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DETERMINATION OF THE BREAKPOINTS AND THE PARENTAL ORIGIN OF A RING 22 CHROMOSOME: AN ANALYSIS BY HIGH-RESOLUTION BANDING TECHNIQUE, QUINACRINE AND SILVER STAININGS

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Summary A 2-year-old girl with r(22) syndrome is presented. High-resolution banding technique and silver staining of nucleolus organizing regions (NORs) were used to define its breakpoints. The breakpoint in the long arm was verified to locate at most distal segment (q13.3) by G- and R-bandings. The breakpoint in the short arm was verified to locate at p13, because the r(22) had an almost normal NOR compared to normal homolog 22. The karyotype was designated as 46,XX,r(22) (p13q13.3). Origin of the ring(22) was estimated to be of paternal by an analysis of Q-band heteromorphism.

INTRODUCTION

The first observation of a ring chromosome 22 was by Weleber *et al.* (1968), which was verified as r(22) later by Magenis *et al.* (1972). Rethoré *et al.* (1976) proposed r(22) syndrome from 14 observations, and Hunter *et al.* (1977) summarized phenotypic correlations on 21 patients with the r(22). We here report a girl with a r(22). Its breakpoints were analyzed by high-resolution banding technique and silver staining of nucleolus organizing regions (NORs), and origin of the r(22) was estimated by Q-band heteromorphism.

CASE REPORT

The propositus was the first child born to healthy nonconsanguineous parents when the mother was 19 and the father was 23 years old. The pregnancy was uncomplicated. There was no known parental exposure to mutagenic agents. She was born at 41th week of gestation. The delivery was complicated with weak labor pains and premature rupture of membrane, but asphyxia was not noted. Birth

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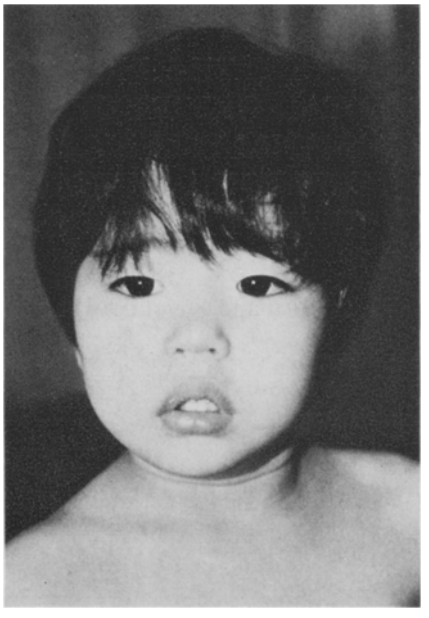

Fig. 1. The propositus at 2 years old. Note full eyebrows, epicanthal folds, low nasal bridge, full lips and micrognathia.

weight was 3,000 g. Cleft soft palate was noted soon after birth. Microcephaly, developmental retardation and hypotonia were pointed out at six months of age. At 18 months of age when she took a verbal training because of speech retardation, plastic surgery for cleft soft palate was recommended. She was referred to our clinic for preoperative examination at two years of age.

At the first visit to our clinic, her physical growth was not so retarded except microcephaly; height was 82.5 cm (−0.6 SD), weight 9,340 g (−1.5 SD) and head circumference 42.2 cm (−3 SD). She could not speak even a word. Unsteady ataxic gait was noted. Physical examination revealed mild synophrys, epicanthal folds, cleft soft palate, high-arched palate, low nasal bridge, thick full lips, micrognathia, small umbilical hernia, clinobrachydactyly of 5th fingers, bilateral cutaneous syndactyly between 2nd and 3rd toes and hypotonia (Fig. 1). Dermatoglyphic examination revealed one arch and nine ulnar loop patterns. Bilateral distal axial triradii(t') was observed. There was no abnormal findings in routine laboratory examinations, brain CT-scanning, electroencephalography, echocardiography and intravenous pyelography.

CYTOGENETICS

Chromosomes of the propositus and the parents were analyzed on lymphocytes obtained by conventional whole blood cultures for 72 hr. The cells for high-resolu-

tion banding were obtained by a modification of ethidium bromide treatment (Ikeuchi and Sasaki, 1979).

In GTG banding preparations, the propositus had 46 chromosomes with a tiny r(22) in 90 cells. In two cells a double sized ring chromosome 22 were observed. 45 chromosomes missing one chromosome 22 were observed in other eight cells. High-resolution GTG, RHG, CBG banding and Ag-NORs staining (Bloom and Goodpasture, 1976) were studied to analyze its breakpoints precisely. Ag-NORs stained preparates were treated with trypsin to distinguish 22 from 21. As shown in partial karyotypes of G and R bandings, a breakpoint in the long arm was verified to locate at most distal segment, 22q13. In Ag-NORs staining size of NOR of r(22) was almost as same as that of its normal homolog. The breakpoint in the short arm seemed to locate at 22p13, because Ag-NORs stain the stalk of acrocentric chromosomes, and Ag-NOR of a r(22) was considered to be intact (Fig. 2). The

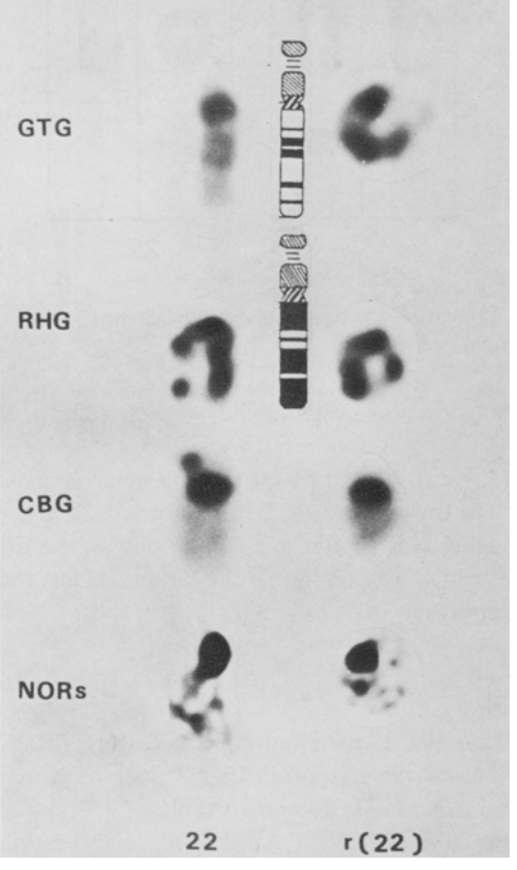

Fig. 2. Partial karyotypes of the propositus. NOR of r(22) was almost as same as NOR of normal homolog.

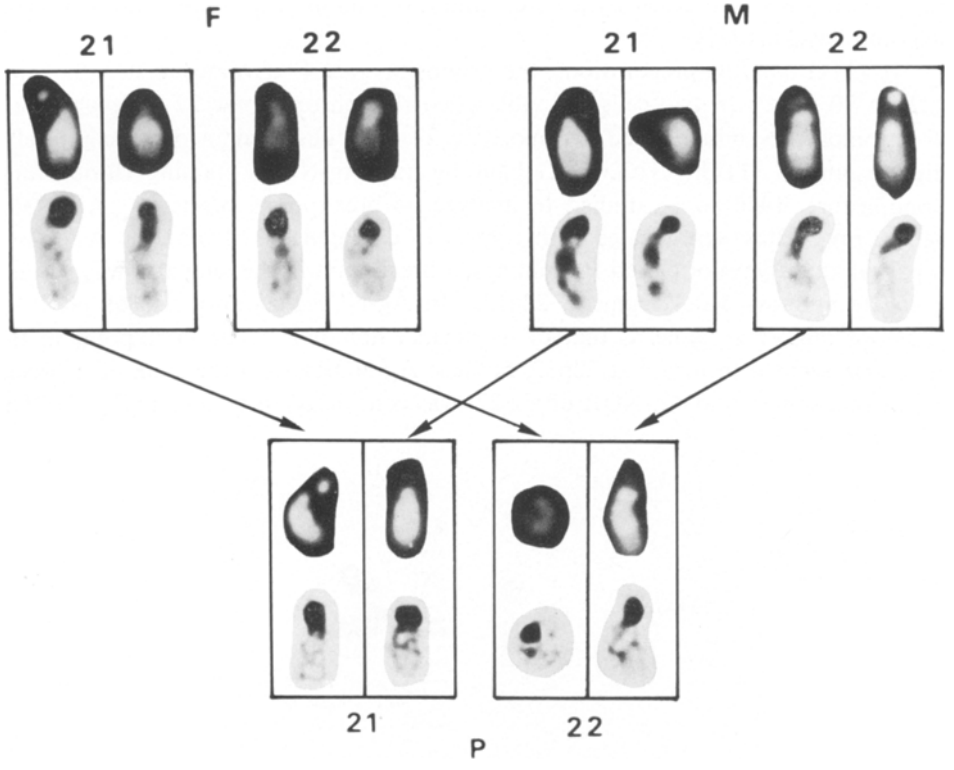

Fig. 3. QFQ and Ag-NOR of the propositus and the parents. Note r(22) seems to be of paternal origin (see text).

karyotype of the propositus was designated as 46,XX,r(22) (p13q13.3). The parents had normal karyotypes.

Origin of this r(22) was studied by QFQ-band heteromorphism of the short arm of chromosome 22. In the propositus, p11 of normal 22 had medium fluorescence with a pale satellite, which was similar to a 22 homolog of the mother. Fluorescence of the r(22) was pale, which was similar to the father. Thus the r(22) was estimated to be of paternal origin (Fig. 3).

DISCUSSION

Since Hunter *et al.* (1977) reviewed clinical features of r(22) syndrome on 21 cases, additional 17 cases have, to our knowledge, been reported including the present case (Palmer *et al.*, 1977; Howard-Peebles, 1977; Funderburk *et al.*, 1979; Faed *et al.*, 1979; Fryns *et al.*, 1979; Aller *et al.*, 1979; Sakuragawa *et al.*, 1979; Fowler *et al.*, 1980; Kondo *et al.*, 1980; Stoll and Roth, 1983; Reeve *et al.*, 1985). As mentioned by Funderburk *et al.* (1979), there is considerable variability in its

expression from normal mentality to profound mental retardation with multiple minor anomalies.

The clinical findings recognized in 50% or more of the patients are mental or developmental retardation, poor weight gain, microcephaly, epicanthal folds, full eyebrows (synophrys), large ears with abnormal auricle, dental malocclusion, thick lips, syndactyly between 2nd and 3rd toes, hypotonia, unsteady gait, hyperreflexia, abnormal EEG findings and distal axial triradius. Findings recognized in 10 to 50% of the patients are feeding difficulty, short stature, abnormal cranium, maxillary hypoplasia, hypertelorism, ptosis of eyelids, low-set ears, low nasal bridge, micrognathia, high-arched palate, vertebral anomaly, brachydactyly, syndactyly (finger), hypertonia, seizures, arch dermal ridge pattern and radial loop pattern (Table 1). Rethoré *et al.* (1976) pointed out almond-shaped palpebral fissures (doe's eyes) as a cardinal finding of r(22) syndrome, but it was not mentioned by other investigators. Major malformations are exceptional.

The r(22) chromosome seems to be stable. All cells had a r(22) in 20 cases

Table 1. Clinical findings of r(22) syndrome in 34 cases reported.

Findings	No. (%)	Findings	No. (%)
Mean maternal age (y/o)	25.83	Low nasal bridge	9/28 (32.1)
Mean paternal age (y/o)	29.72	Large mandible	3/25 (12.0)
Normal gestational age	24/25	Micrognathia	7/25 (28.0)
Mean birth weight (g)	2,960	High-arched palate	11/26 (42.3)
Sex ratio (M/F)	16/18	Dental malocclusion	7/13 (53.8)
Feeding difficulty	9/21 (42.9)	Thick lips	13/17 (76.5)
Developmental delay	27/30 (90.0)	Vertebral anomaly	8
Height (3p)	5/29 (17.2)	Syndactyly (2–3 toes)	12/20 (60.0)
(10p)	10/29 (34.5)	Brachydactyly (5th)	7/22 (31.8)
Weight (3p)	10/30 (29.4)	Syndactyly (finger)	4
(10p)	17/30 (56.7)	Lymphoedema	3
Head circumference (3p)	12/29 (41.4)	Hypotonia	15/20 (75.0)
(10p)	19/29 (65.5)	Hypertonia	3/20 (15.0)
Abnormal cranium	8/23 (34.8)	Unsteady gait	13/15 (86.7)
Maxillary hypoplasia	5	Hyporeflexia	2/13 (15.4)
Epicanthal folds	19/22 (86.4)	Hyperreflexia	7/13 (53.8)
Hypertelorism	7/25 (28.0)	Abnormal EEG	8/16 (50.0)
Full eyebrows	14/14 (100)	Seizures	7/18 (38.9)
Long eyelashes	5/7 (71.4)	Arch pattern	7/23 (30.4)
Ptosis	4	Radial loop pattern	10/23 (43.5)
Large ears	16/22 (72.7)	Distal triradius	15/23 (65.2)
Abnormal auricle	11/13 (84.6)		
Low-set ears	4/23 (17.4)		

Cases 2 to 5 reported by Stoll and Roth was eliminated.

out of 38 cases reported. A double sized r(22) was observed in 1 to 2% of the cells in three cases (Palmer *et al.*, 1977; Aller *et al.*, 1979 and present case). Mosaicism of r(22) with 22 monosomy was highly dominant in most of the cases. Exceptional cases were reported by Stoll and Roth (1983). They made a segregation analysis of r(22) with familial transmission. All five cases with r(22) had a mosaicism with 22 monosomy (ratio was nearly 1/1), and only propositus had mental retardation and microcephaly.

Severity of microcephaly may be related to the ratio of r(22). In 38 cases of r(22) including the present case, 30 had a r(22) in more than 90% of the cells counted. Percentile figures of head circumference were described in 26 cases. Severe microcephaly (head circumference less than 3rd percentile) was found in 12 cases (46%). On the other hand, microcephaly was not noticed in eight cases which showed r(22) in less than 70% of the cells.

Silver staining of NOR in chromosome 22 was studied in three cases (Funderburch *et al.*, 1979; Fowler *et al.*, 1980 and Stoll and Roth, 1983). There was no NOR of a r(22) in all three cases. The breakpoint of the short arm was defined as 22p12. But in the present case, the finding was different from these three cases. NOR was observed in the r(22), and its size was almost as same as that of a normal homolog. The breakpoint was considered to locate at 22p13, most distal segment of the short arm. Ag-NORs staining of the double sized ring could not be permitted to analyze, because its population was very small.

The breakpoint of the long arm was defined at q13 in most of the cases. The clinical findings of r(22) syndrome is presumably due to loss of material from the long arm, because the short arm of chromosome 22 is known to be dispensable without ill effects, *e.g.*, Robertsonian translocation. In the present case, high-resolution GTG and RHG bandings were used to study the breakpoint of the long arm, and it was verified to locate at q13.3. The very distal segment of the long arm of chromosome 22 was deleted. The r(22) of the present case was presumed to arise from two breaks occurred at most distal segments of the short and the long arms and reunion of them.

Origin of a r(22) was studied in only two case (Case 1 of Funderburk *et al.*, 1979 and Fowler *et al.*, 1980) using sequential quinacrine and silver staining. Funderburk *et al.* (1979) could not estimate its origin, but Fowler *et al.* (1980) estimated it was of maternal origin. In the present case, however, the origin of a r(22) was estimated to be of paternal.

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