Familial partial trisomy 8p without dysmorphic features and only mild mental retardation

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Abstract

We report on a mother and her two sons who had a direct duplication of chromosome region 8p22-8p23.1 without dysmorphic features and only mild mental retardation. The patients have been studied using G banding, chromosome painting, and FISH using cosmid probes specific for the region 8p23.1-8pter. Comparison of the phenotypes of our patients and of published patients with an inversion duplication of the short arm of chromosome 8 indicates that trisomy for chromosome band 8p21 causes the more severe clinical picture in the latter.

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Duplication of the short arm of chromosome 8, in which chromosome region 8p11.1 to 8p23 is involved, is associated with characteristic clinical manifestations.^{1,2} Up to now 47 patients have been published with an inversion duplication 8p²⁻¹⁸ with a distal breakpoint in 8p22 (19 cases) or in 8p23 (28 cases) and a proximal breakpoint in 8p11.2 (11 cases), 8p12 (22 cases), or 8p21 (14 cases), as well as three patients with a tandem duplication 8p¹⁹ with breakpoints in 8p22 and 8p23.1 or 8p21.3 and 8p22 (table 1). In 19 of the 47 patients with an inversion duplication 8p, a deletion of (part of) 8p23 has been proved with molecular techniques.¹¹⁻¹³⁻¹⁷⁻²⁰ In the present study we report on three new patients with direct duplication 8p and no apparent deletion and compare their clinical features with the previously reported cases, who definitely have more severe clinical features. A chromosome 8 specific paint was used to visualise the normal and aberrant chromosome 8 and probes specific for chromosome region 8p23.1-8pter were used to determine the origin of the extra material on the short arm of chromosome 8.

Table 1 Cytogenetic findings in published cases with inversion or direct duplication 8p

Duplicated segment	Patients	References
8p11-8p22 (inv dup)	2	5
8p11-8p23 (inv dup)	9	2, 3, 7, 17, 18
8p12-8p22 (inv dup)	9	14
8p12-8p23 (inv dup)	13	2, 4, 6, 11, 12, 15, 16, 18
8p21-8p22 (inv dup)	8	9, 10, 13, 14, 15, 18
8p21-8p23 (inv dup)	6	2, 4, 8
8p21.3-8p22 or 8p22-8p23.1	3	19
8p22-8p23.1 (dir dup)	3	Present study

Materials and methods

CASE REPORTS

Patient 1 is the proband of the family. He was born at term as the second child of non-consanguineous parents. Pregnancy and delivery were uncomplicated. Birth weight was 4000 g (97th centile). His motor development was delayed. He walked independently at the age of 2½ years and speech development was also retarded. Psychological evaluation at the age of 3 years showed an IQ of 70 (Stutsman scale). At evaluation at the age of 5 years he presented as a healthy boy with growth parameters within normal limits. He showed no phenotypic anomalies and attended a school for mildly retarded children.

Patient 2 is the older brother of patient 1. He was born at term, after an uneventful pregnancy and delivery. Birth weight was 2800 g (10th-25th centile). In the first year development was normal, but delay started at the age of 2 years, requiring special education. Now at the age of 8 years he is a cooperative boy with normal social skills. No psychological test data are available. His motor and mental level is similar to that of his younger brother. On physical examination he shows no anomalies, particularly no facial dysmorphism.

Patient 3 is the healthy mother of patients 1 and 2. She had regular education and worked as a saleswoman before her marriage. On physical examination she shows slight hypertelorism (ICD 3-8 cm) and mild proptosis of the eyes, but no further facial dysmorphic signs. She is the only child of healthy parents of normal intelligence. Her husband had special education and now works in sheltered employment. Two brothers out of his seven sibs and two of their sons are of borderline normal intelligence.

CYTOGENETICS

Chromosomes were prepared from peripheral blood lymphocyte cultures, using a modification of the synchronisation method of Dutrillaux and Viegas-Pequignot,²¹ by treatment overnight with thymidine, followed by incubation with 5-BrdU for six hours and ethidium bromide for 1.5 hours before harvesting. High resolution banding was performed by treatment with trypsin followed by staining with Giemsa to obtain a GTG banded pattern. No cell line is available.

PROBES, FISH, AND CHROMOSOME PAINTING

Probe pJM128 (D8Z2) is specific for the cen-

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Figure 1 Partial karyotype representing a normal chromosome 8 (left) and a duplication chromosome 8p (right), a prometaphase GTG banded normal chromosome 8 and a chromosome 8 with duplication in the short arm.

trastere of chromosome 8.²² Cosmids 59C1, 83D9, and 16E12 that have been mapped to chromosome region 8p23.1–8pter²³ were biotin labelled by nick translation, according to the manufacturer's specifications (Gibco BRL). Metaphase spreads were prepared from PHA stimulated lymphocytes using the standard 3:1 (v/v) methanol:acetic acid fixation. Slide treatment and hybridisation was performed according to the protocol of Lichter *et al*²⁴ with minor modifications. A paint specific for chro-

mosome 8 (Gibco BRL) was applied to the metaphase spreads according to the manufacturer's specifications.

Results

Analysis of high resolution prometaphase chromosomes showed additional material on the short arm of one of the chromosomes 8 of all three patients. The mother of patient 3 had normal chromosomes and her father had died. A chromosome 8 specific paint appeared to hybridise to the structurally normal chromosome 8 as well as to all parts of the aberrant chromosome 8. Despite using high resolution GTG banding, it was difficult to detect in the banding pattern whether we were dealing with a direct duplication or with an inverted duplication (fig 1). Cohybridisation of a centromere specific probe and cosmid probes specific for chromosome region 8p23.1–8pter to metaphases of the three patients showed fluorescent spots at the centromere and at the end of the short arm of the normal chromosome 8. The abnormal chromosome 8 showed a fluorescent centromere spot and two signals at the end of the short arm with all three probes. The results of FISH with the cosmid probe 59C1 on a metaphase of case 1 is shown in fig 2.

Discussion

In most cases, GTG banded high quality chromosome preparations produce a banding pattern sufficient for chromosome identification. In the present study, cytogenetic band as-

Figure 2 Metaphase chromosomes of patient 1 after in situ hybridisation with the centromere probe pJm128 and probe 59C1 specific for region 8p23.1–8pter. Normal chromosome 8(left) with a single spot on the short arm and aberrant chromosome 8(right) with double spots on the short arm.

Table 2 Clinical features in patients with duplication 8p

Clinical findings	Reported patients		Present patients
	inv dup (8p) (n = 35)	dir dup (8p) (n = 3)	dir dup (8p) (n = 3)
Neonatal hypotonia/feeding problems	21/27	2/2	0/3
Mental retardation	35/35	3/3	2/3
Residual hypotonia	34/35	0/3	0/3
Kyphosis/scoliosis	12/33	0/3	0/3
Short stature	9/26	3/3	0/3
Brachycephaly/frontal bossing	14/25	0/3	0/3
Hypertelorism	2/26	3/3	1/3
Broad nasal bridge	18/33	2/3	0/3
High arched palate	20/32	0/3	0/3
Prominent mandible	13/31	0/3	0/3
Large mouth with prominent lips	23/33	0/3	0/3
Malformed or low set ears	30/33	0/3	0/3
Abnormal dentition	11/22	1/3	0/3

signment was difficult and the application of FISH using a centromere specific probe, site specific cosmid probes, and a whole chromosome paint was necessary for the identification of the extra chromosomal material. The results of FISH with specific probes provide good evidence that the short arm of the aberrant chromosome 8 has a direct duplication of region 8p22–8p23.1, which gives the three patients a 46, XX(XY), dir dup (8) (pter–p22::p23.1–qter) karyotype. Multicolour or two colour FISH with probes lying within the duplication would produce definitive evidence for direct or inverted duplication.

To date, 50 patients with partial duplication 8p have been reported (table 1). Sufficient clinical data on 38 of them exist (one patient reported by Mitchell *et al.*¹⁶ one by Barber *et al.*¹⁷ 33 patients reviewed by de Die-Smulders *et al.*¹⁸ and three patients described by Dhooge *et al.*¹⁹). Table 2 lists the clinical data of the present cases and the three cases of Dhooge *et al.*¹⁹ together with the clinical features of 35 patients with inversion duplication 8p. The clinical picture of the patients with an inversion duplication 8p comprises hypotonia and neonatal feeding problems, profound mental retardation, structural brain abnormalities, facial anomalies including a large mouth with a thin upper lip, a high arched palate, a broad nasal bridge, an abnormal maxilla or mandible, and malformed, low set ears. Various orthopaedic problems are also often reported. As inversion duplications in 8p coexist with terminal deletions^{11 13–17 20} an effect on the phenotype might be expected. However, as reported earlier,¹⁵ patients in whom the abnormality is restricted to a deletion of 8p23–8pter are only mildly mentally retarded and have a normal phenotype²⁵ or minimal dysmorphic features.^{26–29}

The clinical picture of the three patients described by Dhooge *et al.*¹⁹ is characterised by small stature, facial dysmorphism, and mild mental retardation. In contrast to the patients with inversion duplication 8p, the clinical picture of the present patients is very mild. They show no dysmorphism and have no congenital anomalies.

The shortest region of overlap in patients with 8p duplication listed in table 1 is 8p21.1–8p22. As our patients (and possibly also those

of Dhooge *et al.*¹⁹) are only trisomic for chromosome region 8p22–8p23.1, the critical chromosome region determining the clinical picture in inversion duplication 8p would be 8p21.1–8p21.3.

Several mechanisms have been proposed to explain the origin of inversion duplications and direct duplications leading to partial trisomy 8p. Weleber *et al.*³ proposed a U type exchange causing an end to end fusion of the short arms of chromosome 8, subsequently leading to the formation of an inversion duplication with loss of chromosomal material distal to the initial breakpoint. With DNA studies and FISH this terminal deletion has been illustrated.^{11 13–17 20} A second mechanism that gives rise to an inversion duplication has been suggested by Mitchell *et al.*³⁰ Two abnormal events (paracentric inversion and subsequent U type exchange) would result in a chromosome with an inverted and duplicated region and a deleted region but with normal telomeres. Patient EM of Feldman *et al.*² would fit this mechanism. To explain the formation of a direct duplication three possibilities are proposed.³¹ A direct duplication could originate in a meiotic cell by unequal crossing over between chromatids, by a two break reciprocal translocation between homologues, or by a three break insertion between sister or non-sister chromatids.

The chromosomal and clinical data reported in patients with duplications in 8p lead to the conclusion that involvement of band 8p21 in the duplication is of major importance, because the triplicate state of this region contributes to the severe clinical picture and profound psychomotor retardation. For this reason in situ hybridisation techniques should be used to identify accurately the chromosomal contents of an 8p duplication.

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