Letters to the Editor

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Familial pericentric inversion of chromosome 1 (p36.3q23) and Bardet-Biedl syndrome

EDITOR—We report a familial pericentric inversion (PEI) of chromosome 1 (p36.3q23) in six patients with Bardet-Biedl syndrome (BBS). The proband (III.6, fig 1), an 11 year old Libyan female, was referred for chromosomal analysis because of obesity, polydactyly, and poor vision. She was clinically diagnosed as having BBS. After clinical examination and investigations of her family members, another two sibs (III.3 and III.8) and three maternal cousins (III.10, 11, 12) were ascertained as having BBS. The clinical findings in these patients are presented in table 1. Chromosomal analysis of 100 metaphase spreads using Giemsa trypsin (GTG) banding showed that the proband had PEI (1) (fig 2) with karyotype 46,XX,inv(1)(p36.3 q23). The family members with BBS (III.3, 8, 10, 11, 12) all had the same inversion with the same breakpoints, which was inherited from the phenotypically normal proband's mother (II.4) and her sister (II.6) (fig 1).

Pericentric inversions (PEI) have been observed in all chromosomes except chromosome 20. Different chromosomes and breakpoints are involved non-randomly.[1] The prevalence of inversions varies between 0.3 and 5.0 per 1000. It was estimated to be 1.4/1000 by the French collaborative study based on the analysis of 305 cases of inversions among 221 263 karyotypes.[2] The present study reports the first case of PEI (1) with breakpoints at (p36.3q23) and it is one of the largest reported inversions (involving about 64% of the total length of chromosome 1). Most of the reported cases of PEI (1) were ascertained because of male infertility.[3–5] These inversions involved different breakpoints with no clear relationship between the specific chromosomal breakpoints and the degree of spermatogenic failure.[6] A few cases of PEI (1) have been associated with multiple congenital anomalies or developmental delay, such as Goldenhar syndrome (p13q21),[7] Fanconi anaemia (p13q21),[8] mucopolysaccharidoses (p13q23),[9] and microtia, cleft palate, and meningomyelocele (p36.3q42).[10]

BBS is an autosomal recessive disorder characterised by mental retardation, obesity, pigmentary retinal dystrophy, postaxial polydactyly, and hypogenitalism. Hypertension, diabetes mellitus, and renal and cardiac abnormalities have frequently been observed.[11] Previous clinical suggestions of heterogeneity in BBS were recently confirmed by the identification of four different chromosome loci linked to the disease on chromosomes 3p13 (BBS3), 11q13 (BBS1), 15q22.3q23 (BBS4), and 16q21 (BBS2, MIM 209900 and 209901),[12–14] but some families failed to show linkage to any of these loci.[15] Beales *et al*[15] came to the conclusion that the lack of established linkage in four consanguineous families (28% of their study) from the Middle East and Asia to any of the four BBS loci suggests the presence of at least a fifth BBS locus, and it would seem that locus distribution is subject to regional variation. They also added that the most promising strategy for identifying BBS genes is to adopt a combined candidate gene and positional cloning approach and such efforts may be enhanced by a chance finding of a gross rearrangement. The correlation between PEI (1) and BBS in the patients in the present study may be coincidental

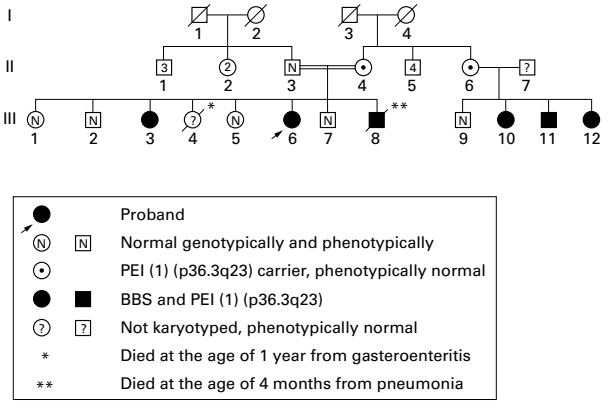

Figure 1 Pedigree of the family with PEI (1).

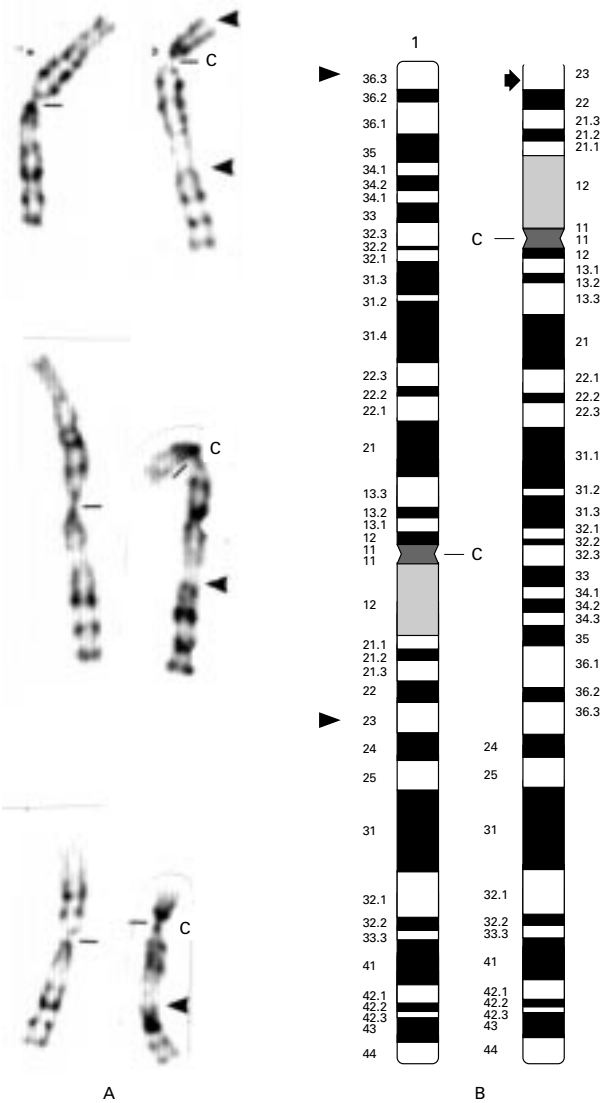

Figure 2 Partial karyotype of the proband showing the pericentric inversion 1 involving breakpoints (p36.3q23). C=centromere.

Table 1 Clinical findings of the BBS patients in the present study (heterozygous PEI (1) (p36.3q23))

Clinical finding	Proband III.6	Proband's sibs III.3	Proband's sibs III.8‡	Proband's cousins III.10	Proband's cousins III.11	Proband's cousins III.12‡
Age	11 y	17 y	4 mth	5 y	3 y	1 y
Sex	F	F	M	F	M	F
Obesity	+	+	?	+	+	+
Bilateral hexadactyly						
Hands	++	++	++	++	++	++
Feet	+L	++	++	+L	+R	++
Mental retardation	+	+	?	+	?	?
Pigmentary retinopathy	+	Blind	?	+	+	?
Nystagmus	−	+	−	−	−	−
Hypogonadism	?	?	?	?	+	?
Renal anomalies*	−	−	Atrophied left kidney	−	−	−
Cardiac defects†	VSD	−	−	−	VSD	−
Hirsutism	+	+	−	−	−	−

? = not assessed, L = left side, R = right side, + = present, − = absent.
*Diagnosed by abdominal ultrasonography. †Diagnosed by echocardiography. ‡Under 2 years of age it is difficult to evaluate night blindness, and developmental delay is usually mild so that the diagnosis is based on the existence of polydactyly.[14]

and DNA linkage analysis is required to investigate a possible BBS gene locus on chromosome 1. Tommerup,[16] although all the 22 cases of BBS of his study had normal karyotypes, reported how in several Mendalian disorders specific constitutional chromosome rearrangements have facilitated the localisation of the relevant locus. Familial translocations and inversions can predispose to the formation of uniparental disomy, whereby autosomal recessive mutations can be reduced to homozygosity.[17]

In conclusion, our observation of PEI (1) and review of published reports suggest that PEI carriers do not appear to be free of risks of abnormalities and caution is recommended when counselling. It also emphasises the importance of cytogenetic investigation in a familial Mendelian disorder to exclude possible chromosomal abnormalities and to understand the significance of familial inversions/variants or polymorphisms.

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Duplication of 8p with minimal phenotypic effect transmitted from a mother to her two daughters

EDITOR—There are many reports of partial trisomy 8p in the offspring of balanced translocation carriers.[1–3] However, in these cases the effect of the partial trisomy is usually masked by the phenotypic consequences of partial monosomy of the partner chromosome.

Partial trisomy for 8p also results from the well known inverted duplication of 8p usually described as inv dup(8)(p11.2p23); this rearrangement, however, also results in partial monosomy for the segment 8p23.1→8pter.[4–6] The inv dup(8) is associated with a well defined clinical syndrome,[5–9] the childhood phenotype of which includes neonatal feeding problems, hypotonia, structural brain abnormalities, facial dysmorphology, malformed, low set ears, and severe developmental delay. In older patients the facial traits are less characteristic, mental retardation is profound, and spastic paraplegia and orthopaedic problems are frequent. It is known that patients with deletion of 8p23→pter as their sole chromosome abnormality have a near normal phenotype with only mild mental retardation and minimal dysmorphology.[10–12] The phenotypic findings of inv dup(8)(p11.2p23) are therefore considered to arise primarily as a result of the duplicated segment 8p21.

More recent reports have described smaller, more distal duplications of 8p in which there is no evidence of any monosomic segment.[13–17] Dhooge *et al*[13] described the transmission of a duplication dup(8)(p22→p23.1) or (p21.3→p22) from a mother to her two children. The associated clinical features were mild mental retardation, short stature, and hypertelorism. Engelen *et al*[14] described a similar case of transmission of partial trisomy 8p resulting from dup(8)(p22→p23.1) from a mother to her two sons. In this family, mental retardation was mild and there was no growth retardation, only the mother showed slight facial dysmorphology. Barber *et al*[15] recently described seven families with small duplications of 8p23.1 and reviewed five families previously reported in abstract form.[16 17] In 10 of the 12 families and 25 of 27 duplication carriers, no phenotypic abnormality was recorded and it

Figure 1 Photograph showing the facial appearance of the proband, patient 1 (on right), with her mother and younger sister.

was suggested that duplication of 8p23.1 should be considered a cytogenetic anomaly of no established significance. Barber *et al*[15] described fluorescence in situ hybridisation (FISH) studies with YAC HTY3020 which suggested that this apparent duplication may involve amplification of a small part of 8p23.1.

In this report we describe a mother and her two daughters (fig 1) with minimal dysmorphology and no significant mental retardation, all of whom had duplication of chromosome region 8p23.1. The chromosomes have been studied with G banding and FISH with whole chromosome paint, a subtelomeric probe for 8p, and YAC HTY3020 which maps to 8p23.1.[18 19]

Patient 1 is the proband who was born in 1995 at term after an uneventful pregnancy and delivery. Birth weight was 3350 g, length 50 cm, and occipitofrontal circumference (OFC) 32 cm. It was noted that she had a smallish head with very mild facial dysmorphism. Karyotyping was requested and chromosome analysis showed an abnormal chromosome 8 with extra material on the short arm. She is the first child of non-consanguineous parents. The healthy father was 32 years old and the mother 30 years old at the time of the proband's birth. When seen in 1998 at 3 years of age, her weight was 11.6 kg (25th centile), height 88.5 cm (25th centile), and OFC 46 cm (10th centile). She was noted to have bilateral clinodactyly and prominent medial epicanthi. Developmental milestones were within normal limits.

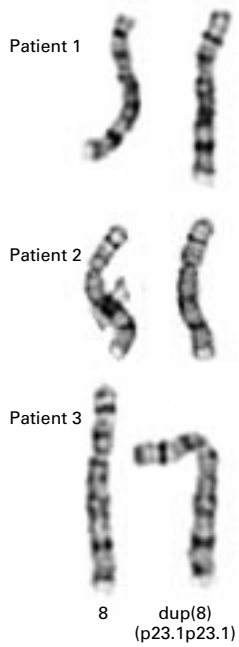

Figure 2 Partial karyotypes from the three patients showing extra material on the end of 8p.

Patient 2 is the younger sister of patient 1. She was born in 1996 at term, following an uncomplicated pregnancy with a birth weight of 3720 g, length 48 cm, and OFC 33 cm. It was noted that she had a smallish head with mild facial dysmorphism and upward slanting palpebral fissures. She also had clinodactyly, bilateral simian creases, and deep skin creases between the first and second toes. She was karyotyped and found to have an abnormal chromosome 8 which was identical to that of her sister. In 1998 at the age of 14 months, she was referred to the Paediatric Clinic at KK Hospital, Singapore for assessment of microcephaly. The developmental assessment was satisfactory; she gained head control at 3 months, sat at 8 months, was walking unsupported at 1 year, and started saying single words at the same time. When seen aged 14 months, her weight was 10 kg (50th centile), height 77 cm (50th centile), and OFC 42 cm (0.7 cm less than the 3rd centile). She was noted to have a small, flattened nose, prominent medial epicanthi, and bilateral clinodactyly. No other dysmorphic features were noted.

Patient 3 is the healthy mother of patients 1 and 2. She had regular education and worked as a sales clerk. Her father, aged 60 years, is healthy and her mother died of a "stroke" at the age of 50. She has four sisters and two brothers of normal intelligence. Her first pregnancy resulted in a spontaneous abortion at 2 months' gestation. She was karyotyped after the birth of her first child and found to be carrying the same abnormal chromosome 8. On examination in 1998 she was noted to have bilateral clinodactyly and no other dysmorphic features.

Karyotyping was performed on G banded metaphase chromosomes after routine PHA stimulated peripheral blood culture. Synchronisation by thymidine block[20] was used to obtain high resolution chromosomes. Chromosome analysis of the three patients showed in each case a karyotype with extra material on the end of the short arm of one chromosome 8 (fig 2). The father of patients 1 and 2 had a normal male karyotype.

FISH with a whole chromosome paint for chromosome 8 (WCP 8) (Cytocell) was performed following the manufacturer's instructions. The paint hybridised over the total length of both copies of chromosome 8 and not to any other chromosomes, showing that the extra material was derived from chromosome 8.

FISH with a subtelomere probe mapping to locus D8S596 (Oncor) which hybridises to band 8p23→pter was performed according to the manufacturer's instructions. Results with this probe showed two sets of signals in each of the 20 cells examined, one set of signals on the tip of the normal 8p and one set on the tip of the abnormal 8p. Our interpretation is that both the abnormal and the normal 8 have one copy of the locus D8S596. These results suggest that the rearrangement is interstitial and that telomeric sequences are not involved.

YAC HTY3020 which maps to 8p23.1 was hybridised as previously described[15] to metaphases from patients 2 and 3 (fig 3). It showed significant contrast in signal intensity between the homologues of chromosome 8, suggestive of amplification in the abnormal chromosome.

We have described a family showing transmission of a small duplication, dup(8)(p23.1p23.1) from a mother to her two daughters. G banded analysis suggested that the abnormality was a duplication, and application of WCP 8 confirmed that the extra material was indeed derived from chromosome 8. Application of the 8p subtelomere probe mapping to locus D8S596 suggested that the 8p telomeric sequences were not deleted and that the additional material

Figure 3 Dual colour FISH with YAC HTY3020 (red signals) and alphoid centromeric probe D8Z2 (green signals) to metaphase chromosomes from patient 2. Note the contrast in signal strength which was consistently found in each cell examined.

was interstitial. FISH with YAC HTY3020 confirmed the involvement of 8p23.1, the contrast in signal strength between homologues suggesting the possibility of amplified sequences.

The duplication we report is smaller and extends more distally than those described by Dhooge *et al*[13] and Engelen *et al*[14] (fig 4). In the family described by Dhooge *et al*,[13] the duplication of 8p was characterised by G banded analysis and FISH with a whole chromosome paint. In the family described by Engelen *et al*,[14] the duplication of 8p was confirmed by FISH with cosmid probes specific for the region 8p23.1→pter. There was no cytogenetic evidence for deletion of the telomeric sequences in either of these families, neither was it possible to confirm whether the duplication was inverted or direct, although Engelen *et al*[14] favoured the interpretation of a direct duplication. Barber *et al*[15] showed gain of distal 8p material by comparative genomic hybridisation which was localised to band 8p23.1 using FISH with YAC HTY3020. The duplication which we report appears identical to the cases described by Barber *et al*[15] and the karyotype in all three patients has been interpreted as 46,XX,dup(8)(p23.1p23.1).ish dup(8)(p23.1p23.1)(HTY3020++). However, involvement of distal p22 or proximal p23.2 cannot be excluded, especially as this might account for the fine G dark band seen midway between p22 and p23.2 on the duplicated chromosome.

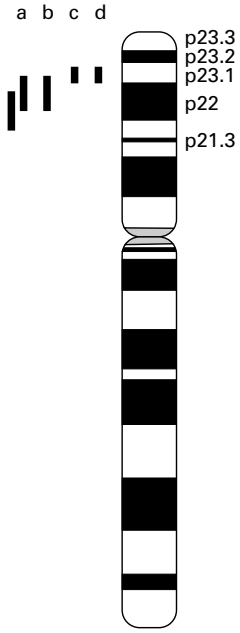

Figure 4 Idiogram of chromosome 8 showing the duplications of Dhooge et al[13] (a) with two bars for the alternative interpretations, Engelen et al[14] (b), Barber et al[15] (c), and Gibbons et al (this report) (d).

The clinical features noted in the three patients of Dhooge *et al*[13] included mild mental retardation, short stature, and hypertelorism, whereas Engelen *et al*[14] reported mild mental retardation as the only constant finding. Barber *et al*,[15] reviewing 27 carriers of duplication 8p23.1, reported only two subjects with phenotypic abnormality (short stature and developmental delay) and concluded that dup(8)(p23.1) is a cytogenetic anomaly of no established significance. The family we describe shows mild phenotypic features with no mental retardation. Microcephaly, small flattened nose, and prominent medial epicanthi were seen in patient 2 at the age of 14 months. Patient 1 at the age of 3 years showed only prominent medial epicanthi and bilateral clinodactyly. The mother of the two girls had no facial dysmorphology or mental retardation; the only feature seen in adulthood appeared to be bilateral clinodactyly. It would therefore appear that this small duplication has minimal, if any, phenotypic effect and may be unrelated to the slight dysmorphology seen in this family.

Small distal duplications of 8p are an entity quite distinct from the inv dup(8)(p11.2p23) syndrome. The inv dup(8)(p11p23) results in duplication of 8p21→p22 and a clinically recognisable multiple congenital anomalies/mental retardation syndrome with severe clinical effect and reduced reproductive fitness such that transmission does not occur. In contrast, the smaller and more distal duplications of 8p22 and 8p23 result in a much milder phenotype with unaffected reproductive fitness. If the duplication extends no further than 8p23.1 it seems unlikely that there is any associated clinical effect and it should probably be considered a cytogenetic variant of no clinical significance. However, caution is needed in interpretation as duplications extending proximally into band 8p22 are associated with mental retardation.

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Cloverleaf skull anomaly and de novo trisomy 4p

EDITOR—Cloverleaf skull deformity (CS, Kleeblattschaedel, MIM 148800) is a severe form of craniosynostosis rarely associated with chromosomal aberrations.[1 2] Recently we observed a newborn male presenting with multiple congenital anomalies including a cloverleaf skull and a de novo partial 4p trisomy. He was a 12 day old male, born at 35 weeks of gestation to healthy, non-consanguineous parents. Respiratory distress was present at birth. At 12 days, his weight was 2650 g (5th centile), length 45 cm (<5th centile), and head circumference 30.5 cm (<<5th centile). On clinical evaluation, multiple congenital anomalies were observed, including cloverleaf skull, orbital hypoplasia with proptosis, hypertelorism, right iris coloboma, depressed nasal bridge, anteverted nostrils, low set ears, wide superior alveolar ridge, and pointed chin. Furthermore, inverted nipples, camptodactyly of the hands, club feet, overlapping toes, shawl scrotum, cryptorchidism, and generalised hypertonia were noted. Skeletal *x* rays showed vertebral anomalies, including hypoplasia of the 5th cervical vertebra, the presence of hemivertebrae of the lumbar spine, and eleven ribs bilaterally. Echocardiography showed a mild atrial septal defect and PDA. Cranial 3D CT scan (fig 1) showed protruding temporal bones and fusion of the coronal, lambdoidal, and temporoparietal sutures with temporoparietal bone ridges. The craniosynostosis partially spared the sagittal and metopic sutures. MRI showed asymmetrically enlarged temporal horns and a hypoplastic corpus callosum. Renal scan was normal; the testes were found by ultrasound inside the inguinal canal bilaterally. EEG was characterised by mild brain electric hypoactivity. Visual evoked potentials were delayed. At 6 months of age, the patient died of cardiac and respiratory failure.

Standard R banding of the patient's chromosomes disclosed the presence of supernumerary chromosomal bands on 2q. This segment was later identified as part of chromosome 4 by FISH, carried out according to Pinkel *et al*[3] and using a whole chromosome 4 painting library (Oncor) (fig 2, left). Prometaphase RHG banded chromosomes, prepared as described elsewhere,[4] defined the extension of the 4p trisomic region as a 4p15.1→pter segment (fig 2, right). No apparent deletion of 2q bands was observed. The patient appeared trisomic for the distal part of 4p, without any apparent deletion of the 2q region apart from the probable loss of the 2q telomere. The patient's karyotype was 46,XY,−2,+der(2)t(2;4)(q37.3;p15.1). Paternal and maternal karyotypes were normal.

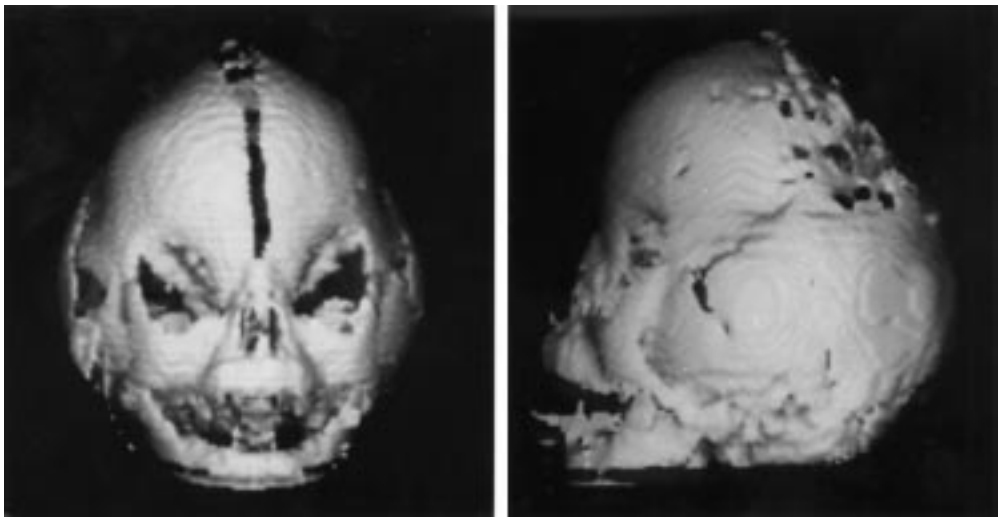

Figure 1 Cranial 3D CT scan of the patient, front and lateral view.

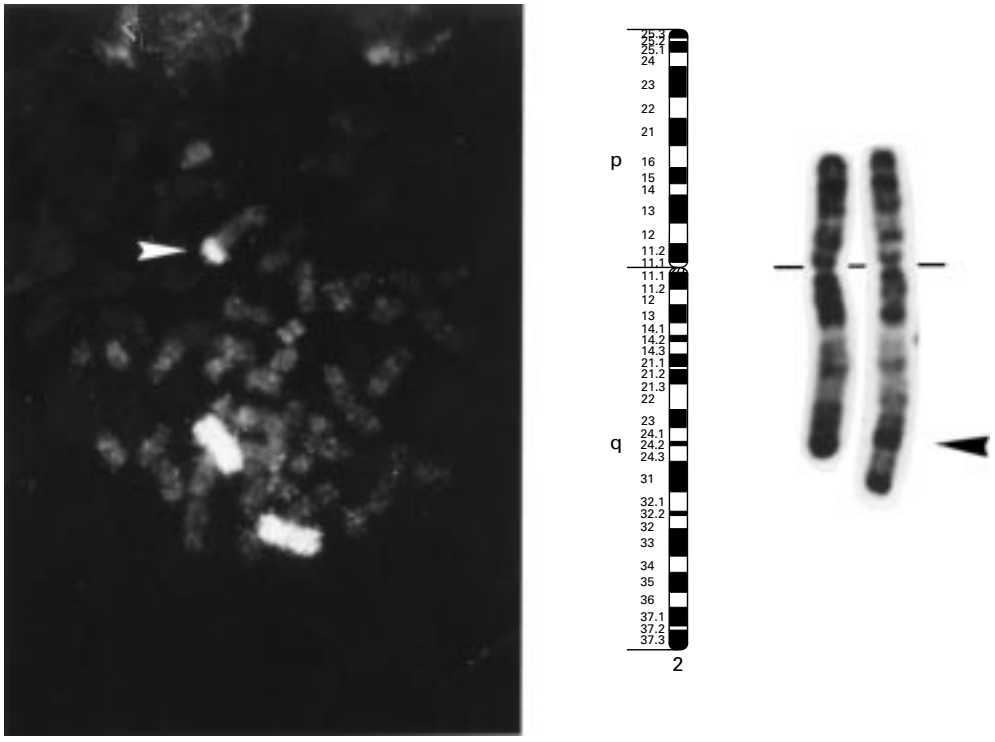

Figure 2 FISH performed in the patient using chromosome 4 painting library: arrowhead indicates part of chromosome 4 translocated onto chromosome 2 (left). RHG banded partial karyotype of the proband: chromosome translocation 2;4 (right).

Molecular analysis (data not shown) performed using PCR on DNA from a lymphoblastoid cell line of the proband and from the parents' peripheral lymphocytes showed that the patient was monosomic for a distal 2q telomeric marker, D2S125 (GDB ID:187994),[5] and heterozygous for a $(AC)_n$ repeat of the ALPP gene (PLAP, GDB ID:180439),[5] which maps to 2q37.1. On the other hand the analysis of the 4p markers disclosed the presence in the patient of three alleles for both a HOX7 $(CA)_n$ repeat (GDB ID:176982)[5] and D4S126 (GDB ID:197926),[5] respectively mapping to 4p16.1 and 4p16.3, the extra allele deriving from an error in paternal meiosis I.

Both environmental and genetic causes have been proposed for the pathogenesis of CS[1] but only three cases of CS have been found associated with cytogenetic aberrations so far. The first two cases reported CS associated with partial trisomy of 15q and 13q,[1] whereas, more recently, Van Allen *et al*[2] reported a child with multiple congenital anomalies, including CS, and an unbalanced 2q;15q translocation. This resulted in trisomy for the 15q26→qter region, and the authors hypothesised a cluster of genes crucial for the closure of cranial sutures located on chromosome 15q. Our patient and that reported by Van Allen *et al*[2] seem to share the same deletion of band 2q37. Even if microcephaly has been observed in some patients with 2q terminal deletion, this cytogenetic aberration has never been correlated with cloverleaf skull anomaly.[6 7] Furthermore, these patients exhibited a breakpoint more proximal than that found in our patient,[6] whereas deletions mapped distally to 2q37.1 were associated with normal cranial circumference or even macrocephaly.[7]

On physical examination, our patient exhibited many clinical features found in the classical trisomy 4p syndrome,[8] including iris coloboma, depressed nasal bridge, pointed chin, vertebral and rib abnormalities, camptodactyly, club feet, cryptorchidism, generalised hypertonia, cardiac defect, and respiratory problems, which can be explained by the presence of trisomy of a large part of the 4p region. However, his overall phenotype appears more severe than that described in the classical trisomy 4p syndrome. Specifically, CS is an extreme form of craniosynostosis compared to the variable pattern of sutural fusion observed in the classical trisomy 4p syndrome, which ranges from protruding glabella (30%) or prominent continuous supraorbital ridge fused across the glabella (23%), to microcephaly, prominent forehead, or other less specific cranial abnormalities, which have been described with a variable degree of severity even within the same family members.[8]

Several of the classical findings of the trisomy 4p syndrome can be caused by the duplication of about 2.1 Mb extending from the telomere and involving the 4p16.1→16.3 region.[9] In order to understand how cranial involvement is a main feature of the trisomy 4p syndrome, particularly in our patient, some of the genes located in the critical region should be taken into account. The gene encoding the fibroblast growth factor receptor 3 (FGFR3) maps to 4p16.3[10] and mutations within its different domains have recently been associated with syndromic and non-syndromic craniosynostosis.[10] A constitutive FGFR3 activation seems to be the mechanism underlying the craniosynostosis.[10] In this respect, three copies of the wild type FGFR3 gene might result in a greater production of protein, thus mimicking the effect of FGFR3 mutations as observed in some craniosynostoses.

The same activating mechanism could be envisaged for MSX1, which maps to 4p16.1. Even if MSX1 has never been associated with craniosynostosis, MSX2, one of its homologues,[11] has been found associated with the Boston type of craniosynostosis through a gain of function mutational mechanism.[12] Furthermore, MSX1 deficiency has been recently associated with failure of development of alveolar bone and teeth in man[13]; overexpression of MSX1 could result in hypertrophy of the alveolar process, as observed in our patient.

Further studies on both FGFR3 and MSX1 will probably clarify the effective involvement of these genes in the determination of craniosynostosis in the trisomy 4p syndrome.

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