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Protelomeric Sequences Are Deleted in Cases of Short Arm Inverted Duplication of Chromosome 8

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We present a patient with a de novo inverted duplication of the short arm of chromosome 8. Molecular analysis confirmed the cytogenetic suspicion of a simultaneous deletion of the tip of the short arm and indicated the maternal origin of the abnormality. This deletion made no detectable contribution to the phenotype of the patient which was comparable to that of previous cases of 8p duplication.

Similar investigations of inverted duplications involving other chromosomes may reveal unexpected deletions with significant phenotypic consequences.

KEY WORDS: chromosome abnormality, chromosome 8, inverted duplication 8p, terminal deletion 8p

INTRODUCTION

"Mirror" or inverted duplications have a particular fascination for cytogeneticists because of their visual symmetry and uncommon occurrence. Eighteen cases of inverted duplication of the short arm of chromosome 8 have been reported previously [Jensen et al., 1982; Fryns et al., 1985; Dill et al., 1987; Nevin et al., 1990; Gorinati et al., 1991; Mitchell et al., 1991] and among these, Dill et al. [1987] were the first to report an inverted duplication of chromosome 8 with a simultaneous deletion of the short arm distal to the duplication itself. Since then, Gorinati et al. [1991] have presented cytogenetic evidence and Mitchell et al. [1991] cytogenetic and molecular evidence for similar concomitant deletions.

Here we present the cytogenetic, molecular, and phenotypic findings in a further patient in whom a duplication-deficiency of 8p was found.

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CLINICAL REPORT

NL (laboratory reference no. 92/4754) was born at term with a birth weight of 3.6 kg. At 20 hours she presented with signs of obstruction and required emergency surgery for malrotation of the gut. She was slow to thrive and generally hypotonic. A CT scan of the brain showed agenesis of the corpus callosum. She developed a thoracic scoliosis convex to the right. Spine roentgenograms documented multiple hemivertebrae from C3 to T4. The other vertebrae appeared normal.

Now at the age of 5 years she shows significant developmental delay. She moves by commando crawling and is unable to walk. She has no speech. On examination her weight is on the 10th centile and her head circumference on the 75th centile. She has dry curly hair. She has a high nasal bridge and long philtrum (Fig. 1). Her palate is high with a deep central groove. Ears are large and posteriorly angulated with bilateral ear pits. Fingers are long and thin with 5th finger clinodactyly. She has increased tone in both legs with bilateral equinovarus deformity of the feet. The rest of the clinical examination was unremarkable. An abdominal ultrasound study showed a small mesenteric cyst to the left of the midline but other internal organs were normal.

CYTOGENETIC AND MOLECULAR FINDINGS

An 8p+ karyotype was reported when the patient was first referred. Subsequently an inverted duplication [inv dup(8)(p23.1p21.1)] was suggested but the distal region of the duplicated chromosome could not be resolved. Both parental karyotypes were normal. Recently a further repeat was requested as part of a systematic recall of all patients whose karyotypes contained material of uncertain origin which might be identified by chromosome painting.

Chromosomes were prepared by standard techniques after semi-synchronization with FdU and release with thymidine and a modification of the technique described by Pinkel et al. [1988] was used for chromosome painting. The normal chromosome 8 together with all of the abnormal 8 was painted after hybridization with the Cambio chromosome 8 library indicating that the extra material was of chromosome 8 origin. Subsequent analysis of high-resolution G-banded cells confirmed an in-

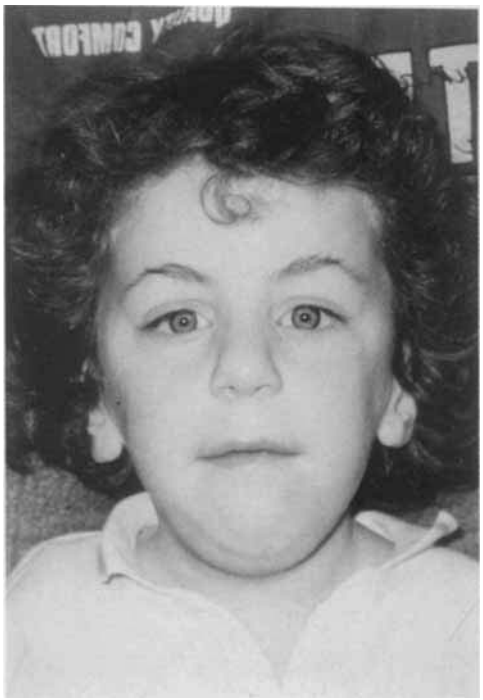

Fig. 1. NL age 5 years. Note her high nasal bridge and long philtrum.

Fig. 2. PCR amplification of DNA microsatellite repeat sequences from the patient NL (lane P) and her parents. Alleles present are indicated by arrows. Primers are Mfd199. NL 1, –; Mother 2, 3; Father 1, 2.

verted duplication of most of the short arm of chromosome 8 but also suggested a simultaneous deletion of the pro-terminal short arm.

Molecular analysis of the short arm of chromosome 8 was by PCR amplification of DNA microsatellite repeats [Weber and May, 1989]. The primer sequences were Mfd199(D8S201) and LPL3GT at the lipoprotein lipase locus [Tomfohrde et al., 1992], PCR conditions were those described by Hudson et al. [1991], and results were visualised using a 6% denaturing polyacrylamide gel followed by autoradiography. The Mfd199 primers which amplify a dinucleotide repeat which maps to 8p22→8pter showed that only the paternal allele had been inherited (Fig. 2); this probe therefore confirms the cytogenetic findings and in addition indicates that the inverted duplication has a maternal origin. The LPL3GT primers which amplify a dinucleotide repeat which maps to 8p21.3→8p22 showed two alleles indicating that the deletion does not extend to this locus.

Careful cytogenetic analysis suggests that the major part of the short arm is followed distally by the inverted duplication itself (p11.23→p23.1) but that distal to this, a short unduplicated segment (p23.1→p23.2) between the distal border of the duplication and the deletion breakpoint is retained (Figs. 3A, 4A). The karyotype is then: 46,XX,inv dup del (8) (qter→p23.1::p23.1→p11.23::p23.1→p23.2:) de novo. An alternative and simpler interpretation is an inverted duplication of the major part of the short arm (p11.22→p23.1) with the distal border of the duplication and the deletion breakpoint coinciding at p11.22 (Figs. 3B, 4B). The karyotype would then be 46,XX,inv dup del (8) (qter→p23.1::p23.1→p11.22:) de novo. Distinguishing between these alternatives requires a nested set of PCR primers or cosmid probes which are not yet available to us.

DISCUSSION

In the subject of this report an inverted duplication of the short arm of chromosome 8 was associated with a deficiency distal to the duplication itself. Three analogous cases which share many clinical similarities have been previously reported [Dill et al., 1987; Gorinati et al., 1991; Mitchell et al., 1991; Henderson et al., 1992]. Molecular analysis confirmed the deletion predicted on cytogenetic grounds and it is probable that further cases of deletion would be found if other patients with inverted duplications of 8p were investigated in this way.

Two mechanisms for the production of such duplication-deficient chromosomes have been proposed; Weleber et al. [1976] and Dill et al. [1987] favour an aberrant U-type recombination event leading to a dicentric chro-

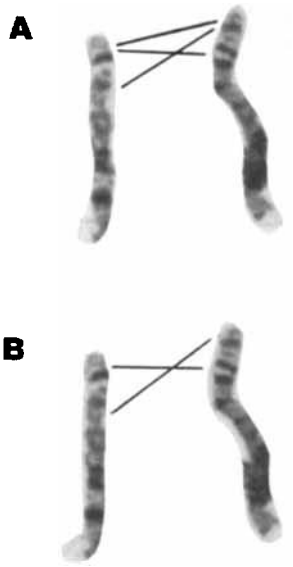

Fig. 3. G-banded partial karyotypes illustrating the alternative cytogenetic interpretations. The normal chromosomes are on the left. **A**: Inverted duplication-deficiency with retention of a segment (p23→p23.2) between the distal duplication border and the deletion breakpoint. **B**: Inverted duplication deficiency in which the deletion and distal duplication breakpoints coincide.

mosome joined at the centre of symmetry of the inversion duplication, followed by breakage of the dicentric and telomere restitution. Our alternative interpretation of the cytogenetics is consistent with this model. The second mechanism, proposed by Mitchell et al. [1991], involves a U-type exchange within a paracentric inversion which leads to a duplication-deficient chromosome in which a normal telomeric region is retained. Such an inversion has not been detected in the parents of this or any of the previous cases with distal deletions. Neither model is consistent with the complex duplication-deficiency of our best interpretation of the cytogenetics which requires three breakpoints as well as telomere restitution. Stabilization of breaks by the addition of telomeric sequences has already been demonstrated in two patients with terminal deletions [Wilkie et al., 1990; Lamb et al., 1993]; in each case the deleted chromosomes had broken at a point which retained 3–4 base pairs which shared homology with the normal human telomeric hexamer.

The lack of any detectable additional phenotypic effect as a result of the concomitant deletion is less surprising in view of its small cytogenetic size in relation to the duplication of the major part of the short arm and the nonspecific phenotype in cases where a similar distal region of chromosome 8 alone is deleted [Fryns et al., 1989; Pecile et al., 1990; Blennow and Brøndum-Nielsen, 1990; Hutchinson et al., 1992; Pettinati et al., 1992]. The same situation has been reported in inverted duplications of chromosome 21 where distal 21q22.3 deletions do not add to the phenotype of Down syndrome [Pangalos et al., 1992]. However, if concomitant deletion is an

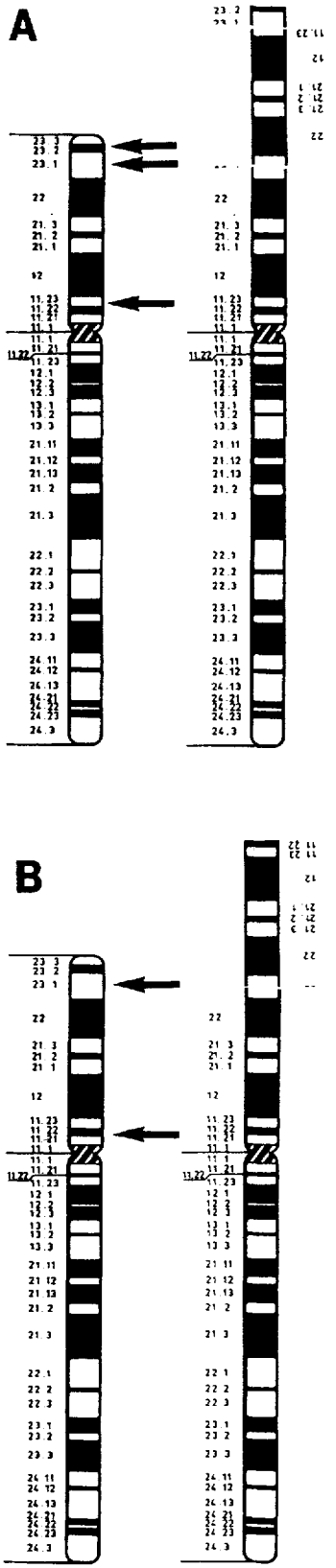

Fig. 4. Idiograms of the partial karyotypes **A** and **B** as defined in Figure 3. Arrows indicate the breakpoints on the normal chromosomes 8.

intrinsic result of the process leading to inverted duplication, the same may not be true for inverted duplications of other chromosome arms where the loss of protelomeric sequences may have significant and distinct phenotypic consequences. In a recent example, mild manifestations of 9p- in addition to the abnormalities of dup 9p were found in a patient with an inverted duplication of chromosome 9 (p13→p22) and a simultaneous distal deletion (p22→pter) [Teebi et al., 1993]. Cases of known inverted duplication where the phenotype is unexpectedly severe or where the phenotype includes abnormalities of a deletion syndrome associated with an immediately adjacent telomeric region may therefore be prime candidates in searching for further unexpected cases of duplication deficiency.

NOTE ADDED IN PROOF

Ten analogous patients with inverted duplications of the short arm of chromosome 8 and distal deletions including the D8S7 locus have been reported while this paper was in press [Minelli et al., 1993]. The duplications were of maternal origin in the 3/10 cases which were informative. It appears that a distal deletion has now been found in every reported case in which it has been looked for.

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