Electronic letter

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Partial tetrasomy 21 in a male infant

EDITOR—Tetrasomy 21 without mosaicism has previously been described in four liveborn children, two of whom had physical features consistent with Down syndrome. We describe a male infant with partial tetrasomy 21 whose examination was consistent with but not typical of trisomy 21.

The clinical features of the proband are summarised in table 1. The proband was the second child born to healthy white parents. Their first child was a normal son aged 18 months. There was no significant family history and no consanguinity. The mother was 34 years old at the time of the birth and the father was 33 years old.

During the pregnancy poor weight gain was reported, although the mother stated that the fetal movements were normal. A detailed ultrasound scan at 18 weeks of gestation was normal. A repeat scan at 30 weeks of gestation showed intrauterine growth retardation.

Labour occurred spontaneously at 33 weeks of gestation and an emergency caesarian section was performed for fetal distress and growth retardation. The birth weight was 1420 g (3rd centile), length 38 cm (1.8 cm less than the 3rd centile), and head circumference 27 cm (0.4th centile). The proband required special care because of his prematurity and small size. Hypospadias, a bifid scrotum, and microcephaly were noted and blood was sent for chromosome analysis on the second day of life (see below). The proband was tube fed for the first 4 weeks of life because of nasal regurgitation and difficulty coordinating sucking and breathing. There were no reports of neonatal hypotonia. A small umbilical hernia was noticed at 1 week of age. He was discharged at 6 weeks of age feeding well with a weight of 2000 g (200 g less than 0.4th centile).

On examination at 5 months (fig 1), length was 55 cm (just below the 0.4th centile), weight 5.8 kg (25-50th centile), and head circumference 37 cm (1 cm less than the 0.4th centile). The anterior fontanelle was small. He had a high anterior hairline with a widow's peak and small, delicate eyebrows, different from other family members. There was bilateral ptosis and he held his head back to improve his vision. There were small epicanthic folds and he had hypertelorism (table 2), but his irides appeared normal. The nasal bridge was wide and he had a short, grooved philtrum with a small, triangular shaped mouth and a small chin. His ears were normally sited and had pointed helices. The tongue was large but the palate was normal. He had a short neck with redundant folds of skin. His hands and feet were small with brachydactyly of the digits and short, proximally inserted thumbs. The palmar and digital flexion creases were normal. There were two 1-2 cm strawberry naevi on the posterior aspect of the right scalp and on the right shoulder. There was a large, soft, reducible umbilical hernia. His penis was small with hypospadias and a shawl scrotum. Both testes were descended. He had mild truncal hypotonia. Cardiac auscultation was normal.

Table 1 Summary of the physical characteristics of the proband

Skull and facial bones	Brachycephaly
	Plagiocephaly
	Midface hypoplasia
Eyes	Bilateral ptosis
	Left divergent strabismus
	Hypertelorism
	Epicanthic folds
Ears	Pointed helices
Nose and mouth	Wide nasal bridge
	Short philtrum
	Small, triangular mouth
	Large, protruding tongue
	Small chin
	Delayed eruption of teeth
Neck	Short
	Redundant neck skin
Hands	Small hands
	Short, proximally inserted thumbs
	Brachydactyly
	Fifth finger clinodactyly
Feet	Small feet
	Hypoplastic toenails
Abdomen	Umbilical hernia
Genitalia	Small penis
	Hypospadias
	Shawl scrotum
Skin	Strawberry naevi

He has had frequent "absences" thought to be seizures since the neonatal period. An EEG has been normal and trial of medications has not been undertaken. A CT scan of his head at 4 months showed cerebral hypodevelopment, the degree of which was greater than expected for corrected age, but there were no structural lesions.

At 18 months (fig 2), he had good head control and was able to roll over and to babble. He had plagiocephaly and there was marked brachycephaly with a flat, underdeveloped midface. His hypertelorism and ptosis were unchanged and there was a left divergent squint. His tongue remained large and protruded from his mouth. His fifth toenails were hypoplastic. The remainder of the examination was unchanged. At 34 months of age, he had been sitting independently for 1 month, weight bearing for several months, and could understand his name but no other words. His tone was normal and there were no epicanthic folds or redundant neck skin. He had seven teeth. Investigations have included a normal renal ultrasound scan and electrocardiogram but echocardiography has not been performed.

Chromosome analysis of peripheral blood lymphocytes showed a small supernumerary chromosome in all cells (fig 3A, B). Fluorescence in situ hybridisation (FISH) studies with a whole chromosome paint for chromosome 21 (Cambio) showed that the extra material was derived from chromosome 21 (fig 4A) and a 13/21 centromere probe (Oncor) showed that the derivative chromosome was an isodicentric chromosome 21 (fig 4B). The proband was therefore tetrasomic for the proximal part of the long arm of chromosome 21 to band 21q22.1 (G banded karyotype 47,XY,+psu idic(21)(q22.1)). Parental chromosomes were normal. Repeat cytogenetic analysis at 18 months showed that the isodicentric chromosome 21 was present in 30/30 peripheral blood lymphocytes and in 30/30 skin fibroblasts. FISH studies on peripheral blood lymphocytes showed no signal from the cosmid clones ICRFc102E0275, ICRFc102H01108, and ICRFc102F01129 mapped to chromosome band 21q22.3 (fig 4C, Imperial Cancer Research Fund Reference Library Database, Lincoln's Inn Fields, London WC2A 3PX).[1 2] The duplicated region in this child therefore did not include the Down syndrome critical region.

To determine the extent of the tetrasomic region, a total of 12 DNA probes were tested using FISH analysis (table 3). These probes included four cosmids and eight BACs. All of the BAC probes were obtained by screening a BAC library

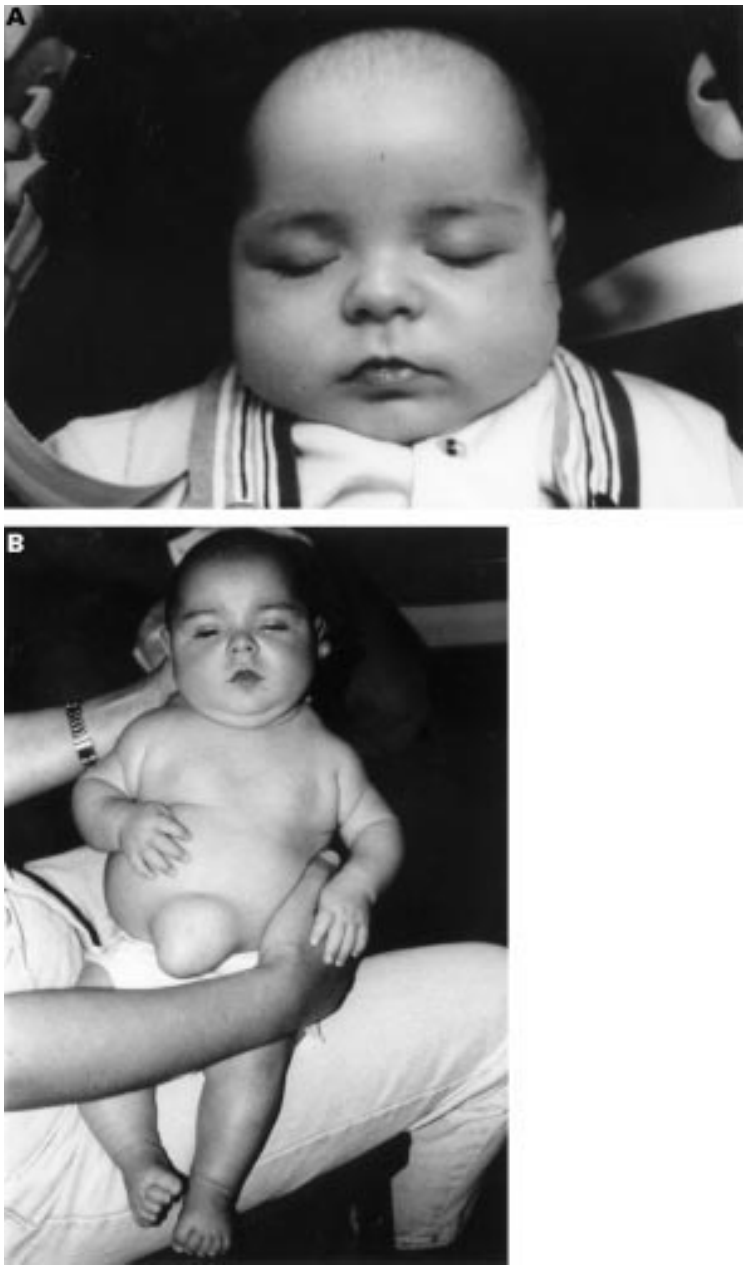

Figure 1 (A) Face of the proband aged 5 months. (B) The body of the proband.

Table 2 Summary of the growth characteristics of the proband

Physical parameter	*Birth*	*Age 5 months*	*Age 18 months*
Weight	1420 g (3rd)	5800 g (25th–50th)	8880 g (0.4th–2nd)
Length	38 cm (<3rd*)	55 cm (<0.4th)	68 cm (<3rd)
Head circumference	27 cm (0.4th)	37 cm (<0.4th)	40.3 cm (<3rd)
Inner canthal distance	—	2.4 cm	2.7 cm
Outer canthal distance	—	8.0 cm	10.0 cm
Interpupillary distance	—	4.8 cm (75th–90th)	—
Right foot length	—	—	8.0 cm (<3rd)
Right palm length	—	—	5.0 cm (3rd–25th)
Right middle finger length	—	—	3.2 cm (<3rd)

*For measurements less than the 0.4th or 3rd centile at birth and 5 months of age, the degree of growth retardation is specified in the text.

(A and B)[3 4] using DNA from chromosome 21 as a probe[5] or from the Integrated Molecular Cytogenetic BAC Resource.[6] Their chromosome locations were confirmed by testing on normal control metaphase chromosomes and by using PCR with custom designed primers as indicated in table 3.

BAC and cosmid DNA preparations, probe labellings, and FISH were performed according to the procedure described by Korenberg *et al*[6] with some modification. Briefly, 10 µl of hybridisation mix (50% deionised formamide, 10% dextran sulphate, and 2 × SSC) containing 200 ng of each Cy3-dUTP or Cy5-dUTP (Amersham Life Science) directly labelled DNA probes, 5 µg of human Cot1 DNA, and 5 µg of sonicated salmon sperm DNA was applied to denatured chromosomes after preannealing the

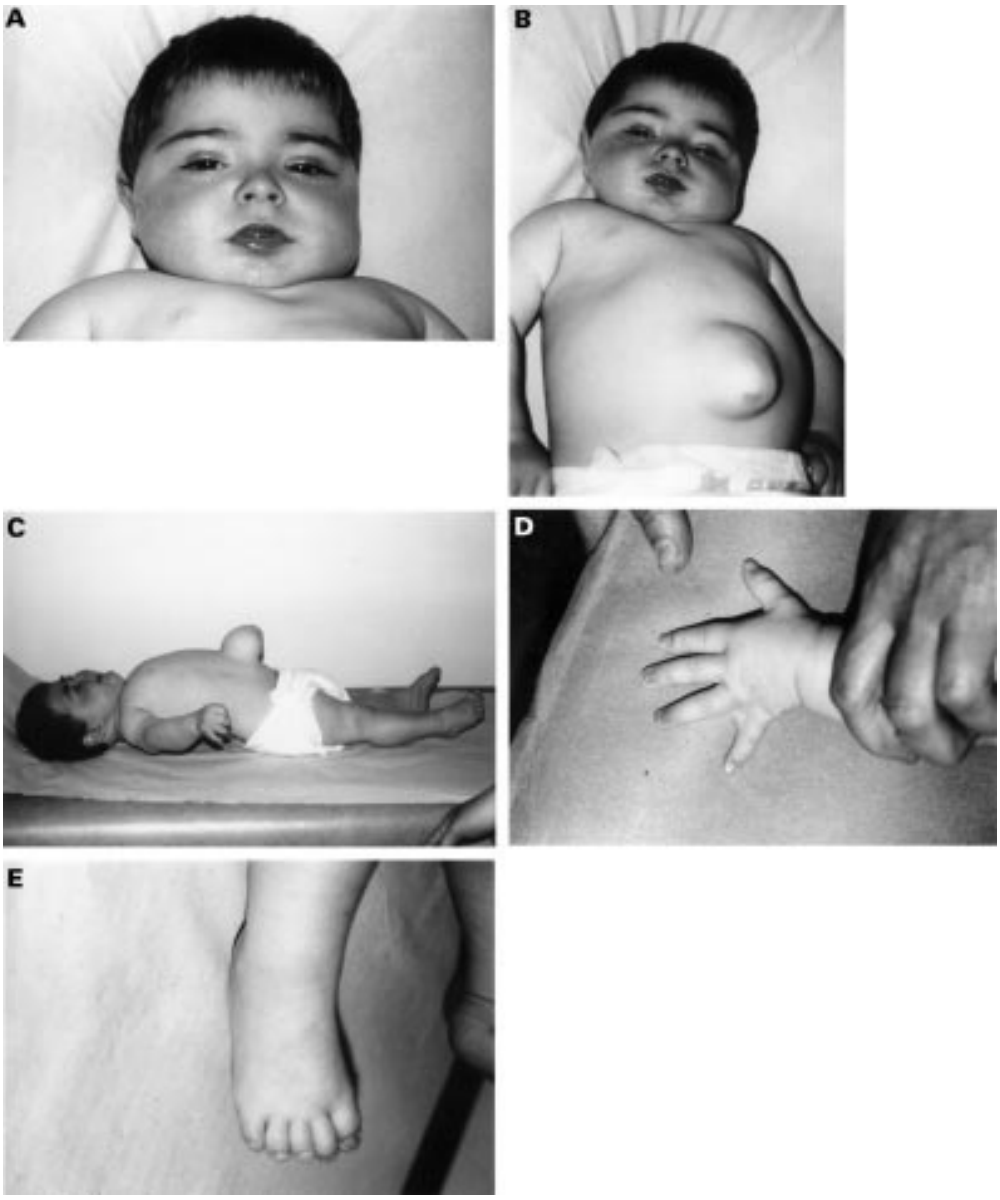

Figure 2 (A) Face of the proband aged 18 months. (B) The body of the proband. (C) Side view of proband. (D) The hand of the proband. (E) The foot of the proband.

probe for 20 minutes at 37°C. Post-hybridisation washes were at 44°C in 2 × SSC and 50% formamide for five minutes three times and followed by washing once in 1 × SSC at 50°C. Chromosomes were identified by reverse banding generated with chromomycin A3 followed by distamycin A[5] and the hybridisation signals were directly reviewed after the counterstain. The images were captured with a Photometrics cooled CCD camera mounted on Zeiss Axiovert 135 microscope using BDS image software (Oncor Imaging, Gaithersburg, MD).

Characterisation of the pseudoisodicentric chromosome 21 of the proband showed that the duplicated segment extended to the distal region of band 21q21, excluding the genes for *APP*, *GRIK*, and *SOD* (fig 5). The distal breakpoint was located between BAC 839E9 showing four copies (fig 4D) and 427H6 showing two copies. The three copies of probe E9 suggest that this probe maps close to or at the proximal breakpoint of the isodicentric chromosome. BAC 839E9 corresponds to YAC 221B7, carrying the markers D21S383, D21S384, and D21S385 and BAC 427H6 is positive for YAC 193G12 and carries markers D21S18 to D21S281. The duplicated region therefore covers the region from D21S16 to D21S1/S11 and possibly extends to D21S281. The distal breakpoint of the isodicentric chromosome characterised by FISH revises the karyotype to 47,XY,+psu idic(21)(q21.1).

We have reported a 34 month old boy with tetrasomy for the short and proximal long arm of chromosome 21 to band 21q21.16 without evidence of mosaicism in peripheral blood lymphocytes and skin fibroblasts. His phenotype was suggestive of but not entirely consistent with Down syndrome. Brachycephaly, epicanthic folds, a flat nasal bridge, an open mouth and protruding tongue, short neck with excess skin, small hands and feet, fifth finger brachydactyly and clinodactyly, and an umbilical hernia gave a Jackson score of 12/25 at 18 months of age, consistent with an 84% probability of Down syndrome (table 4).[7] However, his examination at 5 months of age showed fewer signs of Down syndrome and he had a high anterior hairline, non-slanting palpebral fissures, bilateral ptosis, and a triangular shaped mouth (fig 1). At 34 months of age, epicanthic folds and redundant neck skin were absent and his tone was normal. His Jackson score at this age was 9/25.

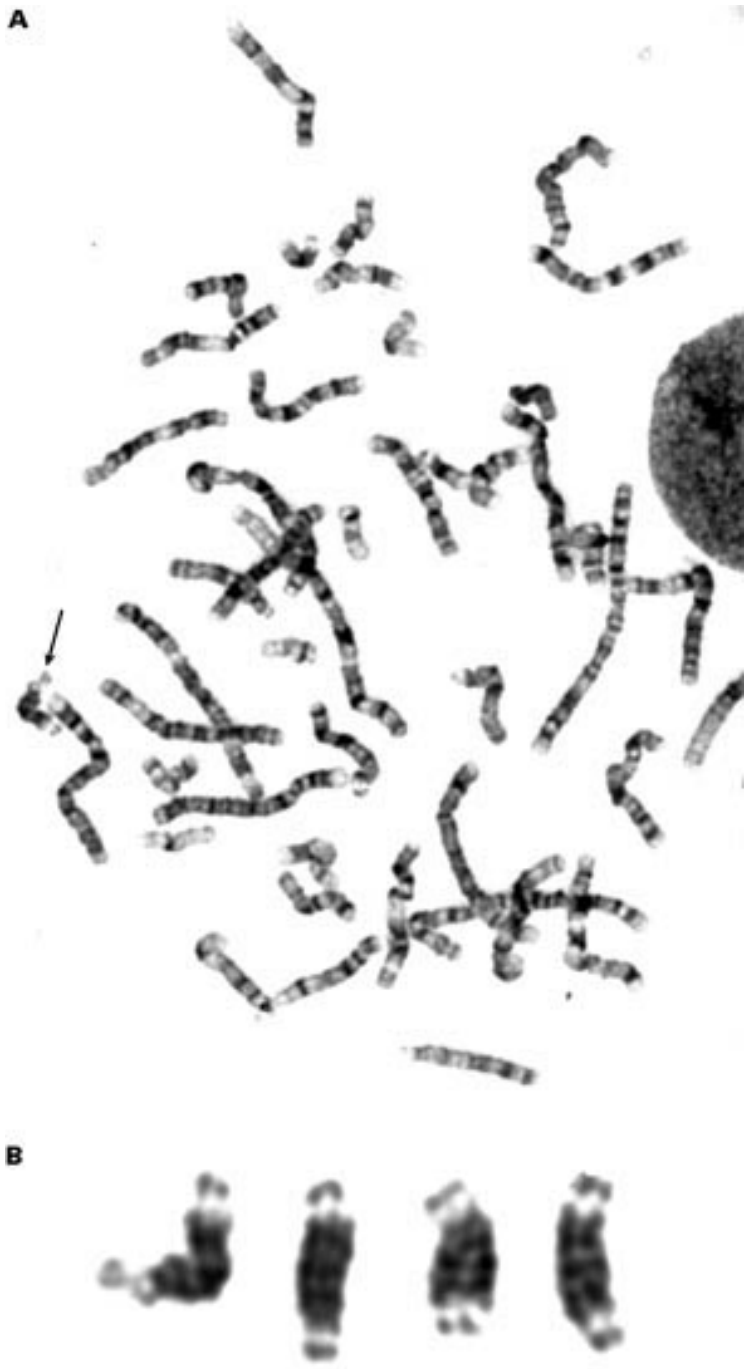

Figure 3 (A) Partial G banded karyotype of the proband, showing small, supernumerary chromosome. (B) G banded examples of the pseudoisodicentric chromosome 21.

Tetrasomy for an autosomal chromosome region has been described for only a few chromosomes.[8] Autosomal tetrasomies are almost always de novo aberrations, with tetrasomy resulting from an additional isochromosome or isodicentric chromosome. More rarely, two extra copies of an acrocentric chromosome may be joined in a Robertsonian translocation or there can be two supernumerary whole chromosomes. Mosaicism is frequent and can result from mitotic instability of the isodicentric chromosome, in vivo growth disadvantage of the aberrant chromosome(s), or failure of the tetrasomic cells to divide in vitro.[8 9]

Partial tetrasomy 21 without mosaicism has previously been described in four liveborn infants (tables 4 and 5). A 33 month old girl with tetrasomy extending to chromosome band 21q22.1 had brachycephaly, a third fontanelle, short, upward slanting palpebral fissures, and a protruding tongue. Single transverse palmar creases, brachydactyly, an umbilical hernia, joint laxity, and hypotonia were also noted (table 4).[10] A baby born at 32 weeks had congenital monocytic leukaemia and two additional copies of the whole of chromosome 21 in peripheral blood lymphocytes.[11] No karyotyping was performed on skin or bone marrow cells. The child had a flat occiput, small, upward slanting palpebral fissures, epicanthic folds, small ears with unfolded helices, a flat nasal bridge, a short neck with redundant skin, and marked hypotonia consistent with Down syndrome. Death occurred at 4 days of age from hyaline membrane disease and septicaemia and necropsy showed a patent ductus arteriosus and a bilobed right lung.[11] Recently, a male aged 30 months was reported

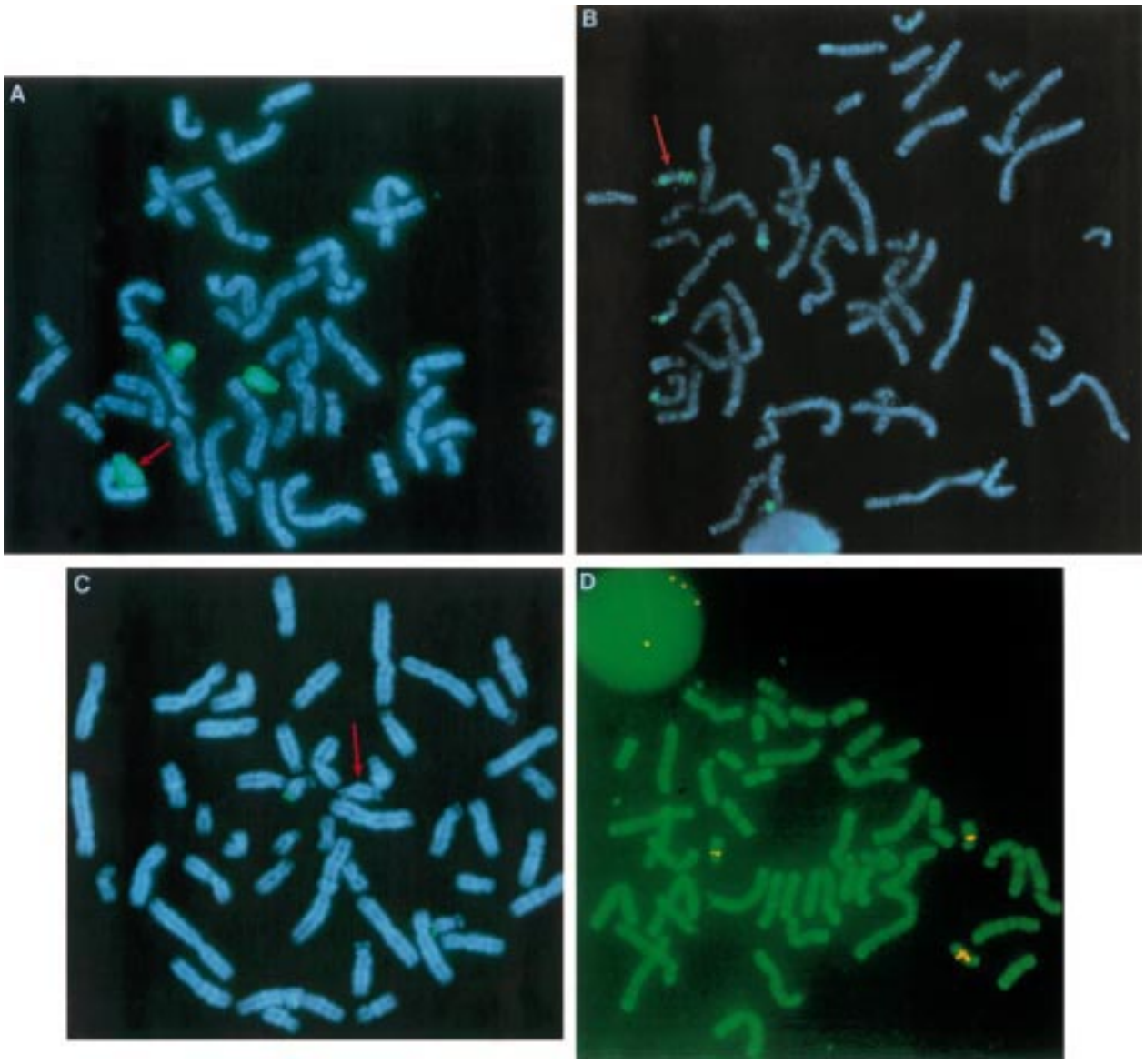

Figure 4 (A) FISH study with a whole chromosome painting probe for chromosome 21 showing that the supernumerary chromosome is derived from chromosome 21. (B) FISH study with a centromere probe for chromosomes 13 and 21 showing two signals on the supernumerary chromosome. (C) FISH study with cosmid clones ICRFc102E0275, ICRFc102H01108, and ICRFc102F01129 (see text) showing absent signals on the supernumerary chromosome. (D) A human chromosome preparation made from the proband hybridised with a Cy3-dUTP direct labelled probe, B839E9. Four copies (shown in red) are present in both metaphase and interphase cells.

with a pseudoisodicentric chromosome 21.[12] He had developmental delay, microcephaly, hypotonia, joint laxity, a flat occiput, downward slanting palpebral fissures, Brushfield spots, telecanthus, and midface hypoplasia.[12] The authors concluded that the child had some but not all of the features of Down syndrome and that genes outside the Down syndrome critical region (DCR) were influential in the trisomy 21 phenotype.[12] Tetrasomy 21 was also described in an 11 year old male with psychomotor and speech retardation (but with normal growth), brachycephaly, a prominent lower lip, macrogenitalism, and idiopathic precocious puberty.[13] The Jackson score for this child was not consistent with Down syndrome.[13]

There are five reports of tetrasomy 21 in mosaic form.[14–18] However, several of these cases were reported before confirmatory FISH studies were available, and one case[15] has subsequently been shown to have Pallister-Killian syndrome with mosaic tetrasomy for chromosome 12p rather than for 21q.[19] The diagnosis in three of the other cases[14 16 17] has also been challenged in view of the phenotypic similarity of these cases to Pallister-Killian syndrome.[20 21] One case of partial tetrasomy 21 in mosaic form verified by FISH had some features consistent with Down syndrome (table 4) with increased nuchal skin, mild brachycephaly, Brushfield spots, a right epicanthic fold, a depressed nasal bridge, bilateral fifth finger clinodactyly, and a wide sandal gap.[18]

Our case provides additional evidence that the phenotype associated with non-mosaic tetrasomy 21 may include physical features uncommon in trisomy 21. Findings in previously reported cases of both mosaic and non-mosaic tetrasomy 21 include a flat occiput,[10] (our patient) a high, broad forehead,[12 18] (our patient), hypertelorism or telecanthus,[12] (our patient), and a short and anteverted nose[13] (our patient). Our patient also had a carp shaped mouth. Non-specific genitourinary anomalies were more frequent (table 4), and cryptorchidism,[11 12] hypospadias, and a small scrotum (our patient) and a large penis[13] have been reported. A patent ductus arteriosus was described in one premature infant,[11] but no other case had a cardiac abnormality. Two cases had an umbilical hernia[10] (our patient) and one had diastasis recti,[10] but there were no

Table 3 DNA probes used for characterisation of the tetrasomic region of chromosome 21

Probe names	*Gene markers*	*Mapping location†*	*Test results (copy number)*
E9 (cosmid)	D21S16	21q11.2	3
B76H7*	D21S11/S1	21q11.2-21.1	4
B839E9	D21S383-S385 Y221B7*	21q21.1	4
B427H6	D21S18-S281 Y193G12	21q21.1	2‡
APP (cosmid)	APP	21q21.2	2
B96E9	D21S303-S129 Y760H5	21q21.2-21.3	2
B8B3	D21S390-S262	21q21.2-21.3	2
5F4	D21S217-S299	21q21.3	2
B19C7	D21S58-S216 Y876D4	21q21.3	2
B417D6	D21S296-S82 Y62G5	21q22.1	2
ICRF0251 (cosmid)	D21S299 (JG108)	21q22.1	2
138H8 (cosmid)	D21S82-S302 Y814C1	21q22.3	2

*B = BAC, Y = YAC.
†The probes have been ordered according to their cytogenetic mapping location.
‡The two signals from this and subseqent probes were located on the q arms of the normal chromosomes 21 of the proband.

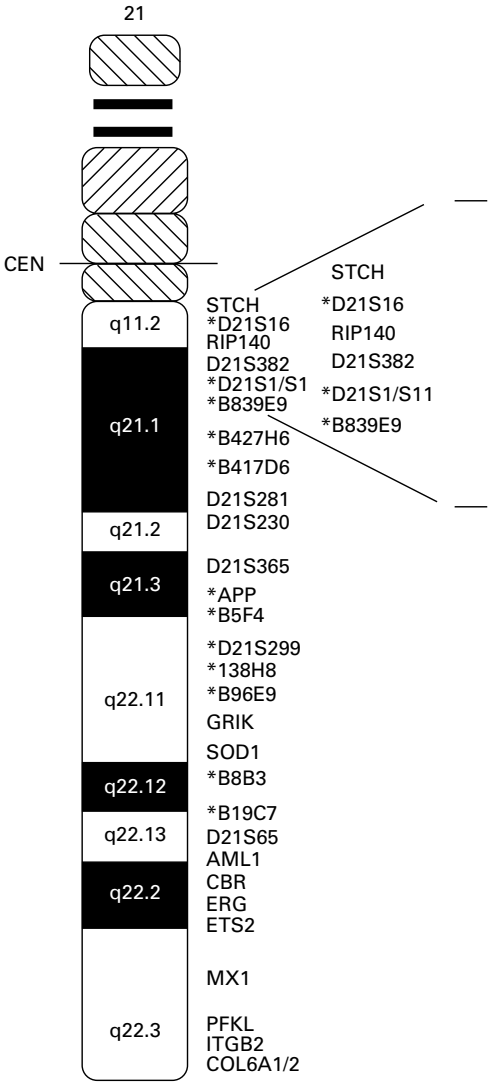

*Figure 5 Molecular cytogenetic analysis with BAC and cosmid probes. A human chromosome 21 ideogram showing the duplicated region and genes. The probes tested are indicated with *.*

other gastrointestinal abnormalities. One child had pectus excavatum.[12] It seems likely that the phenotype associated with tetrasomy 21 will prove to contain features distinct from Down syndrome, although the few cases described to date and the short time period of follow up do not yet allow characterisation of a tetrasomy 21 phenotype.

The phenotypic effects of four copies of an autosomal chromosome region are likely to be more severe than for trisomy of the same chromosome segment as a result of a correspondingly greater imbalance in gene dosage.[7 22 23] This hypothesis holds true for the majority of cases of tetrasomy 21 described to date, as development and growth were more severely impaired than would have been expected with trisomy 21 (table 4). However, there was considerable variation in both growth and development and one girl with non-mosaic tetrasomy 21 walked independently at the age of 18 months and had single words at the age of 33 months.[10] A child with normal growth parameters[13] and a child with normal height and weight but with microcephaly have also been reported.[10]

Molecular cytogenetic characterisation of the tetrasomic region in this patient showed that the duplicated region covers the region from D21S16 to D21S1/S11. This region contains only two known genes, *STCH* and *RIP140*. *STCH* represents the stress-70 chaperone family, consisting of proteins that bind to denatured or incorrectly folded polypeptides and play a major role in the processing of cytosolic and secretory proteins.[24] *RIP140* represents the receptor interacting protein 140 by virtue of its direct association with a transcriptional activation domain of the oestrogen receptor (ESR, 133430) in the presence of oestrogen.[25] Therefore it is likely that some of the physical features and delayed development derive from the overexpression of these genes during development. It is also of interest that subjects who are neither monosomic nor partially trisomic for these regions exhibit such abnormal phenotypes. It will be of interest to determine if more general effects on transcription may result from the overexpression of regulators such as *RPI140*.

Cytogenetic and molecular studies have been used to delineate specific regions of chromosome 21 responsible for the pathogenesis of different features in Down syndrome. Reports of subjects with partial duplications of chromosome 21 and a subset of the physical manifestations of trisomy 21 have identified a critical region for the phenotype at distal 21q21.3 to 21q22.[26–28] Further molecular studies have identified a 5 Mb region between markers D21S58 and D21S42 with duplication of this region sufficient to cause the facial features and mental retardation associated with trisomy 21.[29–33] However, duplications of chromosome 21 distinct from this region also produce phenotypic features of Down syndrome and it is likely that there is a significant contribution from other genes on chromosome 21 and elsewhere in the genome.[12 33 34] Our case is in keeping with the involvement of genes outside this "critical" region in the trisomy 21 phenotype as the tetrasomic region in our patient does not include this chromosome region and yet examination showed many features in accordance with Down syndrome.

Tetrasomy 21 has been well documented as an acquired cytogenetic aberration in subjects with a normal constitutional karyotype who have developed haematological malignancies. The conditions associated with tetrasomy 21 include acute lymphoblastic leukaemia (ALL), acute megakaryoblastic leukaemia (AML), erythroleukaemia, and transient myeloproliferative disorders.[35–37] Children with Down syndrome are also well known to be at increased risk of leukaemia, most specifically ALL and AML.[31] The link between the additional copies of chromosome 21 and haematological malignancy is therefore undoubted, although

Table 4 Clinical features of cases with tetrasomy 21 and tetrasomy 21 mosaicism

Case report	*Jabs et al*[11]	*Daumer-Haas et al*[10]	*Nagarsheth and Mootabar*[18]	*Gutiérrez-Angulo et al*[12]	*Cerretini et al*[13]	*Our patient*
Mother's age	24 y	32 y	38 y	33 y	46 y	34 y
Father's age	36 y			32 y	52 y	33 y
Pregnancy	Spotting/cough	Normal	Bleeding	Bleeding		↓ weight gain
Gestation	32 wk	Term	38+ wk	38 wk		33 wk
Birth weight (g)	2020	2940	2040	3600		1420 (3rd)
Age	4 d	33 mth	1 d	29 mth	11 y	18 mth
Length		89.5 cm (25th)		90 cm (50th)	NAD	68 cm (<<3rd)
Weight		12.0 kg (10th)		10.9 kg (10th)	NAD	8.88 kg (<3rd)
Head circumference		45.5 cm (<3rd)		45.5 m (<3rd)	NAD	40.3 cm (<<3rd)
Motor development		Normal		Unable to walk		Unable to sit
Speech		Single words			IQ=37	Babble
Jackson score						
Brachycephaly		+	+	+	+	+
Oblique eye fissures	+	+		+		
Epicanthic folds	+		+			+
Blepharitis						
Brushfield spots			+	+		
Nystagmus						
Flat nasal bridge	+		+	+		+
Open mouth		+		+		+
Abnormal teeth						+
Protruding tongue		+		+		+
Furrowed tongue						
High arched palate						
Narrow palate						
Folded ear						
Short neck	+	+				+
Loose skin of neck	+		+			+
Short and broad hands		+				+
Short fifth finger		+				+
Incurved fifth finger			+			+
Transverse crease		+				
Gap between 1st/2nd toe			+			
Congenital heart defect	PDA					
Murmur	+					
Joint hyperflexibility	+	+				
Muscular hypotonia	+	+		+		+
Jackson score	9	10	7	7	1	12

Table 5 Karyotypes of cases with tetrasomy 21 and tetrasomy 21 mosaicism

Karyotype	*Jabs et al*[11]	*Daumer-Haas et al*[10]	*Nagarsheth and Mootabar*[18]	*Gutiérrez-Angulo et al*[12]	*Cerretini et al*[13]	*Our patient*
Mosaic/non-mosaic	Non-mosaic	Non-mosaic	Mosiac	Non-mosaic	Non-mosaic	Non-mosaic
Peripheral blood lymphocytes	48,XY,+21,+21	47,XX,+idic(21)(q22.1)	Mos47,XY,+mar.ish i(21)(q10)[1 cell]/ 46,XY[119 cells]	47,XY,+psu idic(21)(q22.1)	47,XY,+psu dic(21;21) (q22.11;q22.11)	47,XY,+psu idic(21)(q22.1)
Skin fibroblasts						47,XY,+psu idic(21)(q22.1)
Amniocytes			Mos47,XY, +i(21q)/46,XY			
Parental chromosomes	Normal	Maternal studies normal	Normal	Normal	Normal	Normal

the pathogenesis of the malignant proliferation is still unknown. A role has been suggested for overexpression of the human *ets-2* and *erg* proto-oncogenes mapped to 21q22.2 to 21q22.3.[38] In tetrasomy 21, the one child described with four whole copies of chromosome 21 in peripheral blood lymphocytes (thus including the region containing the proto-oncogenes) was born with monocytic leukaemia.[11] The other surviving cases either had mosaicism largely confined to skin fibroblasts or did not have additional copies of the 21q22.2 to 21q22.3 region (table 5).

Finally, in our case, the pseudoisodicentric chromosome contained material from both the short and the long arm of chromosome 21 and could have been formed from a U loop mechanism.[39] In the surviving cases of tetrasomy 21 with mosaicism, the aberrant cells are almost always confined to the fibroblast cell lineages and are absent from peripheral blood lymphocytes (table 5). This is similar to the pattern of mosaicism in tetrasomy 12p[40] and is thought to be because of selective loss of the isochromosome during cell division in vitro.[9]

We report a 34 month old child with tetrasomy for the short and long arm of chromosome 21 to band 21q22.1 determined cytogenetically, but only to 21q21.1 as determined by FISH analysis. There was no evidence of mosaicism in peripheral blood lymphocytes and skin fibroblasts. His facial features of brachycephaly, a high and broad forehead, epicanthic folds, bilateral ptosis, hypertelorism, a flat nasal bridge and small nose with anteverted nares, a small mouth, protruding tongue, and a small chin were consistent with but not classically in keeping with the trisomy 21 phenotype. A short neck with loose skin, brachydactyly with fifth finger clinodactyly, umbilical hernia, and small hands and feet were also present. A review of previously reported cases with non-mosaic tetrasomy 21 and mosaicism for tetrasomy 21 suggests that the phenotype for tetrasomy 21 will prove to contain features distinct from Down syndrome.

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1 Lehrach H, Drmavac R, Hoheisel J, Larin Z, Lennon G, Monaco AP, Nizetic D, Zehetner G, Poustka A. Hybridization fingerprinting in genome mapping and sequencing. In: Davies KE, Tilghman SM, eds. *Genome analysis*. Volume 1. *Genetics and physical mapping*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1990: 39-81.
2 Davies AF, Barber L, Murer-Orlando M, Bobrow M, Adinolfi M. FISH detection of trisomy 21 in interphase by the simultaneous use of two differentially labelled cosmid contigs. *J Med Genet* 1994;**31**:679-85.
3 Shizuya H, Birren B, Kim UJ, Mancino V, Slepak T, Tachiiri Y, Simon M. Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in Escherichia coli using an F-factor-based vector. *Proc Natl Acad Sci USA* 1992;**89**:8794-7.
4 Kim UJ, Birren BW, Slepak T, Mancino V, Boysen C, Kang HL, Simon MI, Shizuya H. Construction and characterization of a human bacterial artificial chromosome library. *Genomics* 1996;**2**:213-8.
5 Korenberg JR, Chen XN, Mitchell S, Fannin S, Gerwehr S, Cohen D, Chumakov I. A high-fidelity physical map of human chromosome 21q in yeast artificial chromosomes. *Genome Res* 1995;**5**:427-43.
6 Korenberg JR, Chen XN, Sun Z, Shi ZY, Ma S, Vataru E, Yimlamai D, Weissenbach JS, Shizuya H, Simon MI, Gerety SS, Nguyen H, Zemsteva IS, Hui L, Silva J, Wu X, Birren BW, Hudson TJ. Human genome anatomy: BACs integrating the genetic and cytogenetic maps for bridging genome and biomedicine. *Genome Res* 1999;**9**:994-1001.
7 Jackson JF, North ER III, Thomas JG. Clinical diagnosis of Down's syndrome. *Clin Genet* 1976;**9**:483-7.
8 Schinzel A. Karyotype-phenotype correlations in autosomal chromosomal aberrations. In: *The phenotypic mapping of Down syndrome and other aneuploid conditions*. New York: Wiley Liss, 1993:19-31.
9 Thornberg Reeser SL, Wenger SL. Failure of PHA-stimulated I(12p) lymphocytes to divide in Pallister-Killian syndrome. *Am J Med Genet* 1992;**42**: 815-19.
10 Daumer-Haas C, Schuffenhauer S, Walther JU, Schipper RD, Porstmann T, Korenberg JR. Tetrasomy 21 pter→q22.1 and Down syndrome: molecular definition of the region. *Am J Med Genet* 1994;**53**:359-65.
11 Jabs WE, Stamberg J, Leonard CO. Tetrasomy 21 in an infant with Down syndrome and congenital leukemia. *Am J Med Genet* 1982;**12**:91-5.
12 Gutiérrez-Angulo M, Ramos AL, Dávalos N, Sánchez-Corona J, Rivera H. An extra idic(21)(q22.1) in a child with some features of Down's syndrome. *Clin Genet* 1999;**55**:203- 6.
13 Cerretini R, Luccerini V, Stivel M, Bañares V, Aranda I, Alba L, Pivetta O, Slavutsky I. Tetrasomy 21pter-q22.1: molecular, cytogenetic, and clinical findings. *Clin Genet* 1999;**55**:283-6.
14 Fryns JP, Petit P, Vinken L, Geutjens J, Marien J, van den Berghe H. Mosaic tetrasomy 21 in severe mental handicap. *Eur J Pediatr* 1982;**139**:87-9.
15 Hunter AGW, Clifford B, Speevak M, MacMurray SB. Mosaic tetrasomy 21 in a liveborn male infant. *Clin Genet* 1982;**21**:228-32.
16 Kwee ML, Barth PG, Arwert F, Madan K. Mosaic tetrasomy 21 in a male child. *Clin Genet* 1984;**26**:150-5.
17 Lopes V, Mak E, Wyatt PR. Prenatal diagnosis of tetrasomy 21. *Prenat Diagn* 1985;**5**:233-5.
18 Nagarsheth NP, Mootabar H. Prenatal diagnosis of mosaic tetrasomy 21q confirmed by fluorescence *in situ* hydridization. *Clin Genet* 1997;**51**:260-3.
19 Hunter AGW, Clifford B, Cox DM. The characteristic physiognomy and tissue specific karyotype distribution in the Pallister-Killian syndrome. *Clin Genet* 1985;**28**:47-53.
20 Hall BD. Mosiac tetrasomy 21 is mosaic tetrasomy 12p some of the time. *Clin Genet* 1985;**27**:284-5.
21 McLean S, Stanley W, Stern H, Fonda-Allen J, Devine G, Ellingham T, Rosenbaum K. Prenatal diagnosis of Pallister-Killian syndrome: resolution of cytogenetic ambiguity by use of fluorescent in situ hybridization. *Prenat Diagn* 1992;**12**:985-91.
22 Nuessle NS, Miles JH. Proximal 14q tetrasomy syndrome. *Am J Hum Genet* 1988;**43**:A63.
23 Flint J, Wilkie AOM. The genetics of mental retardation. *Br Med Bull* 1996; **52**:435-64.
24 Otterson GA, Flynn GC, Kratzke RA, Coxon A, Johnston PG, Kaye FJ. Stch encodes the 'ATPase core' of a microsomal stress 70 protein. *EMBO J* 1994;**13**:1216-25.
25 Cavailles V, Dauvois S, Horset LF, Lopez G, Hoare S, Kushner PJ, Parker MG. Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. *EMBO J* 1995;**14**:3741-51.
26 Aula P, Leisti J, von Koskull H. Partial trisomy 21. *Clin Genet* 1973;**4**:241-51.
27 Pellissier MC, Laffage M, Philip N, Passage E, Mattei MG, Mattei JF. Trisomy 21q223 and Down's phenotype correlation evidenced by in situ hybridization. *Hum Genet* 1988;**80**:277-81.
28 Epstein CJ, Korenberg JR, Annerén G, Antonarakis SE, Aymé S, Couchesne E, Epstein LB, Fowler A, Groner Y, Huret JL, Kemper TL, Lott IT, Lubin BH, Magenis E, Opitz JM, Patterson D, Priest JH, Pueschel SM, Rapoport SI, Sinet PM, Tanzi RE, de la Cruz F. Protocols to establish genotype-phenotype correlations in Down syndrome. *Am J Hum Genet* 1991;**49**:207-35.
29 Rahmani Z, Blouin JL, Créau-Goldberg N, Watkins PC, Mattei JF, Poissonnier M, Prieur M, Chettouh Z, Nicole A, Aurias A, Sinet PM, Delabar JM. Down syndrome critical region around D21S55 on proximal 21q22.3. *Am J Med Genet Suppl* 1990;7:98-103.
30 Korenberg JR, Kawashima H, Pulst SM, Allen L, Magenis E, Epstein CJ. Down syndrome: toward a molecular definition of the phenotype. *Am J Med Genet Suppl* 1990;7:91-7.
31 Korenberg JR, Bradley C, Disteche CM. Down syndrome: molecular mapping of the congenital heart disease and duodenal stenosis. *Am J Hum Genet* 1992;**50**:294-302.
32 Delabar JM, Theophile D, Rahmani Z, Chettouh Z, Blouin JL, Prieur M, Noel B, Sinet PM. Molecular mapping of twenty-four features of Down syndrome on chromosome 21. *Eur J Hum Genet* 1993;**1**:114-24.
33 Korenberg JR, Chen XN, Schipper R, Sun Z, Gonsky R, Gerwehr S, Carpenter N, Daumer C, Dignan P, Disteche C, Graham JM Jr, Hugdins L, McGillivray B, Miyazaki K, Ogasawara N, Park JP, Pagon R, Pueschel S, Sack G, Say B, Schuffenhauer S, Soukup S, Yamanaka T. Down syndrome phenotypes: the consequences of chromosomal imbalance. *Proc Natl Acad Sci USA* 1994;**91**:4997-5001.
34 Opitz JM, Gilbert-Barness EF. Reflections on the pathogenesis of Down syndrome. *Am J Med Genet Suppl* 1990;7:38-51.
35 Dewald GW, Diez-Martin JL, Steffen SL, Jenkins RB, Stupca PJ, Burgert EO Jr. Hematologic disorders in 13 patients with acquired trisomy 21 and 13 individuals with Down syndrome. *Am J Med Genet Suppl* 1990;7:247-50.
36 Potocki L, Townes PL, Woda BA, Rao SK, Shearin JP, Schwenn MR. Tetrasomy 21 in megakaryoblastic leukaemia. *Cancer Genet Cytogenet* 1994;**74**: 66-70.
37 Udayakumar AM, Sundareshan TS. Tetrasomy 21 as a sole abnormality in erythroleukemia. *Cancer Genet Cytogenet* 1995;**85**:85-7.
38 Papas TS, Watson DK, Sacchi N, Fujiwara S, Seth AK, Fisher RJ, Bhat NK, Mavrothalassitis G, Koizumi S, Jorcyk CL, Schweinfest CW, Kottaridis SD, Ascione R. ETS family of genes in leukaemia and Down syndrome. *Am J Med Genet Suppl* 1990;7:251-61.
39 Gardner RJM, Sutherland GR. *Chromosome abnormalities and genetic counselling*. 2nd ed. Oxford: Oxford University Press, 1996:265.
40 Turleau C, Simon-Bouy B, Austruy E, Grisard MC, Lemaire F, Molina-Gomes D, Siffroi JP, Boué J. Parental origin and mechanisms of formation of three cases of 12p tetrasomy. *Clin Genet* 1996;**50**:41-6.