European Journal of Human Genetics (1999) 7, 873–883

http://www.stockton-press.co.uk/ejhg

ARTICLE

Recombinant balanced and unbalanced translocations as a consequence of a balanced complex chromosomal rearrangement involving eight breakpoints in four chromosomes

Benno Röthlisberger[1], Dieter Kotzot[1], Lukrecija Brecevic[1], Michael Koehler[2], Damina Balmer[1], Franz Binkert[1] and Albert Schinzel[1]

[1]*Institut für Medizinische Genetik, Universität Zürich, Zürich, Switzerland*
[2]*Applied Spectral Imaging GmbH i.Gr., Edingen-Neckarhausen, Baden-Württemberg, Germany*

We report on a family with a balanced complex chromosomal rearrangement (CCR) involving eight breakpoints between chromosomes 6, 7, 18, and 21 in the father. All three sons inherited one derivative chromosome from the father and in addition each inherited a different recombinant chromosome resulting in a partial trisomy 6q in the first, an apparently balanced karyotype in the second, and a partial trisomy 7q in the third son. Fluorescence *in situ* hybridisation (FISH) and microsatellite analysis were essential for the identification of the breakpoints. In addition, the results were confirmed by a 24-colour FISH experiment using the spectral karyotyping (SKY™) system. Paternal origin of the *de novo* CCR in the father was demonstrated for the first time by haplotype analysis. This is the second report of a CCR leading to simpler but unbalanced translocations in offspring as a consequence of recombination during gametogenesis, and the first report of a family case of CCR exhibiting as many as eight breakpoints in the transmitting carrier. The initial prediction that viable offspring would be quite unlikely had to be revised after the birth of three children. Genetic counselling of carriers of balanced complex rearrangements has to consider a higher probability for unbalanced recombinations than has been so far commonly assumed.

Keywords: complex chromosomal rearrangement (CCR); meiotic recombination; FISH; spectral karyotyping (SKY™); Partial trisomy 6q; Partial trisomy 7q

Introduction

Complex chromosome rearrangement (CCR) is a general term used for structural rearrangements involving three or more chromosomes. Different types of classification have been proposed. Three major categories are recognised by Gardner and Sutherland.[1]

1. The most common is the 'three-way exchange', in which three segments from three chromosomes break off, translocate, and unite.
2. The simplest CCR is the 'double two-way exchange', in which there is a coincidence of two separate simple reciprocal translocations.
3. More complicated CCRs with more than one breakpoint per chromosome.

Correspondence: Benno Röthlisberger, Institute of Medical Genetics, Rämistrasse 74, CH-8001 Zürich, Switzerland. Tel.: 0041 1 634 2522; Fax: 0041 1 634 4916; E-mail: bennor@medgen.unizh.ch
Received 21 May 1999; revised 22 July 1999; accepted 3 August 1999

At present at least 114 cases of CCR have been documented.[2] Thirty-five of them were familial cases, reviewed by Batista *et al*.[3] The following conclusions were drawn:

1 Familial CCRs tend to have fewer breakpoints than do *de novo* CCRs.

2 Familial transmission is mainly observed through female carriers although the origin of *de novo* cases is mostly paternal.

3 An apparent excess of balanced female carriers over balanced male carriers among the offspring of index carriers was noted.

4 Meiotic segregation resulting in malformed live-born infants is most frequently due to adjacent-1 segregation.

Only six families with recombination derived from a parental CCR have been reported[4–9] In the first five reports all offspring had an apparently balanced karyotype showing maternal transmission, with the exception of one report with paternal transmission.[6] Zahed *et al*[9] were the second authors to report recombination with paternal transmission leading, for the first time, to offspring with an unbalanced karyotype.

We report another family with a complex chromosomal rearrangement with paternal transmission and with various meiotic recombinations leading to balanced and to unbalanced karyotypes. To our knowledge this is the first familial case of CCR exhibiting as many as eight breakpoints (involving four chromosomes) in the transmitting carrier. In addition, for the first time, origin and formation of the balanced paternal CCR in the grandpaternal germline could be demonstrated by molecular analysis.

Patients and Methods

Patients

Patient 1 This boy of 11 years 7 months is the first son of healthy, unrelated parents. At birth the father was 25 years old and the mother 27 years old. The boy was born at 41 3/7 weeks of gestation after an uneventful pregnancy except for bleeding during the first 12 weeks. Weight was 2 300g (< 3rd centile) and length was 48 cm (< 10th centile). Apgar scores after 1, 5, and 10 minutes were 8, 9 and 9, respectively. At the age of 1 month the boy displayed the following anomalies: occipitofrontal circumference (OFC) (33.5 cm) below the 3rd centile, shallow orbits with exophthalamos, small nose, prominent upper lip, prominent philtrum, umbilical hernia, inguinoscrotal hernia on the right side, small thorax with thin ribs, single palmar creases, syndactyly between toes 4 and 5 on both sides, and partial syndactyly between 2/3 on the right side. By echocardiography a perimembraneous ventricular septum defect was detected. Clinical re-examination at the age of 4 years 11 months revealed height (109 cm) and weight (16.6 kg) within normal ranges. OFC (49.3 cm) was in the 3 rd centile. Total hand length (11.7 cm) was in the 10th centile and middle finger length (4.3 cm) was in the 3rd centile. Inner canthal distance (3.0 cm) and ear length (5.4 cm) were within normal ranges. Additional findings were brachycephaly, low frontal hairline, epicanthic folds, slightly upslanting palpebral fissures, myopia (–8 dioptries), small nose with a bulbous tip, diastema of the lower incisors, prominent ears of normal size, small penis, clinodactyly 5, and reduced supination on both arms (Figure 1a). The patient was standing at 9 months and walking at 16 months of age. He spoke his first word at 8 months. At present he can ride a bicycle and is able to swim but has problems with precise movements and with balance. Motor skill is decreased, particularly pronation, supination, and diadochokinesis. His global IQ is 85 with slightly reduced abstract thinking and with clear difficulties in visual perception.

Patient 2 This boy of 8 years 11 months is the second of the three brothers born at 39 weeks after an uneventful pregnancy. Weight (3 550 g) and length (51 cm) were around the

Figure 1a *Patient 1 at the age of 4 11/12 years. Note epicanthic folds, upslanting palpebral fissures and small nose with bulbous tip.*

50th centile. At the age of 6 years 10 months height (121 cm), weight (22.8 kg), and OFC (51.2 cm) were around the 50th centile. Except for hypertelorism and prominent upper lip he displayed no dysmorphic features (Figure 1b). Psychomotor development is in the normal range, and he attends normal primary school.

Patient 3 This boy of 5 years 4 months is the third brother and born after an uneventful pregnancy at term by Caesarean section. He was walking at the age of 12 months. At the age of 3 years 3 months, height (92 cm) and OFC (48.5 cm) were in the 3rd centile, weight (13.6 kg) was in the 10th centile. Clinical re-examination at the age of 5 years 2 months showed height (104.2 cm) in the 3rd centile, weight (17.3 kg) between the 10th and 25th centile, and OFC (49.3cm) below the 3rd centile. Hand length (11.5cm), middle finger length (4.6cm), and foot length (16.1cm) were all between the 3rd and 10th centiles. Inner canthal distance (2.6 cm) was in the 25th centile and ear length (5.9 cm) between the 75th and 97th centiles. Additional findings were disappearing epicanthic folds, slightly depressed nasal bridge, thick alae nasi, anteverted nares, postaxial hexadactyly of both hands (removed by surgery), tapering fingers, short proximal phalanges of the 5th fingers, and a small umbilical hernia (Figure 1c). Psychomotor development lies in the normal range and he attends kindergarten.

Patient 4 This is the father of patients 1, 2 and 3. He is a 36-year-old healthy man with academic education. He has no further offspring; probably unbalanced outcome caused 4 or 5 losses in early pregnancy. Genetic counselling after the birth of the first son was predicted that both balanced and unbalanced viable offspring would be unlikely; nevertheless, prenatal cytogenetic examination was offered, but the couple preferred to decline. The patients' father (the paternal grandfather of patients 1–3) was working with radioactive material around the time of conception.

Conventional Cytogenetic Investigations

Metaphase chromosome preparations were obtained from PHA-stimulated lymphocyte cultures from patients 1–3, both parents, and the paternal grandparents according to standard procedures. Chromosome banding was produced by the trypsin-Giemsa technique. Cell lines of patients 1–3 and the father are available.

Fluorescence in situ *Hybridisation*

FISH analysis was performed according to Lichter *et al*[10] using specific libraries of all chromosomes (from Laurence Livermore Laboratory, USA) labelled with digoxigenin and biotin, and directly labelled whole chromosome painting probes for chromosomes 6, 7, 18, and 21 (WCP™, VYSIS,

Figure 1b *Patient 2 at the age of 2 4/12 years.*

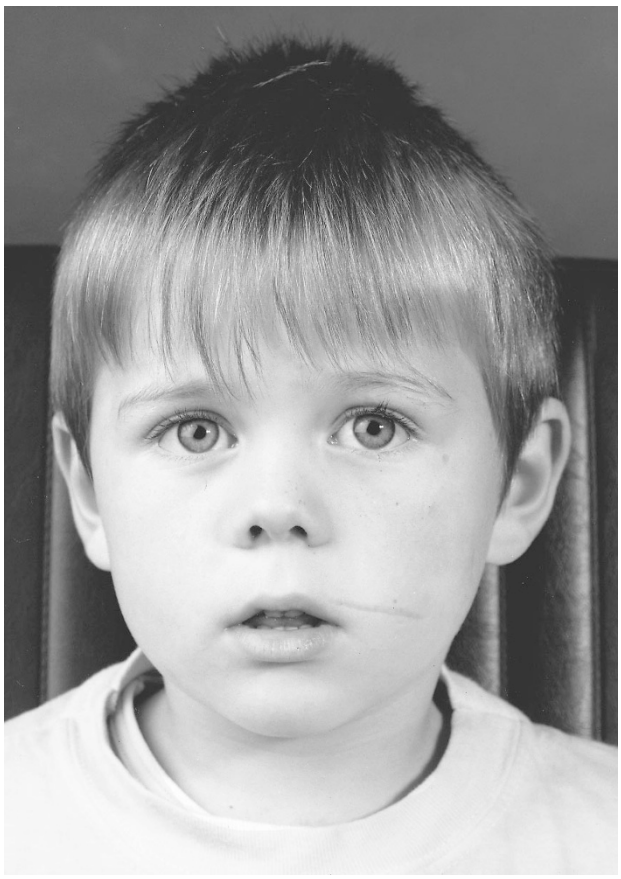

Figure 1c *Patient 3 at the age of 5 2/12 years. Note thick alae nasi and anteverted nares*

Downers Grove, IL, USA). The hybridised metaphases were examined with a Zeiss Axioplan epifluorescence microscope. Subsequent analyses were performed by image acquisition using a CCD camera and digital image analysis (QUIPS™, VYSIS).

Spectral Karyotyping (SKY™)

SKY hybridisation was achieved using the SkyPaint™ probe kit from Applied Spectral Imaging (ASI), Migdal Ha'Emek, Israel, which consists of flow-sorted, combinatorially labelled, human chromosome-specific painting probes for 24 colour FISH.[11] In the SkyPaint probe mixture each chromosome is labelled with a unique combination of five fluorochromes (1 green, 2 red, 2 infrared) which, after *in situ* hybridisation, results in a highly specific emission spectrum characteristic for each chromosome of the complement.

Pretreatment of chromosome preparations, denaturation of probe mixture, chromosome slides, posthybridisation washing, and detection steps were carried out according to the protocol of the manufacturer and as described previously.[12,13] After hybridisation, slides were counterstained with 4'-6'-diamidino-2-phenylindole (DAPI) and embedded in paraphenylendiamine (Sigma) to reduce photobleaching.

Spectral images of hybridised metaphases were captured in a one-step acquisition through a single triple-band pass fluorescence filter (SKY-1, Chroma Technology, Brattleboro, VT, USA) using the SkyVision cytogenetic workstation (Applied Spectral Imaging, Israel) attached to a Zeiss Axioskop1 microscope equipped for epifluorescence. The SkyVision system consists of the SpectraCube™ optical system coupled to a cooled CCD camera (Hamamatsu C4880-85) and a high speed personal computer. This technology combines the application of Fourier spectroscopy, CCD-imaging, microscopy, and computing thus allowing the simultaneous measurement of the precise spectral curve for each point of the image.[14] Corresponding DAPI images were captured through a DAPI specific fluorescence filter cube (Zeiss filter No. 01). Spectral images were analysed using the SkyView software. SkyView first displays the raw spectral image data as a red–green–blue (RGB) display image by assigning the red (R), green (G), and blue (B) display colour ranges to specific spectral emission ranges (R = near infrared (IR) emission, G = red emission, B = green emission). Finally, using spectrally-based algorithms all points of the image with identical (emission) spectra are assigned a unique pseudocolour. This classification procedure is the basis for Spectral Karyotyping and for definitive chromosome recognition.[15] The DAPI counterstained image of the same metaphase can be band-enhanced and displayed side by side with the spectral image which greatly facilitates the integration of chromosome band information within the analysis.

Molecular Genetics Investigations

To determine the formation of the CCR, genomic DNA from patients 1–3, both parents and the paternal grandparents was amplified by standard PCR with commercially available microsatellites (Research Genetics®, Huntsville, USA), loaded on to a 6% polyacrlyamide/urea gel, and visualised by silver staining.

Results

The family was ascertained through patient 1, who displayed several dysmorphic features at birth. Chromosome preparations from blood lymphocyte cultures showed a translocation between chromosome 7 and chromosome 18. The breakpoints could not be determined cytogenetically. Chromosomal analysis of the mother showed a normal 46,XX karyotype.

The father was found to be carrier of a balanced CCR involving chromosomes 6, 7, 18, and 21. In order to determine the breakpoints and exclude other chromosomes involved, bicolour-FISH with all chromosome libraries was performed. It could be shown that the translocation was an eight-break rearrangement involving chromosomes 6, 7, 18, and 21 (Figures 2a–c and Figure 4). The karyotypes of both his parents were normal. Following the detailed system of the nomenclature for such rearrangements[16] his translocation may be written as: 46,XY,t(6;7;18;21)(6pter- > 6q22::6q25- > 6qter;7pter- > 7q21.3::21q21.3- > 21qter;7qter- > 7q32.1::18p11.21- > 18q21.3::7q31.3- > 7q32.1::6q22- > 6q25::18q21.3- > 18qter;21pter- > 21q21.3::7q21.3- > 7q31.3::18p11.21- > 18pter)dn. It should be mentioned that the direction of the inserted segments 6(q22- > q25) and 7(q31.3- > q32.1) towards the centromere is not clear. To confirm the position of the two cytogenetically not distinguishable small terminal segments of chromosome 18 (18pter- > 18p11.21 and 18qter- > 18q21.3, respectively) we performed FISH with a subtelomeric probe for the long arm of chromosome 18 (Figure 2c). Finally the results were confirmed by spectral karyotyping (SKY™). Except for the very small insertion 7;18 and the very small translocation 18;21 which could not be detected (since their size is probably below the detection limit of 2.5 megabases), our preliminary experiments with bicolour-FISH were confirmed (Figure 3). It could be shown that no further chromosome is involved in the CCR.

In Patient 1 bicolour-FISH performed with libraries 6, 7, 18, and 21 (WCP™, VYSIS) revealed a CCR involving chromosomes 6, 7, and 18 (Figure 4). The derivative chromosome 18 and in addition a 'new' aberrant chromosome 7 were found which could only be explained as a result of a crossing-over during meiosis. Since a normal paternal chromosome 6 and the derivative chromosome 18 with an insertion of a segment of chromosome 6 were present, the boy had a small duplication of chromosome 6(q22- > q25). The markers D6S270, D6S292, and D6S311 showed two paternal alleles and one maternal allele. The maximum

size of the duplicated segment spans D6S407 to D6S473, which both show only one paternal allele. The final karyotype was 46,XY,t(6;7;18)(7pter- > 7q31.3:: 18p11.21- > 18pter;7qter- > 7q32.1::18p11.21- > 18q21.3:: 7q31.3- > 7q32.1::6q22- > 6q25::18q21.3- > 18qter)pat.

In Patient 2 a simple reciprocal (7;21)(q21.3;q21.3)pat translocation was found and proved by bicolour-FISH (Figure 4). Thus he inherited from his father the der(7) and in addition a 'new' or recombinant chromosome 21. Given the mechanism leading to this simple reciprocal translocation in this case, the short system of the nomenclature for such rearrangements [ISCN, 1995] might not be appropriate.

In Patient 3 bicolour-FISH revealed a CCR involving chromosomes 6, 7, and 18 (Figure 4). Since a normal paternal chromosome 7 and a 'new' or recombinant chromosome 18 with an insertion of chromosome 7 were present, the boy has a duplication of a small segment of chromosome 7(q31.3- > q32.1). This was confirmed by microsatellite analysis. The markers D7S480 and D7S1517 showed two paternal alleles; proximally of this segment, CF(322), and distally, D7S504, are not duplicated. The final karyotype of this boy was 46,XY,t(6;7;18)(6pter- > 6q22::6q25- > 6qter; 18pter- > 18q21.3::7q31.3- > 7q32.1::6q22- > 6q25:: 18q21.3- > 18qter)pat. These results were confirmed by spectral karyotyping (SKY™).

To determine the parental origin of the CCR in the father, we constructed a chromosome 6 haplotype. Son 2, who inherited the normal chromosome from his father, showed a grandmaternal allele of all investigated microsatellites localised proximal to the proximal breakpoint (D6S261), within the two breakpoints

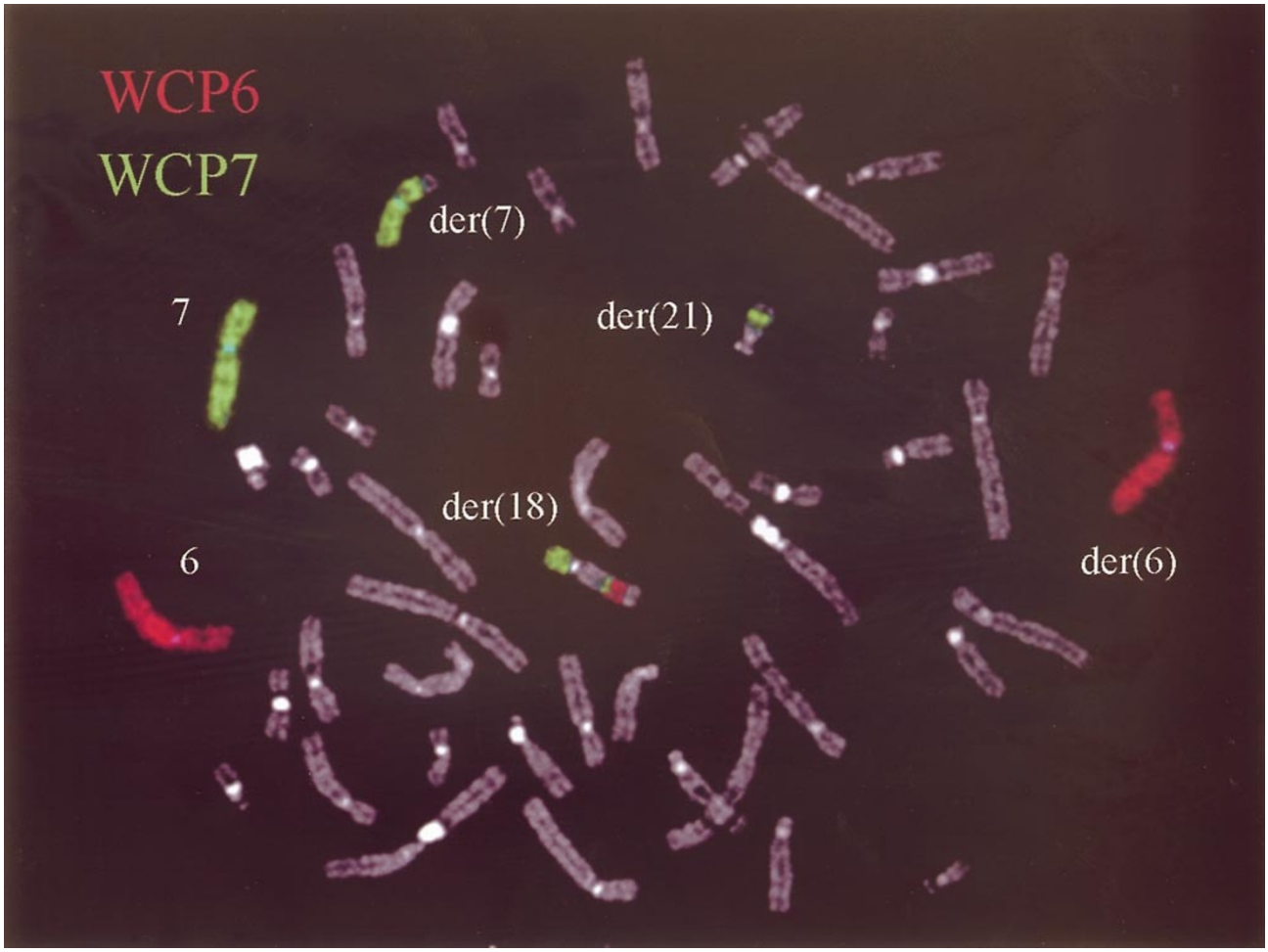

Figure 2a *Dual FISH of a metaphase of the father. Chromosome 7 is painted in green and chromosome 6 in red. Note the derivative chromosome 18 with a tiny segment of chromosome 7 inserted.*

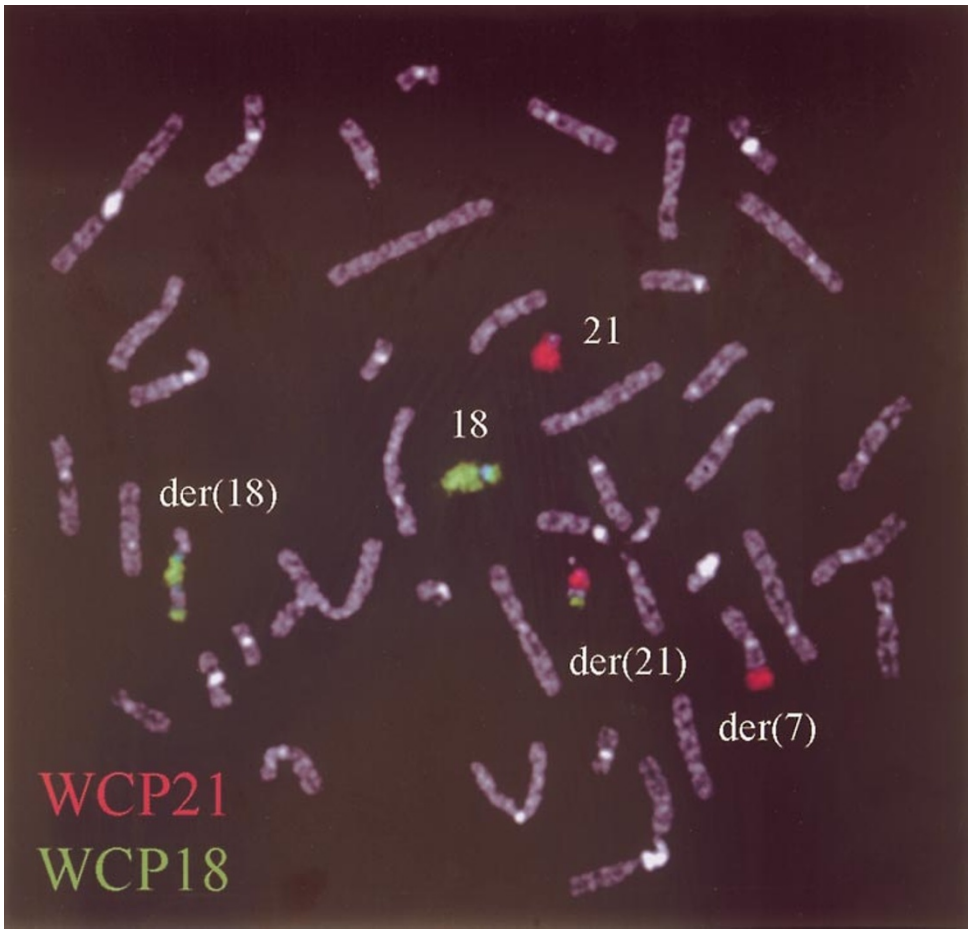

Figure 2b *Chromosome 18 is painted in green and chromosome 21 in red. Note the derivative chromosome 21 with a tiny segment of chromosome 18 translocated.*

(D6S270) and distal to the distal breakpoint (D6S473) respectively. Son 3, who inherited the deleted chromosome 6 and a recombinant chromosome 18 with the insertion of the deleted segment of chromosome 6, showed a grandpaternal allele of all these microsatellites. Son 1, who inherited a normal chromosome 6 and the derivative chromosome 18 with an insertion of chromosome 6, showed one grandpaternal and one grandmaternal allele for D6S270 and a grandmaternal allele for the microsatellites localised outside the duplicated segment (D6S261, D6S473). All these results clearly show the grandpaternal origin of the *de novo* CCR in the father (Figure 5).

By combination of conventional cytogenetics, FISH, microsatellite analysis, and spectral karyotyping (SKY™) we were able to show that all three sons inherited one derivative chromosome from the father, and in addition each had a different recombinant chromosome, resulting in partial trisomy 6q in the first, an apparently balanced karyotype in the second, and partial trisomy 7q in the third son.

Discussion

A recombinant chromosome is a structurally rearranged chromosome with a new segmental composition resulting from meiotic crossing-over between a displaced segment and its normally located counterpart in certain types of structural heterozygotes.[16] At least 255 cases with a recombinant chromosome originating from a crossing-over in inversion or insertion heterozygotes are documented.[17] This almost always results in unbalanced chromosome complements and phenotypically abnormal subjects; to our knowledge only one extraordinary exception, resulting in a balanced karyotype, has been described.[18] In only six reports recombinant chromosomes have been reported in

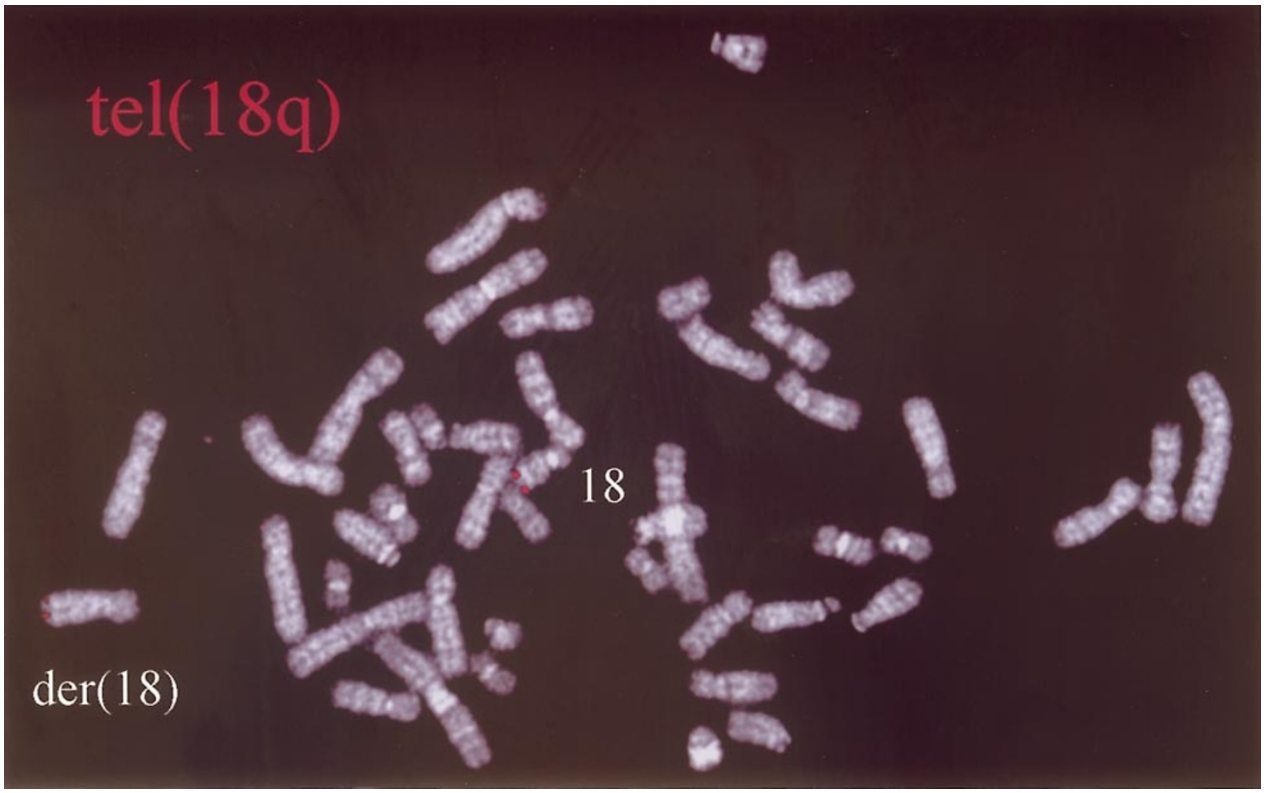

Figure 2c *FISH with a subtelomeric probe of the long arm of chromosome 18. Note a signal on the derivative chromosome 18 and one on the normal homologue*

families with a complex chromosomal rearrangement (CCR) resulting in altogether 11 offspring.

In only one report[9] the recombination resulted in two differently unbalanced offspring in the third generation. The remaining nine cases show an apparently balanced karyotype and normal phenotype in eight of the nine cases. In one of them the paternal recombination between the der(7) and the der(9) resulted in a normal female karyotype (46,XX).[6] However, the same karyotype could be explained by non-paternity or uniparental disomy. At the moment recombinant products following meiotic pairing of CCR are evident in only 10 cases of six families. We report three additional cases in a family of a CCR resulting in one balanced offspring and, for the second time, in two differently unbalanced offspring, all through recombination.

The father shows a balanced translocation between the chromosomes 6, 7, 18, and 21 with breakpoints in 6q22, 6q25, 7q21, 7q31, 7q32, 18p11, 18q21, and 21q21 resulting in four derivative chromosomes. The breakpoint at 18q21 gives further evidence that this is a hot spot for breakpoints in CCR.[2] Considering only 4:4 segregation, 16 different outcomes are possible with only two of them balanced. The derivative chromosomes 18 and 21 are made of five and three segments, respectively, and thus allow the formation of recombinant chromosomes. Two of the inserted segments (6q22->q25 and 7q31->32) are very small with their orientation toward the centromere not distinguishable. We consider it possible that these two small insertions do not take part synapsing during meiosis and thus do not allow for crossing-over. Crossing-over in the two other 'inserted' segments (18p11->q21 and 7q21->q31) will result in the possible formation of four different recombinant chromosomes. This gives an additional 32 possibilities of 4:4 segregation when only one recombinant chromosome is considered with only one of the possibilities being balanced. When we consider a recombination event in both of these two insertions during the same meiosis there are 16 additional segregation possibilities with none of them being balanced. Altogether, only three of 64 meiotic outcomes are balanced when we consider only 4:4 segregation. In addition, we also should take into consideration the possibilities of 5:3, 6:2, and 7:1 segregation, even if most commonly (78%) abnormal liveborns observed in

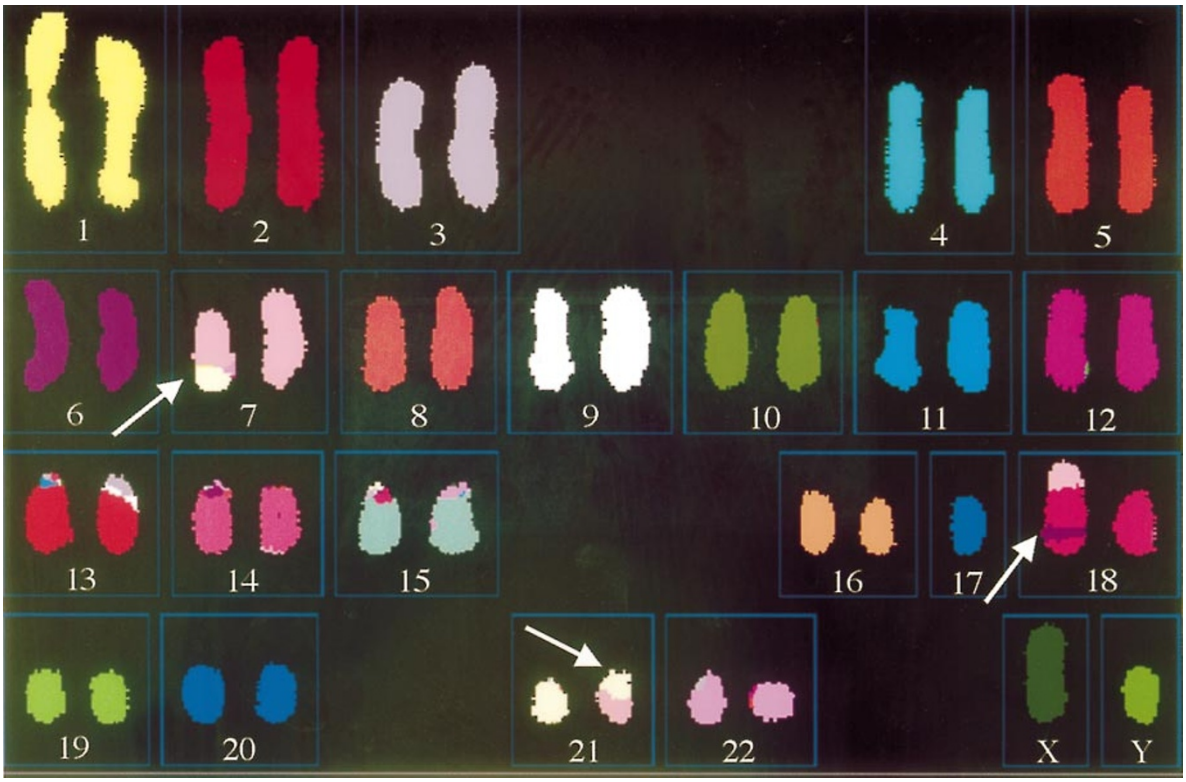

Figure 3 *SKY™ multicolour analysis of a metaphase of the father*

CCRs are due to adjacent-1 segregation.[3] In conclusion, the calculated probability of normal offspring for the father is very low.

Patient 1 inherited the derivative chromosome 18 and in addition a 'new' recombinant chromosome 7. This can only be explained by meiotic crossing-over having taken place in the segment 7q21->q31 between the der(21) and the normal chromosome 7. As a result, Patient 1 has dup(6)(q22->q25).

Patient 2 inherited the derivative chromosome 7 and in addition a 'new' recombinant chromosome 21. The crossing-over occurred again in the segment 7q21->q31 between the der(21) and the normal chromosome 7. As a result, Patient 2 has a simple balanced reciprocal translocation and it should be noted that this is the only possibility of a balanced karyotype with a recombinant chromosome in this CCR.

Patient 3 inherited the chromosome 6 with the interstitial deletion and a 'new' recombinant chromosome 18. The crossing-over took place in the segment 18p11->q21 between the der(18) and the normal chromosome 18. As a result, patient 3 has a dup(7)(q31->q32). Although rare, such events might be big steps and landmarks in eukaryotic evolution. One single event is followed by a completely new composition of much genetic material. In conclusion, the three sons all inherited one derivative and in addition each one a different recombinant chromosome.

Patient 2 is a further example of a balanced rearrangement involving an insertion giving rise to a simpler balanced translocation as a result of crossing-over. Patients 1 and 3 are the third and fourth case to be described of a CCR resulting in simpler but unbalanced translocations as a result of crossing-over. Many reported CCR cases cannot lead to recombinant chromosomes in offspring because they are of the 'simple' type with only one break per chromosome. Recombinants resulting from a CCR are only possible when the number of breakpoints is higher than the number of chromosomes; that means at least one chromosome must have undergone a doublebreak. So far, at least 60 such cases have been reported.[2,9] These cases can be divided into three groups.[8]

1 simple three-way translocations with an additional pericentric inversion in one of the chromosomes;

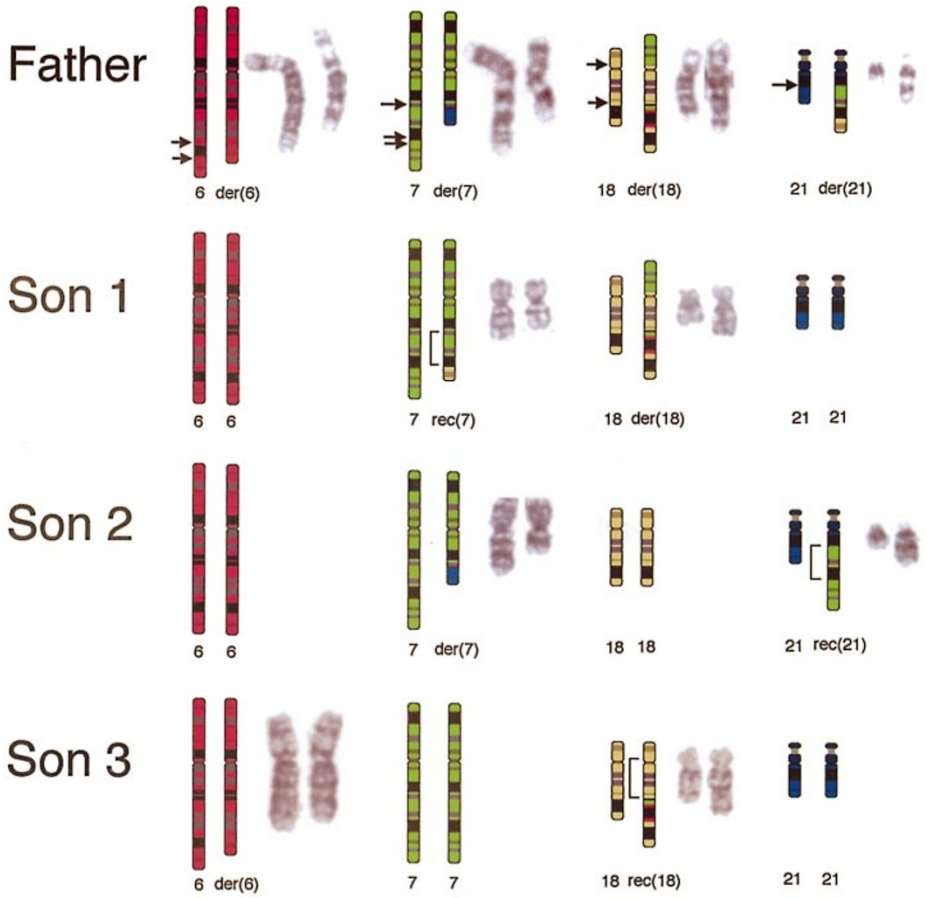

Figure 4 *Partial karyograms and ideograms of the familial complex chromosomal rearrangement. Breakpoints are indicated by arrows on the normal homologues of the ideogram. Note that each son inherited one recombinant chromosome. The sites of recombination are indicated by brackets*

2 translocations between two chromosomes and an insertion of a segment into a third;

3 translocations in which at least one chromosome is made up of segments from three or four chromosomes involved.

Actually, recombination has been observed in only six of the 60 reports with the possibility of producing recombinant offspring, and all six belong to the group 3 described by Madan *et al*.[8] The karyotype of the father described in our report also belongs to this group with as many as two chromosomes made up of segments of three different chromosomes (derivative 18 with segments of chromosomes 6, 7, and 18; derivative 21 with segments of chromosomes 7, 18, and 21). The breakpoints are such that a cross-over in the inserted segments would usually lead to an unbalanced and, with only one exception, a balanced karyotype. This family is another extraordinary example of recombination in a certain type of complex chromosomal rearrangement which involves an insertion leading to simpler translocation (balanced and unbalanced) in the offspring. In three of the families described so far recombinant chromosomes have been observed in more than one offspring, in two out of two siblings,[6,9] and in two out of three siblings, respectively.[5] In two other families no siblings are mentioned[4,7] and in the sixth family[8] not all family members have been investigated. In our family, recombination has been observed in three out of three siblings. Even if recombination in CCR is a very rare finding it seems to be highly recurrent in families, especially in the one described in this paper. What is true for inversions, might be also true for CCR: that the longer the inverted segment, the more likely it is that recombination will occur, and that each individual chromosomal inversion carries its own individual risk. Our family is not only extraordinary because of the recombination event in all three

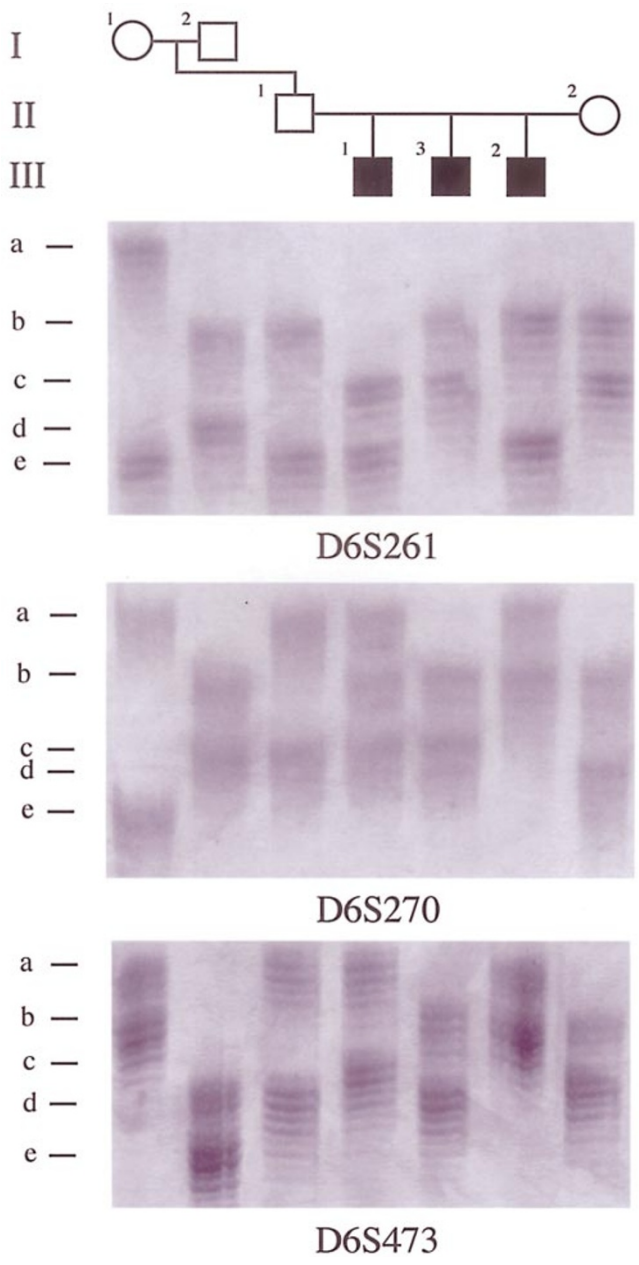

Figure 5 *Results of haplotype analysis of chromosome 6 with the microsatellite markers D6S261 (mapping proximal to the proximal breakpoint), D6S270 (within the duplicated region) and D6S473 (distal to the distal breakpoint). Son 2, who inherited the normal chromosome from his father, showed the grandmaternal allele of all investigated microsatellites. Son 3, who inherited the deleted chromosome 6 and a recombinant chromosome 18 with the insertion of the deleted segment of chromosome 6, showed the grandpaternal allele of all these microsatellites. Son 1, who inherited a normal chromosome 6 and the derivative chromosome 18 with an insertion of chromosome 6, showed one grandpaternal and one grandmaternal allele for D6S270 and a grandmaternal allele for the microsatellites mapping proximal and distal to the duplicated segment respectively*

offspring but also by comparison with the conclusions made for CCRs by Batista *et al*.[3] A review of 35 familial CCRs led to the following observations. 1. Familial CCRs tend to have fewer chromosomes involved and fewer breakpoints than do *de novo* CCRs. In our family we observed eight breakpoints with four chromosomes involved. No family with so many breakpoints was included in Batista's study. 2. Familial transmission is mainly observed through female carriers although the origin of *de novo* cases is mostly paternal; transmission through spermatogenesis occurred in only four out of 30 families. We report an additional family with paternal inheritance, and for the first time we demonstrate paternal origin of a *de novo* CCR, without involvement of a sex chromosome, by molecular analysis. Haplotype analysis is an elegant approach to determining parental origin of a *de novo* CCR if unbalanced offspring is found in the following generation. 3. An apparent excess of balanced female carriers among the offspring of index carriers was noted. We report a family with three sons and no daughter. 4. Meiotic segregation resulting in malformed liveborn infants is most frequently due to adjacent-1 segregation. As described above we report two unbalanced offspring through recombination in meiosis and not 'simple' malsegregation. A better evaluation of individual potential risks for the offspring might be possible by studying the meiotic segregation patterns in spermatozoa from CCR carriers. Different elegant studies using two, three and four colour FISH have been undertaken for inversions and reciprocal translocations.[19] Multicolour FISH or Spectral Karyotyping (SKY™) approaches with locus specific probes will help to study more complicated translocations and to elucidate meiotic behaviour in such cases. This should be helpful in leading to a better understanding of the mechanisms during meiosis and the assessment of the reproductive risk associated with complex chromosomal translocations.

At genetic counselling after the birth of the first child and subsequent detection of the complex paternal rearrangement, it was predicted that further viable offspring would be somewhat unlikely. This was based on the following assumptions:

1. a child with a normal karyotype or the same balanced rearrangement as present in the father could emerge only if, for all four chromosomes involved, always either the normal homologues or the rearranged homologues would be transmitted and no recombination would occur;

2. unbalanced transmission for only one or two rearranged chromosomes in almost all instances would not be viable;
3. also almost all cross-overs would not be viable.

Thus, the subsequent birth of two viable children, one normal and the other with only minor phenotypic alterations, was a great surprise to all participants. For similar rearrangement, the possibility of recombination at any sites leading to only minor unbalanced or, as in Patient 2, even 'correcting' the complex balanced rearrangement to a less complex one, has to be taken into consideration.

Acknowledgements

We are grateful to the family for their co-operation and would like to thank Mrs Janine Steullet for excellent technical assistance and Mr Louis Burger for photos of the patients. Applied Spectral Imaging (ASI) is indebted to Dr Evelin Schröck and Dr Thomas Ried for their continuous support. Furthermore, ASI is grateful to Professor Dr Hansjakob Müller and Dr F Wenzel of the Children's Hospital in Basel, Switzerland, for providing the opportunity to perform a SKY demonstration workshop in their laboratory, and to the Carl Zeiss company (Göttingen, Germany) for providing the microscopic equipment. The study was supported by the Swiss National Foundation, grant No 32-56053.98.

References

1 Gardner RJM, Sutherland GR: *Chromosome Abnormalities and Genetic Counseling*. Oxford University Press: New York, 1996, pp 182–190.
2 Batanian JR, Eswara MS: De novo apparently balanced complex chromosome rearrangement (CCR) involving chromosomes 4, 18, and 21 in a girl with mental retardation: report and review. *Am J Med Genet* 1998; **78**: 44–51.
3 Batista DAS, Pai GS, Stetten G: Molecular analysis of a complex chromosomal rearrangement and a review of familial cases. *Am J Med Genet* 1994; **53**: 255–263.
4 Tabor A, Kieler Jensen L, Lundstein C, Niebuhr E: A 5;7 double reciprocal translocation in a normal mother and a 5;7 translocation with a recombinant chromosome 5 in her normal child. *J Med Genet* 1981; **18**: 307–309.
5 Bass HN, Sparkes RS, Lessner MM, Fox M, Phoenix B, Bernar J: A family with three independant translocations associated with 7q32- > 7qter syndrome. *J Med Genet* 1985; **22**: 59–63.
6 Walker S, Howard PJ, Hunter D: Familial complex autosomal translocations involving chromosomes 7, 8, and 9 exhibiting male and female transmission with segregation and recombination. *J Med Genet* 1985; **99**: 484–491.
7 Masuno M, Asano J, Yasuda K, Kondo T, Orii T: Balanced complex rearrangement involving chromosomes 8, 9, and 12 in a normal mother, derivative chromosome 9 with recombinant chromosome 12 in her daughter with minor anomalies. *Am J Med Genet* 1993; **45**: 65–67.
8 Madan K, Nieuwint AWM, van Bever Y: Recombination in a balanced complex translocation of a mother leading to a balanced reciprocal translocation in the child. Review of 60 cases of balanced complex translocations. *Hum Genet* 1997; **99**: 806–815.
9 Zahed L, Der Kaloustian V, Batanian JR: Familial complex chromosome rearrangement giving rise to balanced and unbalanced recombination products. *Am J Med Genet* 1998; **79**: 30–34.
10 Lichter P, Ried T: Molecular analysis of chromosome aberrations. In situ hybridization. *Methods Mol Biol* 1994; **29**: 449–478.
11 Schröck E, du Manoir S, Veldman T *et al*: Multicolor spectral karyotyping of human chromosomes. *Science* 1996; **273**: 494–497.
12 Ried T, Baldini A, Rand TC, Ward DC: Simultaneous visualization of seven different DNA probes using combinatorial fluorescence and digital imaging microscopy. *Proc Natl Acad Sci USA* 1992; **89**: 1388–1392.
13 Knutsen T, Veldman T, Padilla-Nash H, Schröck E, Liyanage M, Ried T: Spectral karyotyping: chromosomes in color. *Appl Cytogen* 1997; **23**: 26–32.
14 Garini Y, Macville M, du Manoir S *et al*: Spectral karyotyping. *Bioimaging* 1996; **4**: 65–72.
15 Garini Y, Gil A, Bar-Am I, Cabib D, Katzir N: Signal to noise analysis of multiple color fluorescence imaging microscopy. *Cytometry* 1999; **35**: 214–226.
16 ISCN: Mitelman F (ed). *An International System for Human Cytogenetic Nomenclature*. S Karger: Basel, 1995.
17 Schinzel A: Human cytogenetic database. Oxford University Press: Oxford, 1994.
18 Allderdice PW, Ali M, McAlpine PJ: Complementation by two nonhomologous recombinant chromosomes 3. *Am J Med Genet* 1991; **39**: 396–398.
19 Martini E, von Bergh ARM, Coonen E *et al*: Detection of structural abnormalities in spermatozoa of a translocation carier t(3; 11)(q27.3;q24.3) by triple FISH. *Hum Genet* 1988; **102**: 157–165.