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PALLISTER-KILLIAN SYNDROME: CYTOGENETIC AND BIOCHEMICAL STUDIES

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Summary Pallister-Killian syndrome is characterized by specific dysmorphic features and tissue-limited mosaicism for tetrasomy 12p. We describe an additional case of a stillborn neonate, who had not only the specific craniofacial features seen in the syndrome but also various internal malformations. Cytogenetic study showed that an extra F-like chromosome was found in 43% of lymphocytes and in 90% of fibroblasts. The high resolution G-banded pattern of the extra chromosome was consistent with an interpretation of an i(12p). The diagnosis of tetrasomy 12p was further confirmed by four-fold gene dosage effects in fibroblasts for GAPD and LDH-B, whose locus was both assigned to the 12p. The proportion of tetrasomic cells in fibroblasts decreased remarkably during long-term cultures. These results suggest that the tissue specific mosaicism in the syndrome is not simply a result of preferential selection against lymphocytes carrying the marker but may be related to the time of mosaic formation as well as the somatic selection of different intensity in different tissues.

INTRODUCTION

Pallister-Killian syndrome is an eponymous designation honoring the initial discoverers (Pallister *et al.*, 1977; Teschler-Nicola and Killian, 1981). It is a rare clinical entity based on a tissue-limited mosaicism for tetrasomy 12p: an isochromosome of chromosome 12p [i(12p)] is found in a high percentage of skin fibroblasts but virtually absent from blood lymphocytes. The similarity of banding patterns between the 12p and 21q has sometimes led to a misinterpretation of i(12p) as i(21q)

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(Fryns *et al.*, 1982; Hunter *et al.*, 1982; Kwee *et al.*, 1984; Lopes *et al.*, 1985). More than two dozen of cases have so far been reported (Reynolds *et al.*, 1987). Patients with Pallister-Killian syndrome invariably show characteristic clinical features, including normal intrauterine growth, severe mental retardation, pigmentary dysplasia, aberrant scalp hair pattern in infancy and coarse face (Buyse and Korf, 1983). The occurrence of serious internal malformations is rare, and the life prognosis is usually excellent. We describe here another case of a stillborn neonate associated with various internal malformations, where tetrasomy 12p mosaicism were confirmed by cytogenetic and gene dosage studies. The observation of i(12p) in a hitherto unreportedly high proportion of lymphocytes and the substantial reduction in percentages of tetrasomic cells during long-term cultures of fibroblasts provides an important insight into the cytogenetic mechanism of the distinctive pattern of chromosome abnormality in the syndrome.

CASE REPORT

The patient, a male infant with 32 weeks' gestational age, was a product of the fourth pregnancy of unrelated 34-year-old parents, which was complicated by polyhydramnios (*ca.* 5,000 ml of amniotic fluid) and premature onset of labor. The death had occurred during the delivery. There was no family history of mental

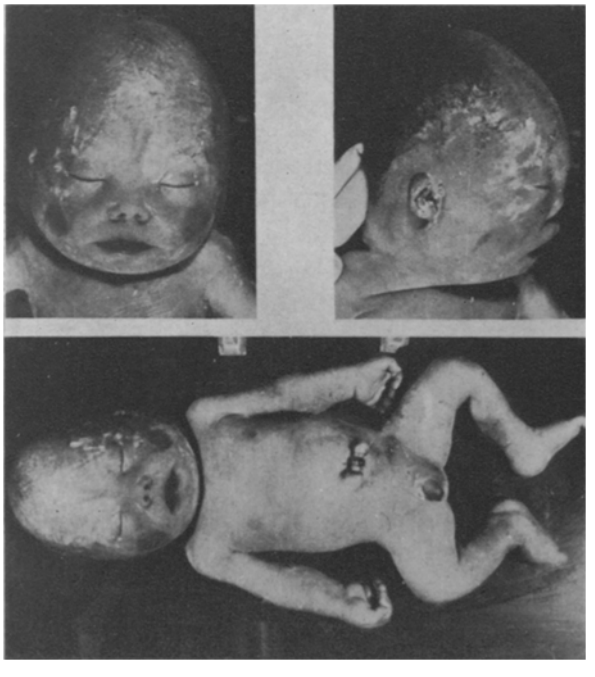

Fig. 1. The patient at autopsy.

retardation or multiple congenital anomalies. The first and third pregnancies yielded two normal siblings, while the second pregnancy ended in a first-trimester spontaneous abortion. The patient was appropriate for the gestational age (2,290 g in weight and 42.0 cm in height). Scalp hairs, thin and hypopigmented, were absent or very sparse in the frontal and bitemporal regions. The head was brachycephalic with a prominent forehead. Facial features consisted of microphthalmia with hypertelorism and epicanthal folds, short upturned nose with broad alae nasi and flat nasal bridge, long philtrum, downturned mouth and full cheeks (Fig. 1). Cleft palate and hypoplasia of ears with low settings were also noticed. The neck was very short with redundant skin. There was shortening of the humeri, broad hands with stubby fingers and postaxial polydactyly on the left foot. Congenital lymphedema was present on the eyelids, the neck and the backs of hands and feet. There was no pigmentary dysplasia of skin. Penis was small, and testes were undescended bilaterally. There was also a sacral dimple and an imperforate anus. Additional findings at autopsy included the left diaphragmatic agenesis with associated herniation of abdominal content into the left hemithorax, defect of the pericardium, the rightward displacement of the heart, juxtaductal coarctation of the aorta, profound hypoplasia of the left lung, accessory left lobe of the liver, common mesenterium and cerebral atrophy with dilated lateral ventricles.

CYTOGENETIC STUDY

Cytogenetic study was performed on a blood sample obtained by cardiac paracentesis at autopsy. Fibroblast cultures were also established from a skin sample taken from the anterior chest. According to the method of Ikeuchi and Sasaki (1979), phytohemagglutinin stimulated lymphocytes were treated with ethidium bromide 2 hr before harvest. Chromosomes were analyzed after GTG-, CBG- and QFQ-bandings and Ag-I staining. Table 1 shows the results of cytogenetic studies. Analysis of blood lymphocytes revealed an extra F-like chromosome in 51 of 119 (42.9%) cells examined. The marker chromosome was found to possess a symmetric structure on QFQ-banding and a single centromeric heterochromatin

Table 1. Results of cytogenetic studies.

	46,XY	47,XY,+i(12p)	48,XY,+i(12p) +mar[a]	Total
Blood lymphocytes	68 (57.1%)	51 (42.9%)	0	119 (100%)
Skin fibroblasts				
3rd passage	9 (9.6%)	84 (89.4%)	1 (1.0%)	94 (100%)
5th passage	4 (10.3%)	35 (89.7%)	0 (0 %)	39 (100%)
11th passage	12 (34.3%)	23 (65.7%)	0 (0 %)	35 (100%)
16th passage	18 (47.4%)	20 (52.6%)	0 (0 %)	38 (100%)

[a] A minute chromosome of unknown origin.

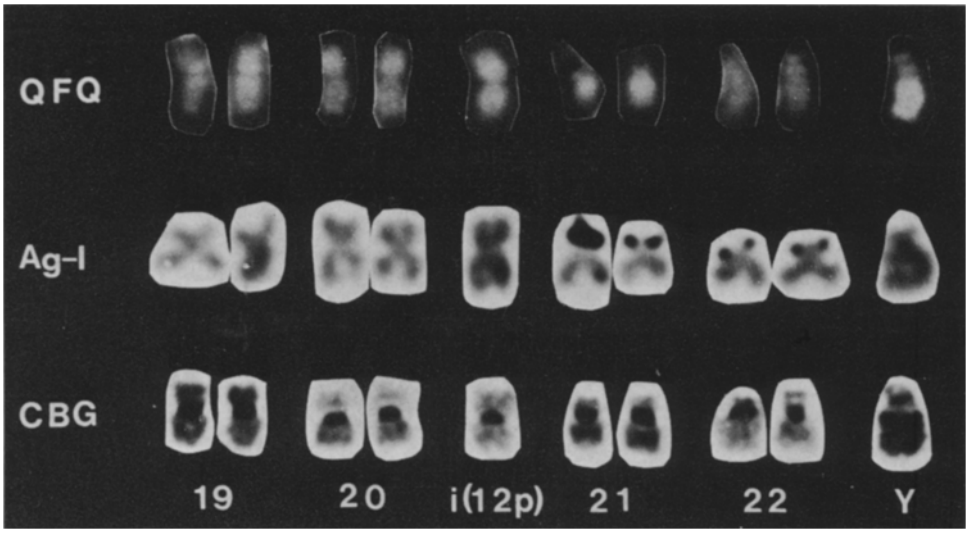

Fig. 2. Partial karyotypes of the patient (QFQ-banding, Ag-I staining and CBG-banding). The extra chromosome, i(12p), is shown in the middle of each row.

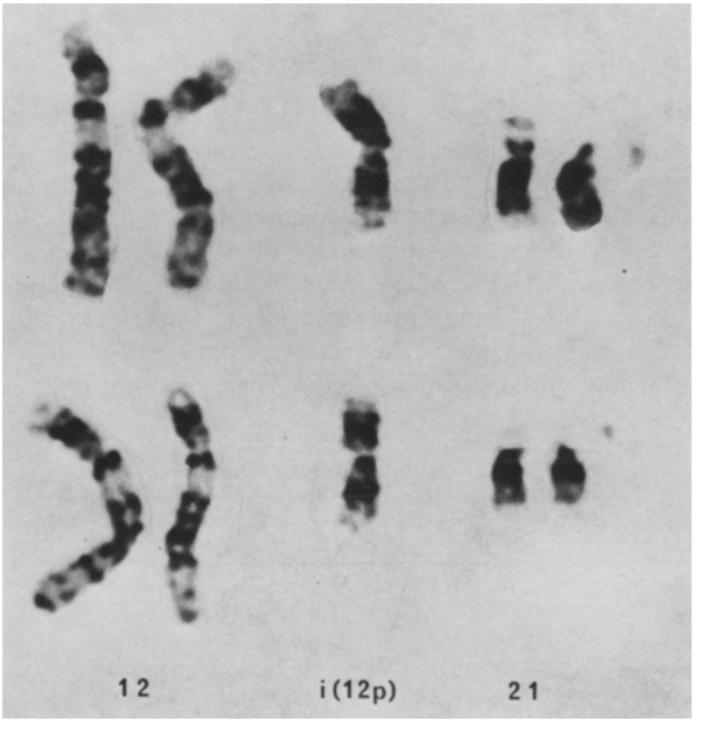

Fig. 3. High-resolution G-banding analysis of the extra chromosome. The banding pattern was compatible with an interpretation of i(12p).

on CBG-banding but no NOR-region on Ag-I staining (Fig. 2). The high-resolution G-banding pattern was compatible with an interpretation of i(12p) (Fig. 3). The same marker chromosome was present in 120 of 133 (90.2%) skin fibroblasts analyzed at early passages (the 3rd and 5th passages), but after long-term cultures the proportion of the abnormal cell line decreased significantly. The G-banded karyotypes of both parents were completely normal.

BIOCHEMICAL STUDIES

In order to confirm biochemically the origin of the extra chromosome, we studied gene dosage effects for glyceraldehyde-3-phosphate dehydrogenase (GAPD), lactate dehydrogenase component B (LDH-B) and soluble superoxide dismutase (SOD1). In Human Gene Mapping 8, GAPD, LDH-B and SOD1 have been assigned to 12p13.1→p13.32, 12p12.1→p12.2 and 21q22.1, respectively. These enzyme activities were estimated on cultured fibroblasts from the patient at the 3rd and 5th passages, using the method of Jaworek *et al.*, 1974, for GAPD, that of Wroblewski and La Due (1955) for LDH and that of McCord and Fridovich (1969) for SOD. As described by Homer *et al.*, 1969, LDH isoenzymes were separated on cellulose acetate. They were stained with nitro blue tetrazolium and quantified by a densitometer (Hiranuma, HAD-501). Fibroblasts harvested on near confluence were lysed by freezing and thawing thrice in distilled water containing 20 mM 2-mercaptoethanol. Controls consisted of cultured skin fibroblasts from 8 individuals with normal karyotypes, ranging from 2 months to 7 years in age. Proteins were assayed according to the method of Lowry *et al.*, 1951.

Results of enzyme assays are shown in Table 2. GAPD activity in fibroblasts from the patient was increased 1.77 times as much as in those from the normal controls, while LDH and SOD activities were within the normal ranges. The increment of the GAPD value in the patient was in good agreement with four-fold gene dosages for GAPD in 90% of cells tested, thus demonstrating that the extra chromosome

Table 2. Results of gene dosage studies on fibroblasts.

	GAPD[a]	LDH[a]	SOD[b]
Patient	7.69 U/mg protein (n=2)	4.80 U/mg protein (n=2)	15.4 U/mg protein (n=2)
Normal controls	4.34±0.78 U/mg protein (n=8)	4.75±0.69 U/mg protein (n=8)	14.7±2.9 U/mg protein (n=5)
Ratio of patient to normal value	1.77	1.01	1.05

[a] One unit is expressed in μM/min. [b] One unit is defined as an activity inhibiting 50% of reduction of cytochrome *c* in a SOD-free reaction system.

Table 3. Results of densitometric assays of LDH isozymes in fibroblasts.

	Patient	Control 1	Control 2	Control 3	Mean of controls
LDH isozymes [a]					
B_4	0%	0%	0%	0%	0%
A_1B_3	5.9%	1.5%	1.4%	1.2%	1.4%
A_2B_2	29.5%	20.9%	19.1%	21.7%	20.6%
A_3B_1	37.4%	41.2%	38.5%	35.9%	38.5%
A_4	27.2%	36.4%	41.0%	41.2%	39.5%
LDH total activity (U/mg protein)	4.80	4.86	4.25	5.49	4.87

[a] A mean of four densitometric assays performed in each subject.

is actually an i(12p). LDH electrophoresis disclosed more prominent staining of A_1B_3 and A_2B_2 isozymes but less distinctive staining of an A_4 isozyme in the patient than seen in the normal controls. The visual impression of the LDH zymograms was substantiated by the densitometric assays (Table 3). If the quantity of LDH component B and A in cells is denoted as x and $1-x$ for controls and y and $1-y$ for the patient, the ratios y/x and $1-y/1-x$ will represent a rough estimate of gene dosage for LDH-B and LDH-A in the patient, respectively. Solving the equations set up between densitometric data of isozymes A_3B_1 and A_2B_2, A_3B_1 and A_1B_3, and A_1B_3 and A_2B_2 gave $y/x=1.75$, 1.72 and 1.87, and $1-y/1-x=0.90$, 0.83 and 0.64, respectively. The ratios also supported our cytogenetic interpretation.

DISCUSSION

The present case was found to have not only the specific craniofacial features seen in Pallister-Killian syndrome but various internal malformations which have been rarely reported in the previous surviving cases. It should be noted that tetrasomy 12p mosaicism has been increasingly ascertained at birth or by prenatal diagnosis. Review of autopsy findings in 8 such cases (Gilgenkrantz *et al.*, 1985; Lopes *et al.*, 1985; Hiraishi *et al.*, 1987; Pauli *et al.*, 1987; Reynolds *et al.*, 1987; Steinbach and Rehder, 1987; Warburton *et al.*, 1987) showed that internal malformations are not at all rare and lung hypoplasia is a common pathological finding (Table 4). This type of malformation may explain why neonatal asphyxia is frequently associated with Pallister-Killian syndrome. Interestingly, 3 cases which did not survive birth and 2 prenatally diagnosed cases were also noted to have massive diaphragmatic hernias. As indicated by Kawashima (1987), the rhizomelic type micromelia involving the humeri has to be regarded as the specific phenotypic features for this syndrome in early infancy.

Table 4. Pathological findings in cases [a] with tetrasomy 12p mosaicism ascertained at birth or prenatally.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Present case
Prenatal diagnosis	+	+		+	+		+	+	
Neonatal death			+			+			+
Polyhydramnios	+	−	−	+	−	+	−	ND	+
Gestational age in weeks	29	ND [b]	31	22	24	38	20	19	32
Body weight in grams	1,920	ND	1,590	825	368	3,100	ND	475	2,290
Diaphragmatic hernia	−	+	+	−	−	+	+	ND	+
Lung hypoplasia	+	+	+	+	+	+	+	ND	+
Cardiac defect	+	−	−	ND	+	−	ND	ND	+
Intestinal malformations	−	−	−	+	+	−	+	ND	+
Imperforate anus	−	−	+	+	−	−	ND	ND	+
Urinary tract malformations	−	−	+	−	+	ND	ND	+	−
Rhizomelic type micromelia	+	ND	+	ND	+	ND	ND	ND	+
Brain malformations	+	ND	ND	ND	+	ND	ND	+	+

[a] Case 1: Gilgenkrantz *et al.*, 1985; Case 2: Lopes *et al.*, 1985; Case 3: Pauli *et al.*, 1987; Case 4: Reynolds *et al.*, 1987; Case 5: Steinbach and Rehder, 1987; Cases 6 and 7: Warburton *et al.*, 1987; and Case 8: Hiraishi *et al.*, 1987. [b] Not described.

The discrepancy in cytogenetic findings between fibroblasts and lymphocytes has been believed to be a hallmark of Pallister-Killian syndrome. The exact mechanism by which the tissue specific mosaicism for i(12p) occurs is unclear. The most plausible hypothesis proposed for the formation of i(12p) is a meiotic error with concurrent isochromosome formation due to a centromere misdivision involving a desynaptic univalent and nondisjunction (Van Dyke *et al.*, 1987). The isochromosome itself is also prone to malsegregate during the early cleavage mitosis, yielding two new cell lines with a chromosome constitution of 46 and 48,+i(12p), +i(12p) in addition to that with 47,+i(12p). Some selective process is presumed to lead to the eventual distinctive cytogenetic pattern.

The tissue-limited mosaicism, however, is not always consistent in the syndrome. In a number of cases (Lopes *et al.*, 1985; Raffel *et al.*, 1986; Pauli *et al.*, 1987; Reynolds *et al.*, 1987; Warburton *et al.*, 1987), i(12p) was found in 10–20% of blood lymphocytes. The percentage of tetrasomy 12p in lymphocytes in our case (43%) was even higher. Furthermore, as indicated in two other reported cases (Peltomäki *et al.*, 1987; Warburton *et al.*, 1987), the proportion of the abnormal cell line in fibroblasts decreased substantially during long-term cultures. These findings suggest that the occurrence of the tissue specific mosaicism in the syndrome is not simply a result of preferential selection against lymphocytes carrying the marker (Hunter *et al.*, 1985) but may be related to the time of mosaic formation as well as the somatic

selection of different intensity in different tissues. Perhaps, the mitotic nondisjunction occurs between the first cleavage division and early embryonal stage. The case misinterpreted as hexasomy 21 mosaicism (Ketupanya *et al.*, 1984) and ours may be the extreme examples concerning the time of mosaic formation.

The diagnosis of tetrasomy 12p in the present case was also supported by the four-fold gene dosage effects for GAPD and LDH-B in cultured fibroblasts. Biochemical confirmation of tetrasomy 12p has previously been made by the use of LDH-B determinations (Vine *et al.*, 1984; Gilgenkrantz *et al.*, 1985; Kelly *et al.*, 1985; Steinbach and Rehder, 1987; and Warburton *et al.*, 1987). On electrophoresis of LDH, extracts of fibroblasts containing an i(12p) in nearly all cells were shown to produce a strong A_2B_2 band and a distinct A_1B_3 band. In those containing less than 30% of the tetrasomic cells, however, an A_1B_3 isozyme is barely recognized, limiting the sensitivity of LDH-B determinations (Warburton *et al.*, 1987). The tetrameric structure of LDH and the reduced expression of LDH-B gene products in fibroblasts may be responsible for the inaccuracy of LDH-B determinations. We wish to emphasize that the assay of GAPD is simpler and more reliable in the biochemical confirmation of tetrasomy 12p.

Recently, tetrasomy 12p has been proven by Southern blot hybridizations using a KRAS 2 gene probe localized in the 12p (Peltomäki *et al.*, 1987). Though this study failed to determine when i(12p) had been formed or from which parent the isochromosome had originated, such a methodological approach may be promising to solve the problems if an fully informative restriction fragment length polymorphism of a gene localized in the 12p is available.

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