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Short Communication

Prenatal diagnosis and molecular cytogenetic characterization of concomitant familial small supernumerary marker chromosome derived from chromosome 4q (4q11.1–q13.2) and 5q13.2 microdeletion with no apparent phenotypic abnormality

Chih-Ping Chen [a, b, c, d, e, f, *], Schu-Rern Chern [b], Yen-Ni Chen [a], Shin-Wen Chen [a], Peih-Shan Wu [g], Chien-Wen Yang [b], Chen-Chi Lee [a], Meng-Shan Lee [a], Chen-Wen Pan [a], Wayseen Wang [b, h]

[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] Gene Biodesign Co. Ltd, Taipei, Taiwan
[h] Department of Bioengineering, Tatung University, Taipei, Taiwan

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ABSTRACT

Objective: We present prenatal diagnosis and molecular cytogenetic characterization of concomitant familial small supernumerary marker chromosome 4 [sSMC(4)] derived from 4q11.1–q12 and q13.2, and 5q13.2 microdeletion with no apparent phenotypic abnormality.
Materials and methods: A 32-year-old woman underwent amniocentesis at 21 weeks of gestation because of absent nasal bone on fetal ultrasound. Amniocentesis revealed a karyotype of 47,XX,+mar[13]/46,XX[3]. Array comparative genomic hybridization analysis on the cultured amniocytes revealed a 2.752-Mb duplication at 4q11–q12, a 1.949-Mb duplication at 4q13.2, and a 1.65-Mb deletion at 5q13.2. The woman underwent repeat amniocentesis at 24 weeks of gestation for molecular cytogenetic characterization. The phenotypically normal parents and their elder son underwent genetic analysis.
Results: At repeat amniocentesis, interphase fluorescence *in situ* hybridization analysis on uncultured amniocytes revealed 79.25% (84/106) mosaicism for the sSMC(4), and metaphase fluorescence *in situ* hybridization analysis on cultured amniocytes revealed that all 20 cells examined (100%) had the sSMC(4). Polymorphic DNA marker analysis on uncultured amniocytes excluded uniparental disomy 4. The father had a karyotype of 47,XY,+mar[2]/46,XY[38], and interphase fluorescence *in situ* hybridization revealed 2.91% (3/103) mosaicism for the sSMC(4) in his peripheral blood. The mother carried the 5q13.2 microdeletion. The elder son had a karyotype of 47,XY,+mar[27]/ 46,XY[13] with duplications of 4q11–q12 and 4q13.2. A 3105 g female baby was delivered at term with no apparent phenotypic abnormality.
Conclusion: Prenatal diagnosis of concomitant sSMC and microdeletion should raise a suspicion of familial inheritance.

Introduction

We previously reported prenatal diagnosis and molecular cytogenetic characterization of mosaicism for a small supernumerary marker chromosome 4 [sSMC(4)] with phenotypic abnormality [1] and maternal transmission of 5q13.2 microdeletion with coarctation of aorta [2]. Here, we present an additional case with familial inheritance of an sSMC(4) and concomitant familial 5q13.2 microdeletion with no apparent phenotypic abnormality. Our presentation may provide useful information of genetic counseling for

* Corresponding author. Department of Obstetrics and Gynecology, Mackay Memorial Hospital, 92, Section 2, Chung-Shan North Road, Taipei 10449, Taiwan.
E-mail address: cpc_mmh@yahoo.com (C.-P. Chen).

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prenatally detected sSMC(4) derived from 4q11–q13.2 and 5q13.2 microdeletion.

Materials and methods

Clinical description

A 32-year-old, gravida 2, para 1 woman underwent amniocentesis at 21 weeks of gestation because of absent nasal bone on fetal ultrasound. Her husband was 34 years old. She and her husband were healthy, and there was no family history of congenital malformations. The couple had a 3-year-old healthy son with no phenotypic abnormality. Amniocentesis revealed a karyotype of 47,XX,+mar[13]/46,XX[3]. Array comparative genomic hybridization (aCGH) analysis on the cultured amniocytes revealed a 2.752-Mb duplication at 4q11–q12, a 1.949-Mb duplication at 4q13.2, and a 1.65-Mb deletion at 5q13.2. The woman underwent repeat amniocentesis at 24 weeks of gestation. The phenotypically normal parents and their elder son underwent genetic analysis.

Conventional cytogenetic analysis

Routine cytogenetic analysis by G-banding techniques at the 550 band of resolution was performed on cultured amniocytes and cultured lymphocytes obtained from the peripheral bloods of the parents and their elder son.

Quantitative fluorescent polymerase chain reaction assays

Quantitative fluorescent polymerase chain reaction analysis was performed on the DNAs extracted from uncultured amniocytes and parental bloods. Briefly, primers specifically flanking short tandem repeat markers on chromosome 4 region, such as D4S2366 (4p16.1) and D4S2362 (4p15.32), were applied to undertake polymorphic marker analysis to exclude uniparental disomy 4.

Array comparative genomic hybridization

Whole-genome aCGH on the DNAs extracted from uncultured amniocytes derived from 10 mL of amniotic fluid and the peripheral blood of the parents and their elder son was performed using CytoChip ISCA (Illumina, San Diego, CA, USA). The CytoChip ISCA array has 60,000 probes and a median resolution of 51 kb.

Fluorescence in situ hybridization

Fluorescence *in situ* hybridization (FISH) analysis was performed on 106 interphase uncultured amniocytes, 20 metaphase cultured amniocytes, 103 interphase cultured lymphocytes of paternal blood, and 20 metaphase cultured lymphocytes of paternal blood using a 4q12-specific bacterial artificial chromosome probe of RP11-535C7 (fluorescein isothiocyanate, spectrum green) and a 4q13.2-specific bacterial artificial chromosome probe of RP11-977E16 (Texas Red, spectrum red) according to the standard FISH protocol.

Spinal muscular atrophy multiplex ligation-dependent probe amplification gene dosage analysis

Cultured amniocytes were analyzed by spinal muscular atrophy (SMA) multiplex ligation-dependent probe amplification (MLPA) to examine common deletion of exon 7 of *SMN1*.

Results

At repeat amniocentesis, conventional cytogenetic analysis of cultured amniocytes revealed a karyotype of 47,XX,+mar in 23/23 colonies (Figure 1), and interphase FISH analysis on uncultured amniocytes revealed 79.25% (84/106 cells) mosaicism for the sSMC(4) (Figure 2A and 2B). Metaphase FISH analysis on cultured amniocytes revealed that 100% (20/20) cells had the sSMC(4)

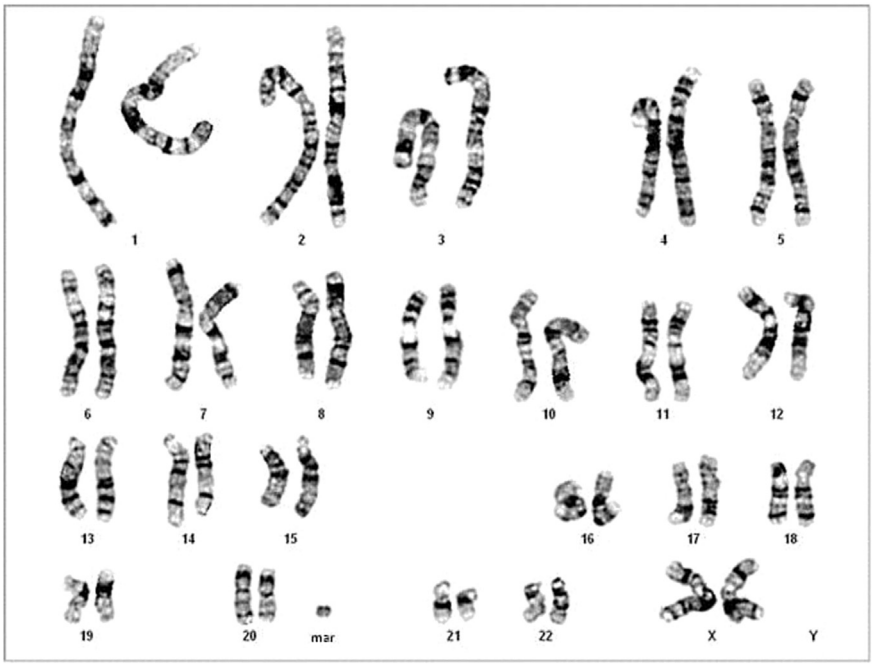
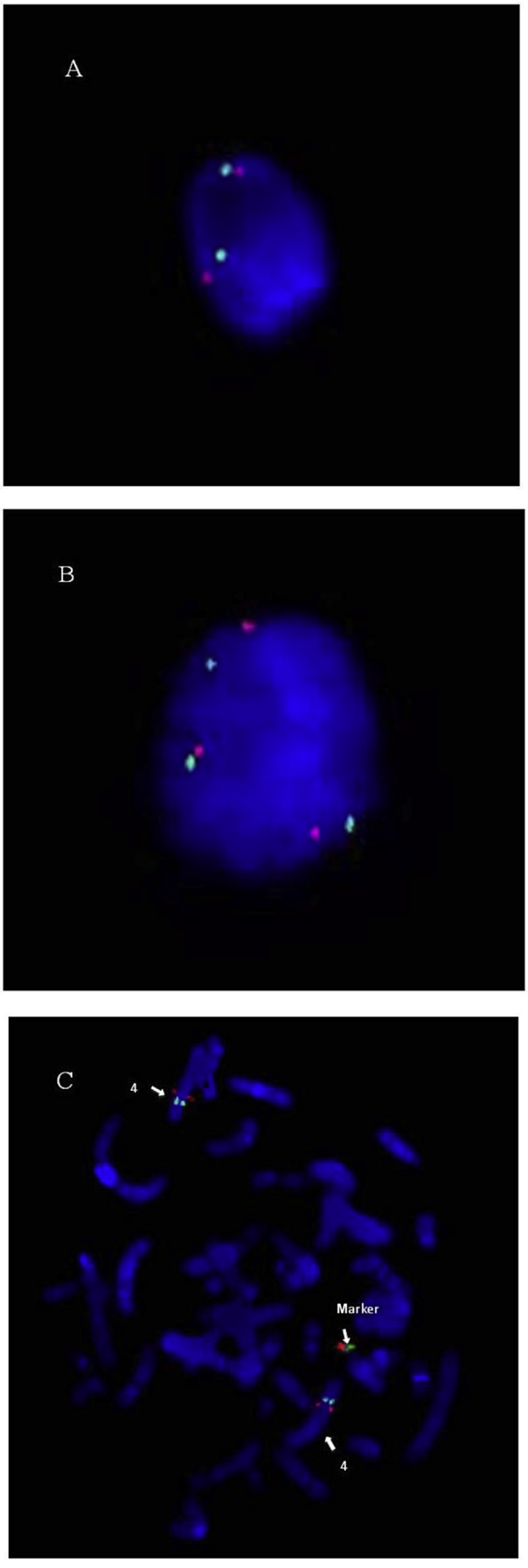


Figure 1. A karyotype of 47,XX,+mar. mar = marker chromosome.



derived from 4q12–q13.2 (Figure 2C). Interphase FISH analysis on cultured lymphocytes of paternal blood revealed 2.91% (3/103 cells) mosaicism for the sSMC(4). Metaphase FISH analysis of paternal blood on cultured lymphocytes revealed no mosaicism for the sSMC(4) in all 20 cells examined. The father had a karyotype of 47,XY,+mar[2]/46,XY[38], the mother had a karyotype of 46,XX, and the elder son had a karyotype of 47,XY,+mar[27]/46,XY[13] in the peripheral blood. The cord blood had a karyotype of 47,XX,+mar[28]/46,XX[12]. Quantitative fluorescent polymerase chain reaction analysis of uncultured amniocytes excluded uniparental disomy 4. SMA MLPA gene dosage analysis excluded homozygous deletion of *SMN1* gene and compound heterozygotes for deletion of *SMN1* exon 7. The aCGH analysis of uncultured amniocytes revealed a result of arr 4q11q12 (52,685,219–55,508,553) × 3.0, 4q13.2 (67,239,638–69,188,444) × 3.0, 5q13.2 (68,936,020–70,586,989) × 1.3 (Figure 3). The 2.823-Mb 4q11–q12 duplication encompasses 31 genes including 13 Online Mendelian Inheritance in Man (OMIM) genes of *DCUN1D4*, *SGCB*, *SPATA18*, *USP46*, *DANCR*, *MIR4449*, *SNORA26*, *RASL11B*, *FIP1L1*, *LNX1*, *CHIC2*, *GSX2*, and *PDGFRA*. The 1.949-Mb 4q13.2 duplication encompasses 27 genes including eight OMIM genes of *CENPC*, *STAP1*, *UBA6*, *CNRHR*, *TMPRSS11D, TMPRSS11A*, *GCOM2*, and *SYT14P1*. The 1.65-Mb 5q13.2 deletion encompasses 35 genes including five OMIM genes of *SMN2*, *SERF1A*, *SMN1*, *NAIP*, and *GTF2H2*. The aCGH analysis of maternal blood revealed a result of arr 5q13.2 (69,238,707–70,586,989) × 1.3 (Figure 4). The aCGH analysis of paternal blood revealed a result of arr (1–22) × 2, X × 1, Y × 1. The aCGH analysis of the peripheral blood of the elder son revealed a result of arr 4q11q12 (52,685,219–55,508,553) × 3.0, 4q13.2 (67,450,575–69,188,444) × 3.0 (Figure 5). A 3105 g female baby was delivered at term with no apparent phenotypic abnormality.

Discussion

The present case had a maternal transmission of the 1.65-Mb 5q13.2 microdeletion encompassing *SMN2*, *SERF1A*, *SMN1*, *NAIP*, and *GTF2H2*. Homozygous deletion of *SMN1* (OMIM 600354) and expression changes for *SMN2* (OMIM 601627) cause the SMA phenotype. *SERF1A* (OMIM 603011) and *GTF2H2* (OMIM 601748) are associated with SMA. *NAIP* (OMIM 600355) is associated with apoptosis. Huang et al [3] previously reported a 5q13.2 microdeletion encompassing *OCLN*, *SMN2*, *SERF1A*, *SMN1*, *NAIP*, and *GTF2H2* in a child with oculoauriculovertebral spectrum (OMIM 164210). Chen et al [2] previously reported a 5q13.2 microdeletion encompassing *OCLN*, *SMN2*, *SERF1A*, *SMN1*, *NAIP*, and *GTF2H2* in a fetus with coarctation of aorta. The present case did not have the deletion of *OCLN*. Compound heterozygous mutations or homozygous mutations of *OCLN* have been associated with autosomal recessive band-like calcification with simplified gyration and polymicrogyria (OMIM 251290).

The present case had a familial transmission of proximal 4q duplication involving 4q11–q12 and 4q13.2. Individuals with very small proximal duplications of 4q11–q13 are usually healthy and of normal height, with only the effects of developmental delay and learning disability [4]. Duplications involving proximal 4q have been associated with variable phenotypic abnormalities [5–11]. Mattei et al [5] reported a 6-year-old girl with psychomotor retardation, microcephaly, facial dysmorphism, and clinodactyly of the fifth fingers, and a 4q12–q13 duplication. Fang et al [6] reported a 27-year-

Figure 2. Fluorescence *in situ* hybridization analysis using the bacterial artificial chromosome probes of RP11-535C7 (4q12, spectrum green) and RP11-977E16 (4q13.2, spectrum red) shows (A) an interphase cell with disomy 4, (B) an interphase cell with the sSMC(4), and (C) a metaphase cell with the sSMC(4). sSMC = small supernumerary marker chromosome.

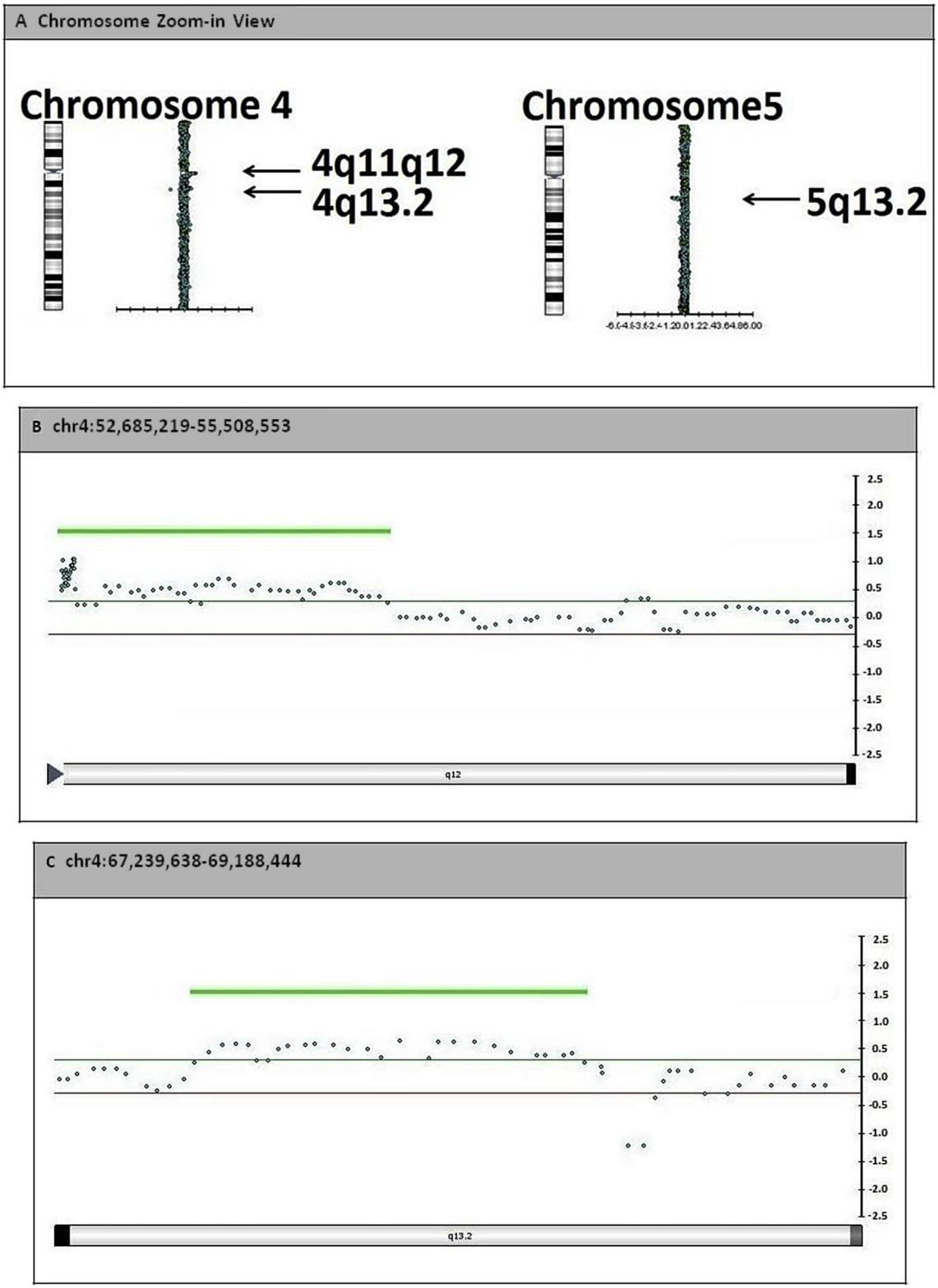


Figure 3. Array comparative genomic hybridization analysis of uncultured amniocytes shows a 4q11–q12 duplication, a 4q13.2 duplication, and a 5q13.2 deletion. (A) Chromosome zoom-in view, (B and C) chromosome 4 (52,685,219–55,508,553 and 67,239,638–69,188,444, respectively), and (D) chromosome 5 (68,936,020–70,586,989).

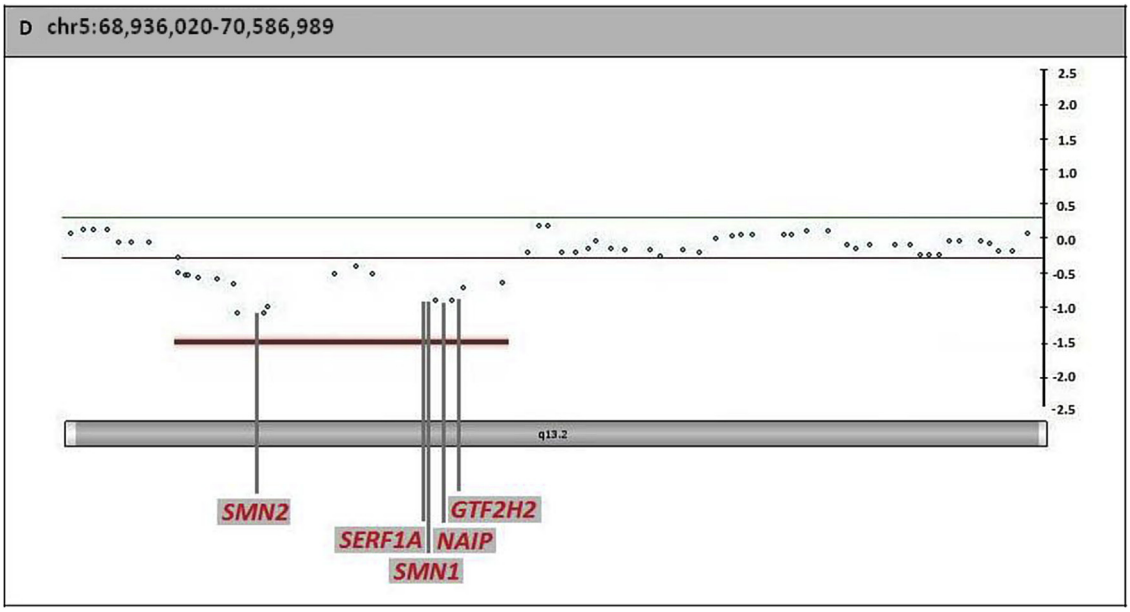


Fig. 3. (*continued*).

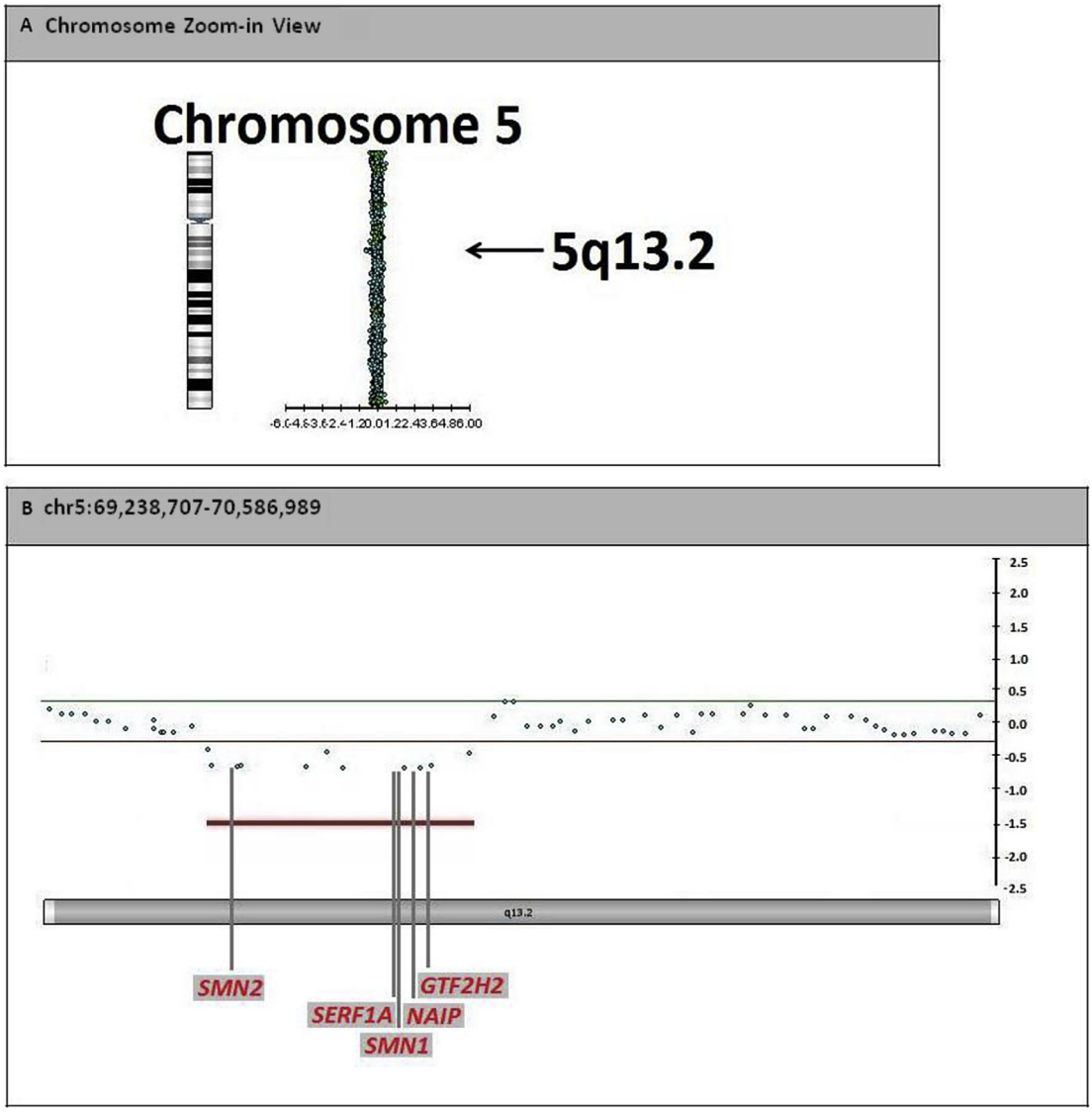


Figure 4. The aCGH analysis of maternal blood shows a 5q13.2 deletion. (A) Chromosome zoom-in view and (B) chromosome 5 (68,936,020–70,586,989). aCGH = array comparative genomic hybridization.

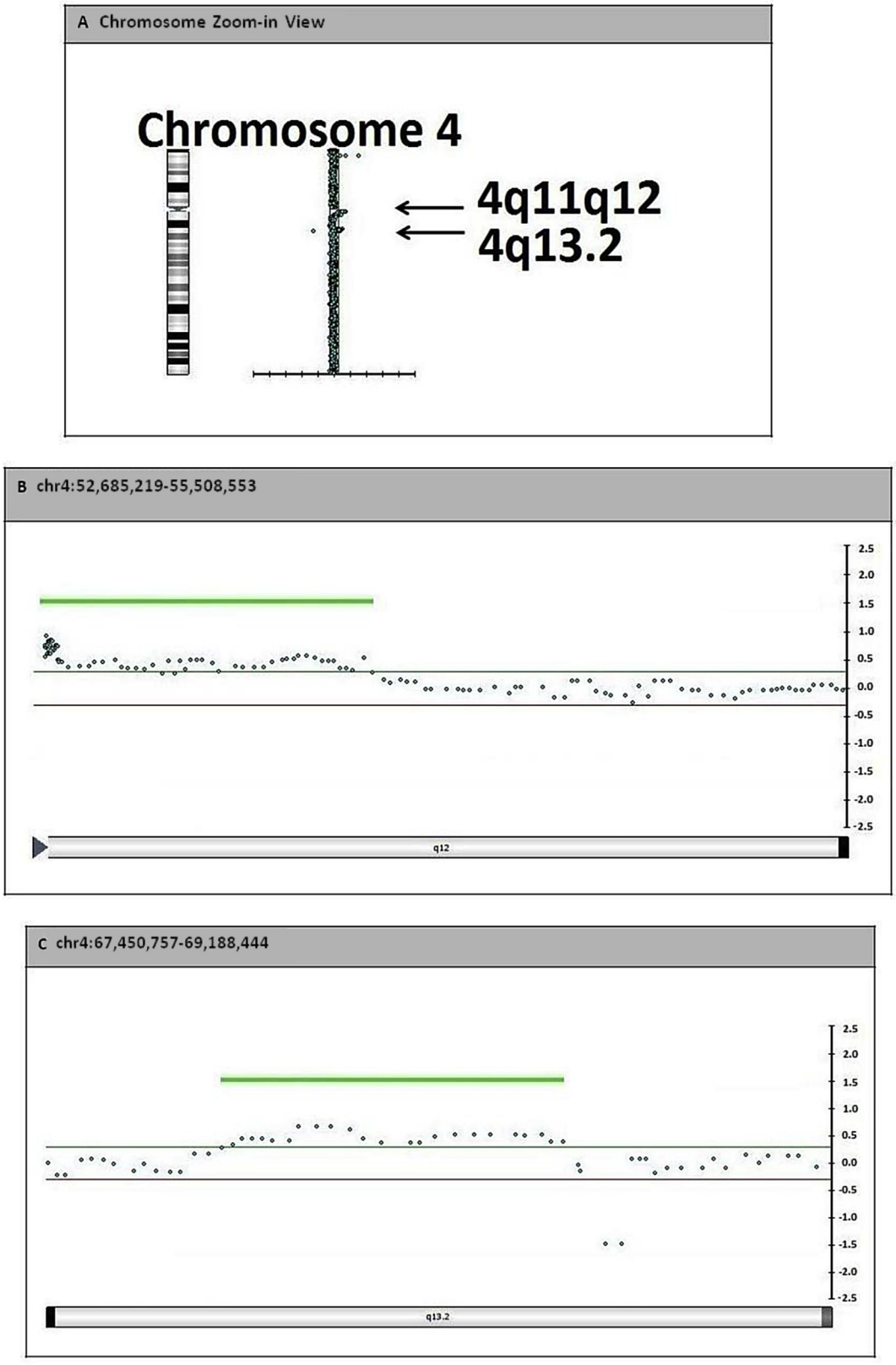


Figure 5. The aCGH analysis of the blood of the elder son shows a 4q11–q12 duplication and a 4q13.2 duplication. (A) Chromosome zoom-in view, and (B and C) chromosome 4 (52,685,219–55,508,553 and 67,450,757–69,188,444, respectively). aCGH = array comparative genomic hybridization.

old male with severe mental retardation, language disability, obesity, facial dysmorphism, clinodactyly of the hand, and syndactyly of foot, and a karyotype of 47,XY,+r(4)(::p10→q12::). Zollino et al [7] reported a 15-year-old girl with moderate intellectual disability, minor physical anomalies, and destructive behavior, and a 4q13.1–q22.2 duplication. Shashi et al [8] reported a 2-year-and-8-month-old boy with microcephaly, mental retardation, and mild facial dysmorphism, and a duplication of 4q12–q13. Bonnet et al [9] reported a 6-year-old girl with developmental delay, tall stature, and obesity, and 82% mosaicism for an sSMC(4) derived from 4q10–q13 in peripheral lymphocytes. Matoso et al [10] reported an 8-year-old girl with developmental delay, speech disability, and attention-deficit hyperactivity disorder, and an 8.6-Mb duplication of 4q13.1–q13.3. Marle et al [11] reported prenatal diagnosis of 47,XX,+mar by amniocentesis with an sSMC(4) derived from 4p11–q12 in a fetus with only hypertelorism and long philtrum at termination of pregnancy. Liehr [12] reported a 3-year-old boy with developmental delay and an sSMC(4) derived from 4p11–q12, and a male with maternally transmitted mosaic sSMC(4) derived from 4p11–q12 presenting only mild motor retardation and mild hypotonia.

In summary, we performed prenatal diagnosis and molecular cytogenetic characterization of concomitant familial sSMC(4) derived from 4q11.1–q12 and q13.2, and 5q13.2 microdeletion with no apparent phenotypic abnormality. We suggest that prenatal diagnosis of concomitant sSMC and microdeletion should raise a suspicion of familial inheritance.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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