Trisomy 10qter confirmed by in situ hybridisation

V Briscioli, G Floridia, E Rossi, A Selicorni, F Lalatta, O Zuffardi

Abstract

We report a boy with multiple congenital anomalies compatible with trisomy for the distal region of the long arm of chromosome 10 and a male karyotype with one 18p+. In situ hybridisation with a cDNA for ornithine aminotransferase (OAT), whose locus maps to 10q26, confirmed the clinical suspicion of distal trisomy 10q. Subterminal localisation of the labelling signals on chromosome 10 and on the der(18) indicated the localisation of the OAT locus in the proximal part of 10q26. Two clusters of labelling signals were also found on the pericentromeric and proximal portion of the X chromosome short arm, thus confirming the presence in this region of two non-adjacent OAT pseudogenes. The phenotypic similarities of this patient to previously reported cases provide further support for the delineation of trisomy 10qter as a specific, clinically recognisable syndrome.

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Clinica Pediatrica II 'De Marchi', Via Commenda 9, 20122 Milano, Italy.
V Briscioli

Biologia Generale e Genetica Medica, Università di Pavia, CP 217, I-27100 Pavia, Italy.
G Floridia
E Rossi
O Zuffardi

Laboratorio di Citogenetica, ICP, Via Commenda 12, 20122 Milano, Italy.
A Selicorni
F Lalatta

Correspondence to Dr Zuffardi.

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De novo unbalanced structural anomalies have a frequency of 0·038%,[1] most of them being unbalanced translocations. When detected in liveborns, these imbalances can pose problems in karyotype interpretation since the chromosome region transposed to the recipient chromosome is usually short enough to allow viability of the zygote and thus it can be difficult to identify it by banding patterns. We present a boy with multiple congenital anomalies, in whom chromosome analysis showed a de novo 18p+ male karyotype. Clinical characterisation of the patient when 6 years old led to the suspicion of trisomy for the distal region of 10q. In situ hybridisation with a probe for the region confirmed the clinical diagnosis.

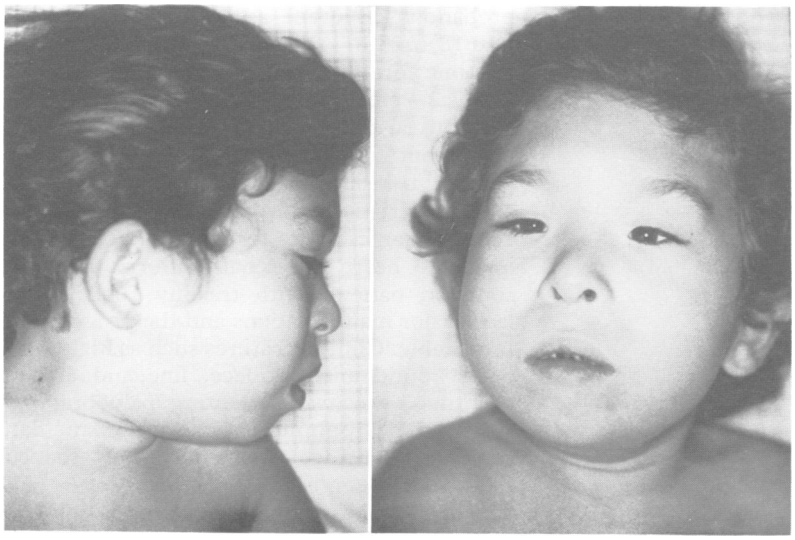

Figure 1 The patient at 6 years.

Case report

The proband was the first child of healthy, unrelated parents; the mother was aged 29 years and the father 34 years. The mother had previously had two spontaneous abortions. The proband was born by normal delivery after an uneventful pregnancy at 41 weeks. Birth weight was 3250 g (50th centile), length 48 cm (50th centile), and head circumference 34 cm (50th centile). At birth he had respiratory distress and severe hypotonia. At 7 months of age he was evaluated because of poor feeding, psychomotor retardation, and craniofacial dysmorphism. Cardiological and ophthalmological examination was normal, but skeletal *x* ray showed marked thoracolumbar kyphoscoliosis and a dysplastic hip. Clinical examination at the age of 6 years showed height and weight below the 3rd centile, head circumference on the 50th centile, inner canthal distance on the 97th centile, outer canthal distance on the 90th centile, total hand and foot length below the 3rd centile, and middle finger length on the 50th centile. The face (fig 1) was round with a high and large forehead and bitemporal constriction. The eyebrows were fine and arched, the palpebral fissures were downward slanting with blepharophimosis, the nose was short with a flat nasal bridge, the mouth was bow shaped, the ears were low set, and the neck was short. Cleft palate, severe kyphoscoliosis, and coxa valga were present. The fingers were long and thin with camptodactyly. He had moderate mental retardation (psychological tests showed an IQ of 63).

CHROMOSOME ANALYSIS

Chromosome analysis was performed on trypsin G banded preparations from lymphocyte cultures. The proband had a male karyotype with extra material on the short arm of a chromosome 18, which appeared slightly larger than a chromosome 16. No cell line is available from this patient. Parental karyotypes were normal. Q, R, DA-DAPI and high resolution banding[2] were performed but no unequivocal interpretation could be made although it was clear that the extra material was entirely euchromatic. Since the malformations were consistent with a partial duplication of 10q, we decided to perform in situ hybridisation with a probe mapping to the distal region of 10q.

Results of in situ hybridisation of hu OAT 6 to the proband's metaphases.*

No of mitoses with at least one FISH signal at 10q26, Xp11, or der(18)distal p/ total no of mitoses	FISH signals at		
	10q26	der(18)distal p	Xp11
50/64	43 in 32 mitoses	20 in 17 mitoses	42 in 27 mitoses

* Only mitoses with one to eight FISH signals have been considered.

IN SITU HYBRIDISATION

In situ hybridisation was performed with a probe for the ornithine-δ-aminotransferase (OAT) gene whose structural locus maps to 10q26 though OAT related sequences are also present on Xp.[3,4] The human cDNA hu OAT 6[5] in pGEM-4 vector was biotin-16-dUTP labelled by nick translation according to the Boehringer-Mannheim protocol. The probe (final concentration 6 ng/μl) was hybridised in 50% formamide in 2 × SSC at 37°C. Post-hybridisation washes were done in 50% formamide in 2 × SSC for 20 minutes and in 2 × SSC for one hour at 42°C. Detection was done with the ONCOR detection kit with two amplification steps. Chromosomes were counterstained with propidium iodide (200 ng/ml in 2 × SSC, five minutes at room temperature) and banded with diamidinophenylindole (DAPI).[6] After mounting in antifade solution (2% McIlvaine buffer, pH 7·0, containing 1 mg/ml of 1,4-phenylendiamin-dihydrochloride in glycerol), slides were evaluated on a Zeiss Axiophot microscope equipped for conventional epifluorescence microscopy (BP 365, FT 395, LP 397 for DAPI staining; BP 450–490, FT 510, LP 520 for FITC and propidium iodide).

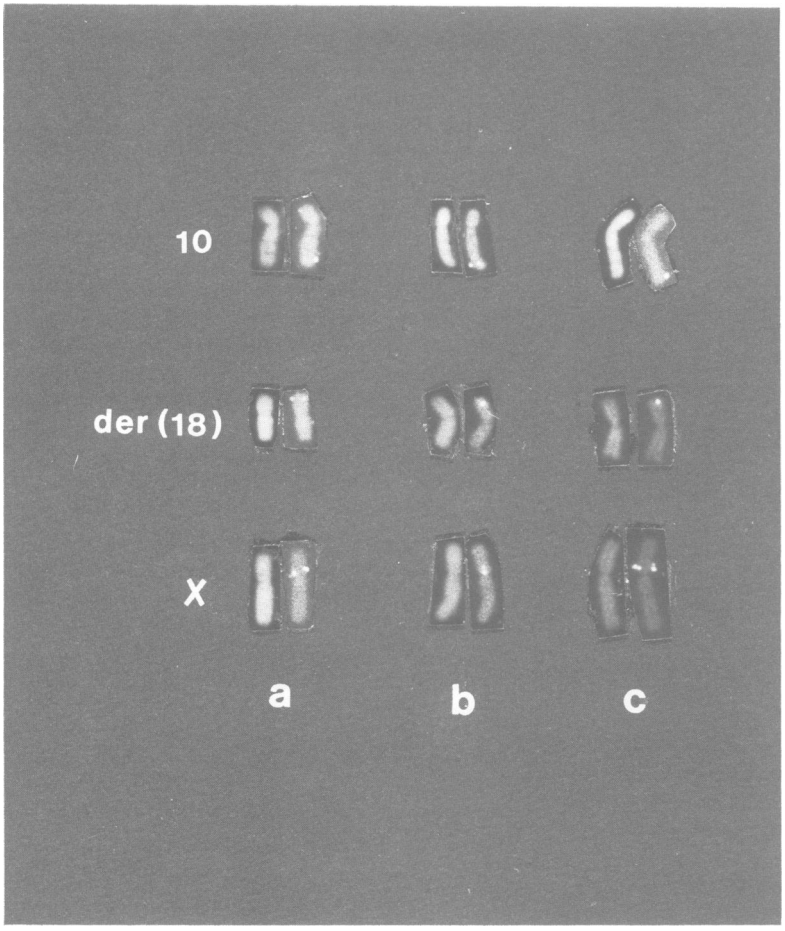

Figure 3 Chromosomes 10, der(18), and X from the proband after in situ hybridisation with hu OAT 6; (left DAPI staining, (right) propidium iodide counterstaining.

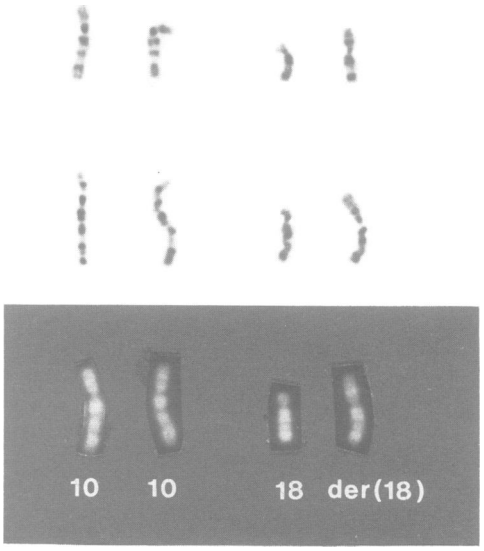

Figure 2 G and Q banded chromosomes 10, 18, and der(18) of the proband.

Results of the in situ hybridisation are given in the table. The distribution of the fluorescent signals shows that the single X chromosome and the two chromosomes 10 had approximately the same number of hybridised spots and this was roughly double the number of spots found in the der(18). The localisation of the spots on the chromosome 10 long arm and on the der(18) short arm was subterminal (fig 2). The spots at Xp11, in those mitoses with more than one signal, were alternatively distributed as two asymmetrical signals, one on each chromatid (fig 3a), two signals one over the other (fig 3b), or as two sets of superimposed symmetrical signals (fig 3c).

Discussion

The distribution of hybridisation signals clearly indicates that the extra piece of the der(18) is constituted by the distal portion of 10q. Together, chromosome banding and hybridisation data led us to interpret the proband's karyotype as 46,XY,-18,+der(18) t(10;18)(q25;p11.2) (fig 1). Thus the patient is trisomic for 10q25→qter and monosomic for 18p11.2→pter.

About 30 cases of distal trisomy for 10q have been reported,[7,8] most of them resulting from a parental translocation. This trisomy results in a characteristic syndrome with more severe clinical manifestations for trisomy 10q24→qter owing to heart and renal malformations. In contrast, patients with trisomy 10q25→qter lack major malformations and the prognosis is favourable. Clinical features such as high forehead, round and flat face, fine and arched eyebrows, downward slanting palpebral fissures, blepharophimosis, hypertelorism, flat, broad nasal bridge, short nose, bow shaped mouth, low set ears, short neck, cleft palate, and kyphoscoliosis are common to both trisomies. In our case all these features were present together with moderate mental

retardation, as in most cases with trisomy 10q25→qter, and in contrast to cases with trisomy 10q24→qter who have profound mental retardation. All these signs prompted us to suspect trisomy for the distal region of 10q and even after the karyotype delineation we could not detect any clinical sign characteristic of monosomy 18p. The same holds true for a patient reported by Forabosco *et al*[9] with a karyotype identical to that of our case.

From the cytogenetic point of view, the OAT locus seems to map to the proximal portion of 10q26, as suggested by the subterminal localisation of the fluorescent spots on chromosomes 10 and der(18). The in situ hybridisation pattern also suggested the presence of two OAT related sequences on Xp. In fact the number of labelling spots on the single X chromosome was double that found on the der(18) and similar to that found on the two chromosomes 10. Moreover, two clusters of spots were identified on Xp, one close to the centromere and a second proximal to Xp21, thus confirming that two OAT related sequences on Xp map to non-adjacent intervals.[4]

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