Molecular Cytogenetic Characterization of the First Reported Case of Inv Dup Del 20p Compatible With a U-Type Exchange Model

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Received 16 May 2008; Accepted 18 September 2008

Inverted duplications with terminal deletions have been reported for an increasing number of chromosome ends. The best characterized and most frequent rearrangement involves the short arm of chromosome 8. It derives from non-allelic homologous recombination (NAHR) between two inverted LCRs (low copy repeats) of the olfactory receptor (OR) gene cluster during maternal meiosis. We report here on the cytogenetic characterization of the first inversion duplication deletion involving the short arm of chromosome 20 (inv dup del 20p) in an 18-month-old boy presenting with clinical signs consistent with 20p trisomy syndrome. This abnormality was suspected on karyotyping, but high-resolution molecular cytogenetic investigations were required to define the breakpoints of the rearrangement and to obtain insight into the mechanism underlying its formation. The duplicated region was estimated to be 18.16 Mb in size, extending from 20p13 to 20p11.22, and the size of the terminal deletion was estimated at 2.02 Mb in the 20p13 region. No single copy region was detected between the deleted and duplicated segments. As neither LCR nor inversion was identified in the 20p13 region, the inv dup del (20p) chromosome abnormality probably did not arise by NAHR. The most likely mechanism involves a break in the 20p13 region, leading to chromosome instability and reparation by U-type exchange or end-to-end fusion. © 2009 Wiley-Liss, Inc.

Key words: inverted duplication, trisomy 20p, NAHR, U-type exchange

INTRODUCTION

Inverted duplications associated with terminal deletion (inv dup del) are complex rearrangements reported for an increasing number of chromosome ends [1p:Ballif et al., 2003; Tonk et al., 2005; 1q:Mewar et al., 1994; De Brasi et al., 2001; 2p:Aviram-Goldring et al., 2000; Thangavelu et al., 2004; Gruchy et al., 2007; 2q:Bonaglia et al., 2000; 3p:Jenderny et al., 1998; Kennedy et al., 2000; 4p:Cotter et al., 2001; Kondoh et al., 2003; Beaujard et al., 2005; 4q:Van Buggenhout et al., 2004; 5p: Sreekantaiah et al., 1999; 7q: Stetten et al., 1997; 9p: Teebi et al., 1993; 10q: Hoo et al., 1995; 11p: Fisher et al., 2002; 14q: Chen et al., 2005; 15q: Genesio et al., 2004; 18p: Morrissette et al., 2005; 18q: Courtens et al., 1998; 21q: Pangalos et al., 1992; Xp: Milunsky et al., 1999; Dupont et al., 2007]. These rearrangements are more frequent than first thought based on banding techniques, as several cases were initially interpreted as terminal duplications [Bonaglia et al., 2000; De Brasi et al., 2001; Beaujard et al., 2005] or terminal deletions [Ballif et al., 2003]. With the advent of molecular techniques, these rearrangements can now be detected with greater sensitivity and have recently been shown to be associated with ring chromosome formation [Knijnenburg et al., 2007; Rossi et al., 2008]. The best characterized and most frequently reported rearrangement involves the short arm of chromosome 8 (inv dup del 8p). Its estimated prevalence is 1/10,000 to 1/30,000 live births [Weleber et al., 1976; Floridia et al., 1996; Giglio et al. 2001; Vermeesch et al., 2005].

How to Cite this Article:

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It arises through non-allelic homologous recombination (NAHR) between two LCRs (low copy repeats) from the olfactory receptor (OR) gene cluster during maternal meiosis, leading to the formation of an intermediate dicentric chromosome, which breaks during anaphase. A polymorphic inversion between OR clusters, present in 26% of the population and detected in all mothers of children with an inv dup del (8p), abrogates correct pairing and causes susceptibility for this rearrangement [Gigliò et al., 2001; Sugawara et al., 2003]. In this case, there is a region of normal copy number between the duplicated and the deleted fragment.

We report here on the cytogenetic characterization of the first inversion duplication deletion involving the short arm of chromosome 20 (inv dup del 20p) in an 18-month-old boy who presents delayed developmental milestones and craniofacial dysmorphism. Fine molecular characterization of this rearrangement provided insight into the mechanism underlying this abnormality.

MATERIALS AND METHODS

Patient

The patient, an 18-month-old boy, is the second child of healthy nonconsanguineous parents. His 4-year-old sister is also healthy. The pregnancy was uneventful and birth occurred at 36 weeks of gestation, by spontaneous vaginal delivery. At birth, the child weighed 2.880 kg (median), measured 46 cm (−1 SD) and had a head circumference of 35.5 cm (+2 SD). He was referred to our department at the age of 18 months, due to delays in achieving developmental milestones and craniofacial dysmorphism.

Clinical examination revealed slight hypoplasia of the antehypophysis. Echocardiogram and audiogram results were normal. Now at the age of 30 months, the child is beginning to pronounce monosyllables, but not words. He has no sleep or appetite disorders.

Karyotype Analysis

Chromosome analyses of the patient and his parents were carried out with standard procedures on peripheral blood lymphocytes, using GTG, RHG, CBG banding and high-resolution techniques after cell culture synchronization and BrdU (5-bromo-2'-deoxyuridine) incorporation.

Fluorescence In Situ Hybridization (FISH) Analysis

FISH analysis was performed on metaphase spreads obtained by standard protocols, using a whole-chromosome paint probe for chromosome 20 (WCP20, QBiogen®) and subtelomeric 20p and 20q chromosome probes (Subtelomeric-specific 20pter, 20qter Cytocell®). For identification and definition of the derived chromosome 20, several BACs covering the region from 20pter to 20p11 were selected with the UCSC genome browser database (http://genome.ucsc.edu/cgi-bin/hgGateway, March 2006) and the Ensembl genome browser (http://www.ensembl.org/). These clones were kindly provided by the Sanger Institute (http://www.sanger.ac.uk/).

CGH Array Analysis

CGH array analysis was carried out with an approximately 1.0 Mb-spaced whole-genome clone array (Cytochip®, Bluegnome, UK). Genomic DNA was isolated from the patient with the Qiagen mini kit (Qiagen®, Courtaboeuf, France) and 400 ng was labeled with the Bioprime® DNA labeling system (Invitrogen, Paris, France), either with Cy3-dCTP or Cy5-dCTP (GE Healthcare, Velizy, France), in a dye-swap protocol, with 400 ng of reference DNA. Slides were hybridized and washed according to the Cytochip® protocol. The arrays were scanned with a Genepix® 4100A scanner (Axon Instruments, Molecular Devices, St. Gregoire, France) and the images were processed with GenePix software. Intensity ratios were determined for the hybridized DNA, using Bluefuse for Microarrays software (Bluegnome®, Cambridge, UK). For further high-resolution analysis of the rearrangement, CGH oligo-array analysis was performed with the NimbleGen® high-density oligo-array HG18-WG Tiling 385K CGH v1.0 (NimbleGen Systems, Inc., Madison, WI), tiling the full genome at a median probe spacing of 6,000 bp. Data were analyzed with the NimbleScan® and SignalMap® software platform.

RESULTS

Conventional Cytogenetic and FISH Analysis

Initial cytogenetic studies showed the de novo addition of new material to the short arm of one chromosome 20 in the proband (Fig. 2A). As confirmed with the whole-chromosome painting
probe, this additional material originated from chromosome 20. Subtelomeric probes for the short and long arms of chromosome 20 showed a 20pter deletion (data not shown).

Analysis of the rearrangement was refined by selecting two BACs in the 20p13 and 20p12 regions (RP4-686C3, 20p13, bp position: 2,440,424–2,601,595; RP11-829A12, 20p12.2, bp position: 10,523,017–10,706,907). Both were found to be duplicated in an inverted position (Fig. 2B).

BAC array-CGH analysis was performed to map further the deleted and duplicated regions. The duplication corresponded to a 17.6–20.3 Mb fragment from BAC clones RP4-686C3 to RP5-1096J16, whereas the deletion corresponded to a 1.2–2.5 Mb fragment from BAC clones RP5-852M4 to RP11-314N13.

Additional high-resolution CGH oligo array analysis was carried out to extend previous findings and to search for a single-copy segment between the two duplicated regions. The size of the duplicated region was estimated to be 18.16 Mb in size, extending from 20p13 to 20p11.22, and the size of the terminal deletion was estimated to be 2.02 Mb in size in the 20p13 region (Fig. 2C). No single copy region was detected with an average resolution of 6 kb.

**FIG. 2.** A: Partial karyotype, showing the normal and derivative chromosome 20 with two different chromosome banding techniques. B: DiAmino Phenyl Indol (DAPI) staining image and FISH analysis with BACs RP4-686C3, 20p13 (red) and RP11-829A12 (green) 20p12.2 showing inverted duplicated signals on der(20). C: Oligo-array log 2 ratio plot of whole chromosome 20 and high magnification view of 20p13 region centered on the breakpoint between the deleted and duplicated segments. Log 2 ratios obtained for the most proximal deleted oligomer (red arrow) and the most distal duplicated oligomer (green arrow) are shown: absence of normal copy number region between the deleted and duplicated regions. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
DISCUSSION

Rearrangement

We report here on the first case of inv dup del (20p) in a boy with delayed developmental milestones and dysmorphism. High-resolution oligonucleotide CGH array analysis revealed an 18.16 Mb duplication of 20p11.22p13 and a 2.02 Mb telomeric deletion of 20p13.

Inverted duplications associated with terminal deletions have now been observed for various chromosomal ends and, in a few cases, inv dup del rearrangements are cryptic [Ballif et al., 2003; Dupont et al., 2007; Knijnenburg et al., 2007].

Several mechanisms have been proposed to explain the origin of inv dup del. The best known case involves the short arm of chromosome 8. Inv dup del (8p) arise through non-allelic homologous recombination, during maternal meiosis, between two misaligned olfactory receptor gene clusters situated 6 Mb apart at 8p23 [Giglio et al., 2001]. This meiotic recombination event is promoted by the presence of a polymorphic inversion between these OR clusters. This inversion is detected in 26–27% of the population and in all mothers of children with an inv dup del (8p) [Giglio et al., 2001; Sugawara et al., 2003]. The dicentric chromosome intermediate is the first product of the abnormal meiotic recombination. It contains two duplicated regions separated by a single copy region flanked by the two homologous OR gene clusters. From the distal to the proximal 8p region, the inv dup del (8p) comprises three segments: one deleted, one present as a single copy and one inverted and duplicated.

We investigated whether the 8p model could be extended to our case, by searching for these two major factors (LCR regions and polymorphic inversion) potentially favoring unequal recombination in the 20p region.

Theoretically, as the deleted and duplicated segment breakpoints are located almost 2.0 Mb from telomere 20p, any LCR involved should be present in this specific region. However, in silico analysis (http://projects.tcag.ca/variation/) identified no LCRs in this area.

Inversion polymorphism has been demonstrated for various chromosomal regions through statistical methods based on unusual linkage patterns in high-density SNP analysis [Bansal et al., 2007]. However, no case of microinversion has ever been described in the 20p13 region.

Neither the 1 Mb BAC array nor the high-density oligoarray gave conclusive results concerning the identification of a possible fragment with a normal copy number between the deleted and duplicated regions. These results suggest that the inv dup del (20p) was formed through a mechanism different from that described for inv dup del (8p).

FIG. 3. Suggested mechanism leading to the inv dup del 20p in our patient [from Ballif et al., 2003]. a: double-strand break at 2.0 Mb from the telomere in the 20p13 region (premeiotic state). b: Terminal deletion 20p13. c: NHEJ repair by sister chromatid fusion. d: Formation of a dicentric chromosome and breakage within the 20p11.2 band during anaphase. e: inv dup del(20p). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
The second proposed mechanism involves a U-type exchange, leading to end-to-end fusion and the formation of a symmetric dicentric chromosome [Weleber et al., 1976; Mitchell et al., 1994; Hoo et al., 1995; Kondoh et al., 2003; Van Buggenhout et al., 2004]. The breakage of this dicentric chromosome during anaphase, followed by and telomere-healing/capture leads to the formation of an inv dup del chromosome [Ballif et al., 2003]. In 2003, Ballif et al. described this mechanism in their detailed breakpoint analysis of three patients with an 1p36 deletion. In two cases, a duplicated region followed the deleted region. As the breakpoints were similar to those seen in tumor cell lines, Ballif suggested that breakage-fusion-bridge (BFB) cycles may play an important role in terminal deletion associated with inverted duplication. The initial event would thus be a DNA double-strand break in the 1p subtelomeric region, leading to chromosome instability. The deleted chromosome is then repaired by sister chromatid fusion (non-homologous end joining: NHEJ), generating an intermediate dicentric chromosome. As DNA repair by NHEJ is repressed during meiosis to favor repair by homologous recombination, this mechanism probably occurs before the initiation of meiosis. As the formation of the inv dup del (20p) chromosome does not seem to be due to NAHR between LCR regions, the mechanism involved may be NEJH, as described by Ballif (Fig. 3).

Genotype–Phenotype Correlation

We report here the first case of inv dup del involving the short arm of chromosome 20. Molecular cytogenetic analysis showed the patient to be trisomic for the 20p13–p11.2 segment and monosomic for the terminal 20p13 region.

Trisomy for the short arm of chromosome 20 is a rare chromosomal abnormality. Thirty-nine cases have been reported, all but two cases [Zumel et al., 1989; Molina-Gomes et al., 2006] diagnosed postnatally. Most partial trisomies result from reciprocal translocation [Taylor et al., 1976; Marcus et al., 1979; Funderburk et al., 1983; Lurie et al., 1985; Vamos et al., 1985; Zumel et al., 1989; Grammatico et al., 1992; LeChien et al., 1994; Belin et al., 1999; Oppenheimer et al., 2000; Wieczorek et al., 2003; Thomas et al., 2004; Tümer et al., 2005; Brenk et al., 2007]. In a few cases, trisomy 20p results from parental inversion [Lucas et al., 1985; Bown et al., 1986; Molina-Gomes et al., 2006; Chaabouni et al., 2007] or the formation of isochromosomes [Sidwell et al., 2000]. The genotype–phenotype correlation is therefore not very clear, because these trisomies of 20p are heterogeneous and are frequently associated with segmental aneuploidies of other chromosomes. Only a few cases may be considered to correspond to pure partial trisomy 20p [Sidwell et al., 2000].

A recognizable phenotype has emerged from these postnatal observations. Grammatico et al. [1992] reported eight typical features associated with trisomy of 20p (frequency 67–96%): mental retardation, psychomotor acquisition delay, normal growth, round face with prominent cheeks, dental abnormalities, vertebral abnormalities, poor motor coordination and poor speech. Oppenheimer added new phenotypic elements to this list in 2000, mostly relating to dysmorphic features, with a frequency of 34–54%. The developmental phenotype and dysmorphic features of our patient are consistent with these clinical features of trisomy 20p (Table I). As vertebral abnormalities are a key feature of this condition, an X-ray was included in subsequent follow-up of the patient. Finally, plagiocephaly was observed in this patient. This feature was reported in two previous clinical descriptions of trisomy 20p [Lucas et al., 1985; Bown et al., 1986].

The patient described here also presented a 2 Mb terminal deletion of the 20p13 region. Delineation of the monosomy

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**TABLE I. Main Traits Reported in Previous Studies [Grammatico et al., 1992; Oppenheimer et al., 2000] and in Our Case of Trisomy 20p Syndrome**

<table>
<thead>
<tr>
<th>Main phenotypic traits of trisomy 20p syndrome</th>
<th>Our case</th>
<th>Reported frequency [Oppenheimer et al., 2000]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical signs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[1] Psychomotor retardation</td>
<td>+</td>
<td>96%</td>
</tr>
<tr>
<td>[3] Poor motor coordination</td>
<td>+</td>
<td>95%</td>
</tr>
<tr>
<td>[4] Poor speech</td>
<td>+/-</td>
<td>95%</td>
</tr>
<tr>
<td>[6] Vertebral abnormalities</td>
<td>n.e.</td>
<td>77%</td>
</tr>
<tr>
<td>[7] Dental abnormalities</td>
<td>-</td>
<td>81%</td>
</tr>
<tr>
<td>[8] Round face with prominent cheeks</td>
<td>+</td>
<td>82%</td>
</tr>
<tr>
<td>Other frequent findings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[1] Mongoloid slanting of palpebral fissures</td>
<td>-</td>
<td>67%</td>
</tr>
<tr>
<td>[2] Coarse hair</td>
<td>+</td>
<td>74%</td>
</tr>
<tr>
<td>[3] Short nose with large nostrils</td>
<td>+</td>
<td>55%</td>
</tr>
<tr>
<td>[4] Hypertelorism</td>
<td>-</td>
<td>54%</td>
</tr>
<tr>
<td>[5] Strabismus</td>
<td>-</td>
<td>40%</td>
</tr>
<tr>
<td>[7] Epicanthic folds</td>
<td>+</td>
<td>35%</td>
</tr>
<tr>
<td>[8] Clinodactyly and other abnormalities of finger and toes</td>
<td>+</td>
<td>21–34%</td>
</tr>
</tbody>
</table>

n.r., not reported; n.e., not evaluated.
TABLE II. Clinical Phenotypes of Terminal Deletion of 20p

<table>
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</thead>
<tbody>
<tr>
<td>Mosaicism</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>1.5</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Developmental retardation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>n.r.</td>
<td>+</td>
</tr>
<tr>
<td>Growth retardation</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>n.r.</td>
<td>−</td>
</tr>
<tr>
<td>Dysmorphic features</td>
<td>Strabism</td>
<td>−</td>
<td>−</td>
<td>+ (not detailed)</td>
<td>−</td>
<td>−</td>
<td>n.r.</td>
</tr>
<tr>
<td>Broad nasal bridge</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Prominent forehead</td>
<td>+</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Long face</td>
<td>−</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deep set eyes</td>
<td>−</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Short philtrum</td>
<td>−</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small mouth</td>
<td>−</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other abnormalities</td>
<td>Slight antehypophyseal hypoplasia</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>n.r., not reported.</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

n.r., not reported.
20p13-pter phenotype remains difficult because pure 20p terminal deletions are rare events. Most of the described 20p deletions concern a more proximal region (20p11p12) associated with Alagille–Watson syndrome (AWS). The JAG1 gene, mutations in which cause AWS, was mapped to the 20p12 subband [Li et al., 1997] and was not part of the region deleted in our patient, consistent with his phenotype as he presented no features of AWS. However, this gene maps to the duplicated region. Alagille syndrome has been reported in one family with 20p duplication [Moog et al., 1996] and was caused by disruption, by the duplication breakpoints, of the gene usually deleted in Alagille patients. The few reported cases of pure terminal deletions in the 20p13 region were identified through subtelomere screening studies in patients presenting idiopathic mental retardation and developmental delay [Baker et al., 2002; Adeyinka et al., 2005; Ravan et al., 2006] or patients presenting a non-supernumerary ring chromosome 20 with isolated loss of the 20p13 region [Zou et al., 2006]. To our knowledge, no interstitial deletion overlapping our deleted region has ever been reported. The genotype–phenotype correlation of monosomy 20ppter is presented in Table II. Most subjects presenting a terminal 20p deletion display developmental retardation (8 of 12 cases). In our case, it is difficult to determine whether this feature is related to the loss of the 20p subtelomeric region or interstitial trisomy of 20p. Three familial cases of deletion 20pter have been reported [Baker et al., 2002; Adeyinka et al., 2005; Ravan et al., 2006]. Clinical findings reported for two of these cases suggested that the phenotypic consequences of this chromosomal imbalance were variable. In one case, the phenotype of the transmitting mother was much milder (isolated hearing loss; Table II) than that of the child, whereas, in the second case, the transmitting mother and the affected child had much more similar phenotypes. It is therefore difficult to evaluate the phenotypic repercussions of monosomy 20pter.

In silico analysis of the deleted region of 20p13 revealed that several genes known to be selectively expressed in the brain, including those encoding NRSN2, syntaphilin and SIRP, had been deleted. NRSN2 is a small membrane protein present in various neurons [Nakanishi et al., 2006], whereas syntaphilin is a presynaptic membrane protein that regulates synaptic vesicle exocytosis and endocytosis [Lao et al., 2000; Das et al., 2003]. The p84 or SIRP protein is a neural adhesion molecule that may play an important role in synaptogenesis [Eckert et al., 1997]. These genes have not previously been implicated in any disease, but their absence could be involved in the developmental retardation observed in our patient.

In conclusion, we describe here the first patient presenting a de novo inv dup del (20p) with clinical manifestation mostly consistent with partial trisomy 20p. This abnormality was suspected on karyotyping, but high-resolution molecular cytogenetic investigations made it possible to define the breakpoints of the rearrangement and to establish the most likely mechanism.

ACKNOWLEDGMENTS

We thank the parents of our patient for supporting this publication.

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