Mosaic Ring Chromosome 18, Ring Chromosome 18 Duplication/Deletion and Disomy 18: Perinatal Findings and Molecular Cytogenetic Characterization by Fluorescence *In Situ* Hybridization and Array Comparative Genomic Hybridization

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SUMMARY

Objective: To present the perinatal findings and molecular cytogenetic analysis of a rare chromosomal abnormality involving structural and numerical abnormalities of chromosome 18.

Materials, Methods and Results: A 36-year-old woman, gravida 5, para 3, underwent amniocentesis because of her advanced maternal age. Amniocentesis revealed a karyotype of 46,XY,r(18) [27]/45,XY,-18[5]/46,XY[5]. The parents decided to continue the pregnancy. Level II ultrasound revealed ventriculomegaly. At 38 weeks of gestation, a 3,725 g male fetus was delivered. The fetus had microcephaly, hypertelorism, epicanthal folds, cleft palate, a broad flat nose, simian creases, broad hands, tapered fingers, clubfeet, micropenis, a sacral dimple, hypotonia, ventriculomegaly, and a ventricular septal defect. The peripheral blood lymphocytes revealed a karyotype of 46,XY,r(18)[81]/45,XY,-18[3]/46,XY,idic r(18)[3]/46,XY[13]. Fluorescence *in situ* hybridization using chromosome 18 centromeric probe (cep18) and subtelomeric (18pter, 18qter) identified four types of cells, r(18), idic r(18), monosomy 18, and disomy 18. Array comparative genomic hybridization analysis of the blood demonstrated a 14.9-Mb deletion at chromosome 18p [arr cgh 18p11.32p11.21 (0–14,941,330)×1] and a 29.6-Mb deletion at chromosome 18q [arr cgh 18q21.2q23 (46,533,430–76,117,153)×1]. The proband's karyotype was 46,XY,r(18)(p11.21q21.2)[81]/45,XY,-18[3]/46,XY,idic r(18)(p11.21q21.2;p11.21q21.2)[3]/46,XY[13].

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Conclusion: Array comparative genomic hybridization is useful to determine the breakpoints of a ring chromosome, particularly in cases where the ring chromosome comprises the majority of the mosaicism. [*Taiwan J Obstet Gynecol* 2010;49(3):327–332]

Key Words: array comparative genomic hybridization, fluorescence *in situ* hybridization, mosaic ring chromosome, prenatal diagnosis, ring chromosome 18 duplication/deletion

Introduction

The 18q deletion syndrome (OMIM 601808) is caused by variable deletions ranging from 18q21.2, 18q21.3, or 18q22.2 to 18qter [1–3]. The diverse phenotypic features of the 18q deletion syndrome include low birth weight, short stature, microcephaly, midfacial hypoplasia, prognathism, a carp-shaped mouth, a protuberant lower lip, dysplastic ears with prominent antihelix and antitragus, abnormal skull, vertebrae and ribs, atretic ear canals, clubfeet, vertical tali, tapered fingers, dimples over limb joints, hypoplasia of labia or scrotum, micropenis, cryptorchidism, hypospadias, nystagmus, strabismus, glaucoma, tapetoretinal degeneration, bilateral optic atrophy, hypotonia, seizures, deafness, enlarged ventricles, hydrocephalus, porencephaly, holoprosencephaly (HPE), cerebellar hypoplasia, decreased white matter, impaired or delayed myelination, and congenital heart defects [3–12].

The clinical phenotype of the 18p deletion syndrome (OMIM 146390) usually includes growth and mental retardation, hypotonia, epicanthic folds, ptosis, a low nasal bridge, a rounded face, micrognathia, a short neck, abnormal ears, small hands and feet, clinodactyly of the fifth finger, cardiac defects, abnormal genitalia, and cerebral malformations [13–15].

A ring chromosome 18, or r(18) exhibits breakage and reunion at the breakpoints on the long and short arms of chromosome 18, with deletions of the chromosomal segments distal to the breakpoints. The r(18) phenotype is associated with the anomalies of 18p deletion and 18q deletion, and can be associated with the features of both 18p deletion and 18q deletion syndromes [16]. Here, we present the perinatal findings and array comparative genomic hybridization (aCGH) characterization of a rare chromosomal abnormality associated with different cell lines involving structural and numerical abnormalities of chromosome 18.

Materials, Methods and Results

A 36-year-old woman, gravida 5, para 3, underwent amniocentesis at a community obstetric clinic because of her advanced maternal age. Her husband was 40 years old. She and her husband were both healthy and unrelated, and there was no family history of congenital malformations. The parents had two healthy daughters, one was 12 years old and the other was 9 years old. Amniocentesis at 16 weeks of gestation revealed a male fetus with mosaic ring chromosome 18 [r(18)], monosomy 18 and disomy 18, or 46,*XY*,r(18)[27]/45,*XY*,-18[5]/46,*XY*[5]. The parents decided to continue the pregnancy. Level II ultrasound was unremarkable, except for ventriculomegaly. A 3,725 g male fetus was delivered uneventfully at 38 weeks of gestation. The baby had a head circumference of 33 cm (15^{th} centile) and a body length of 51 cm (60^{th} centile). On examination, the baby manifested microcephaly, hypertelorism, epicanthal folds, cleft palate, a broad flat nose, simian creases, broad hands, tapered fingers, clubfeet, micropenis, a sacral dimple, and hypotonia. Brain ultrasound revealed ventriculomegaly, and echocardiography revealed a ventricular septal defect (VSD). Conventional cytogenetic analysis of peripheral blood lymphocytes revealed a karyotype of 46,XY,r(18)[81]/45,XY,-18[3]/46,XY,idic r(18)[3]/46,*XY*[13] (Figures 1–4). The deletion of distal 18p and distal 18q on r(18) and idic r(18), and the duplication of the centromere of chromosome 18 were demonstrated by fluorescence *in situ* hybridization (FISH) using an 18p terminal probe (18pter, RP11-324G2)

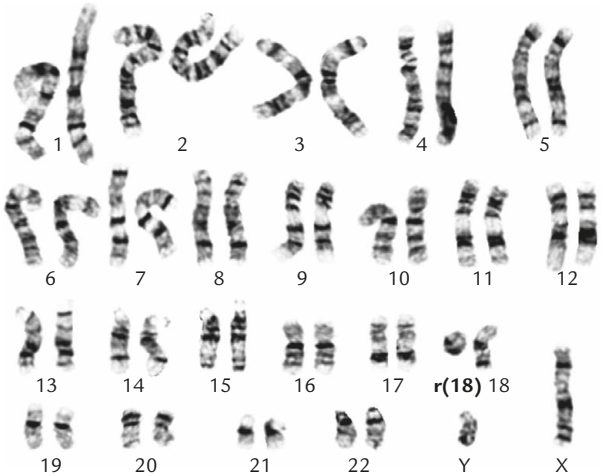

Figure 1. A karyotype of 46,XY,r(18)(p11.21q21.2).

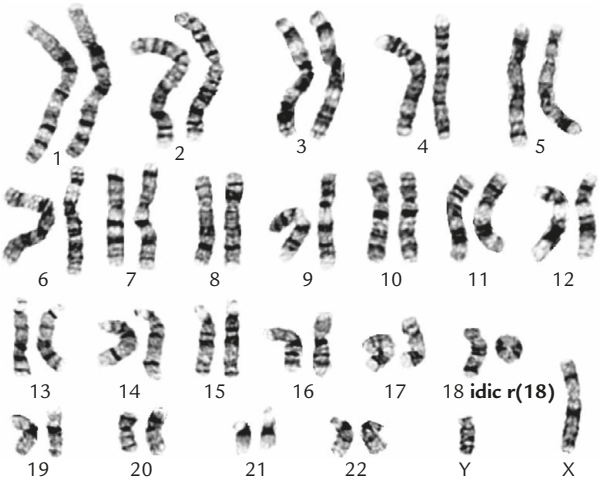

Figure 2. A karyotype of 46,*XY*,idic r(18)(p11.21q21.2; p11.21q21.2).

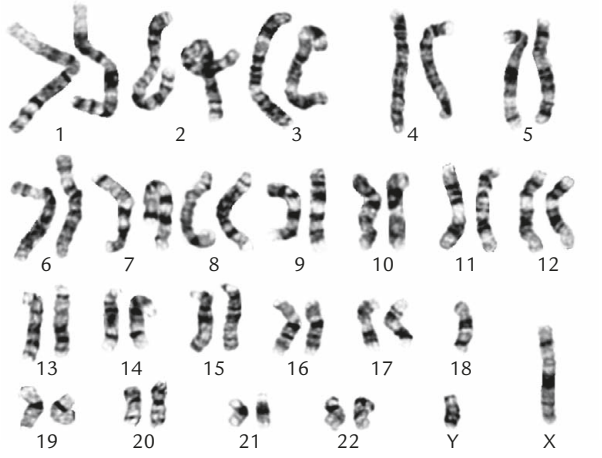

Figure 3. A karyotype of 45,*XY*,-18.

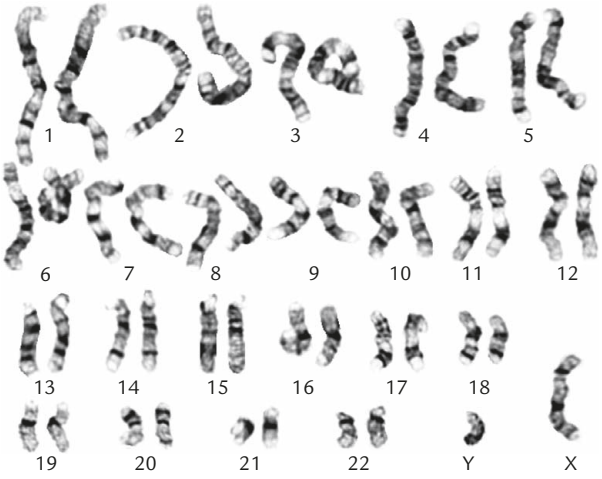

Figure 4. A karyotype of 46,*XY*.

(spectrum green) at 18p11.32, an 18q terminal probe (18qter, RP11-89N1) (spectrum yellow) at 18q23, and an 18 centromeric probe (cep18) (spectrum red) (TelVysion, Downers Grove, IL, USA) (Figure 5). A normal chromosome 18 contained one cep18 signal (red), one 18pter signal (green), and one 18qter (yellow). The r(18) contained only one cep18 signal (red) and lacked the 18pter signal (green) and the 18qter signal (yellow). The idic r(18) contained two cep18 signals (red) and lacked the 18pter signal (green) and the 18qter signal (yellow). A monosomy 18 cell contained only one normal chromosome 18. A disomy 18 cell contained only two normal chromosomes 18. Oligonucleotide-based aCGH of the blood using Oligo HD Scan (CMDX, Irvine, CA, USA) revealed a 14.9-Mb deletion at chromosome 18p [arr cgh 18p11.32p11.21 (0–14,941, 330)×1] and a 29.6-Mb deletion at chromosome 18q [arr cgh 18q21.2q23 (46,533,430–76,117,153)×1] (Figure 5). Therefore, the proband's karyotype was 46, *XY*,r(18)(p11.21q21.2)[81]/45,*XY*,-18[3]/46,*XY*,idic r(18)(p11.21q21.2;p11.21q21.2)[3]/46,*XY*[13].

Discussion

We previously demonstrated the usefulness of aCGH for prenatal detection of microdeletions and unbalanced translocations [17,18]. In this report, we also demonstrate the use of aCGH to determine the breakpoints of a ring chromosome in a case in which the ring chromosome comprises the majority of the mosaicism. The present case had high-level mosaicism for r(18) and low-level mosaicism for disomy 18, r(18) duplication and r(18) deletion. The aCGH findings were consistent with a deletion of 18p11.21→pter and a deletion of 18q21.2→qter in the r(18). aCGH can detect DNA dosage imbalances, including deletions and duplications, but shows limitations for the detection of low-level mosaicism, balanced translocations, inversions and polyploidy. Recent studies have suggested that aCGH can detect as little as 20% mosaicism in peripheral blood cells [19,20]. In this study, aCGH was unable to detect low-level mosaicism.

The present case had a 29.6-Mb 18q deletion, a 14.9-Mb 18p deletion, and phenotypic abnormalities included microcephaly, ventriculomegaly, clubfeet, abnormal external genitalia, ventricular septal defect, cleft palate, hypotonia, and facial dysmorphism. The 18q 21.2→qter deletion in this case encompassed the critical regions for orofacial cleft, microcephaly, and the typical 18q deletion phenotype. Dostal et al [21] suggested that the potential critical region for orofacial cleft is at 18q22.3, between markers D18S879 and D18S1141, and contains the orofacial cleft candidate genes, *SALL3* and *TSHZ1*. Feenstra et al [12] suggested the critical region for the typical phenotype of 18q deletion syndrome is 4.3-Mb region located within 18q22.3-q23,

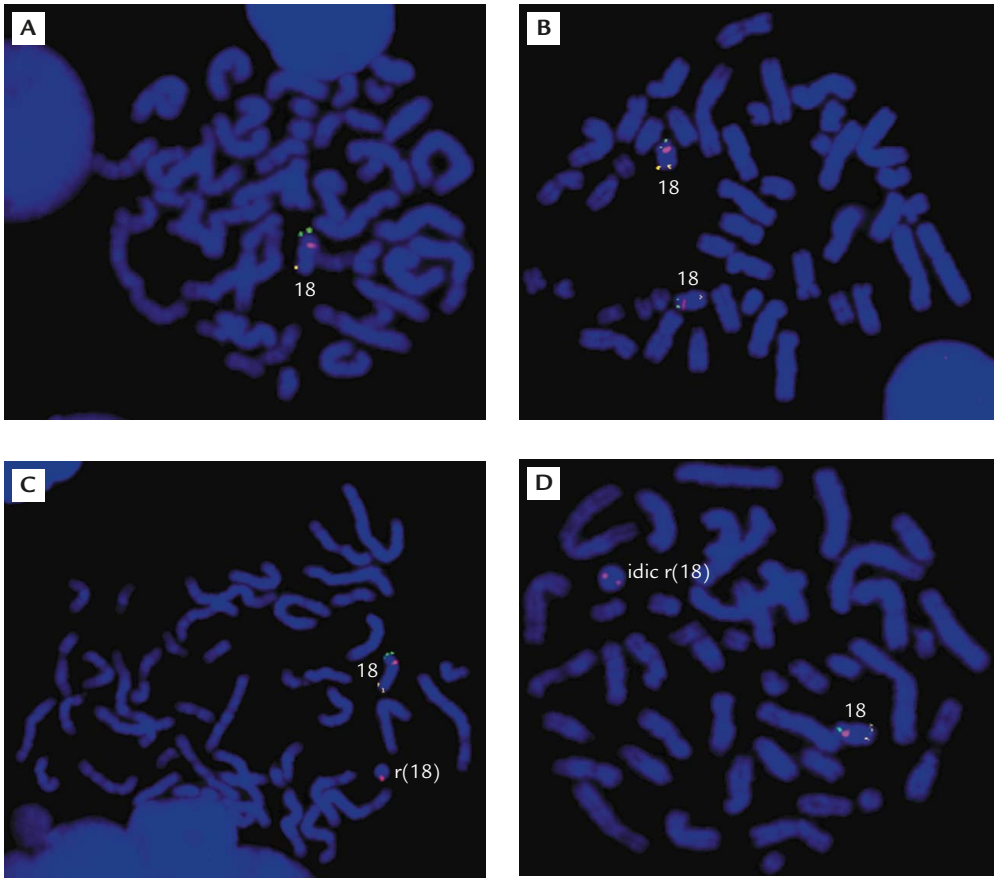

Figure 5. Fluorescence *in situ* hybridization studies of four types of cells with an 18p terminal probe RP11-324G2 (spectrum green), an 18q terminal probe RP11-89N1 (spectrum yellow) and an 18 centromeric probe (spectrum red). (A) A metaphase cell with monosomy 18 and one chromosome 18 with three signals (green, red and yellow). (B) A metaphase cell with disomy 18 and two chromosomes 18. (C) A metaphase cell with r(18) and one chromosome 18 and one r(18), which has only one red signal. (D) A metaphase cell with idic r(18) and one chromosome 18 and one idic r(18), which has two red signals. r(18) = ring chromosome 18; idic r(18) = isodicentric ring chromosome 18; 18 = chromosome 18.

and the critical region for microcephaly is at 18q21.33. The 18p11.21→pter deletion in this case encompassed *HPE4*. However, the present case lacked the HPE phenotype. An HPE critical region on 18p11.3 has been defined as *HPE4* (OMIM 142946) [22] and the responsible gene is *TGIF* (OMIM 602630) [23]. Only ~10% of cases with 18p deletion have HPE [13]. The low incidence of HPE in patients with 18p deletion and *TGIF* haploinsufficiency is because of the autosomal-dominant inheritance pattern of *TGIF* with low penetrance [23], and the requirement for other genetic or environmental factors [24]. Nanni et al [25] suggested that either maternal retinoic acid levels or altered activity in another protein can modify the effects of *TGIF*, and thus lead to phenotypic variability among patients with a *TGIF* deletion.

Prenatal diagnosis of mosaic r(18) with r(18) deletion/duplication is rare [26–29]. Eiben et al [26] reported prenatal diagnosis of 45,XX,-18/46,XX,r(18)(p11q12) by amniocentesis in a fetus with cebocephaly and HPE. The skin fibroblasts had 26.7% (8/30) of monosomy 18 and 73.3% (22/30) of r(18). Fischer et al [27] reported prenatal diagnosis of 46,XY,r(18)(p11q23)[103]/45,XY,-18[13] by amniocentesis in a fetus with amniotic band syndrome. The cord blood had a karyotype of 46,XY,r(18). Carreira et al [28] reported prenatal diagnosis of mosaic r(18) and r(18) deletion/duplication in two cases, and used multicolor banding to determine the breakpoints. In the first case, a fetus with HPE, 46,XY,r(18)(p11.1q22)[36]/45,XY,-18[7]/47,XY,-18, +r(18)(p11.1q22) × 2[1]/46,XY,dupr(18)(p11.1q22)[1] was diagnosed by amniocentesis. Postnatal cytogenetic analysis of fetal tissue revealed a karyotype of 46,XY,r(18)[89]/45,XY,-18[22]/46,XY,dupr(18)[2]/46,XY,mar(18)[4]. In the second case, a fetus with congenital heart defects and obstructive uropathy, 46,XY,r(18)(p11.22q21.2)[12]/46,XY,r(18;18)(p11.22q21.2;p11.22q21.2)[4]/47,XY,-18, +r(18)(p11.22q21.2) × 2[1]/del(18)(:p11.21→q11.2:)[1]/ace(18)(:q11.2→q12.2:)[1] was diagnosed by amniocentesis. Mello et al [29] reported prenatal diagnosis of 46,XY,t(3;6)(p26.2;q15),r(18)[22]/45,XY,t(3;6)(p26.2;

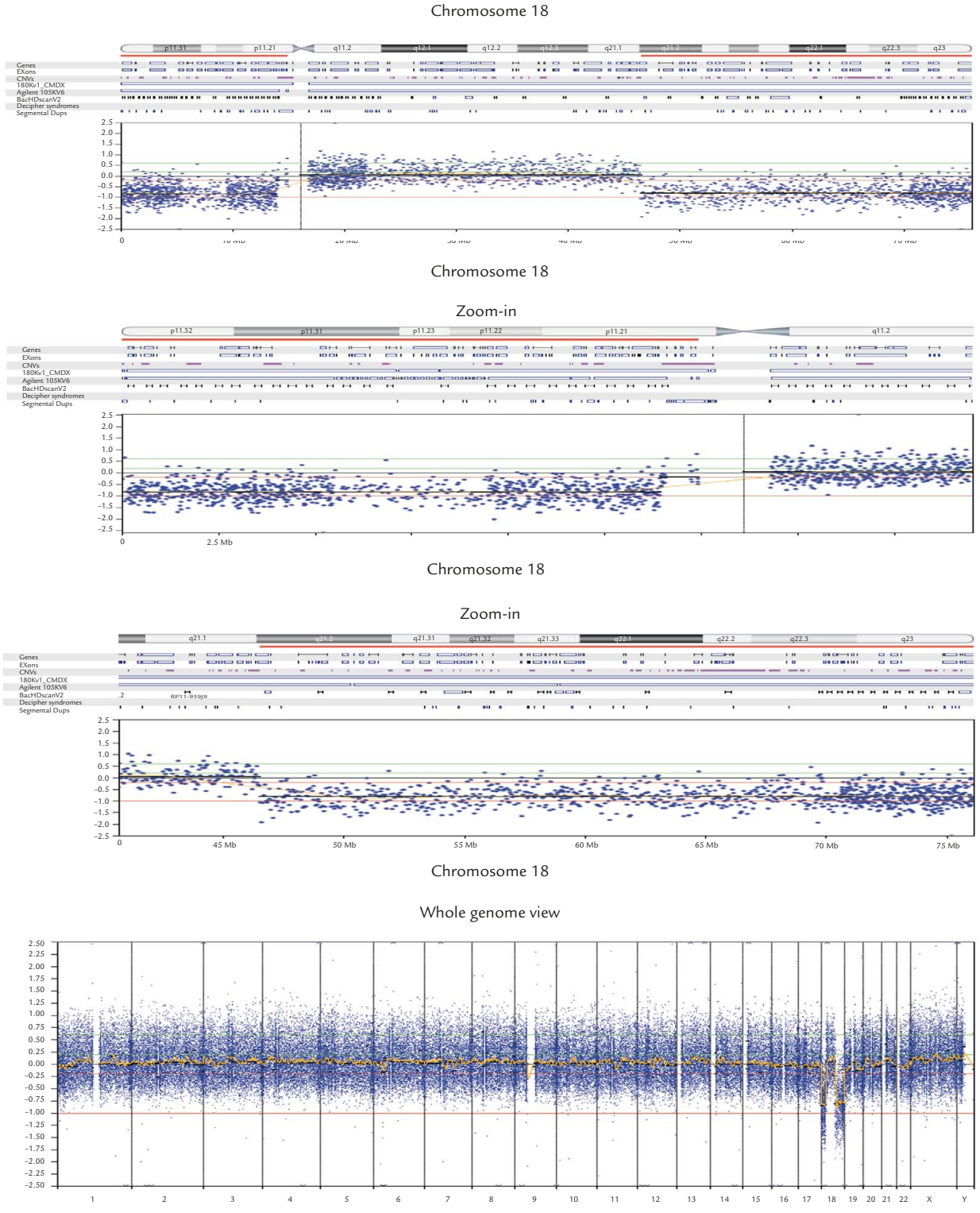

Figure 6. Oligonucleotide-based array comparative genomic hybridization shows a 14.9-Mb deletion (0–14,941,330 bp) of the short arm of chromosome 18 and a 29.6-Mb deletion (46,533,430–76,117,153 bp) of the long arm of chromosome 18.

q15),-18[6] by amniocentesis in a fetus with a single umbilical artery, an increased nuchal fold thickness and tricuspid regurgitation. aCGH revealed a 2.8-Mb of 18p11.31p11.32 deletion and a 22.5-Mb 18q21.3 q23 deletion. The fetus was carried to term and had microcephaly, micropenis, a short stature, congenital heart defects, a high-arched palate, abnormal hands, and facial dysmorphism.

To our knowledge, this is the first report of mosaic r(18), r(18) deletion/duplication and disomy 18 in which

the baby was carried to term. Perinatally, this case represents a difficulty for genetic counseling because of the complexity of the karyotype and the molecular evidence of large deletions of 18p and 18q. The parents elected to continue the pregnancy based on a desire for a son. Although the proband has many features of both 18p and 18q deletion syndromes, he is making great progress and surviving the neonatal period.

Acknowledgments

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