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A COMPLEX MOSAIC WITH tdic(13 ; 18) (p11 ; p11), +13p−, +18p−, r(13) *etc.* IN A MALE INFANT

I. CENTROMERE INACTIVATION AND DISSOCIATION OF DICENTRIC CHROMOSOME

Mariko UEHARA and Mitsushiro KIDA

Department of Pediatrics, Teikyo University School of Medicine, Kaga, Itabashi-ku, Tokyo 173, Japan

Summary A complex chromosomal mosaic was observed in cultured lymphocytes and skin fibroblasts of a male infant with multiple congenital malformation. The clinical features of the patient overlapped with those of 18p−, 13 trisomy and 18 trisomy syndromes. The karyotype of the modal cell line was 45,XY,t(13;18)(p11.1-2;p11.2), in which the translocation chromosome has one or two functional centromeres; minor cell lines were 46,XY,t(13;18),+13p−; 46,XY,t(13;18),+18p−; 46,XY,r(13), 18p−; 46,XY,13p−,18p−; *etc.* This chromosomal mosaicism may have been derived from breakage-fusion-bridge (BFB) cycles and centromere inactivation in the early cleavage stage of the embryo.

INTRODUCTION

Generally, in dicentric (dic) chromosomes, either of the centromeres can become inactivated so that the chromosome changes to one which has only one functional centromere and behaves like a monocentric chromosome. If both centromeres are active, they form anaphase bridges, which cause dissociations of dic, aneusomies by non-disjunction or anaphase lagging, or tetraploidies by nuclear fusion. This tendency toward unstable cell divisions usually leads to cell death.

In 42 reported cases of autosomal tdic (translocation dicentric) summarized by Dewald *et al.* (1979), the tdic chromosome consisted of at least one acrocentric chromosome with one exception. In the reported cases of 18p− syndrome, the deletion of the short arm of no. 18 resulted from translocation between no. 18 and acrocentric chromosomes involving no. 13, and there has been no report about dic chromosome and mosaic constitution of chromosomal structural changes (Funderburk *et al.*, 1977; Kistenmacher *et al.*, 1974; Leisti *et al.*, 1973; Moedjone *et al.*, 1979).

We describe a rare case with a complex chromosomal mosaic of 18p− syndrome

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with tdic which has one or two functional centromeres. The majority of karyotype is 45,XY,t(13;18)(p11.1-2;p11.2), and other abnormal karyotypes observed are 46,XY,t,+13p−, 46,XY,t,+18p−, 46,XY,r(13),18p−, 46,XY,13p−,18p−, 4n(= 90, 92), *etc.* The mechanism of occurrence of the mosaic and clinical and karyotypical aspects are discussed.

CASE REPORT

The patient was a male infant born at full term on the 18th June 1982, as a first birth to a 24-year-old father and a 20-year-old mother. The parents were healthy and non-consanguineous marriage. The birth weight of the patient was 2,700 g, length 48 cm, head circumference 33 cm, and chest circumference 31.5 cm. On 23 June, he was referred to a Department of Pediatrics because of attacks of cyanosis from two days before.

The main clinical features were microcephaly, prominent forehead, hypertelorism, prominent bridge and broad-based nose, carp mouth, micrognathia, large and low-set ears, clinodactyly of the fifth finger, and simian crease of the left palm. Computed tomography showed colpocephaly (Fig. 1). At seven months of age his weight was 6.9 kg (−1.7 SD), his height 64.5 cm (−6.4 SD), and head circumference 43.5 cm (−2.7 SD).

MATERIALS AND METHODS

Peripheral lymphocytes from the patient were cultured at different time intervals at the ages of four, six, seven and thirteen months. The lymphocytes were grown in RPMI 1640 medium with 20% fetal calf serum, 2% PHA, and penicillin (100 IU) in an incubator with a 5% CO_2 atmosphere for two and three-day. Each culture was harvested with colcemid at a final concentration of 0.04 μg/ml for 2 hr, hypotonic treatment with KCl (0.075 M) and methanol-acetic acid fixation. Chromosomes were prepared and stained by the techniques of G-bands with trypsin (Seabright, 1971), C-bands with $Ba(OH)_2$ (Sumner, 1972), Cd-bands (Eiberg, 1974), and Ag-bands (Bloom and Goodpasture, 1976). High resolution banding was obtained with the use of ethidium bromide at a final concentration of 10 μg/ml for the final 2 hr (Ikeuchi and Sasaki, 1979). Anaphase chromosomes were obtained without colcemid. Lymphocytes from the parents were also cultured and chromosomes were prepared using the same method.

Chromosome study was also made by skin fibroblasts established from the patient at the age of one year and one month after the second subculture.

RESULTS

Analyses of metaphase chromosomes in lymphocytes from the patient at the ages of 4, 6, 7 and 13 months using G, C, Cd and Ag banding methods revealed

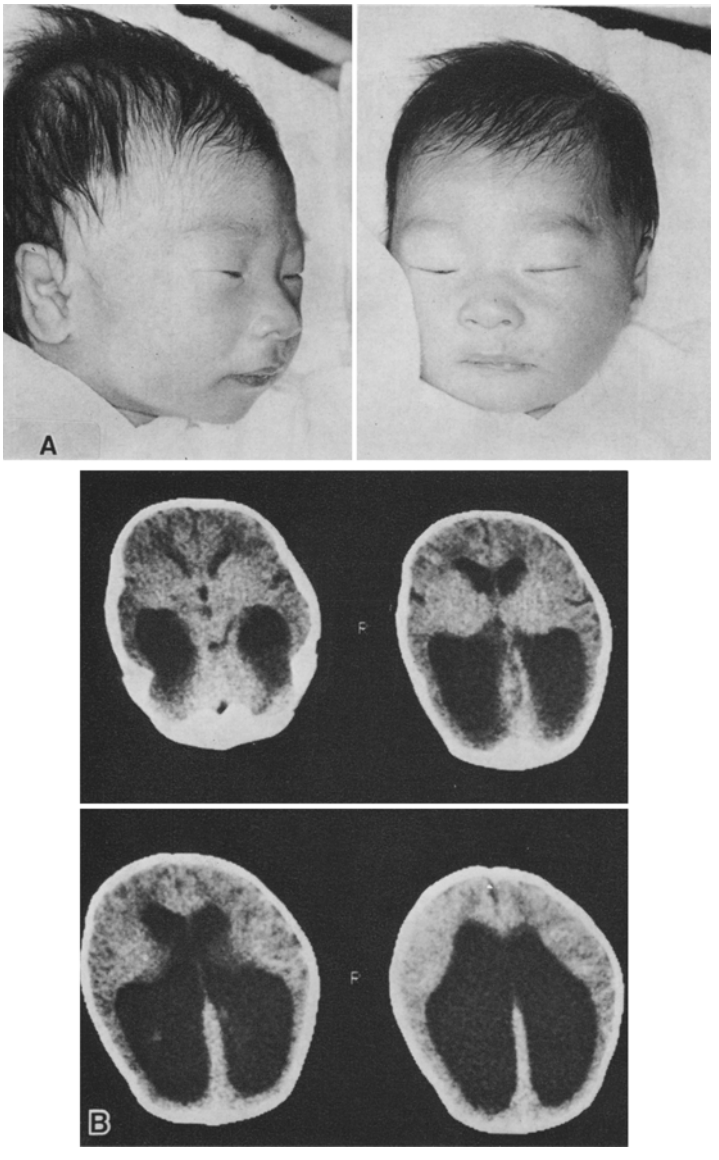

Fig. 1. (A) The patient. Note the prominent bridge and braod-based nose, carp mouth, hypertelorism, large and low-set ears, and prominent forehead. (B) Computed tomography of the patient's head showing colpocephaly.

a variety of abnormal karyotypes (Table 1). Most cells had the karyotype 45,XY, $-13,-18,+t(13;18)(p11.1\text{-}2;p11.2)$. In about 50% of these cells, chromosome t(13;18) had two constrictions and two Cd-bands at both centromeres, which seemed to be functional centromeres (Fig. 2A). In about 30% of cells the chromosome

Table 1. Percentage of cells with abnormal karyotypes in cultured lymphocytes at different ages of the patient.

Karyotypes or number of chromosomes	Percentage of cells with abnormal karyotypes at different ages[a]			
	4 mo. (3)	6 mo. (2)	7 mo. (3)	13 mo. (3)
[Diploid]				
45,XY,t(13;18)	85	93	83	84
Trisomy				
46,XY,t(13;18),+13p−	4	2	3	4
46,XY,t(13;18),+18p−	1	1	1	2
Dissociation				
46,XY,13p−,18p−	4	0	2	6
Other anomalies				
46,XY,r(13),18p−	4	1	0	1
46,XY,t(13;18),+i(13)	0	0	0	1
45,XY,t(13;18)[b]	—	—	2	2
[Tetraploid]				
90	0	0	3	1
92	2	0	1	0
Total no. of cells	91	94	93	101

[a] Duration of days of cell culture is shown in parentheses. [b] In this karyotype t(13;18) had large intercentromeric region.

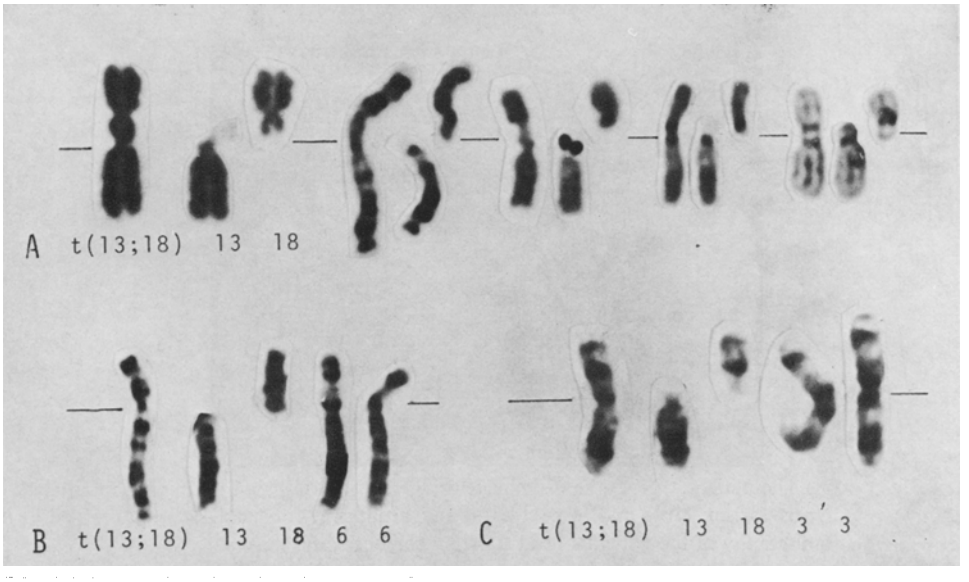

Fig. 2. Variation of constriction at sites of centromeres of t(13;18) chromosomes in cells with 45,XY,t(13;18). (A) Two constrictions at both centromeres. From left to right: with conventional staining, G-bands, Ag-bands, C-bands and Cd-bands. (B) One constriction at centromere of chromosome 18. The latter two chromosomes are no. 6. (C) One constriction at the centromere of chromosome 13. The latter two chromosomes are no. 3.

had only one constriction at the centromere of chromosome 18, which seemed to be a functional centromere, and the shape of the chromosome was similar to that of chromosome 6 (Fig. 2B). In the remaining cells the chromosome had only one constriction at the centromere of chromosome 13, which seemed to be a functional centromere, and the shape of the chromosome was similar to that of chromosome 3 (Fig. 2C). Other abnormal karyotypes observed were as follows: 46,XY,t(13;18),+13p−; 46,XY,t(13;18),+18p−; 46,XY,13p−,18p− (Fig. 3, left) in which double minute fragments were sometimes observed; 46,XY,r(13),18p− (Fig. 3, center); 46,XY,t(13;18),+i(13); 45,XY,t(13;18) with a large intercentromeric region with two constrictions (Fig. 3, right), and tetraploidy. Anaphase bridges and breakages were observed once in 50 anaphases (Fig. 4). Analysis of metaphases in skin fibroblasts from the patient showed a similar complex mosaic, but a higher percentage of tetraploidy was found in the skin fibroblasts (31%) than in lymphocytes (1%).

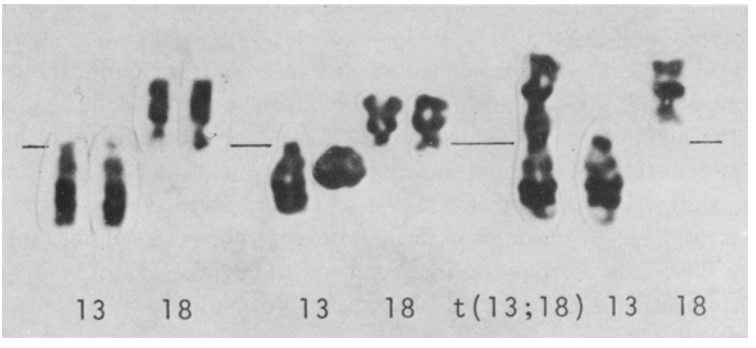

Fig. 3. Partial karyotypes of abnormal chromosomes in the patient. Left: 46,XY, 13p−,18p−. Center: 46,XY,r(13),18p−. Right: t(13;18) chromosomes with large intercentromeric region.

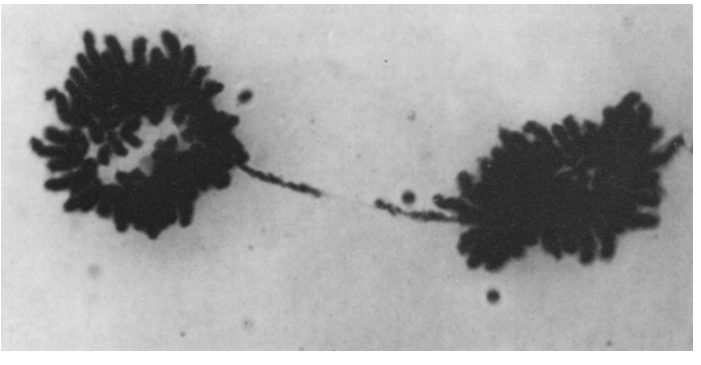

Fig. 4. Anaphase bridge and break.

DISCUSSION

In most of the reported cases with dicentric chromosomes showing one morphologic centromere, the same centromere is always constricted from one cell to another within an individual, indicating the occurrence of the nonfunction of the centromere (Daniel and Lam-Po-Tang, 1976; Daniel, 1979; Dewald *et al.*, 1979). In some cases, however, there are multiple cell lines in which either centromere or both centromeres are functional (Niebuhr 1972; Daniel and Lam-Po-Tang, 1976; Daniel, 1979; Dewald *et al.*, 1979). In tdic chromosomes consisting of an acrocentric chromosome and a non-acrocentric chromosome, there is a general tendency for centromere inactivation of the acrocentric chromosome to be dominant. The centromere inactivation can be detected as the centromere separates and by negative Cd-bands (Eiberg, 1974; Evans and Ross, 1974).

The present case is a mosaic of centromere inactivation of tdic, in which the centromere of chromosome 13 is dominant to chromosome 18. Furthermore, cells with karyotypes of 46,XY,t(13;18),+13p− and 46,XY,t(13;18),+18p−, and 46,XY, r(13),18p− are observed. These lines seemed to arise from non-disjunctions after dissociations of t(13;18) chromosomes, or a breakage at or near the centromere and fusion to the telomere of chromosome 13. Thus, the centromere of chromosome 13 was more unstable than that of chromosome 18. The t(13;18) chromosome which had a large intercentromeric region seemed to arise as a result of breakages and fusions of t(13;18) chromosomes in both centromeres. Double minute fragments may have arisen from the intercentromeric region. Tetraploidy may have arisen from nuclear fusions after anaphase bridge formation.

Table 2 summarizes reported cases of chromosomal mosaic which show the same type of structural chromosomal anomaly as the present case that derived from one original translocation. No case of multiple mosaics as seen in the present study has been reported. It is known that in dic chromosomes breakage-fusion-bridge (BFB) cycles lead to such multiple anomalies. McClintock (1950 and 1953) reported a dissociation element (Ds) sited in heterochromatin such as centromeres in maize; the Ds gene carries a tendency for the chromosome to dissociate, leading to multiple chromosomal anomalies through a BFB cycle in early development. A similar mechanism may be applied to the present case.

As shown in Fig. 5, clinical features overlapped with those of cases with 18p−, 13 trisomy and 18 trisomy reported in the literature. Furthermore, the cases of 18p− syndrome are divided into two groups: one of these shows only minor anomalies; the other shows not only such minor anomalies but also cerebral malformation sometimes with harelip and cleft palate, which are observed in 13 trisomy and 18 trisomy syndromes (Lurie and Lazjuk, 1972; Shinzel *et al.*, 1974; Faust *et al.*, 1976). The present case also shows features observed in 18p− syndrome, 13 trisomy and 18 trisomy syndromes including cerebral malformation (*in B, D, E and F of Fig.

Table 2. Reported cases of chromosomal mosaic derived from an original anomaly.

References	Original anomaly	Percentage of cells with original(*) and derived types of anomaly					
		(A)	(B)	(C)	(D)	(E)	(F)
Singh-Kahlon *et al.*	1977 (15; 18) (p ; p)	95*		5			
Niebuhr	1972 (13; 5) (p13; p11)	99*	1				+
Dewald *et al.*	1979 (15; 5) (p11; p13)	96*	4				
Jenkins *et al.*	1981 (14; 15) (q ; q)	89*			10		
Vianna-Morgante and Nunesmaia	1978 (15; 21) (p13; p11)	68*			32		
Guanti and Maritato	1978 (21; 21) (p ; q)	20*	50	30			
Fryns *et al.*	1979 (13; 13) (q11; p11)	25*	75				
Oka *et al.*	1977 (13; 13) (p ; q)	75*				25	
Madan *et al.*	1981 (18; 18) (p11; p11)			2	64*	34	
Schwartz *et al.*	1983 (13; 13) (q ; q)	55*					45
Present case	(13; 18) (p11; p11)	84*	6	6	1	1	2

(A), translocation of chromosomes; (B), dissociation of chromosomes; (C), non-disjunction of chromosomes; (D), isochromosomes; (E), ring chromosomes; (F), another derived dicentric chromosome. +: Present but percentage unknown.

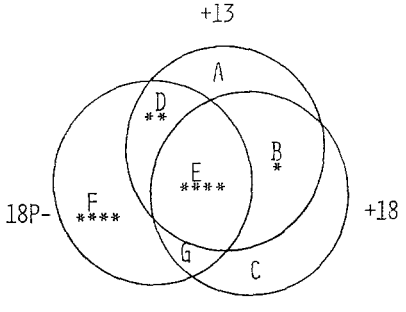

Fig. 5. Overlapping of clinical features in the cases of 18p−,13 trisomy and 18 trisomy syndrome reported in the literature, and also in the present case. (*Clinical features observed in the present case.)

The three circles represent the clinical features in 18p− (left), 13 trisomy (center) and 18 trisomy syndrome (right) reported in the literature; From A to G, the divided areas in the circles represent the following groups of clinical features. A: microphthalmos and polydactyly found in 13 trisomy syndrome. B: simian crease*, distal axial triradius, cryptochism (more frequent in 13 trisomy) flexion deformity of fingers, rocker bottom feet, ventricular septal defect, renal anomaly and hypertonic (more frequent in 18 trisomy) found in both 13 trisomy and 18 trisomy syndrome. C: elongated skull and overlapping finger found in 18 trisomy syndrome. D: microcephaly*, hypertelorism* and hypotonia found in both 13 trisomy and 18p− syndrome. E: epicanthus, micrognathia*, syndactyly, clinodactyly*, malformed low-set ears*, short neck, harelip, cleft palate and cerebral malformation* commonly found in 13 trisomy, 18 trisomy and 18p− syndrome. F: short stature*, strabismus, short broad-based nose*, large ears* and short fingers* found in 18p− syndrome. G: ptosis, broad chest and webbed neck found in 18 trisomy and 18p− syndrome. The numbers of stars (*) in the divided areas (A–G) within the circles represent the numbers of features observed in the present case.

5). One explanation for the overlapping clinical features in the present case is that centromere inactivation and nondisjunction have occurred at two different sites in the early cleavage stage of the embryo. The phenomenon of overlapping clinical features may be explained by chromosomal mosaicism which might be resulted from BFB-cycles not only in the present case but also in some of the other reported cases of 18p− syndrome (Lurie and Lazjuk, 1972; Shinzel *et al.*, 1974; Faust *et al.*, 1976), when a selective disadvantage in growth of minor cell lines such as 13 trisomy or 18 trisomy cells are assumed, or if those cell lines are overlooked.

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