HPE-PMA. *TGIF* or *TGIF1* (OMIM 602630) encodes transforming growth factor-β-induced factor which belongs to evolutionarily conserved atypical homeodomain proteins that act as transcriptional repressors and co-repressors in retinoid and transforming growth factor signaling pathway (Shen and Walsh, 2005). *TGIF* encodes for a transcription factor that