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Interstitial Deletion of Proximal 8q Including Part of the Centromere from Unbalanced Segregation of a Paternal Deletion/Marker Karyotype with Neocentromere Formation at 8p22

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Key Words
Centromere misdivision · Microarray · Neocentromere

Abstract
Background/Aims: The 'McClintock mechanism' of chromosome breakage and centromere misdivision, in which a deleted chromosome with its concomitant excised marker or ring chromosome is formed, has been described in approximately one dozen reports. We report a case of a girl with short stature, developmental delay, and dysmorphic features. ***Methods:*** Analysis was performed on the proband and father using cytogenetic chromosome analysis and the Affymetrix 6.0 SNP microarray. Fluorescence in situ hybridization (FISH) using a chromosome 8 alpha-satellite probe and immunofluorescence with antibodies to CENP-C were used to examine the centromere positions in these chromosomes. ***Results:*** An abnormal chromosome 8 with a cytogenetically visible deletion was further defined by SNP array as a 10.6-Mb deletion from 8q11.1→q12.1. FISH with a chromosome 8 alpha-satellite probe demonstrated that the deletion removed a significant portion of the pericentromeric alpha-satellite repeat sequences and proximal q arm. The deleted chromosome 8 appeared to have a constriction at 8p22, suggesting the formation of a neocentromere, even though alpha-satellite sequences still appeared at the normal location. Chromosome analysis of the phenotypically normal father revealed the same deleted chromosome 8, as well as an apparently balancing mosaic marker chromosome 8. FISH studies revealed that the majority of the chromosome 8 alpha-satellite DNA resided in the marker chromosome. Immunofluorescence studies with antibodies to CENP-C, a kinetochore protein, proved the presence of a neocentromere at 8p22. The excision of the marker from the deleted chromosome 8 likely necessitated the formation of a new kinetochore at the 8p22 neocentromere to stabilize the chromosome during mitosis. ***Conclusion:*** This case clearly illustrates the utilization of classic cytogenetics, FISH, and array technologies to better characterize chromosomal abnormalities and provide information on recurrence risks. It also represents a rare case where a neocentromere can form even in the presence of existing alpha-satellite DNA.

The 'McClintock mechanism' of ring/marker chromosome formation postulates an initiating break in the alpha-satellite repeat sequences of a centromere, followed by a second break in the p or q arm of a chromosome with subsequent excision of a ring or marker chromosome

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[Baldwin et al., 2008; Mantzouratou et al., 2009]. This generates a chromosome with a deletion and a supernumerary ring/marker chromosome, either of which may be passed on to the next generation in an unbalanced fashion. A key aspect of this mechanism is that 2 functional centromeres are produced, one on each chromosome, from the 'misdivision' of the centromere [Perry et al., 2004]. Approximately one dozen case reports exist in the literature documenting patients with unbalanced karyotypes due to inheritance of an abnormal chromosome from a parent in whom a marker has formed by such a mechanism [Fryns et al., 1985; Krauss et al., 1987; Andersen et al., 1990; Pfeiffer et al., 1991; Friedman et al., 1992; Quack et al., 1992; Schuffenhauer et al., 1996; Lasan Trcic et al., 2003; Baldwin et al., 2008; Mantzouratou et al., 2009]. Patients who inherit an excised marker chromosome often have mosaic karyotypes, perhaps due to inherent mitotic instability of ring chromosomes.

Here we present a case in which the proband inherited a deleted chromosome 8 from her father who carries the balanced del/marker karyotype. G-banded chromosome analysis identified an abnormal chromosome 8 which was further characterized using SNP microarray analysis, demonstrating a proximal 8q deletion. Further fluorescence in situ hybridization (FISH) and immunofluorescence studies demonstrated that most of the centromeric alpha-satellite repeat sequences were deleted, and a neocentromere had formed at 8p22 which provided mitotic stability.

Material and Methods

Clinical Report

The proband was a 6-year-old female referred for genetic analysis for short stature, dysmorphic features, and developmental delay. Prenatal history was significant for severe intrauterine growth retardation (IUGR) and a 2-vessel umbilical cord. Neonatally, she was diagnosed with a ventricular septal defect (VSD), which was surgically repaired. Dysmorphic features included narrow facies, protuberant ears and a beaked nose. A skeletal exam was significant for bilateral fifth finger cinodactyly, broad thumbs and great toes. She is the only child of non-consanguineous parents and was not conceived by artificial means. There was no reported family history of recurrent miscarriages.

Cytogenetic and FISH Analyses

Chromosome analysis was performed on cultured blood lymphocytes at approximately the 500-band level from the proband, her mother, and father using standard techniques. The chromosomes were analyzed and the karyotype described according to the International System for Cytogenetic Nomenclature [ISCN, 2009].

The FISH probes used included an alpha-satellite probe for chromosome 8 (Abbott Molecular, Downers Grove, Ill., USA), and slide hybridization and washes were performed using standard FISH protocols. Slides were counterstained with DAPI and analyzed with a Zeiss Axio Imager.M1 microscope (Carl Zeiss, Inc., Thornwood, N.Y., USA) equipped with an 8200 series filter combination and a CCD camera, and coupled to the Applied Spectral Imaging analysis system (Applied Spectral Imaging, Vista, Calif., USA). Ten metaphases each were analyzed for the proband and her father.

Immunofluorescence and FISH analyses of the neocentromere were performed as described [Warburton et al., 2000]. Immunofluorescence was performed using antibodies to CENP-C on 3:1 methanol:acetic acid fixed chromosomes from primary lymphocytes. FISH was performed using the alpha-satellite probe for chromosome 8 (Vysis, Downers Grove, Ill., USA).

SNP Microarray Analysis (CMA)

SNP microarray analysis was performed using the Affymetrix v.6.0 platform. The gene chip contains over 900,000 SNP probes and 900,000 non-polymorphic copy number probes with a median spacing of 0.7 kb. Briefly, 500 ng of total genomic DNA extracted from peripheral blood lymphocytes was digested with *Nsp*I and *Sty*I and then ligated to *Nsp*I or *Sty*I adaptors, respectively, and amplified using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, Calif., USA). PCR products were purified using AMPure beads (Agencourt Biosciences, Beverly, Mass., USA) and quantified using NanoDrop 8000 (Thermo Fisher, Wilmington, Del., USA). Purified DNA was fragmented and biotin labeled and hybridized to the Affymetrix 6.0 GeneChip (Affymetrix, Santa Clara, Calif., USA). Data was analyzed using Affymetrix Genotyping Console Browser v.3.01.

Results

GTG-banded chromosome analysis of the proband revealed an abnormal chromosome 8 with a proximal deletion around the centromere (fig. 1). It was not apparent by chromosome analysis whether the short (p) or long (q) arm was deleted, and it appeared that at least part of the centromere was also deleted. In addition, a distal constriction was noted at 8p22 that suggested neocentromere formation. In order to determine whether centromeric alpha-satellite sequences had been deleted, metaphase FISH analysis using a commercially available chromosome 8 alpha-satellite probe was performed and showed a diminished hybridization signal on the deleted chromosome 8 (fig. 1). Since it could not be determined from standard G-banded chromosome analysis which arm was deleted, SNP microarray using the Affymetrix 6.0 platform was performed on DNA extracted from blood lymphocytes. Results revealed that 10.6 Mb of the proximal q arm was deleted, from linear position 47,038,426 to 57,620,091 (8q11.1→q12.1) (fig. 2), including centromeric

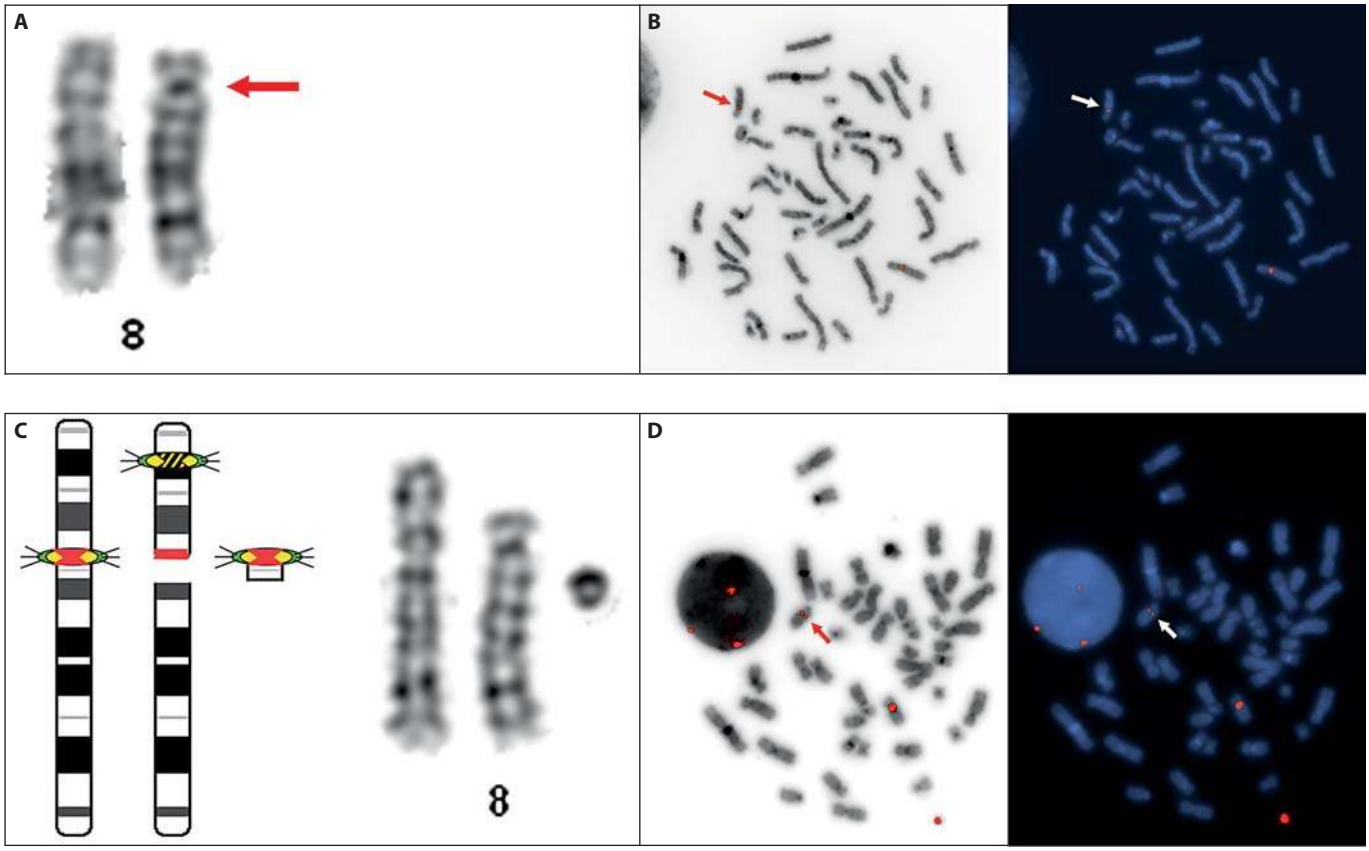

Fig. 1. A, **C** Partial karyotype and composite of the proband (**A**) and her father (**C**) showing a distal constriction on the deleted chromosome 8 (red arrow in **A**). Note the corresponding excised marker in the father. **B**, **D** Chromosome 8 alpha-satellite FISH image of the proband (**B**) and father (**D**) showing deletion of most of the pericentromeric sequences from the deleted homologue (red arrows in the reverse images, white arrows in the DAPI images) and retention of the alpha-satellite sequences on the excised marker.

material and 42 RefSeq genes, 25 of which are OMIM annotated. No other copy number changes were observed in the SNP analysis.

Chromosome analysis of the father revealed the same deletion of the proximal q arm of chromosome 8, as well as a mosaic marker chromosome, which we presumed was the excised portion of the deleted chromosome. The 2 cells in which a normal karyotype was found did not appear to have a deleted chromosome 8, suggesting the possibility of a post-zygotic event and gonadal mosaicism. Chromosome analysis of the mother revealed a normal 46,XX karyotype. Although most such deletion chromosomes are rings, we did not formally demonstrate this for the marker chromosome in this report. The father was noted to be phenotypically normal consistent with his balanced karyotype. FISH analysis using the same chromosome 8 alpha-satellite probe confirmed that the marker chromosome contained most of the chromosome 8 centromeric alpha-satellite DNA sequences, with a diminished signal on the deleted chromosome 8, as was seen in the proband. It is interesting to note that the excised marker chromosome was mosaic since it contained much of the centromeric repeat sequences. The observed mosaicism of the marker chromosome was likely due to the inherent mitotic instability of marker chromosomes in general [Kosztolanyi, 1987], despite the fact that it has an almost intact centromere.

In order to confirm that the distal constriction at 8p22 is a neocentromere, we performed immunofluorescence using antibodies to CENP-C, along with FISH using the chromosome 8 alpha-satellite probe (fig. 3). On the normal chromosome 8 the alpha satellite and CENP-C colocalized. On the deleted chromosome 8, residual alpha satellite was seen at the inactive centromere, while CENP-C

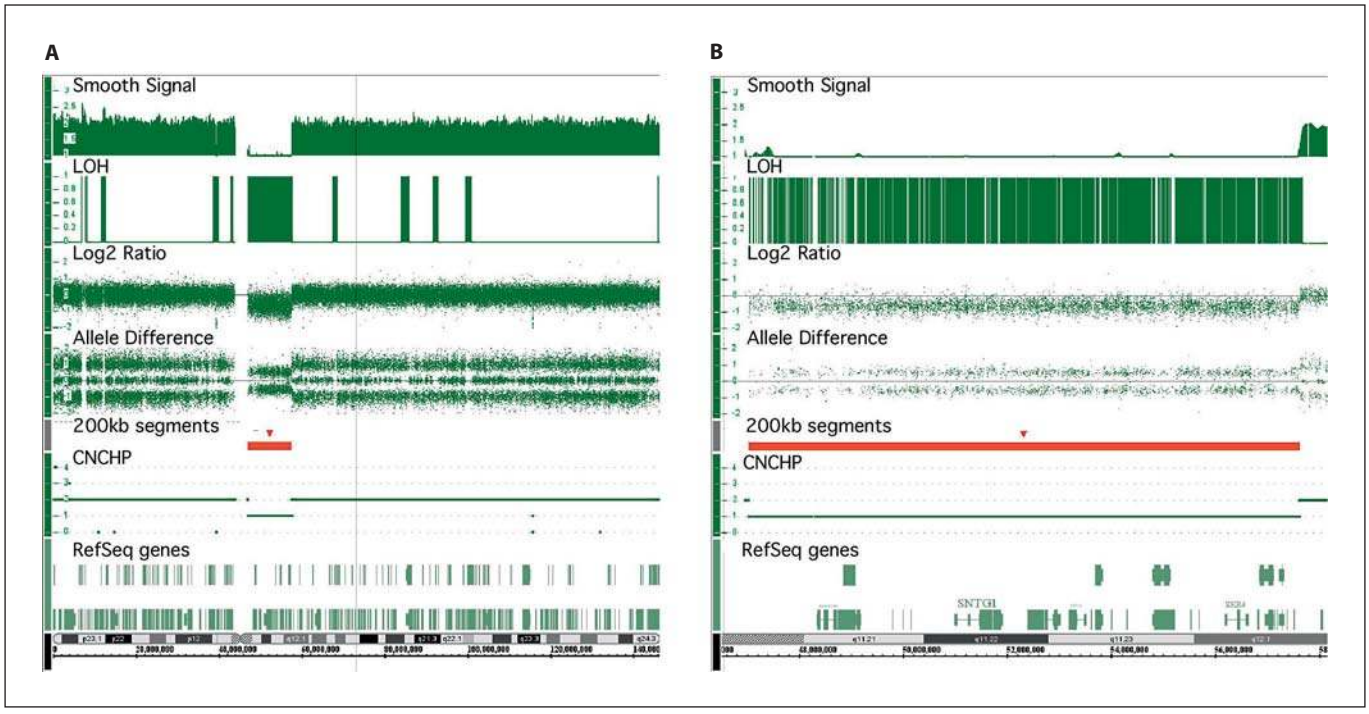

Fig. 2. Affymetrix GTC 3.0.1 screen shot of deleted chromosome 8 in the proband. **A** Entire chromosome 8. **B** Close-up of proximal 8q showing the deletion extending into 8q11.1.

was seen in 8p22. This confirmed that the kinetochore was localized to the distal constriction and did not contain chromosome 8 alpha-satellite DNA. The final karyotype of the proband is: 46,XX,del(8)(q11.1q12.1).ish del(8)(q11.2q11.2)(D8Z2 dim)neo(8)(p22).arr 8q11.1q12.1 (47,038,426–57,620,091)×1 pat. The final karyotype of the father is: 47,XY,del(8)(q11.1q12.1),+r(8)(q11.1q12.1). ish del(8)(q11.2q11.2)(D8Z2 dim)neo(8)(p22)+r(8)(q11.2 q11.2)(D8Z2+)[18]/46,XY[2].

Discussion

Approximately one dozen reports in the literature demonstrate inheritance of an unbalanced karyotype from a parent with a deleted chromosome and excised marker [Fryns et al., 1985; Krauss et al., 1987; Andersen et al., 1990; Pfeiffer et al., 1991; Friedman et al., 1992; Quack et al., 1992; Schuffenhauer et al., 1996; Lasan Trcic et al., 2003; Baldwin et al., 2008; Mantzouratou et al., 2009]. These excised markers are generally ring chromosomes and tend to be mitotically unstable, leading to a mosaic karyotype, as was the case in the father of the proband of this report. This is the first report to our knowledge demonstrating inheritance of a deletion of the proximal q arm of chromosome 8 from such a deletion/excised marker karyotype. The 6-year-old proband presented with short stature, dysmorphic features, and developmental delay. Other significant features included a prenatal history of severe IUGR and a 2-vessel umbilical cord, as well as postnatal findings of a VSD, narrow facies, protuberant ears, a beaked nose, bilateral fifth finger clinodactyly, broad thumbs and great toes. One report of a male infant with a de novo deletion of proximal 8q (q11.2q13) showed IUGR, craniofacial, ear, genital, heart, and skeletal abnormalities [Asamoah et al., 2004]. Schinzel et al. [1994] reported a patient with a deletion of proximal 8q (q11q13) and a phenotype similar to Russell-Silver syndrome with prominent ears and cinodactyly, similar to our patient; while the adolescent patient reported by Kazukawa et al. [1986] presented with no dysmorphic features and mild mental retardation (8q11.2q13). Based on the band designations reported for each of these cases, the deletions of proximal 8q in these patients encompasses approximately 24 Mb, much larger than the 10.6-Mb deletion in our patient, possibly contributing to the variation in phenotypes seen, although no molecular studies were performed.

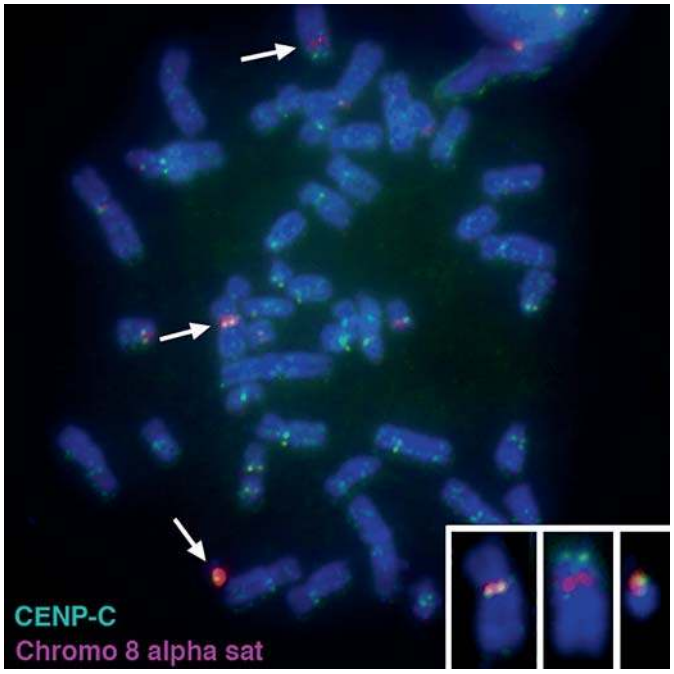

Fig. 3. Immunofluorescence and FISH analysis of the father. FISH using chromosome 8 specific alpha-satellite probe (red) and immunofluorescence using antibodies to CENP-C (green). **Inset:** left – normal chromosome 8, middle – neocentric deleted chromosome 8, right – marker chromosome.

Neocentric chromosomes are found when chromosomes have lost their endogenous centromere but maintain mitotic stability because of neocentromere formation [Warburton, 2004]. The centromere and kinetochore provide the necessary structure and function for proper mitotic disjunction. In our case, a neocentromere formed in the father when the excision of the ring chromosome removed most of the alpha-satellite repeat sequences from the endogenous chromosome 8 centromere region. The region in which the neocentromere formed was a G-band-positive, relatively gene-poor region of chromosome 8. While endogenous kinetochores form on alphoid repeat sequences that are recognized by the centromeric protein B (CENP-B), neocentromeres do not seem to require such repetitive sequences in order to form, and indeed the neocentromere in this case is not associated with any repetitive sequences. Kinetochore formation at centromeres requires the binding of many essential proteins, particularly the histone H3 variant, CENP-A, and another DNA binding protein, CENP-C [Warburton, 2004; Marshall et al., 2008; Santaguida and Musacchio, 2009]. The presence of CENP-C was detected in our patient and her father at 8p22, confirming the formation of a neocentromere at this site.

There have been 4 reported cases of intact chromosomes that retain the array of alpha-satellite DNA, in which the endogenous centromere has been inactivated and a neocentromere formed within the euchromatin of either the long or short arm [Amor et al., 2004; Ventura et al., 2004; Warburton, 2004; Liehr et al., 2009]. This neocentromere case is unique in that it represents an example of a centromere fission (splitting of the alpha-satellite array), with inactivation of the remaining centromere on the deleted chromosome 8. It is likely that the small amount of alpha-satellite DNA remaining may not be sufficient to support an active centromere.

In conclusion, we present a case of a patient with multiple congenital anomalies due to the inheritance of a paternally deleted chromosome 8 from an unbalanced meiotic segregation of the deleted chromosome without the concomitant excised marker. This case also provides information regarding recurrence risk, since the deleted chromosome 8 segregated without the excised marker. It is therefore possible that the marker chromosome may segregate with the normal chromosome 8, producing a fetus trisomic for proximal 8q. Since the father also had what appeared to be a normal cell line, there also exists the possibility of gonadal mosaicism for normal gametes. The excision of the marker chromosome deleted most of the alpha-satellite centromeric repeat sequences from the endogenous centromere, likely necessitating the formation of a neocentromere at 8p22 for mitotic stability. This is the first case that we are aware of in which a neocentromere has formed at 8p22, and is an example of the benefit of microarray analysis to define cytogenetically visible but uncertain abnormalities.

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