A NEW CASE OF CHROMOANASYNTHESIS, A NEW MECHANISM FOR COMPLEX CHROMOSOMAL REARRANGEMENTS

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INTRODUCTION

We report the case of a child of 2.5 years old with syndromic developmental delay, due to a complex chromosome 18 rearrangement, diagnosed by conventional cytogenetics. In order to characterise this rearrangement, a SNP-array was performed and revealed a highly complex chromosomal rearrangement with more than 15 breakpoints, located both on the short arm and the long arm of chromosome 18. We detail the clinical presentation of the patient, the results of conventional and molecular cytogenetic analyses.The mechanism of formation of this complex chromosomal rearrangement, which strongly suggests a chromoanasynthesis is discussed.

CASE REPORT

Clinical report

The child is the only one of a young couple from Morocco, unrelated and without any family history. He was born eutrophic, after unremarkable obstetric history, with increased head circumference (HC) + 3 SD. The neonatal period was marked by initial feeding difficulties, spontaneously regressive and the management of tracheomalacia. Motor acquisitions were delayed: an outfit head at 9 months, walking at 26 months. He mastered only a few words, without real association. Social interactions were of good quality. There were a unilateral isolated pyelyc duplicity without further visceral malformation. At two and a half years, weight and height growths were satisfying and the HC was measured at -0.5DS. The neurological examination was norma. There was a craniofacial dysmorphism (**Fig. 1**).

Chromosomal rearrangement

The conventional karyotype found a complex chromosome 18 rearrangement (**Fig. 2**).

The complexity of this rearrangement did not allow an accurate analysis with conventional cytogenetics, and a SNP-array (whole genome Affymetrix microarray, CytoscanHD, resolution 40Kb) was performed (**Fig. 3**). This highlighted a complex chromosome 18 rearrangement with seven regions of the long arm duplicated and one region of the short arm triplicated, summarized in **Table 1**. The sizes of these regions ranged from 200 Kb to 5 Mb, with a total gain of approximately 21 Mb of chromosomal material. The FISH techniques using BAC probes confirmed and clarified the positions of the regions involved.

A particular focus is placed on the region 2: The use of molecular cytogenetic Bac probes has highlighted a more complex rearrangement comprising three sub-regions: 2a was duplicated on the short arm and the long arm, 2b was not duplicated and positioned on the short arm, 2c was duplicated and only positioned on the short arm.

The maternal karyotype was normal. The paternal karyotype could not be performed. The expected phenotype was compatible with a partial trisomy 18.

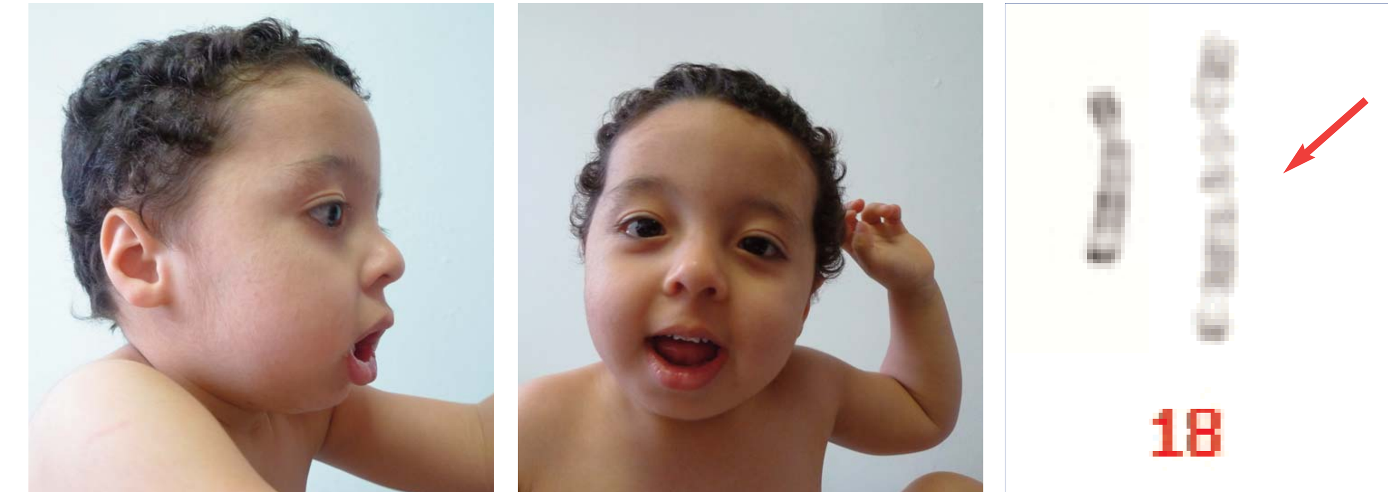

Figure 1. Picture of the patient at 2 and half years old: the face was round with facial hypotonia, telecanthus, down slanting palpebral fissures, low-set and slightly dysplasic ears.

Figure 2. Conventionnal karyotpe (RHG banding, resolution of 550 bands for a haploid karyotype): chromosome 18 with a complex rearrangement.

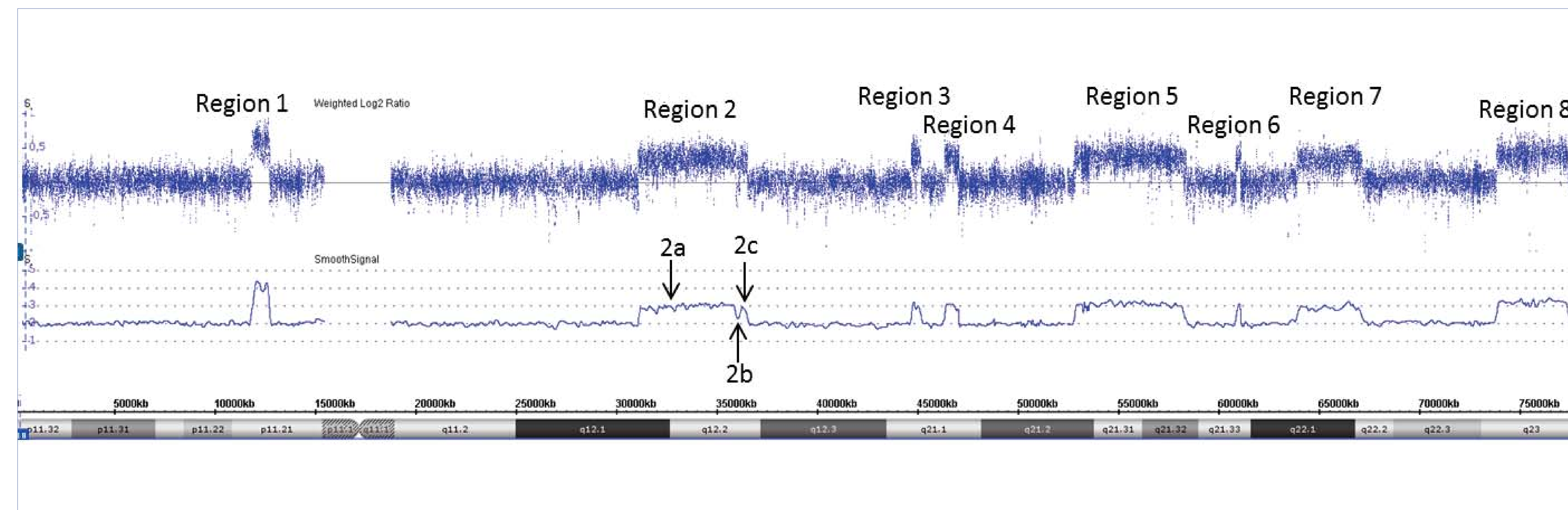

Figure 3: CGH array (Affymetrix): Highlighting eight rearrangements: a triplicated region in 18p11.21 (smooth signal ≈ 4) and 7 duplicated regions in the long arm (smooth signal ≈ 3).

	Localisation	Size	CGH rearrangement	FISH rearrangement
Region 1	p11.21 (11,585,369-12,482,080)	896 Kb	Triplication	Amplification of the spot in 18p
Region 2	q12.1-q12.2 (30,852,180-36,260,132)	5 407 Kb	Duplication	3 sous-régions (QS texte)
Region 3	q21.1 (44,426,209-44,898,600)	472 Kb	Duplication	Duplication sur le bras long, à distance
Region 4	q21.1 (46,076,777-46,787,156)	710 Kb	Duplication	Duplication sur place
Region 5	q21.2-q21.32 (52,567,865-58,008,305)	5 440 Kb	Duplication	Duplication sur place
Region 6	q21.33 (60,595,227-60,819,311)	224 Kb	Duplication	Duplication sur place
Region 7	q22.1 (63,614,315-66,856,964)	3 242 Kb	Duplication	Duplication sur le bras court et le bras long
Region 8	q23 (73,554,500-77,125,545)	3 571 Kb	Duplication	Duplication sur place

Table 1. Chromosome rearrangement by CGH array and molecular cytogenetics (BACs probes)

MECHANISM

Two new mechanisms, inferred from carcinogenesis, have been described to explain such events: chromothripsis and chromoanasynthesis.

Their description is based on three main features: 1) the occurrence of remarkable numbers of rearrangements in localized chromosome areas, that suggest that the rearrangement occurred when chromosomes are largely condensed, such as during mitosis ; 2) a low number of copy number states (generally between one and three) across the rearranged region, that implies that those rearrangements occurred within a relatively short time period ; 3) an alternation of regions where heterozygosity is preserved with regions presenting loss of heterozygosity (LOH), that suggests that it took place early in development, at a time when both parental copies were present before LOH.

This supports to the idea that chromothripsis or chromoanasynthesis are linked to a single causative event, taking place in one cell cycle, concerning one nuclear domain usually involving a single chromosomal region or rarely several chromosomal regions closed in the spatial organization of the nucleus.

Chromothripsis (**Fig. 4**) corresponds to a chromosome explosion in many fragments (DNA double-strand breaks (DSBs)) and subsequent break-repair errors linking non-specific fragments. The link require little or no sequence homology between them (NHEJ). The consequences are essentially multiple inversions and deletions.

Chromoanasynthesis is based on serial replication slippage during a single cell cycle through mechanisms involved in the restoration of collapsed replication forks, such microhomology-mediated break-induced replication (MMBIR). The MMBIR is a mechanism that allows restarting replication after the blocking of the replication fork by breaking of the template strands. For this, the process used new DNA templates with micro-homologies with the free 3' end generated and then create new loop replication. There may be several matrix changes before returning to the original strand (**Fig. 5a**).

Collapsed replication forks are usually restored by template switching inside the same ongoing fork. However, erroneous switching between different forks sharing regions of micro-homology can result in complex chromosomes rearrangements like deletion, triplication, inversion and translocation (**Fig. 5b**).

It is likely that the mechanism of complex rearrangement of our patient is a chromoanasynthesis. The sequencing of breakpoints to highlight these micro-homologies would prove it. Our case is the second case reported in the literature relevant to both the short arm and the long arm of chromosome (Liu P et al, 2011).

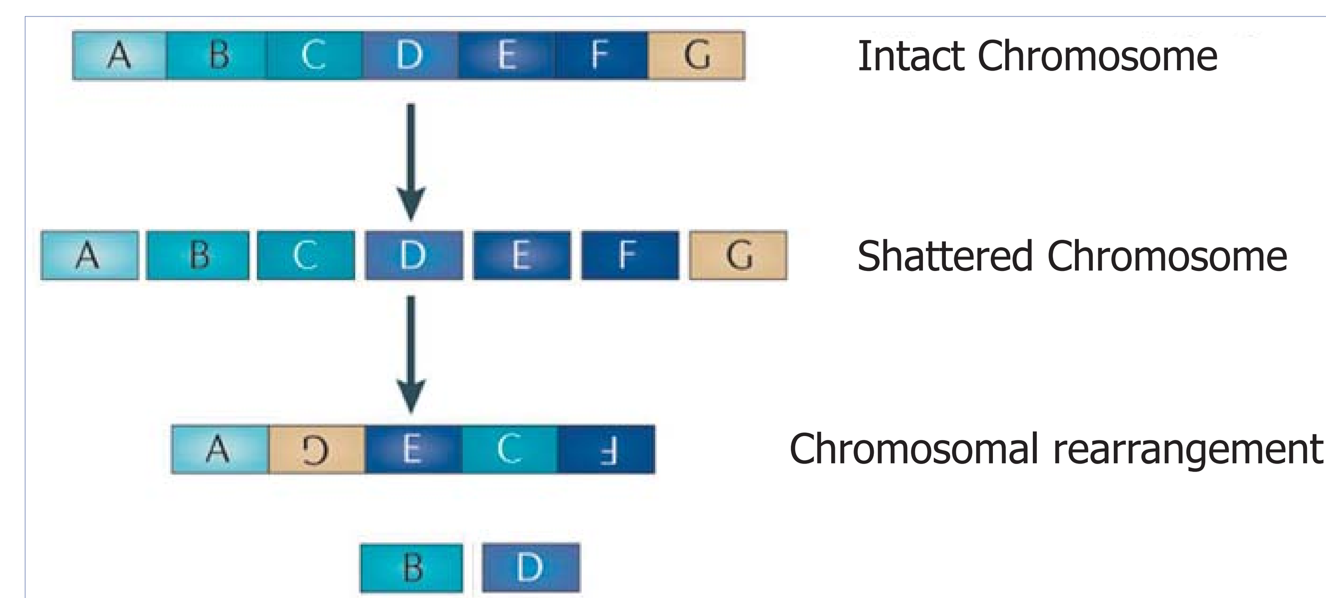

Figure 4: Chromothripsis (Schema derived from Forment JV et al, 2012)

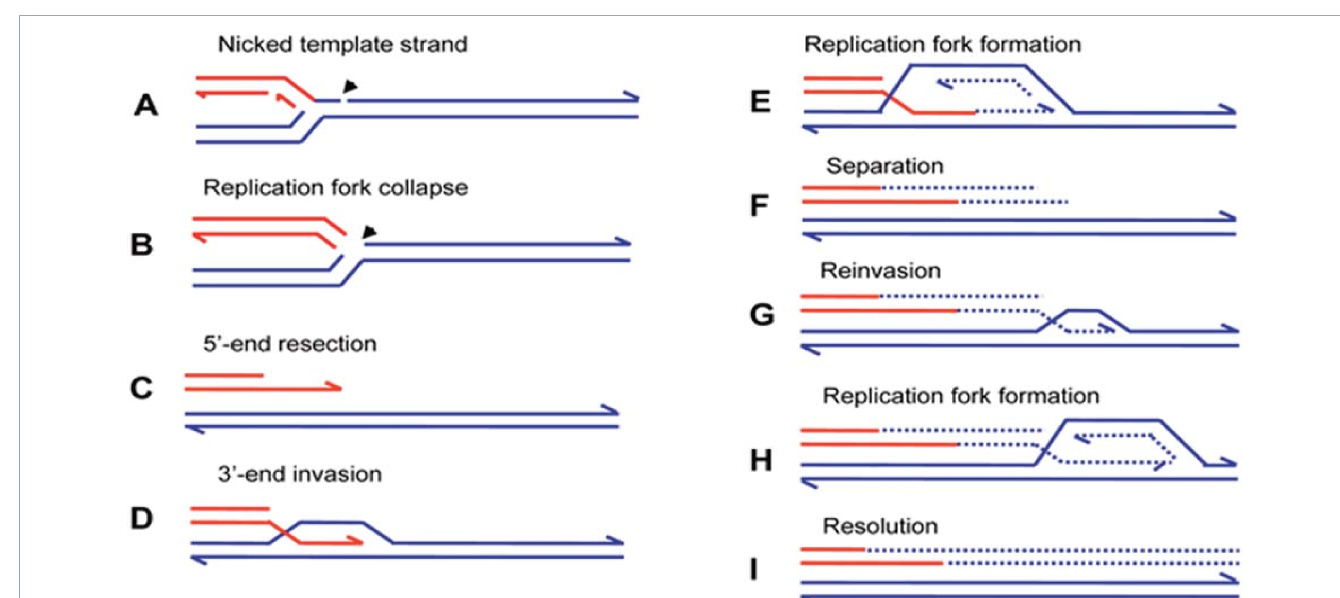

Figure 5a: MMBIR (Schema from Hasting PJ et al, 2009)

When a replication fork encounters a nick in a template strand (A) (arrowhead), one arm of the fork breaks off (red), producing a collapsed fork (B). At the single double-strand end, the 5′ strand is resected, giving a 3′ overhang (C). The 3′ single-strand end invades the sister molecule (blue), forming a D-loop (D), which subsequently becomes a replication fork with both leading and agging strand replication (E). There is a Holliday junction at the site of the D-loop. Migration of the Holliday junction, or some other helicase activity, separates the extended double-strand end from its templates (F). The separated end is again processed to give a 3′ single-strand end, which again invades the sister, and forms a replication fork (G). Eventually the replication fork becomes fully processive, and continues replication to the chromosome end (H and I). This process is shown here with the Holliday junction following the fork so that newly formed strands are segregated together (conservative segregation) (H). Each line represents a DNA nucleotide chain (strand). Polarity is indicated by half arrows on 3′ end. New DNA synthesis is shown by dashed lines.

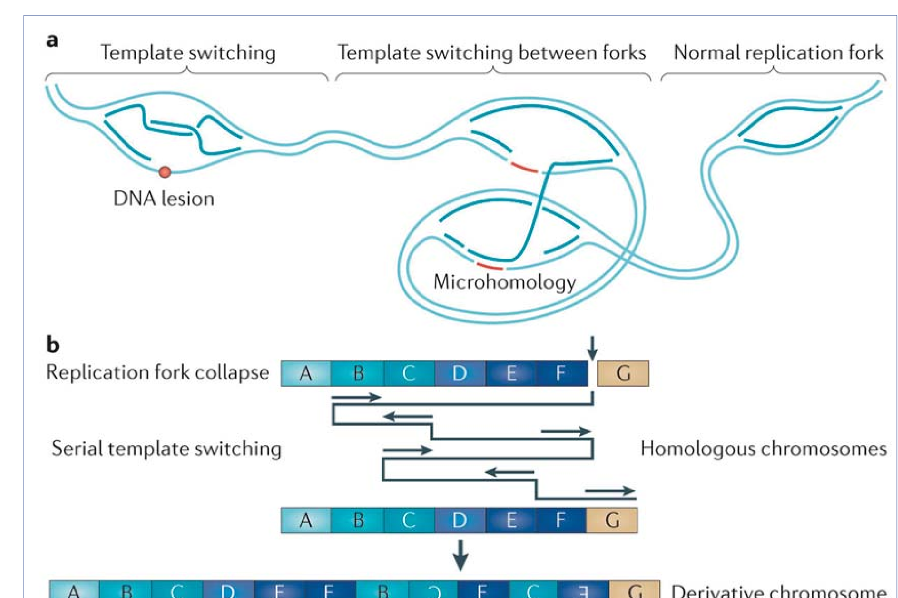

Figure 5b: Chromoanasynthesis (Schema from Forment JV et al, 2012)

CONCLUSION

This is a patient with developmental delay and craniofacial dysmorphism, due to a complex chromosome 18 rearrangement.

The molecular cytogenetic techniques (SNP-Array) allowed to characterise this rearragement, with seven duplications and one triplication.

As the possibly underlying mechanism of formation we propose chromoanasynthesis, resulting in several types of rearrangements (deletion, duplication, triplication, inversion, translocation,...). The use of deep sequencing will allow to furtherinvestigate the mechanism of formation of this complex genomic reorganization.

The complexity of this rearrangement has been demonstrated in our patient through the use of a high resolution SNP array. There is no doubt that the use of this technique will highlight such phenomenon more frequently than we suppose with conventional cytogenetic techniques.

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