Hum Genet (1993) 91:121–127

human genetics

Molecular cytogenetic study of patients with Pallister-Killian syndrome

Marcelo Larramendy[1,2], Mervi Heiskanen[1], Maija Wessman[1], Annukka Ritvanen[3], Päivi Peltomäki[1], Kalle Simola[4], Helena Kääriäinen[1], Harriet von Koskull[6], Marketta Kähkönen[5], Sakari Knuutila[1]

[1] Department of Medical Genetics, University of Helsinki, Haartmaniukatu 3, SF-00290 Helsinki, Finland
[2] Laboratorio de Citogenética (Facultad de Ciencias Naturales y Museo de La Plata) y Cátedra de Genética y Mejoramiento Animal y Vegetal (Facultad de Ciencias Agrarias y Forestales), Universidad Nacional de La Plata, La Plata, Argentina
[3] Department of Medical Genetics, Finnish Population and Family Welfare Federation, Helsinki, Finland
[4] Department of Clinical Genetics, Tampere University Central Hospital, Tampere, Finland
[5] Clinical Genetic Unit, Oulu University Central Hospital, Oulu, Finland
[6] Laboratory of Prenatal Genetics, Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Helsinki, Finland

Received: 23 March 1992 / Revised: 17 June 1992

Abstract. The Pallister-Killian syndrome (PKS) is characterized by tissue limited chromosomal mosaicism, i.e. the presence of a supernumerary metacentric chromosome [i(12p)] often confined to skin fibroblasts while the karyotype of cultured lymphocytes is normal. In the present study, chromosome painting by chromosomal in situ suppression (CISS) hybridization and interphase cytogenetic procedures employing biotinylated or digoxigenin labelled probes was carried out. These probes comprised a chromosome 12 specific library (LA 12NSO1) and chromosome 12 centromere specific α-satellite (pSP12-1). They were used to analyse and quantify the presence of i(12p) in lymphocytes, granulocytes/monocytes, skin fibroblasts and buccal mucosal cells from five patients and one aborted fetus with PKS, and ten normal donors. CISS hybridization on mitotic skin fibroblasts reliably indicated the presence of i(12p) cells, even when metaphases of poor quality were included in the analysis. Two of the five patients showed i(12p) in a small proportion (≤0.5%) of the cultured lymphocytes too. The interphase cytogenetics procedure did not reveal the isochromosome in lymphocytes or granulocytes/monocytes in any of the patients. Two of the six patients had a twofold increase in the number of buccal mucosal cells with three hybridization signals over control values. However, for mucosal cells, methodological improvements are required. For cytogenetic diagnosis of PKS, cultured fibroblasts subjected to chromosome painting by CISS hybridization with a chromosome 12 specific library probe are recommended.

Introduction

Pallister-Killian syndrome (PKS) is a rare sporadic human disorder. The affected individuals show highly typical coarse facies, pigmentary skin anomalies, unusual hair pattern and severe psychomotor retardation with autistic features (Gorlin et al. 1990).

The diagnosis of PKS is based on a characteristic cytogenetic abnormality, i.e. the presence of an extra metacentric chromosome, i(12p), which is usually confined to skin fibroblasts. Nevertheless, it is sometimes observed in cultured peripheral blood lymphocytes, but at a very low frequency (for review see Gorlin et al. 1990). Thus, the correct diagnosis has probably been missed in a number of patients since skin fibroblasts are not routinely included in karyotyping (Fryns et al. 1982; Gorlin et al. 1990 and references therein; Hall 1985; Hunter et al. 1982; Kwee et al. 1984; López et al. 1985). Nevertheless, over 40 PKS cases with documented i(12p) have been reported so far (Chrzanowska and Fryns 1989; Greig et al. 1991; Soukup and Neidich 1990; Speleman et al. 1991; Steinbach and Rehder 1987).

During the past few years, library and human α-satellite probes specific to individual chromosomes as molecular markers have emerged as new tools for cytogenetic and genetic studies of both metaphase chromosomes and interphase nuclei (Cremer et al. 1988; Greig et al. 1991; Moyzis et al. 1987; Pérez Losada et al. 1991; Pinkel et al. 1988; Zhang et al. 1989).

We report molecular cytogenetic results obtained from various cells of five living patients and one aborted fetus with PKS. We used in situ hybridization with chromosome 12 centromere specific α-satellite and chromosome 12 specific library probes, which recognize specifically human chromosome 12.

Materials and methods

Patients and cell samples

Five patients (1–5) known to have PKS with a karyotype of mos46/47,+i(12p) in cultured skin fibroblasts were studied (Table 1). The pa-

Correspondence to: S. Knuutila

tients came from three different genetic counselling units. The diagnosis had been suspected owing to typical facial features and severe retardation in each case. In addition, several tissues obtained from a fetus diagnosed at amniocentesis and aborted at 17 weeks of gestation, were also included in the analysis. Previous cytogenetic analysis of cultured amniocytes of this fetus (patient 6) had revealed i(12p) in at least 28% of the metaphases analysed. For the present study, cultured skin fibroblasts obtained from patients 1–5 were recovered from liquid nitrogen after 3 to 5 years of storage. Similarly, skin and liver cell cultures from the fetus were restarted from cells stored in liquid nitrogen for 1 year. In all cases, the molecular cytogenetic analysis of fibroblasts was performed on exponentially growing cells after an overnight Colcemid (0.1 µg/ml, Gibco, Grand Island, N.Y., USA) treatment and conventional harvest. Slides were processed according to in situ hybridization procedures (see below) after an air drying period of at least 18 h.

Buccal mucosal cells and heparinized venous blood (20 ml) were obtained 6–18 h before processing. Prior to mucosal sampling, the donors rinsed their mouth twice with running tap water. Cells were scraped from the buccal mucosa with a wooden tongue depressor. These scrapings were smeared onto precleaned microscope slides and air dried. The smears were processed by in situ hybridization techniques after fixation in 94% ethanol (6–14 h) and air drying for at least 4 h.

Control blood and buccal mucosal samples were obtained from ten nonsmoking, healthy donors (20–30 years old) who had not had X-ray exposure or drug therapy in 6 months prior to the sampling.

Mononuclear leucocytes from whole blood were isolated with the Ficoll-Hypaque density gradient centrifugation technique (Pharmacia Fine Chemicals, Uppsala, Sweden). The mononuclear cells were resuspended in complete culture medium [80% RPMI 1640 (Gibco), 20% fetal calf serum (Gibco), 0.29 mg/ml L-glutamine (Gibco), 100 units/ml penicillin (Gibco), and 100 µg/ml streptomycin (Gibco)]. A part of this suspension was used to make cytospin preparations. The remaining cells were cultured at a density of 1.5×10^6 cells/ml in TC25 Falcon flasks. The in vitro lymphocyte stimulation was done either with phytohaemagglutinin P (PHA, 0.5 µg/ml, Gibco) or with pokeweed mitogen (PWM, 100 µg/ml, Gibco). The cultures were incubated at 37°C in a 5% CO_2 atmosphere for 96 h. During the final 4 h, the cells were treated with Colcemid (0.1 µg/ml, Gibco). Thereafter they were collected by centrifugation, washed twice with RPMI 1640 medium without serum, and divided into two aliquots. One aliquot was used for conventional cytogenetic chromosome spreads using hypotonic treatment (0.075 *M* KCl, 15 min, 37°C), fixation with methanol:acetic acid (3:1), and air drying. Afterwards these slides were processed according to the in situ hybridization technique described below. The remaining aliquot was used to make cytospin preparations by the methodology explained in detail by Knuutila and Teerenhovi (1989).

Granulocyte/monocyte samples obtained from the erythrocyte pellets of the Ficoll-Hypaque gradient samples after removal of red blood cells were used to make cytospin preparations.

The cytospin preparations mentioned above were incubated in methanol:acetic acid (3:1) for 1 to 2 h. After fixation, the slides were air dried for at least 4 h before in situ hybridization.

Chromosome painting by chromosomal in situ suppression (CISS) hybridization using a chromosome 12 specific library probe

The CISS hybridization studies were performed on metaphases of cultured skin fibroblasts and mitogen stimulated conventionally harvested lymphocytes.

Hybridizations were carried out with DNA from a bacteriophage library probe, established from sorted human chromosome 12 (Library LA 12NSO1, American Type Culture Collection, Rockville, Md., USA). The probe was labelled by nick-translation using biotin-11-dUTP (Sigma Chemical Co., St. Louis, Mo., USA) according to the instructions of the kit supplier (Nick-translation Kit, Bethesda Research Laboratories, Bethesda, Md., USA). CISS hybridization and detection of the hybridized probe with fluorescein isothiocyanate (FITC) conjugated avidin (Vector Laboratories, Burlingame, Calif., USA) were performed as described in detail elsewhere (Lichter et al. 1988). Amplification of the signal was performed as described by Pinkel et al. (1986).

The cells were analysed with a Leitz Laborlux D photomicroscope by scoring at least 100 metaphases per sample. Photographs were taken on Kodak Ektachrome 400 ASA colour slide film.

Interphase cytogenetics using a chromosome 12 centromere specific α-satellite probe

Cell preparations of cultured skin fibroblasts from five PKS patients and one fetus, and buccal mucosa cells, granulocites/monocytes, circulating lymphocytes, and mitogen stimulated lymphocytes from either PKS patients or controls were included in the interphase cytogenetic studies.

The slides were incubated in 0.01 *N* HCl containing 0.01 mg/ml pepsin (37°C, 4–10 min, Sigma), washed in distilled water, incubated in a sequential ethanol series (70%, 94%, absolute, 5 min each), and air dried for 1–4 h. Post-fixation incubation in 7% H_2O_2 in methanol for 30 min was included for buccal mucosa, granulocyte/monocyte, and liver cell samples, followed by dehydration in absolute ethanol.

Hybridizations were carried out with a chromosome 12 centromere specific α-satellite probe (pSP12-1, kindly provided by Dr. H. F. Willard, Stanford University, Calif., USA), which was labelled by nick-translation with biotin-11-dUTP (Sigma) or digoxigenin-11-dUTP (Boehringer Mannheim Biochemica, Mannheim, FRG) according to the instructions of the kit supplier (Nick-translation Kit, Bethesda Research Laboratories; DIG DNA Labeling Kit, Boehringer Mannheim). Digoxigenin-labelled probe was used only for hybridization of liver cells because these cells contain endogenous biotin preventing the use of a biotin-labelled probe.

Denaturation, hybridization, washing conditions, indirect immunological detection of hybridization of the biotin-labelled probe, and counterstaining of cells with Harris' haematoxylin were performed as described by Pérez Losada et al. (1991). Signals of the digoxigenin labelled probe were detected by an indirect immunofluorescence method using monoclonal mouse anti-digoxigenin antibody (Sigma) followed by incubations with FITC conjugated sheep anti-mouse antibody (Sigma), and finally with FITC conjugated donkey anti-sheep antibody (Sigma). Cells were counterstained with propidium iodide.

For the buccal mucosa, different pre-hybridization enzymatic treatments were assessed, including pepsin (Gibco, 0.01–1.0 mg/ml) and trypsin (Gibco, 0.1–1.0 mg/ml) digestions for different periods. In addition, several modifications of the denaturation, hybridization, and post-hybridization stringency conditions were used without achieving improvement on the results by the methods described above.

The slides were coded and analysed in a double blind manner, and the number of hybridization signals was evaluated and determined by analysing at least 500 interphase cells and 100 metaphase spreads per sample. Only non-overlapping cells with good morphology were included in the score. When necessary, photographs were taken on Kodak Ektachrome 64T colour slide film and on Kodak Imagelink HQ black-and-white film.

Results

Karyotype analysis by G-banding of metaphase cells

Previous diagnostic G-banding analyses had shown the presence of i(12p) in skin fibroblast samples from patients 1–5 (Table 1). Similar analysis on various cells from the fetus had revealed the isochromosome in amniocytes (28%), chorion villus cells (25%), skin fibroblasts (8%), bone marrow cells (70%), and cultured liver cells (59%) (Table 3).

Chromosome painting by CISS hybridization with a chromosome 12 specific library probe

CISS hybridization with the recombinant LA 12NSO1 library probe was used to recognize complete individual chromosome 12 within a metaphase plate. This technique

Table 1. Cytogenetic characteristics of the Pallister-Killian syndrome (PKS) patients

Patient	Sex	Age (years)	i(12p) present (%)[b]	
			PHA-stimulated lymphocytes	Skin fibroblasts
1[a]	F	9	0 (100)	96 (150) [5] Passages 1 and 5
2	M	14	0 (113)	100 (10) [6] Passage 1
3	M	18	0 (100)	45 (50) [3] Passage 3
4	F	14	0 (20)	81 (32) [5] Passage 2
5	F	7	0 (20)	15 (40) [5] Passage 2

[a] From Peltomäki et al. (1987)
[b] Number of G-banded metaphase analysed in parentheses; years elapsed prior to the present molecular cytogenetic study in brackets. PHA, phytohaemagglutinin

Table 2. Percentage of mitotic cells with two and three signals detected by chromosomal in situ suppression (CISS) hybridization with the biotinylated chromosome 12 specific library LA 12NSO1 probe in skin fibroblasts and mitogen-stimulated lymphocyte metaphases from PKS patients. At least 100 metaphases were analysed per sample. ND, not done; PWM, pokeweed mitogen

Patient	Skin fibroblasts		Peripheral lymphocytes			
	2 signals	3 signals	PHA stimulation		PWM stimulation	
			2 signals	3 signals	2 signals	3 signals
1	7	86	98	0.5	95	0.5
2	10	90	97	0.3	100	0
3	89	4	95	0	ND	ND
4	60	36	100	0	99	0
5	Failed	Failed	100	0	100	0

gave a very high hybridization efficiency, which was independent of the degree of spreading and morphology of the chromosomes. Chromosomes 12 in metaphases of poor quality, not amenable to conventional cytogenetic study, could reliably be included in the analysis.

Stimulated lymphocytes. In all patients, 95%–100% of the mitotic lymphocytes analysed had two chromosomes 12 (Table 2) and the frequency of lymphocyte metaphases with i(12p) was very low. In patients 1 and 2, three hybridization signals were observed in only a few metaphases (0.3%–0.5%) (Table 2). There were no differences in PHA or PWM stimulated cultures.

Skin fibroblasts. CISS hybridization on mitotic skin fibroblasts from PK patients revealed the i(12p) in 4% (patient 3) up to 90% (patient 2) of the metaphases analysed (Fig. 1A, B) whereas the proportion of cells with two hybridization signals varied from 7% (patient 1) to 89% (patient 3) (Table 2).

Fetal cells. In mitotic skin fibroblasts of the fetus, i(12p) was evident in 4% of the metaphases only and two hybridization signals were observed in 92% of the interphase nuclei (Table 3).

Interphase cytogenetics using a chromosome 12 centromere specific α-satellite probe

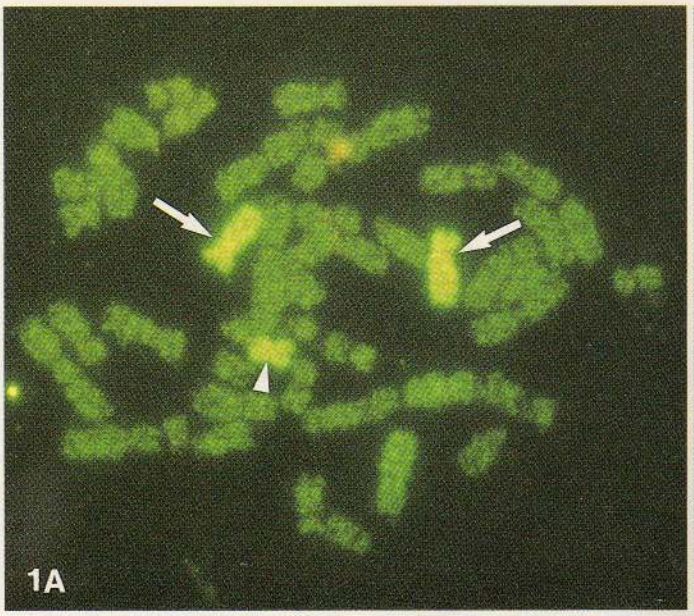
The α-satellite pSP12-1 probe detected the centromeric region of chromosome 12. The sequences recognized by this probe represent repetitive DNA consisting of tandem units of 1.4 kb (Greig et al. 1991).

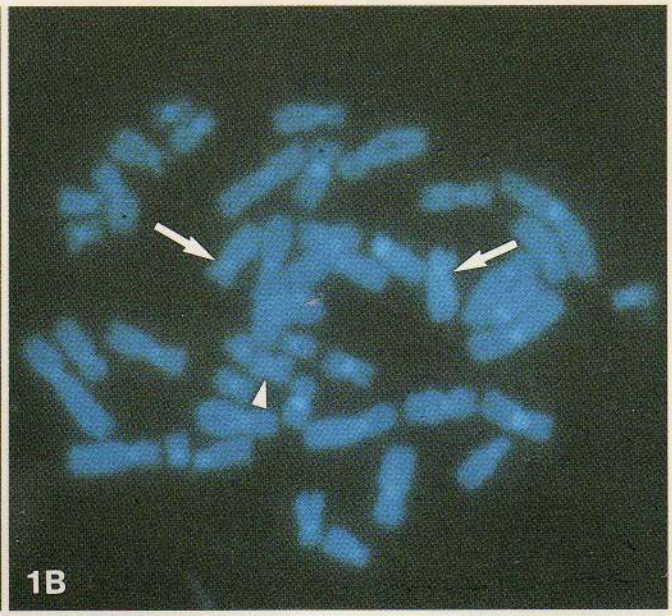
The α-satellite probe was not used in fibroblast cultures since the library probe proved superior in this respect, as shown in the previous section. However, the probe was used to detect a signal size polymorphism in i(12p), i.e. to analyse possible heteromorphisms in i(12p) among the PKS patients.

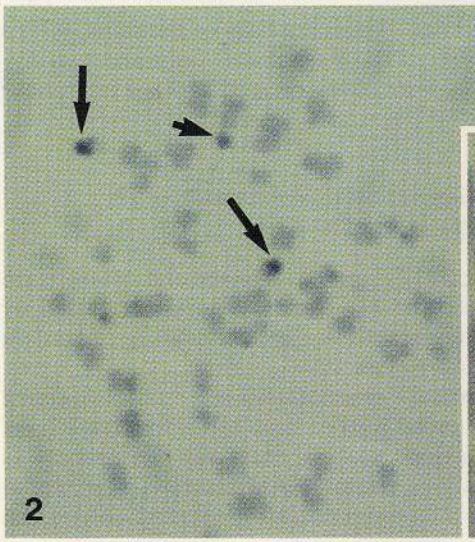
Table 3. Cytogenetic and molecular cytogenetic study of aneuploid i(12p) cells in different tissues of a fetus with PKS aborted at the 17th week of gestation

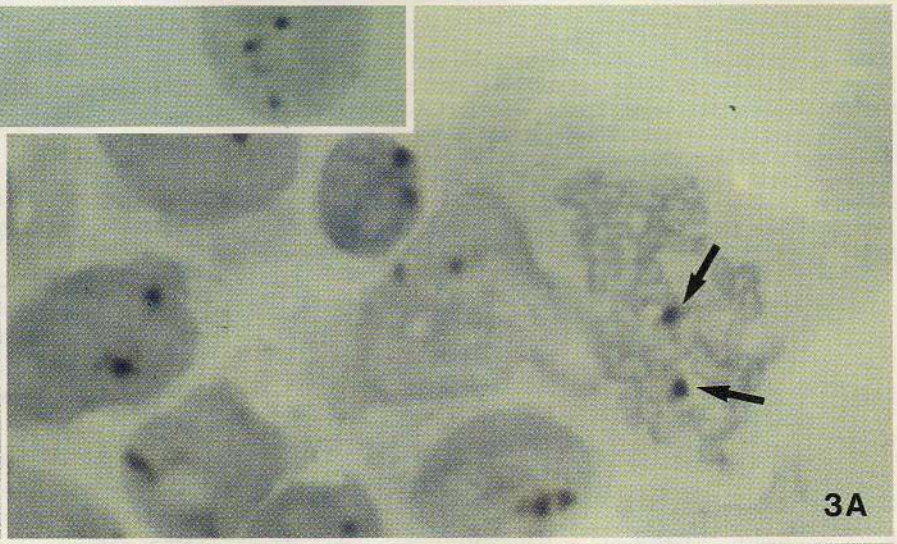
Type of cells	Frequency of cells with i(12p) by G-banding analysis[a]	Frequency of cells with hybridization signal (%)[b]			Chromosome 12 specific probe used
		2 signals	3 signals	No. of cells analysed	
Amniocytes	28 (44)				
Fibroblasts	8 (66)	79	9	268	pSP12-1
		92	4	100	LA 12NSO1
PHA-stimulated lymphocytes (3-day culture)	0 (100)				
Bone marrow (1-day culture)	67 (3)				
Chorion (2-day culture)	25 (4)				
Liver (2-day culture)	59 (32)	73	9	159	pSP12-1

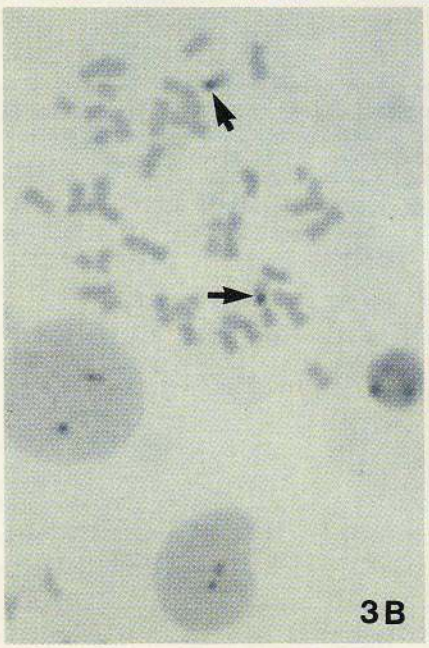
[a] The number of metaphases analysed is given in parentheses
[b] From two passages before freezing and two passages after thawing

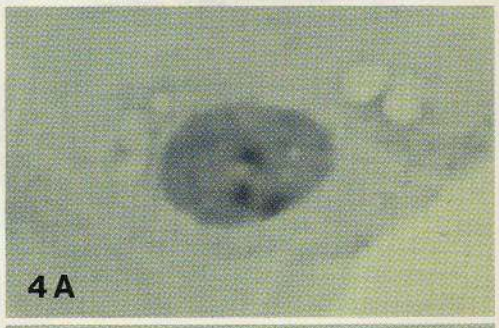

1A

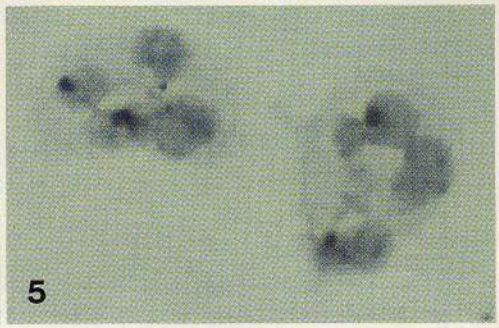

1B


2


3A


3B


4A


5

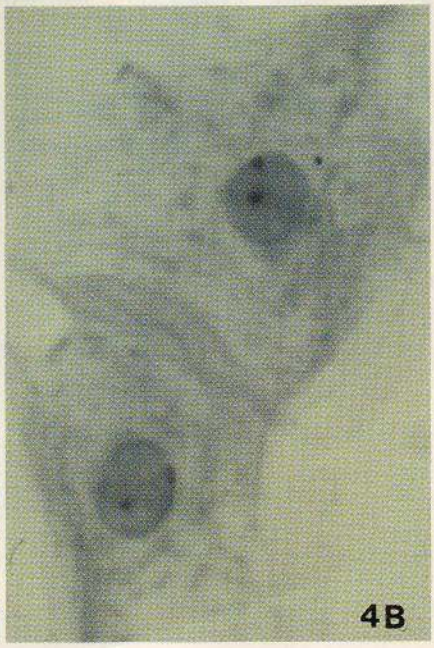

4B

Table 4. Percentages of interphase cells from PKS patients and control donors showing 0, 1, 2, and 3 signals indicating in situ hybridization with the biotinylated chromosome 12 centromere specific α-satellite pSP12-1 probe in buccal mucosal cells, granulocytes/monocytes, and lymphocytes. NE, Not evaluable

Donor	Buccal mucosal cells					Granulocytes/ monocytes		Peripheral lymphocytes culture time			
								0 h		72 h[a]	
	0 signal	1 signal	2 signals	3 signals	NE	2 signals	3 signals	2 signals	3 signals	2 signals	3 signals
Patients											
1	66	5	15	2	12	98	1	97	0	94	0
2	84	2	9	1	4	95	1	99	0	99	0
3	27	2	33	13	25	95	1	99	0.2	98	0.2
4	35	5	38	4	18	92	0.5	98	1	97	1
5	74	2	11	7	6	96	1	96	1	98	0.2
Controls											
7	67	2	8	0	23	95	1	99	0	99	0.1
8		Dense background			100	95	0.5	96	0.3	99	0
9		Dense background			100	83	2	97	0.2	95	1
10	49	0	44	0	7	88	1	96	1	96	1
11	85	2	6	1	6	92	3				
12	96	1	1	0	2	93	2				
13	46	11	31	4	8	89	2				
14	82	2	9	0	7	78	2				
15	61	2	30	0	7	95	2				
16	52	6	33	2	7	95	1				

[a] In the presence of PHA

Fig. 1. A Isochromosome i(12p) in a metaphase of a skin fibroblast from a PKS patient (patient 1) after CISS hybridization with a biotinylated chromosome 12-specific library DNA probe (LA 12NSO1) detected with avidin-FITC (fluorescein isothiocyanate) and propidium iodide chromosome counterstaining. *Arrows* indicate the centromeric regions of chromosome 12; *arrowhead* shows i(12p). **B** Same metaphase spread as in **A** after 4;6-diamidino-2-phenylindole (DAPI) chromosome counterstaining

Fig. 2. Isochromosome i(12p) in a metaphase of a skin fibroblast from a PKS patient (patient 1) after in situ hybridization with a biotinylated chromosome 12-specific α-satellite DNA probe (pSP12-1) detected by the indirect immunoenzymatic method. *Large arrows* indicate the centromeric regions of chromosomes 12; *small arrow* indicates i(12p) with smaller hybridization signal than those in normal chromosomes 12. Note the presence of three hybridization signals in the interphase cell

Fig. 3A, B. PHA stimulated lymphocytes from a PKS patient (patient 2). **A** Cytospin preparation. **B** Cytogenetic preparation made after conventional harvest. Interphase and metaphase cells after in situ hybridization with a biotinylated chromosome 12-specific α-satellite DNA probe (pSP12-1) detected by the indirect immunoenzymatic method. Two hybridization signals are seen in every cell, pointing to the absence of i(12p) in both interphase and metaphase cells. *Arrows* point to the centromeric regions of chromoses 12 in metaphase

Fig. 4 A, B. Buccal mucosal cells from a PKS patient (patient 5) (**A**) and from a control donor (donor 7) (**B**) after in situ hybridization with a biotinylated chromosome 12-specific α-satellite DNA probe (pSP12-1) detected by the indirect immunoenzymatic method. Note the presence of three hybridization signals in the cell of the patient (**A**) indicating the presence of i(12p)

Fig. 5. Cytospin preparation of granulocytes from a PKS patient (patient 3) after in situ hybridization with a biotinylated chromosome 12-specific α-satellite DNA probe (pSP12-1) detected by the indirect immunoenzymatic method. Two hybridization signals can be seen in every cell, indicating the absence of i(12p)

In every PKS patient, metaphases of cultured fibroblasts carrying i(12p) had three hybridization spots. The hybridization signal originating from the i(12p) was, however, smaller than the signals observed at the centromeres of the normal chromosomes 12 (Fig. 2). Similarly, three well-defined hybridization signals were also observed in a proportion of the interphase nuclei. The hybridization pattern consisted of two strong hybridization signals and a weaker one.

Stimulated lymphocytes. Patients 1–5 had two hybridization signals in the nuclei of 96%–99% of lymphocytes (Fig. 3A, B, Table 4). No lymphocytes with three hybridization signals were observed in patients 1 and 2, and their frequency even in the other three patients was very low (0.2%–1%) (Table 4). The results were similar in PKS patients and controls (Table 4). The chromosome 12 specific centromere α-satellite probe did not detect i(12p) in cultured lymphocytes of patients 1 and 2, though the painting technique confirmed its presence at a very low frequency (Table 2). False positive cells with three hybridization signals in control samples, at frequencies reaching 1% (donors 9 and 10), cast doubt on the findings on patients 3–5, too.

Buccal mucosal cells. The hybridization efficiency of the technique was very high with other cells, as pointed out above. However, the percentage of mucosal cells with no hybridization signals was as high as 84% for PKS patients (patient 2) and 96% for controls (donor 12). Nevertheless, in two patients (Fig. 4A) (patients 3 and 5), the proportion

of epithelial mucosal cells with three hybridization signals was two to three times higher than the highest values among controls (donor 13) (Fig 4B, Table 4).

Granulocytes/monocytes. No increase in the number of cells with three hybridization signals was observed in PKS patients compared with controls (Fig. 5, Table 4). The highest frequency of nuclei with three hybridization signals was in fact in a control donor (control 11) (Table 4).

Fetal cells. Both in cultured fibroblasts and liver cells the frequency of interphases with the isochromosome was 9% (Table 3). This value corresponded to the data from G-banded fibroblasts, but, was only a sixth of the frequency of aneuploid cells in G-banded liver metaphases.

Discussion

Our results show that chromosome painting by CISS hybridization with a chromosome 12-specific library probe is a reliable and sensitive method of detecting the extra isochromosome 12 in PKS patients. This methodology is more reliable and rapid than the conventional karyotype analysis of G-banded metaphase chromosomes. Chromosome painting by CISS hybridization with library probes makes it possible to analyse metaphases of poor quality, e.g. those with short and contracted chromosomes or poor spreading. Accordingly, this method allows the use of prolonged colchicine treatment to maximize the number of mitotic cells in the sample. In short, up to 100–500 metaphases can be analysed within 1–2 hours, whereas conventional karyotyping of the same number of G-banded metaphases could take at least 1 working day.

It has been previously demonstrated that the copy number of normal chromosomes 12 can also be determined in interphase cells by means of the α-satellite probe pSP12-1 (Greig et al. 1991; Pérez-Lozada et al. 1991). In our study, this α-satellite probe detects the isochromosome and gives a similar hybridization pattern in the fibroblasts of all patients. Accordingly, our six PKS cases did not show evidence of polymorphism in the size of the signal. However, two recent studies have demonstrated polymorphism in the centromeric region of chromosome 12 and in i(12p) after in situ hybridization (Mukherjee et al. 1991; Speleman et al. 1991). Mukherjee et al. (1991) studied primary cell cultures and established cell lines derived from human male germ cell tumours, using the α-satellite D12Z3 probe for in situ hybridization. They report variation in the signals between i(12p) and normal chromosomes 12. Polymorphism in the centromeric region of i(12p), but not normal chromosomes 12, was detected. Speleman et al. (1991) were unable to detect the centromeric region of the i(12p) in one PKS patient with the pα12H8 probe while subsequent analysis with the M28DNA and F8VWF probes confirmed the presence of i(12p). We emphasize that centromere specific probes should be employed judiciously in the molecular cytogenetic characterization of i(12p) or any other extra chromosome, and suggest that several probes should be used simultaneously whenever possible.

In addition to skin fibroblasts, lymphocytes and granulocytes/monocytes of PKS patients had large numbers of cells with two or more hybridization signals. Furthermore, only a few cells with three hybridization signals were found in the controls in accord with previously published data (Pérez Losada et al. 1991). On the other hand, a high proportion of buccal mucosal cells were without hybridization signals, which was most probably due to a poor penetration of the probe into them. Thus, the sensitivity of the technique is rather low. Even though only two PKS patients in the present study had some buccal mucosal cells containing i(12p), it remains possible that this chromosome abnormality is in fact regularly present in a proportion of these cells.

Our observations support previous findings that mitotic lymphocytes do not contain the isochromosome at all or in very low frequencies only (0.5% or less) (Fryns et al. 1982; Gilgenkrantz et al. 1985; Hunter et al. 1982; Kwee et al. 1984; Pauli et al. 1987; Raffel et al. 1986; Reynolds et al. 1987; Warburton et al. 1987; Ward et al. 1986, 1988). Our results even extend previous findings, showing that neither small lymphocytes nor mature granulocytes/monocytes contain the isochromosome.

The bone marrow cells of the fetus investigated in this study had the isochromosome 12p, confirming the observations of Ward et al. (1986, 1988) and Wenger et al. (1990). Unfortunately, bone marrow from the fetus was not available for our combination study of immunophenotype and in situ hybridization. Thus, we could not determine whether the mitotic bone marrow cells carrying the i(12p) belonged to a particular myeloid lineage, though they were most probably committed to the granulocytic cell lineage. If this assumption is true, the haemopoietic stem cells must have the abnormality, which later disappears during cell maturation into lymphocytes and granulocytes.

The frequency of aneuploid i(12p) cells among metaphases from skin fibroblast cultures of the PKS patients varied from 4% up to 90% in the present study. Previous reports have demonstrated time-dependent selection of the cells with i(12p) when skin fibroblasts are cultured in vitro. In the primary fibroblast cultures, the proportion of aneuploid cells can be 90% or higher, but a marked decline in the percentage of i(12p) cells is observed after a few passages (Peltomäki et al. 1987; Quarrel et al. 1988; Speleman et al. 1991; Warburton et al. 1987). Besides, the rate of this in vitro selection may vary considerably among different PKS patients (Peltomäki et al. 1987; Warburton et al. 1987). Fibroblasts studied by us (Tables 1, 2) came from different culture passages in different patients, which may partly explain the variation in the frequency of aneuploid cells. The possible influence of selective mechanisms makes it difficult to evaluate whether the frequency of the aneuploid cells and the clinical manifestations in PKS patients are correlated.

In conclusion, our study confirms the utmost importance of fibroblast cytogenetic analysis in the diagnosis of PKS. This analysis is greatly enhanced and made more reliable by chromosome painting with CISS hybridization. Though interphase cytogenetics can be successfully applied in buccal mucosal cells, further methodological de-

velopments are needed before these cells can be used in routine diagnosis.

Acknowledgements. This study was supported by grants from the Sigrid Jusélius Foundation, Finnish Academy of Sciences, Finnish Cancer Society, University of Helsinki, Emil Aaltonen Foundation, and from the National Council of Scientific and Technological Research (CONICET) of Argentina. The authors wish to thank Mrs. Heloisa Markkanen, Mrs. Paula Kvick, and Mrs. Tiina Wirtanen for skillful technical assistance.

References

Chrzanowska K, Fryns JP (1989) La tétrasomie 12p (Syndrome de Pallister-Killian): un diagnostic possible avant l'âge d'un an. J Génét Hum 37:259–261

Cremer T, Lichter P, Borden J, Ward DC, Manuelidis L (1988) Detection of chromosome aberrations in metaphase and interphase tumor cells by in situ hybridization using chromosome-specific library probes. Hum Genet 80:235–246

Fryns JP, Petit P, Vinken L, Geutjens J, Marien J, Berghe H van der (1982) Mosaic tetrasomy 21 in severe mental handicap. Eur J Pediatr 139:87–89

Gilgenkrantz S, Droulle P, Schweitzer M, Foliguet B, Chadefaux B, Lombard M, Chery M, Prieur M (1985) Mosaic tetrasomy 12p. Clin Genet 28:495–502

Gorlin RJ, Cohen NN, Levin LS (1990) Pallister-Killian syndrome (mosaic tetrasomy 12p, isochromosome 12p syndrome). In: Syndromes of the head and neck. Oxford University Press, New York Oxford, pp 86–88

Greig GM, Parikh S, George J, Powers VE, Willard HF (1991) Molecular cytogenetics of α satellite DNA from chromosome 12: fluorescence in situ hybridization and description of DNA and array length polymorphisms. Cytogenet Cell Genet 56:144–148

Hall BD (1985) Mosaic tetrasomy 21 is mosaic tetrasomy 12p some of the time. Clin Genet 27:284–286

Hunter AGW, Clifforf B, Speevak M, MacMurray (1982) Mosaic tetrasomy 21 in a liveborn male infant. Clin Genet 21:228–232

Knuutila S, Teerenhovi L (1989) Immunophenotyping of aneuploid cells. Cancer Genet Cytogenet 41:1–17

Kwee ML, Barth PG, Arwet F, Madan K (1984) Mosaic tetrasomy 21 in a male child. Clin Genet 26:150–155

Lichter P, Cremer T, Borden J, Manuelidis L, Ward DC (1988) Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using recombinant DNA libraries. Hum Genet 80:224–234

López V, Mak E, Wyatt PR (1985) Prenatal diagnosis of tetrasomy 21. Prenat Diagn 5:233–235

Moyzis RK, Albright KL, Bartholdi MF, Cram LS, Deaven LL, Hildebrand CE, Longmier JL, Schwarzacher-Robinson T (1987) Human chromosome-specific repetitive DNA sequences. Chromosoma 95:375–386

Mukherjee AB, Murty VVVS, Rodriguez E, Reuter VE, Bosl GJ, Chaganti RSK (1991) Detection and analysis of origin of i(12p), a diagnostic marker of human male germ cell tumors, by fluorescence in situ hybridization. Genes Chromosomes Cancer 3:300–307

Pauli RM, Zeier RA, Sekhon GS (1987) Mosaic isochromosome 12p (Letter to the editor). Am J Med Genet 27:291–294

Peltomäki P, Knuutila S, Ritvanen A, Chapelle A de la (1987) Pallister-Killian syndrome: cytogenetic and molecular studies. Clin Genet 31:399–405

Pérez Losada A, Wessman M, Tiainen M, Hopman AHN, Willard HF, Sole F, Caballin R, Woessner S, Knuutila S (1991) Trisomy 12 in chronic lymphocytic leukemia – an interphase cytogenetic study. Blood 78:775–779

Pinkel D, Strauma T, Gray JW (1986) Cytogenetic analysis using quantitative, high-sensitive, fluorescence hybridization. Proc Natl Acad Sci USA 83:2934–2938

Pinkel D, Landegent J, Collins C, Fusco J, Segraves R, Lucas J, Gray JW (1988) Fluorescence in situ hybridization with human chromosome specific libraries: detection of trisomy 21 and translocations of chromosome 4. Proc Natl Acad Sci USA 85:9138–9142

Quarrell OWJ, Hamil MA, Hughes HE (1988) Pallister-Killian mosaic syndrome with emphasis on adult phenotype. Am J Med Genet 31:841–844

Raffel LJ, Mohandas T, Rimoin DL (1986) Chromosomal mosaicism in the Killian/Teschler-Nicola syndrome. Am J Med Genet 24: 607–611

Reynolds JF, Daniel A, Kelly TE, Grollin SM, Stephan MJ, Carey J, Adkins WN, Webb MJ, Char F, Jimenez JF, Opitz JM (1987) Isochromosome 12p mosaicism (Pallister mosaic aneuploidy or Pallister-Killian syndrome): report of 11 cases. Am J Med Genet 27:257–274

Soukup S, Neidich K (1990) Prenatal diagnosis of Pallister-Killian syndrome. Am J Med Genet 35:526–528

Speleman F, Leroy J, Roy N van, De Paepe A, Suijkerbuijk R, Brunner H, Looijenga L, Verschraegen M-R, Orye E (1991) Pallister-Killian syndrome: characterization of the isochromosome 12p by fluorescent in situ hybridization. Am J Med Genet 41:381–387

Steinbach P, Rehder H (1987) Tetrasomy for the short arm of chromosome 12 with accessory isochromosome (+i(12p)) and a marked LDH-B gene dosage effect. Clin Genet 32:1–4

Warburton D, Anyane-Yeboa K, Francke K (1987) Mosaic tetrasomy 12p: four new cases and confirmation of the chromosome origin of the supernumerary chromosome in one of the original Pallister mosaic syndrome cases. Am J Med Genet 27:275–283

Ward BE, Winchester PD, Robinson A (1986) The Pallister-Killian syndrome and tetrasomy 12p: support for differential developmental potential as a mechanism leading to tissue limited mosaicism. Am J Hum Genet 39:137

Ward BE, Hayden MW, Robinson A (1988) Isochromosome 12p mosaicism (Pallister-Killian syndrome): newborn diagnosis by direct bone marrow analysis. Am J Med Genet 31:835–839

Wenger SL, Boone LY, Steele MW (1990) Brief clinical report: mosaicism in Pallister i(12p) syndrome. Am J Med Genet 35:523–525

Zhang J, Marynen P, Devriendt K, Fryns J-P, van den Berghe H, Cassiman J-J (1989) Molecular analysis of the isochromosome 12p in the Pallister-Killian syndrome. Construction of a mouse-human hybrid cell line containing an i(12p) as the sole human chromosome. Hum Genet 83:359–363