

## Del(18p) shown to be a cryptic translocation using a multiprobe FISH assay for subtelomeric chromosome rearrangements

Sharon W Horsley, Samantha J L Knight, John Nixon, Susan Huson, Maggie Fitchett, Reginald A Boone, David Hilton-Jones, Jonathan Flint, Lyndal Kearney

### Abstract

We have previously described a fluorescence in situ hybridisation (FISH) assay for the simultaneous analysis of all human subtelomeric regions using a single microscope slide. Here we report the use of this multiprobe FISH assay in the study of a patient whose karyotype was reported by G banding analysis as 46,XX,del(18)(p11.2). Although the proband had some features suggestive of a chromosomal abnormality, relatively few of the specific features of del(18p) were present. She was a 37 year old female with mild distal spinal muscular atrophy (SMA), arthritis of the hands, an abnormal chest shape (pectus excavatum), and an unusual skin condition (keratosis pilaris). Reverse chromosome painting with degenerate oligonucleotide primer-polymerase chain reaction (DOP-PCR) amplified del(18p) chromosomes as a probe confirmed the abnormality as del(18p), with no evidence of any other chromosome involvement. Subsequently, the multiprobe FISH assay confirmed deletion of 18p subtelomeric sequence. However, the assay also showed that sequences corresponding to the 2p subtelomeric probe were present on the tip of the shortened 18p. The patient is therefore monosomic for 18p11.2-pter and trisomic for 2p25-pter, and the revised karyotype is 46,XX,der(18)t(2;18)(p25;p11.2). We believe that a proportion of all cases reported as telomeric deletions may be cryptic translocations involving other chromosome subtelomeric regions. Further studies such as this are necessary to define accurately the clinical characteristics associated with pure monosomy in chromosomal deletion syndromes.

(*J Med Genet* 1998;35:722-726)

Keywords: del(18p); fluorescence in situ hybridisation (FISH); subtelomeric chromosome rearrangements; cryptic translocations

Deletions of part or the whole of the short arm of chromosome 18 give rise to the del(18p) syndrome. The majority of reported cases are de novo deletions or inherited unbalanced translocations, with a smaller proportion being de novo unbalanced translocations involving an acrocentric chromosome.<sup>1</sup> This is one of the most common autosomal deletion syndromes.

Growth retardation and short stature are consistent clinical features, with facial dysmorphism such as round face, hypertelorism, flattened nasal bridge, wide mouth with downturned corners, and single maxillary incisor being more variable.<sup>2-7</sup> Holoprosencephaly (HPE) is found in 10% of cases, and the critical region for one of the putative HPE genes, HPE4, has been mapped to 18p11.3.<sup>8</sup> Mental retardation with varying degrees of severity has also been described. To date, there has been no correlation between the extent of the deletion and the severity of clinical features.

We recently derived a full set of cosmid, P1, and PAC clones specific for the subtelomeric regions of all human chromosomes.<sup>9</sup> We have also devised a method for the simultaneous screening of all telomeric chromosome regions using a single microscope slide, based on the commercially available chromosome painting kit (Chromoprobe Multiprobe™, Cytocell Ltd, Banbury), using custom made microscope slides and plastic coverslip devices.<sup>10</sup> Using a combination of differentially labelled short (p) and long (q) arm subtelomeric probes for each chromosome, this permits the simultaneous analysis of all 41 chromosome ends (excluding the p arms of the acrocentrics). We describe here the application of this technique to the investigation of an apparent del(18p) karyotype in a patient with a very mild clinical phenotype. The patient, a 37 year old female of normal intelligence, was initially investigated for a chromosome abnormality because of the presence of distal SMA and an unusual skin condition. The finding of a del(18p) was unexpected, and led us to investigate the possibility of the involvement of a cryptic translocation.

### Case report

The proband (fig 1) was 37 years old at the time of presentation to our department. She had a history of increasing stiffness of her neck over the preceding 10 years, weakness of hand grip for six years, and weakness of shoulder elevation with prominent shoulder blades for three years. She has no children and has had no miscarriages. Her parents are both dead, her mother dying at the age of 66 of breast cancer and her father at the age of 72 of chronic obstructive airways disease. There is no history of other medical problems in her parents. She has one sister aged 39 who is alive and well and who has three normal children.

Apart from the symptoms outlined above, the patient had no other complaints. She had a history of asthma since childhood, but this was

MRC Molecular  
Haematology Unit,  
Institute of Molecular  
Medicine, The John  
Radcliffe, Headington,  
Oxford OX3 9DS, UK  
S W Horsley  
S J L Knight  
R A Boone  
J Flint  
L Kearney

Department of Clinical  
Genetics, The  
Churchill Hospital,  
Oxford, UK  
J Nixon  
S Huson

Oxford Medical  
Genetics Laboratories,  
The Churchill  
Hospital, Oxford, UK  
M Fitchett

Department of Clinical  
Neurology, The  
Radcliffe Infirmary,  
Oxford, UK  
D Hilton-Jones

Correspondence to:  
Dr Kearney.

Received 19 December 1997  
Revised version accepted for  
publication 2 March 1998



Figure 1 (A) Side view and (B) front view of the proband aged 37 years. (Photographs reproduced with permission.)

not severe enough to require hospital admission until the age of 23. She attended normal school until the age of 16 and achieved four CSE passes. She lives independently and works as an assembler of electronic components. On examination her height was 153 cm (3rd-10th centile) and her head circumference was 55.5 cm (90th centile). She was brachycephalic with a broad neck, and her neck movements were reduced with decreased flexion and lateral rotation. Her ears were normal, her teeth were normal apart from multiple caries, and she had a high arched palate. She had pectus excavatum. A rash affecting the distal extensor aspect of her arms was diagnosed as keratosis pilaris and this was confirmed on skin biopsy. She had short toes and little fingers. Examination of her neuromuscular system showed scapular winging but no deltoid weakness. There was weakness and wasting of the upper arm, forearm, and hands. These changes were bilateral, symmetrical, and greater distally than proximally. Her legs were normal.

Neurophysiological studies of the patient's arms were consistent with distal spinal muscular atrophy, with chronic neurogenic changes and no evidence of peripheral neuropathy. Biopsy of the deltoid muscle was normal. A PCR based deletion screen of the SMN gene showed no evidence of SMN deletion.<sup>11 12</sup> Radiological studies of her hands showed osteoarthritic changes in some of the metacarpophalangeal joints, which were thought to be secondary to her chronic neurological condition. MRI of the cervical spine was normal. Her immunoglobulin levels were normal.

### Materials and methods

#### CYTOGENETIC ANALYSIS

Peripheral blood and skin fibroblast metaphase chromosomes were prepared by standard procedures. G banding was carried out using a modified trypsin-Giemsa staining method.<sup>13</sup> An Epstein-Barr virus (EBV) transformed lymphoblastoid cell line is available.

#### CHROMOSOME PAINTING

Forward chromosome painting was performed on metaphases from the EBV transformed cell line using a commercially available biotinylated whole chromosome 18 painting probe, according to the manufacturer's instructions (Cambio, Cambridge, UK). Reverse chromosome painting using DOP-PCR amplification of flow sorted chromosomes was carried out essentially as previously described.<sup>14</sup> Metaphase chromosomes were isolated from an EBV transformed cell line derived from the proband's peripheral blood.<sup>15</sup> DOP-PCR amplification was carried out using the method of Carter *et al.*<sup>16</sup> Approximately 500 flow sorted abnormal chromosomes 18 were added to the initial DOP-PCR reaction. A 5 µl aliquot from this was transferred to the second round of amplification for incorporation of biotin-16-dUTP (final concentration 300 µmol/l). FISH was carried out as previously described.<sup>15</sup> The hybridisation mixture contained 100 ng biotin labelled flow sorted chromosome 18 paint and 6.25 µg unlabelled human Cot-1 DNA, denatured and annealed at 37°C for 15 minutes before hybridisation. Hybridisation to normal male metaphase cells was carried out at 42°C overnight. After stringent washes, the sites of hybridisation were detected with successive layers of fluorescein-isothiocyanate (FITC) conjugated anti-avidin (5 µg/ml, Vector Laboratories) and biotinylated anti-avidin (5 µg/ml, Vector Laboratories). Slides were mounted in Vectashield (Vector Laboratories) containing 1 µg/ml propidium iodide and 1 µg/ml 4'6'diamidinophenylindole (DAPI) to allow concurrent G banded analysis under UV light. Results were analysed and images captured using a BioRad MRC 600 confocal laser scanning microscope.

#### TELOMERIC PROBES

The full set of human telomeric cosmid and P1 clones has been previously described.<sup>9</sup> The probes used to characterise the der(18) were 18p: P1 clone 52M11, 18q: cosmid 2050a6, 2p: cosmid 2052f6, 2q: P1 clone 210E14.<sup>9 10</sup>

#### MULTIPROBE FISH ASSAY

The multiprobe FISH assay was carried out on metaphases from the proband's peripheral blood and from the cell line as previously described.<sup>10</sup> Metaphase chromosomes were harvested from phytohaemagglutinin stimulated peripheral blood and from the EBV cell line by standard procedures.<sup>15</sup> The resultant fixed chromosome suspensions were diluted such that a 2 µl drop contained 10-20 metaphases. Two microlitre aliquots of fixed chromosome suspension were dispensed on to each of 24 demarcated slide sections. Probes were labelled by nick translation with biotin-16-dUTP (p arm probes) or digoxigenin-11-dUTP (q arm probes) (both from Boehringer Mannheim). Each square of the coverslip device received 4 ng of each labelled probe, combined with 0.25 µg (cosmids) or 0.5 µg (P1 clones) human Cot-1 DNA, which was then allowed to dry. Each corresponding square of the device received 2 µl of hybridisation buffer

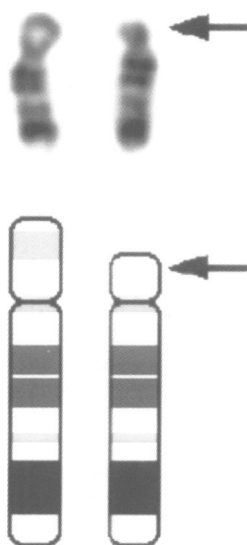
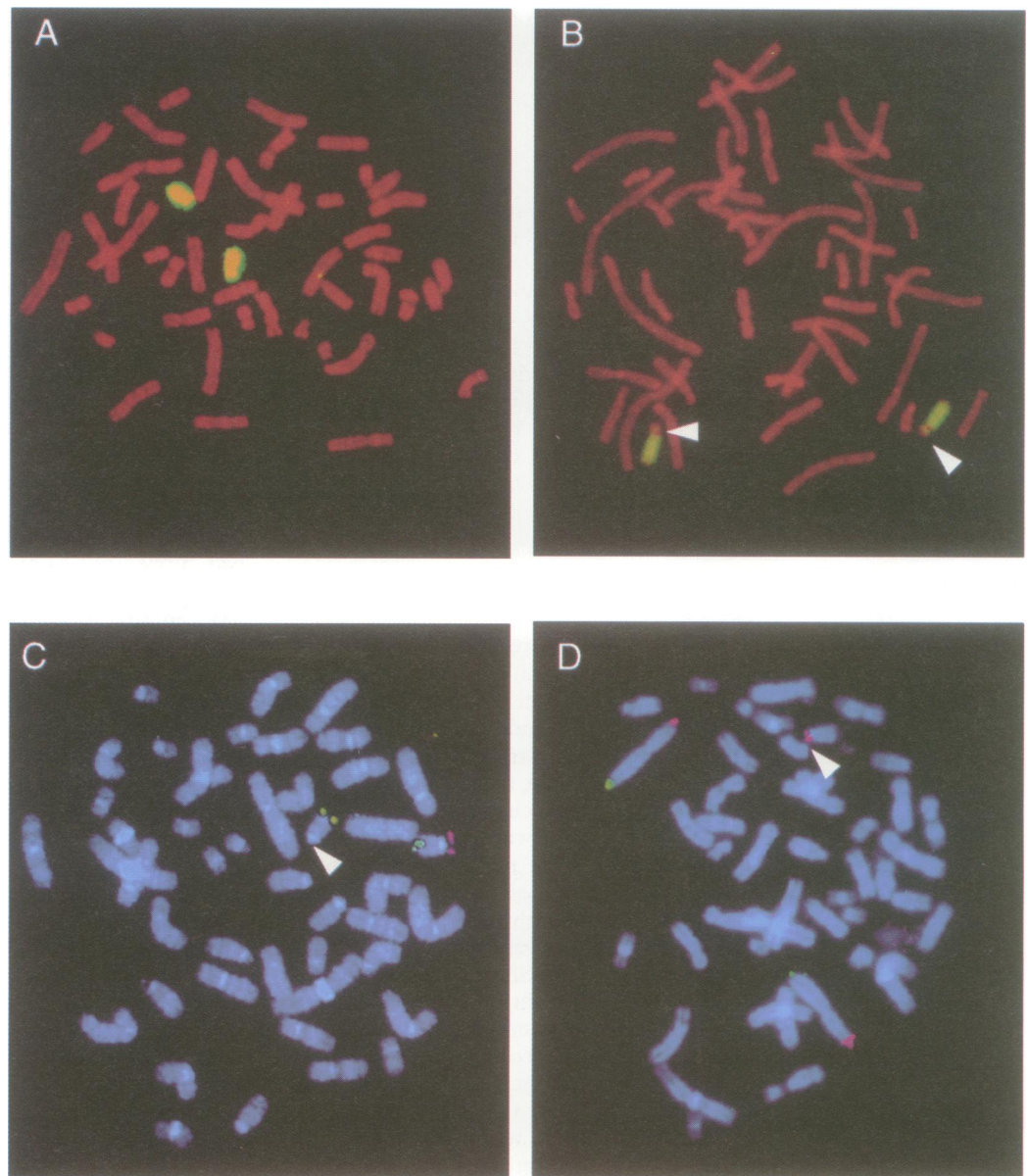


Figure 2 Partial G banded karyotype and ideogram of the proband's chromosomes 18, derived from PHA stimulated peripheral blood cultures. The der(18p) is indicated by an arrow.



**Figure 3** Examples of FISH results. (A) Forward chromosome painting of a whole chromosome 18 painting probe hybridised to a metaphase from the cell line established from the proband's lymphocytes. Both chromosome 18 homologues are fully painted, with no other chromosome 18 material visible on any other chromosome. (B) Reverse chromosome painting using a probe derived from amplified del(18p) chromosomes from the proband, hybridised to a normal male metaphase. Fluorescent signal highlights the entire chromosome 18q arm, and only a small portion of the 18p arm. The region on 18p corresponding to the deletion is unpainted (arrowheads). In both A and B, the fluorescein detected chromosome paint appears yellow, and the propidium iodide stained chromosomes appear red. (C) Representative metaphase from the chromosome 18 square of the multiprobe slide showing two copies of 18q telomeric sequences (greenish-yellow fluorescent signal) and one copy of the 18p telomeric sequences (red fluorescent signal). (D) Representative metaphase from the chromosome 2 square showing three copies of 2p telomeric sequences (red signal) and two copies of 2q telomeric sequences (greenish-yellow signal). The DAPI stained chromosomes appear light blue. The der(18) is indicated by an arrowhead.

before placing the microscope slide on top. Simultaneous denaturation of probe and chromosomal DNA was achieved by heating the slide and coverslip to 75°C on a hotplate for 1 minute 50 seconds. Hybridisation was then allowed to proceed overnight at 37°C. Stringent washes were then carried out to remove unbound, labelled probe and the sites of hybridisation detected with Texas red (biotin labelled p arms) and FITC (digoxigenin labelled q arms) as previously described.<sup>10</sup> Slides were mounted in Vectashield (Vector Laboratories) containing 1 µg/ml DAPI as counterstain. Slides were viewed using an Olympus BX 60 fluorescence microscope equipped with a triple and dual bandpass filter

and representative images captured and stored using a Photometrics ImagePoint cooled charge coupled device (CCD) camera and MacProbe version 3.3 software (Perceptive Scientific International, Chester, UK). Between five and 20 metaphases per square (each corresponding to a chromosome) were scored for the presence or absence of fluorescent signal. Deletions were identified by the presence of a single signal and triplications by the presence of three signals.

## Results

### CYTOGENETIC ANALYSIS

G banded cytogenetic analysis of 53 metaphases from peripheral blood lymphocytes and

30 metaphases from cultured skin fibroblasts found the proband's karyotype to be 46,XX,del(18)(p11.2) in all cells. A representative partial karyotype is given in fig 2.

#### CHROMOSOME PAINTING

Forward chromosome painting with a whole chromosome 18 probe showed both the der(18) and the normal homologue to be fully painted in all cells analysed. No other chromosome 18 material was observed on any other chromosome (fig 3A). Reverse painting with DOP-PCR amplified del(18p) chromosomes as a probe showed both homologues of chromosome 18 to be partially painted, consistent with the G banded assessment of del(18)(p11.2). There was no evidence of fluorescent signal on any other chromosome (fig 3B).

#### MULTIPROBE FISH ASSAY

Hybridisation with telomeric probes 52M11 (18p) and 2050a6 (18q) showed the presence of one fluorescent signal corresponding to 18p telomeric sequences and two signals corresponding to 18q telomeric sequences in all metaphases examined (fig 3C). Hybridisation with the telomeric probes 2052f6 (2p) and 210E14 (2q) showed the presence of two signals corresponding to the 2q telomeric sequences, and three signals corresponding to 2p telomeric sequences, with the additional 2p sequences located on the putative del(18p) in all metaphases examined (fig 3D). The 2q fluorescent signal was apparently reduced in intensity on one homologue (fig 3D). All other telomeric probes were present in two copies. These results confirm that the abnormality is a der(18)t(2;18)(p25;p11.2). In the light of these results we re-examined the reverse painting slides using the CCD imaging system. Despite the increased sensitivity of this system, no fluorescent signal was observed on the 2p telomere.

#### Discussion

The present case was investigated because the clinical features were suggestive of a chromosome anomaly, but did not fit the observed karyotype. We used a set of chromosome specific subtelomeric probes and a multiprobe FISH technique to show the presence of a cryptic translocation of 2p subtelomeric sequences onto an apparently deleted 18p. This unbalanced translocation results in monosomy for 18p11.2-pter and trisomy for 2p25-pter. Chromosome painting with a whole chromosome 18 painting probe confirmed the loss of a large portion of 18p. Reverse painting with DOP-PCR amplified flow sorted der(18) chromosomes from the patient failed to detect the 2p telomeric sequences, even when re-examined after the results with the telomeric probes were known. This latter finding highlights the relative insensitivity of chromosome painting for the detection of rearrangements involving subtelomeric regions. Whole chromosome painting probes are derived either from flow sorted chromosome libraries or from DOP-PCR amplified flow sorted chromo-

somes and all suffer from under-representation of sequences at some sites, especially the centromeric and telomeric regions.

The finding of an unbalanced translocation, however unexpected, does not fully explain the mild clinical phenotype in this patient. The proband showed few dysmorphic features and no mental retardation. However, there was no evidence of mosaicism in a large number of metaphases analysed from both peripheral blood and skin fibroblast cultures. Although there are a number of reports of trisomy 2p, most involve large segments of 2p.<sup>17</sup> Only two cases of trisomy for 2p25-pter have been reported, one a duplication of 2p25.1-2p25.3,<sup>18</sup> and the other a der(17)t(2;17)(p25.1;q23.1).<sup>19</sup> The former patient showed facial dysmorphism and clinical features consistent with distal trisomy 2p syndrome. The latter case was complicated by deletion of 17q23.1-qter. Based on the failure to detect the present translocation by reverse painting, we estimate that our case involves just the terminal portion of 2p25. We also found a consistently reduced fluorescent signal corresponding to the 2q probe on one chromosome homologue. Macina *et al*<sup>20</sup> have presented evidence for a 55 kb length polymorphism at the 2q telomere. We have therefore concluded that the reduced fluorescent signal is because of a subtelomeric polymorphism of 2q in this subject. It was not possible to determine whether the rearrangement was inherited or had arisen *de novo*. Neither of the parents was alive, and the only remaining relative (a sister) had an apparently normal karyotype on conventional G banded analysis. Subsequent multiprobe analysis of the sister's chromosomes has shown no abnormality of subtelomeric regions.

We believe that the multiprobe telomere assay provides a sensitive approach to the detection of subtelomeric chromosome rearrangements. In contrast to alternative approaches based on combinatorial labelling and multicolour detection of whole chromosome painting probes,<sup>21,22</sup> the method is simple and requires no expensive equipment. We suggest that telomere screening of patients with known deletions may show submicroscopic telomeric translocations, particularly in cases where the phenotype is mild or atypical. Such studies will be important in determining the true clinical effects of pure monosomy for these regions.

The authors gratefully acknowledge the support of Action Research (SWH), the Medical Research Council, UK (LK), the Wellcome Trust (SJLK, JF), and the Kay Kendall Leukaemia Research Fund (RB). We would like to thank George Clarke and other cytogeneticists at the Churchill Hospital, Oxford for cytogenetic analysis, and Professor Albert Schinzel for helpful advice on the manuscript.

- Schinzel A. Del(18)(pter→p11 to q11) = "18p- syndrome". In: *Catalogue of unbalanced chromosome aberrations in man*. Berlin: Walter de Gruyter, 1984:604-7.
- Aughton DJ, AlSaadi AA, Transue DJ. Single maxillary central incisor in a girl with del(18p) syndrome. *J Med Genet* 1991;28:530-2.
- Pfeiffer RA, Hertrick K, Cohen M. Single mandibular incisor in a patient with del(18p) anomaly. *Clin Genet* 1994;46:430-2.
- Voiculescu I, Toder R, Back E, Osswald P, Schempp W. A retrospective CISS hybridization analysis of a case with *de novo* translocation t(18;22) resulting in an 18p- syndrome. *Clin Genet* 1993;43:318-20.

- 5 Telvi L, Bernheim A, Ion A, Fouquet F, Le Bocu Y, Chaus-  
sian JL. Gonadal dysgenesis in del(18p) syndrome. *Am J  
Med Genet* 1995;57:598-600.
- 6 Gimelli G, Cinti R, Varone P, Naselli A, Di Battista E, Pez-  
zolo A. The phenotype of a 45,X male with a Y/18 translo-  
cation. *Clin Genet* 1996;49:37-41.
- 7 Schober E, Scheibenreiter S, Frisch H. 18p monosomy with  
GH-deficiency and empty sella: good response to GH-  
treatment. *Clin Genet* 1995;47:254-6.
- 8 Overhauser J, Mitchell HF, Zackai EH, Tick DB, Rojas K,  
Muenke M. Physical mapping of the holoprosencephaly  
critical region in 18p11.3. *Am J Hum Genet* 1995;57:1080-  
5.
- 9 National Institutes of Health and Institute of Molecular  
Medicine Collaboration. A complete set of human  
telomeric probes and their clinical application. *Nat Genet*  
1996;13:86-9.
- 10 Knight SJL, Horsley SW, Regan R, et al. Development and  
clinical application of an innovative fluorescence in situ  
hybridization technique which detects submicroscopic  
rearrangements involving telomeres. *Eur J Hum Genet*  
1997;5:1-8.
- 11 Van der Steege G, Grootshoeten PM, van der Vlies P, et al.  
PCR-based DNA test to confirm clinical diagnosis of auto-  
somal recessive spinal muscular atrophy. *Lancet* 1995;345:  
985-6.
- 12 Lefebvre S, Bürglen L, Reboullet S, et al. Identification and  
characterization of a spinal muscular atrophy-determining  
gene. *Cell* 1995;80:155-65.
- 13 Benn PA, Perle MA. *Chromosome staining and banding  
techniques*. In: Rooney DE, Czepulkowski BH, eds. *Human  
cytogenetics: a practical approach*. Oxford: IRL Press,  
1992:91-118.
- 14 Rack KA, Harris PC, MacCarthy AM, et al. Characterisa-  
tion of three *de novo* derivative chromosomes 16 by 'reverse  
chromosome painting' and molecular analysis. *Am J Hum  
Genet* 1993;52:987-97.
- 15 Buckle VJ, Rack K. Fluorescent in situ hybridization. In:  
Davies KE, ed. *Human genetic disease analysis*. Oxford: IRL  
Press, 1993:59-80.
- 16 Carter NP, Ferguson-Smith MA, Perryman MT, et al.  
Reverse chromosome painting: a method for the rapid  
analysis of aberrant chromosomes in clinical cytogenetics. *J  
Med Genet* 1992;29:299-307.
- 17 Lurie IW, Ilyina HG, Gurevich DB, et al. Trisomy 2p: analy-  
sis of unusual phenotypic findings. *Am J Med Genet* 1995;  
55:229-36.
- 18 Wakita Y, Narahara K, Takahashi Y, et al. Duplication of  
2p25: confirmation of the assignment of soluble acid phos-  
phate (ACP<sub>1</sub>) locus to 2p25. *Hum Genet* 1985;71:259-60.
- 19 Bridge J, Sanger W, Mosher G, et al. Partial deletion of dis-  
tal 17q. *Am J Med Genet* 1985;21:225-9.
- 20 Macina RA, Negorev DG, Spais C, Ruthig LA, Hu XL,  
Riethman HC. Sequence organization of the human 2q telom-  
ere. *Hum Mol Genet* 1994;3:1847-53.
- 21 Schrock E, du Manoir S, Veldman T, et al. Multicolor spec-  
tral karyotyping of human chromosomes. *Science* 1996;273:  
494-7.
- 22 Speicher MR, Ballard SG, Ward DC. Karyotyping human  
chromosomes by combinatorial multi-fluor FISH. *Nat  
Genet* 1996;12:368-75.