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ARTICLE

CGH and direct diagnosis of mosaic structural chromosomal abnormalities: description of a mosaic ring chromosome 17 and review of the literature

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We report the characterisation of a *de novo* supernumerary chromosome marker in a mosaic state (50%) by comparative genomic hybridisation (CGH) in an 8-year-old child with hypotonia, dysmorphia and mild-to-moderate mental retardation. We describe the combined use of CGH and fluorescence *in situ* hybridisation (FISH) to identify the origin of the additional chromosomal material. Visual analysis of 10 CGH-metaphase spreads revealed a gain of green fluorescent signal on pericentromeric region of chromosome 17. The CGH finding was confirmed by FISH analysis using a whole chromosome 17 paint, a chromosome 17 centromeric probe and the probe coding for the Smith–Magenis locus in 17p11.2. These results show that performing both CGH and FISH in combination with classical karyotyping will certainly allow the identification of imbalanced chromosome rearrangements and, by the way, allow the identification of genes involved in mental retardation and/or malformative pathology.
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Introduction

The origin of *de novo* supernumerary chromosome markers is sometimes difficult to determine using standard banding methods, especially when additional chromosomal material is too small. For defining the origin of a chromosomal segment in excess or a supernumerary marker chromosome, fluorescent *in situ* hybridisation (FISH) using successive whole chromosome paints is often required. However, this procedure may be time consuming before the extra material is recognised.

Comparative genomic hybridisation (CGH) is an additional molecular cytogenetic approach that can scan the entire genome and allows a comprehensive analysis of gains and losses of chromosomal material. After being used to detect and map unbalanced chromosome aberrations in tumour cells, CGH has been used successfully to identify the origin of small unbalanced chromosome rearrangements,[1,2] even in a mosaic state.[3,4]

Here we report the clinical and cytogenetic studies of a child with a *de novo* autosomal marker originating from chromosome segment 17p identified by CGH and confirmed by FISH. We compare this case with those described by Stankiewicz *et al*,[5] who reviewed the clinical data and the breakpoints in eight previously published cases with extra chromosome markers derived from chromosome segment 17p.

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Materials and methods

Clinical report

This girl was the second child of nonconsanguineous, healthy parents of Italian-Spanish background, with

unremarkable family history. Pregnancy and delivery occurred normally. Birth weight was 2450 g, birth length 47.5 cm, OFC 34 cm, at 39 weeks of gestation. Anteposed anus was noted in the newborn period. Umbilical cord was reported as short. In infancy, she had recurrent vagal malaises that were because of severe gastro-oesophageal reflux with oesophagitis. She suffered of severe constipation till the age of 18 months. Hypotonia and psychomotor delay were obvious at the age of 9 months. She sat at 14 months, and walked at age 2 years. She had her first words around 2 years, but her expressive skills remained very poor, with limited ability to speak contrasting with good understanding.

An MRI scan at age 2 years showed focal cortical dysplasia of inner part of the left temporal lobe. She never had seizures and EEG was normal. Cardiac and renal ultrasonography, and skeletal survey were normal. Abnormal latencies of auditory-evoked potentials were noted that were compatible with abnormal signal transmission in the brain stem. Abnormal visual-evoked potentials were also recorded. These anomalies were not associated with functional hearing or visual impairment. ERG showed reduced amplitude bilaterally. She suffered of left eye myopia with squint. A first analysis of her karyotype, performed at age 2 years, found a mosaic chromosome in 70% of lymphocytes, but this marker was not otherwise characterised at that time.

When examined at age 8.5 years, she was 125 cm tall (−0.5 SD), weighed 27.4 kg (75th centile) and had an OFC of 52 cm (mean). She had a squared face, somewhat narrow bitemporal diameter with dolichocephalic shape of the skull, mildly downslanting palpebral fissures, prominent nasal bridge, stubby nose with bulbous tip, low set collumella, hypoplastic alae nasi (reminiscent of the shape of a Rubinstein–Taybi syndrome nose), high arched palate and normal ears (Figure 1). There was no external or internal malformations. Neurological examination was normal. She had no specific behavioural disorder. Formal IQ testing was not available, but she functioned in the mild-to-moderate mental retardation.

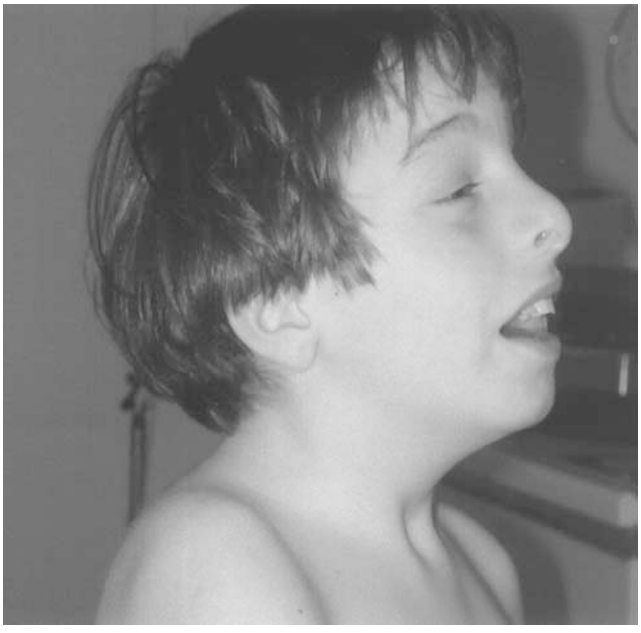

Figure 1 Proband at age 8 years.

Classical cytogenetic investigations

Further chromosome analyses were performed on cultured lymphocytes by standard, RTG, GTG, C-banding, NOR-banding and high-resolution techniques according to standard procedures. Chromosome analyses were also performed on cultured lymphocytes of both parents.

Comparative genomic hybridisation (CGH)

High-molecular test DNA and reference DNA were extracted with the Puregene™ DNA Isolation Kit (Gentra® systems-Minneapolis, MN, USA) according to the manufacturer's instructions. Test and reference DNA were labelled by nick translation using, respectively, spectrum green for the test and spectrum red for the reference, (Adgenix® CGH nick translation reagent kit; Downers Grove, IL, USA), following the supplier's recommendations. The target normal male metaphase slides for CGH were prepared according to standard procedure.[6] Hybridisation was performed according to the protocol described by Kallioniemi *et al*.[7] Chromosomes were counterstained with DAPI (4,6-diamino-2-phenylindole, 0.8 mM) in an antifade solution (Vectashield, Vector Laboratories, Burlingame, CA, USA), which produced bands adequate for chromosome identification.

Fluorescence *in situ* hybridisation (FISH)

Metaphase chromosome spreads from lymphocyte cultures and fluorescent painting probe for human chromosome 17 (Appligene™ Oncor®-Gaithersburg, MD, USA), chromosome 17 centromeric probe (D17Z1-Adgenix®, Downers Grove, IL, USA), Smith–Magenis: 17p11.2 probe (Aquarius™ probes – Cytocell®; Oxon, UK) were prepared according to standard procedures. Before observation, chromosomes were counterstained with DAPI in an antifade solution.

Digital analysis

Both FISH and CGH slides were analysed using a Zeiss Axiophot with a camera and connected to an Imaging System package (Applied Imaging, Newcastle-upon-Tyne, UK). A triple band-pass filter for FITC, Texas red and DAPI was used for the simultaneous visualisation of orange, green and DAPI. The aqua filter was used to visualise the blue spots.

In all, 10 CGH-metaphase spreads were acquired. Fluorescence intensities were measured along the length

of each of the homologue chromosomes. Fluorescence ratio (FR) were then calculated as the quotient of green and red fluorescence intensities and normalised to 1.0, for each chromosome, to obtain the resultant mean FR. Chromosome regions with mean FR values outside 0.8 and 1.2 values were considered to be respectively under- or over-represented.

Results

Classical cytogenetic analysis

Analysis of 20 metaphases revealed a female karyotype with a supernumerary chromosome in 50% of analysed cells (Figure 2a). This additional chromosome marker was too small to be identified by its banding pattern. It was labelled with C-banding (Figure 2b) but was negative with NOR-banding. Parental karyotypes were normal.

CGH analysis

Visual analysis of 10 CGH-metaphase spreads revealed a gain of green fluorescent signal on pericentromeric region of chromosome 17, which indicated an over-representation of this region in the test DNA (Figure 2c). Chromosome imbalance of the test DNA was confirmed and mapped precisely by the measurement of the fluorescence intensity ratio (FR) along each target chromosome.

FISH analysis

Hybridisation using chromosome 17 whole chromosome paint allowed to confirm CGH data and did not reveal any other chromosome material in this marker (Figure 3a). Three spots were observed in 50% of the cells with chromosome 17 centromeric probe (Figure 3b). Moreover, the supernumerary chromosome marker was shown to be labelled by FISH using the Smith–Magenis locus probe in 17p11.2 (Figure 3c). A single fluorescent signal was observed with this probe, which allowed to differentiate between a ring chromosome and an isochromosome 17p.

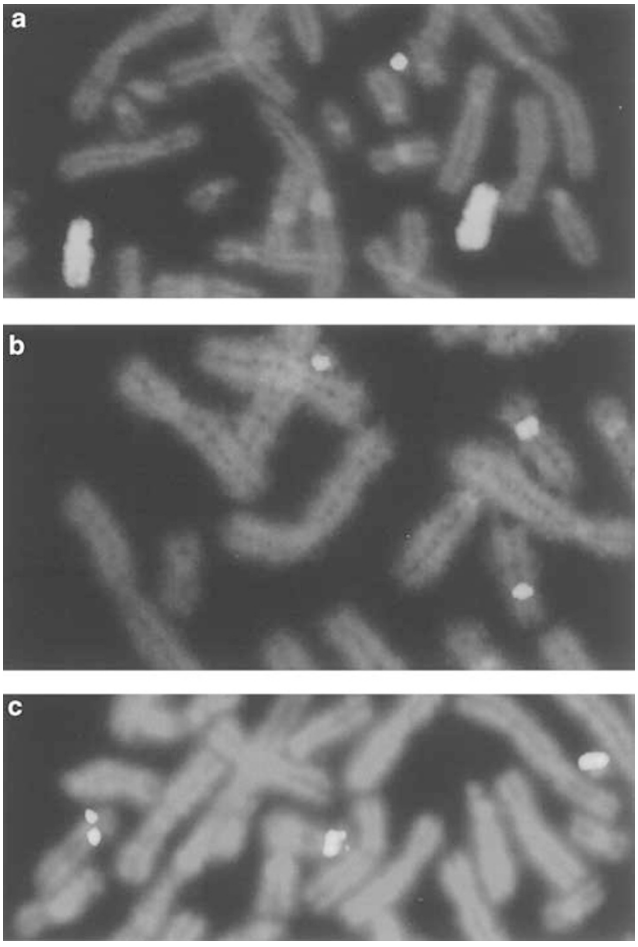

Figure 3 FISH analysis. (**a**) FISH analysis with a chromosome 17 paint showing the positive fluorescence on the chromosome 17 pair and on the small additional marker. (**b**) FISH analysis with a chromosome 17 centromeric probe showing the positive fluorescence on the chromosome 17 pair and on the small additional marker. (**c**) FISH analysis with the Smith–Magenis probe showing a positive fluorescence on the marker meaning it contains 17p11.2 region.

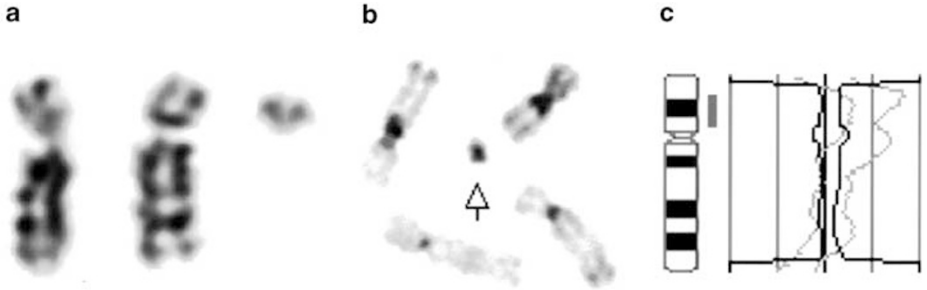

Figure 2 Classical cytogenetic and CGH analysis. (**a**) Results of the cytogenetic analysis from cultured lymphocytes. RTG-banding chromosome 17 pair and the derivative chromosome 17. (**b**) Results of the cytogenetic analysis from cultured lymphocytes: C-banding. The arrow indicates the derivative chromosome 17 with the relative proportions of heterochromatin and euchromatin. (**c**) Results of comparative genomic hybridisation (CGH) on chromosome 17. Note the ratio peak on the chromosome 17p11.1–p12 segment, representing gains of test DNA for this region.

Based on the CGH and FISH data, the proband's karyotype was finally defined as 46,XX [50]/47,XX,+mar. ish der (17)(D17Z1+,wcp17+,17p11.2+)[50].

Discussion

Here we have reported a case of partial trisomy 17p because of the presence, in a mosaic state, of a *de novo* extra chromosome marker. This case emphasises the usefulness of the CGH method, combined with FISH, for the diagnosis of small supernumerary chromosomes. Indeed, although CGH is preferentially used for the identification of homogenous chromosomal imbalance,[8] in our case, it revealed clearly a gain of chromosomal material in 17p11.1–17p12, whereas the abnormal marker was present in half the metaphases analysed. CGH results were then confirmed by FISH using appropriate whole chromosome painting and centromeric probes.

Several methods have been proposed for identifying chromosome markers when standard cytogenetic techniques fail to find their origin. The serial hybridisation of probes coding for the different chromosomes is the simplest way but is cost expensive and time consuming. However, this method can be efficiently used when the banding pattern of the markers suggests their chromosomal nature, which is uncommon because of their frequent short size. Spectral karyotyping (SKY),[9] microdissection of markers, followed by their amplification by PCR and by a reverse *in situ* hybridisation,[10] or multicolour-FISH[11] can also be used but requires special and expensive equipments that only few laboratories can afford. In comparison, CGH requires only a fluorescence microscope connected to an appropriate image analysis system, both materials now found in most cytogenetic laboratories. Moreover, CGH allows to determine from which part of a chromosome a marker is derived. Although the use of CGH is limited by the size of the chromosomal imbalance and/or by the existence of a mosaicism, the case that we have described shows that this technique allows to reach a diagnosis even with small chromosome aberrations present in a mosaic state.

A recent review[5] of eight cases of chromosome markers deriving from chromosome 17 attempted to delineate a common clinical pattern. Although all these cases differed in size, most of them were characterised by mild developmental delay and neurobehavioural features, minor craniofacial anomalies and absence of major organ malformations, especially when chromosomal imbalance was restricted to the 17p11.2 region. Most features described in cases of partial trisomy 17p[5,9,10,12–15] were present in our patient: hypotonia, short stature, developmental delay with moderate mental retardation, abnormal latencies of auditory-evoked potentials and also facial characteristics like downslanting palpebral fissures, prominent nasal bridge, stubby nose with bulbous tip and high arched palate. However, myopia and abnormal visual-evoked potentials are not commonly associated with partial 17p trisomy phenotype. She did not either present clinical findings that are usually found in larger trisomy 17p as congenital heart defects,[9,10] skeletal malformations or external ear deformities.[5,9,16]

Morisson *et al*[14] described a 3-year-old girl with partial trisomy 17 because of a mosaic ring chromosome in 13% of her cells, the chromosomal breakpoints of which were similar to those observed in our case. Like our patient, she presented mild developmental delay with subtle facial abnormalities but also single palmar creases, generalised joint laxity and scoliose that were lacking in our case. Additionally, our patient did not have evidence of demyelineating neuropathy, indicating that her ring chromosome did not include the CMT1A chromosomal locus in 17p12 which is involved in Charcot–Marie–Tooth disease type 1A.[17]

The eight markers previously described[5] and ours differed in size, and the level of mosaicism varied from 2 to 94%. Besides the degree of the mosaicism, the localisation of the abnormal cell line in other organs, and especially in brain, can also modulate phenotype gravity.

In conclusion, this case confirms that CGH alone is able to diagnose mosaic unbalanced structural chromosomal abnormalities. When combined to DNA chip technology, this technique, or array-based CGH, allows the diagnosis of subtle chromosomal rearrangements[18] and represents a powerful tool for future studies dealing with the diagnosis of cryptic interstitial chromosome imbalances, the incidence of which is still unknown. However, direct diagnosis by CGH should always be confirmed by FISH using commercially available probes or probes derived from bacterial artificial chromosomes. Performing both procedures, in combination with classical karyotyping, will certainly allow the identification of genes involved in mental retardation and/or malformative pathology.

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