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Methods paper

3q26.31–q29 duplication and 9q34.3 microdeletion associated with omphalocele, ventricular septal defect, abnormal first-trimester maternal serum screening and increased nuchal translucency: Prenatal diagnosis and aCGH characterization

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ABSTRACT

We present prenatal diagnosis and array comparative genomic hybridization characterization of 3q26.31–q29 duplication and 9q34.3 microdeletion in a fetus with omphalocele, ventricular septal defect, increased nuchal translucency, abnormal first-trimester maternal screening and facial dysmorphism with distinct features of the 3q duplication syndrome and Kleefstra syndrome. The 26.61-Mb duplication of 3q26.31–q29 encompasses *EPHB3*, *CLDN1* and *CLDN16*, and the 972-kb deletion of 9q34.3 encompasses *EHMT1*. We review the literature of partial trisomy 3q associated with omphalocele and discuss the genotype–phenotype correlation in this case.

1. Introduction

Partial 3q duplication involving the minimal critical region of 3q26.3–q29 has been associated with the 3q duplication syndrome which has clinical overlap with Cornelia de Lange syndrome such as facial dysmorphism of hypertrichosis, prominent eyelashes, bushy eyebrows, broad nose with anteverted nares and depressed nasal bridge, hypertelorism, up-slanting palpebral fissures, epicanthic folds, long philtrum, micrognathia, short and webbed neck and low anterior hairline, rhizomelic shortening of the limbs, genital hypoplasia, congenital heart defects (septal defects) and developmental delay (Aqua et al., 1995; Battaglia et al., 2006; Chen et al., 2011a; Deardorff et al., 2011; Faas et al., 2002; Grossmann et al., 2009; Rizzu and Baldini, 1994; Shanske et al., 2010; Steinbach et al., 1981).

Partial trisomy 3q can be associated with major congenital malformations such as Dandy–Walker malformation (de Azevedo Moreira et al., 2005; Jung et al., 2012), cerebellar hypoplasia and corpus callosum agenesis (Roberts et al., 2006), microcephaly (Ballif et al., 2008; Grossmann et al., 2009; Lisi et al., 2008; Meins et al., 2005; Wilson et al., 1978), sacrococcygeal teratoma (Dundar et al., 2011), lumbosacral meningocele (Prabhakara et al., 2008), spina bifida or sacral dimple (Allderdice et al., 1975), cystic hygroma (Chen, 2001; Pires et al., 2005), congenital heart defects (Azar et al., 1999; Ballif et al., 2008; Faas et al., 2002; Grossmann et al., 2009; Lisi et al., 2008; Meins et al., 2005), kidney defects (Grossmann et al., 2009), and omphalocele (Allderdice et al., 1975; Chen, 1999, 2007; Chen et al., 1996, 1997; Cinti

Abbreviations: aCGH, array comparative genomic hybridization; NT, nuchal translucency; del, deletion; MoM, multiples of the median; β-hCG, β-human chorionic gonadotropin; dn, *de novo*; PAPP-A, pregnancy-associated plasma protein-A; der, derivative chromosome; dup, duplication; inv, inversion; t, translocation; trp, triplication; OMIM, Online Mendelian Inheritance in Man; QF-PCR, quantitative fluorescent polymerase chain reaction; STRs, short tandem repeats; VSD, ventricular septal defect; ASD, atrial septal defect.

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et al., 2000; Mulcahy et al., 1979; Õunap et al., 2005; Park et al., 2008; Wilson et al., 1985; Yatsenko et al., 2003).

Pure partial trisomy 3q is rare, and most reported cases with partial trisomy 3q have been associated with a large duplication of 3q and an additional second chromosomal imbalance. Here, we present our experience of prenatal diagnosis and aCGH characterization of *de novo* 3q26.31–q29 duplication and 9q34.3 microdeletion in a fetus with omphalocele, VSD, increased NT, abnormal first-trimester maternal screening and facial dysmorphism with distinct features of the 3q duplication syndrome and Kleefstra syndrome.

2. Clinical description

A 35-year-old, primigravid woman underwent first-trimester maternal serum screening at 12 weeks of gestation. Prenatal ultrasound revealed an increased NT thickness of 4.7 mm and omphalocele (Fig. S1). Maternal serum screening showed the results of an elevated free β-hCG level of 4.04 MoM and a PAPP-A level of 1.069 MoM. The risk calculation was as the following: trisomy 21 = 1/8, trisomy 18 = 1/136, and trisomy 13 = 1/1707. She underwent non-invasive prenatal screening using maternal blood at 15 weeks of gestation, and the result excluded common trisomies. Amniocentesis was performed at 17 weeks of gestation, and conventional cytogenetic analysis revealed a derivative chromosome 9 [der(9)] with additional maternal of unknown origin attached to the distal end of the long arm of chromosome 9. The nature of the der(9) was investigated by aCGH on cultured amniocytes. Level II ultrasound at 23 weeks of gestation revealed omphalocele, VSD and nuchal edema (Fig. S2). The pregnancy was subsequently terminated. The cord blood was collected for cytogenetic analysis.

3. Methods for detection

3.1. Conventional cytogenetic analysis

Routine cytogenetic analysis by G-banding techniques at the 550 bands of resolution was performed. About 20 mL of amniotic fluid was collected, and the sample was subjected to *in situ* amniocyte culture according to the standard cytogenetic protocol (Peakman, 1991). Parental bloods and cord blood were collected, and the samples were subjected to lymphocyte culture according to the standard blood cytogenetic protocol (Jenks and Taplett, 1991).

3.2. Array-CGH

Whole-genome aCGH on the DNA extracted from cultured amniocytes 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's instruction. The DNA from amniocytes 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).

3.3. QF-PCR

QF-PCR analysis was performed by using genomic DNAs extracted from fetal tissues and parental bloods as described elsewhere (Chen et al., 2000). Briefly, primers specifically flanking STRs markers on chromosome 3q region such as D3S1754 (3q26.32), D3S2425 (3q26.31), D3S1744 (3q24) and D3S2440 (3q24) were applied to undertake polymorphic marker analysis and parental origin determination of the distal 3q deletion.

4. Results

Whole-genome aCGH analysis on the DNA extracted from cultured amniocytes detected a 26.61-Mb duplication at 3q26.31–q29, or arr [hg 19] 3q26.31q29 (171,287,090–197,897,268) × 3 and a 972.25-kb deletion at 9q34.3 or arr [hg 19] 9q34.3 (140,047,349–141,019,600) × 1 (Fig. 1). The duplicated 3q26.31–q29 region contains 300 genes including 119 OMIM genes (Table S1). The deleted 9q34.3 region contains 39 genes including 16 OMIM genes (Table S2). The father had a karyotype of 46,XY, and the mother had a karyotype of 46,XX. The karyotype of the cultured amniocytes in 23 colonies was 46,XX,der(9)t(3;9)(q26.31;q34.3)dn (Fig. 2). A 680-g female malformed fetus was delivered with hypertrichosis, synophrys, bushy eyebrows, midface hypoplasia, broad nose with anteverted nares and depressed nasal bridge, hypertelorism, epicanthic folds, long philtrum, low-set ears with malformed pinnas, full everted lower lip, cupid bowed upper lip, prognathism, short and webbed neck, nuchal edema and a small omphalocele (Fig. S3). Postnatal cytogenetic analysis of cord blood revealed a karyotype of 46,XX,der(9)t(3;9)(q26.31;q34.3)dn in 20 cultured lymphocytes. QF-PCR assays showed duplication of the paternal allele in the fetus on the informative markers of D3S1754 (3q26.32) and D3S2425 (3q26.31), indicating a paternal origin of the distal 3q duplication and the unbalanced translocation (Fig. 3).

5. Discussion

An abnormal maternal serum screening and increased NT may result in incidental detection of uncommon fetal aneuploidy (Chen et al., 2011b, 2012, 2013a,b). The present case manifested increased NT and an abnormally high maternal serum free β-hCG level in the first trimester and nuchal edema in the second trimester. This is the first report of partial trisomy 3q pregnancy associated with a distinctive first-trimester maternal serum screening pattern of an extremely high level of maternal serum free β-hCG associated with increased NT. Increased NT and nuchal edema can be prenatal features of partial trisomy 3q. Chen (2001) reported cystic hygroma and unilateral pleural effusion in a 16-gestational-week fetus with partial trisomy 3q (3q22 → qter) and partial monosomy 6q (6q25.3 → qter). Park et al. (2008) reported an increased NT thickness of 3.5 mm and liver herniation in a 10-gestational-week fetus with partial trisomy 3q (3q25.1 → qter) and partial monosomy 9p (9p24.2 → pter). The present case provides evidence that partial trisomy 3q pregnancy may present an abnormally high level of maternal serum free β-hCG in the first-trimester screening associated with increased NT.

The present case has characteristic features of the 3q duplication syndrome such as hypertrichosis, bushy eyebrows, broad nose with anteverted nares and depressed nasal bridge, hypertelorism, long philtrum, low-set ears with malformed pinnas, short and webbed neck and congenital heart defects. The peculiar aspect of the present case is the association with omphalocele. To date, at least 13 cases of partial trisomy 3q associated with omphalocele have been reported. Table 1 shows the reported cases with chromosomal rearrangements involving partial trisomy 3q and omphalocele. Allderdice et al. (1975) reported the association of omphalocele with dup(3)(q21 → qter) and del(3)(p25 → pter) in the children of two families with parental carriers of inv(3)(p25q21). Mulcahy et al. (1979) reported a 3-month-old girl with dup(3)(q24 → qter), del(3)(p25 → pter) and omphalocele born to a carrier mother with inv(3) (p25q24). Chen et al. (1996, 1997) and Chen (1999) reported recurrent omphalocele in three affected siblings with dup(3)(q21 → qter) and del(11)(q23 → qter) born to a carrier mother with t(3;11)(q21;q23). Cinti et al. (2000) reported a 15-gestational-week fetus with dup(3)(q24 → qter), del(20)(p13 → pter)

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