Pallister-Killian syndrome in a preterm newborn who died soon after precipitous delivery: cytogenetic analysis

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Summary

The authors report a preterm neonate with dysmorphic traits and cleft palate who was born preterm because of precipitous delivery and died soon after birth notwithstanding neonatal intensive care unit (NICU) support. The cytogenetic analysis on fibroblasts from post-mortem skin biopsy demonstrated a Pallister-Killian syndrome (PKS). PKS is a cytogenetically syndrome characterized by a tissue limited mosaic distribution of one isochromosome 12p (tetrasomy 12p). Clinical manifestations of PKS are variable, and some symptoms may overlap with other malformative syndromes, thus the correct diagnosis mainly depends on the demonstration of the specific cytogenetic abnormality.

Key words: Pallister-Killian Syndrome; Cytogenetic analysis; FISH.

Introduction

Pallister-Killian syndrome (PKS) is a rare genetic disorder, first described in 1977 by Pallister *et al.* [1], in two adult patients with severe mental retardation, seizures, hypotonia, "coarse" facies, limbs anomalies, multiple visceral malformations, and anomalies of skin pigmentation. In 1981 Killian and Teschler-Nicola reported a similar clinical phenotype in a three-year-old girl [2].

PKS is cytogenetically syndrome characterized by a tissue limited mosaic distribution of one isochromosome 12p (tetrasomy 12p) [3-5]. The supernumerary chromosome is present in a high percentage of fibroblasts and bone marrow cells, whereas lymphocytes are usually normal. Prenatal diagnosis may be achieved from chorionic villus, amniocytes, and other fetal tissues, not including fetal blood.

Clinical manifestations of PKS are variable, and some symptoms may overlap with other malformative syndromes, for instance Frin's syndrome [6], thus the correct diagnosis mainly depends on the demonstration of the specific cytogenetic abnormality.

The authors report a preterm neonate with dysmorphic traits and cleft palate, diagnosed as PKS by cytogenetic analysis on fibroblasts from post-mortem skin biopsy.

Case Report

The authors confirm that the patient described in the case report had given her informed consent for the case report to be published.

The patient was a 41-year-old Caucasian woman who came to the present emergency delivery room for preterm labor and immediately delivered a male neonate at a gestational age of 30 weeks who died soon after birth notwithstanding neonatal intensive care unit (NICU) support. The parents were in good health and unrelated. The mother was 41 years and the father 44 years at the time of the delivery. The family history was unremarkable in the maternal family, while a great number of abortions was referred for the grandmother of the patient's father. The couple had two previous miscarriages in the first trimester, which were not investigated. During the present pregnancy, the sole noted echographic anomaly was polyhydramnios. The woman refused to undergo first trimester ultrasound screening such as combined test with measurement of nuchal translucency.

The newborn, weighing 1,320 grams (25° percentile), showed a coarse face, sparse hair in the frontal area, high forehead, sparse eyebrows, ocular hypertelorism, broad nasal root, short nose, long and smooth philtrum, thin upper lip, cupid bow shape, short neck, diffuse hypertricosis, and sacral dimple (Figure 1). During the autoptic examination the external appearance of the brain was normal, with hyperaemic pial membrane. The palate showed a schisis in the posterior side of the arch; the thoracic, abdominal and pelvic organs were normal.

Standard cytogenetics

All the cytogenetic analysis of the patient were performed on cultured skin fibroblasts. The bioptic tissue was cut into fine pieces by scissors or sterile scalpel in petri dish containing Hank' s solution supplemented with antibiotics (usually penicillin and streptomycin). The tissue fragments were then transferred into a conical tube containing a collagenase A solution and incubated overnight a 37°C in water bath. The resulting cellular suspension was then centrifuged, the cells were suspended in complete culture medium (Chang medium), inoculated in two culture flasks, and incubated at 37°C in 5% CO_2 atmosphere. A good cell growth was observed two to three days later and a complete monolayer was formed in seven to ten days. For chromosome analysis, the cells were detached from the flasks by trypsin treatment, suspended in complete medium, and transferred in suitable aliquots onto 30 mm petri dishes with a coverslip at the bottom. After 24–48 hours the mitotic cells were blocked by colcemid treatment for two hours and harvested by "in situ" method. Briefly, after hypotonic shock with KCl 0,075 M for ten minutes and two passages in fixative methanol: acetic acid 3: 1, the coverslips were removed from dishes and air dried. After 24 hours the slides were stained with Quinacrine mustard for QFQ banding. Routine chromosome analyses of both parents were performed on blood lymphocytes using standard methodologies.

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Fluorescence in situ hybridization (FISH) studies

FISH experiments were performed using a whole chromosome painting probe (wcp) and a specific centromeric probe (CEP) of chromosome 12. The slides for FISH were pre-treated with pepsin in order to remove excessive cytoplasmic background, then they were dehydrated through a decreasing alcohol sequence and air dried. Some slides were immediately used for FISH, others were stored in freezer at -20°C until used.

Probes and slides were co-denatured at 72°C for three minutes and then incubated overnight at 37-42°C using a hybridization system. The post-hybridization washes, according to the manufacturer's protocol, were with 0.4X SSC plus 0.3% Nonidet at 72°C for two minutes, followed by a rapid wash in 2X SSC plus 0.1% Nonidet at room temperature for 30 seconds. The slides were then drained, stained with DAPI/antifade, and observed with fluorescence microscope using suitable filters.

The standard cytogenetic analysis, performed on fibroblasts at first passage in culture, showed the presence of a supernumerary metacentric chromosome, consistent with an isochromosome for the short arms of one chromosome 12, i(12p), in 16/16 cells scored (Figure 2A). Subsequent FISH with the wcp(12) confirmed the origin of the isochromosome from chromosome 12 (Figure 2B). In the same preparation were also present metaphases at 46 chromosomes, lacking in the supernumerary i(12p), hence the karyotype was a mosaic: 46,XY[6]/47,XY,+i(12p) [94] with a percentage of abnormal cells of 94%. Interphase FISH with CEP12 detected three signals (cells with the supernumerary chromosome) in 290/310 nuclei 93%) (Figure 2C). Both parents had normal chromosomes.

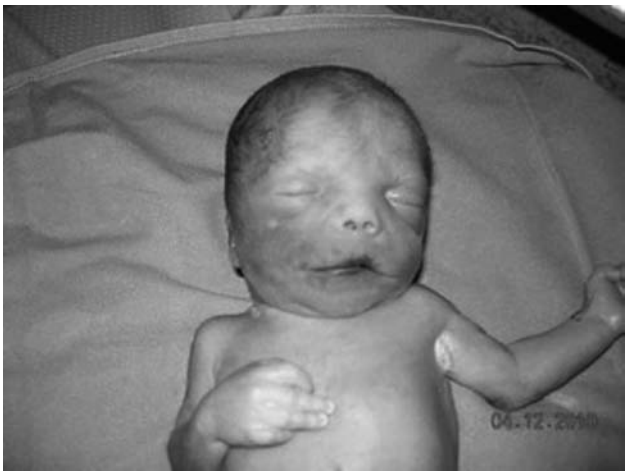

Figure 1. — Frontal view of the neonate.

Discussion

PKS is a rare, sporadic disorder caused by a mosaic supernumerary isochromosome 12p. Isochromosomes are uncommon chromosomal anomalies, which may occur with different mechanisms and more frequently involve 9p, 12p, 15q, 18p, 21q, 22q, Xp, and Xq [3,7-9]. The parental origin of i(12p) is maternal in nearly all cases [9-11]. Recently, Wilkens *et al.* [12] reported a comprehensive evaluation of 59 affected individuals and reviewed the previously reported cases. The authors reported that at least greater than 47% of the PKS pregnancies were associated with polyhydramnios which can be associated with preterm delivery (as in the present case) [12].

The variable mosaic distribution of i(12p) is characteristic of tetrasomy 12p; loss of i(12p) could be explained by

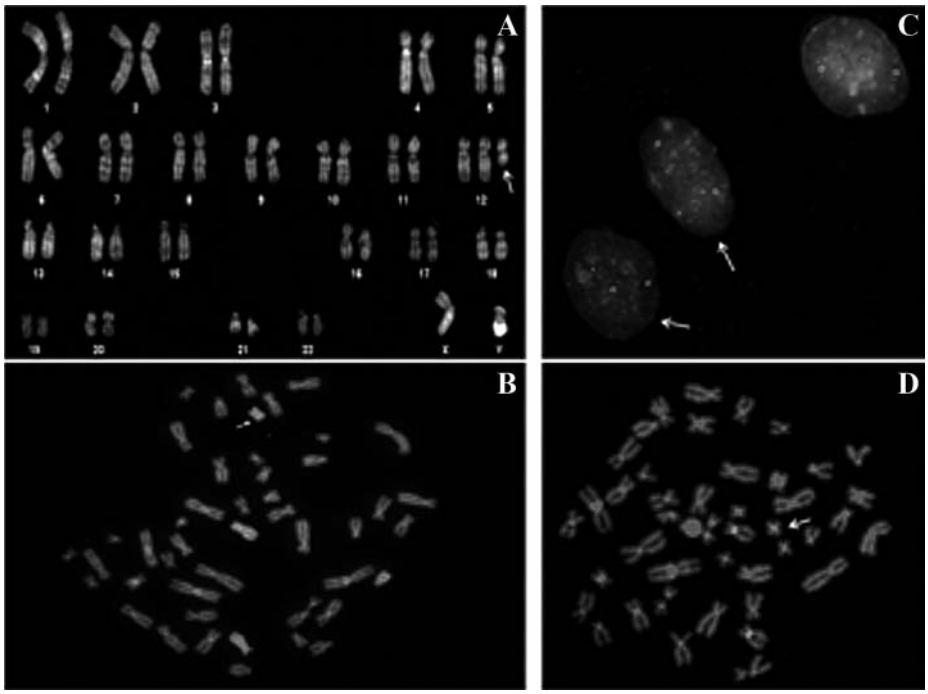

Figure 2. — A: QFQ banded karyotype. The arrow indicates the supernumerary chromosome; B: FISH with wcp of chromosome 12; C: Interphase FISH with centromeric probe of chromosome 12 showing both trisomic and disomic nuclei; D: Metaphase FISH with centromeric probe of chromosome 12. The arrow indicates the i(12p).

a selective advantage of the normal diploid over the tetraploid cells [13]. This mechanism, which is likely active both *in vivo* and *in vitro*, may account for the difficulty of detect i(12p) mosaic in fibroblasts of adult patients and in cultured lymphocytes, which are rapidly proliferating cells, or in amniocytes after so many replications in culture [14].

In postnatal cases, tetrasomy 12p may be found in variable rates in skin fibroblasts of newborns or young patients, while it is typically absent in blood lymphocytes. In alternative to skin biopsy, another tissue which seems to be informative is buccal smear [15].

Postnatal clinical manifestations of PKS are variable and may involve different organs and systems. The commonest symptoms are: coarse facies with a high forehead, sparse scalp hair, hypertelorism, broad nasal bridge, streaks of hypo-hyperpigmentation, hypotonia, congenital heart defects, and diaphragmatic hernia. Profound mental retardation is a constant feature.

The fetal phenotype is variable as well, therefore the ultrasonographic findings are inconstant or may be absent. The main ultrasound anomalies include: polyhydramnios, congenital diaphragmatic hernia, and micromelia, of predominantly rhizomelic type. Less frequently are reported: hydrops fetalis, hygroma colli, increased nucal translucency, fetal overgrowth, ventriculomegaly, dilatation of cavum pellucidum, absence of stomach visualization, and presence of a sacral appendix. Intrauterine growth retardation was never reported [14, 16]. Some authors suggest that the association of diaphragmatic hernia, polyhydramnios, and short femurs in a fetus with normal or increased growth is enough to suggest the PKS [17]. In addition, a fetal profile showing a small nose and a thin upper lip with a protruding lower one may be a further marker of the syndrome [18, 19].

Conclusions

This case suggests that, at the time of birth, diagnosis of PKS could be suspected even in absence of significant congenital malformations, when some dysmorphic signs suggesting the syndrome are noted, particularly in patients with normal chromosomes on peripheral lymphocytes. In these cases the chromosomal complement requires a further control in other tissues, e.g in skin fibroblasts or in buccal smears. A correct cytogenetic diagnosis is indeed essential for genetic counselling, since the recurrence risk may be considered practically absent in PKS.

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