Detecting Rearrangements in Children Using Subtelomeric FISH and SKY

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The etiology of mental retardation (MR), often presenting as developmental delay in childhood, is unknown in approximately one-half of cases. G-banding is the standard method for investigating those suspected of having a chromosomal etiology; however, detection of structural abnormalities is limited by the size and pattern of the G-bands involved. Rearrangements involving subtelomeric regions have been shown to cause MR and this has generated interest in investigating the prevalence of these rearrangements using telomere-specific probes. In addition, because cryptic interchromosomal rearrangements may not be small or confined to chromosomal ends, spectral karyotyping (SKY) using chromosome-specific painting probes may be of value. We report here a study using these two FISH-based techniques in 50 children with idiopathic MR or developmental delay and normal GTG-banded karyotypes. Our objective was to assess the prevalence of cryptic rearrangements in this population using subtelomeric FISH and SKY. Three rearrangements were detected by subtelomeric FISH: a derivative 5 from a maternal t(5;21); a recombinant 11 from a paternal pericentric inversion; and a 2q deletion that was also present in the mother. Only the derivative 5 was detected by SKY. SKY did not detect any interstitial interchromosomal rearrangement. The prevalence of clinically significant cryptic rearrangements by subtelomeric FISH and SKY was thus 4% (95% confidence interval 0.5–13.7) and 2% (95% CI 0.05–10.7), respectively. This study supports the view that G-banding does not detect all clinically significant chromosomal abnormalities and that subtelomeric FISH and SKY can detect some of these abnormalities. © 2001 Wiley-Liss, Inc.

KEY WORDS: cryptic rearrangements; subtelomeric FISH; spectral karyotyping

INTRODUCTION

Mental retardation (MR) affects approximately 1–2% of the general population [reviewed by Curry et al., 1997]. In infancy and early childhood, mental retardation often presents as developmental delay [Aicardi, 1998]. The etiology of intellectual and developmental impairments is unknown in ~30–50% of cases [reviewed by McLaren and Bryson, 1987] and failure to obtain a specific diagnosis limits the effectiveness of counseling about recurrence risk, prognosis, and treatment [Curry et al., 1997; Hunter, 2000].

Chromosomal abnormalities account for up to ~40% of severe and less than 10% of mild cases of MR [reviewed by Raynham et al., 1996; Curry et al., 1997]. Cytogenetic analysis by G-banding is the standard method for investigating chromosomal abnormalities; however, this technique cannot easily detect structural abnormalities that are small (< 4 Mb), within G-negative bands, and/or involve exchanges of segments with similar G-banded patterns.

The subtelomeric ends of chromosomes are considered to be relatively gene-rich [Craig and Bickmore, 1994; Saccone et al., 1992] and rearrangements involving these regions have been shown to cause MR in recognizable syndromes: e.g., α-thalassemia/MR syndrome and Miller-Dieker syndrome [Lamb et al.,

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These findings have generated interest in investigating the implications and prevalence of cryptic subtelomeric rearrangements [Ledbetter, 1992; Flint et al., 1995]. Telomere-specific fluorescence in situ hybridization (FISH) probes have been made available for clinical use [National Institutes of Health and Institute of Molecular Medicine Collaboration, 1996; Knight et al., 2000] and a device that allows the simultaneous hybridization of all telomeric probes on one slide has been developed [Knight et al., 1997]. A 7.4% prevalence of subtle chromosomal abnormalities in children with moderate to severe MR (n = 284) and a 0.5% prevalence in children with mild MR (n = 182) has been reported [Knight et al., 1997, 1999]. Subtelomeric probes and their use in clinical diagnosis has recently been reviewed [Knight and Flint, 2000].

Cryptic interchromosomal rearrangements are not necessarily small and are not confined to chromosomal ends. The prevalence of interchromosomal insertions reported in the literature are very low [van Hemel and Eussen, 2000]; however, they are encountered in a clinical cytogenetics laboratory and the frequency may be dependent on the patient population being tested and the G-band level used. Moreover, the frequency of cryptic interchromosomal insertions is not known because cytogenetic techniques amenable for their detection have not been available until recently. Spectral karyotyping (SKY) is a multicolor cytogenetic technique that uses specific combinations of fluorochromes to generate chromosome-specific painting probes for use in one hybridization step [Schro¨ck et al., 1996]. Visualization is by an interferometer that measures the spectrum of each pixel in the field of the hybridized metaphase chromosomes. Interchromosomal insertions are more readily detected by SKY than by G-banding.

We report here a study using these two FISH-based techniques in 50 children with idiopathic MR or developmental delay, one or more additional criteria, and a normal GTG-banded karyotype. Our objective was to assess the prevalence of cryptic rearrangements in this population using subtelomeric FISH and SKY.

MATERIALS AND METHODS

Patients

Infants, children, and adolescents under age 19 years with idiopathic MR (IQ < 70) or developmental delay of unknown origin and a normal GTG-banded karyotype (550-band level) were recruited by clinical geneticists at the Hospital for Sick Children in Toronto, Canada. Band level was assessed according to Josifek et al. [1991]. Additional inclusion criteria consisted of one or more of the following: abnormal growth (< 3rd or > 97th centile), microcephaly or macrocephaly, unusual craniofacial appearance, multiple congenital anomalies, or a suggestive family history (miscarriages, perinatal death, or the above-mentioned clinical criteria).

Clinical geneticists and genetic counselors explained the study protocol to parents and received written consent to include their child in the study. A clinical checklist was completed for each participant based on clinical assessments and family history to include: severity of MR and developmental delay (assessed by a clinical geneticist, a developmental pediatrician, or psychologist); height, weight, and occipitofrontal circumference (OFC); dysmorphic features; congenital anomalies; birth history; and family history. A 3 mL peripheral blood specimen in sodium heparin was obtained from each patient. Blood specimens from parents of probands with rearrangements were also obtained to determine whether the translocation was inherited or had occurred de novo. All study procedures were approved by the Research Ethics Board of the Hospital for Sick Children.

Procedures

Fixed chromosome suspensions were prepared from peripheral blood specimens according to standard procedures.

Simultaneous FISH for all 41 telomeric regions was performed using the Chromoprobe Multiprobe®-T device according to the manufacturer’s specifications (Cytocell, Oxford, UK). Hybridized metaphase spreads were analyzed using a Zeiss Axioplan 2 epifluorescence microscope equipped with filters for separate detection of DAPI, FITC, and Texas Red fluorescent signals and a triple bandpass filter for simultaneous detection of signals. The p-arm probes had been labeled with FITC and the q-arm probes with Texas Red. The majority of these probes are < 330 kb from the telomere. Chromosomes were counterstained with DAPI. Images were captured by a CCD camera (Sensys, Roper Scientific, Tucson, AZ) and analyzed using an imaging system with MacProbe software v. 4.1 (Applied Imaging, Santa Clara, CA). The presence or absence of p-arm and q-arm signals for each chromosome was recorded for at least three metaphases from each participant.

SKY was performed using 24-color SkyPaintTM probes according to the manufacturer’s specifications (Applied Spectral Imaging, Carlsbad, CA). Hybridized metaphase spreads were viewed with a Zeiss epifluorescence microscope and spectral images were acquired with an SD200 SpectraCube system and analyzed using SkyView software 1.6 (Applied Spectral Imaging). At least three metaphases were karyotyped for each participant.

Abnormalities detected by the Multiprobe®-T or SKY were confirmed by telomeric and paint FISH probes from Vysis (Downers Grove, IL). Parents of probands with rearrangements were studied using subtelomeric FISH and SKY.

RESULTS

Fifty patients with idiopathic MR or developmental delay were studied, 22 males and 28 females. Three rearrangements were detected by subtelomeric FISH: a derivative 5 from a maternal translocation t(5;21); a duplication of 11p and a deletion of 11q derived from a paternal pericentric inversion; and a q2 deletion also present in the mother and considered a polymorphism
Only the derivative 5 was detected by SKY. No interstitial interchromosomal rearrangement was detected by this method. Mosaicism for cryptic rearrangements was not ruled out; however, in one case one metaphase had an extra chromosome 9 by SKY, which prompted additional GTG-banding analyses, and trisomy 9 was found in a total of 3/50 metaphases. The prevalence of clinically significant cryptic rearrangements by Multiprobe 1 and SKY in the sample was thus 4% (95% confidence interval (CI) of 0.5–13.7) and 2% (95% CI 0.05–10.7), respectively.

Patient 1

This patient was a 3-month-old boy with dysmorphic features and multiple congenital anomalies. After a gestation characterized by intrauterine growth retardation, he was born prematurely at 35 weeks of gestation to a 27-year-old G1P0 woman. A prenatal ultrasound had noted the presence of a double bubble, a dysplastic left kidney, and increased nuchal fold thickness. The triple screen had been negative and amniocentesis was declined. Delivery was by cesarean section because of deteriorating parameters in fetal monitoring. He weighed 1,210 g at birth (<3rd centile) and his length and OFC were 38 and 27 cm, respectively (both <3rd centile). Apgar scores were 6 at 1 min and 8 at 5 min. He demonstrated feeding intolerance and reflux and was subsequently diagnosed with duodenal atresia. His left kidney was polycystic. He had pulmonary edema and developed chronic lung disease. The echocardiography was normal.

At age 3 months, his dysmorphic features included small eyes, short palpebral fissures, hypertelorism, small epicanthal folds, and a small nose (Fig. 1A). There was a “subtle gestalt” of Down syndrome. He had a cleft of the soft palate and microstomia. His jowls were prominent and his neck appeared continuous with his chest. His hands were characterized by the 2nd and 5th fingers overlapping the 3rd and 4th, respectively, clinodactyly of the 5th finger, and single palmar creases bilaterally. He had a sandal gap between his 1st and 2nd toes, syndactyly between the 4th and 5th toes, and hypoplastic toe nails bilaterally. He died at age 10 months due to gastrointestinal complications.

His GTG-banded chromosomes were reported as normal from blood and skin biopsy specimens at birth and were normal upon repeat testing of a blood specimen as part of this study (Fig. 1B). Using subtelomeric FISH (Fig. 2A,B) and SKY (Fig. 2C–E), an unbalanced translocation was detected between the short arm of chromosome 5 and the long arm of chromosome 21. Subsequent analyses revealed that the mother and grandmother were translocation carriers. The infant’s karyotype was 46,XY,der(5)t(5;21)(p13;q21.1)mat with a deletion of part of the chromosome 5 short arm and duplication for most of the chromosome 21 long arm. The family history was negative for MR or congenital anomalies.

Patient 2

This patient was a 16-year-old girl with moderate MR, developmental delay, and dysmorphic features (Fig. 3A). She was born at 37 weeks of gestation to a 30-year-old G3P2 woman after a pregnancy characterized by polyhydramnios of unknown etiology. Her
birthweight was 3,280 g (55th centile). As a neonate, she was noted to have dysmorphic features that consisted of a broad nasal root, telecanthus, low-set dysplastic ears, and widely spaced nipples. She had single transverse palmar creases bilaterally, narrow hyperconvex nails, and syndactyly of her 2nd and 3rd toes. A murmur heard at birth was diagnosed as a hemodynamically insignificant ASD. She had mild hepatosplenomegaly, thrombocytopenia, and amino aciduria, but no diagnosis was made. Torch screening was negative. At age 6 months she was reevaluated. Her development was slow and she was noted to have mild hypertelorism and heterochromia of the irises, in addition to the previous findings. Because of the dysmorphic features and persistent hepatosplenomegaly, she was evaluated for storage disorders. Screen-
ing for oligosaccharides and mucopolysaccharides were negative. Her developmental progress included walking at age 17 months and talking at age 2 years. She was hospitalized 2–3 times per year for chronic pneumonia from the ages of 1–6 years and several times for high fevers of unknown etiology. She had a history of severe menorrhagia and ultrasound revealed a heart-shaped uterus.

At age 16 years, her height was 158 cm (25th centile), weight 64 kg (80th centile), and OFC 60.5 cm (>95th centile). She had a relatively short forehead with a long mid-face and mild synophrys. She had a prominent nose with a long philtrum. Her ears were low-set and posteriorly rotated with relatively simple outer helices. She had a narrow palate and microstomia. Her hands were characterized by single transverse palmar creases bilaterally, distally placed palmar triradii, camptodactyly of fingers 4 and 5, and marked clinodactyly of the 3rd toes. There was no hepatosplenomegaly. Her gross motor and fine motor skills were delayed. She attends regular classes with an educational assistant and functions socially at an age-appropriate level despite her intellectual limitations.

Her GTG-banded karyotypes were normal in the newborn period, in both cultured lymphocytes and fibroblasts, and the karyotype was normal upon repeat testing as part of this study. By subtelomERIC FISH, 11p was detected at both ends of one chromosome 11 (Fig. 3B). Her SKY karyotype was normal. Parental cytogenetic investigations indicated that the rearrangement 11 was a recombinant 11 secondary to a paternal pericentric inversion. Her karyotype was 46,XX,rec(11)dup(11p)inv(11)(p15.5q24.3)pat with a duplication of the distal short arm and a deletion of the distal long arm of chromosome 11. The parents were nonconsanguineous and the family had no known history of recurrent miscarriages, stillbirths, or MR/developmental delay; however, the paternal grandfather of the proband had two brothers who died as infants, but the cause of death was not known.

**Patient 3**

This patient was a 12-year-old boy with developmental delay and mildly dysmorphic features (Fig. 3C). He was born at 41 weeks of gestation to a 41-year-old G1P1 woman. The birthweight was 3,300 g. His developmental milestones included walking at 18 months and articulating single words at age 16 months. Concerns about language and fine motor skills were present since age 5 years and prompted neurological, developmental, and genetics evaluations. Investigations revealed normal EEG, CT, and hearing results. He was fragile X negative. At age 12, his height was 142.5 cm (25th centile), weight 3,450 g (25–50th centile), and OFC 51.5 cm (10th centile). His facial features included telecanthus, slightly upslanting palpebral fissures, and a high nasal bridge. He had a small chin, an overbite with crowded teeth, and a narrow high-arched palate. His fingers were tapered with short 5th fingers bilaterally. He had mild hyperextensibility of the small joints of his fingers and elbows. His growth parameters were in the normal range for height, weight, and OFC. His features were suggestive of velocardiofacial syndrome but he did not have a cardiac abnormality and was negative for microdeletion 22 by FISH using the probe for TUPLE1. His school performance was poor. He had difficulty with articulation, verbal comprehension, nonverbal reasoning, and perceptual organizational skills. He had poor fine motor skills, diffuse hypotonia, difficulties with tandem gait, and a mild intention tremor.

His GTG-banded karyotype was normal. SubtelomERIC FISH with PAC clone dJ-1011-017 (locus D2S2986) showed that the signal for 2q was absent in the patient’s metaphases (Fig. 3D) and also absent in the mother’s metaphases. Neither the proband nor his mother were deleted by FISH using the Vysis telomeric probe V1J-Yrm2112 (locus D2S447). His SKY karyotype was normal. Features characteristic of velocardiofacial syndrome were not noted in the mother. She had completed grade 8 education. The parents were nonconsanguineous. There is no reported history of developmental delay or learning difficulties in the extended family. The remainder of the family history is unremarkable except for a stillbirth of unknown etiology in the paternal aunt of the proband.

**DISCUSSION**

We report here on three rearrangements in a sample of 50 infants and children with idiopathic MR or developmental delay and normal G-banded karyotypes, as detected by subtelomERIC FISH and SKY. One rearrangement (deletion of 2q) was present in the child and the mother and interpreted to be clinically benign. Mosaicism for cryptic rearrangements was not ruled out; however, in one patient low-level mosaicism for trisomy 9 was detected by SKY and later confirmed by additional GTG analyses. The prevalence of clinically relevant rearrangements in this population was 4% as detected by subtelomERIC FISH and 2% as detected by SKY. A previous study on the frequency of chromosomal abnormalities as detected by subtelomERIC FISH reported a 7.4% frequency (95% CI 4.4–10.4) in children with moderate to severe MR, and 0.5% (95% CI 0.5–1.6) in children with mild MR in a study of 466 children [Knight et al., 1999]. No similar study using SKY was available for comparison.

SubtelomERIC FISH was found to be more informative than SKY in detecting cryptic rearrangements. In Patient 1, the rearrangement was not detectable at the 550 G-band level because similar bands had been exchanged and the use of the 24-color painting probes made detection of the der(5)t(5;21) possible. Of note are the literature reports of similar der(5)t(5;21) chromosomes that were not detected by G-banding but detected by R-banding, a technique not frequently used in North America [Phelan et al., 1988]. The other two rearrangements were not visualized by SKY because the rearrangements were intrachromosomal and involved small segments. In Patient 2, a duplication of 11p and a deletion of 11q was present as a recombinant
from a paternal pericentric inversion of chromosome 11. In Patient 3, the absence of the 2q signal indicated the presence of a microdeletion. SKY has a reported resolution limit of 0.5–1.5 Mb [Schrock et al., 1996]; however, the smallest interchromosomal insertion that we have detected by SKY and confirmed by chromosome painting in our 3-year experience using SKY was equivalent to one small G-band (~3 Mb).

An important question suggested by this and similar studies is whether a genotype–phenotype causality can be established for the subtelomeric abnormalities that are detected. It is possible that small rearrangements could be without significant phenotypic effect. In 2/3 abnormalities detected in this study, the rearrangements are the likely cause of the children’s phenotypes. In Patient 1, there is little doubt that the der(5) is the cause of the patient’s phenotype. Both partial duplication of 21q and partial deletion of 5p are well-known causes of developmental delay, MR, and multiple congenital anomalies. In addition to the “subtle gestalt” of trisomy 21, the loose skin of the short neck, flat nasal bridge, dysplastic ears, clinodactyly of the 5th finger, gap between 1st and 2nd toes, developmental delay, and duodenal atresia present in Patient 1 are additional features suggestive of trisomy 21 [Epstein, 1989]. The microcephaly and hypertelorism also present in Patient 1 are features consistent with cri du chat syndrome [Niebuhr, 1978]. Lastly, the intrauterine growth retardation, low birthweight, epicanthal folds, and transverse palmar creases present in Patient 1 are common to both syndromes. In Patient 2, the 11p duplication involved ~11p15.5–11pter, the 11q deletion ~11q24.3–11qter, and the abnormality was paternal in origin. The phenotype of Patient 2 was more in keeping with the phenotype described by Fryns et al. [1981] than the phenotype described by Waziri et al. [1983] for a recombinant 11, with duplication of 11p and a deletion of 11q, secondary to a paternal pericentric inversion. Paternal duplication of 11p15.5 and deletion of 11q24 are found in Beckwith-Wiedemann and Jacobsen syndromes, respectively. The patient in Waziri et al. [1983] had features of Beckwith-Wiedemann syndrome [Elliot and Maher, 1994] and the patient in Fryns et al. [1981] had features of Jacobsen syndrome [Jacobsen et al., 1973; Fryns et al., 1986; Lewanda et al., 1995; Penny et al., 1995]. The hypertelorism, telecanthus, broad nasal root, low-set malformed ears, transverse palmar creases, hand anomalies, malformed toes, wide-spaced nipples, mild to moderate psychosocial retardation, ASD, and thrombocytopenia present in Patient 2 are found in Jacobsen syndrome, whereas only the head circumference at the 97th centile was consistent with Beckwith-Wiedemann syndrome. The subtelomeric abnormality detected in Patient 2 was thus considered to be the causal, with the breakpoint in 11p perhaps distal to most of the critical region for Beckwith-Wiedemann syndrome. In Patient 3, the 2q deletion detected by the dJ-1011-O17 probe was also present in the mother, and assuming that the deletions are similar in size, the 2q deletion is not likely the cause of the patient’s phenotype, but rather a familial polymorphism [Knight et al., 2000; Ballif et al., 2000]. This observation demonstrates the importance of parental investigations for all subtelomeric rearrangements detected, before concluding on their clinical significance.

In conclusion, this study supports the view that G-band karyotyping is inadequate for detecting clinically significant chromosomal abnormalities if similar bands are exchanged or if deletions and duplications are small. Subtelomeric FISH and SKY detected abnormalities that were not detectable by G-banding. Although the sample size in this study was small (with overlap of the 95% CIs for subtelomeric FISH and SKY results), subtelomeric FISH was more effective than SKY in detecting cryptic rearrangements. Subtelomeric FISH can detect, both large and small, interchromosomal and intrachromosomal rearrangements involving the chromosome ends, whereas SKY detects only the larger interchromosomal rearrangements involving the ends. Nevertheless, SKY also has the potential to detect interchromosomal insertions and although these rearrangements requiring three breaks occur less frequently than interchromosomal subtelomeric rearrangements requiring two breaks, SKY is technically easier, less costly, and less labor-intensive than subtelomeric FISH. Therefore, our experience is in keeping with the prevalent view that both subtelomeric FISH and SKY can be of value in clinical genetics, where investigations for the evaluation of patients with MR or developmental delay can be costly.

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