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Partial trisomy and monosomy 8p due to inversion duplication

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Fluorescent *in situ* hybridization with probes specific for a chromosomal subregion and chromosome-specific libraries (chromosome painting) are important new methods for assessing chromosome rearrangements. In this paper we present four patients with additional chromosomal material on chromosome 8p who have been studied using G-banding techniques, chromosome painting and FISH with cosmid probes specific for the region 8p23.1→8pter. In all cases we found a partial inversion duplication of 8p along with a deletion of the region 8p23.1→8pter.

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Giemsa (GTG) banding is widely used for the exact identification of metaphase chromosomes, marker chromosomes and chromosomal rearrangements. Nevertheless, many chromosome abnormalities still cannot be fully determined. For additional cytogenetic analysis in these cases it is important to study chromosomes or interphase nuclei by fluorescent *in situ* hybridization (FISH). In recent years, the use of FISH has been expanded with the availability of DNA libraries made from specific chromosomal regions or whole chromosomes (Pinkel et al. 1988, Lichter et al. 1988). This technique, called chromosome painting, allows rapid visualization of the target chromosomes.

In the present study we demonstrate further the usefulness of chromosome painting and FISH in clinical cytogenetics. In four patients, a chromosome 8 specific paint was used to visualize the chromosomes 8 and to confirm the nature of the rearrangements we expected after Giemsa (GTG) banding.

Materials and methods

Clinical reports

Patient 1 was a prematurely born girl. Weight, length and head circumference were within normal limits. At the age of 15 months the patient was evaluated because of hypotonia and psychomotor retardation. She showed facial dysmorphism, i.e. a rectangular headshape with high forehead and chubby cheeks, a large mouth with everted lower lip, a small nose with upturned nostrils, micrognathia and large dysplastic ears. She had small teeth and a high palate.

Patient 2 was a 2.5-year-old girl. She was born after an uncomplicated pregnancy at 34 weeks and

had normal body measurements. At physical examination, dextrocardia and hypoplasia of the right kidney were found. Facial dysmorphism included rectangular headshape with high forehead and a broad nasal bridge with anteverted nostrils. She had a long philtrum and a large mouth with thin lips and an everted lower lip. The ears were large and low set. Psychomotor retardation was severe.

Patient 3 was a 43-year-old male, the only child of healthy unrelated parents. Remarkable physical findings were his excessive height (205 cm, > P97), large skull (OFC 62 cm, > P97), coarse facial features, i.e. a large nose, retrognathia, a large mouth with widely spaced teeth and large ears with poor lobulation. He had severe contractures of the knees and ankles, owing to neurological deficit, and was severely mentally retarded.

Patient 4 was a 42-year-old male with profound mental retardation. He had coarse features, a large beaked nose and a large mouth with eversion of the lower lip. The ears were large and dysplastic. He had contractures of his lower extremities and severe scoliosis.

Cytogenetics

Chromosomes were prepared from peripheral blood lymphocyte cultures. The cells were cultured using a modification of the synchronization method of Dutrillaux & Viegas-Pequignot (1981), by treatment overnight with thymidine, followed by incubation with 5-BrdU for 6 h and ethidium-bromide for 1.5 h before harvesting. High-resolution banding was performed by treatment with trypsine, followed by staining with Giemsa to obtain a GTG banded pattern.

Probes, FISH and chromosome painting

Probe pJM128 (D8Z2) is a DNA probe specific for the centromere of chromosome 8 (Donlon et al. 1986). The cosmids 59C1 (D8S7), 83D9 (D8S11) and 16E12 (D8S11) have been mapped to chromosome region 8p23.1–8pter (Wood et al. 1992). Probes were biotin-labeled by nick-translation, according to the manufacturer's specifications (Gibco BRL). The cosmid probes were ethanol precipitated in the presence of a 500-fold concentration of total human DNA and subsequently dissolved in a hybridization mixture containing 50% formamide

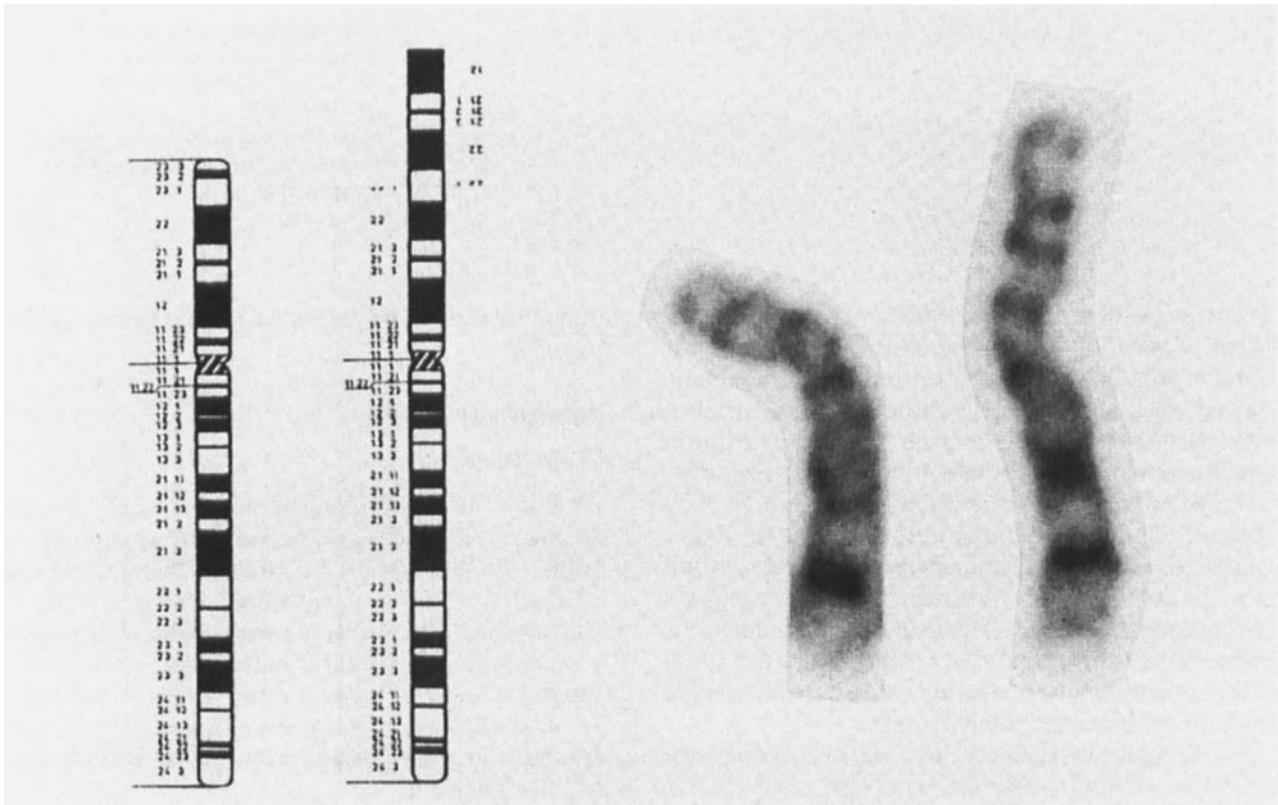

Fig. 1. Partial karyotype depicting diagrammatic representation of a normal chromosome 8 (left) and an inversion duplication chromosome 8p (right), prometaphase GTG-banded normal chromosome 8 and a chromosome 8 with an inversion duplication and a deletion in the short arm.

and 10% dextran sulfate pH 7.0 to a final concentration of 10 ng/μl. The biotin-labeled centromere-specific probe was added to this hybridization buffer in a final concentration of 5 ng/μl. Metaphase spreads were prepared from PHA-stimulated lymphocytes using the standard 3:1 (v/v) methanol:acetic acid fixation protocol. Prior to FISH the preparations were digested with 100 μg/ml pepsin (p-7012, Sigma) in hydrochloric acid pH 1.8 at 37°C for 12 min, followed by two rinses in PBS. Hybridization was performed at 37°C according to the protocol of Lichter et al. (1988) with minor modifications.

For chromosome painting, a paint specific for the total chromosome 8 and directly labeled with Spectrum Orange fluorophore was purchased (Gibco BRL). The probe mixture was denatured at 80°C for 3 min and stored on ice; subsequently it was applied to the slides and sealed with a coverslip (20 × 20 mm). Hybridization was accomplished for 16 h at 37°C; thereafter the slides were washed once with 50% formamide in 2 × SSC (5 min), three times with 2 × SSC (5 min) and once with 0.1% NP-40 in 2 × SSC at room temperature (5 min). After dehydrating and air drying, slides were mounted in medium containing p-phenylenediamine dihydrochloride and 4,6-diamidino-2-phenylindole for antifading and counterstaining of all chromosomal material, and sealed under a cover slip.

Results

Analysis of high resolution prometaphase chromosomes showed additional chromosomal material on the short arm of one of the chromosomes 8 of patients 1, 3 and 4. The aberration was an inverted tandem duplication of the short arm segment 8p12→8p23.1 (Fig. 1). Both parents of patient 1 had normal GTG banded chromosomes, and thus the anomaly is likely to be *de novo*. The parents of patient 3 had died. The mother of patient 4 and his five sibs were chromosomally normal; his father had died. For patient 2, chromosome analysis was performed on cultured blood lymphocytes and an inverted tandem duplication of the region 8p21.1→8p22 was found in all the cells examined. Karyotypes of the parents were normal.

As the GTG banding pattern of the aberrant short arm of chromosome 8 suggested an inversion duplication in all four cases, we decided to verify this interpretation with a chromosome-8-specific paint. In all four cases the chromosome paint hybridized to the structurally normal chromosome 8 as well as to all parts of the chromosome 8 with the abnormal short arm (Fig. 2).

Despite using high resolution GTG banding, it was difficult to detect in the banding pattern whether the terminal positive G band in the aberrant chromosome 8 represented band 8p23.3 or was part of the inverted and duplicated region. If the latter were the case, the aberrant chromosomes would be missing at least the terminal band 8p23.2 and 8p23.3. To examine the possibility of such a terminal deletion in the aberrant chromosome, hybridization with cosmid probes specific for chromosome region 8p23.1→8pter was performed. Cohybridization of the chromosome 8 centromere specific probe and the cosmid probes to metaphases of the four patients resulted in fluorescent spots at the centromeres and at the end of the short arm of the normal chromosomes 8. However, in all four cases the abnormal chromosomes 8, detected by the fluorescing centromere spots, lacked the signals at the end of their short arm. The result of FISH with the cosmid probe 59C1 in a metaphase of case 1 is shown in Fig. 3.

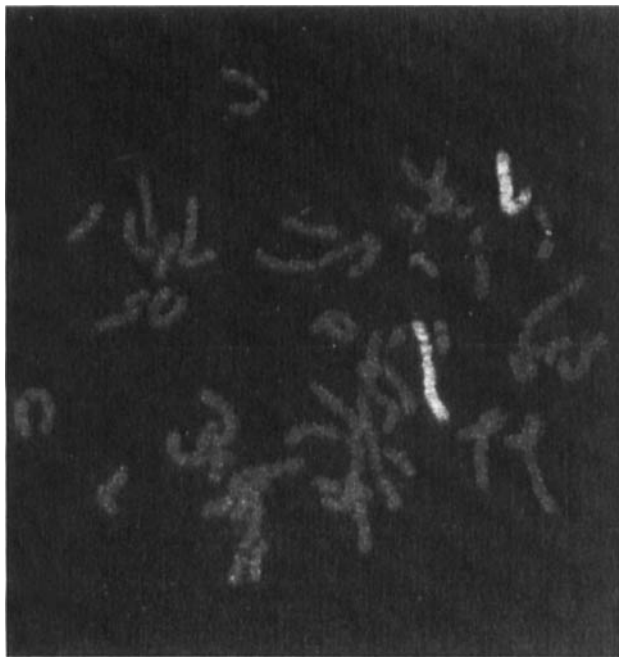

Fig. 2. Metaphase chromosomes after *in situ* hybridization with a Spectrum Orange fluorophore labeled paint for chromosome 8.

Discussion

In most cases, when high-quality chromosome preparations are used, GTG banding alone may produce a banding pattern sufficient for chromosome identification. For cases where cytogenetic band assignment is difficult, the application of FISH using chromosome centromere-specific probes, cosmids or whole chromosome libraries can provide identification of the chromosome (region) involved. Here, we describe the combined use

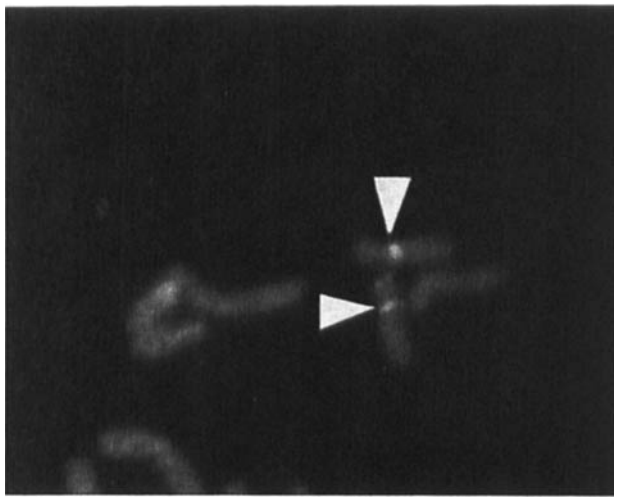

Fig. 3. Metaphase chromosomes after *in situ* hybridization with the centromere probe PJM 128 specific for chromosome 8 and with the cosmid probe 16E12 specific for region 8p23.1→8pter. Arrowheads point to the normal and aberrant chromosome.

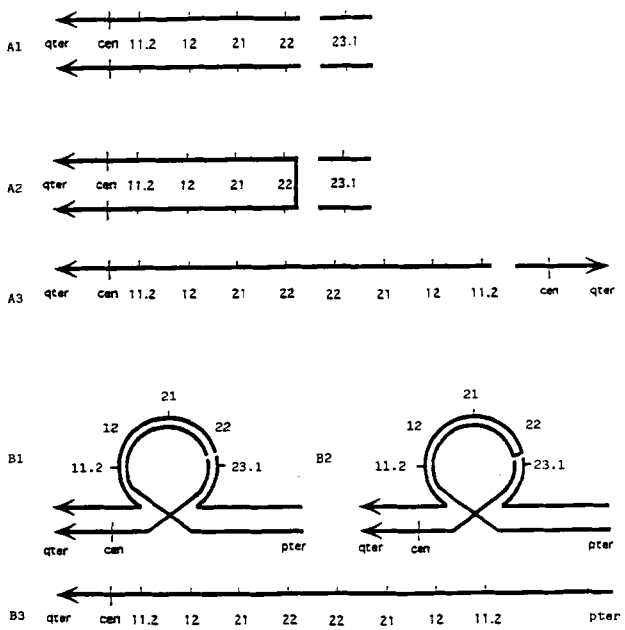

Fig. 4. Formation of an inverted duplication. *A1*: Both strands are broken at band 8p23. *A2*: Repair processes create a U-type exchange at band 8p23. *A3*: During the subsequent anaphase, a break in band 8p11.2 will lead to two aberrant chromosomes, one with an inversion duplication and one with a deletion in the short arm of chromosome 8. *B1*: Two strands of non-sister chromatids of chromosomes, one with a paracentric inversion, are broken at 8p23. *B2*: Repair processes create a U-type exchange at band 8p23. *B3*: Unfolding shows an inverted duplication with loss of chromosome region 8p23→8pter.

of Giemsa banding, whole chromosome painting and chromosome band-specific FISH which enabled the exact determination of the patients' abnormal karyotypes.

To date, a total of 22 patients with a partial duplication 8p due to interstitial inversion duplication has been reported (patients 7 and 8 of Rethoré et al. (1977) and the 20 patients reviewed by Feldman et al. (1993)). The clinical picture emerging from these patients (and the four new cases) comprises severe mental retardation, hypotonia and neonatal feeding problems, structural brain abnormalities and minor facial anomalies, including a broad nasal bridge, a large mouth with a thin upper lip, a high-arched palate, an abnormal maxilla or mandible and malformed, low-set ears. Various orthopedic problems are often also reported and were present in our adult male patients.

In 12 patients, including the present cases 1, 3 and 4, the breakpoints were located in band 8p12 and 8p23; breakpoint 8p22, found in case 2, was reported four times. All patients shared region 8p21→8p22 in the duplicated segment and it is reasonable to assume the clinical features of inversion duplication 8p depend on trisomy for this chromosome region. We have shown that all four present cases lacked the terminal signals of the probes 59C1, 83D9 and 16E12 in the abnormal chromosome 8. Thus, as well as duplication of 8p21→8p22, the patients had a deletion in the region 8p23→8pter at which these probes are located. Of the 22 patients previously described, only two (Dill et al. (1987), Henderson et al. (1992) and Gorinati et al. (1991)) were reported to have a deletion in this region. The contribution of this deletion to the clinical picture is probably less important than the inversion duplication of 8p21→8p22 because patients with deletions of 8p23→8pter are reported to have mild mental retardation with a normal habitus (Fryns et al. 1989) or with minimal dysmorphic features (Blennow et al. 1990, Pettenati et al. 1992).

Two mechanisms can be proposed to explain the *de novo* inversion duplication leading to partial monosomy and trisomy 8p (Fig. 4). One mechanism that requires only a single primary recombination event, an aberrant U-type exchange with loss of chromosomal material, was proposed by Weleber et al. (1976). They suggested as an initial event the end-to-end fusion of the short arms of chromosome 8 homologues, resulting in a dicentric chromosome. During anaphase this dicentric chromosome could break close to one of the centromeres in 8p11, leading to a chromosome with an inversion duplication on the short arm and loss of chromosomal material distal to the site of recombination, the telomeric region included. A much more complicated mechanism has been suggested by Mitchel et al. (1991), in which two abnormal events (paracentric inversion and subsequent U-type exchange) would result in a chromosome with a duplicated region and a deleted region, but with normal telomeres. Both mechanisms could

have led to the aberrant chromosome 8 of the patients of this study. However, as the parents of patients 1 and 2 had normal karyotypes, germ line mosaicism for a paracentric inversion must be assumed in one parent of the couple.

The present study of four patients illustrates the power of fluorescent *in situ* hybridization and whole chromosome painting for the confirmation and characterization of subtle chromosome aberrations. As the karyotypes of the other family members are normal, both techniques are an important adjunct to the conventional cytogenetic methods in providing additional evidence for the origin of unbalanced chromosome aberrations.

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