Cytogenetic characterization of an extra structurally abnormal chromosome associated with severe mental retardation: inv dup (15) (q13)

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We have studied an extra structually abnormal chromosome (ESAC) in a 13 years old boy with profound mental, psychomotor and speech retardation, behavioral problems, seizures and abnormal electroencephalogram. The examination of the bisatellited ESAC with chromosome banding demonstrated that the karyotype was: 47, XY, +inv dup (15) (pter→q13::q13→pter). The cytogenetic characterization of the inv dup (15) is reported with special emphasis on the usefulness of DA/DAPI staining when G-banding is sequentially performed to discard possible heteromorphisms in DA/DAPI positive chromosomes, and the importance of Ag-NOR heteromorphisms to ascertain the maternal origin of the inv dup (15). A U-type exchange between two non-sister chromatids is proposed as its mechanism of formation. The clinical features of the case were consistent with those previously reported in similar cases.

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Sporadic cases of phenotypically abnormal individuals with an extra, structurally abnormal chromosome (ESAC) are not uncommon (STAMBERG and THOMAS 1986). Presumably the phenotype is abnormal in correlation with the extent of euchromatin present in the ESAC. Among the bisatellited supernumerary chromosomes, inv dup (15) chromosomes account for approximately half of them (MATTEI et al. 1984). This rearrangement was first described by WISNIEWSKI et al. (1979), and since then numerous cases have been reported (GRAMMATICO et al. 1994). During the last years, the use of fluorescent in situ hybridization (FISH) as a diagnostic aid in clinical cytogenetics has helped to identify chromosome abnormalities more accurately (KRAKER et al. 1992). Recent reports have focused on the molecular composition of the inv dup (15) in relation to the Prader Willi/Angelman syndromes and the (15) (q11–13) paternal/maternal genetic balance (CHENG et al. 1994; LEANA-COX et al. 1994).

We report a severely mentally retarded boy with an ESAC. The cytogenetic and clinical features of the case are compared with previously reported cases to contribute to a better delineation of the syndrome.

MATERIALS AND METHODS

Case report

This 13 years old boy was the second son of an unrelated couple. His elder sister is healthy. He weighed 3.400 g at birth after an uneventful pregnancy. Perinatal data were vaginal delivery with very slight hypoxia; reanimation maneuvers were not necessary. During the first two years of infancy the parents noticed irritability and hypertonic episodes of few seconds duration. He started to walk at the age of 2 years, after psychomotor stimulation, and he began to pronounce the first words when he was 4 years old. At present he has a vocabulary of no more than 20 words.

Clinical examination showed severe mental, motor and speech retardation and diffuse hypotonia. His face is enlarged and dysmorphic but not particularly unusual, the ears are large and low-sitting (Fig. 1a and b), the hands are large with low implantation of the thumb and has large feet moderately dismorphic in the plantar region.

EEG showed high voltage spike and wave discharges in the left fronto–temporal lobes, at times they spread to the opposite hemisphere. CT was normal and fundoscopic examination of his eyes was normal. He had several tonic nocturnal seizures. He presents behavioral problems, particularly aggression, at the school for mentally handicapped which he frequents.

Cytogenetic

Metaphase spreads were prepared from PHA-stimulated lymphocyte cultures from the patient and his parents. G-banding was obtained by trypsin digestion (SEABRIGHT 1971) and C-banding by alkaline treatment (SUMNER 1972).

To detect DAPI-fluorescent heterochromatic regions (DA/DAPI technique), the preparations were treated with 0.1 mg/ml distamycin A in pH 7 Mc Ilvaines's buffer for 15 min and then stained with DAPI (0.6 ug/ml in the same buffer) for 20–30 min (SCHWEIZER 1980).

Additional cultures for high-resolution chromosome analysis were performed (YUNIS 1981). Cultures were synchronized by FUDR followed by BUDR release and chromosomes were evaluated by G-banding.

Ag-NORs staining was performed in a 50 % aqueous silver nitrate solution using 2 % gelatin colloidal protector, in 1 % formic acid for 2–4 min (HOWELL and BLACK 1980). From the patient and his parents a total of 100 metaphases with clearly defined D and G chromosome groups were scored for the number of Ag-NOR chromosomes and their involvement in satellite associations. The number of association pairs (ASPs) was used as an integrated parameter for estimating the degree of satellite association. Two or more acrocentric chromosomes were considered to form an ASP if they were connected by Ag-stained protein material as a thread-like structure or if the distance between their NORs did not exceed the length of the long arm of the largest G chromosome according to previous criteria (PEDRAZZINI and SLAVUTSKY 1991).

For FISH chromosome painting fixed cell suspensions from the conventional cytogenetic preparations were hybridized with a FITC-labelled paint from chromosome 15 (CAMBIO). The slides were denatured in 70 % formamide in 2 × SSC, pH 7.0 at 70°C for 2 min and then rehydrated in a cold ethanol series and air dried. The painting probe of chromosome 15 was denatured for 5 min at 65°C and then applied to the slide overnight at 42°C in a moist chamber. Three post-hybridization washes in 50 % formamide in 2 × SSC at 42°C were performed. The fluorescence intensity was reinforced with a sandwich amplification according to standard procedures. Slides were counterstained with an antifade solution containing propidium iodide and analyzed through a Zeiss fluorescence microscope equipped with a fluorescein filter package and photographed using Ektachrome 400 Kodak film. Twenty metaphases were scored for hybridization signals with the chromosome 15 painting probe.

RESULTS

The analysis of the patient's G-banded chromosomes revealed a bisatellited extra supernumerary abnormal chromosome with different ends (Fig. 2). Both ends of the ESAC stained brightly with DA/DAPI staining (Fig. 2c). Among human satellited chromosomes, DA/DAPI highlights only the proximal short arm segment of chromosome 15 (SCHWEIZER 1980), and so the ESAC was first supposed to be formed from chromosome 15. To test the specificity of DA/DAPI in our material, we performed sequential DA/DAPI plus G-banding on the preparations of the patients and his parents (Fig. 3), excluding the DA/DAPI heteromorphism on other acrocentrics than 15.

Chromosomal analysis of high-resolution G-banded metaphase spreads revealed that q13 was the chromosome 15 breakpoint (Fig. 2a). Thus, the kary-

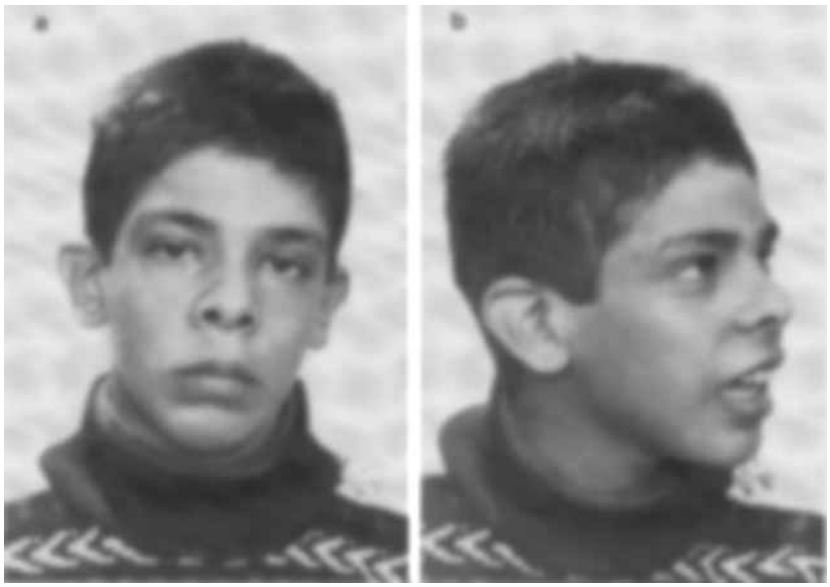

Fig. 1a and b. Patient's clinical phenotype.

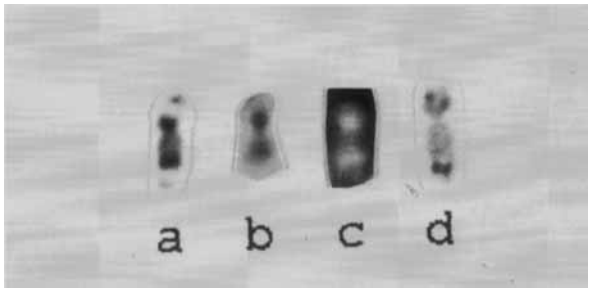

Fig. 2a and d. Partial karyotype of invdup (15). **a** High resolution G-banding. **b** C-banding. **c** DA/DAPI. **d** NOR.

otype was 47, XY, +inv dup (15) (pter→q13::q13→pter). The ideogram of the inv dup (15) at approximately the 2000 band stage with breakpoint at q13 shows that euchromatin is involved. The situation leads to a partial tetrasomy for chromosome 15 (pter::q13), which presumably is responsible for the abnormal phenotype.

FISH with a chromosome 15 painting probe confirmed the exclusive participation of chromosome 15 in this rearrangement, as the painting probe hybridized only to both normal chromosomes 15 and the inv dup (15) (Fig. 4).

In Ag-NORs analysis, the mother revealed a chromosome 15 ph+. This polymorphism showed the maternal origin of the marker. As shown by G, DA/DAPI, C and NOR staining, the inv dup (15) has different ends, suggesting that two different chromosomes 15 participated in its formation (Fig. 2). The patient showed an increase in the total mean number of Ag-NORs per metaphase ($p < 0.001$), compared with his parents, due to the presence of the inv dup (15).

The extra bisatellited chromosome was observed with only one Ag-NOR (+) in 60% of cells, being negative in the polymorphic end. Twenty percent of the cells showed two active NORs whereas the remaining 20 % were negative in both ends.

An increase in the mean ASPs/cell was observed in the patient (1.80) and in the mother (1.40), compared with the father (0.95).

DISCUSSION

Most reported cases of inv dup (15) have been identified because of mental retardation and/or developmental delay (WISNIEWSKI et al. 1979; MARASCHIO et al. 1981). However, considerable clinical variation has been observed. This has led some authors to suggest that the abnormal phenotype may be correlated with the breakpoint involved in the rearrangement q11, q12, q13 or q14 (WISNIEWSKI and DOHERTY 1985; WEBB 1994). G-band analysis showed that q13 was the chromosome 15 breakpoint in the present case. The clinical features coincide with those reported in the literature for this chromosome rearrangement (ROBINSON et al. 1993; GRAMMATICO et al. 1994) and the patient's signs closely resembles the case of inv dup (15) (q13) reported by GRAMMATICO et al. (1994): Mental, motor, developmental and speech retardation, muscular hypotonia, low-set ears, seizures with abnormal EEG, and behavioral problems. In the cytogenetic characterization of the ESAC, the DA/DAPI technique was originally thought to be able to differentiate among the acrocentric human chromosomes as it stained specifically the short arm of chromosome 15. However, later reports showed that 15p does not always fluoresce brightly with DA/DAPI (BAVU et al. 1986) and that

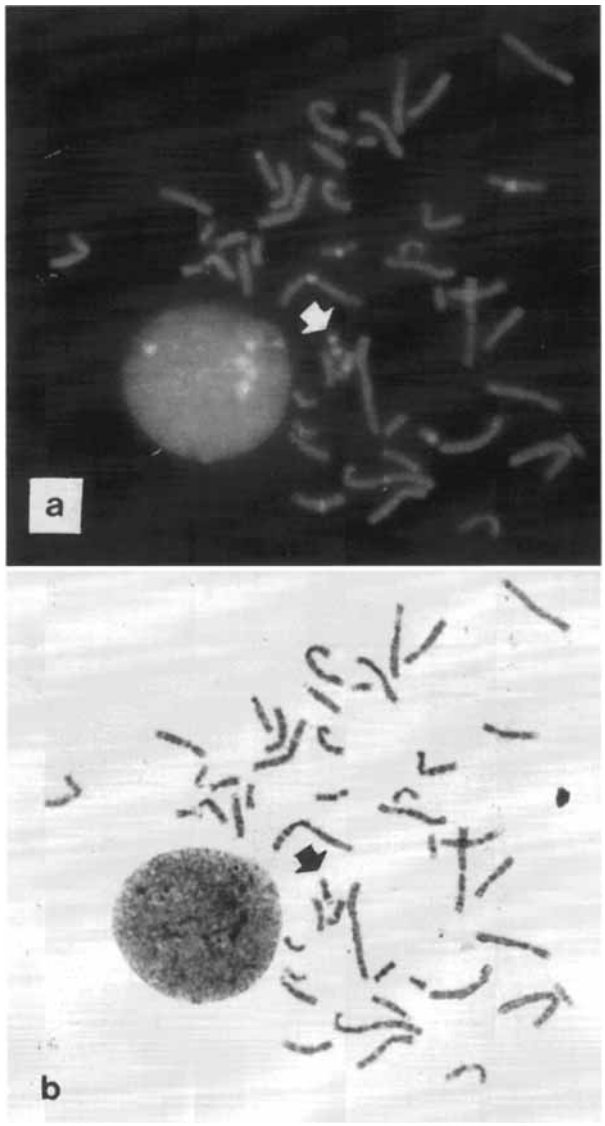

Fig. 3a and b. **a** DA/DAPI and **b** sequential G-banding. The inv dup (15) is indicated by arrows.

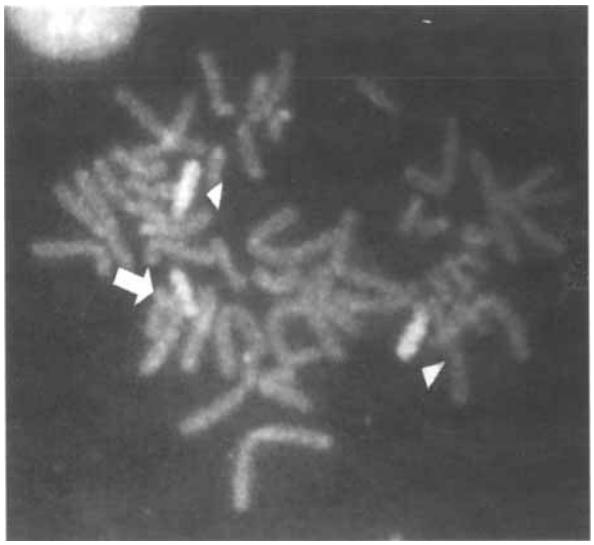

Fig. 4. FISH with a FITC-labelled paint from chromosome 15 (CAMBIO) on conventional cytogenetic preparations from the patient. Notice the two normal chromsome 15 (arrowheads) and the inv dup (15) (arrow).

acrocentric chromosomes other than 15 can present a brightly fluorescing block (PÉREZ-CASTILLO et al. 1987). DA/DAPI plus G-banding was performed on the patient's parents to test the specificity of the technique in this particular case. The absence of fluorescence in other acrocentrics than chromosome 15 in this family, allowed us to confirm that the ESAC was derived from this chromosome. For laboratories without FISH methodology, our findings support the idea that the DA/DAPI technique is useful and informative for identifying markers involving 15q, if both the patient and the parents can be analyzed with this technique plus G-banding.

NOR activity, expressed as the mean number of Ag-NOR chromosomes and the satellite association patterns, showed that the presence of a doubly satellited marker affected neither the silver stainability of NORs nor their possibility to participate in acrocentric associations. TUCK et al. (1983) described a similar situation in a family with a doubly satellited marker originated from chromosome 13. Furthermore, the analysis of the acrocentric chromosomes of our family is also consistent with the Mendelian inheritance of the Ag-NOR pattern (MARKOVIC et al. 1978; ZAKHAROV et al. 1982). NOR polymorphisms were particularly useful to determine the parental origin of the ESAC. The polymorphic 15ph+ endpoint of the ESAC was inherited from the mother, as is usually the case (MARASCHIO et al. 1981).

The mechanism of formation of inv dup (15) could be a pre-meiotic event of breakage and reunion or a U-type exchange between two non-sister chromatids (MARASCHIO et al. 1988). That two different chromosomes 15 participated in this particular ESAC suggests an U-type exchange between two non-sister chromatids in the maternal oogenesis.

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