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Short Communication

Chromosome 22q11.2 deletion syndrome: prenatal diagnosis, array comparative genomic hybridization characterization using uncultured amniocytes and literature review

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ABSTRACT

We present prenatal diagnosis of *de novo* 22q11.2 microdeletion syndrome using uncultured amniocytes in a pregnancy with conotruncal heart malformations in the fetus. We discuss the genotype–phenotype correlation and the consequence of haploinsufficiency of *TBX1*, *COMT*, *UFD1L*, *GNB1L* and *MED15* in the deleted region. We review the literature of chromosomal loci and genes responsible for conotruncal heart malformations and tetralogy of Fallot.

1. Introduction

Chromosome 22q11.2 deletion syndrome occurs in 1:4000–1:8000 live births (Scambler, 2000). Chromosome 22q11.2 deletion syndrome, including DGS (OMIM 188400) and VCFS (OMIM 192430), is caused by a 1.5–3.0-Mb hemizygous deletion of 22q11.2 and is associated with a highly variable phenotype caused mainly by haploinsufficiency of the *TBX1* gene (OMIM 602054) which is located at 22q11.21 (McDonald-McGinn et al., 2013; Molesky, 2011; Yu et al., 2012). DGS is characterized by outflow tract defects of the heart, thymic hypoplasia, parathyroid hypoplasia, hypocalcemia and T-cell immunodeficiency (Scambler et al., 1991). VCFS is characterized by velopharyngeal insufficiency with cleft palate, speech disorders, cardiac defects, microcephaly, short stature, typical facial appearance, auricular anomalies, learning problems, cognitive difficulties, and intellectual disabilities (Driscoll et al., 1992; Shprintzen et al., 1981).

With the advent of ultrasound and molecular genetic technology, prenatal diagnosis of 22q11.2 deletion syndrome is possible by the use of FISH, MLPA, aCGH and next generation sequencing (Chen and Chien, 2008; Chen et al., 2004, 2009, 2013a; Chen et al., 2006; Jensen et al., 2012; Liu et al., 2010; Mademont-Soler et al., 2012, 2013; Vialard et al., 2009). Recently, aCGH has been used prospectively on uncultured amniocytes to detect aneuploidy (Chen et al., 2011, 2012a, 2012b, 2013b, 2013c). Here, we present our experience of prenatal diagnosis and aCGH characterization of 22q11.2 microdeletion syndrome using uncultured amniocytes in a fetus with conotruncal heart malformations.

Abbreviations: aCGH, array comparative genomic hybridization; OMIM, Online Mendelian Inheritance in Man; DGS, DiGeorge syndrome; VCFS, velocardiofacial syndrome; del, deletion; MLPA, multiplex ligation-dependent probe amplification; VSD, ventricular septal defect; FISH, fluorescence *in situ* hybridization; DORV, double-outlet right ventricle; TOF, tetralogy of Fallot; CTHM, conotruncal heart malformations; ASD, atrial septal defect; AVSD, atrioventricular septal defect.

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2. Methods and 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 according to the manufacturer's instruction. Parental bloods were also collected, and the samples were subjected to aCGH analysis using the same array kit.

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 according to the standard cytogenetic protocol.

2.3. FISH

Metaphase FISH analysis on cultured amniocytes was performed using Vysis DiGeorge region probe [Vysis, LSI TUPLE 1 (red spectrum)/ LSI ARSA (green spectrum, FITC)] (Abbott Laboratories, Abbott Park, IL, USA).

2.4. Clinical description

A 29-year-old, primigravid woman was referred for counseling at 23 weeks of gestation because of abnormal ultrasound findings of congenital heart defects and transposition of great arteries in the fetus. Her husband was 30 years old. The woman and her husband were normal and non-consanguineous, and there was no familial history of congenital malformations. Level II ultrasound revealed a singleton fetus with microcephaly, VSD, a large overriding vessel with pulmonary artery branching, persistent truncus arteriosus and DORV (Fig. S1). Amniocentesis was performed at 23 weeks of gestation. Whole-genome aCGH analysis on uncultured amniocytes detected a 3.08-Mb deletion at 22q11.21 (Fig. 1). The parents did not have such a deletion. Cytogenetic analysis of cultured amniocytes revealed a normal male karyotype. FISH analysis confirmed an interstitial 22q11.21 deletion (Fig. 2). The pregnancy was terminated at 24 weeks of gestation, and a malformed fetus was delivered with facial dysmorphism of narrow palpebral fissures, prominent nasal root, bulbous nasal tip, hypoplastic alae nasi, a small mouth, micrognathia and small overfolded ears (Fig. S2).

3. Results

Whole-genome aCGH analysis on uncultured amniocytes detected a 3.08-Mb deletion at 22q11.21, or arr [hg 19] 22q11.21 (18,656,529–21,732,904) × 1 (Fig. 1). The result was obtained within one week of examination. The deleted region encompasses 127 genes and including 43 OMIM genes of *USP18*, *DGCR6*, *PRODH*, *DGCR2*, *DGCR14*, *TSSK2*, *GSC2*, *SLC25A1*, *CLTCL1*, *DVL1L1*, *HIRA*, *MRPL40*, *UFD1L*, *CDC45*, *CLDN5*, *SEPT5*, *GP1BB*, *TBX1*, *GNB1L*, *TXNRD2*, *COMT*, *ARVCF*, *DGCR8*, *TRMT2A*, *RANBP1*, *ZDHHC8*, *RTN4R*, *DGCR6L*, *GGTLC3*, *RIMBP3*, *ZNF74*, *SCARF2*, *MED15*, *PI4KA*, *SERPIND1*, *SNAP29*, *CRKL*, *LZTR1*, *THAP7*, *P2RX6*, *SLC7A4*, *BCRP2* and *GGT2*. Whole-genome aCGH analysis on parental bloods revealed no genomic imbalance. Conventional cytogenetic analysis of cultured amniocytes revealed a karyotype of 46,XY. Metaphase FISH analysis on cultured amniocytes showed the presence of only one red signal of Vysis LSI TUPLE 1 at 22q11.2, and the presence of two green FITC signals of Vysis LSI ARSA, indicating a deletion of DiGeorge syndrome TUPLE 1 locus at 22q11.2 in the fetus (Fig. 2). The karyotype after FISH analysis was 46,XY.ish del(22)(q11.21)(TUPLE 1-).

4. Discussion

The present case had a 3.08-Mb deletion at 22q11.2 encompassing the genes of *TBX1*, *COMT*, *UFD1L*, *GNB1L* and *MED15*, and manifested craniofacial dysmorphism and conotruncal heart malformations.

Patients with 22q11.2 deletion syndrome are associated with a high rate (74%) of congenital heart detects, especially conotruncal malformations, TOF, interrupted aortic arch, VSD and truncus arteriosus; and a high rate (69%) of palatal malformations (McDonald-McGinn et al., 2013). TBX1 is required for inner ear morphogenesis and is expressed in otocyst development in the otic epithelium and in the periotic mesenchyme (Vitelli et al., 2003). In transgenic mice, *Tbx1* deficiency causes cardiovascular defects, abnormal formation and growth of the pharyngeal arch arteries, and abnormal growth and septation of the outflow tract of the heart, interventricular septation and conal alignment (Vitelli et al., 2002). Mutation or haploinsufficiency of *TBX1* has been associated with DGS, VCFS, CTHM (OMIM 217095) and TOF (OMIM 187500). TBX1 functions through wnt11r to regulate heart looping and differentiation (Choudhry and Trede, 2013). In the patients with 22q11.2 deletion syndrome, *TBX1* genotype correlates with cardiovascular phenotype (Guo et al., 2011). TBX1 regulates oral epithelial adhesion and palatal development (Funato et al., 2012]. In the patients with 22q11.2 deletion syndrome, *TBX1* genotype correlates with overt cleft palate phenotype (Herman et al., 2012).

Patients with 22q11.2 deletion syndrome are associated with a high rate (25%) of schizophrenia (Bassett et al., 2011; McDonald-McGinn et al., 2013). van Beveren et al. (2012) identified decreased expression of the genes *GNB1L*, *COMT*, *UFD1L* and *MED15* in patients with 22q11.2 deletion syndrome. *COMT* (OMIM 116790) encodes catechol-O-methyltransferase which plays an important role in dopamine metabolism (Gogos et al., 1998). *COMT* is a strong candidate gene for schizophrenia susceptibility (OMIM 181500) (Ira et al., 2013; Lee et al., 2005; Palmatier et al., 2004; Shifman et al., 2002; van Beveren et al., 2012). *GNB1L* (OMIM 610778) encodes a G-protein β-subunit-like polypeptide. There is strong evidence that the *GNB1L* is associated with schizophrenia (Ishiguro et al., 2010; Li et al., 2011; van Beveren et al., 2012; Williams et al., 2008). *GNB1L* is also associated with autism (Chen et al., 2012c) and bipolar disorder (Li et al., 2011). *UFD1L* (OMIM 601754) encodes ubiquitin degradation 1-like protein that plays a role in degradation of ubiquitin fusion protein (Pizutti et al., 1997). *UFD1L* polymorphism is associated with schizophrenia (Ota et al., 2010; van Beveren et al., 2012; Xie et al., 2008). *MED15* (OMIM 607372) or *PCQAP* encodes a component of the metazoan mediator complex and plays a role in TGFB/Activin/Nodal/Smad2/3 signal transduction (Kato et al., 2002). *MED15* polymorphism is associated with schizophrenia (De Luca et al., 2003; Sandhu et al., 2004; van Beveren et al., 2012). Other possible candidate genes of schizophrenia associated with 22q11.2 deletion syndrome include *ZDHHC8* (OMIM 608784), *PRODH* (OMIM 606810), *RTN4R* (OMIM 605566), *DGCR6* (OMIM 601279), *DGCR6L* (OMIM 609459) and *ARVCF* (OMIM 602269).

Prenatal diagnosis of cardiac defects should raise suspicion of aneuploidy and 22q11.2 microdeletion. In a study of 1510 cases with prenatally detected structural heart defects, Moore et al. (2004) found that 41.3% (624 cases) had chromosome abnormalities including trisomy 18 [36.9% (n = 230)], trisomy 21 [27.9% (n = 174)], trisomy 13 [15.4% (n = 96)], trisomy 14 [0.6% (n = 4)], trisomy 16 [0.3% (n = 2)], trisomy 22 [0.3% (n = 2)], trisomy 9 [0.2% (n = 1)], 45,X [4% (n = 25)], 47,XXY [0.2% (n = 1)], triploidy [4.3% (n = 27)], balanced translocation [5.1% (n = 32)], balanced inversion [0.6% (n = 4)], unbalanced derivative chromosome [3.2% (n = 20)] and deletion [1% (n = 6)], and 58.7% (886 cases) had apparently normal karyotypes of which 3.1% (17 cases) had 22q11.2 microdeletion confirmed by FISH. In a study of 169 pregnancies with prenatal FISH screening for 22q11.2 deletion because of congenital heart defects, most being conotruncal (n = 147), extracardiac ultrasound findings (n = 7), positive family history (n = 10) and unknown causes (n = 5), Bretelle et al. (2010) found that 4.7% (8/169)