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ORIGINAL INVESTIGATION

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ICF syndrome (immunodeficiency, centromeric instability and facial anomalies): investigation of heterochromatin abnormalities and review of clinical outcome

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Abstract A further patient with the ICF syndrome (immunodeficiency, centromeric heterochromatin instability of chromosomes 1, 9 and 16 and facial anomalies) is described. This case is the second to be reported with consanguinity of the parents. This lends support to the theory of autosomal recessive inheritance. The features of the 15 published cases are reviewed. The clinical and cytogenetic characteristics of the syndrome are discussed, and new evidence provided as to the role of centromeres and centric heterochromatin in the production of chromosome aberrations. Correspondence with other authors has made possible a review of the clinical outcome in this condition.

Introduction

Constitutional chromosomal disorders usually consist of rearrangements which are both stable and identical in all cells. Exceptions include mosaicism and a small number of disorders characterised by variable chromosomal instability and rearrangement. Examples of the latter are Bloom's syndrome and Fanconi anaemia, both of which also involve immunodeficiency to some extent. More recently the ICF syndrome, comprising immunodeficiency, centromeric heterochromatin instability and facial anomalies, has been described and 14 cases published to date. Although autosomal recessive inheritance has been suggested, as yet only one other case with parental consanguinity has been reported.

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Materials and methods

Clinical investigations

The proband was born in June 1987 by caesarian section for pregnancy-induced hypertension after 38 weeks gestation, the first child of a 22-year-old mother and 36-year-old father. The pregnancy had been uneventful except for maternal chickenpox in the first trimester. The baby was of very low birth weight (1300 g) and small for gestational age. Oxygen therapy was required for mild respiratory distress in the first 24-h of life; perinatal course was otherwise normal. The proband's grandfathers were twin brothers and her grandmothers were sisters, making her parents first cousins.

She was hospitalised at the age of 25 months following 10 days of night cough and increasing wheeze. Her parents then commented that they had noticed her breathing to be laboured on frequent occasions since birth, but there was no history of any other respiratory symptoms or of gastrointestinal disturbance. On examination she was hypotrophic with a triangular face, upturned nose with flattened bridge, frontal bossing and sparse, dry hair (Fig. 1).

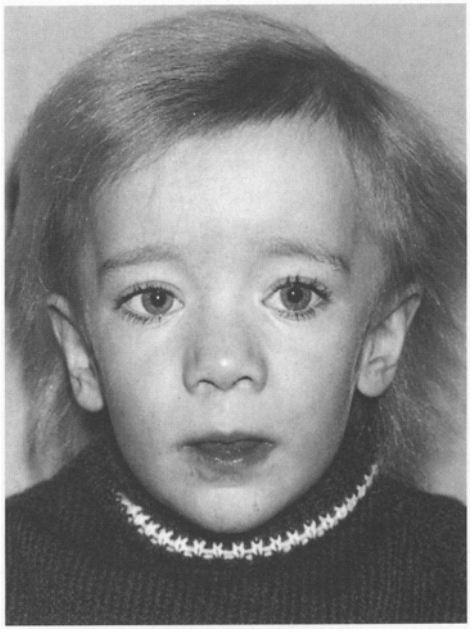

Fig. 1 Facial appearance of proband

Weight, height and head circumference were all below the third centile for age. She was tachypnoeic with widespread expiratory rhonchi. Gross motor development was normal but expressive speech was impaired, with a vocabulary of only three words. Her failure to thrive was investigated and the following normal results were reported: blood counts, biochemistry, liver enzymes, thyroid function, sweat electrolytes, jejunal biopsy and urine culture. Immunoglobulin levels were all decreased; IgA 0.07 g/l (normal range 0.26–1.47), IgG 1.5 g/l (5.0–13.0), IgM 0.22 g/l (0.36–1.92). The results of chromosome analysis are detailed below. She had not been treated with antibiotics prior to collection of the blood sample.

There were two further admissions to hospital for treatment of acute infection during the following 6 months. Pneumocystis carnii pneumonia was treated with high dose co-trimoxazole. Leucopaenia necessitated a change of treatment to nebulized pentamidine. Respiratory syncytial virus pneumonia was treated successfully with small-particle aerosol ribavarin. Intermittent intravenous immunoglobulin therapy was commenced at an initial dose of 3 g every 3 weeks to maintain IgG levels above 5.0 g/l. Following attainment of therapeutic immunoglobulin levels, pentamidine was discontinued. At age 3.5 years she was functioning developmentally at a level of 2.5 years. She continues to measure below the third centile for weight and height.

Cytogenetic studies

Lymphocytes cultured from the initial blood sample twice failed to yield metaphases adequate for analysis and a repeat sample was requested; this gave a satisfactory yield of reasonable quality metaphases using standard techniques. Sister chromatid exchange (SCE) investigations were undertaken using the alkylating agent mitomycin C. Chromosomes were prepared for and examined by scanning electron microscopy as previously described (Sumner 1991).

Results

All metaphases examined had a female karyotype, with 70% showing no abnormality (46,XX). However, in the remaining 30%, a variety of chromosome aberrations was observed, all involving the heterochromatic regions of chromosomes 1 and 16, and to a lesser extent chromosome 9 (Fig. 2). The unusual nature of these findings prompted a request for a further sample, and for samples from both parents to be provided at the same time. Both parents were shown to have apparently normal karyotypes of appropriate sex and with no evidence of aberrations of chromosomes 1, 9 and 16, although these were again detected in cultures from the child. Cultured skin fibroblasts from the child showed no evidence of any abnormality.

After discussions with the clinicians, a tentative diagnosis of Bloom's syndrome was suggested, although it was noted that some of the configurations observed were not entirely typical of that disorder. It was agreed that sister chromatid exchange investigations would be undertaken but these were deferred because the child was, at that time, on a high level of antibiotic therapy for recurrent infection. Following a careful review of published cases it was decided that the available clinical and cytogenetic data were consistent with a diagnosis of ICF syndrome in our patient. We examined a variety of parameters during this investigation, including the possibility of a link between time from first referral and the aberration rate/type (Table 1), the effect of medium composition on aberration rate/type (Table 2) and the effect of time in culture on aberration rate and type (Table 3). The aberration rate was always lowest in RPMI and higher in the low-folate Iscoves and E199 media. Only time in culture showed any profound effect on aberration rate with a striking increase in the rate of associations in the 3-day as compared with the 2-day cultures, although the rate of heterochromatic "stretching" was increased only minimally.

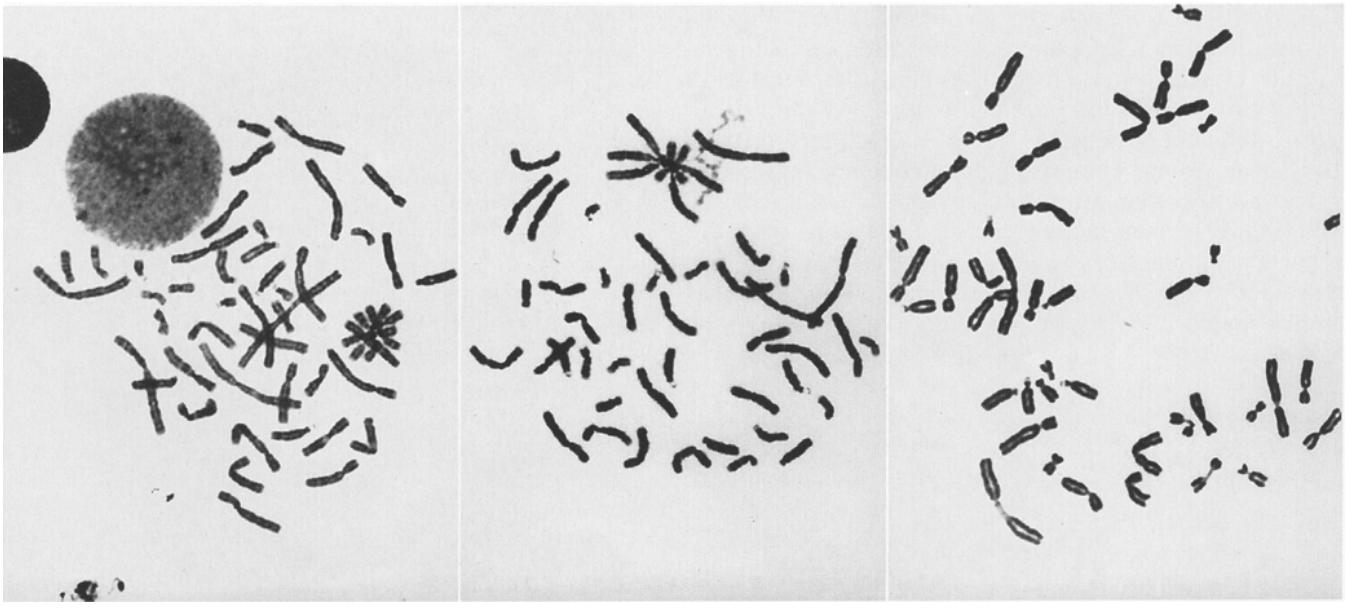

Fig. 2 Metaphases showing: (left) association between the homologues of chromosome 16 with duplication of long and short arms to give a "starburst" effect; (centre) association of chromosomes 1 and 16 at the heterochromatic regions; and (right) undercondensation or "stretching" of the heterochromatic regions of chromosomes 1 and 16 and, to a lesser degree, of chromosome 9

Table 1 Effect of time of sampling on abnormality rate

Age of patient (months)	26	27	29	35
Type of abnormality (%)				
Stretching of qhs	31	45	52	30
Association of qhs	29	17	25	20
Total abnormal cells	60	62	77	50

Table 2 Effect of type of medium on abnormality rate

Type of medium	RPMI	RPMI+ MTX	Iscoves	E199
Type of abnormality (%)				
Stretching of qhs	38	30	47	43
Association of qhs	12	30	29	23
Total abnormal cells	50	60	76	66

Table 3 Effect of time in culture on abnormality rates

Time in culture	2 days	3 days
Type of abnormality (%)		
Stretching of qhs	48	52
Association of qhs	3	25
Total abnormal cells	51	77

Table 4 Sister chromatid exchange rates and the effect of exposure to mitomycin C

Concentration of mitomycin C	Patient	Control
0	11	10
4.2×10^{-8} *M*	17	22
4.2×10^{-7} *M*	37	76

qhs: long arm heterochromatin, MTX: methotrexate

Sister chromatid exchange (SCE) investigations, summarised in Table 4, indicate that the SCE rate/cell was comparable between child and control, and that the increase in SCE rate with increasing concentrations of mitomycin C was lower in the patient than in the control. Studies using bromodeoxyuridine showed a normal response. Parental SCE rates were also within control limits. These findings were against the tentative diagnosis of Blooms's syndrome.

The cytogenetic phenomena visible by light microscopy were apparent in much greater detail using scanning electron microscopy (Fig. 3a). It was clear that in multibranched configurations the heterochromatic segments are intimately fused and not merely in close contact (Fig. 3b, c) while Fig. 3d shows normal chromosomes with blocks of extended heterochromatin lying closely adjacent to one another but clearly not fused. Extended heterochromatin is commonly observed (Fig. 3e, f).

Discussion

Fragility of the paracentromeric heterochromatin of one or more of chromosomes 1, 9 and 16 resulting in multibranched chromosomes and interchanges between homologous and non-homologous chromosomes and associated with immunodeficiency, has now been reported in 15 patients. These have been reviewed to form a phenotypic and cytogenetic overview of the syndrome (Table 5).

The sex ratio is 8:7 (M:F). Mean maternal age is 27 years (for 11 patients), mean paternal age is 36.5 years (for six cases including one 68-year-old father). Mean birth weight is 2.5 kg (11 patients), delivery has usually been at or near term.

All but one of the patients have had facial anomalies. Manifestations most often recorded are hypertelorism, a flat nasal bridge, epicanthic folds, protrusion of the tongue and micrognathia. Mental retardation is variable, from severe neurodegeneration to special educational needs without any delay in motor function. Our patient is typical in that speech development is delayed. There is usually growth retardation.

Immunodeficiency usually manifests as respiratory or gastrointestinal upset. In all but three cases (Tiepolo et al. 1979; Gimelli et al. 1993), IgA and IgM levels were low. Correspondence with other authors (Hulten 1978; Fryns et al. 1981; Maraschio et al. 1988; Turleau et al. 1989) has provided further information on the outcome of several cases. Prognosis is variable, but clinical course appears to have been favourably affected by immunoglobulin transfusion and by the absence of combined-type immunodeficiency (Table 6).

Ours is the second report in which parental consanguinity is documented. In two previously reported cases (Tiepolo et al. 1979; Valkova et al. 1987) siblings had died from infection but no chromosomal analysis had been carried out. There were unexplained deaths following pyrexial illness in early childhood in the extended family in one case (Fasth et al. 1990). Two adult affected siblings were reported recently (Gimelli et al. 1993). These cases suggest autosomal recessive inheritance. The equal sex preponderance and the usually normal parental phenotype and genotype lend further support to this theory.

Chromosomes 1, 9 and 16 were all affected in nine of the cases, only 1 and 16 in four and only 1 in two. In two cases chromosome 2 was also involved, and chromosome 10 in one. The common abnormalities are undercondensation, chromatid and chromosome breaks, somatic pairing and interchanges between homologous and non-homologous chromosomes. Also noted were deletions, triradials and multibranched chromosomes. The possibility of prolonged antibiotic therapy being responsible for an increased frequency of branched configurations was excluded by the case of Howard et al. (1985) and the present case, when chromosomes were first examined before the administration of any antibiotics. Parental chromosomal abnormalities were noted in only one case (Carpenter et al. 1988), amounting to heterochromatin abnormalities in 5% and 2.7% of the metaphases of the mother and father, respectively. Chromosomal analysis of skin fibroblasts has revealed abnormalities in four of the previously published cases (Howard et al. 1985; Fasth et al. 1990; Gimelli et al. 1993). These were slight stretching and despiralisation of the heterochromatic region of chromosome 1.

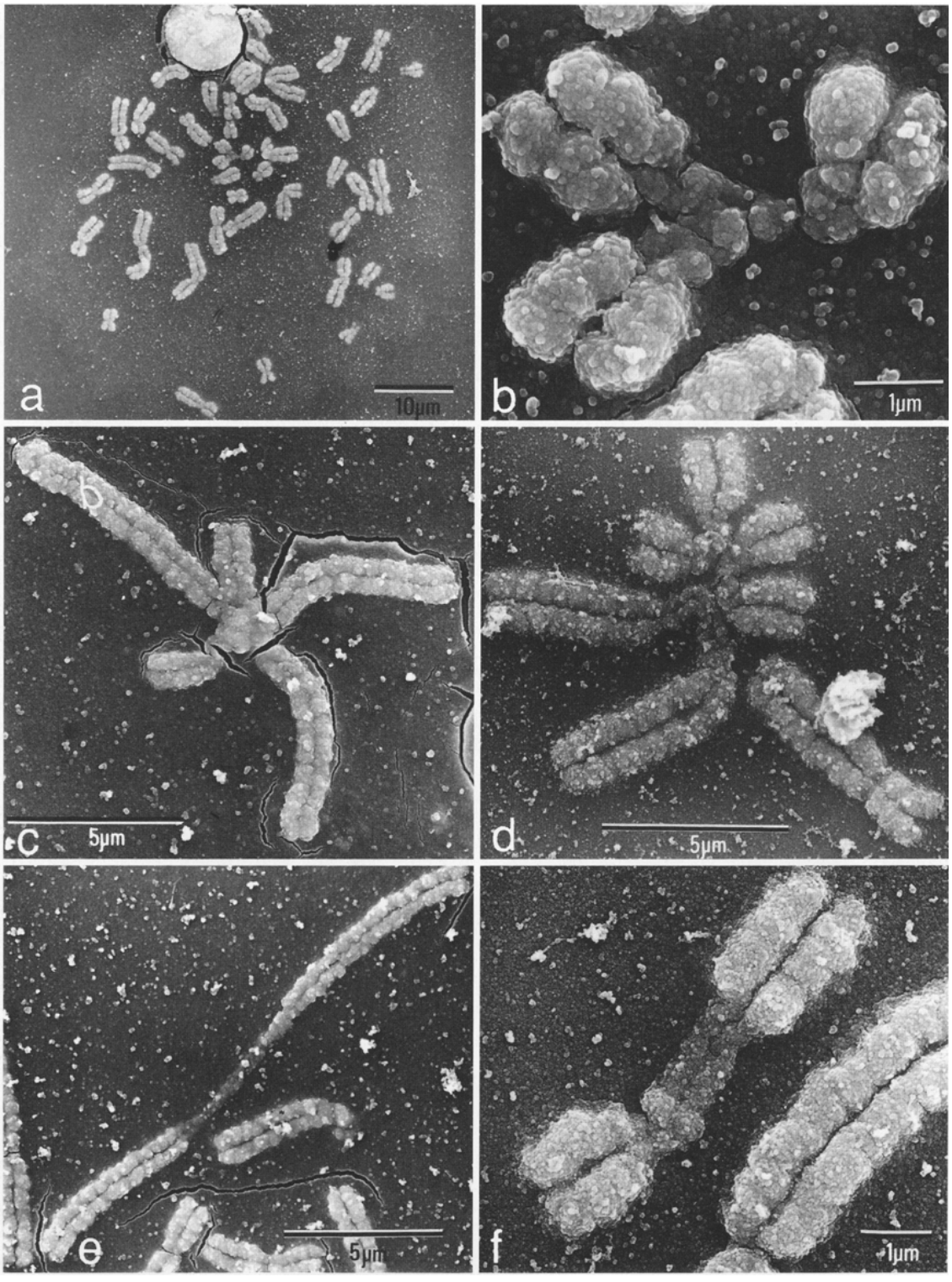

Fig. 3 a–f Scanning electron micrographs showing: (**a**) a whole metaphase containing a triradial configuration; (**b**) the triradial configuration from the metaphase in (**a**); (**c**) a pentaradial configuration; note that there are no gaps between the individual chromosome arms and the central block of material (the obvious cracks are an artefact of preparation); (**d**) chromosomes with extended heterochromatin lying adjacent and parallel, but not fused, possibly representing an intermediate step in the fusion process; (**e**) a chromosome 1 with greatly extended heterochromatin; and (**f**) a chromosome 16 with extended heterochromatin, at one end of which is a condensed block probably representing the centromere itself

Cytogenetic findings in our patient are very similar to those previously described. Only conventional staining methods have been applied in the investigation of most previous ICF patients so that the opportunity to employ SEM offered a new dimension and yielded some interesting information concerning the distinction between the behaviour of paracentomeric heterochromatin in the multibranched configurations and in those situations where chromosomes were in close association only. Gimelli et

Table 5 Phenotypic characteristics of published cases

Author	Sex	Birth weight (kg)	Age of mother	Age of father	Appearance/abnormalities	Chromosomes	Immunodeficiency
Hulten (1978)	M	?	?	?	Facial dysmorphism	1	Combined
Tiepolo et al. (1979)	M	3.4	26	37	Flat face, epicanthus, low nasal bridge	1,9,16	Combined
Fryns et al. (1981)	F	2.6	26	26	Epicanthus, hypertelorism, macroglossia	1,2,9,16	Ig
Howard et al. (1985)	M	2.6	?	?	Micrognathia, cleft palate, epicanthus, hooded prepuce	1,16	Ig
Valkova et al. (1987)	F	3.4	23	29	Umbilical hernia, micrognathia, hydrocephalus	1,2,9,16	Combined
Maraschio et al. (1988)	F	3.1	37	68	Hypertelorism, flat nasal bridge, low-set ears, protruding tongue	1,9,16	Ig
Carpenter et al. (1988)	F	3.5	?	?	Hypertelorism, frontal bossing	1,9,16	Ig
Turleau et al. (1989)	M	2.5	28	23	Macroglossia, hypertelorism, epicanthus, micrognathia, protruding tongue, small upturned nose	1,9,10,16	Ig
Fasth et al. (1990)	M	1.7	? *	?	Antimongolian slant, macrocornea, uneven pigmentation	1	Combined
	F	1.1			Not mentioned	1	Combined
Kieback et al. (1992)	M	?	?	?	Flat nasal bridge, low set ears	1,16	Ig
Gimelli et al. (1993)	F	?	?	?	Round face, hypertelorism, epicanthus, small upturned nose, flat nasal bridge, micrognathia, macroglossia	1,9,16	Ig
	M	?	?	?	Normal	1,9,16	Ig
Smeets et al. (1994)	M	2.1	?	?	Flat nasal bridge, telecanthus, epicanthus, low-set ears, protruding tongue	1,16	Ig
This study	F	1.3	22 *	36	Triangular face, upturned nose, flat nasal bridge, frontal bossing, sparse, dry hair	1,9,16	Ig

* Parental consanguinity

Table 6 Clinical progress of published cases

Author	Clinical progress
Hulten (1978)	Recurrent infection, malabsorption. Died following unsuccessful bone marrow transplantation
Tiepolo et al. (1979)	Growth <3rd centile. "Retarded" from 3 months. Died of pneumonia and respiratory failure at 12 years
Fryns et al. (1981)	Growth <3rd centile. Not sitting at 8 months. Staphylococcal septicaemia and pneumonia from 3 to 8 months
Howard et al. (1985)	Normal growth. Special education. By 4 years had bronchopneumonia and empyema
Valkova et al. (1987)	Poor weight gain. Assumed psychomotor retardation. Died aged 4 months after pertussis, bronchopneumonia and colitis
Maraschio et al. (1988)	Growth 10th centile. Pneumonia aged 2–3 years. Normal school. Aged 9 years, well on Ig therapy
Carpenter et al. (1988)	Poor weight gain. Moderate global delay. Seven episodes of pneumonia before age 15 months. At 3 years on Ig therapy
Turleau et al. (1989)	Growth ≤ 3rd centile. Aged 5.5 years, well on Ig therapy
Fasth et al. (1990)	Severely retarded. Died age 5 months of thrush, CMV and bronchopneumonia Mentally retarded. Died age 15 months of colitis and interstitial pneumonitis
Kieback et al. (1992)	Mental retardation and neurological defects
Gimelli et al. (1993)	Delayed motor development and speech, recurrent respiratory infections and diarrhoea. Twenty-nine years old. Healthy aged 30 years
Smeets et al. (1994)	Failure to thrive and malabsorption. Delayed speech
This study	Growth <3rd centile. Mild developmental delay. Aged 7 years, well on Ig therapy

al. (1993) applied fluorescence in situ hybridisation (FISH) using alphoid sequences specific to the centromeric regions of chromosomes 1 and 16 to investigate the behaviour of centromeres in their ICF patients, and found that the hybridisation signal was always present as an unique spot in the centromeric region of these chromosomes. There was never any evidence of hybridisation in the duplicated long arms of multibranched chromosomes. These authors also employed CD (Kinetochore) staining to confirm the presence of active centromeres on the short arms of multibranched chromosomes. As a consequence they were able to refute the hypothesis of Fryns et al. (1981)

that centromeres involved in multibranched configurations split into two or more parts. It is now clear that centromeres are not directly involved in the origin of multibranched chromosomes but that it is the paracentromeric heterochromatin, particularly of chromosomes 1, 9 and 16, which plays the "active" role.

This theory is apparently supported by the SEM studies on our patient which reveal greater details of the configurations described, with genuine fusion of the heterochromatin in the long arms of multibranched chromosomes, and no involvement of centromeres. It has been suggested that extended (or "undercondensed") heterochromatin may be associated with undermethylation of the chromosomal DNA, and recently it has been shown that satellite, but not alphoid, DNA sequences are undermethylated in ICF patients (Jeanpierre et al. 1993). When normal lymphocyte cultures are treated with 5-azacytidine, a DNA demethylating agent, not only is the heterochromatin extended but fusion of heterochromatin comparable to that in ICF patients is also seen (Schmid et al. 1983), suggesting that fusion of heterochromatin may also be connected with undermethylation of DNA in some way.

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