RESEARCH NOTE

De novo dir dup/del of 18q characterized by SNP arrays and FISH in a girl child with mixed phenotypes

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Introduction

About seven critical regions along the whole 18q have been proposed for Edwards syndrome (ES) / trisomy 18 (T18) phenotype (Boghosian-Sell *et al*. 1994; Nguyen-Minh *et al*. 2013). In contrast, hemizygosity for 18q22.3-18qter region has been linked to most of the features of 18q− syndrome (Feenstra *et al*. 2007; Cody *et al*. 2009). Moreover, combination of both a partial gain and deletion within the same 18q seems to be fairly rare. Here we report on the first case of *de novo* 18q direct duplication (∼34.9 Mb) / deletion (∼8.8 Mb) in a girl child with combined phenotypes related to both syndromes as seen in other duplication/deletion complex rearrangements (Neira *et al*. 2012). Since these complex rearrangements usually exhibit an inv dup/del configuration derived from a U-type exchange, our findings and observations suggest variations of that mechanism for leading to the configuration observed here.

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Material and methods

Clinical report

The girl child is the third child of a G3P2C1 mother and an unrelated father. Prenatal ultrasonography (USG) studies on two different occasions were normal. The child was delivered by Caesarian section at 9 months due to breech presentation and preterm labour. At birth, she breathed and cried, and her body weight and length were 2600 g (Pc 10–25) and 50 cm (Pc 50–75), respectively; occipital frontal circumference was not recorded. At 5 months (figure 1), she was reactive, active, had adequate colouration of integuments, and exhibited growth retardation, hypotonia, skull with sagittal suture ride, normotense anterior fontanel, coarse face, broad forehead with hypertrichosis, bilateral epicanthus and upslanting palpebral fissures, depressed nasal bridge, midface hypoplasia, downward lip corners, sparse hair, low-set ears with posterior rotation, short neck with nuchal fold, widely spaced nipples, cubitus valgus, distinctive hands showing T18 syndrome, hip dislocation, clubfoot, rocker-bottom feet, hirsutism in back and arms and umbilical hernia. A renal USG showed only mild left hydronephrosis. Heart and liver were structurally and functionally normal. Her record shows

Keywords. 18q complex rearrangements; noninverted duplication; compound phenotypes; SNP arrays; fluorescence *in situ* hybridization.

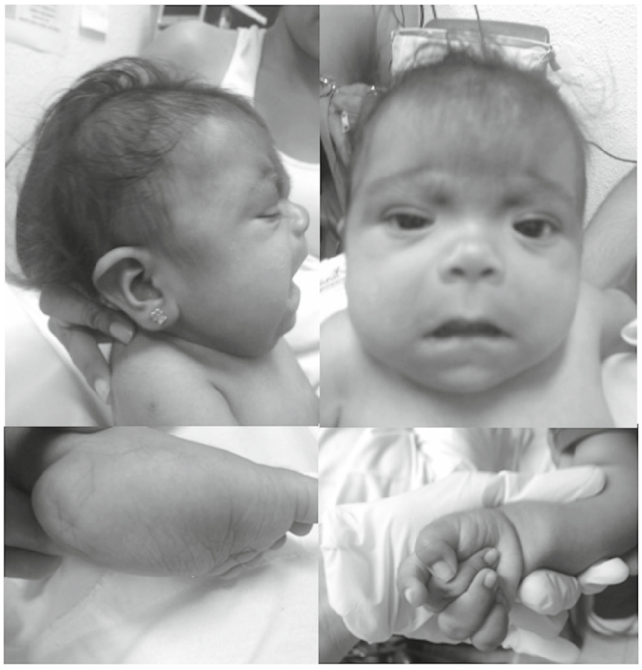

Figure 1. Patient's craniofacial and extremity features at five months of age.

electrolyte abnormalities and bronchiolitis at 14 days, with a further nine days hospitalization due to pneumonia and gastroenteritis at two months of age.

Cytogenetic analyses

The initial cytogenetic analyses of the patient and her parents were made on G-banded chromosomes obtained from 72 h lymphocyte cultures.

SNP microarray analysis

To determine the chromosomal origin and complete gene content of the patient's 18q+, and to search for other genomic unbalances, a high-resolution genomic scan using Affymetrix GenomeWide SNP 6.0 platform (NCBI36/hg18) was performed on the patient. In brief, patient's genomic DNA (∼250 ng) from peripheral blood was digested with *Sty*I and *Nsp*I restriction enzymes, and the digested products were linked by an adapter that recognizes sticky ends. Using a generic primer that binds to the adapter, amplicons of 200–1100 bp were generated and purified by isopropanol precipitation method and fragmented by a *DNA*seI. Fragmented DNA was labelled and hybridized to a chip which was stained, washed and scanned. Analysis of the array was carried out using Genotyping Console v4.0 software. This sample was taken under signed informed consent.

Fluorescence in situ hybridization (FISH) analysis

To explore the orientation of the duplication detected by array studies, a MALT (18q21) Break probe (Kreatech, Amsterdam, The Netherlands, Cat. # KBI-10608) was used on cytogenetic preparations obtained from the patient and her parents. Such a dual-colour split probe contains two portions, a proximal (red) and a distal (green), flanking the *MALT1* gene which maps at positions 54,489,598–54,568,350 and spans ∼79 kb (NCBI36/hg18).

Results

The patient had, as the sole abnormality, an 18q+ of unknown composition in all 50 G-banded cells scored. Parental chromosomes in 100 metaphases per individual were normal.

Microarray results documented two 18q imbalances, namely, a proximal duplication of ∼34.9 Mb at 18q12.2–18q22.3 (genomic position chr18: 32,371,790–67,274,247; NCBI36/hg18) and a concomitant terminal deletion of ∼8.8 Mb at 18q22.3–18q23 (genomic position chr18: 67,274,767–76,116,029; NCBI36/hg18) (figure 2). The maximal distance between both imbalances was just 520 bp (according to the resolution capability of the used microarray) or even less. The duplication included ∼126 genes and the deletion, 27 genes (OMIM and RefSeq genes; cDNAs, miRNAs and hypothetical proteins were disregarded). The duplication proximal breakpoint partially spanned the *FHOD3* gene, but this result may differ on using the GRCh37/hg19 assembly as reference.

FISH assays with the dual MALT probe revealed a direct duplication in the patient's 18q+ chromosome ($n = 5$ metaphases); i.e., the expected two pairs of signals were wide apart and showed a noninverted pattern: red–green, red–green from centromere to telomere (figure 3). The number and orientation of probe signals in the normal homologue as well as in all four parental chromosomes 18 were normal (data not shown). According to the ISCN 2013, the patient's final karyotype was 46,XX,add(18)(q22.3)dn.ish der(18)(MALT++).arr[hg18] 18q22.3q23(67,274,767–76,116,029) × 1,18q12.2q22.3 (32,371,790−67,274,247) × 3.

Discussion

Including the patient, only six bona fide cases with dup/del complex 18q rearrangements, whose imbalances are heterogeneous in size, have been reported (figure 2). Among these, a set of monozygotic twins had overlapping phenotypes reminiscent of both T18 and 18q− syndromes (Courtens *et al*. 1998). Two other patients showed no clinical features of T18 (Lee *et al*. 2009; Nguyen-Minh *et al*. 2013) but the boy described by Nguyen-Minh *et al*. (2013) showed a conspicuous 18q− phenotype. The feotus described by Lee *et al*. (2009), could not be assessed in detail for deletion features. Another patient (Rowe *et al*. 2009) was recruited due to developmental delay (Prof. S. T. South, e-mail communication, September 2013, Institute for Clinical and Experimental Pathology, Associated Regional and University Pathologists

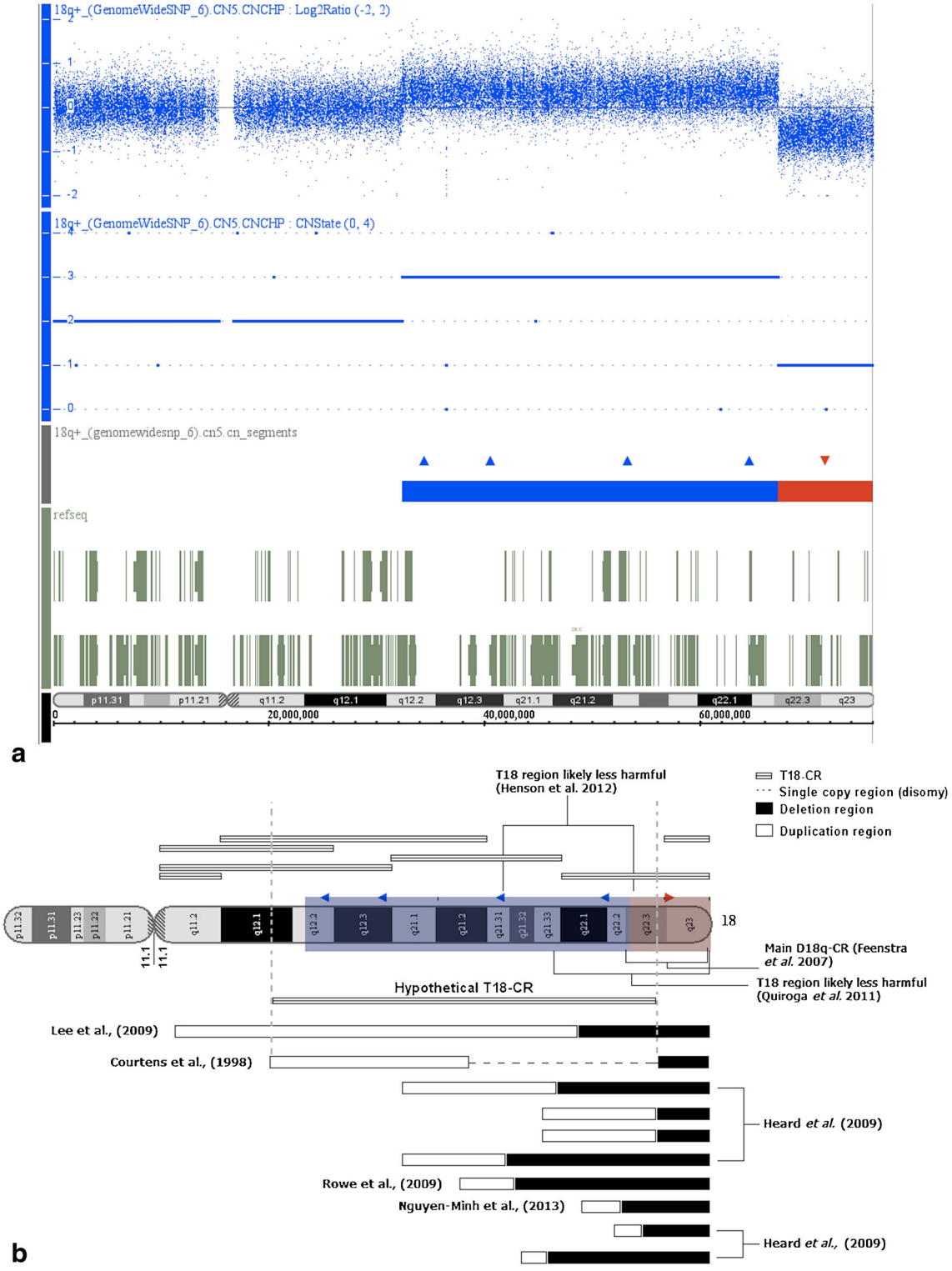

Figure 2. (a) Affymetrix microarray results scheme from the patient: log2 and copy number state visualization indicated by blue dots (upper part) and thin, blue horizontal lines, respectively. Thick, horizontal blue and red bars represent amplified and deleted regions, respectively. Note that there are no probes in normal dosage between both chromosomal lesions. (b) Representation (not to scale) of the overlapping of amplified and deleted regions reported so far from patients with a duplication–deletion combination (lower part) including the present case (chromosome 18 ideogram: duplication in blue and deletion in red shading). On the upper part are represented the proposed critical regions for T18 syndrome (T18-CR). D18q-CR = 18q– syndrome critical region. Gray dotted lines delimitate the hypothetical critical region for full T18 syndrome. Inclusion of Heard's cases (2009) as 'legitimate' duplications was by visual appraisal. The rearrangement described by Chia *et al*. (1992) and cited by Courtens *et al*. (1998), was not included in this scheme because of scanty information available.

(ARUP) Laboratories, Salt Lake City, Utah, USA). Noticeably, our patient's compound phenotype visibly evokes both T18 and 18q− syndromes.

Although the twins' duplication led to most key features of T18 syndrome (i.e. growth, cardiac, genitourinary, palate and hand defects and umbilical hernia) (Courtens *et al*. 1998), other overlapping large duplications failed to cause major (present case) or any (Lee *et al*. 2009) features of T18. This apparent inconsistency may be explained by some effect of the maternal paracentric inversion that gave rise to the twins' duplication and whose distal breakpoint nearly coincides with the duplication/deletion junction of the present rearrangement. Since the present duplication lacks a small proximal portion of the twins' duplication and that the large duplication of Lee *et al*. (2009) does not essentially embrace any T18 terminal critical segment, it is tempting to speculate that the segment between the 18q12.1 breakpoint in the twins' recombinant and near the distal breakpoint at 18q22.3 in the present duplication and in the maternal inversion aforementioned (Courtens *et al*. 1998) (figure 2) contains genes and even regulatory elements related to the full symptomatology of T18. Moreover, unknown 'interactions'or regulatory mechanisms involving two noncontiguous chromosomal segments (proximal and distal) may contribute to the full T18 syndrome (Turleau and de Grouchy 1977; Boghosian-Sell *et al*. 1994; Quiroga *et al*. 2011; Nguyen-Minh *et al*. 2013). In fact, position effects derived from the duplication orientation in similar rearrangements have previously been suggested to influence the clinical presentation (Hulick *et al*. 2009).

We are aware of ∼17 cases of complex rearrangements entailing an 18q proximal duplication and a terminal deletion within the same arm (Chia *et al*. 1992 as cited by Courtens *et al*. 1998; Courtens *et al*. 1998; Heard *et al*. 2009; Lee *et al*. 2009; Rowe *et al*. 2009; Nguyen-Minh *et al*. 2013). Yet, this figure may be greatly reduced if 11 cases considered as 'segmental duplications' (SD), but not as partial trisomies or common duplications, are excluded (Heard *et al*. 2009). In fact, Prof. J. Cody (e-mail communication, September 2013, Division of Genetics and Metabolic Disorders, Chromosome 18 Clinical Research Center, UT Health Science Center, MSC 7820 7703 Floyd Curl Drive) states that the *de novo* 18q SD found proximal to 18q deletions (Heard *et al*. 2009) were not part of a 'normal' genomic architecture but inherent to the chromosome healing process. It seems that size, gene content and occurrence (inherited or *de novo*) are essential in distinguishing a SD from a pathogenic duplication. Accordingly, six out of those 11 amplifications may correspond to 'true' partial trisomies (figure 2).

Even though a duplication immediately proximal to a terminal deletion is commonly inverted (Heard *et al*. 2009; Hulick *et al*. 2009; Lee *et al*. 2009; Rowe *et al*. 2009; Zuffardi *et al*. 2009), our FISH results revealed a 'direct' duplication (figure 3). Similar configurations for other rearranged chromosomes have hardly been reported (Zollino *et al*. 1999). In fact, the orientation of several duplications, including those for 18q, has been difficult to establish or simply not confirmed (Heard *et al*. 2009; Rowe *et al*. 2009; Nguyen-Minh *et al*. 2013). The most frequent mechanism leading to a *de novo* inv dup/del rearrangement appears to involve breakage of an unstable dicentric chromosome created by a U-type exchange (Rowe *et al*. 2009). Although, the lack of SD (at UCSC genome browser) flanking the 520 bp maximal distance (genomic position chr18:

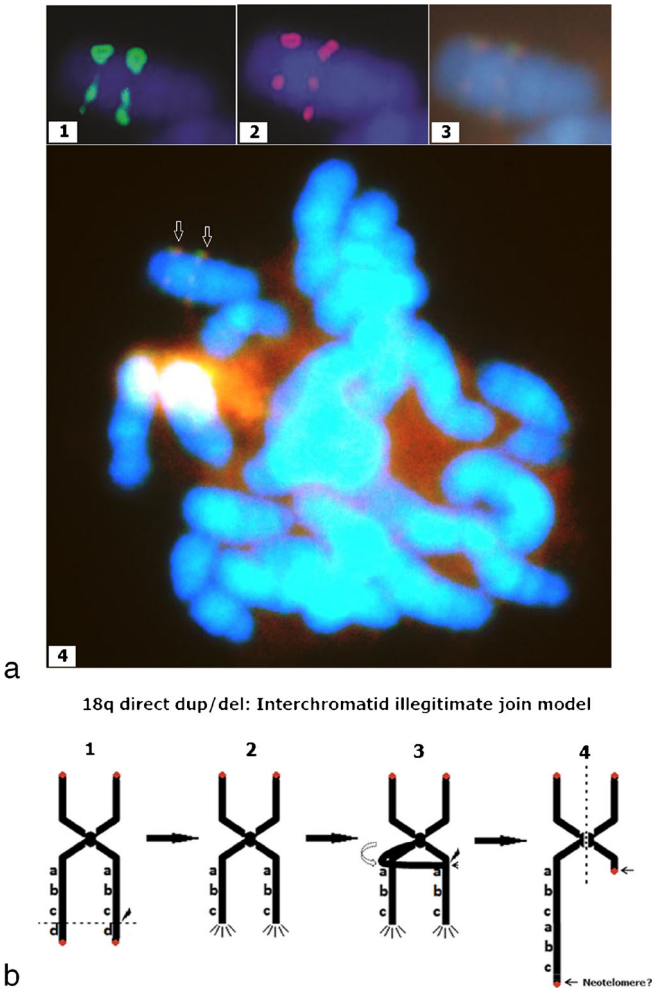

Figure 3. (a) The 18q direct duplication as revealed by the dual probe MALT. Note that the distal green signal (1) is more telomeric whereas the proximal red signal (2) is more centromeric than the counterpart signals. In spite of its suboptimal quality, the combined image (3 and 4) is consistent with a direct duplication (FISH probes distribution: red–green, red–green from centromere to telomere). (b) Potential mechanism for leading to direct duplication following a terminal deletion: (1) lightning figure indicates the breakpoint site; (2) chromosome with terminal deletion and 'exposed' ends; (3) 'healing' by interchromatid illegitimate rejoining and sister chromatid fragment pulling without dicentric formation (lightning figure indicates the breakpoint site, and arrowhead the potential nonallelic homologous sequences); (4) formation of a new recombinant chromatid with both the tandem direct duplication and the terminal deletion; chromatids separation (dotted line). The original mechanism implies reversed-ends transposition on transposon particular target sequences (Zhang *et al*. 2013).

67,274,247–67,274,767) between duplication and deletion in a radius of ∼1 Mb, the absence of a disomic region between them and the *de novo* occurrence in the present case (figure 2) agree with such mechanism (Zuffardi *et al*. 2009; Rowe *et al*. 2009), the latter cannot explain the direct orientation of the present duplication. To explain direct dup/del rearrangements we recall the mechanism proposed in maize for formation of a tandem direct duplication (Zhang *et al*. 2013) following a terminal deletion. In short, this hypothetic mechanism would imply a double-strand break at 18q22.3 (described as fragile site by Debacker *et al*. 2007) leaving both sister chromatids with 'exposed' ends; in turn, the illegitimate rejoining of one 'exposed' end with the sister chromatid at 18q12.2 would lead to a new recombinant chromatid with both the tandem direct duplication and the terminal deletion. Finally, given the risk of DNA erosion inherent to telomereless ends (Courtens *et al*. 1998; Heard *et al*. 2009), we assume healing of the broken 18q by formation of a neotelomere (figure 3).

The present complex rearrangement appears to represent just the first case of 18q direct duplication/terminal deletion described so far (of course, such orientation could remain undetected in other cases). In conclusion, we believe that it would be useful to analyse in detail those mechanisms underlying dup/del rearrangements.

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