Trisomy 8 syndrome owing to isodicentric 8p chromosomes: regional assignment of a presumptive gene involved in corpus callosum development

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Abstract

Two patients with trisomy 8 syndrome owing to an isodicentric 8p;8p chromosome are described. Case 1 had a 46,XX/46,XX,-8,+idic(8)(p23) karyotype while case 2, a male, had the same abnormal karyotype without evidence of mosaicism. In situ hybridisation, performed in case 1, showed that the isochromosome was asymmetrical. Agenesis of the corpus callosum (ACC), which is a feature of trisomy 8 syndrome, was found in both patients. Although ACC is associated with aneuploidies for different chromosomes, a review of published reports indicates that, when associated with chromosome 8, this defect is the result of duplication of a gene located within 8p21-pter. Molecular analysis in one of our patients led us to exclude the distal 23 Mb of 8p from this ACC region.

(J Med Genet 1994;31:238-241)

Mosaicism for trisomy 8 results in a characteristic syndrome reported in more than 100 patients.¹⁻⁴ We report on two patients with the clinical phenotype of trisomy 8 syndrome owing to isodicentric 8p;8p chromosome.

Case reports

CASE 1

Case 1, a female, was born to healthy, unrelated parents. She was the product of the second pregnancy, the first having ended in spontaneous abortion at 6 months. Vaginal delivery of the proband occurred at 7 months. Birth weight was 2700 g. The perinatal period was complicated by respiratory distress and jaundice. Feeding difficulties were reported in the first months. Psychomotor development was delayed. At 14 months of age weight was 7100 g (<3rd centile), height 75 cm (25th centile), and head circumference 46 cm (between the 25th and the 50th centile). The hair was fair and thin. The palpebral fissures were horizontal. There was an alternating internal strabismus. A flat angioma was noted on the right cheek. The nose was saddle shaped and the philtrum long. The mouth was small with a thick lower lip and high arched palate. The ears were low set with a flat helix, wide concha, and prominent antitragus. The

neck was short with mild webbing. The shoulders were narrow with epitroclear dimples. The fingers were long and showed camptodactyly and the feet were large with deep plantar furrows. Routine laboratory investigations were normal. A cerebral CT scan showed agenesis of the corpus callosum. Echocardiography showed valvular pulmonary stenosis and secundum atrial septal defect.

CASE 2

Case 2, a male, was the first child of healthy, unrelated parents. At birth the mother was 20 years old and the father 21. The family history was unremarkable. One previous pregnancy resulted in a spontaneous abortion in the second month of gestation.

First and second trimester ultrasound investigations were normal, but a reduced biparietal distance was reported in the third trimester. The baby was born at term by vaginal delivery. Birth weight was 3150 g (25th centile), length 53 cm (50th centile), and head circumference 35 cm (50th centile). The early weeks were complicated by stunted growth. The patient was referred to us at the age of 2 months, when weight was 3500 g (<3rd centile) and head circumference 37 cm (10th centile).

Physical examination (fig 1A) showed an asymmetrical skull with sparse hair and hypertrichosis of the frontotemporal region. The forehead was high and prominent with slightly depressed temples. The eyebrows were sparse and elongated and the eyes deep set with upward slanting palpebral fissures. Inner canthal distance was 3.1 cm (>97 centile). The nose had a characteristic "boxer appearance" with a wide nasal bridge and anteverted nostrils. The philtrum was normal. The upper lip was prominent and thick. The lower lip and jaw were retracted and the chin was rounded. The face was covered with a fine lanugo. A 2 × 1.5 cm cutaneous haemangioma was noted on the left cheek. The ears were low set and dysmorphic, with a large lobule, an incompletely folded helix, and a prominent antihelix. The neck was short. There were umbilical and bilateral inguinal hernias. The scrotum was empty. The fingers were flexed and the distal phalanges were short especially in the third and fifth fingers. The nails were hypoplastic, the fifth finger nails being 1.5 × 1.5 mm in size. Deep palmar furrows were present bilaterally

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Received 2 September 1993
Accepted for publication
7 October 1993

Figure 1 Case 2. (A) Facial appearance, (B) deep palmar furrows, (C) cutout of chromosomes 8 from two selected cells. The normal chromosome 8 is reproduced in duplicate on the lower left and upper right of the idic(8) chromosome.

Figure 2 Case 1. (A) Cutout of Q banded chromosomes 8. (B) C banding of two idic(8). The idic(8) on the left has one centromere active and one inactive, that on the right has both centromeres active. (C) FISH with 11E1 shows that this cosmid hybridises only to the normal chromosome 8. The chromosome with hybridisation signals is counterstained with propidium iodide, that on the right is stained with DAPI. (D) FISH with CRI-L40 and pZ8.4 shows signals of CRI-L40 at 8p22-23 both in the normal chromosome 8 and the idic(8). The large signals of pZ8.4 are localised at the centromere of the normal chromosome 8 and at the active and inactive centromere of the idic(8). Chromosomes are positioned as in (C).

(fig 1B). Lower limb anomalies included varus deformity of the right foot, short metatarsals with hypoplastic toes and toenails, cutaneous syndactyly between the second and third toes and marked plantar furrows. The patient was moderately hypertonic and developmentally retarded. Routine laboratory analyses were normal. A CT scan of the brain showed agenesis of the corpus callosum and a cystic tumour in the occipital region. Echocardiography and angiocardiology disclosed a ventricular septal defect with a persistent left superior vena cava.

A skeletal x ray survey at 2 months showed advanced bone age (in the range of 6 months to 2 years), scaphocephaly with synostosis of the sagittal suture, 13 pairs of ribs with a hypoplastic left first rib, rarefaction of the proximal humeral metaphyses, a “bone within bone” image in the vertebral bodies, and asymmetrical ossification of the femoral heads. The distal phalanges were hypoplastic and absent in the right fifth finger and fifth toes. Renal ultrasound was unremarkable.

The patient died at home of congestive heart failure at 4 months.

Cytogenetic studies

CASE 1

Chromosome analysis was performed on lymphocyte and fibroblast cultures. In both tissues mosaicism was detected with the normal cell line present in 11 out of 40 and five out of 25 cells respectively. In the abnormal cell line, one chromosome 8 was replaced by a long submetacentric chromosome interpreted as an isodicentric 8 with a breakpoint at p23. The abnormal chromosome showed a single primary constriction with inactivation of the second centromere in the great majority of the cells though two centromere constrictions were clearly identified in a few cells (fig 2 A,B). Thus the proband’s karyotype was interpreted as 46,XX/46,XX,-8,+ idic(8)(qter-p23::p23-qter). A fibroblast cell line (652/91) is available. The parents had normal karyotypes.

In situ hybridisation was performed with cosmid 11E1 (D8S7 locus mapping to 8p23⁵) isolated from a LA08NC01 flow sorted cosmid library⁶ and with probe CRI-L40 (locus D8S35 mapping at about 23 cM from the tip of 8p proximally to D8S7⁵). In some experiments quicker detection of chromosome 8 was achieved by using CRI-L40 together with the alphoid probe pZ8.4 specific for the chromosome 8 centromere. The probes were biotin-16-dUTP labelled by nick translation according to the Boehringer Mannheim protocol. Conditions for cosmid 11E1 in situ hybridisation were as described by Lichter *et al*⁷ using *Cot*I DNA (BRL) instead of human placental DNA to block repetitive sequences. Hybridisation with CRI-L40 (200 ng/slide) plus pZ8.4 (50 ng/slide) was performed in 50% formamide/2 × SSC with 500 × salmon sperm DNA at 37°C for 12 to 16 hours. Post hybridisation washes were made at 42°C in 50% formamide/2 × SSC (three cycles for five minutes), and then in 0.1 × SSC (three cycles for five

minutes). Under these conditions the identification of the *idic(8)* and of the normal chromosome 8 was immediate, though other chromosomes showed alphoid hybridisation signals at the centromeres. In all cases the detection was done according to the ONCOR detection kit with three amplification steps. Chromosomes were counterstained with propidium iodide (1 µg/ml) and banded with DAPI (0.5 µg/ml). Microscopic analysis was performed on metaphase chromosomes with no more than 10 hybridisation signals and with at least one spot on one chromosome 8. In the 50 informative cells analysed after FISH with 11E1, 28 hybridisation spots were clustered at 8p23.3 in the normal chromosome 8 while three and eight signals were spread along the normal and the abnormal chromosome 8 respectively (fig 2C). No signals were found in the central portion of the *idic(8)* corresponding to band 8p23. This was taken as an indication that the distal portion of chromosome 8, from pter to D8S7, was missing from the dicentric chromosome 8. FISH analysis in 50 informative metaphases with CRI-L40 and pZ8.4 showed signals relative to CRI-L40 both in the normal (53 signals clustered at the subterminal short arm 8p22–p23) and in the dicentric chromosome 8 (60 signals lying between the two alphoid ones). In some cells the normal or the isodicentric chromosome 8 or both showed two CRI-L40 signals that were localised at the same height, one on each chromatid (fig 2D). Signals on the *idic(8)* did not indicate the presence of more than one D8S35 locus in any cell. Unclustered signals were spread along the normal (seven) and the abnormal (16) chromosomes 8. Altogether, a similar number of CRI-L40 signals was found in the normal 8 and the *idic(8)* and the distribution of the signals was taken as an indication that sequences homologous to CRI-L40 were present in a single copy in the dicentric chromosome 8.

CASE 2

Chromosome studies performed on lymphocyte (40 mitoses) and fibroblast cultures (30 mitoses) showed a modal number of 46, with a structurally abnormal, long, submetacentric chromosome replacing one chromosome 8. This abnormality was interpreted as an isodicentric resulting from terminal fusion of the short arms of two chromosomes 8 with functional suppression of one centromere. Thus, the karyotype was designated 46,XY,-8,+*idic(8)(qter-p23:p23-qter)* (fig 1C). The parents had normal chromosome constitutions.

Discussion

Most if not all subjects with trisomy 8 are mosaics.⁴ Thus, complete trisomy 8 is a rare abnormality in postnatal life, while it accounts for 12% of autosomal trisomies found in spontaneous abortions.⁸ Structural anomalies of chromosome 8 resulting in full blown trisomy 8 syndrome are very rare. To the best of our knowledge only one patient has been reported

with mosaic trisomy of a portion of the short arm and the entire long arm: *dic(8)(qter-p21::p21-qter)*.⁹

Our case 2 seems to be the first example of a non-mosaic *idic(8p;8p)*. The distal location of the break in 8p produced an apparently stable *idic(8)* chromosome and thus the patient was in fact functionally trisomic for nearly the whole of chromosome 8. If mosaicism with a normal cell line is not present, the chromosome anomaly must have originated either prezygotically, possibly during parental meiosis, or in the zygote itself after sister chromatid exchange. The association with a normal cell line in the case reported by Ray and Hunter⁹ and in our case 1 suggests a postzygotic origin of the dicentric chromosome 8. FISH findings in case 1 indicate that the isochromosome is not symmetrical, having one breakpoint between D8S7 and D8S35 and the other one proximal to D8S35. Presumptive isochromosomes X^{10,11} and Y¹²⁻¹⁴ have been reported to be asymmetrical after quantitative DNA analysis. Thus, asymmetrical exchanges between sister or homologous chromatids could be a frequent mechanism of formation of those chromosome rearrangements usually designated isochromosomes.

Trisomy 8 syndrome is characterised by craniofacial dysmorphism, skeletal abnormalities, cardiovascular and urogenital malformations, deep palmar and plantar grooves, mental retardation, and agenesis of corpus callosum.²⁴ Our cases show most of these features. Interestingly, case 2 had several features found in the Coffin-Siris syndrome.¹⁵ They include sparse scalp hair, coarse facial appearance, facial lanugo, thick upper lip, micrognathia, camptodactyly, hypoplastic fifth finger nails, haemangioma, congenital heart disease, and mental retardation. Thus, at least in the neonatal period, there seems to be a striking overlap of dysmorphic features between Coffin-Siris and non-mosaic trisomy 8 syndrome, in which deep palmar and plantar furrows are distinguishing features.

ACC is a common finding in patients with trisomy 8 syndrome. The origin of this anomaly is heterogeneous and it has been reported to be the result of autosomal recessive, X linked dominant, and X linked recessive mutations.¹⁶ Moreover, the defect is found in association with different chromosome abnormalities¹⁷ including aneuploidies of chromosome 8 as mosaic trisomies,⁴ short arm tetrasomies owing to *i(8p)*,^{18,19} duplications of different portions of 8p secondary to unbalanced translocations,²⁰⁻²⁴ or the so called *inv dup(8p)*.²⁵ With the exclusion of these latter cases, in which duplication of 8p was concurrent with distal 8p deletion whose limits are not yet defined,²⁵ these chromosome 8 imbalances is trisomy 8p21–pter (fig 3). Thus, it is likely that this region contains a gene (ACC-8) whose duplication can interfere with normal brain development. In this respect, NEFL (neurofilament, light polypeptide), whose locus is at 8p21,³² could be a candidate. However, among the 12 informative cases trisomic for different portions of 8p as

Figure 3 Extent of 8p duplication in patients with and without agenesis of the corpus callosum (ACC). Below: patients with ACC and 8p duplication secondary to translocation (—) or to mosaic *i*(8p) (—). Above: patients without ACC and 8p duplication secondary to translocation (-----). M and F indicate that the patient is male or female, mat and pat indicate maternal and paternal origin of the duplication. Reference numbers are in parentheses. Only patients in whom specific brain investigations have been performed were considered.

products of unbalanced translocations, only five display ACC (fig 3). If we exclude the case reported by Stengel-Rutkowski *et al.*,³⁰ trisomic for 8p23–pter, in which the absence of ACC could be because of the location of the ACC-8 gene proximal to 8p23, the proportion of cases trisomic for the postulated ACC-8 gene and with ACC is 5/11. Conversely, all the three properly investigated supernumerary *i*(8p) cases,^{18,19} tetrasomic for the entire 8p, display ACC (fig 3). To explain these observations we are forced to assume that three alleles of the presumptive gene produce a product in a concentration that can be critical for ACC development while four alleles produce a concentration that is always above the critical threshold. It must be noted that the presence or absence of ACC is independent of the parental origin of the gene, thus excluding an imprinting effect, and it is also independent of the sex of the patient (four females:four males with ACC; three females:four males without ACC) (fig 3). Interestingly, ACC has also been reported in one patient with distal 8p deletion.³³ Since imprinting of a gene located in the unbalanced region cannot account for this finding and abnormalities of the corpus callosum have never been reported in the numerous patients with 8p deletion,⁴³ this case could have a more complex rearrangement also involving a partial duplication. The finding that our case 1, although duplicated for nearly the whole of chromosome 8, is not duplicated for the distal 23 Mb from D8S35 to 8pter, indicates that the ACC-8 gene lies in the distal half of the chromosome 8 short arm, proximally to D8S35.

The authors wish to thank Dr S Wood, Vancouver, and Dr M Rocchi, Bari, for providing probes 11E1 and pZ8.4. They also thank Professor M Fraccaro for helpful discussions. This work was supported by Telethon-Italy (project "Correlation between cerebral malformations, neurological impairment and extension of chromosomal duplication in patients with inv dup (8p)") and by the Associazione Italiana per lo Studio delle Malformazioni, ASM Milano.

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