We report on a familial cryptic (20;21) translocation \([t(20;21)]\) that was initially suspected with the observation of a single chromosome 21 specific signal in an interphase nuclei by in situ hybridization (FISH) study performed on a 34-week gestation amniotic fluid specimen. The genetic amniocentesis was prompted by the presence of fetal anomalies detected by ultrasound. In addition, there was a family history of a maternal uncle with mental retardation and multiple malformations and an apparently normal karyotype. No obvious aberration could be detected in the G-banded karyotype prepared from the amniotic fluid specimen. A FISH study using a chromosome 21 specific long arm probe and chromosome 20 whole chromosome paint, however, showed an unbalanced rearrangement in the fetus \([46,XY, \text{der}(21)t(20;21)(q13.2;q22.13 \text{ or } 22.2)\text{ mat}]\). The mother and maternal grandmother were demonstrated to be balanced translocation carriers. These results were confirmed by multicolor karyotyping. This familial aberration was discovered by chance in the interphase FISH analysis. Our experience with this case, however, serves to emphasize the importance of the reevaluation of patients with mental retardation and congenital malformations of unknown cause and prudent use of multicolor karyotyping in the detection of cryptic cytogenetic rearrangements. Am. J. Med. Genet. 93:273–277, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: cryptic translocation; multi-target FISH

INTRODUCTION

Banded chromosome preparations have been the traditional technique for investigating patients suspected of having chromosomal aberrations. The development and application of fluorescent in situ hybridization (FISH) and, more recently multicolor karyotyping have served to increase our ability to identify subtle aberrations or more completely characterize complex rearrangements. FISH studies, however, have been typically used in adjunct to confirm or further clarify aberrations that have been identified in banded chromosome preparations, the exception being the use of cosmid probes to detect microdeletions known to be associated specific syndromes.

Recent investigations using chromosome specific subtelomeric analysis performed by FISH or microsatellite markers [Flint et al., 1995; Viot et al., 1998; Slavotinek et al., 1999] have shown that an unexpectedly high fraction of individuals with nonsyndromic mental retardation harbor cryptic unbalanced chromosomal abnormalities. We report here the serendipitous identification of just such a cryptic aberration that could not be recognized in G-banded preparations of a banding level routinely achieved in genetic amniocentesis specimens. The aberration was first suspected in an interphase FISH study and then identified and characterized in metaphase studies. The results were readily confirmed by a multicolor karyotyping system that allowed simultaneous identification and visualization of all 24 chromosomes [Schrock et al., 1996]. As we discuss below, cases such as this illustrate the need to define clinical criteria for the most effective application of the advanced in situ technologies such as 24 color karyotyping or subtelomeric analysis for the identification and confirmation of suspected subtle unbalanced chromosomal aberrations.
CLINICAL REPORT

At approximately 34 weeks of gestation, an ultrasound evaluation was performed for possible IUGR on a pregnancy to a 19-year-old gravida 1 woman. The mother described the pregnancy as unremarkable and was not aware of exposure to teratogenic substances. Ultrasound evaluation showed a cleft lip, IUGR, dilated posterior fossa and a two-vessel umbilical cord. Based on these findings, a genetic amniocentesis was performed. At 39 weeks of gestation, labor was induced because of pregnancy-induced hypertension. A male infant was delivered normally without complications. Apgar scores were 4 and 7 at 1 and 5 min respectively. Birth weight was 1.678 gm (<5th centile), length 45 cm (3rd–10th centile) and OFC 30.5 cm (<5th centile).

At birth the right cleft extended to the level of the right ala (Fig.1A). He also had multiple small furrows of the forehead, deep-set eyes with short downward slanting palpebral fissures, and broad nasal bridge. The left ear was cupped shaped and measured 3.5 cm in length and the right ear had a notch in the outer helix and measured 3.1 cm in length. Both ears were posteriorly angulated. There was a midline dimple in the chin. Penis was small with a stretched length of 1.2 cm. There was a midline cleft in the scrotum. The infant had generalized hypotonia and a high-pitched cry.

A maternal uncle was born in 1976 to a then 29-year-old gravida 5, para 1, SAB 3 mother after a 42-week gestation with microcephaly, growth retardation (all growth parameters <5th centile), left sclerocornea, deep set eyes with short palpebral fissures, low set simple ears, webbed neck, hemivertebrae at T9, and mild hypospadias with a hooded foreskin (Fig. 1B). After the identification of a heart murmur in the neonatal period, ECG and echocardiogram studies documented the probable presence of an atrioventricular canal. His infancy and childhood were characterized by the emergence and worsening severity of multiple medical problems and developmental delays that included kyphoscoliosis, tonic clonic seizures, poor growth (height, weight and head circumference <5th centile), absence of speech, and significantly impaired motor skills with only limited uses of his hands. His highest developmental level was estimated at approximately 7–8 months. Recurrent pulmonary edema and pneumonia were the cause of death at the age of 16 years. Evaluations performed to determine an underlying etiology for this patient’s findings included urine metabolic screen and urine organic acids both of which were normal. An R-banded cytogenetic study performed in 1977 was interpreted as 46,XY without demonstrable abnormalities.

The maternal grandmother of the propositus had 3 early pregnancy losses and three liveborn children including the mother of the propositus, the maternal uncle described, and another healthy daughter.

MATERIALS AND METHODS

Cytogenetic studies were performed using standard techniques for both amniocyte and peripheral blood samples. FISH probes used for analysis include LSI 13, CEP 18, LSI 21, CEP X and CEP Y all obtained from Vysis Inc. (Downers Grove, IL). LSI 13 localizes to band 13q13 and LSI 21 localizes to bands 21q22.13–q22.2. The CEP probes are the alpha satellite repeats from the specific chromosome designated. A chromosome 20 whole chromosome paint probe (Oncor Inc., Gaithersburg, MD) was initially used to confirm the rearrangement was between chromosomes 20 and 21. All hybridizations were performed following the manufacturer’s instructions. Multicolor karyotyping was performed using the “SKY” system of Applied Spectral Imaging (Carlsbad, CA) per the manufacturer’s recommendations.

RESULTS

Initial interphase FISH studies were performed on uncultured amniocyte (Ward et al., 1993) from the pro-
positus. Fifty interphase nuclei were evaluated for each probe that showed 2 signals each from the chromosome 13 probe and the chromosome 18 probe. However, there was a only single signal from the chromosome 21 probe, however, in every interphase nuclei examined (Fig. 2A). Analysis of the X and Y centromeric probes showed a single signal from each probe, consistent with a male fetus. G-banded cytogenetic preparations from the amniocyte showed an apparently normal cytogenetic complement with two homologues of chromosome 21 present in a total of 33 metaphase cells analyzed from 31 different clones (Fig. 3A). FISH analysis performed using the same LSI 21 probe on metaphase cells showed hybridization signals present on only one homologue of chromosome 21 in 20 metaphase cells examined; there was complete absence of hybridization signals on the other chromosome 21 homologue identified by DAPI counterstaining in each of the metaphase cells scored.

A peripheral blood sample was obtained from the mother of the propositus because of the suggestive family history. Prometaphase chromosome preparations at approximately the 700 band level showed a minimal discrepancy by G-banding between the chromosome 21 homologues but no detectable difference between the chromosome 20 homologues (Fig. 3B). All other chromosomes appeared intact without any evidence of rearrangement. FISH analysis using the chromosome LSI 21 probe showed one set of signals on the distal long arm of one homologue of chromosome 21 and another set of signals present on the distal long arm of one homologue of chromosome 20. The chromosome 20 whole chromosome paint probe showed complete hybridization to one homologue of chromosome 20, hybridization to all but the distal long arm of the other chromosome 20 homologue, and translocated material to the distal long arm of one chromosome 21 homologue (Fig. 2B). The G-banding and FISH results were most consistent with the cytogenetic diagnosis, 46,XX,t(20;21)(q13.2;q22.13 or q22.2). Cytogenetic analysis of maternal aunt, maternal grandmother and maternal grandfather showed that only the maternal grandmother was a carrier of this same rearrangement. Cytogenetic evaluation of the maternal grandmother’s parents were normal indicating that the t(20;21) was likely a de novo rearrangement in the maternal grandmother of the proband.

Given the difficulty detecting this translocation on G-banded chromosome preparations, particularly in the unbalanced form, we utilized the multicolor karyotyping system, “SKY” (Applied Spectral Imaging, Carlsbad, CA) to test whether this rearrangement could be readily identified. In the 5 metaphase cells examined from both the patient’s amniotic fluid sample and his mother’s peripheral blood sample, the small translocation between chromosome 20 and 21 was clearly detectable in both the derivative (Fig. 3C) and the balanced (Fig. 3D) forms by multicolor karyotyping.

**DISCUSSION**

We describe a balanced cryptic familial translocation that likely resulted in two cytogenetically abnormal offspring from their carrier mothers. The propositus inherited the derivative 21 as the result of adjacent-1 segregation from his balanced t(20;21)(q13.2;q22.13 or 22.2) carrier mother resulting in duplication of the distal long arm of chromosome 20 and deletion of the distal long arm of chromosome 21. This cytogenetic rearrangement was cryptic by G-banded analysis, hence the difficulty with the breakpoint assignment. Given that the LSI p21 probe localizes to 21q22.13–q22.2, the translocation breakpoints of t(20,21)(q13.3;q22.13) are most compatible with the FISH results that showed the total absence of hybridization signals from this probe on the derivative 21. The alternative breakpoint of

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**Fig. 2.** (A) Interphase nuclei from the amniotic fluid sample obtain from the mother of the propositus. The interphase nuclei showed a single hybridization signal from the LSI 21 probe (Vysis, Inc., Downers Grove, IL) that localizes to the long arm of chromosome 21 at band q13.22–22.13. (B) Chromosome 20 whole chromosome paint probe (Oncor, Inc.) hybridized to a metaphase spread from the propositus’ mother showing the translocation between chromosomes 20 and 21. One homologue of chromosome 20 was entirely coated by hybridization signals from this probe. The other chromosome 20 homologue has a portion of the distal long arm that lacks hybridization signals (large arrow) whereas there was a small area of hybridization signals present on the distal long of chromosome 21 (small arrow).
21q22.2 was given based on the G-banding results suggesting that the breakpoint on chromosome 21 was more distal than band 21q22.13.

The exact nature of the cytogenetic abnormality in the maternal uncle could not be determined. The other cytogenetic configuration from adjacent-1 segregation, however, would cause constitutional deletion for the distal long arm of chromosome 20 and duplication for the distal long arm of chromosome 21. Patients with constitutional deletions of the distal long arm of chromosome 20 are extremely rare. There is one report by Frasse et al. [1981] with a nonmosaic deletion of the long arm of chromosome 20 between bands q13.11–qter in a 3-month-old with adenosine deaminase deficiency, developmental delays, severe hand malformations and distinctively different craniofacial anomalies than those observed in our patient and his maternal uncle. Moreover, duplication for the distal long arm of chromosome 21 results in findings typical of Down syndrome [Korenberg et al., 1990] that were certainly not present in the maternal uncle. Therefore, we think it is most likely that the maternal uncle has the same cytogenetic configuration that was present in the propositus.

There are no reports of patients with duplications of the distal long arm of chromosome 20 as their only cytogenetic abnormality. The phenotype shared by the propositus and maternal uncle include growth retardation, minor facial anomalies with deep set eyes and small palpebral fissures, anomalous ears, and genital abnormalities are findings previously reported in other patients with duplications involving the distal long arm of chromosome 20 as part of their duplication-deletion syndrome [Pawlowitzki et al., 1979; Nielson et al., 1986; Sax et al., 1986; Pierquin et al., 1988; Herens et al., 1990; Waters, et al. 1990].

This case highlights the importance or re-evaluating patients with nonsyndromic mental retardation or multiple congenital malformations and the need to develop criteria for the application of advanced in situ techniques as part of their cytogenetic testing. Guidelines published by a consensus conference on the evaluation of patients with mental retardation [Curry et al., 1997], suggest that if a patient with mental retardation of unknown cause has a normal chromosome study more than 5 years old, the patient should be re-examined with strong consideration of repeating the cytogenetic study. In a recent study, of patients with suspected constitutional chromosome abnormality [Leppig et al., 1997], repeat cytogenetic studies detected an unbalanced cytogenetic abnormality in 9% of the cases. The factors that enhanced the detection rate of a cytogenetic abnormality were re-evaluation of the patient by a clinical geneticist, a directed study to a specific chromosome location based on the patient’s phenotype, a positive family history, and the combined use of high resolution chromosome analysis and FISH studies.

With the improved resolution in banded chromosome preparations, subtle chromosome rearrangements have been identified for individual familial cases of mental retardation and malformations as well as more commonly recognized syndromes [Herens et al., 1997; Lindeman-Kusse et al., 1996]. In addition, there has been significant advances in FISH technology that most recently has included subtelomeric analysis and multicolor karyotyping. The detection rate for unbalanced rearrangements using subtelomeric analysis on patients with undiagnosed mental retardation ± malformations has been reported to range from 3% to 23%, most likely based on the selection of the study population [Flint et al., 1995; Viot et al., 1998; Slavotinek et al., 1999]. The detection rate for 24 color karyotyping for similar populations is currently unknown. This case report illustrated the utility of multicolor karyotyping for evaluating cases with suspected cytogenetic rearrangements. Until studies are completed to determine the sensitivity of multicolor karyotyping, the selected use of this technique for patients without a recognized monogenic syndrome whose phenotype includes mental retardation, multiple congenital malformations, poor growth, or minor anomalies, may be extremely helpful for identifying cryptic chromosome abnormalities. In addition, families such as these where the first individual with an abnormal phenotype is deceased, prudent use of multicolor karyotyping in conjunction with cytogenetic analysis may be helpful for identifying individuals at risk for having a child with a chromosome abnormality.

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