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THE CRITICAL MONOSOMIC SEGMENT INVOLVED IN 4p− SYNDROME: A HIGH-RESOLUTION BANDING STUDY ON FIVE INHERITED CASES

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Summary In an attempt to determine the critical monosomic segment involved in 4p− syndrome, we studied the precise breakpoints of five inherited cases with the syndrome using a high-resolution banding technique. The 5 patients ranged in age at diagnosis from newborn to 15 months, 4 of whom could be clinically diagnosed as having 4p− syndrome. Common clinical features included mental retardation, low birth weight, growth failure, hypotonia, microcephaly, peculiar facial dysmorphia and ear malformations. Karyotypes of the 5 were 46,XX,−4,+der(4),t(4;21)(p16.1;q22.3)pat; 46,XX,−4,+der(4), inv ins(4;9)(p15.32p16.3;q34.3)pat; 46,XX,rec(4),del p,inv(4)(p15.2q35)pat; and 46,XX,−4,+der(4),t(4;18)(p15.2;p11.21)mat (two cases, related). The results suggested that monosomy for the proximal half of the 4p16 band is sufficient to express 4p− syndrome.

INTRODUCTION

The partial deletion of the short arm of chromosome 4 (4p−) has long been established as a clinically recognizable syndrome (Wolf *et al.*, 1965; Hirschhorn *et al.*, 1965; Centerwall *et al.*, 1975). Up to the present, more than 120 cases have been documented (Lurie *et al.*, 1980). The majority of those had a *de novo* deletion (simple deletions, unbalanced translocations involving 4p or ring chromosomes 4), but a small proportion of those were the results of parental chromosome aberrations. Although no exact breakpoints were given in most of those cases, it has been suggested that monosomy for the 4p16 band is responsible for the main clinical

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features of 4p− syndrome (Lejeune *et al.*, 1975; Fryns *et al.*, 1979; Wilson *et al.*, 1981).

The development of high-resolution banding techniques has provided a more precise identification of chromosome abnormalities (Yunis, 1976). The purpose of this paper is to present clinical and cytogenetic findings from five inherited cases with 4p− syndrome. High-resolution banding studies in these cases indicated that the critical monosomic segment involved in 4p− syndrome lies in the proximal half of the 4p16 band.

CASE REPORT

The subjects consisted of 5 female infants, ranging in age at diagnosis from newborn to 15 months (Fig. 1). They all were born as the first child to healthy nonconsanguineous parents. The pregnancies were uneventful in all cases, and there was neonatal asphyxia in two cases (cases 1 and 4). Family history for spontaneous abortions and congenital malformations was present in cases 4 and 5. Pedigree studies showed that these two cases were of the same ancestral origin (Fig. 2). The other cases were unrelated. Clinical findings of the 5 cases were summarized in Table 1. Despite the full-term delivery, their birth weights were inappropriately low (from 1,400 g to 2,360 g). All cases but one (case 5) were clinically suspected as having 4p− syndrome before the chromosome identification. The clinical diagnosis of case 5 was not feasible, since the coexistence of microphth-

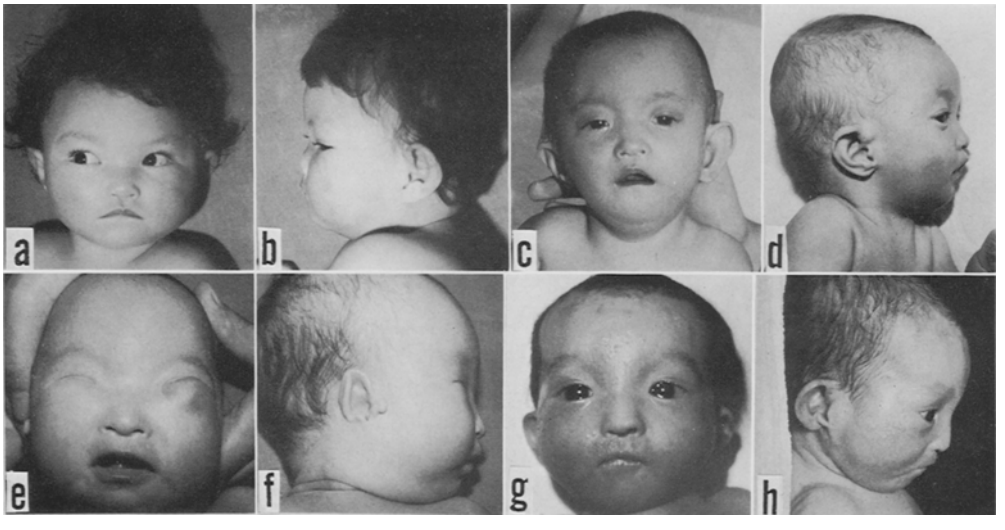

Fig. 1. Frontal and lateral views of the patients: case 1 (a and b), case 2 (c and d), case 4 (e and f) and case 5 (g and h). No photograph of case 3 was available.

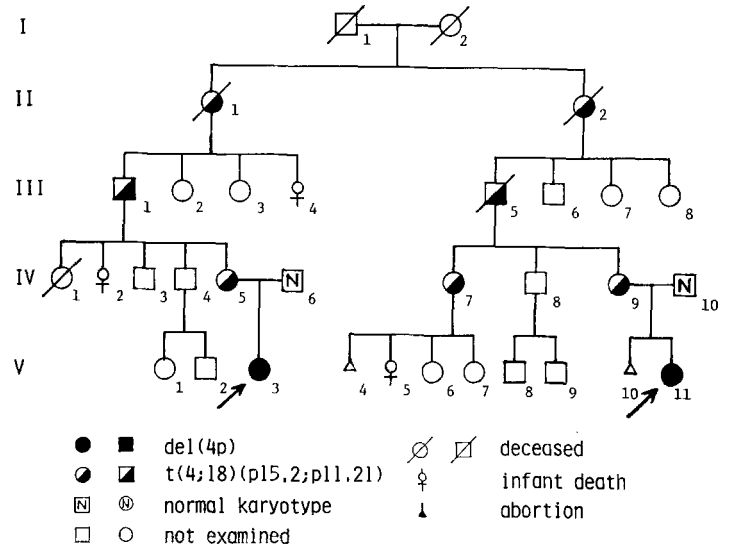

Fig. 2. Family pedigree of cases 4 (V-3) and 5 (V-11). Three decreased individuals (II-1, II-2 and III-5) were considered to be a obligate carrier of the balanced translocation.

almia, gross cavernous hemangioma of the eyelid and hypoplastic auricles obscured the characteristic phenotype of 4p− syndrome. Clinical features shared by the 5 cases were low birth weight, delayed psychomotor development, growth failure, marked hypotonia, microcephaly, low-set and simply formed ears, frontal bossing, prominent glabella, arched eyebrows, hypertelorism, and downturned corners of the mouth. In contrast to the report by Centerwall *et al.* (1975), broad or beaked nose was found less often. Additional malformations frequently seen in the subjects included prominent bulbi and epicanthic folds (4 cases), micrognathia (4 cases), short philtrum (3 cases), strabismus (3 cases), congenital heart disease (3 cases), obstruction of the nasolacrimal duct (2 cases), pes equinovarus (2 cases), hemangioma (2 cases), and cleft lip and/or palate (2 cases). Furthermore, intractable epileptic seizures were noticed in 3 cases: psychomotor seizures in case 1 and grand mal seizures in cases 2 and 5. Computerized tomography of the head and examinations of acoustic brain stem response demonstrated severe brain atrophy and sensorineural deafness in cases 1 and 2. The complete dermatoglyphic study was available only in case 1. The dermatoglyphics in the remaining cases were difficult to analyze because of the ridge hypoplasia and dissociation. Digital patterns of case 1 were W.W.UL.UL.UL on the right hand and W.W.UL.W.UL on the left, and a total finger ridge count was 88. There was a simian crease on the right palm and a Sydney line on the left. The main line formula were 11.10.8.3–t′–L^r/A^r.O.O.L^d.O on the right palm and 9.7.5″.1–t′–A^u.O.O.O.L^d on the left. With regard to the life prognosis, four out of the 5 cases were alive (4 to 22 months old when last seen).

Table 1. Clinical findings in the 5 patients with 4p− syndrome.

	Case 1	Case 2	Case 3	Case 4	Case 5	Centerwall *et al.*, 1975
Sex	F	F	F	F	F	
Age at diagnosis	15 m	7 m	Newborn	Newborn	Newborn	
Parental karyotype	t(4;21)pat	ins(4;9)pat	inv(4)pat	t(4;18)mat	t(4;18)mat	
Maternal age at birth	27	23	22	21	25	
Paternal age at birth	27	28	25	27	26	
Gestational age	42 w	39 w	39 w	42 w	40 w	
Birth weight	2,360 g	1,400 g	1,635 g	1,770 g	1,815 g	
Mental retardation	+	+	+	+	+	50-100%
Growth failure	+	+	+	+	+	50-100%
Epileptic seizures	+	+	−	−	+	50-100%
Hypotonia	+	+	+	+	+	50-100%
Microcephaly	+	+	+	+	+	50-100%
Frontal bossing	+	+	+	+	+	
Prominent glabell[a]	+	+	+	+	+	50-100%
Arched eyebrows	+	+	+	+	+	
Hypertelorism	+	+	+	+	+	50-100%
Prominent bulbi	+	+	+	+	microph-thalmia	
Epicanthic folds	−	+	+	+	+	50-100%
Strabismus	+	+	−	+	−	50-100%
Obstruction of the nasolacrimal duct	−	−	+	+	−	10-50%
Broad or beaked nose	−	+	−	+	−	50-100%
Short philtrum	+	+	−	+	−	10-50%
Downslanting mouth	+	+	+	+	+	50-100%
Cleft lip or palate	−	−	+	+	−	50-100%
Micrognathia	−	+	+	+	+	50-100%
Low-set ears	+	+	+	+	+	50-100%
Simply formed ears	+	+	+	+	+	50-100%
Preauricular tags	−	−	+	−	+	10-50%
Accessory ribs	−	−	−	+	−	<10%
Hip dislocation	−	+	−	−	−	
Pes equinovarus	+	−	+	−	−	<10%
Sacral sinus or dimple	−	−	+	+	−	10-50%
Hemangioma	−	−	+	−	+	10-50%
Congenital heart disease	ASD+PS	−	VSD?	VSD	−	10-50%
Deafness	+	+	?	?	?	<10%

Case 3 died from aspiration pneumonia at 3 months of age. The surviving cases showed severe failure to thrive, and two cases (cases 4 and 5) still underwent tube feedings.

CYTOGENETIC STUDIES

Peripheral blood lymphocytes were cultured using Eagle's minimum essential medium supplemented with 15% fetal calf serum and phytohemagglutinin. Cells in prometaphase or prophase were collected according to the method of Ikeuchi and Sasaki (1979). Briefly, ethidium bromide (10 μg/ml) and Colcemid (0.03 μg/ml) were added to the culture flasks two hours prior to harvest. Chromosome preparations were aged for one week and then processed by GTG banding. In 4 of the 5 cases, high-resolution banding analysis was successful at the level of more than 550 bands in a haploid set (ISCN, 1981).

Case 1. Although repeated conventional chromosome examinations failed to detect any cytological abnormalities, the high-resolution banding technique showed a subtle deletion involving the 4p16 band (Fig. 3a). The mother had normal chromosome, while the father was found to have a balanced translocation between chromosomes 4 and 21 (Fig. 3b). The breakpoints were at 4p16.1 and 21q22.3. The patient's karyotype was 46,XX,−4,+der(4), t(4;21)(p16.1;q22.3)pat.

Case 2. Chromosome analysis of this patient in other laboratory was normal. High-resolution banding, however, disclosed the presence of an interstitial deletion of 4p (Fig. 4a). The father was found to have an inverted insertion of the segment

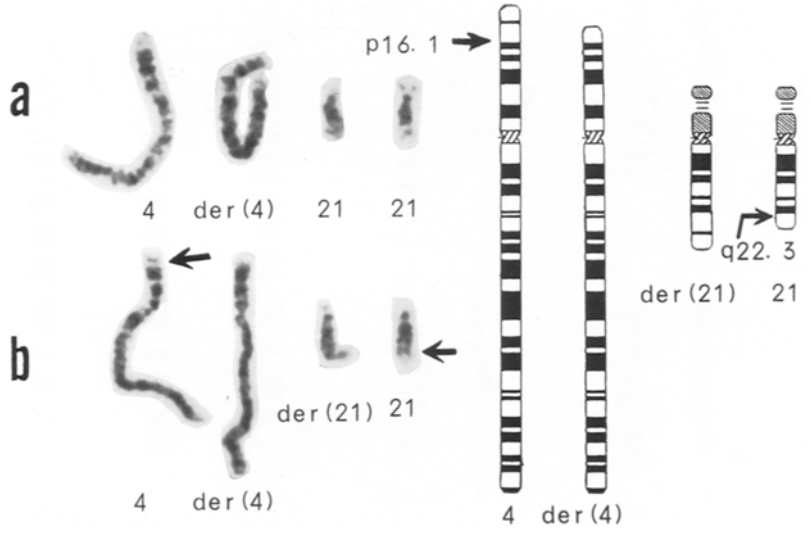

Fig. 3. Partial karyotypes of case 1 (a) and the father (b). Arrows indicate breakpoints.

4p15.32→p16.3 into the 9q34.3 (Fig. 4b). The patient's karyotype was 46,XX,−4, +der(4),inv ins(4;9)(p15.32p16.3;q34.3)pat. The mother had a normal karyotype.

Case 3. In this patient, high-resolution banding analysis was unsuccessful. The conventional chromosome study showed that the short arm of one chromosome

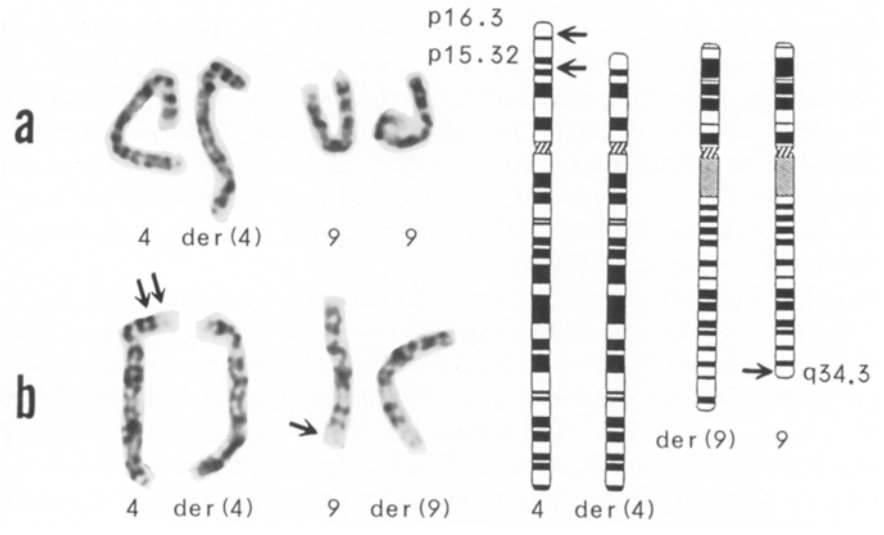

Fig. 4. Partial karyotypes of case 2 (a) and the father (b). Arrows indicate breakpoints.

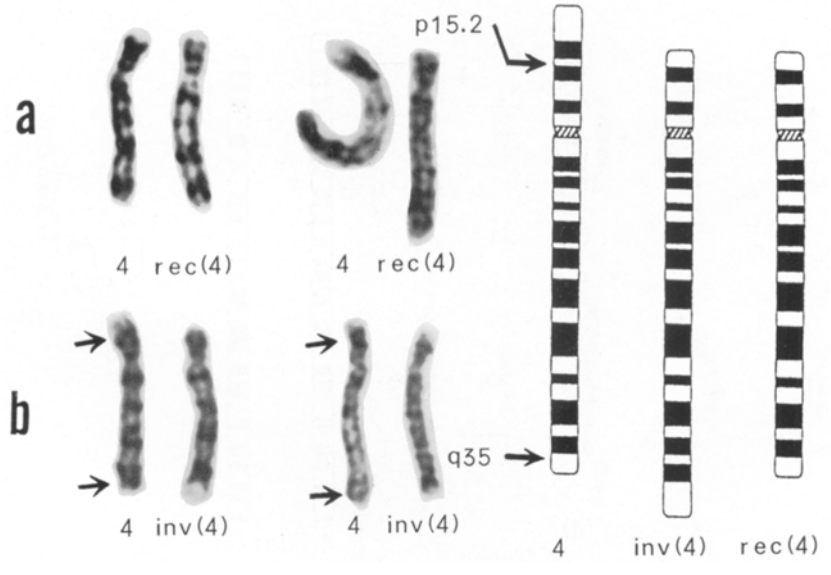

Fig. 5. Partial karyotypes of case 3 (a) and the father (b). Arrows indicate breakpoints.

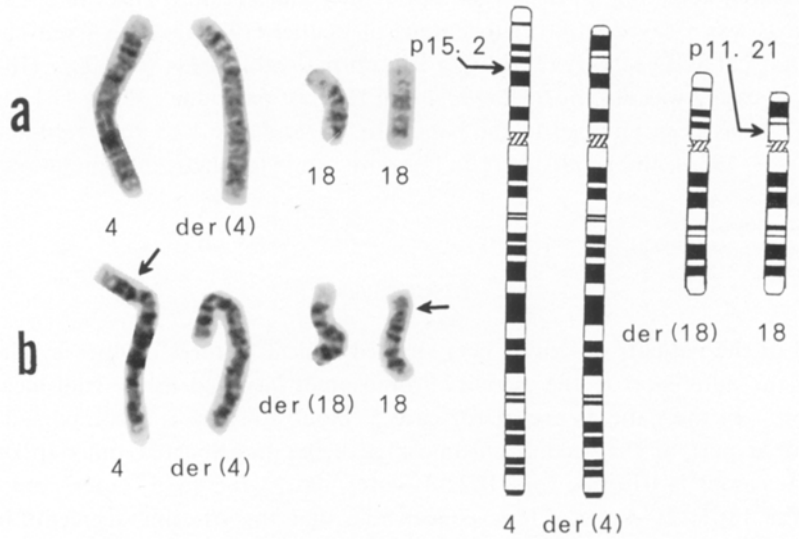

Fig. 6. Partial karyotypes of case 4 (a) and the mother (b). Arrows indicate breakpoints.

4 was shorter than that of the homologue (Fig. 5a). The father was found to be a carrier of a pericentric inversion of chromosome 4, in which the breakpoints appeared to occur at 4p15.2 and 4q35 (Fig. 5b). The deletion in the patient was considered to result from aneusomic recombination between the inverted chromosome 4 and the normal homologue during meiosis I of the father. The mother had a normal karyotype. The patient's karyotype was 46,XX,rec(4), del p,inv(4)(p15.2 q35)pat. Five months after the patient's death, the mother became pregnant. Prenatal diagnosis at 18 weeks' gestation demonstrated that the fetus had the same inverted chromosome 4 as did the father. The pregnancy was elected to continue, resulting in the delivery of a phenotypically normal boy infant.

Cases 4 and 5. The two patients initially seemed unrelated. Pedigree analysis, however, revealed that the two were really related. Both cases had the identical deletion of 4p, which resulted from maternal balanced translocations between chromosomes 4 and 18 (Figs. 6a and 6b, there presented only karyotypes of case 4 and the mother). High-resolution banding study showed the breakpoints at 4p15.2 and 18p11.21. The karyotypes of the patients were 46,XX,−4,+der(4), t(4;18)(p15.2; p11.21)mat. In the pedigree (Fig. 2), III-1 and IV-7 were also found to be a carrier of the balanced translocation.

GENE MARKER STUDIES

Gene dosage studies of superoxide dismutase 1 (SOD 1) and adenylate kinase 1 (AK 1) were carried out in cases 1 and 2, respectively. SOD of red blood cells

was measured according to the method of Winterbourn *et al.*, 1975, while AK of red blood cells was assayed with the technique of Beutler (1975). Case 1 was shown to have a normal SOD activity (3,534 U/g Hb, normal value 3,434±486 U/g Hb). AK activity in case 2 was also normal (192.4 U/g Hb, normal value 232.6±42.1 U/g Hb). As SOD 1 has been mapped to the band 21q22.1 and AK 1 to 9q34 (Human Gene Mapping 7, 1984), the results were in line with our cytogenetic interpretations of the two cases.

DISCUSSION

All of the patients described here showed typical features of 4p− syndrome in spite of the differences in the second chromosomes involved in the translocation or inversion. In the patients except for case 2, monosomy 4p is accompanied by trisomy for a part of the second chromosome being translocated onto 4p or being inverted: case 1 is trisomic for 21q22.3→qter, case 3 for 4q35→qter, and cases 4 and 5 for 18p11.21→pter. It is conceivable that the trisomic segments in these cases are too short to produce any specific phenotypic abnormalities. Of particular interest, however, is some discordance between the phenotypes of cases 4 and 5, of which karyotypes are wholly identical. The phenotype of case 4 is essentially of 4p− syndrome, whereas that of case 5 is complicated by the presence of microphthalmia, a feature never described in the previous cases with 4p− syndrome. This leads us to assume that 'epistasis' of monosomy 4p over trisomy 18p (Cantú *et al.*, 1981) is involved in the former case but 'phenotypic hybridization' between monosomy 4p and trisomy 18p (Stengel-Rutkowski *et al.*, 1984) occurs in the latter case. Which mechanism operates on the phenotypic expression in cases with translocation 4p− syndrome appears to depend for the most part upon the size of the translocated segment of another chromosome. When the translocated segment is long, a 'hybrid phenotype' is likely to take place.

Since the first description of a case with translocation 4p− syndrome by Neu *et al.*, 1975, 16 such cases including ours have been published (Table 2). Second chromosomes involved in the translocations are varied and unspecific. The sex ratio among the translocation cases (females to males 14 : 8) is similar to that (2 : 1) among the *de novo* deletion cases (de Grouchy and Turleau, 1977). In all cases but two (cases 1 and 12), monosomy 4p occurred as a result of adjacent-1 segregation from parental translocations. Cases 1 and 12 had the apparently same balanced translocations as the mothers. Banding analysis in case 1 substantiated the loss of the 4p16 band from the translocated chromosome 1 (Stoll *et al.*, 1981). The occurrence of monosomy 4p was interpreted by the author as a consequence of an unequal crossing-over during meiosis in the mother. As far as we know, our case 3 is the second example of a patient with 4p− syndrome resulting from a parental pericentric inversion of chromosome 4. The first reported case with a recombinant chromosome 4 due to a parental inversion, on the other hand, had gross trisomy for

4q25 → qter in addition to monosomy for 4p14 → pter (van der Linden *et al.*, 1975).

The previous cases with translocation or *de novo* 4p− syndrome in which the breakpoints were identified usually had terminal deletions of the 4p segments (Fig. 7), including p13 → pter (a), p14 → pter (b), p15.1 → pter (c), p15.2 → pter (d), p15.3

Table 2. Translocation 4p− syndrome.

Case No.	Sex	Parental karyotypes	References
1	F	t(1;4)(q11;p16)mat	Stoll *et al.*, 1981
2	M	t(1;4)(q42;p14)pat	Wilson *et al.*, 1981
3	2F	t(2;4)(q37;p14)mat	Ohdo *et al.*, 1976
4	M	t(4;8)(p15.3;p22)mat	Stengel-Rutkoswki *et al.*, 1984
5	F	t(4;9)(p15.1;p23)mat	Aurias *et al.*, 1978
6	F	ins(4;9)(p15.2p16.3;q34.3)pat	Present case 2
7	F	t(4;10)(p13;q26)mat	Hedner *et al.*, 1977
8	M	t(4;12)(p14;p13)pat	Mortimer *et al.*, 1978
9	2F 1M	t(4;12)(p15.3;q26)mat	Levy *et al.*, 1976
10	M	t(4;18)(p16;p11)mat	Chiyo *et al.*, 1981
11	2F	t(4;18)(p15.2;p11.21)mat	Present cases 4 and 5
12	M	t(4;19)(? ; ?)mat	Neu *et al.*, 1975
13	F	t(4;20)(p15;p12)pat	Lejeune *et al.*, 1975
14	F	t(4;21)(p16.1;q22.3)pat	Present case 1
15	1F 1M	t(4;22)(p15.2;p11)mat	Lurie *et al.*, 1980
16	1F 1M	t(4;22)(p16.1;q13.33)mat or pat	Curry *et al.*, 1982

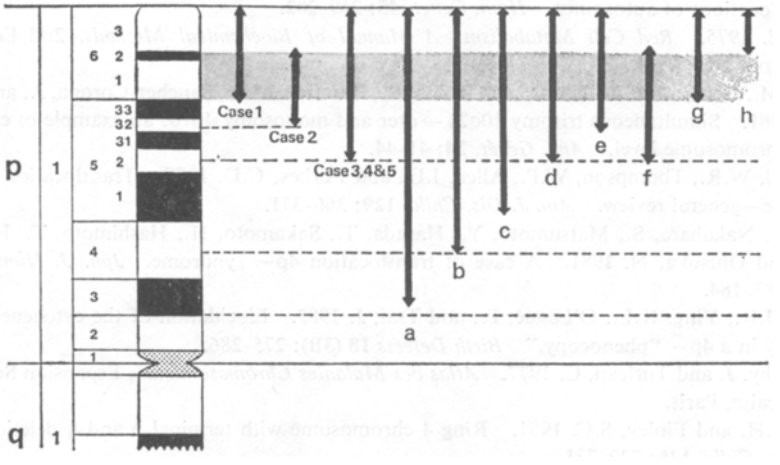

Fig. 7. Schematic representation of deleted segments of 4p in the present and previous cases with 4p− syndrome (see the references of Lurie *et al.*, 1980; Wilson *et al.*, 1981; and Stengel-Rutkowski *et al.*, 1984). The shadowed band represents the critical monosomic segment involved in 4p− syndrome.

→pter (e), p16.1→pter (g), and p16.3→pter (h). The breakpoint in the last (h) seems questionable, since the translocation of an undetermined chromosome segment onto 4p made the exact identification of the breakpoint difficult (Wilson *et al.*, 1981). An interstitial deletion of 4p as in our case 2 is exceptional, and there has been only one such case (Takata *et al.*, 1983). This case had the breakpoints at 4p15 and 4p16 (f). It is generally acknowledged that the variations in deletions of 4p do not produce any fundamental phenotypic differences and that the deletion of the terminal 4p16 band is sufficient to express the main clinical symptoms of 4p− syndrome. The results of the high-resolution banding studies in our subjects, especially in cases 1 and 2, suggest strongly that the critical monosomic segment involved in 4p− syndrome is within the proximal half of te 4p16 band. This concept is further supported by the observation that one case with r(4)(p15q35) showed 4p− syndrome (Fraisse *et al.*, 1977), while another case with r(4) (p16q35) did not (Finley and Finley, 1981). In the latter case, the deletion did not appear to involve the critical monosomic segment.

The application of the high-resolution banding technique has first made it possible to identify the subtle deletions of 4p in our two cases. There is a possibility that the critical segment is even shorter than the proximal half of the 4p16 band. This provides an important clinical implication that the extensive cytogenetic investigation using a high-resolution banding technique is warranted for diagnosis and genetic counseling of a case with a 'phenocopy' of 4p− syndrome.

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