Gene 536 (2014) 425–429

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short Communication

Prenatal diagnosis and molecular cytogenetic characterization of *de novo* pure partial trisomy 6p associated with microcephaly, craniosynostosis and abnormal maternal serum biochemistry

Chih-Ping Chen [a,b,c,d,e,f,*], Ming Chen [g,h,i], Chen-Yu Chen [a], Schu-Rern Chern [b], Peih-Shan Wu [j], Shun-Ping Chang [g,h], Yu-Ling Kuo [k], Wen-Lin Chen [a], Chen-Wen Pan [a], Wayseen Wang [b,l]

[a] Department of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei, Taiwan
[b] Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan
[c] Department of Biotechnology, Asia University, Taichung, Taiwan
[d] School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan
[e] Institute of Clinical and Community Health Nursing, National Yang-Ming University, Taipei, Taiwan
[f] Department of Obstetrics and Gynecology, School of Medicine, National Yang-Ming University, Taipei, Taiwan
[g] Department of Medical Research, Center for Medical Genetics, Changhua Christian Hospital, Changhua, Taiwan
[h] Department of Genomic Medicine, Center for Medical Genetics, Changhua Christian Hospital, Changhua, Taiwan
[i] Department of Obstetrics and Gynecology, Changhua Christian Hospital, Changhua, Taiwan
[j] Gene Biodesign Co. Ltd, Taipei, Taiwan
[k] Department of Obstetrics and Gynecology, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[l] Department of Bioengineering, Tatung University, Taipei, Taiwan

ARTICLE INFO

Article history:
Accepted 12 December 2013
Available online 25 December 2013

Keywords:
BMP6
Craniosynostosis
Microcephaly
Partial trisomy 6p
Prenatal diagnosis

ABSTRACT

We present prenatal diagnosis and molecular cytogenetic characterization of *de novo* pure trisomy 6p22.3 → p25.3 encompassing *BMP6* in a fetus associated with microcephaly and craniosynostosis on prenatal ultrasound, abnormal maternal serum biochemistry of a low PAPP-A level in the first-trimester combined test, and a karyotype of 46,XX,der(22)t(6;22)(p22.3;p13)dn. The present case demonstrates the usefulness of rapid prenatal identification of the origin of the extra chromosome material on the short arm of an acrocentric chromosome by spectral karyotyping, fluorescence *in situ* hybridization and array comparative genomic hybridization. We review the phenotypic abnormality of craniosynostosis in previously reported patients with partial trisomy 6p. We discuss the genotype–phenotype correlation of the involved gene of *BMP6* in this case.

1. Introduction

Partial trisomy 6p is a rare chromosome aberration with variable clinical features including characteristic craniofacial manifestations of craniosynostosis, prominent forehead, flat occiput, abnormal fontanelles, blepharophimosis, blepharoptosis, short flat bulbous nose, tiny anteverted nares, hypotelorism, epicanthic folds, small mouth with pointed chin and thin lips, micrognathia, long philtrum, choanal atresia, high-arched palate, gingival hypertrophy, cataracts, strabismus, microcornea and low-set malformed ears, low birth weight, growth retardation, developmental delay, mental retardation, autism, hearing loss, feeding problems, immunodeficiency, recurrent infections, and cardiac, renal and musculoskeletal abnormalities (Bart et al., 2011; Breuning et al., 1977; Castiglione et al., 2013; Delatycki et al., 1999; Fogu et al., 2007; Fryns et al., 1986; Giardino et al., 2002; Smith and Pettersen, 1985; Su et al., 2012; Varvagiannis et al., 2013; Villa et al., 2000).

Prenatal diagnosis of pure trisomy 6p22.3 → p25.3 has not previously been described. Here, we present our experience of prenatal diagnosis and molecular cytogenetic characterization of *de novo* pure trisomy 6p22.3 → p25.3 in a fetus associated with prenatal findings of microcephaly, craniosynostosis and abnormal maternal serum biochemistry.

2. Clinical description

A 33-year-old, gravid 2, para 1, woman underwent first-trimester combined test at 13^{+1} weeks of gestation. Prenatal ultrasound revealed

Abbreviations: aCGH, array comparative genomic hybridization; NT, nuchal translucency; t, translocation; MoM, multiples of the median; β-hCG, β-human chorionic gonadotrophin; dn, *de novo*; PAPP-A, pregnancy-associated plasma protein-A; der, derivative chromosome; dup, duplication; FISH, fluorescence *in situ* hybridization; SKY, spectral karyotyping; OMIM, Online Mendelian Inheritance in Man; TGF-β, transforming growth factor-β.

* Corresponding author at: Department of Obstetrics and Gynecology, Mackay Memorial Hospital 92, Section 2, Chung-Shan North Road, Taipei, Taiwan. Tel.: +886 2 25433535; fax: +886 2 25433642, +886 2 25232448.
E-mail address: cpc_mmh@yahoo.com (C.-P. Chen).

0378-1119/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2013.12.036

an NT thickness of 1.8 mm. Maternal serum screening showed the results of a free β-hCG level of 0.908 MoM and a low PAPP-A level of 0.176 MoM. The risk calculation was as the following: trisomy 21 = 1/259, trisomy 18 = 1/632, and trisomy 13 = 1/1362. The patient hesitated at invasive prenatal diagnostic procedures and preferred to wait for second-trimester ultrasound examinations. However, second-trimester ultrasound examinations revealed intrauterine growth restriction and microcephaly. At 24 weeks of gestation, following genetic counseling of possible chromosomal abnormalities in the fetus, the woman underwent amniocentesis. Conventional cytogenetic analysis at amniocentesis revealed a derivative chromosome 22 [der(22)] with extra chromosomal material on the short arm of the chromosome 22. The nature of the der(22) was investigated by SKY, FISH and aCGH on cultured amniocytes. Level II ultrasound at 24 weeks of gestation revealed microcephaly, craniosynostosis (Fig. S1) and decreased amniotic fluid amount. The biparietal diameter was 5.25 cm (<5th centile), the head circumference (HC) was 19.95 cm (<5th centile), the abdominal circumference (AC) was 19.12 cm (50th centile), the femur length was 3.92 cm (25th centile), and the HC/AC ratio was 1.04 (5th centile).

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. SKY and FISH

The aberrant chromosome was characterized by SKY using 24-color SKY probes (Applied Spectral Imaging, Carlsbad, CA, USA). Metaphase FISH was performed using the chromosome 6 whole chromosome painting probe (WCP6) (Cytocell, Adderbury, Oxfordshire, UK), and the chromosome 22q subtelomere-specific probe (D22S1726).

3.3. 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, 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).

4. Results

SKY using 24-color SKY probes showed that all the metaphase cells examined contain a der(22) with a chromosome 6 segment on the short arm of chromosome 22 (Fig. 1). Metaphase FISH using WCP6 probe (spectrum green) and a chromosome 22q subtelomere-specific probe (D22S1726) (spectrum red) showed a der(22) containing the segment of chromosome 6 (Fig. 2). Whole-genome aCGH analysis on the DNA extracted from cultured amniocytes detected a 20.88-Mb duplication at 6p25.3–p22.3, or arr [hg 19] 6p25.3p22.3 (1–20,875,000) × 3.05 (Fig. 3). The duplicated 6p25.3–p22.3 region contains 190 genes including 67 OMIM genes (Table S1). The father had a karyotype of 46,XY. The mother had a karyotype of 46,XX. The karyotype of the amniocytes was 46,XX,der(22)t(6;22)(p22.3;p13)dn (Fig. 4). Cord blood sampling confirmed a karyotype of 46,XX,der(22)t(6;22)(p22.3;p13)dn. An informed consent for feticide because of fetal chromosomal and structural abnormalities was obtained from the parents. At 27 weeks of gestation, a dead 898-g female fetus was delivered with craniosynostosis, closed anterior fontanelles, prominent forehead, microcephaly, short bulbous nose, long philtrum, small mouth, micrognathia, low-set ears and clinodactyly. Postnatal skull X-ray showed fusion of cranial sutures consisting with the diagnosis of craniosynostosis (Fig. S2).

5. Discussion

Abnormal maternal serum biochemistry during early pregnancy screening may incidentally detect uncommon chromosome aberrations (Chen et al., 2012, 2013a,b). The present case provides evidence that partial trisomy 6p pregnancy may present an abnormally low level of maternal serum PAPP-A in the first trimester.

Pure partial trisomy 6p is a very rare and can be caused by tandem duplications, inverted duplications, a supernumerary marker chromosome, interchromosomal insertions and unbalanced chromosome rearrangements in association with the short arm of an acrocentric chromosome or the long arm of Y chromosome (Andrieux et al., 2006, 2008; Bart et al., 2011; Chiyo et al., 1975; Domínguez et al., 2003; Engelen et al., 2001; Fogu et al., 2007; Giardino et al., 2002; Karamanov et al., 2001; Mefford et al., 2010; Morton et al., 1980; Nakajima et al., 1995; Ng et al., 2001; Pearson et al., 1979; Phelan et al., 1986; Scott et al., 2007; Stohler et al., 2007; Varvagiannis et al., 2013; Villa et al., 2000, 2007). An increase in the length of satellite stalk or satellite on the short arm of an acrocentric chromosome may be interpreted as a variant of an acrocentric chromosome with a large satellite stalk (Chiyo et al., 1975; Engelen et al., 2001; Nakajima et al., 1995). The present case had extra chromosome material on the short arm of one chromosome 22 that might be mistaken as a chromosome 22 variant with a giant satellite. The present case provides evidence for the usefulness of rapid quantitative identification of the origin of extra chromosome material on the short arm of an acrocentric chromosome by SKY and FISH, and rapid quantitative identification of pure partial trisomy 6p by aCGH.

The present case had a 20.88-Mb gene dosage increase over the region of 6p25.3–p22.3 encompassing the gene of *BMP6* which may contribute to the phenotype of microcephaly and craniosynostosis in this case because of overexpression of *BMP6* under the circumstance of partial trisomy 6p. *BMP6* (OMIM 112266) is located at 6p24.3 and encodes bone morphogenetic protein 6 which belongs to TGF-β superfamily and has osteogenic activity and involvement in bone formation (Cheng et al., 2003; Kugimiya et al., 2005). BMPs together with TGF-β and activins/inhibins constitute the TGF-β superfamily of ligands, and concerted interactions of different ligands and receptors generate highly specific cellular signals required during development and tissue homeostasis (Mueller and Nickel, 2012). *BMP6* overexpression has been shown as the effect on adipose stem cell chondrogenesis to upregulate the expression of *COL2A1* and aggrecan and inhibiting the expression of *COL10A1* (Diekman et al., 2010; Estes et al., 2006a,b). Castiglione et al. (2013) suggested that *BMP6* is responsible for the partial trisomy 6p-associated phenotypic expression of craniofacial abnormalities such as craniosynostosis, choanal atresia and other mild to severe dysmorphic features. Craniosynostosis and microcephaly have been observed in patients with dup(6)(pter → p21) (Pearson et al., 1979; Phelan et al., 1986; Schinzel, 2001), dup(6)(p21.1 → p12.3) (Mefford et al., 2010; Varvagiannis et al., 2013) and dup(6)(p21.1 → p10) (Villa et al.,