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**RESEARCH NOTE**

# A *de novo* marker chromosome 15 in a child with isolated developmental delay

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**Abstract.** We report a rare case of a 14-month-old male child who was referred for developmental delay. Clinical examination revealed a hypotonic infant with speech delay and no dysmorphic features. The banding cytogenetics revealed a small supernumerary marker chromosome. Upon silver staining, the marker showed the presence of satellite regions on either ends. Further, analysis using fluorescence *in situ* hybridization on marker chromosome revealed its origin from chromosome 15.

**Keywords.** cytogenetics; satellite chromosome; fluorescence *in situ* hybridization; marker chromosome; uniparental disomy.

## Introduction

Small supernumerary marker chromosome (sSMC) is a structurally abnormal chromosome fragment of unknown origin (ISCN 2013). In general, sSMC is unique in its size, and banding pattern and characterization of sSMC using routine banding cytogenetics is not achievable (Liehr *et al*. 2004). sSMC can either be present as part of 46 chromosomes or as additional material in a karyotype (Stefanou and Crocker 2004).

Based on its structure, sSMC is categorized into five groups (i) satellite/bisatellited (ii) small metacentrics (iii) small supernumerary ring (iv) minutes and (v) neocentric chromosomes. sSMC can be routinely detected by various banding techniques like G-bands using trypsin digestion with Giemsa stain (GTG), reverse, constitutive heterochromatin and nucleolar organizer region (NOR) banding (Crolla *et al*. 1995). However, they can be best characterized by molecular techniques like fluorescence *in situ* hybridization (FISH), array comparative genomic hybridization (CGH), and uniparental disomy (UPD) studies. Hence, in the present study, we used banding cytogenetics, NOR staining and FISH to classify the sSMC detected in the patient's sample.

## Materials and methods

### *Clinical report*

A 14-month-old boy was referred to clinical genetic evaluation of developmental delay. The three generation pedigree analysis was not contributory. He was born after 40 weeks of uncomplicated gestation by normal vaginal delivery and is the first child from natural conception of nonconsanguineous parents. There is no history of antenatal problems, exposure to drugs or medical illness during pregnancy and there are also no perinatal issues. However, there is a history of speech delay (figure 1a). The proband was advised for cytogenetic analysis. The analysis is consistent with a marker chromosome. After genetic counselling, banding cytogenetic analysis of the parents was carried out. The chromosome analysis of the parents was normal.

### *Sample collection*

After getting informed consent, we collected 2-mL blood sample in a sodium heparin tube (BD Vacutainer, USA) from

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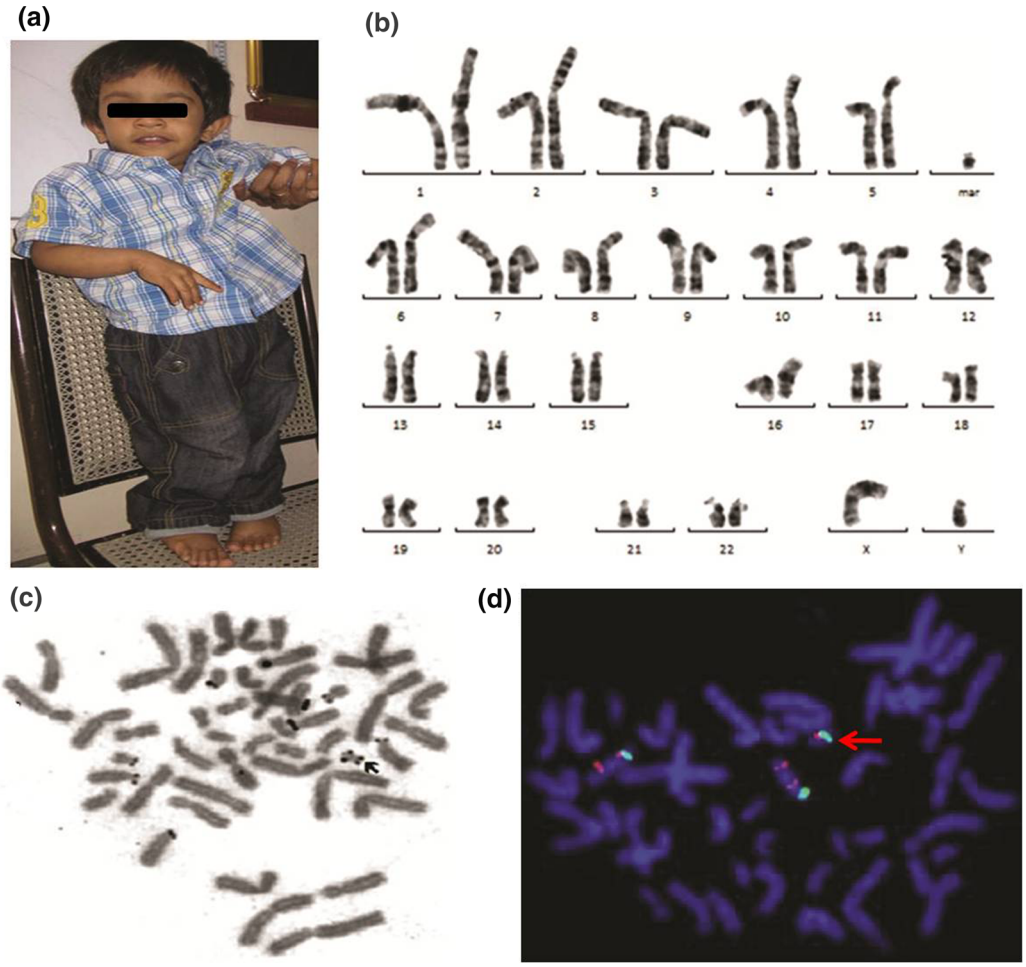


**Figure 1.** (a) Clinical photograph of the proband, (b) karyotype of the proband showing marker chromosome. (c) NOR staining of the metaphase spread showing bisatellite maker chromosome. (d) Metaphase FISH with CEP 15 (green)/SNRPN (red)//PML (red)/probe.

the patient and his parents. The chromosomes were obtained using standard procedures and investigated using various banding techniques like GTG, NOR, and FISH analysis.

### *Banding cytogenetics*

Phytohemagglutinin (PHA) stimulated human leukocyte culture was established by adding 0.5 mL of peripheral venous blood sample obtained from the patient into a 30 mL culture vial containing 5 mL of RPMI 1640 medium and 2 mL of foetal bovine serum (FBS). The blood culture was incubated at 37°C for 69 h in $CO_2$ incubator. The cells were then arrested in metaphase by adding 0.1 mL of colchicine and reincubating the vials for 12 min at 37°C. The cell suspension was then centrifuged at 1000 rpm for 10 min. After discarding the supernatant, pellet was resuspended in 10 mL of 0.075 M prewarmed hypotonic solution (KCl) and incubated at 37°C for 15 min. The cell suspension was then subjected to successive washing with fresh carnoy's fixative (3:1 ratio of methanol and glacial acetic acid) until a clear cell pellet was observed. Glass slides soaked in ice cold water was used for metaphase slide preparation, the slides were baked at 58°C for one week before the staining procedure.

### *GTG banding*

The prepared slides were suspended in 0.005% trypsin for 2 s, stained with Giemsa for 5 min and washed with distilled water. Metaphase spreads were observed under light microscope (BH-2, Olympus, Japan) using oil immersion lens. The metaphase spreads were captured and analysed using Cytovision software v. 3.1 (Applied Imaging, California, USA).

### *NOR staining*

Two drops of gelatin and four drops of silver nitrate were added to three week old slides and evenly spread with cover glass. The slides were then kept on a hot plate until the solution turned golden brown. Cover glass was removed and the slides were rinsed thoroughly with distilled water. The slides were counterstained using Giemsa stain for 20 min before observing under light microscope for NOR bands.

***FISH technique***

The metaphase FISH analysis is performed using the metaphase slides prepared from 72 h culture. About 10 μL probe (promyelocytic leukemia (PML)red/small nuclear ribonucleoprotein polypeptide N (SNRPN) SpectrumRed/centromere enumeration probe (CEP) 15 SpectrumGreen) was added to the slide, and sealed with a rubber solution. The slides were then denatured for 5 min at 73°C, followed by hybridization at 37°C for 16–24 h. Further, the slides were washed with 0.4 x saline-sodium citrate (SSC)/0.3% NP 40 at 73°C for 2 min and 2x SSC/0.1% NP 40 at 37°C for 2 min. The slides were counter stained with 10 μL of 4′-6-diamidino-2-phenylindole (DAPI) (Abbott Molecular) and viewed under fluorescence light microscope (BX-60, Olympus) using appropriate filters (DAPI, fluorescein-5-and/or-6 isothiocynate (FITC), tetramethyl rhodamine isothiocyanate (TRITC), dual band pass filter, multiband pass filter). The images were captured using the Cytovision FISH software (v. 3.1).

## Results

Chromosomal analysis using GTG banding revealed a marker chromosome (karyotype: 47,XY,+mar) in all the cells analysed (figure 1b). Further analysis with NOR staining showed that the marker chromosome to be bisatellited (figure 1c). Hybridization using chromosome CEP 15 (green)/SNRPN (red)//PML (red)/ probe showed one signal for CEP 15 (green) and one signal for SNRPN (red) on the marker chromosome, this was separate from the normal hybridization pattern on two copies of chromosome 15 (figure 1d). The banding cytogenetic analysis of the parents was normal indicating the *de novo* origin of the sSMC (15) in the child.

## Discussion

sSMC are structurally abnormal chromosomes that occur in addition to the other 46 chromosomes in a cell (Crolla *et al*. 1997). They are rare chromosomal abnormalities resulting in partial trisomy of specific genomic regions with characteristic phenotypic effects (Kolialexi *et al*. 2006). sSMCs may occur as familial or *de novo* in origin. They are usually found postnatally in children investigated for developmental delays. sSMC show an overall incidence of 3/1000 against the live birth rate of 0.5/1000 (0.05%), demonstrating that many markers have very mild phenotypic effects (Jane 2017).

sSMC can be in different sizes and shapes, such as inverted duplicated fragments, ring chromosomes, and minute chromosomes. They can be derived from either acrocentric or nonacrocentric chromosomes. In general, the incidence of sSMC derived from acrocentric chromosomes is higher (Liehr *et al*. 2004). Banding cytogenetics have shown that over 80% of sSMC are derived from the short arm and pericentromeric regions of acrocentric chromosomes of about which approximately half are derived from chromosome 15 (Crolla *et al*. 1995). Since banding cytogenetics has an inherent limitation in the elucidation of marker chromosome in metaphase. With this background, in the present case, we used chromosome 15 FISH probe as a baseline analysis for categorizing marker chromosome.

Supernumerary markers are classified into different groups such as bisatellited and dicentric, bisatellited and tricentric, bisatellited and monocentric, monosatellited and monocentric, ring, markers, acentric, isochromosomes (nonacrocentric), and isodicentric (Crolla *et al*. 1995). In general, individuals with bisatellite and monocentric marker chromosome show about 17% risk for abnormal phenotype (Graf *et al*. 2006).

Fourteen cases with sSMCs were identified in a large case series of 2871 patients. Of the 14 cases three SMCs (one *de novo*, two familial) were derived from chromosome 15 and are associated with developmental delay and failure to thrive (Jang *et al*. 2016). In another case series of 32 patients with sSMC derived from chromosome 15, six cases (1 ring, 5 minute) with monocentric SMC were identified. Of the five patients with minute sSMC, three were familial and two were *de novo* in origin. Further the study showed that sSMC derived from chromosome 15 was due to homologous recombination between two individual chromosomes 15 leading to a pseudodicentric marker or inversion duplication. Generally, the monocentric markers are unstable during meiosis (Eggermann *et al*. 2002). Hence there are very few reports on bisatellite and monocentric marker derived from chromosome 15. In the present case study, the latter type was observed. However, sSMCs derived from chromosome 15 has not been observed in a case series of 31 prenatal and postnatal samples identified with sSMCs using banding cytogenetics, FISH and chromosome microarray analysis (CMA). Further, of the 22 cases with positive CMA the chromosomal origin was identified mostly on chromosomes 2, 7, 9, 10, 12, 18, 22, X and Y (Xue *et al*. 2019).

The risk for phenotypic abnormality associated with sSMC depends on several factors like inheritance, mode of ascertainment, origin, shape, euchromatin content and structure (Graf *et al*. 2006). The euchromatin content in the marker chromosome determines the extent of phenotypic abnormality. In general, sSMC derived from chromosome 15 with euchromatin leads to intellectual and psychomotor disability. Whereas, individuals with sSMCs derived from chromosome 15 without euchromatin do not have phenotypic effects. However, they are often found in males with infertility (Eggermann *et al*. 2002).

sSMC derived from chromosome 15 are classified in two major groups. First group include acrocentric shaped chromosomes including inverted duplications of bands q11→q13 which are normally sporadic in origin. They are associated with mild to severe mental retardation without phenotypic

effects. The second group includes small familial or *de novo* metacentrics which do not have inverted duplications. They are associated with normal phenotype (Crolla *et al*. 1995). The present study belongs to the second group.

Phenotypic effect increases with additional copies of Prader–Willi/Angelman critical region (PWACR) on the sSMC derived from chromosome 15 (Dennis *et al*. 2006). Interestingly, our patient was found to have three copies of SNRPN upon FISH analysis, yet presented with only developmental delay. Marker chromosomes cause abnormal phenotype either by dosage effects or specific imprinting ratios of maternal and paternal genes (Crolla *et al*. 1992; Melo *et al*. 2015). Increase in maternal copies of the proximal 15q imprinted region results in moderate to severe intellectual disability. Whereas, markers derived from paternal copies, the resulting dosage compensation might lead to a normal phenotype (Crolla *et al*. 1995). Thus, further analysis using UPD studies for completely understanding of this marker chromosome is the future prospects of this study.

Although there are high-throughput techniques like microarray-based comparative genomic hybridization (array CGH), chromosomal microarray (CMA) and multiplex ligation-dependent probe amplification (MLPA), still banding cytogenetics remains the first step in the diagnosis and classification of the sSMC detected in the patient sample. The present case report also stresses the importance of chromosomal analysis in all cases with isolated developmental delay. This study explores the utilization of probes for FISH in the characterization of marker chromosomes.

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