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Short communication

# Post-zygotic breakage of a dicentric chromosome results in mosaicism for a telocentric 9p marker chromosome in a boy with developmental delay

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ABSTRACT

Chromosomal rearrangements resulting in an inverted duplication and a terminal deletion (inv dup del) can occur due to three known mechanisms, two of them resulting in a normal copy region between the duplicated regions. These mechanisms involve the formation of a dicentric chromosome, which undergo breakage during cell division resulting in cells with either an inverted duplication and deletion or a terminal deletion. We describe a mosaic 3 year old patient with two cell lines carrying a chromosome 9p deletion where one of the cell lines contains an additional telocentric marker chromosome. Our patient is mosaic for the product of a double breakage of a dicentric chromosome including a centric fission. Mosaicism involving different rearrangements of the same chromosome is rare and suggests an early mitotic breakage event.

Chr9p terminal deletions associated with duplications have previously been reported in 11 patients. We compare the clinical features of all 12 patients including the patient that we report here. To the best to our knowledge this is a first case reported where the double breakage occurred in the dicentric derivative chromosome 9.



## 1. Introduction

Inv dup del chromosomes occur due to three known mechanisms, the most common is a U-type exchange mechanism which does not result in a normal copy region in between the duplicated regions. This occurs when there is a random DNA double strand break and fusion of the broken chromatid ends. The other two mechanisms result in duplicated regions separated by a normal copy region. This can occur when one of the parents carry a paracentric inversion in one of their chromosomes, or by non-allelic homologous recombination (NAHR) between inverted low copy repeats (LCR) present at the borders of the region that is in normal copy in the resulting inv dup del chromosome (Rowe et al., 2009). All three mechanisms involve unstable dicentric chromosomes, which will undergo breakage during cell division. In contrast to the U-type exchange mechanism the latter two mechanisms result in one chromosome with a terminal deletion and one with inv dup del with a normal copy region between the duplicated regions (Rowe et al., 2009; Yu et al., 2010).

Chromosome breakage and fusions occur frequently during human embryogenesis leading to a formation of a dicentric chromosome and an acentric fragment at the zygote or the first two cleavages (Voet et al., 2011). Later, when the dicentric chromosome is pulled to opposite poles during the subsequent mitoses, a monocentric chromosome with a terminal deletion is generated in one blastomere and a chromosome with terminal deletion with an inverted duplication separated by a normal copy region in between the duplication in the other daughter cell (Voet et al., 2011). Chromosomal rearrangements resulting in an interstitial inverted duplication with a terminal deletion (inv dup del) is a rare complex rearrangement. Chromosome 9 deletion associated with duplication has previously been described in 11 patients (Al Achkar et al., 2010; Chen et al., 2011; Di Bartolo et al., 2012; Faas et al., 2007; Hauge et al., 2008; Hulick et al., 2009; Krepischi-Santos and Vianna-Morgante, 2003; Recalcati et al., 2012; Swinkels et al., 2008; Teebi et al., 1993) and most of them share features associated with the 9p deletion/duplication syndromes, such as developmental delay, speech delay, mid face hypoplasia, low set of ears, long philtrum, trigonocephaly, genital disorders, microcephaly and hypotonia.

We present a 3 year old boy with two cell lines both containing a chr9p terminal deletion and one of the cell lines harboring a telocentric marker chromosome with a duplicated and a normal copy region derived from chr9p. We propose that NAHR between inverted LCRs caused the aberration seen in this patient. So far only a few cases have

*Abbreviations:* aCGH, Array comparative genomic hybridization; BAC, Bacterial artificial chromosome; *CER1*, Cerberus 1; Chr9, Chromosome 9; CT, Computerized tomography; *DMRT 1,2,3*, Doublesex and Mab.3 related transcription factor *-1*, *-2* and *-3*; *DOCK8*, Dedicator of cytokinesis 8; FISH, Fluorescence in situ hybridization; *FOXD4*, Forkhead box D4; *FREM1*, FRAS1 related extracellular matrix 1; inv dup del, Inverted duplication and a terminal deletion; LCR, Low copy repeats; NAHR, Non-allelic homologous recombination; OFC, Occipital Frontal Circumference; *SLC1A1*, Solute carrier family 1; *VLDLR1*, Very low density lipoprotein receptor 1.

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been reported with stable centric fission leading to complete or partial trisomy of a chromosome arm.

## 2. Chromosome anomaly

G-banding at 500 band resolution was performed on metaphases from peripheral blood from the patient showing a mosaic karyotype (47,XY,del(9)(p22.2),+mar[24], 46,XY,del(9)(p22.2)[14]). One cell line, observed in 14/38 cells showed a chr9p22.2pter deletion (Fig. 1A). The other cells carried the same chr9p22.2pter deletion and an additional small marker chromosome (Fig. 1B).

Array comparative genomic hybridization (aCGH) was carried out using a SurePrint G3 Unrestricted CGH ISCA v2, 4 × 180K Agilent oligo array according to manufacturer's instructions and the data was analyzed by Cytogenomics v2.0.6.0 (Agilent Technologies, CA, USA). All genomic positions were based on the February 2009 human reference sequence (GRCh37/hg19) produced by the Genome Reference Consortium. aCGH showed a 17.2 Mb deletion on Chr9:204,104–17,466,907 bp, a 561 kb region of normal copy number on Chr9:17,512,109–18,073,357 bp and a 20.6 Mb duplication on Chr9:18,092,732–38,763,958 bp (Fig. 2). The karyotype was revised according to the aCGH results: 46,XY.arr 9p24.3p22.2(204,104–17,466,907 bp)×1/ 47,XY,+mar.arr 9p24.3p22.2(204,104–17,466,907 bp)×1, 9p22.1p13.1(18,092,732–38,763,958 bp)×3.

## 3. Characterization of the chr9p inv dup del

To verify the aCGH findings and further characterize the derivative chromosomes, fluorescence in situ hybridization (FISH) was performed on metaphase chromosomes from patients and parents using standard methods (Lichter et al., 1990). A commercial centromeric probe (Vysis CEP9), a designed telomeric repeat probe and BAC clones were used as FISH probes (probes are shown in Figs. 3a and b). HYBriteTM station (Vysis, Downer Grove, Il, USA) was used to run the hybridization (2 min at 73 °C, 37 °C overnight). Digital images were obtained using a Nikon Eclipse 80i epifluorescence microscope. BAC clones were selected using BACPAC Resources CHORI library (http://bacpac.chori.org) based on the aCGH results. Terminal deletions on chr9p in both the cell lines were confirmed by the probe RP11-105H18 (Probe 1, Fig. 3ci). RP11-751J3 (Probe 7) was used as a control probe to identify chromosome 9. We selected the FISH probes RP11-601F21, RP11-379M22 and RP11-732M1 (Fig. 3a) in the 571 kb normal copy region detected by aCGH. RP11-601F21 (Probe 2) confirmed the presence of the normal copy region in chromosome 9, and in the marker chromosome (Fig. 3cii). The probes RP11-379M22 and RP11-732M1, however, verified the presence of a duplicated region detecting signal on chromosome 9, der(9) and the marker chromosome (data not shown). Thus, the normal copy region was approximately 300 kb in size. The probes mapping at the two extremes of the duplicated region according to aCGH, RP11-1A10 (Probe 3) and RP11-113O24 (Probe 4), were co-hybridized giving signal on chr9, der(9) and the marker chromosome demonstrating the presence of the duplicated segment on the marker chromosome (Fig. 3ciii). Probes 3 and 4, which are located 20 Mb apart on chromosome 9, give two distinct signals on the marker chromosome. This result indicates that the marker is a linear chromosome. Since there were no oligos from the chr9p13.2 to chr9q21.11 region on the Agilent array, additional FISH experiments were performed to more precisely map the break points of the duplication. The most proximal chr9p11.2 probe RP11-416F19 (data not shown) and the centromeric probe CEP9 (Probe 5) gave signal on the marker chromosome (Fig. 3civ), while the chr9q11.2 probe RP11-151G22 (Probe 6, Fig. 3cv) gave signal only on chr9 and der(9) confirming the absence of chr9q sequences in the marker chromosome. These FISH results revealed that the duplication was 30 Mb extending to the centromere of chromosome 9. This confirms that the breakage occurred at the centromere and the duplicated region detected by Probes 4–6 is present in the marker chromosome, chr9 and der9 (Fig. 3a). A telomeric probe (Probe 8) designed using two sets of primers $(TTAGGG)_5$ and $(CCCTAA)_5$ for the telomeric region $(TTAGGG)_5$ (Ijdo et al., 1991) gave FISH signals on both the p and the q arm on der9 and only one signal in the marker chromosome opposite to the telocentromere, confirming that the marker is not a ring chromosome (Fig. 3cvi). FISH experiments on chromosomes from the parents revealed that the aberrations were de novo in this child.

## 4. Clinical description

The patient, a 3 year old boy, was the second child of healthy, unrelated parents from Sri Lanka. The mother was 34 and the father was 29 years old at the time of the boy's birth. The pregnancy was complicated by recurrent bleeding in the first trimester and premature rupture of the membrane in the 34th week. He was born after a normal delivery at 37th week of gestation. Birth weight was 2300 g (<3rd centile), length 50 cm (50th centile) and occipital frontal circumference (OFC) 33 cm (25–50th centile). He was resuscitated at birth and showed some dysmorphic features. Omphalocele surgery was done soon after

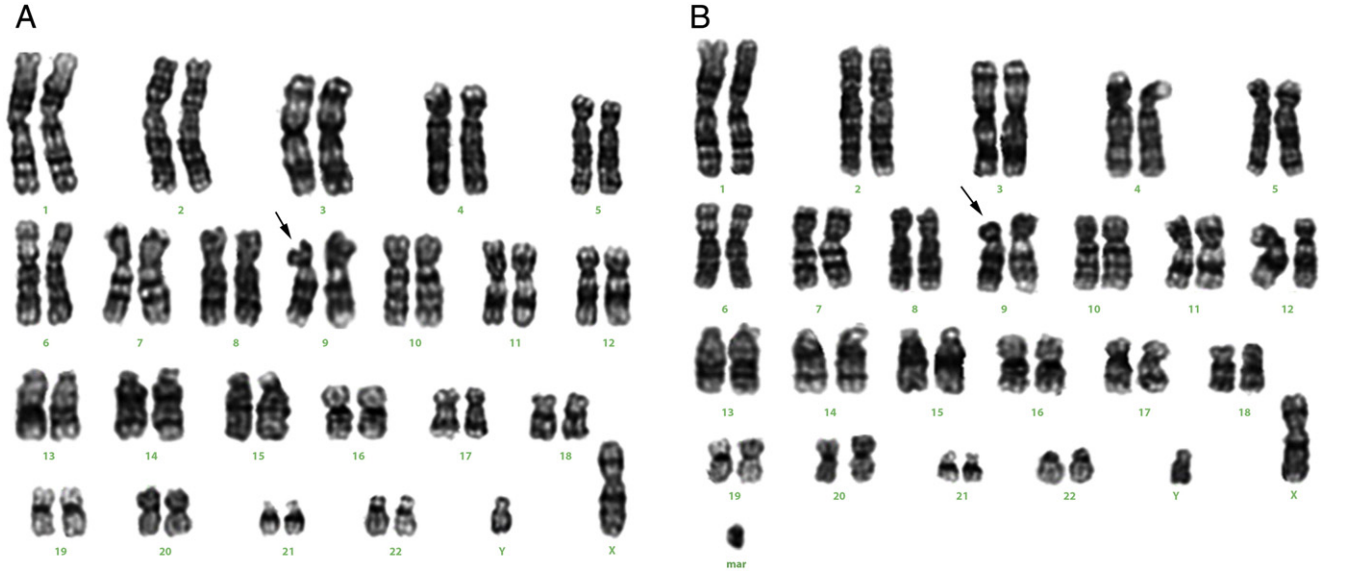


**Fig. 1.** G-band karyotyping of the patient identified one cell line with karyotype 46,XY,del(9)(p22.2) (A), and one cell line with karyotype 47,XY,del(9)(p22.2),+mar (B). The arrows indicate the deletion.