Clinical Report
Small Supernumerary Marker Chromosome Causing Partial Trisomy 6p in a Child With Craniosynostosis

Olaya Villa,1,2,3 Miguel del Campo,1,3,4 Marta Salido,2 Blanca Gener,1 Laura Astier,2 Jesus del Valle,1 Fátima Gallastegui,1,3 Luis A. Pérez-Jurado,1,3,4* and Francesc Solé2

1Genetics Unit, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain
2Laboratory of Cytogenetics and Molecular Biology, Hospital del Mar, URTTS/IMAS-IMIM, Barcelona, Spain
3Center for Biomedical Research on Rare Diseases, Barcelona, Spain
4Program in Molecular Medicine and Genetics, Hospital Vall d’Hebron, Barcelona, Spain

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We report on a child with a small supernumerary marker chromosome (sSMC) causing partial trisomy 6p. The child showed a phenotype consisting of neonatal craniosynostosis, microcephaly, and borderline developmental delay. By molecular techniques the sSMC has been shown to contain ~16 Mb of genomic DNA from 6p21.1 to 6cen, being de novo and of maternal origin. © 2007 Wiley-Liss, Inc.

Key words: craniosynostosis; small supernumerary marker chromosome; FISH; CGH; SKY-FISH; chromosome 6p; developmental delay


INTRODUCTION

Small supernumerary marker chromosomes (sSMC) are defined as structurally abnormal chromosomes that cannot be identified or characterized unambiguously by conventional banding cytogenetics alone and are, in general, equal or smaller in size than a chromosome 20 of the same metaphase spread [Liehr et al., 2004]. These marker chromosomes, including inverted duplicated, ring, and minute chromosomes, are present in 4.5/10,000 newborns. An almost double frequency (7.9/10,000) is detected in prenatal studies, most likely due to the higher risk of that population (advanced maternal age and other factors such as previous spontaneous abortion or malformed fetuses) as well as the reduced viability of the pregnancy in some cases. sSMC have been found for all chromosomes with different frequencies: about 30% are derived from chromosome 15, 20% from 22, 9% from 12, and only 1% from chromosome 6. The characterization of several sSMC has led to the description and better definition of specific disorders such as Pallister-Killian syndrome caused by i(12p), the cat-eye syndrome due to invdup(22), or the i(9p) and i(18p) syndromes [Liehr et al., 2006a]. In addition to the potential consequences of partial trisomy for the region contained in the sSMC, uniparental disomy (UPD) for the entire sSMC sister chromosomes has been reported in association with sSMC. The overall risk for abnormal phenotype when an uncharacterized sSMC is detected prenatally is in the range of 26%, but if high resolution ultrasound studies are normal, the risk decreases to 18% [Graf et al., 2006]. About one third of cases of prenatal detection of sSMC are electively interrupted [Bartsch et al., 2005].

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*Correspondence to: Luis A. Pérez-Jurado, M.D., Ph.D., Unitat de Genètica, Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Dr. Aiguader 80, 08003 Barcelona, Spain. E-mail: luis.perez@upf.edu
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Partial trisomy 6p is an uncommon alteration, and generally secondary to an unbalanced segregation of a familial reciprocal translocation. There are approximately 40 cases described in which the trisomy extends to the telomere with variable proximal breakpoints from 6p25 to 6p11 [Villa et al., 2000; Engelen et al., 2001; Ng et al., 2001; Domínguez et al., 2003]. To the best of our knowledge, isolated trisomy for the most centromeric portion of the short arm has never been reported.

Craniosynostosis is defined as the premature fusion of one or several sutures of the skull. It is one of the most common craniofacial anomalies at birth with a prevalence of 1:2,100–3,000 newborns. There are three main mechanisms involved in this premature fusion of the sutures: (1) as a consequence of primary microencephaly with deficient brain growth, usually reflected in a small head circumference (2) as a primary bone growth disorder (craniosynostosis syndromes) or (3) as a consequence of external factors having a deformational compression effect on the skull [Aleck, 2004].

Herein we present a report of a child with a mild phenotype including craniosynostosis and a marker chromosome causing partial trisomy 6p (p21.1 → cen).

**MATERIALS AND METHODS**

**Clinical Report**

The patient is the first child born to healthy nonconsanguineous parents. Family history was non-contributory. The pregnancy was normal, with no prenatal or perinatal complications. He was born at term with birth weight and length in the 50th centile. Head circumference was 32.5 cm (10th centile), showing marked brachycephaly with mild plagiocephaly, apparently closed anterior fontanel and clinical ridging of the sagittal suture. Ongoing global craniosynostosis was suspected on cranial X-ray, but could not be diagnosed with certainty since all cranial sutures seemed to be at a similar stage of fusion. Ophthalmologic examination was normal.

His growth and psychomotor development have been followed closely since birth. Medical history is unremarkable except for an advanced dentition. At 2 years 8 months, his height was 93 cm (50th centile), weight 15.1 kg (75–90th centile) and head circumference 46 cm (3rd centile, −3.5 SDS). Microcephaly was more obvious, still with brachycephaly, but the asymmetry was then almost undetectable. Other features included round flat face with midface hypoplasia, unusually shaped ear tragus and thin upper lip, (Fig. 1). A generalized mildly hyperpigmented pattern of the skin (clearly showing a distribution along Blaschko lines in several areas), not seen immediately at birth, has become clearly visible with time. Psychomotor development is mildly delayed or borderline with a global developmental age of 2 years 3 months at a chronological age of 2 years 10 months. Using the “McCarthy scale of children abilities,” the full-scale IQ was 81 (normal range of 80–120) with a motor component of 44 (normal range 40–60). However, the presence of increased activity, distractibility, and poor attention span suggested a diagnosis of attention deficit disorder with hyperactivity (ADHD). Computerized tomography (CT) scans and magnetic resonance imaging (MRI) at 2 years of age detected a global reduction in brain volume but no specific brain abnormalities.

**Cytogenetics and FISH**

Routine cytogenetics was performed from peripheral blood lymphocytes after phytohemagglutinin (PHA) stimulation and Wright staining (G banding) by conventional procedures at 3 days, 10 and 18 months of age.

Spectral karyotyping (SKY) technique was applied as manufacturer’s instructions (SKYPaint™, Applied Spectral Imaging Ltd., Migdal Ha’Emek, Israel). Ten metaphases were analyzed with Nikon Eclipse E600 microscope using a custom designed optical filter (SKY-1; Chroma Technology, Brattleboro, VT).

Whole chromosome paint (WCP) specific for chromosome 6 (Metasystems GmbH, Altlussheim, Germany) was used for FISH analysis in the patient. A gain of material was detected on chromosome 6 in the patient, which was confirmed with WCP specific for chromosome 6 (Fig. 1).

**FIG. 1.** Patient at age 8 months showing round and flat face with high forehead and a thin superior lip (**a**: frontal view), along with a flat occiput and brachycephaly with slight hemifacial protrusion of the right side (**b** and **c**: upper view). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
Germany) with FITC labeling was performed as manufacturer's protocol with minimal changes.

Comparative genomic hybridization (CGH) was performed as previously reported with minimal changes [Kallioniemi et al., 1993]. At least 10 metaphases were analyzed with CytoVysion software (Applied Imaging Systems, New Castle, UK).

Fluorescence in situ hybridization (FISH) using bacterial artificial chromosomes (BACs) as probes was performed in order to precisely delimitate the trisomic region. BAC clones mapped from 6p25 to 6q11 were selected from the 32k library (www.chori.org/bacpac), and DNA was isolated using standard alkaline lysis techniques (Qiagen GmbH, Hilden, Germany). The cloned DNA was labeled with SpectrumRed-dUTP or SpectrumGreen-dUTP by nick translation (Vysis, Chicago, IL). Images were captured using the CytoVision software (Applied Imaging Systems, UK).

**Genotyping**

Genotyping of microsatellite markers was carried out on peripheral blood DNA from the patient and their parents. Three polymorphic microsatellite repeats from 6p21.2, 6p11.2 and 6q15 (loci D6S1610, D6S257 and D6S462) were applied. PCR amplifications were done by standard protocols and the products were analyzed on a Genescan ABI 3100 genotyper (Applied Biosystems). To define the degree of mosaicism in different tissues, we analyzed DNA extracted from peripheral blood, buccal smears and hair roots. To obtain relative quantification of alleles, we compared the ratios of peak heights from the Genescan analyses.

**RESULTS**

The neonatal GTG-banding karyotype was 47,XY,+mar[14]/46,XY, showing mosaicism for a small marker chromosome. Both parents had a normal karyotype in peripheral blood. SKY revealed that the origin of the extra material was from chromosome 6 (sSMC(6)), which was confirmed with specific WCP6: the entire length of both homologues 6 and the marker chromosome were painted. Chromosomal CGH showed a gain of the region from 6p21.1 to the centromere (Fig. 2). Repeated cytogenetic and FISH studies on interphase nuclei from blood samples at the age of 18 months revealed a decrease in the level of mosaicism for the sSMC(6), from 70% of cells (neonatal) to 30% (18 months).
Genotyping of microsatellite markers D6S1610, D6S257, and D6S462, located at 6p21.2, 6p11.2, and 6q15, respectively, revealed the presence of an extra allele of maternal origin exclusively at D6S257 (6p11.2). The presence of a normal paternal allele at all loci ruled out the existence of UPD. To compare the degree of mosaicism in tissues from different origin, we obtained DNA from buccal smear, peripheral blood and hair at age 18 months. In buccal smear the presence of the marker chromosome was estimated in ~55% of cells, ~30% in peripheral blood and ~76% in hair. However, the suboptimal quality of the DNA obtained from hair decreased the reliability of the technique.

In order to delineate the expression patterns, we performed FISH analysis using multiple BACs clones as probes (Table I, supplemental). One of the breakpoints was mapped in 6p21.1 at Mb 43.5 (between clones RP11-400E17 and RP11-444C15) while the other was located close to the centromere at Mb 59.5, since no signal was obtained with any clone from 6q. Therefore, the length of the sSMC(6) is ~16 Mb from centromere into the p arm (Fig. 2), containing ~60 genes (from SPATS1 to C6orf216) for which the patient is trisomic in the cells carrying the sSMC(6).

DISCUSSION

It is important to characterize in detail as many sSMC cases as possible and also report them, to have the basis for a more proper genotype–phenotype correlation. In addition to identify genes for specific phenotypes, this information can be used for prenatal counseling and for more specific management and monitoring of patients. sSMC are more commonly found in patients with congenital anomalies and/or mental disability as well as in subfertile persons. An sSMC(6) is present in 33% of cases with multiple sSMC, but in only ~1% of cases with a single sSMC [Liehr et al., 2006b].

To the best of our knowledge, this is the first report of a case presenting an isolated partial trisomy of a proximal fragment of the short arm of chromosome 6 (p21.1 → cen), in the form of sSMC(6), and a phenotype consisting of neonatal craniosynostosis, microcephaly and developmental delay.

Partial 6p trisomies that partially overlap with the one we describe here have been reported in patients with different phenotypes (Table I). Duplication of an almost entire 6p in association with 9p deletion had been described in a patient with psychomotor delay, low birth weight, microcephaly, high forehead, small mouth or thin lips, and genital and heart anomalies [Lytle et al., 1989]. A male patient with a tandem duplication of 6p21.3p12 was reported with a phenotype including growth retardation, psychomotor delay and craniofacial, brain, limb, and genital anomalies. Although craniosynostosis was not mentioned, a premature closure of the anterior fontanel was evident at 7 months of age. The child died at the age of 16 months [Villa et al., 2000]. A girl with developmental delay, craniofacial anomalies, deficit of language skills, moderate difficulties with fine and gross motor skills, lack of attention and hyperactivity presented an interstitial tandem duplication of 6p22.2p21.1 [Ng et al., 2001].

Interestingly, involvement of the cranial sutures leading to a premature craniosynostosis of the sagittal and basal parts of the coronal sutures has also been reported in a patient with two sSMC, one of them originating from chromosome 6 and the other from chromosome 11 [Maurer et al., 2001]. The sSMC(6) was a larger dicentric ring structure, containing from 6p22 to 6p10 and part of 6p24. Notwithstanding and in contrast to our case, this patient had a normal head circumference at birth (37 cm), distinctive dysmorphic features and considerable motor difficulties although verbal abilities were appropriate for his age. Furthermore, even though the sSMC(11) was reported as to containing only heterochromatin, the putative presence of a partial trisomy of chromosome 11 could mask the phenotype caused by gain of 6p.

A sSMC(6) likely similar to the one described here including from 6p21 to 6p11 was reported in a girl with intrauterine growth retardation, genitourinary anomalies, mild mental retardation and transient neonatal diabetes (TNDM). Paternal isodisomy for the normal chromosomes 6 and maternal origin for the sSMC was demonstrated. Therefore, the clinical consequences of the partial 6p trisomy in this case were confounded by the effects of UPD 6. Specifically, the transient neonatal diabetes was most likely due to the paternal UPD 6 since the TNDM gene is known to be imprinted and located at 6q24, and this phenotype is commonly associated to paternal UPD for chromosome 6 [Crolla et al., 1998].

The finding of the sSMC(6) as the only cytogenetic alteration in our patient and the fact that it occurred de novo suggest a causal relationship with the phenotype. In the absence of other external factors with deformational effects, the craniosynostosis of this patient could be a primary bone defect, but both the involvement of all sutures on X-ray as well as the evolving microcephaly suggested that the main cause was most likely primary microencephaly. Therefore, we propose that there are dosage sensitive genes in the trisomic region that may lead to microcephaly with global craniosynostosis and abnormal brain development through overexpression. To date, no loci associated with craniosynostosis or microcephaly have been found in the chromosome region 6p21.1 → cen, and the region is quite large (16 Mb, ~60 genes) to define just one or a few candidates for these alterations. Nonetheless, based on the known function and expression pattern, the gene BMP5 coding for the bone...
### Table I. A Comparison of Phenotypes of the Reported Cases With Partial Proximal Trisomy 6p.

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<tbody>
<tr>
<td>Additional disbalance/</td>
<td>Almost entire 6p</td>
<td>6p21 3-6p12</td>
<td>6p22.2-6p21.1</td>
<td>6p22-6p10</td>
<td>6p21-6p11</td>
<td>6p21-6p10</td>
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<tr>
<td>rearrangements</td>
<td>Monosomy 9pter-9p24</td>
<td>—</td>
<td>—</td>
<td>Trisomy 6p24 and sSMC(11) (only heterochromatin?)</td>
<td>Paternal UPD(6) (isodisomy)</td>
<td>—</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
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<tr>
<td>Age at last evaluation</td>
<td>4.5 m</td>
<td>16 m</td>
<td>7 y 4 m</td>
<td>5 y</td>
<td>2 y 8 m</td>
<td>2 y 10 m</td>
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<td>Craniofacial anomalies</td>
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<td>Frontal bossing</td>
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<td>Broad nasal bridge</td>
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<tr>
<td>Long/smooth philtrum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>—</td>
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<tr>
<td>Thin upper lip</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>—</td>
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<tr>
<td>Down-turned mouth</td>
<td>?</td>
<td>? (microstomia)</td>
<td>+</td>
<td>+</td>
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<td>—</td>
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<tr>
<td>Abnormal ears</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Micrognatia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>—</td>
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<td>Thick lips and prominent</td>
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<td>checks</td>
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<td>—</td>
<td>+</td>
<td>—</td>
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<tr>
<td>Craniosynostosis</td>
<td>—</td>
<td>+/- (premature closure of anterior fontanel)</td>
<td>—</td>
<td>+ (sagittal and basal parts of the coronal sutures)</td>
<td>—</td>
<td>+ (closed anterior fontanel; premature fusion of sagittal sutures)</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Prenatal growth retardation</td>
<td>+</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Postnatal growth retardation</td>
<td>+</td>
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| Developmental delay      | +                   | +                   | +                | +                    | +/-                           | + (mild) +/
|                          |                     |                     |                  |                      |                               |              |
| Cardiac anomalies        | + (VSD, PDA, and PS)| + (cryptorquardium) | —                | — (ASD and PDA)      | —                             | —            |
| Genital anomalies        | + (microphallus)    | + (distal arthrogryposis) | —                | —                    | + (prominent clitoris)        | —            |
| Distal contractures      | + (camptodactyly)   | (tight heel cords)  | —                | —                    | —                             | —            |
| Attention deficit/ hyperactivity | NA             | NA                  | —                | —                    | —                             | +            |
| Other features           | Trigonocephaly     |                     | Cynodactily      | Left kidney hypoplasia, mit-tagus, strabismus | Transient neonatal diabetes | Blaschko lines, advanced dentition |
|                          |                     |                     |                  |                     | Died at 16 months             | Tapered fingers |

+, present; -, absent; ?, unknown; +/-, borderline; UPD, uniparental disomy; m, months; y, years; ASD, atrial septal defect; VSD, ventriculoseptal defect; PDA, persistent ductus arteriosus; PS, valvular pulmonic stenosis; NA, not applicable.
morphogenetic protein 5 (OMIM #112265), located at 6p12.1, could be a good candidate for the premature fusion of the cranial sutures.

In order to establish specific correlations between the cytogenetic anomaly and the phenotype, the presence of mosaicism precludes the precise definition of the clinical picture for sSMC(6) when present in all cells, if this situation were viable. As in many cases of mosaic chromosome anomalies, the effects on cognition and neurodevelopment are present but remain subtle. This child has attention deficit and hyperactivity along with mild difficulties in language development. However, his motor skills are excellent when his attention is properly driven. A need for behavioral intervention was identified early thanks to this cytogenetic diagnosis, and the predicted improvement in attention will very likely have a very positive influence in his learning abilities and his final cognitive outcome. As a consequence and until more cases are reported, prenatal or presymptomatic counseling of cases of sSMC affecting the same region will always have to use this information with caution, owing to the important limitations imposed by mosaicism in genetic counseling.

INTERNET RESOURCES

http://www.ensembl.org/Homo_sapiens/index.html
http://bacpac.chori.org/
http://mti-n.mti.uni-jena.de/~huwww/MOL_ZYTO/sSMC/00START.htm

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