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Short Communication

Prenatal diagnosis and molecular cytogenetic characterization of mosaicism for a small supernumerary marker chromosome derived from ring chromosome 4

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Abstract

Objective: To present prenatal diagnosis and molecular cytogenetic characterization of mosaicism for a small supernumerary marker chromosome (sSMC) derived from ring chromosome, or r(4) by spectral karyotyping (SKY), fluorescence *in situ* hybridization (FISH), and array comparative genomic hybridization (aCGH).

Materials, Methods, and Results: A 37-year-old, primigravid woman underwent amniocentesis at 18 weeks of gestation because of advanced maternal age. Amniocentesis revealed a *de novo* ring-shaped sSMC in 16 of 31 amniocyte colonies. The parental karyotypes were normal. Level II ultrasound findings were unremarkable. Repeated amniocentesis revealed a karyotype of 47,XX,+mar[17]/46,XX[19]. The sSMC was characterized by SKY and FISH, which showed a chromosome 4 origin of the sSMC. aCGH demonstrated a 21.7-Mb gain in the gene dosage encompassing the region of 4p12→q13.2. The sSMC was r(4)(p12q13.2). The fetal karyotype was 47,XX,+r(4)(p12q13.2)[17]/46,XX[19]. The pregnancy was subsequently terminated. The fetus postnatally manifested hypertelorism, epicanthic folds, a prominent nose, a triangular face, low-set ears, clinodactyly of the fingers, and small big toes. Postnatal cytogenetic analyses of fetal and extraembryonic tissues revealed the karyotypes of 47,XX,+r(4)[18]/46,XX[21] in cord blood, 47,XX,+r(4)[20]/48,XX,+r(4),+r(4)[1]/46,XX[9] in umbilical cord, 47,XX,+r(4)[14]/47,XX,+dic r(4)[1]/46,XX[25] in skin, 47,XX,+r(4)[15]/46,XX[25] in amnion, and 47,XX,+r(4)[12]/47,XX,+dic r(4)[1]/46,XX[2] in placenta.

Conclusion: SKY, FISH, and aCGH are helpful in genetic counseling of prenatally detected sSMCs by providing the immediate and thorough information on the origin and genetic component of the sSMC.

Keywords: Array comparative genomic hybridization (aCGH); Chromosome 4; Mosaicism; Prenatal diagnosis; Ring chromosome; Small supernumerary marker chromosome (sSMC); Spectral karyotyping (SKY)

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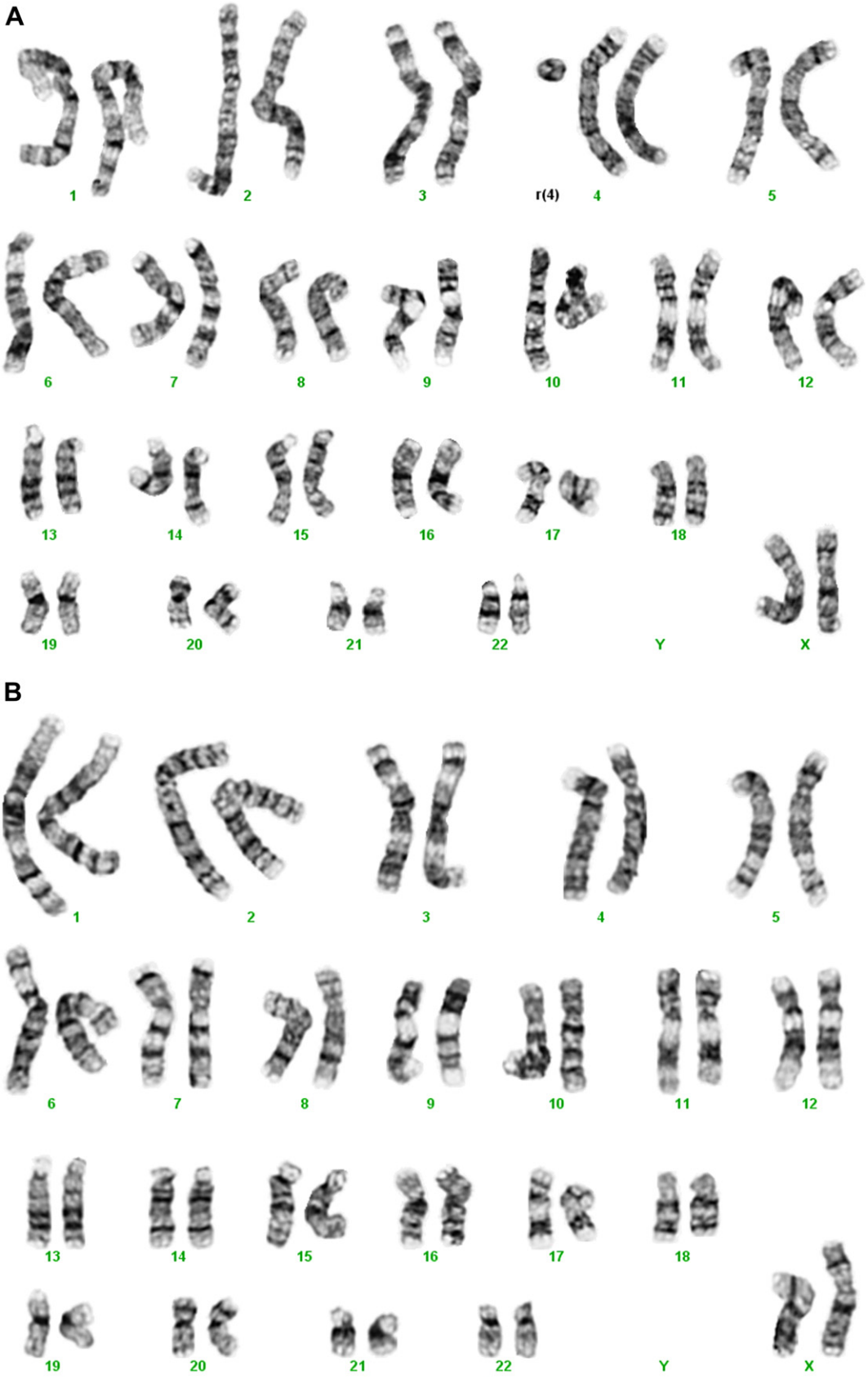

Fig. 1. (A) A karyotype of 47,XX,+r(4) and (B) a karyotype of 46,XX.

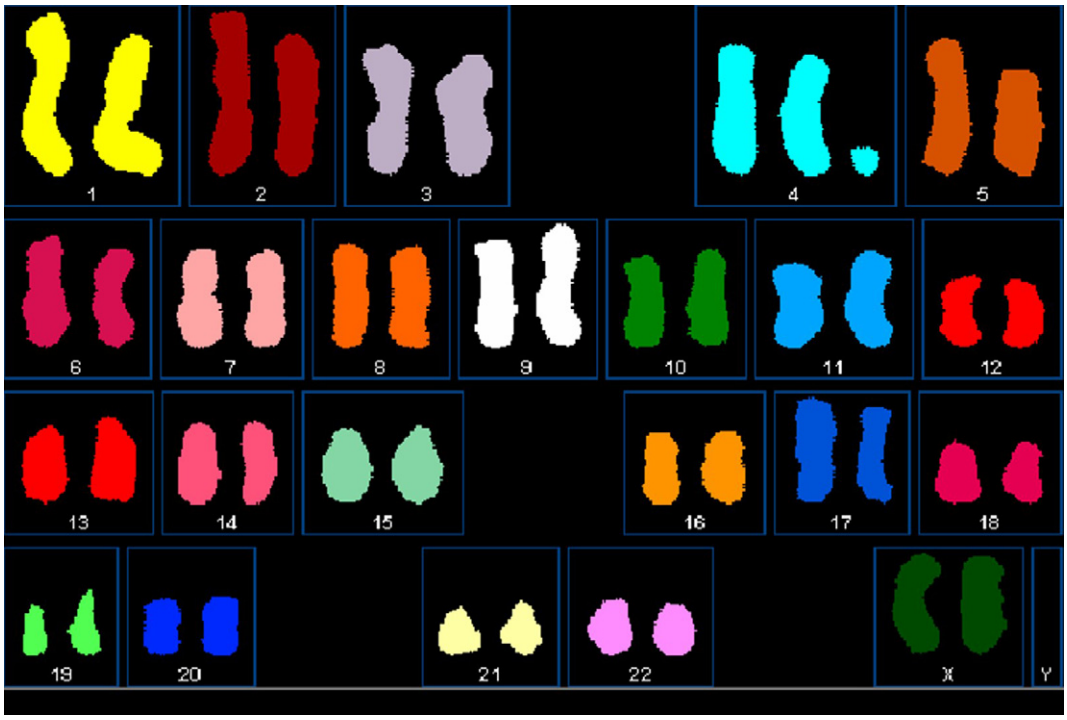

Fig. 2. Spectral karyotyping (SKY) using 24-color SKY probes shows a small supernumerary marker chromosome derived from chromosome 4.

Introduction

Small supernumerary marker chromosomes (sSMCs) are structurally abnormal chromosomes that are generally equal in size or smaller than a chromosome 20 and cannot be identified or characterized by conventional banding techniques [1–6]. sSMCs can present in 0.044% of newborn infants and in 0.075% of prenatal fetal cases [1,3,7]. About 70% of sSMCs arise *de novo*, about 70% of sSMCs belong to acrocentric chromosomes, and about 70% cases of *de novo* sSMCs have no phenotypic effects [1,7–9]. Ring chromosomes are structurally abnormal chromosomes arisen by breakage in the short and long arms of a chromosome and by fusion at the breakpoints with loss of the distal segments [6,10,11]. Ring chromosomes occur in 1:25,000 recognized conceptions and can originate from all human chromosomes [12]. Prenatal diagnosis of a supernumerary ring chromosome 4, or r(4), is uncommon [13–16]. The r(4) may be instable and can be associated with loss of r(4), double r(4), and dicentric r(4) [10,17]. Herein, we present our experience of prenatal diagnosis and molecular cytogenetic characterization of mosaicism for an sSMC derived from r(4) by means of spectral karyotyping (SKY), fluorescence *in situ* hybridization (FISH), and array comparative genomic hybridization (aCGH).

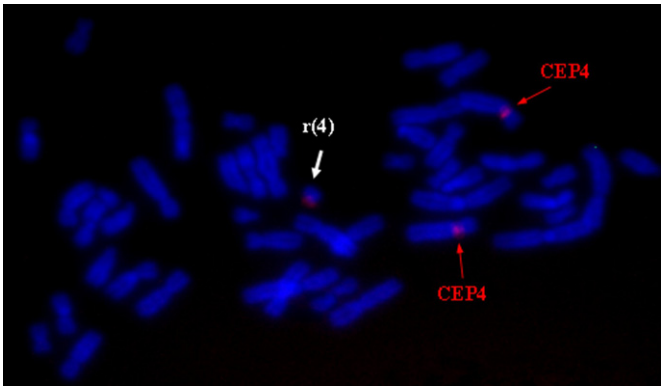

Fig. 3. Fluorescence *in situ* hybridization using a chromosome 4 centromeric probe (CEP4) shows that the supernumerary r(4) contains a CEP4 signal (pink color) and additional chromosomal materials.

Materials, methods, and results

A 37-year-old, primigravid woman underwent amniocentesis at 18 weeks of gestation because of advanced maternal age. Cytogenetic analysis of the cultured amniocytes using *in situ* cultured method showed mosaicism for a ring-shaped sSMC. Of 31 colonies of cultured amniocytes, 16 colonies had an sSMC, whereas the rest 15 colonies were normal. The karyotype was 47,XX,+mar[16]/46,XX[15]. The parental karyotypes were normal. Level II ultrasound findings were unremarkable. At 21 weeks of gestation, the parents requested repeated amniocentesis, which revealed a karyotype of 47,XX,+mar[17]/46,XX[19] (Fig. 1). The ring-shaped sSMC was characterized by SKY using 24-color SKY probes (Applied Spectral Imaging, Carlsbad, CA, USA) and by FISH using a chromosome 4 centromeric probe (4p11.1-q11.1; D4Z1 locus) (Cytocell, Adderbury, Oxfordshire, UK). SKY showed that the sSMC was derived from r(4) (Fig. 2). FISH

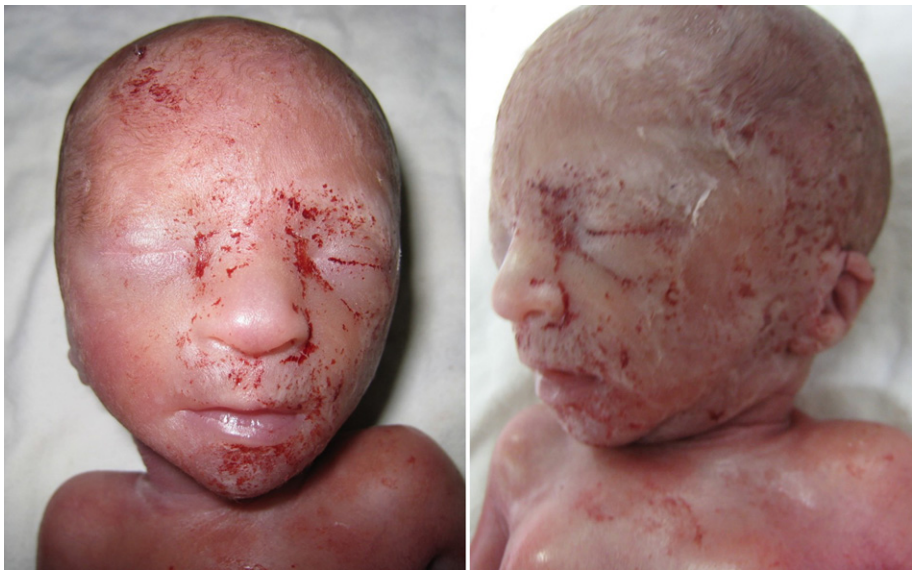

Fig. 4. Craniofacial appearance of the proband at birth.

analysis showed that the r(4) contained a positive chromosome 4 centromeric probe signal and an additional chromosomal segment (Fig. 3). The fetal karyotype was 47,XX,+r(4) .ish r(4)(SKY+,D4Z1+)[17]/46,XX[19]. The pregnancy was subsequently terminated. A 566-g malformed female fetus was delivered with hypertelorism, epicanthic folds, a prominent nose, a triangular face, low-set ears, clinodactyly of the fingers, and small big toes (Fig. 4). Postnatal cytogenetic analyses of fetal and extraembryonic tissues revealed a karyotype of 47,XX,+r(4)[18]/46,XX[21] in the cord blood, a karyotype of 47,XX,+r(4)[20]/48,XX,+r(4),+r(4)[1]/46,XX [9] in the umbilical cord, a karyotype of 47,XX,+r(4)[14]/ 47,XX,+dic r(4)[1]/46,XX[25] in the skin, a karyotype of 47,XX,+r(4)[15]/46,XX[25] in the amnion, and a karyotype of 47,XX,+r(4)[12]/47,XX,+dic r(4)[1]/46,XX[2] in the placenta (Figs. 5 and 6). aCGH analysis of the umbilical cord using bacterial artificial chromosome (BAC)-based aCGH (CMDX BAC-aCGH CA3000 chips) (CMDX, Irvine, CA, USA)

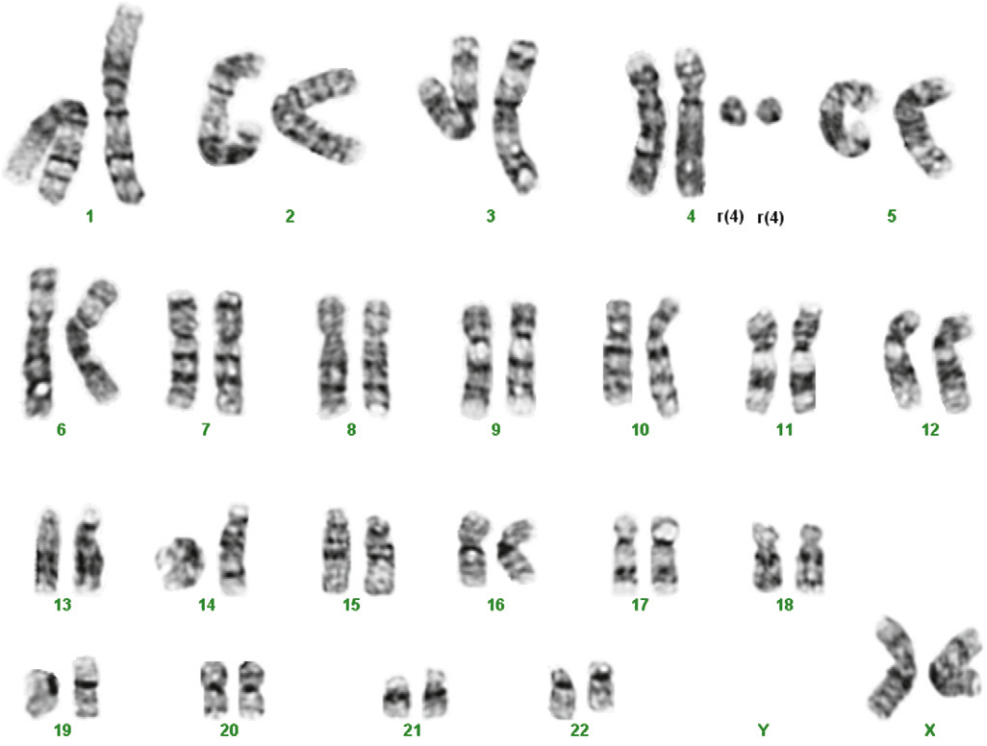

Fig. 5. A karyotype of 48,XX,+r(4),+r(4).

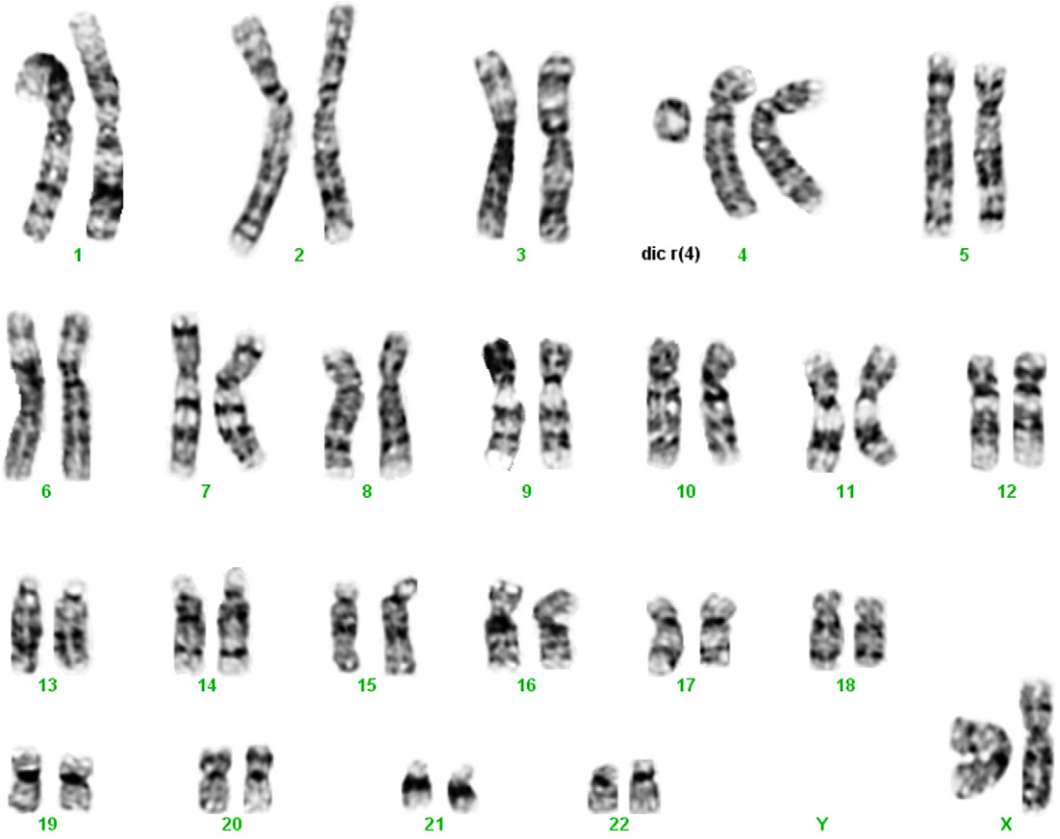

Fig. 6. A karyotype of 47,XX,+dic r(4).

and oligonucleotide-based aCGH (CytoChip Oligo) (BlueGnome, Cambridge, UK) demonstrated genomic imbalance with a gain in the gene dosage on the pericentric euchromatic region of chromosome 4 (Figs. 7 and 8). There was a 21.7-Mb duplicated segment encompassing 4p12→q13.2 (Fig. 8). The sSMC was r(4)(p12q13.2). Polymorphic DNA marker analysis excluded uniparental disomy 4 and the maternal origin of the r(4).

Discussion

The present case had mosaicism for supernumerary r(4), double r(4), and dicentric r(4), a 21.7-Mb duplication of 4p12-q13.2, and phenotypic abnormalities. Duplications involving the proximal region of 4q have been reported to be associated with a mild phenotype [18,19]. Mattei et al [18] reported a 6-year-old girl with psychomotor retardation, microcephaly, bilateral epicanthic folds, a broad nose, a large mouth, poorly formed ears, clinodactyly of the fifth fingers, and a duplication of 4q12-q13 resulted from an unbalanced segregation of a maternal insertional translocation. Shashi et al [19] reported a 2-year and 8-month-old boy with microcephaly, mental retardation, minor facial anomalies, and a karyotype of 46,XY,dup(4)(q12q13).

sSMC(4) with duplication of 4p12→q13 can be associated with clinical findings. Fang et al [20] reported a 27-year-old male with 100% supernumerary r(4)(::p10→q12::) or 47,XY,+r(4). The man manifested severe mental retardation, obesity, gynecomastia, kyphosis, a narrow forehead with ridged occiput, downslanting palpebral fissures, a downturned mouth, short philtrum, narrow pinna, bilateral clinodactyly of the fifth fingers, and syndactyly of toes 2 and 3. Pappas et al [21] reported a 26-year-old mother with 67% mosaicism for r(4)(::p12→q12::) and a 19-year-old son with 70% mosaicism for r(4)(::p12→q12::). Both the mother and son manifested relative microcephaly, triangular shape of face, downslanted and prominent eyes, a broad tip of nose, broad palms and soles, hyperpigmented skin, and wide sandal gaps. Baldwin et al [22] reported a father with 60% mosaicism for r(4)(::p12→q13.2::) (4.2 Mb in 4p and 18 Mb in 4q; total 22.2 Mb) and a child with 60% mosaicism for r(4)(::p12→q13.2::). The father had mild intellectual disability, and the child manifested developmental delay, attention deficit hyperactivity disorder, unilateral partial vision loss, mild to moderate dysmorphic features, and Torette syndrome. Bonnet et al [23] reported a 6-year-old girl with developmental delay; tall stature; obesity; and 82% mosaicism for 47,XX,+r(4),48,XX,+r(4),+r(4), and 49,XX,+r(4),+r(4),+r(4) in the peripheral blood lymphocytes. aCGH analysis revealed a gain of BAC-clones spanning a 16-Mb region at 4q11-q13.2, including the *IGFBP7* gene. Liehr [24] reported a male newborn with 100% supernumerary ring chromosomes, including r(4)(::p11→q12::)[8] and r(4;4)(::p11→q12::p11→q12::)[2]. The infant manifested cerebellar atrophy, agranulocytosis, hematuria, and developmental delay.

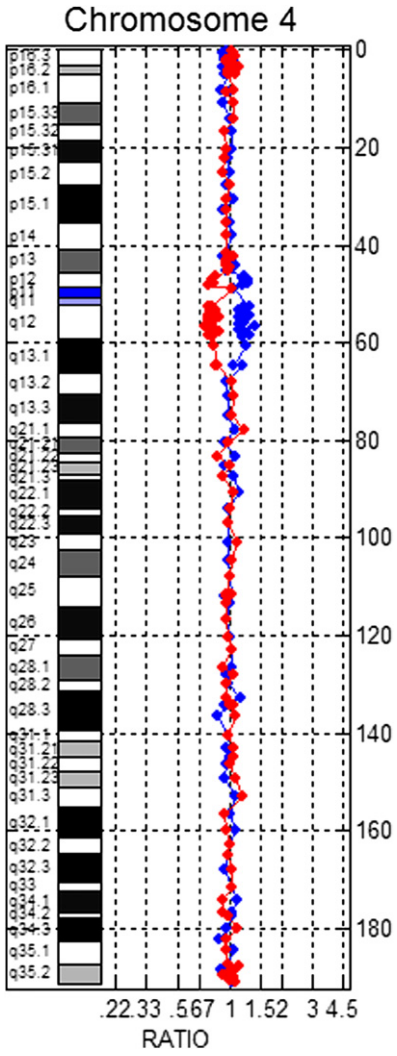

Fig. 7. Bacterial artificial chromosome-based array comparative genomic hybridization demonstrates a duplication in 4p12→q13.1 (RP11-380K10→RP11-21I19).

The genes most likely duplicated in the pericentromeric region of Chromosome 4 in this case were *COX7B2*, *GABRA4*, *GABRB1*, *COMMD8*, *ATP10D*, *CORIN*, *TXK*, *TEC*, *SLAIN2*, *SLC10A4*, *ZAR1*, *FRYL*, *DCUN1D4*, *SPATA18*, *RASL11B*, *SCFD2*, *FIPIL1*, *LNX1*, *LOC402176*, *CHIC2*, *PDGFRA*, *KIT*, *SRD5A3*, *TMEM165*, *CLOCK*, *EXOC1*, *KIAA1211*, *HOPX*, *SPINK2*, *REST*, *POLR2B*, *IGFBP7*, *SRIL*, *LPHN3*, *SRP5A2L2*, and *EPHA5*.

GABRA4 (GABA-A receptor, α-4 polypeptide) (OMIM 137141) (4p12) and *GABRB1* (GABA-A receptor, β-1 polypeptide) (OMIM 137190) (4p12) encode γ-aminobutyric acid (GABA) receptors and are involved in GABA-ergic neurotransmission of the mammalian central nervous system. Four GABA-A receptor subunit genes *GABRG1*, *GABRA2*, *GABRA4*. and *GABRB1* are located on 4p12. Ma et al [25] suggested the contribution of GABA-A receptor genes and the interaction between *GABRA4* and *GABRB1* in the etiology of autism. Kakinuma et al [26] reported three copies of *GABRA4* and two copies of *GABRB1* in an autistic patient

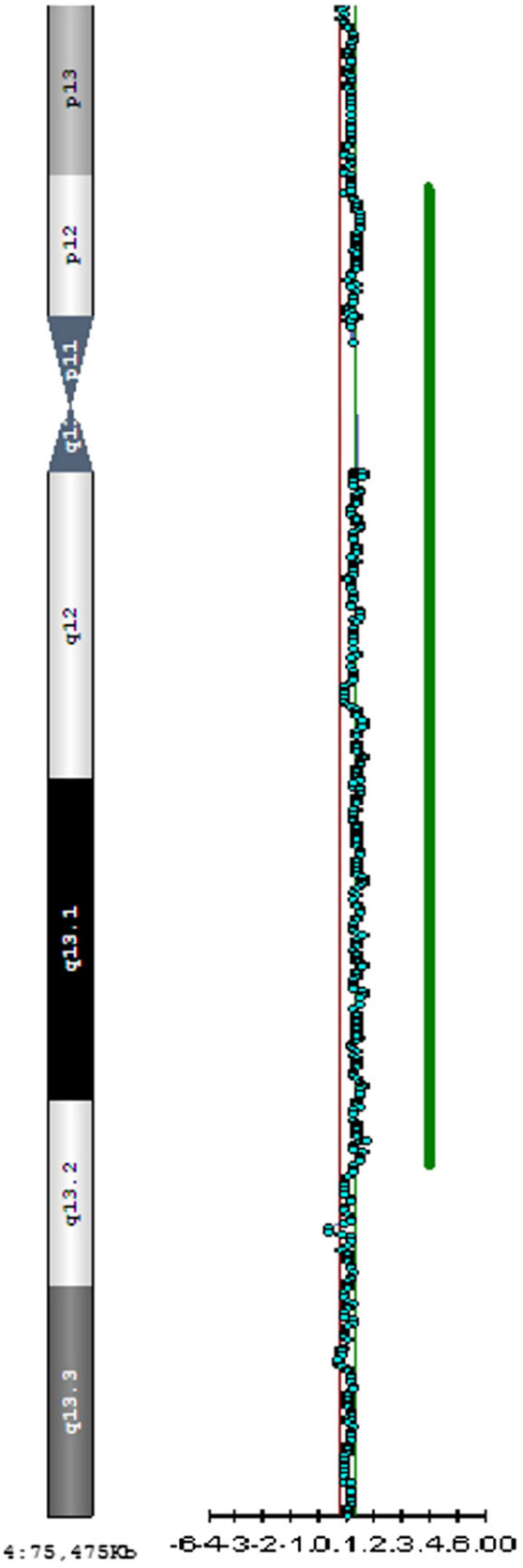

Fig. 8. Oligonucleotide-based array comparative genomic hybridization demonstrates a 21.7-Mb duplication in 4p12→q13.2 (46,178,756–67,852,719).

with mosaic 4p (4p12-p16) duplication and suggested that aberrant copy number of the GABA-A receptor subunit genes may contribute to the etiology of autism. GABA-A receptors also mediate tonic inhibition, and increased tonic inhibition has been associated with a decrease in inhibitory synaptic activity and an increase in seizure susceptibility [27−29].

Obesity or postnatal overweight can be a prominent phenotype in patients with a supernumerary r(4) [20,23,30]. Fang et al [20] reported obesity in a 27-year-old male with a supernumerary r(4). Vermeesch et al [30] reported postnatal overweight in a 22-year-old female with a mosaic supernumerary r(4). The present case had a genomic gain of the *IGFBP7* gene. A gene dosage effect of *IGFBP7* (insulin-like growth factor-binding protein 7) (OMIM 602867) (4q13) has been proposed to be related to obesity and tall stature in a 6-year-old girl with mosaic supernumerary r(4) and a proximal 4q duplication involving the *IGFBP7* gene [23].

With the advent of SKY, FISH, and aCGH, *de novo* non-acrocentric sSMCs can be well characterized by molecular cytogenetic technologies. We conclude that SKY, FISH, and aCGH are helpful in genetic counseling of prenatally detected sSMCs by providing the immediate and thorough information on the origin and genetic component of the sSMC.

Acknowledgments

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References

[1] Liehr T, Claussen U, Starke H. Small supernumerary marker chromosomes (sSMC) in humans. Cytogenet Genome Res 2004;107:55−67.

[2] Liehr T, Mrasek K, Weise A, Dufke A, Rodríguez L, Martínez Guardia N, et al. Small supernumerary marker chromosomes—progress towards a genotype-phenotype correlation. Cytogenet Genome Res 2006;112:23−34.

[3] Liehr T. Characterization of prenatally assessed de novo small supernumerary marker chromosomes by molecular cytogenetics. Methods Mol Biol 2008;444:27−38.

[4] Chen C-P, Lin C-C, Ko T-M, Tsai F-J, Chern S-R, Lee C-C, et al. Prenatal diagnosis and molecular cytogenetic characterization of a small supernumerary marker chromosome derived from chromosome 21. Taiwan J Obstet Gynecol 2010;49:377−80.

[5] Chen C-P, Lin C-C, Su Y-N, Tsai F-J, Chern S-R, Lee C-C, et al. Prenatal diagnosis and molecular cytogenetic characterization of a small supernumerary marker chromosome derived from chromosome 22. Taiwan J Obstet Gynecol 2010;49:381−4.

[6] Chen C-P, Chen M, Ko T-M, Ma G-C, Tsai F-J, Tsai M-S, et al. Prenatal diagnosis and molecular cytogenetic characterization of a small supernumerary marker chromosome derived from chromosome 8. Taiwan J Obstet Gynecol 2010;49:500−5.

[7] Liehr T, Ewers E, Kosyakova N, Klaschka V, Rietz F, Wagner R, et al. Handling small supernumerary marker chromosomes in prenatal diagnostics. Expert Rev Mol Diagn 2009;9:317−24.

[8] Viersbach R, Engels H, Gamerdinger U, Hansmann M. Delineation of supernumerary marker chromosomes in 38 patients. Am J Med Genet 1998;76:351−8.

[9] Liehr T, Weise A. Frequency of small supernumerary marker chromosomes in prenatal, newborn, developmentally retarded and infertility diagnostics. Int J Mol Med 2007;19:719−31.

[10] Soysal Y, Balci S, Hekimler K, Liehr T, Ewers E, Schoumans J, et al. Characterization of double ring chromosome 4 mosaicism associated with bilateral hip dislocation, cortical dysgenesis, and epilepsy. Am J Med Genet 2009;149A:2782−7.

[11] Chen C-P, Lin C-C, Su Y-N, Tsai F-J, Chen J-T, Chern S-R, et al. Prenatal diagnosis and molecular cytogenetic characterization of a small supernumerary marker chromosome derived from chromosome 18 and associated with a reciprocal translocation involving chromosomes 17 and 18. Taiwan J Obstet Gynecol 2010;49:188−93.

[12] Jacobs PA. Mutation rates of structural chromosome rearrangements in man. Am J Hum Genet 1981;33:44−54.

[13] Blennow E, Anneren G, Bui TH, Berggren E, Asadi E, Nordenskjöld M. Characterization of supernumerary ring marker chromosomes by fluorescence *in situ* hybridization (FISH). Am J Hum Genet 1993;53:433−42.

[14] Blennow E, Bui TH, Kristoffersson U, Vujic M, Anneren G, Holmberg E, et al. Swedish survey on extra structurally abnormal chromosomes in 39,105 consecutive prenatal diagnoses: prevalence and characterization by fluorescence *in situ* hybridization. Prenat Diagn 1994;14:1019−28.

[15] Blennow E, Nielsen KB, Telenius H, Carter NP, Kristoffersson U, Holmberg E, et al. Fifty probands with extra structurally abnormal chromosomes characterized by fluorescence *in situ* hybridization. Am J Med Genet 1995;55:85−94.

[16] Bartsch O, Loitzsch A, Kozlowski P, Mazauric ML, Hickmann G. Forty-two supernumerary marker chromosomes (SMCs) in 43,273 prenatal samples: chromosomal distribution, clinical findings, and UPD studies. Eur J Hum Genet 2005;13:1192−204.

[17] Chen C-P, Hsu C-Y, Tzen C-Y, Lee C-C, Chen W-L, Chen L-F, et al. Prenatal diagnosis of mosaic ring chromosome 4. Prenat Diagn 2007;27:485−7.

[18] Mattei MG, Mattei JF, Bernard R, Giraud F. Partial trisomy 4 resulting from a complex maternal rearrangement of chromosomes 2, 4, and 18 with interstitial translocation. Hum Genet 1979;51:55−61.

[19] Shashi V, Berry MN, Santos C, Pettenati MJ. Partial duplication of 4q12q13 leads to a mild phenotype. Am J Med Genet 1999;86:51−3.

[20] Fang Y-Y, Eyre HJ, Bohlander SK, Estop A, McPherson E, Träger T, et al. Mechanisms of small ring formation suggested by the molecular characterization of two small accessory ring chromosomes derived from chromosome 4. Am J Hum Genet 1995;57:1137−42.

[21] Pappas JG, Havens G, Bhatt J, Babu A. Familial chromosomal mosaicism for a small ring originated from chromosome 4. Cytogenet Cell Genet 1999;85:160. Abstract no: P674.

[22] Baldwin EL, May LF, Justice AN, Martin CL, Ledbetter DH. Mechanisms and consequences of small supernumerary marker chromosomes: from Barbara McClintock to modern genetic-counseling issues. Am J Hum Genet 2008;82:398−410.

[23] Bonnet C, Zix C, Grégoire MJ, Brochet K, Duc M, Rousselet F, et al. Characterization of mosaic supernumerary ring chromosomes by array-CGH: segmental aneusomy for proximal 4q in a child with tall stature and obesity. Am J Med Genet 2006;140A:233−7.

[24] Liehr T. Small supernumerary marker chromosome database. sSMC derived from chromosome 4. Available at, http://www.med.uni-jena.de/fish/sSMC/00START.htm. Accessed date December 7, 2010.

[25] Ma DQ, Whitehead PL, Menold MM, Martin ER, Ashley-Koch AE, Mei H, et al. Identification of significant association and gene-gene interaction of GABA receptor subunit genes in autism. Am J Hum Genet 2005;77:377−88.

[26] Kakinuma H, Ozaki M, Sato H, Takahashi H. Variation in GABA-A subunit gene copy number in an autistic patient with mosaic 4 p duplication (p12p16). Am J Med Genet B Neuropsychiatr Genet 2008;147B:973−5.

[27] Wisden W, Cope D, Klausberger T, Hauer B, Sinkkonen ST, Tretter V, et al. Ectopic expression of the $GABA_A$ receptor alpha6 subunit in hippocampal pyramidal neurons produces extrasynaptic receptors and an increased tonic inhibition. Neuropharmacology 2002;43:530−49.

[28] Sinkkonen ST, Vekovischeva OY, Möykkynen T, Ogris W, Sieghart W, Wisden W, et al. Behavioural correlates of an altered balance between synaptic and extrasynaptic $GABA_A$ergic inhibition in a mouse model. Eur J Neurosci 2004;20:2168–78.

[29] Roberts DS, Raol YH, Bandyopadhyay S, Lund IV, Budreck EC, Passini MA, et al. Egr3 stimulation of *GABRA4* promoter activity as a mechanism for seizure-induced up-regulation of $GABA_A$ receptor alpha4 subunit expression. Proc Natl Acad Sci USA 2005;102: 11894–9.

[30] Vermeesch JR, Petit P, Dutra A, Schröck E, Ried T, Fryns JP. A mosaic extra ring chromosome 4 in a female patient with postnatal overgrowth. Genet Couns 1999;10:195–6.