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Spectral karyotyping refines cytogenetic diagnostics of constitutional chromosomal abnormalities

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Abstract Karyotype analysis by chromosome banding is the standard method for identifying numerical and structural chromosomal aberrations in pre- and postnatal cytogenetics laboratories. However, the chromosomal origins of markers, subtle translocations, or complex chromosomal rearrangements are often difficult to identify with certainty. We have developed a novel karyotyping technique, termed spectral karyotyping (SKY), which is based on the simultaneous hybridization of 24 chromosome-specific painting probes labeled with different fluorochromes or fluorochrome combinations. The measurement of defined emission spectra by means of interferometer-based spectral imaging allows for the definitive discernment of all human chromosomes in different colors. Here, we report the comprehensive karyotype analysis of 16 samples from different cytogenetic laboratories by merging conventional cytogenetic methodology and spectral karyotyping. This approach could become a powerful tool for the cytogeneticists, because it results in a considerable improvement of karyotype analysis by identifying chromosomal aberrations not previously detected by G-banding alone. Advantages, limitations, and future directions of spectral karyotyping are discussed.

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Introduction

Since the introduction of chromosome banding in 1969 by Caspersson and Zech (Caspersson et al. 1969; Caspersson et al. 1970), karyotyping has become the standard diagnostic procedure for identifying chromosomal aberrations involved in human diseases. The search for constitutional chromosomal abnormalities in pre- and postnatal laboratories is of the utmost importance for precise diagnostics, risk assessment, and genetic counseling. In numerous examples, the identification of recurrent cytogenetic aberrations has also provided the first evidence for a disease locus (Rowley 1973; Francke and Kung 1976; Greenstein et al. 1977) and, hence, an entry point for positional cloning strategies (Collins 1995). G-banding is a particularly useful procedure for initial screening for chromosomal aberrations, because the entire genome can be evaluated in a single experiment. In some instances, however, chromosome-banding patterns are difficult to interpret. In particular, this shortcoming holds true in cases in which subtle chromosomal translocations, small marker chromosomes, or complex chromosomal aberrations confound a comprehensive karyotype analysis. Molecular cytogenetic techniques, e.g., fluorescence in situ hybridization (FISH),

have alleviated this problem to some degree (Jauch et al. 1990). Various probe sets can be used to confirm the presence of suspected chromosomal aberrations with a high degree of sensitivity and specificity (Le Beau 1993). Microdissection procedures and reverse chromosome painting have been successfully applied to analyze aberrant chromosomes and chromosomal regions (Lüdecke et al. 1989; Müller-Navia et al. 1996). However, a tailored analysis with region-specific probes covers only a fraction of the genome and, consequently, requires a previous notion of chromosome abnormalities. Cytogenetic analyses would therefore greatly benefit from hybridization-based screening approaches that combine the sensitivity and specificity of FISH with the potential to analyze all chromosomes simultaneously. This goal has been achieved recently (Speicher et al. 1996; Schröck et al. 1996). Speicher and colleagues have reported the simultaneous color differentiation of all human chromosomes by using combinatorial labeling and sequential exposure through fluorochrome-specific filters (Speicher et al. 1996). We have developed an approach that is based on the measurement of the entire emission spectrum through a single custom-designed optical filter (Schröck et al. 1996) by means of spectral imaging. Spectral imaging is based on a combination of fluorescence microscopy, Fourier spectroscopy, and charge-coupled device imaging (Malik et al. 1996). Spectral karyotyping (SKY) refers to the application of spectral imaging to the differential color display of all human chromosomes. SKY 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. In contrast to filter-based technologies, spectral imaging allows one to measure the whole spectrum of the emitted light in a single exposure of the entire image by generating an interferogram for each pixel that is specific for a certain fluorochrome or fluorochrome combination in the image (Garini et al. 1996). A spectral classification then assigns a discrete color to all pixels with identical spectra. The spectral classification is the basis for chromosome identification and SKY of human (Schröck et al. 1996) and mouse (Liyanage et al. 1996) chromosomes.

Table 1 Phenotype information, G-banded karyotypes, and results of SKY analysis of 16 clinical cases from seven different laboratories

Case no.	Cytogen. lab-	Indication for chromosome analysis	Karyotype based on G-banding	Karyotype based on SKY-analysis
LC1	LabCorp	Multiple miscarriages	45, XX,dic(13;15)(p12;p12)	45,XX,dic(13;15)(p12;p12)
EK1	Odense	Multiple miscarriages	46,XX,t(2;8;15)(q24.3;q23;q26.2)	46,XXt(2;8;15)(q24;q23;q26)
TE1	AML	Multiple miscarriages	46,XY,t(5;7)(q15;q11.23)ins(7;5)(q31.2 or q32;q13q15)	46,XY,t(5;7)(q15;q11.2)ins(7;5)(q22 or q31;q13q15)
LC2	LabCorp	Dysmorphic features, developmental delay	45,XY,der(7)t(7;15)(q32.2;q11.2),–15	45,XY,der(7)t(7;15)(q32;q11.2),–15
LC3	LabCorp	Mild dysmorphic features, moderate mental retardation	45,XY, dic(21;22)(p11;q13.3)	45,XY,der(22)t(21;22)(p11;q13.3)
LC4	LabCorp	Dysmorphic features, developmental delay	46,XY,t(5;7;9;18)	46,XY,t(5;9)(q22.1;p22),t(7;18)(p14;q21)inv?(7q)
CK1	GU	Dysmorphic features, developmental delay	46,XY	46,XY,der(18)(X;18)(?;q23)
MP1	GGC	Dysmorphic features, developmental delay	G-bands: 46,XY HRG: 46,XY,add(4)(p15)	46,XY,der(4)t(4;8)(p15;?)
MP2	GGC	Dysmorphic features, developmental delay	47,XY,+mar	47,XY, +der(8)
MP3	GGC	Dysmorphic features, more prominent on the right	47,XX,+ring[30]/46,XX[20]	47,XX,+r(4)[4]/46,XX[2]
JS1	Mayo Clinic	Dysmorphic features and developmental delay	46,XY,der(2)t(2;8)(q33;q24.1),add(3)(p21)der(8)t(2;8)(q33;q24.1)ins(8;9)(q23.2;q22.1q31),del(9)(q21.2q32)	46,XY,der(2)t(2;8)(q33;q24),der(8)t(2;8;9)(q33;q24;q31),del(9)(q?)
JS2	Mayo Clinic	Mother of a child with cognitive learning problems and mild dysmorphic features	46,XX,del(5)(p13.3p14.2) or p14.2p15.) or add(5)(p13.3)	46,XX
LS1	Baylor	Developmental delay	46,XY,add(14)(p13)	46,XY,der(14)t(5;14)
LS2	Baylor	Not available	46,X,der(Y)	46,X,der(Y)t(X;Y)(?;q11.2)
LS3	Baylor	Developmental delay	46,XY,t(7;21;22)(q21.2;q21;q13.3)	46,XY,t(7;21;22)(q21;q21;q13)
LS4	Baylor	Speech delay and hyperactivity	46,X,add(Y)(p11.3)	46,X,der(Y)t(Xor3;Y)(?;p11.3)

Here, we describe the application of SKY to identify chromosomal aberrations in 16 postnatal clinical samples. Special emphasis has been given (1) to exploring the routine applicability of SKY to metaphase preparations collected in different laboratories, and (2) to the methodological improvement that combines conventional cytogenetic analysis (G-banding) with SKY. In one case, an unambiguous chromosomal aberration was identified by SKY, whereas the G-banded karyotype was interpreted as being normal.

Materials and methods

Patient samples

Metaphase chromosomes were prepared, with informed consent, from peripheral blood lymphocytes taken from patients referred to clinical laboratories for karyotype analysis. Samples were coded to assure confidentiality, and no identifier occurred on the slides. The relevant laboratories, clinical information, and karyotypes are summarized in Table 1.

Metaphase chromosome preparations and G-banding analysis

Metaphase chromosome preparations and G-banding analyses were performed in all cases by using routine procedures in the respective laboratories. Specific protocols or variations can be provided upon request.

Spectral karyotyping

Twenty-four human-chromosome-specific DNA libraries were generated by bivariate high-resolution flow sorting and were amplified by using a degenerate oligonucleotide primed polymerase chain reaction (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 10μl hybridization buffer consisting of 50% formamide, 10% dextran sulfate, 2 × SSC (1 × SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0). The probe cocktail was denatured for 5 min at 80° C and was then allowed to preanneal for 1 h at 37° C. Metaphase chromosome slides were denatured separately at 75° C for 1.5–2 min in 70% formamide, 2 × SSC, and dehydrated through an ethanol series. When G-banding and SKY were combined on the same spreads, G-banded metaphases were imaged and the XY-coordinates recorded. Slides were destained in ethanol and processed as described. The probe cocktail was applied to the slides and hybridized for 2 days at 37° C. Posthybridization washes were performed as follows: 3 × 5 min in 50% formamide, 2 × SSC at 45° C; 3 × 5 min in 1 × SSC at 45° C; 1 × 30 min in blocking solution (4 × SSC, 3% bovine serum albumin) at 37° C. The biotinylated probe sequences were detected by incubation in avidin-Cy5 (Amersham Life Sciences), and the digoxigenin probe sequences were visualized by 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 × SSC Tween, dehydrated through an ethanol series, counterstained with DAPI (4,6-diamidino-2-phenylindole), and embedded in paraphenylendiamine (Sigma) to reduce photobleaching.

Spectral images were acquired with the SD200 Spectracube system (Applied Spectral Imaging) coupled via a c-mount adapter to a Leica DMRBE microscope. The samples were illuminated with a Xenon lamp (150 W, Optiquip). The use of a custom-designed triple-bandpass filter (SKY, v.3, Chroma Technology, Brattleboro, Vt.) permitted 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 an optical path difference as a function of the emission wavelength was generated simultaneously at all image points (pixels). The spectrum was recovered by Fourier transformation as described (Malik et al. 1996). The spectral information was visualized by assigning an RGB look-up table to produce discrete spectral ranges (display colors). The spectra-based classification was obtained by using an algorithm that assigns a spectra-specific pseudocolor to all pixels that have the same spectrum in the image. This spectra-based classification is the basis for SKY and definitive chromosome identification. The algorithm and details of the image acquisition procedure are described in Garini et al. (1996) and Schröck et al. (1996). The acquisition of DAPI images for all metaphases facilitated the assignment of chromosomal bands during SKY analysis. Some 5–10 metaphase spreads per case were examined.

Results

Here, we report the comprehensive cytogenetic analysis of 16 patient samples collected from seven different clinical cytogenetic laboratories using chromosome banding analysis and SKY. All samples were initially analyzed by G-banding; the karyotype interpretation is summarized in Table 1. SKY was performed without prior knowledge of the chromosome aberrations detected by G-banding. Results are presented in Figs. 1–3 and Table 1.

In 5 cases (TE1, LC1, LC2, EK1 and LS3), karyotype analysis by G-banding and SKY revealed concordant results (Table 1). One of these cases (TE1) is presented in Fig. 2A. The proband was referred for cytogenetic analysis after multiple miscarriages. SKY corroborated the translocation t(5;7) and an insertion of chromosome 5 material into the translocated section of chromosome 7.

In 9 cases (CK1, LC3, LC4, LS1, LS2, LS4, MP1, MP2, MP3), the cytogenetic interpretation was either specified, completed, or modified by SKY (e.g., Fig. 1). In case LS1, an abnormal chromosome 14, diagnosed as add(14)(p13) was shown to be a der(14)t(5;14). In case LS2, which had been diagnosed as 46,X,der(Y), the cytogenetic interpretation was refined to der(Y)t(X;Y). High resolution chromosome-banding analysis of case MP1 revealed an add(4)(p15), which was not detectable on metaphase chromosomes (Hannig et al. 1984). SKY, however, unambiguously identified chromosome 8 material translocated onto the terminal band of chromosome arm 4p at a 400-band resolution. The phenotype of the patient was similar to Wolf-Hirschhorn-syndrome. The small marker chromosome in case MP2 was readily identified by SKY as being derived from chromosome 8. An as yet unidentified ring chromosome contained chromosome 4 material (MP3). In this case, mosaicism was detected by both SKY and G-banding. This observation could explain the unilateral predominance of dysmorphic features in the patient. In case LC4, a translocation involving four chromosomes, which could only be described as t(5;7;9;18) after G-banding, was shown to comprise reciprocal translocations t(5;9) and t(7;18). The different size and the ab-

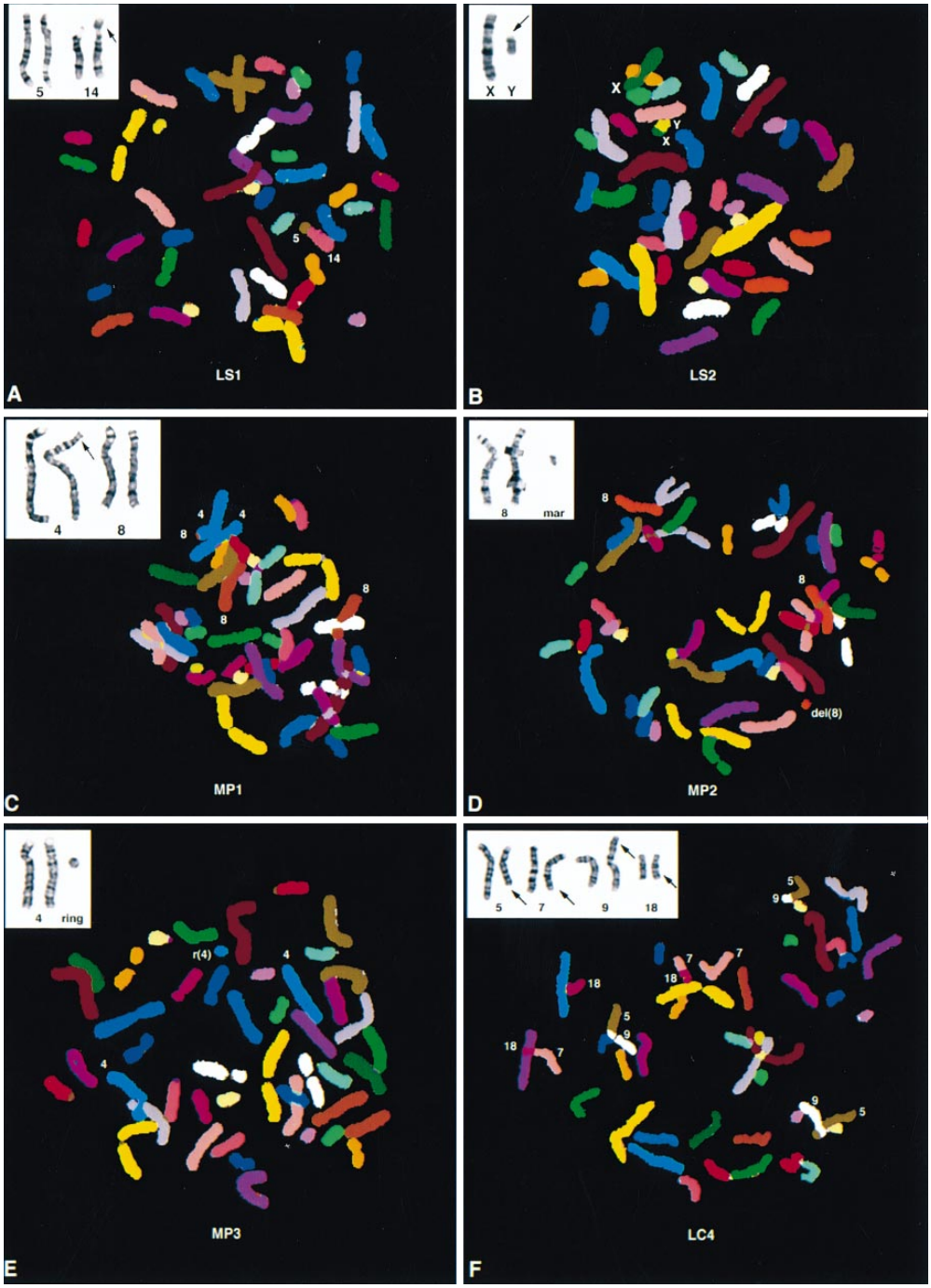

Fig. 1 A–F SKY of 6 clinical cases previously analyzed by G-banding. The chromosomal origin of additional material (**A–C**), a marker (**D**) and a ring (**E**) chromosome could be identified unambiguously. A complex rearrangement involving chromosomes 5, 7, 9, and 18 (**F**) could be clarified as t(5;9),t(7;18)inv(7). The G-banded metaphase chromosomes with questionable interpretation (*arrows*) and their normal homologs are shown as *insets* and the spectrally karyotyped metaphases are displayed. Note that, in all instances, the origin of additional or rearranged chromosomal segments could be identified (compare Table 1)

normal DAPI-banding pattern of the long arm of chromosome 7 involved in the translocation suggests an inversion in this chromosomal region. Figure 1 also displays the G-banding pattern of the involved chromosomes for comparison. In most cases, SKY results were confirmed independently by using conventional dual color FISH experiments with chromosome painting probes (data not shown) and chromosome-specific telomeric probes (Ning et al. 1996).

In case LS4, we had been unable to assign the origin of a small additional band on the short arm of chromosome Y to a single chromosome. The additional material, however, was classified by SKY as either chromosome 3 or X (Table 1). Conventional multicolor FISH was performed based on this suggestion and revealed the X-chromosomal origin of the additional material. The karyotype was therefore refined to 46,X,der (Y)t(X;Y).

In two instances (cases JS1 and JS2), G-banding revealed a subtle change of the banding pattern that could not be resolved by SKY (Fig. 2B, C). In case JS1, complex rearrangements involving chromosomes 2, 8 and 9 were detected by G-banding and SKY. In addition,

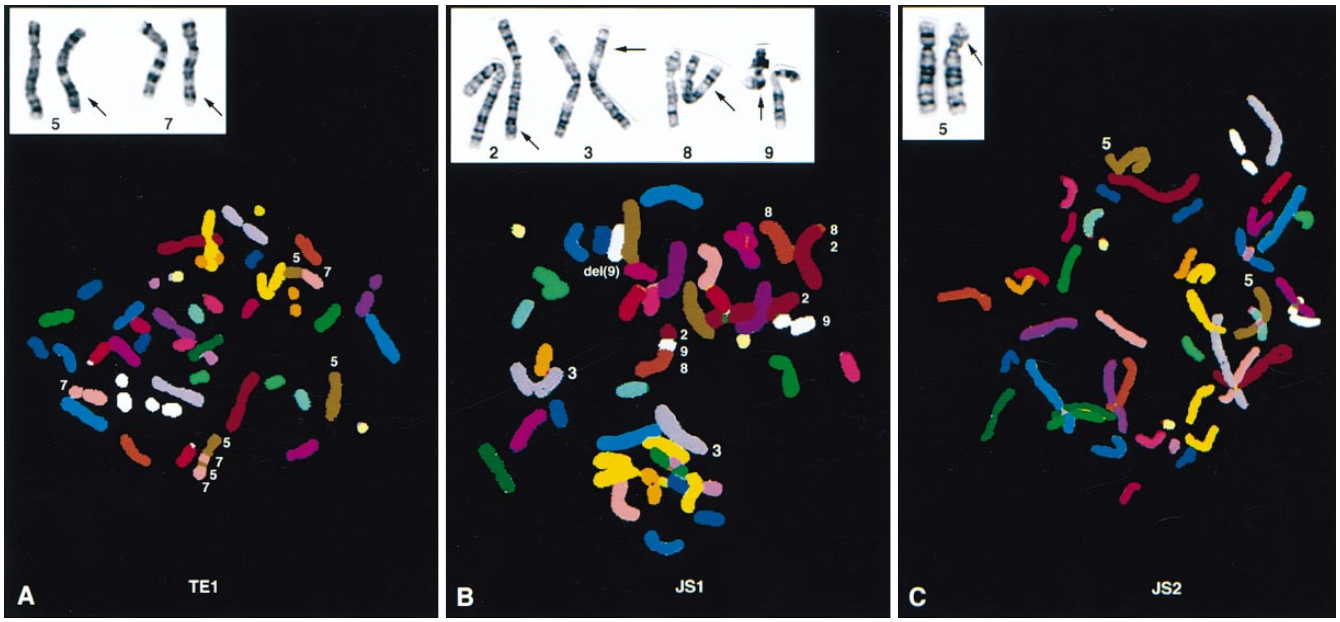

Fig. 2 A–C G-banding analyses and SKY of 3 cases demonstrating subtle chromosomal aberrations. In one case (**A**), a small insertion of chromosome 5 material was detected by G-banding (*arrows*) and SKY, in addition to the translocation t(5;7). In case JS1 (**B**), a slight shift in banding pattern on chromosome 3p was observed by karyotype analysis (*arrows*). SKY, however, showed two normal chromosomes 3. The complex rearrangement involving chromosomes 2, 8, and 9 was detected by both methods. Subsequently, FISH with a cosmid probe for the c-myc oncogene revealed a small insertion on chromosome 3p. A slightly aberrant banding pattern was detected in case JS2 (**C**) on the short arm of one chromosome 5 (*arrow*). This aberration was suspected to be a small interstitial deletion or a translocation. SKY detected two normal chromosomes 5

slightly different banding pattern was observed on the short arm of chromosome 3 by G-banding analysis. This chromosome was classified as a normal chromosome 3 using SKY (Fig. 2B). However, FISH with a probe for the c-myc oncogene detected a subtle insertion of chromosome 8 material into chromosome arm 3p. In case JS2, a different G-banding pattern on the short arm of chromosome 5 suggested a small interstitial deletion on the short arm of chromosome 5; this was not visible by SKY (Fig. 2C).

In selected cases, previously G-banded slides were destained and subsequently analyzed by SKY. Figure 3 shows, as an example, the combined cytogenetic results for case CK1. The G-banded metaphase spread (arranged in Fig. 3A) was destained and hybridized with the SKY-probe cocktail, containing all 24 human chromosomes labeled with various fluorochromes (Fig. 3B). The G-banded spread was karyotyped as 46,XY, but SKY revealed a translocation involving chromosomes X and 18. This translocation was confirmed by FISH with telomeric probes for chromosomes Xq and 18q (Ning et al. 1996).

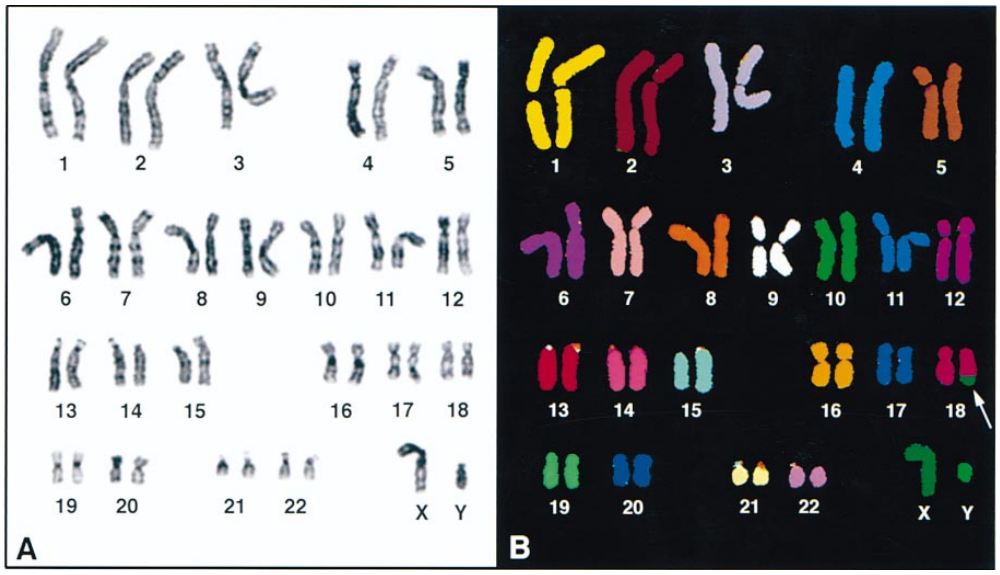

Fig. 3A, B Comprehensive cytogenetic analysis of a metaphase spread from a child (CK1) with dysmorphic features and developmental delay resembling an 18q- syndrome. **A** G-banded metaphase chromosomes. The karyotype interpretation is 46,XY (normal male karyotype). **B** SKY was performed on the same metaphase spread as in **A** after destaining, hybridization, and relocation. The multicolor hybridization clearly reveals an aberrant chromosome (*arrow*) that contains chromosomes 18 (*red*) and X (*dark green*) material. The G-banding interpretation of a normal male karyotype (46,XY) was therefore corrected after SKY to 46,XY, der(18)t(X;18)(?;q23)

The correct karyotype was therefore 46,XY,der(18)t(X;18)(q28;q23). This case was of special interest, because the G-banding analysis, displaying a normal karyotype, was performed twice, viz., the first time during pregnancy, because of abnormal maternal alphafetoprotein values, and the second time, after birth. The child was afflicted with developmental delay, microcephaly, hypogonadism, and other unspecific physical abnormalities. Retrospectively, however, the phenotype of the patient is comparable to an 18q-syndrome. Our data taken together therefore suggest that the combination of conventional G-banding and SKY provides a refinement of clinical cytogenetic diagnoses.

Discussion

The standard of care in the cytogenetic diagnostic laboratory currently relies on chromosome-banding analysis. Karyotype analysis serves as a screening test for aberrations where previous knowledge of the chromosomal rearrangements is not required. Karyotyping has its limitations with regards to specific aberrations. For example, the interchange of small chromatin parts, particularly telomeric chromatin (i.e., cryptic translocations and insertions), poses a severe problem, because the exchanged chromatin may have similar banding patterns, thus precluding the visualization of such aberrations by conventional chromosome-banding procedures (Ledbetter 1992). Secondly, the characterization of complex chromosomal aberrations is often difficult, as the origin of translocated chromosomal bands cannot be established unambiguously. Thirdly, the long perceived goal of a full and reliable automation of karyotype analysis has not been reached to date (Lundsteen and Piper 1989).

Whereas FISH with chromosome painting probes, centromere-specific probes, or regional or gene-specific probes has greatly assisted the identification of known chromosomal aberrations, such as the Down syndrome (e.g., Lichter et al. 1988), single gene deletions (e.g., Ried et al. 1990), or tumor-specific chromosomal translocations (Tkachuk et al. 1990; Arnoldus et al. 1990; Ried et al. 1992), FISH is not a suitable experimental approach for screening for chromosomal aberrations. Its value lies mainly in the confirmation or clarification of previously identified rearrangements (e.g., Spikes et al. 1995). In order to overcome these limitations of FISH analysis, we have recently developed a hybridization-based karyotyping approach that allows one to color-display all human chromosomes differentially (Schröck et al. 1996). Color distinction is based on spectral imaging, a methodology that enables the measurement of discrete emission spectra, with high spectral resolution, simultaneously at all image points of a sample (Garini et al. 1996). The feasibility of using SKY to discern all human and mouse chromosomes has been reported (Schröck et al. 1996; Liyanage et al. 1996), and its application to the analysis of hidden chromosomal aberrations in hematological malignancies has been demonstrated (Veldman et al. 1997). In previous experiments, we have shown that the sensitivity of SKY in detecting interchromosomal changes is in the range of 1.5 Mb (Schröck et al. 1996).

Here, we explore the value of SKY in improving cytogenetic diagnosis of constitutional chromosome abnormalities. In order to address whether SKY is broadly applicable to complement G-banding analysis, we have purposely chosen to analyze cases that were made available to us from seven cytogenetic laboratories throughout the country. All cases could be hybridized and analyzed successfully, thus demonstrating the robustness of SKY and the possibility of transferring SKY to clinical laboratories to complement routine cytogenetic diagnostics.

Readily identified chromosomal aberrations, in 5 cases previously diagnosed by G-banding analyses, were confirmed by SKY. In 9 cases, however, the karyotype interpretation was modified and improved after SKY, and the nature of the chromosomal aberrations determined more precisely. Notably, one case (CK1) diagnosed as normal by G-banding (46,XY), was shown to be a der(18)t(X;18). Pre- and postnatal G-banding analysis had failed to identify the aberration, because the exchange of chromosomal material did not result in a different banding pattern. Retrospectively, the phenotype of the child was comparable with an 18q- syndrome and was thus in accordance with the SKY data. The chromosomal aberrations detected by SKY were confirmed in most of the cases by conventional dual color FISH with chromosome painting probes (data not shown) or by using specific telomeric probes (Ning et al. 1996). In one of these cases, SKY could only suggest the involvement of either chromosome 3 or chromosome X in an aberration resulting in a small additional band on the short arm of chromosome Y (LS4). Based on this assumption, the origin of this additional material from chromosome X could be determined by conventional FISH analysis. SKY analysis was therefore useful in decreasing the number of possibly involved chromosomes from 24 to 2.

In 2 cases (JS1 and JS2) where G-banding results were suggestive of subtle rearrangements, SKY could not identify any material from other chromosomes involved. Regarding case JS1, however, the metaphase chromosomes available for SKY analysis were relatively condensed, thus limiting the resolution. SKY and G-banding analysis detected complex rearrangements involving chromosomes 2, 8, and 9. In addition, G-banding detected a subtle difference in the banding pattern on chromosome arm 3p; this was not visible with SKY. Dual color FISH experiments, previously performed with chromosome painting probes for chromosomes 2, 8, and 9 to confirm the complex rearrangements found by G-banding, showed a signal that was derived from chromosome 8 on chromosome arm 3p, but the signal was barely above background. Since the breakpoint on the aberrant chromosome 8 was mapped to chromosome band 8q24.1 (the position of the c-myc oncogene), FISH was repeated with a single-copy probe for the c-myc oncogene and revealed a subtle insertion of chromosome 8 material into chromosome arm 3p. We conclude that the resolution for detecting inser-

tions when comparing conventional FISH with SKY by using painting probes is of the same order of magnitude and depends mainly on the degree of chromatin condensation. With respect to case JS2, the cytogenetically visible aberration was interpreted as an interstitial deletion or translocation on chromosome arm 5p. This anomaly was detected by G-banding in a normal female proband (case JS2) and her son showing mild dysmorphic features (not analyzed). A subtle deletion is not expected to be detectable by means of painting probes with SKY or conventional FISH. It should be emphasized that the sensitivity is not limited by spectral imaging, but, as in any other FISH experiment, by the nature of the probe itself. The probes of choice for the detection of submicroscopic intrachromosomal or telomeric deletions must therefore be well-defined locus-specific probes.

We have shown that SKY has a definite value in identifying hidden chromosomal aberrations and in refining karyotype interpretation. It is evident from the results of this study that SKY with chromosome painting probes will complement, but not replace, chromosome-banding-based cytogenetic diagnostics. The detection of intrachromosomal rearrangements, such as deletions, duplications, and inversions, requires simultaneous banding analysis, and the identification of intrachromosomal or telomeric deletions will greatly benefit from the combination of specific probe sets for commonly deleted regions and chromosome-specific subtelomeric sequences (Ning et al. 1996).

We conclude that an approach that combines conventional chromosomal banding analysis, i.e., G-banding, with the hybridization-based color discrimination by SKY provides the most comprehensive cytogenetic diagnostic approach. Ideally, such a combination should be performed on the same metaphase spread in order to exclude the possibility of non-clonal abnormalities, thus preventing their detection. The technical feasibility of such an approach has been demonstrated here in case CK1 (Fig. 3), which involves the translocation der(18) t(X;18). G-banding and SKY analysis, in combination, greatly benefit from microscope hardware and software that provides the automated relocation of G-banded metaphase spreads after hybridization. The combination of G-banding and SKY, together with the interpretation of the respective results, should enable the automated karyotype analysis of the many normal cases referred to pre- and postnatal diagnostic laboratories. SKY will also refine breakpoint determination, because the identification of translocated chromosomal bands will be facilitated substantially if the origin of the aberrant chromatin can be identified.

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References

Arnoldus EPJ, Wiegant J, Noordermeer IA, Wessels JW, Beverstock GC, Grosveld GC, Ploeg M van der, Raap AK (1990) Detection of the Philadelphia chromosome in interphase nuclei. Cytogenet Cell Genet 54:108–111

Caspersson T, Zech L, Modest EJ, Foley GE, Wagh U (1969) Chemical differentiation with fluorescent alkylating agents in *Vicia faba* metaphase chromosomes. Exp Cell Res 58:128–140

Caspersson T, Zech L, Johansson C (1970) Differential banding of alkylating fluorochromes in human chromosomes. Exp Cell Res 60:315–319

Collins FS (1995) Positional cloning moves from perditional to traditional. Nature Genet 9:347–350

Francke U, Kung F (1976) Sporadic bilateral retinoblastoma and 13q- chromosomal deletion. Med Pediatr Oncol 2:379–385

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

Hannig V, Schroer RJ, Martens P, Phelan MC (1984) Chromosome 4p deletion with substitution of unknown chromosomal segment and clinical signs of Wolf syndrome. Proc Greenwood Genet Center 3:19–21

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

Le Beau MM (1993) Detecting genetic changes in human tumor cells: have scientists "gone fishing?" Blood 81:1979–1983

Ledbetter DH (1992) Cryptic translocations and telomere integrity. Am J Hum Genet 51:451–456

Lichter P, Cremer T, Tang C-JC, Watkins P, Manuelidis L, Ward DC (1988) Rapid detection of human chromosome 21 aberrations by in situ hybridization. Proc Natl Acad Sci USA 85: 9664–9668

Liyanage M, Coleman A, du Manoir S, Veldman T, McCormack S, Dickson RB, Barlow C, Wynshaw-Boris A, Janz S, Wienberg J, Ferguson-Smith MA, Schröck E, Ried T (1996) Multicolour spectral karyotyping of mouse chromosomes. Nat Genet 14:312–315

Lüdecke HJ, Senger G, Claussen U, Horsthemke B (1989) Cloning defined regions of the human genome by microdissection of banded chromosomes and enzymatic amplification. Nature 23: 348–350

Lundsteen C, Piper J (1989) Automation of cytogenetics. Springer, Berlin Heidelberg New York

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

Müller-Navia J, Nebel A, Oehler D, Theile U, Zabel B, Schleiermacher E (1996) Microdissection and DOP-PCR-based reverse chromosome painting as a fast and reliable strategy in the analysis of various structural chromosome abnormalities. Prenat Diagn 16:915–922

Ning Y, Roschke A, Smith ACM, Macha M, Precht K, Riethman H, Ledbetter D (Group 1), Flint J, Horsley S, Regan R, Kearney L, Knight S, Kvaloy K, Brown WRA (Group 2) (1996) A complete set of human telomeric probes and their clinical application. Nat Genet 14:86–89

Ried T, Mahler V, Vogt P, Blonden L, Ommen GJB van, Cremer T, Cremer M (1990) Direct carrier detection by in situ suppression hybridization with cosmid clones for the Duchenne/Becker muscular dystrophy locus. Hum Genet 85:581–586

Ried T, Lengauer C, Cremer T, Wiegant J, Raap AK, Ploeg M van der, Groitl P, Lipp M (1992) Specific metaphase and interphase detection of the breakpoint region in 8q24 of Burkitt lymphoma cells by triple-color fluorescence in situ hybridization. Genes Chromosom Cancer 4:69–74

Rowley JD (1973) A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. Nature 243:290–293

Schröck E, Manoir S du, 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

Speicher MR, Ballard SG, Ward DC (1996) Karyotyping human chromosomes by combinatorial multi-fluor FISH. Nat Genet 12:368–375

Spikes A, Hegmann K, Smith JL, Shaffer L (1995) Use of fluorescence in situ hybridization to clarify a complex chromosomal rearrangement in a child with multiple congenital anomalies. Am J Med Genet 57:31–34

Telenius H, Pelear 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 Chromosom Cancer 4:257–263

Tkachuk DC, Westbrook CA, Andreeff M, Donlon TA, Cleary ML, Suryanarayan K, Homge M, Redner A, Gray J, Pinkel D (1990) Detection of bcr-abl fusion in chronic myelogeneous leukemia by in situ hybridization. Science 250:559–562

Veldman T, Vignon C, Schröck E, Rowley JD, Ried T (1997) Hidden chromosome abnormalities in haematological malignancies detected by multicolour spectral karyotyping. Nat Genet 15:406–410