

# Pallister–Killian Syndrome Caused by Mosaicism for a Supernumerary Ring Chromosome 12p

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Pallister–Killian syndrome (PKS) is a rare but distinctive chromosomal syndrome distinguished by severe intellectual impairment, characteristic facial features, and variable structural anomalies. The characteristic cytogenetic abnormality in PKS is a supernumerary isochromosome 12p that confers mosaic tetrasomy. We describe a female child with PKS in whom tetrasomy 12p resulted from a supernumerary ring chromosome containing two copies of chromosome 12cen → p13, a novel cytogenetic finding. The ring chromosome exhibited tissue-limited mosaicism, being absent in blood but detected in 38% of buccal mucosa cells and 41% of skin fibroblasts. Our patient demonstrated the typical dysmorphic characteristics of PKS, but her development was relatively advanced in comparison to children with isochromosome PKS. Her milder developmental phenotype may be attributable to differences in the mosaic distribution or the genomic content of the ring chromosome compared to mosaic isochromosome 12p. © 2009 Wiley-Liss, Inc.

**Key words:** Pallister–Killian syndrome; chromosome 12; tetrasomy 12p; ring chromosome

## INTRODUCTION

Pallister–Killian syndrome (PKS) is a rare sporadic disorder caused by mosaicism for tetrasomy of chromosome 12p [Pallister et al., 1977; Killian and Teschler-Nicola, 1981]. Clinical features include intellectual impairment that is usually severe, streaks of altered skin pigmentation, and facial dysmorphism that includes prominent forehead with temporal balding, hypertelorism, short nose, and wide mouth. All organ systems can be affected, but prominent malformations are cleft palate, supernumerary nipples, diaphragmatic hernia, congenital heart defects, and genital abnormalities. Birth weight and length can be normal or increased but growth subsequently slows [Schinzel, 1991].

The characteristic cytogenetic abnormality that causes PKS is a supernumerary isochromosome 12p i(12p) [Peltomaki et al., 1987; Zhang et al., 1989; Cormier-Daire et al., 1997; Struthers et al., 1999]. Tissue specific mosaicism is characteristic, with the proportion of cells containing i(12p) reported to be 0–2% in lymphocytes,

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50–100% in skin fibroblasts and buccal mucosal cells, and 100% in amniocytes and bone marrow cells [Ward et al., 1988; Ohashi et al., 1993]. Diagnosis of PKS is usually confirmed by fluorescence in situ hybridization (FISH), using DNA probes specific to chromosome 12p applied to a buccal mucosa sample [McLeod et al., 1991; Manasse et al., 2000] or to skin fibroblasts [Speleman et al., 1991].

In the great majority of patients the i(12p) is maternal in origin, with the underlying mechanism thought to involve a combination of centromere misdivision and nondisjunction at meiosis [Rivera et al., 1986; Van Dyke et al., 1987; Los et al., 1995; Turleau et al., 1996; Cormier-Daire et al., 1997; Schubert et al., 1997; Struthers et al., 1999]. As a result, the i(12p) is present at conception and mosaicism results from postzygotic mitotic loss [Peltomaki et al., 1987]. There is no apparent correlation between the proportion of tetrasomic cells and the severity of clinical presentation [Schinzel, 1991].

Here we report on an unusual patient with PKS in whom the underlying cytogenetic mechanism is a supernumerary ring chromosome comprising two copies of 12p, a finding not previously reported.

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## CLINICAL REPORT

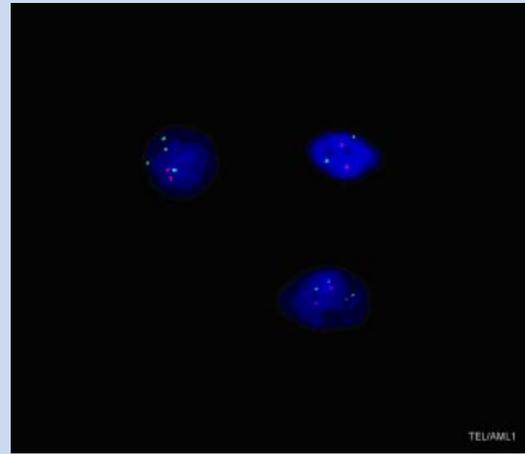
The proposita is the first child of non-consanguineous Caucasian parents. Family history and antenatal history were unremarkable. There was no known teratogen exposure and antenatal ultrasounds were reported as normal. Delivery was by vacuum-assisted delivery at term with birth weight of 3.44 kg (50th centile) and length 50 cm (>50th centile). Dysmorphic features and a cleft palate were noted at birth; however results of peripheral blood karyotype and FISH for 22q11 deletion were normal.

At age 7 months, the patient was referred to the genetics service with developmental delay and dysmorphic features. She was noted to have temporal alopecia, periorbital fullness, ptosis, nystagmus, wide mouth, long philtrum, and cleft palate (Fig. 1). She had bilateral single palmar creases, bilateral accessory nipples, small hands and feet with dorsal edema, and hypoplasia of the labia with a common anal and vaginal opening. Growth parameters were now all below the third centile for age, and there was moderate hypotonia and global developmental delay. The patient was unable to roll and had persistent head-lag.

Subtelomere MLPA analysis on lymphocytes and magnetic resonance imaging of the brain were both normal. The patient's development was monitored and considerable progress was observed over the ensuing year. By 9 months, she was lifting her head and sitting with support. At 11 months she was rolling and grasping



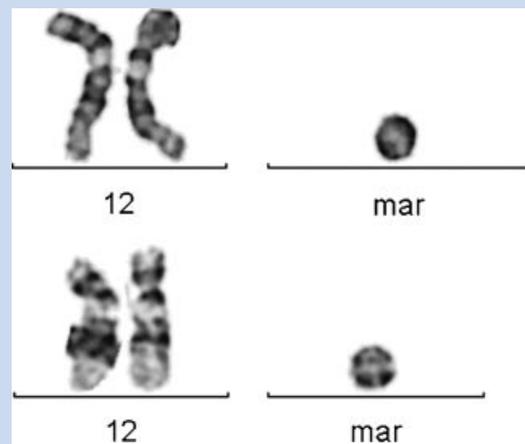
**FIG. 1.** Patient at 2 years 4 months: Note temporal alopecia, wide mouth, small hands. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]



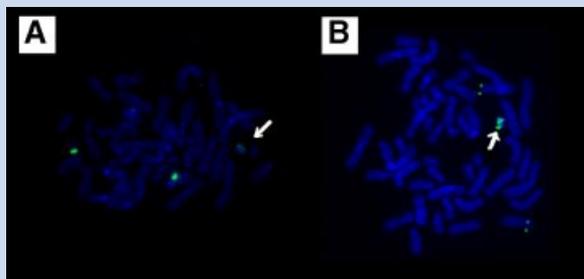
**FIG. 2.** Interphase FISH of buccal mucosa cells, showing both the ring chromosome 12 cells (four green signals) and normal cells (two green signals) using the TEL probe. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

objects, and by 16 months, she was able to stand and vocalize. Unfortunately, this rate of progression did not continue, and a review at 2 years of age revealed little further gain in developmental milestones. Growth parameters at this time revealed height 77 cm (<3rd centile), weight 10.5 kg (10th centile), and head circumference 48.5 cm (75th centile).

A diagnosis of PKS was suspected on the basis of the patient's clinical features. Interphase FISH studies were performed on buccal mucosa cells utilizing a chromosome 12 probe specific for the 12p13 region (TEL, Vysis, Downers Grove, IL). Four signals from the chromosome 12p13 (TEL) probe were observed in 72 of 189 (38%) interphase nuclei examined (Fig. 2); the remaining nuclei contained two signals.



**FIG. 3.** Partial karyotypes of cultured fibroblast cells (GTL-banding) showing the two normal chromosomes 12 and the chromosome 12 derived ring.



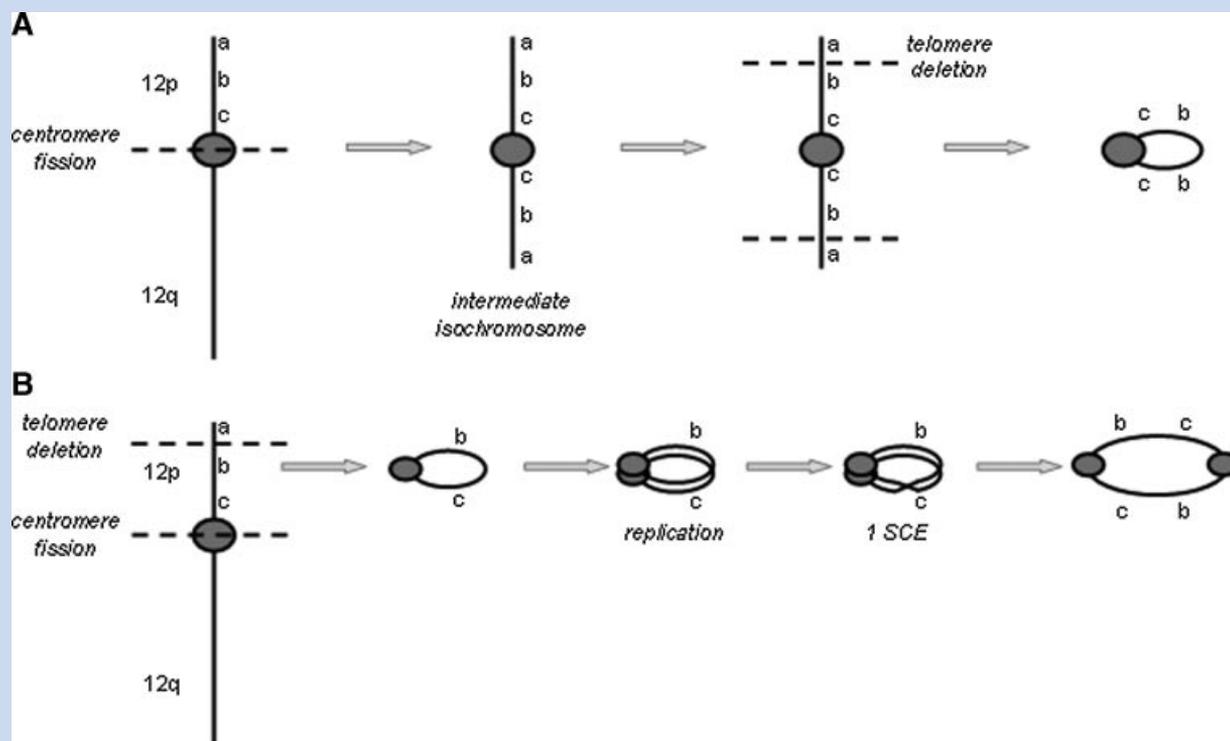
**FIG. 4.** A: Metaphase FISH analysis of cultured fibroblast cells with the chromosome 12alpha satellite centromere probe, D12Z1 [green]. Arrow indicates ring chromosome with signal. B: Metaphase FISH analysis of cultured fibroblast cells with the TEL probe localized to 12p13 [green]. Arrow indicates ring chromosome with signal. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

Chromosomal analysis was performed on fibroblasts derived from a skin biopsy. Analysis of fibroblast mitoses by GTL-banding showed the presence of a supernumerary ring chromosome in 11/27 (41%) cells examined (Fig. 3). Metaphase FISH studies using the

12p13 (TEL) probe and a chromosome 12 centromere-specific probe (D12Z1) showed that the ring chromosome contained two copies TEL and two copies of D12Z1 (Fig. 4). The ring chromosome resulted in tetrasomy for at least the region 12cen → p13: 47,XX,+r(12)::p?13 → cen → p?13 → cen::). ish r(12)(TEL++, D12Z1++)[11]/46,XX[16]. The rapid loss of the ring from cultured cells prevented further molecular analysis.

## DISCUSSION

Our patient presents many of the classical phenotypic features of PKS, including typical facial features, cleft palate, supernumerary nipples, genital abnormalities, and postnatal growth delay. Yet developmental progress has been more advanced than would be expected for typical PKS. Most reported adult patients with PKS are nonverbal, and only 30% walk independently [Reynolds et al., 1987; Mathieu et al., 1997]. Two possible mechanisms are proposed to explain the milder developmental phenotype in our patient. First, the tetrasomic segment in the ring chromosome of our patient may be reduced in size compared to an isochromosome 12p. This may occur because in the ring chromosome there is deletion of the 12p telomere and presumably some subtelomeric sequence. Meanwhile, comparative genomic hybridization (CGH) studies have shown not only the presence of this expected terminal deletion in ring chromosomes, but also that deletions are often larger than expected and



**FIG. 5.** Two possible mechanisms for formation of a ring chromosome comprising two copies of 12p. In A there is breakage and reunion of the arms of an intermediate isochromosome. In B chromosome breakage generates a fragment comprising most of 12p and at least part of the centromere followed by fusion of the ends to produce a single ring. Following replication, a sister-chromatid exchange generates a double-sized and symmetrically dicentric ring. The cytogenetic results in our patients are consistent with mechanism B.

can occur with additional interstitial deletions or duplications which impact the phenotype [Rossi et al., 2008]. Examination using CGH array analysis would identify the absence of segments of 12p not visible on banded examination; however an adequate amount of DNA could not be extracted from the early passages of our patient to allow this. Second, the tissue distribution of the ring chromosome in our patient may differ from that of the i(12p) in other PKS patients. This hypothesis is consistent with the ring chromosome being found in 41% of fibroblasts and 38% of buccal mucosa cells, a proportion lower than is typical for i(12p) PKS. Tissue distribution would be expected to differ if the ring chromosome arose during a postzygotic mitotic division, rather than at meiosis as is typical for isochromosome PKS. Furthermore, even if the ring was present at conception, it may be less stable than an i(12p), leading to more rapid loss in some tissues.

A milder developmental phenotype has been reported in several other PKS patients, including a 5-year-old child with only moderate delay in gross motor and language development and normal social skills [Bielanska et al., 1996], a 7-year-old patient with a relative lack of physical manifestations and only mild speech delay who was able to attend mainstream schooling [Schaefer et al., 1997], a 15-year-old who attended a normal school until the age of 14 years and a child who at age 13 years had a full-scale IQ of 93 and was later diagnosed with an autism spectrum disorder [Genevieve et al., 2003; Stalker et al., 2006]. It is possible that in these patients with a milder phenotype, the isochromosome 12p has originated from a postzygotic event.

Duplicated ring chromosomes can arise by one of two possible mechanisms (Fig. 5). The first mechanism involves generation of an intermediate isochromosome, followed by breakage and reunion of the two arms to form a ring [Wong et al., 1989]. Alternatively, chromosome breakage can initially generate a single copy ring chromosome, with subsequent replication combined with a single sister-chromatid exchange generating a double-sized and symmetrically dicentric ring [McGinniss et al., 1992]. The latter mechanism is the most likely in our patient because the ring chromosome appeared symmetrical and was dicentric; however, due to rapid loss of the ring from culture, it cannot be determined whether the ring originated in meiosis or mitosis.

Recent evidence suggests that many small ring chromosomes contain unique sequence from only one arm, consistent with their origin involving centromere misdivision [Baldwin et al., 2008]. The FISH results in our patient are also consistent with centromere misdivision, with the centromeric alpha-satellite signal appearing of lower intensity in the ring chromosome compared to the normal chromosome 12s, consistent with partial deletion of alpha satellite.

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