

ORIGINAL INVESTIGATION

**Bassem R. Haddad · Evelin Schröck · Jeanne Meck
Janet Cowan · Hannah Young
Malcolm A. Ferguson-Smith · Stanislas du Manoir
Thomas Ried**

Identification of de novo chromosomal markers and derivatives by spectral karyotyping

Received: 2 June 1998 / Accepted: 16 June 1998

Abstract Despite major advances in molecular cytogenetics during the past decade and the important diagnostic role that fluorescence in situ hybridization (FISH) plays in the characterization of chromosomal abnormalities, the usefulness of this technique remains limited by the number of spectrally distinguishable fluorochromes or fluorochrome combinations. Overcoming this major limitation would allow one to use FISH to screen the whole human genome for chromosomal aberrations which, until recently, was possible only through conventional karyotyping. A recently described molecular cytogenetics technology, 24-color FISH using spectral karyotyping (SKY), permits the simultaneous visualization of all human chromosomes in 24 different colors. Most chromosomal aberrations detected during cytogenetic evaluation can be resolved using routine cytogenetic techniques alone or in combination with single- or dual-color FISH. However, some cases remain unresolved, in particular de novo supernumerary marker chromosomes and de novo unbalanced structural rearrangements. These findings cause particular diagnostic and counseling concerns when detected during prenatal diagnosis. The purpose of this report is to demonstrate the application of SKY in the characterization of these de novo structural chromosomal abnormalities. Eight cases are described in this report. SKY has considerable diagnostic applications in pre-

natal diagnosis because of its reliability and speed. The identification of the chromosomal origin of markers and unbalanced translocations provides the patient, physician, and genetic counselor with better predictive information on the phenotype of the carrier.

Introduction

Accurate identification of small de novo markers and structural rearrangements remains a challenge to the cytogenetic laboratory. The problem is accentuated when these findings are detected during prenatal cytogenetic evaluation due to time constraints and the limited amount of material available for analysis. We have recently reported the use of spectral karyotyping (SKY) to refine cytogenetic diagnosis of constitutional chromosomal abnormalities (Schröck et al. 1997). In the present study, we focus on the use of SKY in the characterization of several cases with marker chromosomes as well as a de novo unbalanced translocation. The present study confirms that this technique provides an accurate cytogenetic characterization of these chromosomal aberrations in a timely fashion and we propose that SKY is particularly useful when these aberrations are detected during the course of prenatal cytogenetic evaluation.

De novo marker chromosomes and structural rearrangements, detected during prenatal diagnosis, represent major diagnostic and counseling dilemmas. Marker chromosomes, also known as extra structurally abnormal chromosomes (ESACs), half of which are de novo in origin, are detected in up to 0.15% of prenatal cytogenetic investigations (Ferguson-Smith and Yates 1984; Hook and Cross 1987; Sachs et al. 1987; Warburton 1991). Structural rearrangements are detected in up to 0.1% of prenatal cases (Gardner and Sutherland 1996). The risk of congenital abnormalities in the familial cases of chromosomal markers is low or absent, but it may be as high as 13% in de novo cases (Warburton 1991). The risk of a serious congenital anomaly

B.R. Haddad (✉) · J. Meck · H. Young
Institute for Molecular and Human Genetics and
Department of Obstetrics and Gynecology, Georgetown University
Medical Center, M-4000, 3800 Reservoir Road NW,
Washington, DC 20007, USA
e-mail: haddadb1@gunet.georgetown.edu, Tel.: +1-202-7840759,
Fax: +1-202-7841770

E. Schröck · S. du Manoir · T. Ried
Genome Technology Branch, NHGRI/NIH,
Bethesda, Maryland, USA

J. Cowan
New England Medical Center, Tufts University,
Boston, Massachusetts, USA

M.A. Ferguson-Smith
Department of Pathology, Cambridge University, UK

ly is estimated to be 6.1% for de novo reciprocal translocation (Warburton 1991). Risk estimates are currently based on large population surveys, where the chromosomal findings have been characterized primarily by conventional cytogenetic methods such as G- and C-banding and NOR staining. While G-banding is a particularly useful procedure for an initial screening for chromosomal aberrations, because the entire genome can be evaluated in a single experiment, it is limited in its ability to clearly identify the origin of the markers and unbalanced de novo translocations. This results in the use of non-specific risk figures, which can create a great deal of anxiety and uncertainty on the part of the consultands (Blennow et al. 1994; Brondum-Nielsen and Mikkelsen 1995). The ability to accurately determine the genetic constitution associated with different markers and rearrangements will provide more precise prediction of the phenotype (Brondum-Nielsen and Mikkelsen 1995). In addition, in many cases, recurrent cytogenetic aberrations associated with a particular disease have been instrumental in the identification of a disease locus (Rowley 1973; Francke and Kung 1976; Greenstein et al. 1977), making it possible to use positional cloning (Collins 1995).

Molecular cytogenetic techniques, namely fluorescence in situ hybridization (FISH), have provided a valuable method to accurately characterize the majority of chromosomal aberrations, including many ESACs (Jauch et al. 1990; Trask 1991). Different types of probes are available for this use, providing a high degree of sensitivity and specificity. However, until recently, these probes have been used in a targeted approach where the choice of a test probe relied on previous knowledge of the chromosome abnormality to be characterized. Overcoming this major limitation allows the concomitant use of multiple probes to characterize chromosomal aberrations. This represents a major step towards providing an accurate diagnosis in a timely fashion, which is important in cytogenetic analysis in general and in prenatal diagnosis in particular. This goal was achieved recently when Speicher and colleagues (1996) and Schröck and colleagues (1996) described two different molecular cytogenetic approaches that allow the simultaneous visualization of the 24 different human chromosomes in unique colors.

Speicher et al. (1996) reported the simultaneous color differentiation of all human chromosomes using combinatorial labeling and sequential exposure through fluorochrome-specific filters. Schröck et al. (1996) developed an approach that is based on the measurement of the entire emission spectrum through a single, custom-designed optical filter by means of spectral imaging. Spectral imaging is based on a combination of fluorescence microscopy, Fourier spectroscopy, and CCD imaging. SKY refers to the application of spectral imaging to the differential color display of all human chromosomes and is based on the simultaneous hybridization of 24 chromosome-specific painting probes. Each probe is labeled with one or more fluorochromes, either singly or in combinations and, therefore, is characterized by a specific spectral signature. After hybridization, the emission spectra are measured for each image point. Based on the spectral information, a classification algorithm permits the identification of normal and aberrant

chromosomes. This spectral classification assigns a discrete color to all pixels with identical spectra.

Many chromosomal aberrations detected during prenatal diagnosis can be resolved using routine cytogenetic techniques, alone or in combination with single- or dual-color FISH. However, some cases remain unresolved, leaving patients with anxiety and uncertainty about the fetal phenotype. We have used SKY to characterize seven postnatal chromosomal markers of different sizes and origins. We then successfully applied this approach to characterize a prenatal case from amniotic fluid found to have extra material of unknown origin attached to the short arm of chromosome 21. The SKY results were confirmed in all cases using specific FISH probes. The purpose of this work is to demonstrate the application of SKY for the characterization of de novo rearrangements. This is particularly useful for prenatal cytogenetic evaluation since it provides a rapid and definitive identification of chromosomal aberrations in a single FISH experiment.

Materials and methods

A total of eight cases of de novo unbalanced structural chromosomal abnormalities were analyzed by SKY after ascertainment by G-band studies in two different laboratories (Georgetown University and Tufts University).

The clinical information, conventional cytogenetic findings and SKY results for each case are described in Table 1. Metaphase chromosome preparations and G-banding were performed by routine methods. Metaphase slides were freshly prepared for SKY using chromosome suspensions that were stored at -20°C in methanol/acetic acid (3:1) for up to 4 years. Cases 1–7 were from lymphocyte harvests; case 8 was an amniocyte culture. SKY was performed without prior knowledge of the clinical cytogenetic diagnosis based upon G-banding. A minimum of five metaphase spreads were examined from each case before a chromosome assignment was made for each marker.

Spectral karyotyping

Twenty-four human chromosome-specific DNA libraries were generated by bivariate, high-resolution flow sorting and amplified using degenerate oligonucleotide primed PCR (Telenius et al. 1992). DNA labeling was performed by directly incorporating haptenized or fluorochrome-conjugated dUTPs as described (Schröck et al. 1996). The differentially labeled probe sets were combined and precipitated in the presence of an excess of unlabeled human Cot-1 DNA (Bethesda Research Laboratories) and resuspended in 10 μl of hybridization buffer (50% formamide, 10% dextran sulfate, 2 \times SSC). The probe cocktail was denatured for 5 min at 80°C , and allowed to preanneal for 1 h at 37°C . Metaphase chromosomes were denatured separately at 80°C for 2 min in 70% formamide, 2 \times SSC and dehydrated through an ethanol series. The probe cocktail was applied to the slides and hybridized for 2 days at 37°C . Posthybridization washes were performed as follows: 3 \times 5 min in 50% formamide, 2 \times SSC at 45°C ; 3 \times 5 min in 1 \times SSC at 60°C ; 1 \times 30 min in blocking solution (4 \times SSC, 3% BSA) at 37°C . The biotinylated probe sequences were detected by incubation with avidin–Cy5 (Amersham Life Sciences) and the digoxigenin probe sequences were visualized using a mouse anti-digoxin antibody (Sigma Chemicals) followed by a goat anti-mouse antibody conjugated to Cy5.5 (Amersham Life Sciences). Slides were washed in 4 \times SSC, 0.1% Tween-20 at 42°C , counterstained with DAPI, and embedded in anti-fade (200 mM DABCO, 90% v/v glycerol, 20 mM TRIS-HCl, pH 8) to reduce photobleaching.

Spectral images were acquired with a SD200 SpectraCube system (Applied Spectral Imaging) coupled via a c-mount adapter to a Leica

Table 1 Clinical information, conventional cytogenetic findings, and determination of chromosome origin by spectral karyotyping (SKY) (ESAC extra structurally abnormal chromosome, FISH fluorescence in situ hybridization)

Case	Clinical presentation	Conventional cytogenetic findings	Chromosomal origin of ESAC or translocation by SKY	Confirmation by FISH
1	Significant psychomotor and growth retardation	Deleted acrocentric. C-banding: positive and negative regions. NOR: positive. Karyotype: 47,XX,+mar	Chromosome 15	Confirmation with chromosome 15 painting probe
2	Abnormal facies, hypoplastic nails, tetralogy of Fallot, VSD, bilateral cystic renal disease, seizures, poor growth	Small acrocentric marker. Karyotype: 47,XX,+mar	Chromosome 15	Confirmation with chromosome 15 painting probe
3	History not available	Small acrocentric marker. Karyotype: 47,XX,+mar	Chromosome 15	Confirmation with chromosome 15 painting probe
4	Significant developmental delays, autistic behavior, minor anomalies of hands and feet, normal motor development	Deleted acrocentric. C-banding: positive and negative regions. NOR: positive. Karyotype: 47,XY,+mar[13]/46,XY[7]	Chromosome 13	Confirmation with chromosome 13 painting probe
5	Normal at birth except for significant hyperpigmentation	Medium sized metacentric marker. Karyotype: 47,XX,+?i(9p)[8]/46,XX[27]	Chromosome 9	Confirmation with chromosome 9 painting probe
6	23-year-old male diagnosed with Klippel-Trenaunay-Weber syndrome	Small ring chromosome. Karyotype: 47,XY,+mar	Chromosome 18	Confirmation with chromosome 18 painting probe
7	Dysmorphic features, early onset scoliosis, developmental delay, microcephaly	Small non-mosaic marker (mar1) which appeared to consist only of satellites and a larger mosaic non-satellited marker (mar2). C-banding: mar1, inconclusive; mar2, negative. Karyotype: 48,XX,+mar1,+mar2[9]/47,XX,+mar1[11]	mar1: not identified by SKY mar2: chromosome 6	Confirmation with chromosome 6 painting probe
8	Amniocentesis for advanced maternal age	Extra material of unknown origin attached to the short arm of chromosome 21 in all cells examined. Karyotype: 46,XX,der(21)t(?:21)(?:p11.2)	Extra material on 21p originated from chromosome 4	Confirmation with chromosome 4 painting probe

DMIRBE microscope. The samples were illuminated with a xenon lamp (150 W, Optiquip). The use of a custom-designed triple band-pass filter (SKY, ver 3; Chroma Technology) permits the excitation of all dyes and the measurement of their emission spectra simultaneously, without the need for subsequent exposure through fluorochrome-specific optical filters. The emitted light was sent through a Sagnac interferometer where optical path differences were generated as a function of the emission wavelengths. The resulting interferograms were measured simultaneously for all image points (pixels) with a high-resolution cooled CCD camera. The spectrum was recovered by Fourier transformation as described (Malik et al. 1996). Spectra-based classification was obtained by using an algorithm that assigns a spectra-specific pseudocolor to all pixels in the image that have the same spectrum. This spectra-based classification permits the identification of normal and rearranged chromosomes. The algorithm, as well as details of the image acquisition procedure, are described elsewhere (Garini et al. 1996; Schröck et al. 1996). DAPI images for all metaphases were acquired and then electronically inverted to give a G-band like appearance in order to facilitate the assignment of chromosomal bands during SKY analysis.

FISH using chromosome-specific painting probes

To confirm the SKY results in each of the eight cases, standard FISH using chromosome-specific painting probes was performed in a clinical cytogenetic laboratory (at Georgetown University or Tufts University) approved by CLIA (Clinical Laboratory Improvement Amendments) using routine procedures.

Results

We conducted this study to evaluate the potential applications of SKY in prenatal cytogenetic studies for the characterization of de novo chromosomal abnormalities detected by conventional cytogenetic analysis.

All eight samples were originally analyzed by G-banding and some were also characterized by C-banding and/or silver staining (NOR) (Table 1). Results of the conventional cytogenetic evaluation were blinded to us prior to SKY evaluation. In each of the eight cases analyzed, the diagnosis obtained from the SKY experiment was confirmed by standard single-color FISH using chromosome-specific painting probes, demonstrating that the SKY result was accurate in all cases.

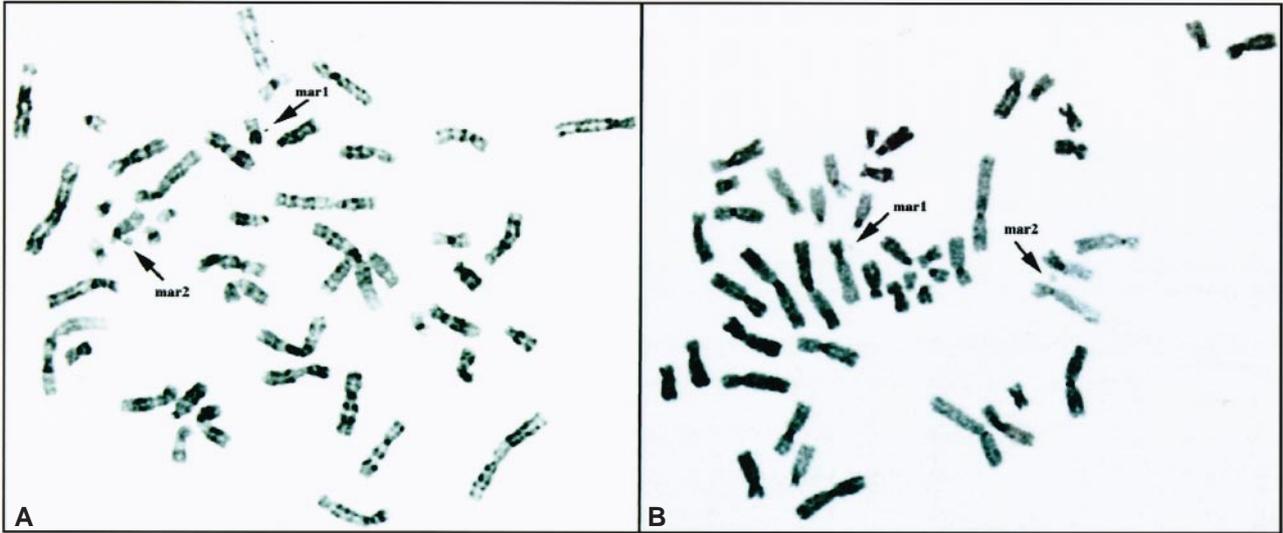
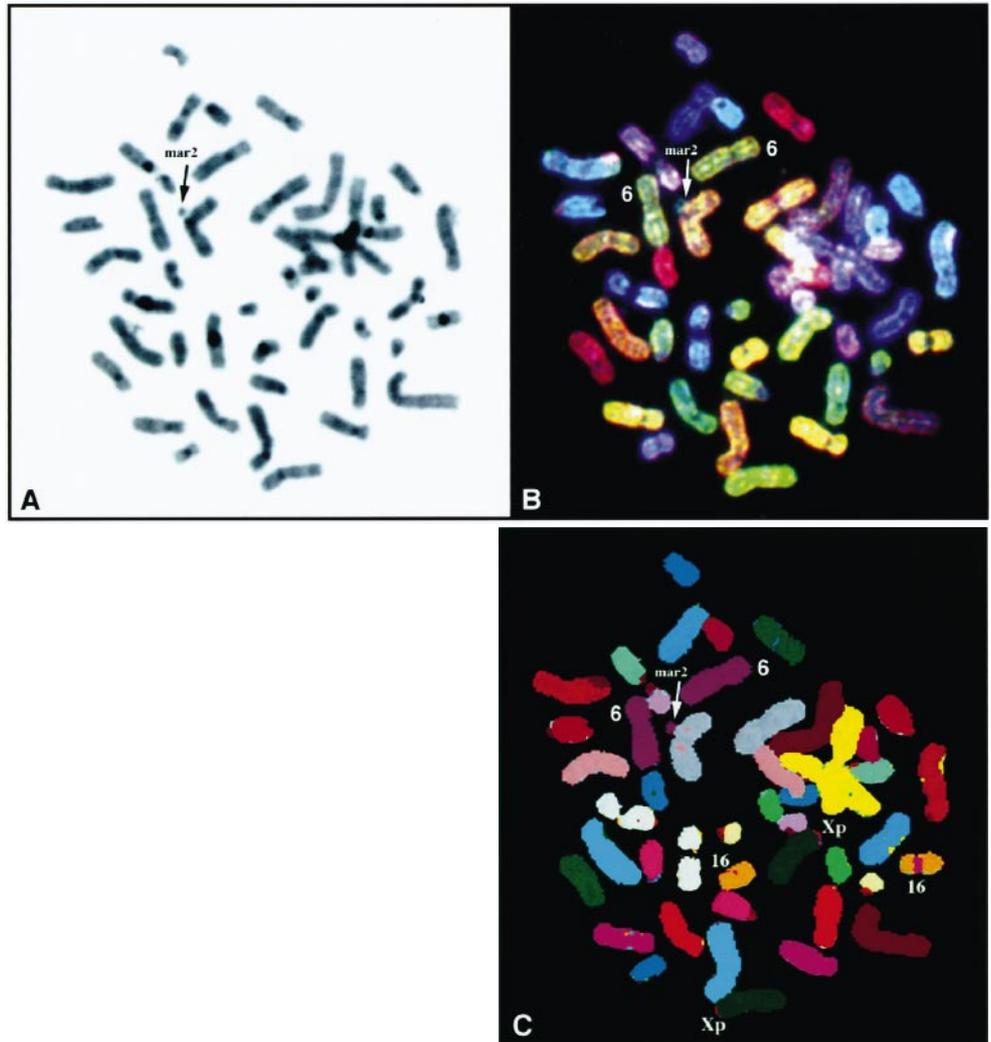


Fig. 1A,B Case 7. G-banding image of one of the metaphase spreads (A) and C-banding image of another metaphase spread (B), both showing the two markers (mar1 and mar2)

Fig. 2A–C Spectral karyotyping analysis of a metaphase spread from case 7. **A** Inverted DAPI image of one of the metaphase spreads analyzed. *Arrow* indicates mar2. mar1 was DAPI negative and therefore is not seen. (On G-banding, mar1 appeared to consist only of small satellites.) **B** Presentation of display colors. **C** Presentation of spectra-based classification colors. The marker chromosome (*arrow*) is shown to originate from chromosome 6. Note that the qh region on one of the chromosomes 16 shows non-specific hybridization. Note also that the pseudoautosomal region on both X chromosomes shows some cross-hybridization with Y chromosomal material



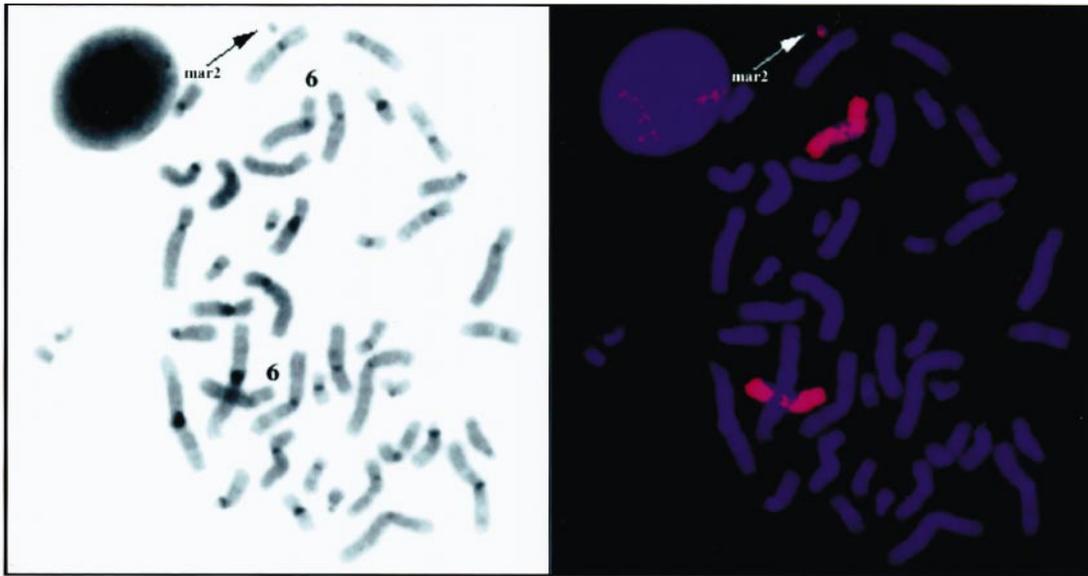


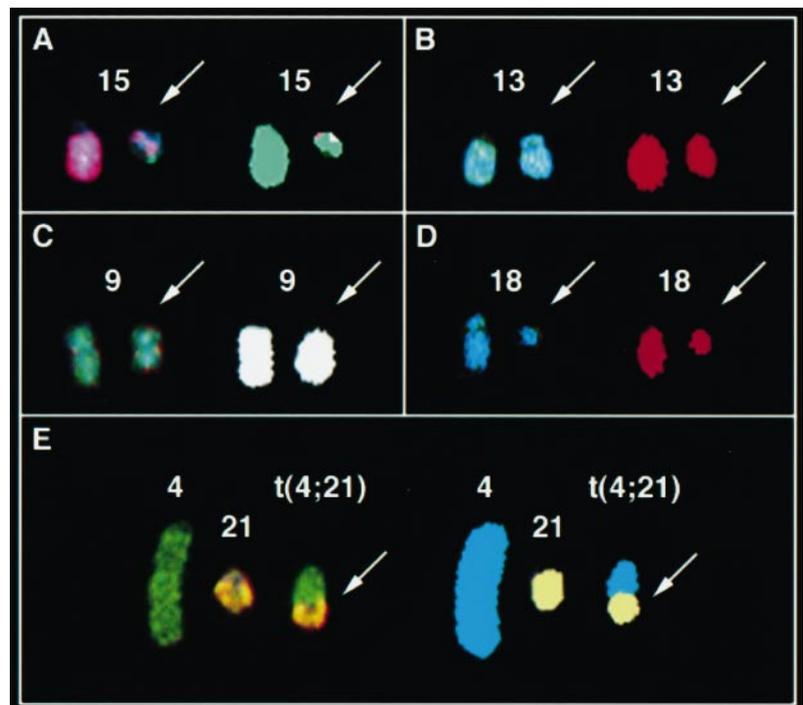
Fig. 3A,B Confirmation of the chromosomal origin of mar2 in case 7 using chromosome 6-specific painting probes. **A** Inverted DAPI image of one of the metaphase spreads analyzed. *Arrow* indicates mar2. **B** FISH image of the same metaphase using a chromosome 6-specific painting probe. mar2 shows hybridization with these probes, confirming its origin to be from chromosome 6 as detected by spectral karyotyping

In three cases (cases 1–3), the markers were found by SKY to originate from chromosome 15. Case 1 was referred for significant psychomotor and growth retardation. G-banding revealed a non-mosaic ESAC, 47,XX,+mar. On C-banding, the marker had positive and negative regions

and was NOR positive by silver stain. Case 2 was referred because of abnormal facies, hypoplastic nails, tetralogy of Fallot, VSD, bilateral cystic renal disease, seizures, and poor growth. G-banding revealed an ESAC in all cells (47,XX,+mar). The clinical history was not available on case 3 but G-banding revealed a 47,XX,+mar karyotype.

Case 4 was referred because of significant developmental delays, autistic behavior, minor anomalies of hands and feet, but otherwise normal motor development. G-banding showed the following mosaic karyotype: 47,XY,+mar[13]/46,XY[7]. The marker showed C-band negative and positive regions and was NOR positive by sil-

Fig. 4A–E Presentation of the chromosomal markers and derivatives (indicated by *arrows*) and their chromosomes of origin from cases 1 (**A**), 4 (**B**), 5 (**C**), 6 (**D**), and 8 (**E**). For each case, the chromosomes are shown in display colors on the left and in spectra-based classification colors on the right



ver staining. SKY revealed the marker to originate from chromosome 13.

Case 5 was normal at birth except for areas of significant hyperpigmentation. G-banding showed mosaicism for a medium sized metacentric marker suspected of being an isochromosome of the short arm of chromosome 9. The karyotype was 47,XX,+9p[8]/46,XX[27]. SKY revealed results consistent with the suspected G-band finding of the origin of the marker as derived from chromosome 9.

Case 6 was clinically diagnosed with Klippel-Trenaunay-Weber syndrome. G-banding revealed a ring chromosome in all cells (47,XY,+r). SKY showed that the ring chromosome originated from chromosome 18.

Case 7 was referred because of dysmorphic features, early onset scoliosis, developmental delay, and microcephaly. G-band analysis showed a small non-mosaic marker (mar1), which appeared to consist only of satellites, and a larger mosaic non-satellited marker (mar2). The karyotype was: 48,XX,+mar1,+mar2[9]/47,XX,+mar1[11] (Fig. 1A). On C-banding, mar1 showed inconclusive results while mar2 was C-banding negative (Fig. 1B). Mar1 was DAPI negative, which means that the marker did not contain the proximal short arm of chromosome 15 nor the heterochromatic regions of chromosomes 1, 9, 16 or Y. This DAPI finding was consistent with the G-banding impression that the marker consisted of satellites. SKY failed to determine the origin of the satellited marker (mar1) while the second marker (mar2) was found to originate from chromosome 6 (Fig. 2), a finding which was confirmed by chromosome 6 painting probes (Fig. 3).

In case 8, amniocentesis was performed because of advanced maternal age. G-band analysis showed extra material of unknown origin attached to the short arm of chromosome 21 in all cells examined. The karyotype was 46,XX,der(21)t(?;21)(?;p11.2). SKY revealed that the extra material on 21p originated from chromosome 4. A combination of the G-band findings and the FISH results indicated that the trisomic region was from the 4q31.3→q terminus.

Chromosomal markers and derivatives and their chromosomes of origin from cases 1, 4, 5, 6, and 8 are shown in Fig. 4.

Discussion

Conventional cytogenetic techniques using chromosome banding remain the standard method for chromosome evaluation. Karyotype analysis is the technique of choice because it allows the evaluation of the whole genome and screening for chromosomal aberrations in a single experiment. In some instances, however, chromosome banding patterns are difficult to interpret. This is especially true in cases with subtle chromosomal rearrangements such as cryptic translocations and small insertions, complex structural abnormalities, and small markers.

The use of molecular cytogenetic techniques (FISH) in clinical diagnostic laboratories has made it possible to accurately characterize many marker chromosomes

(Brondum-Nielsen and Mikkelsen 1995). Their origin can be determined through the use of single- or dual-color FISH using centromeric and/or whole chromosome paint probes (Callen et al. 1992; Brandt et al. 1993; Popp et al. 1993; Blennow et al. 1995; Wegner et al. 1996). Characterization of these markers involves testing a number of probes, either singly or two at a time, to determine the origin of each marker. The choice of probes is usually made according to their reported frequency in the literature or according to any G-banded patterns that may be present. While in many instances the origin of the marker is accurately determined, it is a time-consuming approach which may not lead to a diagnosis within the time limits imposed by the gestational age in a prenatal case. In addition, the lack of full characterization of complex markers prohibits their correlation with a specific phenotype, which causes many counseling difficulties in prenatal diagnosis (Blennow et al. 1994; Brondum-Nielsen and Mikkelsen 1995). While the value of standard FISH analysis lies mainly in the clarification or confirmation of previously identified chromosomal aberrations, it is not a suitable tool to screen for these aberrations. The recently developed SKY technology allows for the unique color display of all human chromosomes (Schröck et al. 1996, 1997; Veldman et al. 1997). The sensitivity of SKY with chromosome painting probes in detecting chromosomal aberrations was shown previously to be in the range of 1.5 Mb (Schröck et al. 1996).

In this study we describe the application of SKY for the characterization of structural chromosomal abnormalities that had been previously detected by conventional cytogenetic analysis. This is particularly important in prenatal cases where their identification is needed in a timely fashion. All the cases we evaluated in this study were successfully hybridized and all markers, with the exception of the one with the small satellited marker (case 7, mar1), were positively identified, thus demonstrating the application of this technology for the characterization of unknown marker chromosomes.

In case 5, SKY confirmed the suspected finding by G-banding (iso 9p). In the six other cases, however, SKY was instrumental in identifying the origin of the marker chromosome. In case 7, there were two markers: a small non-mosaic satellited marker (mar1) and a larger mosaic non-satellited one (mar2). Using SKY, mar2 was correctly identified; however, mar1 did not show a hybridization signal. By G-banding, mar1 appeared to consist solely of satellites. To date no abnormal phenotype has been reported in a case with a marker originating from satellite regions. Since these regions consist of highly repetitive DNA, which is suppressed by Cot-1 DNA during the SKY hybridization step, no information can be obtained from SKY. Therefore, SKY is helpful in cases where the genetic material consists partially or completely of euchromatin. A negative SKY result is also useful. If the marker is not euchromatic it is less likely to be clinically significant. Case 8 was a prenatal diagnosis for advanced maternal age which had a derivative chromosome 21 with extra material unidentified by G-banding alone. SKY was performed and yielded a result within 48 h of the time of detection of the abnormality by

G-banding. This result was confirmed using a chromosome 4 painting probe 24 h later. SKY was very useful for the rapid characterization of the G-band findings and the timely counseling of the couple.

As discussed earlier, many cytogenetic findings can be characterized by conventional methods alone or in combination with single- or two-color FISH. Spectral karyotyping is an important technique in that it can be used in reference cytogenetic laboratories for those cases that remain unresolved using standard approaches. This is particularly true for prenatal cases where the use of SKY permits full characterization of the chromosomal aberrations in a single FISH experiment, thus overcoming the delays created by sequential hybridizations and also overcoming the problems of the limited amounts of available material. In addition, this technology can lead to greater precision in karyotype-phenotype correlations, which would be beneficial for genetic counseling.

We conclude that an approach that uses conventional cytogenetic techniques to screen for prenatal chromosomal aberrations, coupled with hybridization-based color discrimination by SKY when indicated, will allow the reliable characterization of most aberrations involving segments larger than 1.5 Mb (Schröck et al. 1996).

Acknowledgements The authors wish to thank Drs Chahira Kozma, Mira Irons, and Ellen Elias for referring patients for cytogenetic evaluation.

References

- Blennow E, Bui TH, Kristoffersson U, Vujic M, Anneren G, Holmberg E, Nordenskjöld M (1994) Swedish survey on extra structurally abnormal chromosomes in 39 105 consecutive prenatal diagnoses: prevalence and characterization by fluorescence in situ hybridization. *Prenat Diagn* 14:1019–1028
- Blennow E, Brondum-Nielsen K, Telenius H, Carter N, Kristoffersson U, Holmberg E, Gillberg C, Nordenskjöld M (1995) Fifty probands with extra structurally abnormal chromosomes characterized by fluorescence in situ hybridization. *Am J Med Genet* 55:85–94
- Brandt CA, Hindkjaer J, Stromkjaer H, Pederson S, Sunde L, Kolvraa S (1993) Molecular cytogenetics: applications in clinical genetics. *Eur J Obstet Gynecol Reprod Biol* 50:235–242
- Brondum-Nielsen K, Mikkelsen M (1995) A 10-year survey, 1980–1990, of prenatally diagnosed small supernumerary marker chromosomes, identified by FISH analysis. Outcome and follow-up of 14 cases diagnosed in a series of 12 699 prenatal samples. *Prenat Diagn* 15:615–619
- Callen DF, Eyre H, Yip M-Y, Freemantle J, Haan EA (1992) Molecular cytogenetic and clinical studies of 42 patients with marker chromosomes. *Am J Med Genet* 43:709–715
- Collins FS (1995) Positional cloning moves from prediagnostic to traditional. *Nat Genet* 9:347–350
- Ferguson-Smith MA, Yates JRW (1984) Maternal age-specific rates for chromosome aberrations and factors influencing them: a report of a collective European study on 52 965 amniocenteses. *Prenat Diagn* 4:5–44
- Francke U, Kung F (1976) Sporadic bilateral retinoblastoma and 13q chromosomal deletion. *Med Pediatr Oncol* 2:379–385
- Gardner RJM, Sutherland GR (1996) Chromosome abnormalities and genetic counseling. Oxford University Press, Oxford, pp 206–208, 365–371
- Garini Y, Macville M, Manoir S du, Buckwald RA, Lavi M, Katzir N, Wine D, Bar-Am I, Schröck E, Cabib D, Ried T (1996) Spectral karyotyping. *Bioimaging* 4:65–72
- Greenstein RM, Reardon MP, Chan TS (1977) An X-autosomal translocation in a girl with Duchenne muscular dystrophy (DMD): evidence for DMD gene location. *Pediatr Res* 11:457
- Hook EB, Cross PK (1987) Extra structurally abnormal chromosomes (ESAC) detected at amniocentesis: frequency in approximately 75 000 prenatal cytogenetic diagnoses and association with maternal and paternal age. *Am J Hum Genet* 40:83–101
- Jauch A, Daumer C, Lichter P, Murken J, Schroeder-Kurth T, Cremer T (1990) Chromosomal in situ suppression hybridization of human gonosomes and autosomes and its use in clinical cytogenetics. *Hum Genet* 85:145–150
- Malik Z, Cabib D, Buckwald RA, Talmi A, Garini Y, Lipson SG (1996) Fourier transform multipixel spectroscopy for quantitative cytology. *J Microsc* 182:133–140
- Popp S, Jauch A, Schindler D, Speicher MR, Lengauer C, Donis-Keller H, Riethman HC, Cremer T (1993) A strategy for the characterization of minute chromosome rearrangements using multiple color fluorescence in situ hybridization with chromosome-specific DNA libraries and YAC clones. *Hum Genet* 92:527–532
- Rowley JD (1973) A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. *Nature* 243:290–293
- Sachs ES, Van Hemel JO, Den Hollander JC, Jahoda MGJ (1987) Marker chromosomes in a series of 10 000 prenatal diagnoses: cytogenetics and follow up studies. *Prenat Diagn* 7:81–89
- Schröck E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, Ning Y, Ledbetter DH, Bar-Am I, Soenksen D, Garini Y, Ried T (1996) Multicolor spectral karyotyping of human chromosomes. *Science* 273:494–497
- Schröck E, Veldman T, Padilla-Nash H, Ning Y, Spurbeck J, Jalal S, Shaffer LG, Papenhausen P, Kozma C, Phelan MC, Kjeldsen E, Scharberg SA, O'Brien P, Biesscker L, du Manoir S, Ried T (1997) Spectral karyotyping refines cytogenetic diagnostics of constitutional chromosomal abnormalities. *Hum Genet* 101:255–262
- Speicher MR, Ballard SG, Ward DC (1996) Karyotyping human chromosomes by combinatorial multi-fluor FISH. *Nat Genet* 12:368–375
- Telenius H, Pelmeur AH, Tunnacliffe A, Carter NP, Behmel A, Ferguson-Smith MA, Nordenskjöld M, Pfragner R, Ponder BAJ (1992) Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow-sorted chromosomes. *Genes Chromosomes Cancer* 4:257–263
- Trask BJ (1991) Fluorescence in situ hybridization: applications in cytogenetics and gene mapping. *Trends Genet* 7:149–154
- Veldman T, Vignon C, Schröck E, Rowley J, Ried T (1997) Hidden chromosome abnormalities in haematological malignancies detected by multicolour spectral karyotyping. *Nat Genet* 15:406–410
- Warburton D (1991) De novo balanced chromosome rearrangements and extra marker chromosomes identified at prenatal diagnosis: clinical significance and distribution of breakpoints. *Am J Hum Genet* 49:995–1013
- Wegner RD, Schröck E, Obladen M, Becker R, Stumm M, Sperling K (1996) Partial trisomy/monosomy 6q in fetal cells and CVS long-term culture not present in CVS short-term culture. *Prenat Diagn* 16:741–748