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Inverted tandem duplication generates a duplication deficiency of chromosome 8p

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An adult female with severe mental retardation and dysmorphic features is described. A *de novo* chromosomal aberration involving 8p was found. The karyotype was 46, XX, inv dup (8) (p12→p23.1). Dosage studies with the DNA probe D8S7, which is located at 8p23→8pter, showed that the patient was monosomic for this marker. Thus the *de novo* rearrangement generated a duplication-deficiency chromosome. The possible mechanisms of formation of this abnormal chromosome are discussed.

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The occurrence of imbalance of the short arm of chromosome 8 is well documented. This includes simple monosomy and trisomy involving all or part of 8p as well as segmental trisomies for different regions within 8p (Feinman et al. 1979, Reiss et al. 1979). In addition, a number of patients have been described with a chromosomal abnormality involving 8p that is variously described as an interstitial inversion duplication or an inverted tandem, or mirror duplication (Weleber et al. 1976, Taylor et al. 1977, Jensen et al. 1982, Fryns et al. 1985).

We now report a severely mentally retarded patient with a *de novo* inv dup (8)(p12→p23.1). We have used a DNA probe derived from the terminal short arm to demonstrate a deficiency within the region 8p23→8pter. This *de novo* inversion duplication, which produces a duplication-deficiency chromosome, provides new information relating to the mechanism of formation of these chromosomes.

Material and Methods

Case Report

G.S. is a 27-year-old profoundly retarded female who resides in an institution. She was born to a 20-year-old mother and a 23-year-old father. Her birth weight was 3100 g. Developmental milestones were markedly delayed. At age 3 years 4 months she was noted to be grossly retarded, hypotonic and had heel cord contractures. In childhood she had screaming spells and periodic vomiting.

Presently her height is 146 cm, weight 32.4 kg and head circumference 51 cm. She has pronounced scoliosis, which measures 105 degrees, and extends from T8 to L4. Dysmorphic features include a quadrangularly shaped head with flat occiput, short webbed neck, antimongoloid slant, large ears with prominent helix, narrow nasal bridge, long hooked nose, maxillary hypoplasia, large mouth with prominent lower lip and prominent mandible (Fig. 1). Her

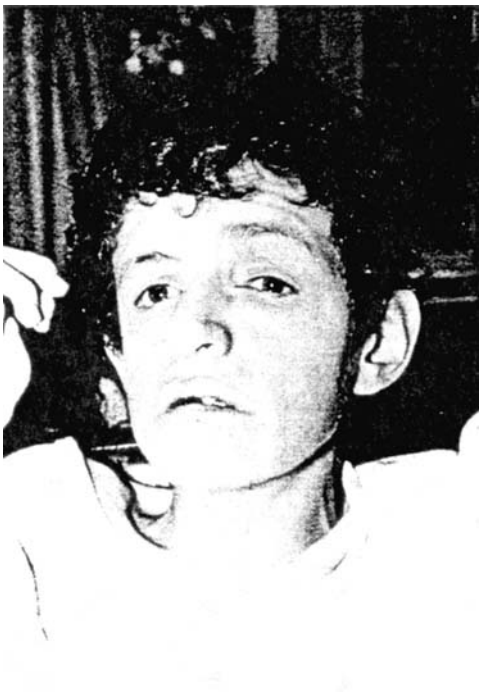

Fig. 1. The patient.

fingers and toes are long and hyperextensible. She has cutis marmorata, a number of pigmented nevi and partial syndactyly of the second and third toes.

Cytogenetic Studies

Chromosome analysis of the patient and her parents was carried out on cultured lymphocytes using conventional G-banding. Subsequently prometaphase patient chromosomes were analyzed.

Molecular Genetic Studies

DNA was isolated from white cells of the patient, from control placentae and from the cultured fibroblast cell strain GM3255 (obtained from the NIGMS Human Genetic Mutant Cell Repository, Camden, NJ, USA). The GM3255 cell strain is trisomic for 8p21→8pter [46, XY, −15, +der(15), t(8;15)(15pter→15q26::8p21→8pter)mat].

These DNA samples were restriction-digested with HindIII, the resulting fragments separated by electrophoresis in 0.7% agarose gels and blotted (Southern 1975) onto Nytran membranes. Random primed labelled probes (Feinberg & Vogelstein 1984) were prepared using the 3.3 kb HindIII fragment contained within the cloned 8.0 kb EcoRI fragment of the human thyroglobulin gene probe pcHT16/8.0 (Baas et al. 1985) and the D8S7 3.1 kb HindIII fragment of plasmid pSW50 (Wood et al. 1986). The blots were prehybridized in 5X SSPE, 5X Denhardt's, 0.3% SDS and 100 μg/ml salmon sperm DNA overnight at 62°C, and then hybridized overnight after adding 30 ng of each probe labelled to a specific activity in excess of 10^9 cpm/μg. After washing in 0.2× SSC, 0.1% SDS for 1 h the membranes were dried and exposed to Kodak XAR X-ray film for 1–2 days using Dupont Cronex Lightening Plus intensifying screens. The autoradiograph was scanned with a Helena scanning densitometer.

Results

Initial analysis of the patient's karyotype revealed additional bands on 8p. The parental chromosomes were normal. Prometaphase analysis of the abnormal chromosome 8 suggested that the extra bands represented an inverted duplication of a large part of 8p. There was marked banding symmetry around a point distal to band p22 (Fig. 2). The duplicated segment comprised bands p12→p23.1.

The results of DNA hybridization studies are shown in Fig. 3. The human thyroglobulin (TG) gene probe was used as a control for the amount of DNA present in each lane. The 3.3 kb HindIII TG probe detects 3.3 kb genomic fragments in all DNA samples. The TG gene has been mapped to

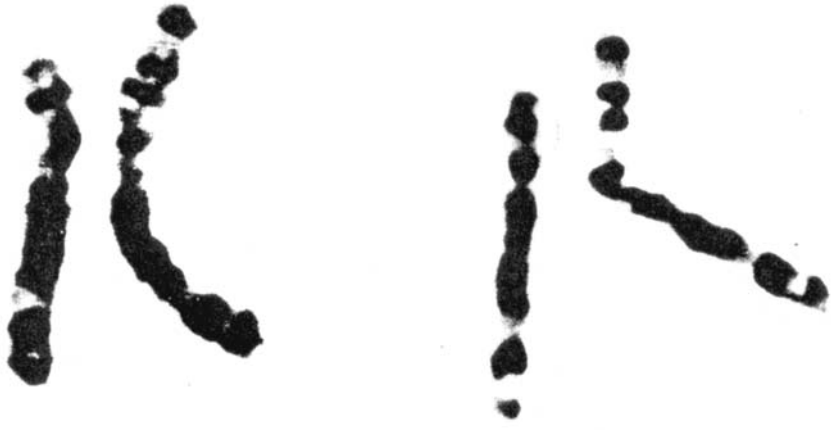

Fig. 2. Partial G-banded karyotype showing chromosomes 8 from two cells. The inverted duplication is on the right.

8q24 (Bass et al. 1985). The anonymous probe D8S7, cloned in the plasmid pSW50, has been localized to 8p23→8pter (Wood et al. 1986). This probe detects HindIII alleles of 3.1 kb and 2.7 kb. The placental DNA sample in lane 3 is homozygous while the placental DNA sample in lane 4 is heterozygous for D8S7. The GM3255 DNA sample in lane 1 is trisomic for 8p21→8pter and hence for the D8S7 probe. The hybridization intensity ratio (D8S7 (A1 allele):TG) was calculated from the scanning densitometer tracing and normalized to 1.0 for the placental sample in lane 3. The ratio for GM3255 (lane 1) was 1.3, for the patient (lane 2) 0.53, and for the placental sample (lane 4) 0.44. The patient sample shows a similar intensity of hybridization of the D8S7-A1 fragment, as does the placental sample in lane 4. This placental sample is heterozygous for the D8S7 marker and hence monosomic for the A1 allele. Thus the patient is monosomic for D8S7.

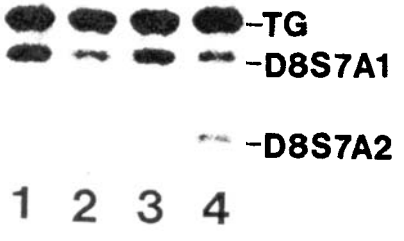

Fig. 3. Five μg of HindIII-digested DNA from GM3255 (lane 1), the patient (lane 2), and placentae (lanes 3 and 4) probed with TG and D8MGV7.

Discussion

The patient described in this report has an inversion duplication involving the short arm of chromosome 8. We have shown that patient DNA is monosomic for the probe D8S7 that is located at 8p23→8pter. Thus the patient has a deletion involving this region of chromosome 8. The chromosome 8 that appears to be cytologically normal may have a microscopic deletion involving D8S7 in this patient. Although we have not found such a deletion allele in control individuals, we are unable to formally exclude this possibility. However, we consider it more likely that the structurally abnormal chromosome 8 is nullisomic for D8S7 as a result of the process that produced the inversion duplication.

Thus our patient has a partial monosomy and a partial trisomy for 8p, both of which may influence the phenotype. We have noted a striking similarity in phenotype between our patient and the patient described by Fryns et al. (1985) with a remarkably similar inv dup 8p. The partial monosomy found in our patient was unsuspected from cytogenetic analysis and illustrates the value of molecular probe analysis in patients with structural chromosomal aberrations.

The generation of an inversion or mirror duplication is a complex event. It has been proposed that the event may occur between chromosomes or chromatids as a result of three breaks (Taylor et al. 1977). Two breakpoints in the donor would produce a fragment that when inverted could be inserted into a breakpoint in the homologue. This could either occur between homologous chromosomes or between sister chromatids of a single chromosome. This mechanism yields a mirror duplication and conserves telomeres but requires three events that are probably independent to produce the interstitial inversion duplication. This mechanism would not lead to loss of chromosomal material.

An alternative mechanism of end-to-end fusion between the short arms of chromosome 8 homologues has been proposed (Weleber et al. 1976). The resulting dicentric chromosome would subsequently break during anaphase to generate a duplication-deficiency chromosome with an inversion duplication. The telomeres would need to be reconstituted in some manner.

We favour this second hypothesis and suggest that an end-to-end fusion between chromosome homologues could be the result of unequal recombination between homologous DNA sequences that lie in an opposite orientation to each other. This event would occur at either meiosis I between homologous chromosomes or alternatively at meiosis II between sister chromatids. The resulting dicentric chromosome after breakage at anaphase would have an inversion duplication and a loss of sequences distal to the recombination point. We find this mechanism has a greater appeal, since it requires a single anomalous primary event (unequal and maloriented recombination), which is then followed by secondary events of anaphase breakage and telomere restitution.

At the molecular level, any two members of the numerous families of repeat sequences could undergo unequal and maloriented recombination, provided one member is oriented toward the centromere and the other toward the telomere.

This mechanism of generating an inversion duplication will always lead to loss of sequences distal to the point of unequal maloriented recombination. Thus the model predicts that the most distal sequences that are retained are those that correspond to the centre of symmetry.

We hope the observation of duplication deficiency in this patient will prompt others to examine similar cases of inversion dupli-

cation using the variety of DNA probes that are now available in order to determine whether these observations seem to be generally applicable.

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