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Brief Clinical Report

Complex Familial Rearrangement of Chromosome 9p24.3 Detected by FISH

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We describe a newborn male with minor facial anomalies, pyloric stenosis, and a chromosome rearrangement that involves deletion and addition of material at 9p24.3. Routine studies showed a 46, XY, add (9) (p24) karyotype. Fluorescence in situ hybridization (FISH) with two different whole chromosome probes for chromosome 9 failed to identify whether the additional material was derived from that chromosome. FISH with single copy YAC probes from 9p24 (D9S1858, D9S1813 and D9S54) showed a more complex rearrangement involving a deletion at D9S1858 but not at D9S1813 or D9S54. Parental chromosome studies demonstrated an apparently identical 9p abnormality in the patient's mother. This report describes a familial chromosome rearrangement in an abnormal child and his normal mother and demonstrates the use and limitations of FISH in characterizing chromosomal abnormalities. Am. J. Med. Genet. 76:306–309, 1998. © 1998 Wiley-Liss, Inc.

KEY WORDS: chromosome 9p24; monosomy 9p syndrome; FISH

INTRODUCTION

Rearrangements involving the distal region of the short arm of chromosome 9 (9p22 to 9p24) are well described and often involve deletions or duplications resulting in the partial monosomy 9p (9p-) [Huret et al., 1988] or the partial trisomy 9p syndromes [Young et al., 1982; Wilson et al., 1985], respectively. Both syndromes include mental retardation and characteristic craniofacial findings. Individuals with deletions characteristically have trigonocephaly and long fingers. In contrast, those with partial trisomy have microcephaly, hypertelorism, external ear anomalies and short fingers. Recent data have attributed the 9p- syndrome to a region in 9p22-p23 [Wagstaff and Hemann, 1995] but many of the described deletion patients also had other chromosome imbalances [Huret et al., 1988], therefore making it difficult to assign clinical traits to a particular chromosome band. Sex reversal has been seen in some males with terminal rearrangements of 9p24 [Hoo et al., 1989; Bennet et al., 1993] and the critical region appears to be sublocalized to 9p24.3 [Flejter et al., 1996].

Chromosome rearrangements are detected initially by routine cytogenetic methods in clinical laboratories. Now, they are often characterized further by molecular methods such as fluorescence in situ hybridization (FISH). This characterization is possible given the availability of whole chromosome probes and locus-specific sequences (either commercially or through genome data bases). FISH is an important adjunct to routine chromosome analysis. We discuss the classical and molecular cytogenetic findings of a chromosome 9p24 rearrangement in an abnormal child and his phenotypically normal mother.

CLINICAL REPORT

This boy was born at 32.5 weeks to a 30-year-old G3P1 mother and 31-year-old father. Parents are second cousins with no personal or extended family history of congenital anomalies, minor anomalies, learning disabilities or frequent pregnancy losses. They had a first trimester miscarriage and have a healthy 4-year-old daughter. The third pregnancy was complicated by oligohydramnios and intrauterine growth retardation. The infant was born via cesarean section due to breech presentation. Birth weight was 1,780 g, length 41 cm (both at the 50th centile for gestational age) and head circumference (OFC) 28.5 cm (25th centile). At birth, he had a flat occiput, upslanting palpe-

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bral fissures and telecanthus. An anterior polar cataract was noted in his right eye. He had protruding ears with prominent ear lobes, a small upturned nose and a long philtrum. He had mild microretrognathia and redundant nuchal skin. He had male external genitalia with undescended testes. He had flat, square, long palms (length at the 97th centile for gestational age) and short fingers. His tone and reflexes were normal.

Pyloric stenosis was diagnosed at one month. At 4 months (Fig. 1), his length, weight, and OFC were at the 25-50th centile for corrected gestational age. No significant change was noted in his craniofacial findings. His testes had descended spontaneously. His development was consistent with his corrected age. At 11 months, he was diagnosed with mild to moderate conductive hearing loss. He has mild speech delay, but has age-appropriate social, fine and gross motor skills.

CYTOGENETIC AND MOLECULAR CYTOGENETIC FINDINGS

GTG-banded chromosome analysis [Seabright, 1972] of peripheral lymphocytes from the propositus showed a 46, XY, add (9)(p24) karyotype. A partial karyotype of chromosome 9 is presented in Figure 2A. The additional material at the end of the chromosome 9 was light staining with GTG-banding (Fig. 2A) and negative by CBG-banding (Fig. 2B) [Sumner, 1972]. Therefore, this region was not comprised of constitutive heterochromatin. Routine chromosome analysis of lymphocytes from the parents showed a normal 46, XY karyotype in the father and 46, XX, add (9)(p24) in the mother.

The rearrangement was suspected of being a duplication of (9)(p24) or a translocation involving another chromosome. FISH with two whole chromosome 9 probes (WCP-9, Gibco BRL and Coatasome 9, Oncor, Inc. [Gaithersburg, MD]) and three 9p24 sequences (YACs 765H2 [D9S1858], 727D12 [D9S1813], and 783G1 [D9S54]) were performed to determine if the patient's phenotype was the result of a chromosomal imbalance due to aberrant segregation of a cryptic translocation in the mother. YACs 765H2 and 727D12 map to 9p24.3 with YAC 765H2 being most telomeric; YAC 783G1 maps to 9p24.2 [Collins et al., 1996]. WCP-9 was directly labeled with Spectrum Green and Coatasome 9 with digoxigenin-11-dUTP. FISH was performed according to the manufacturer's specifications for the whole chromosome probes. The YAC clones were amplified with polymerase chain reaction (PCR) using inter-Alu primers AGK34, Alu 3′ and Alu 5′ [Baldini et al., 1992; Tagle and Collins, 1992] before

Fig. 1. The patient at 4 months of age. Facial features are nonspecific with telecanthus and prominent ear lobes present.

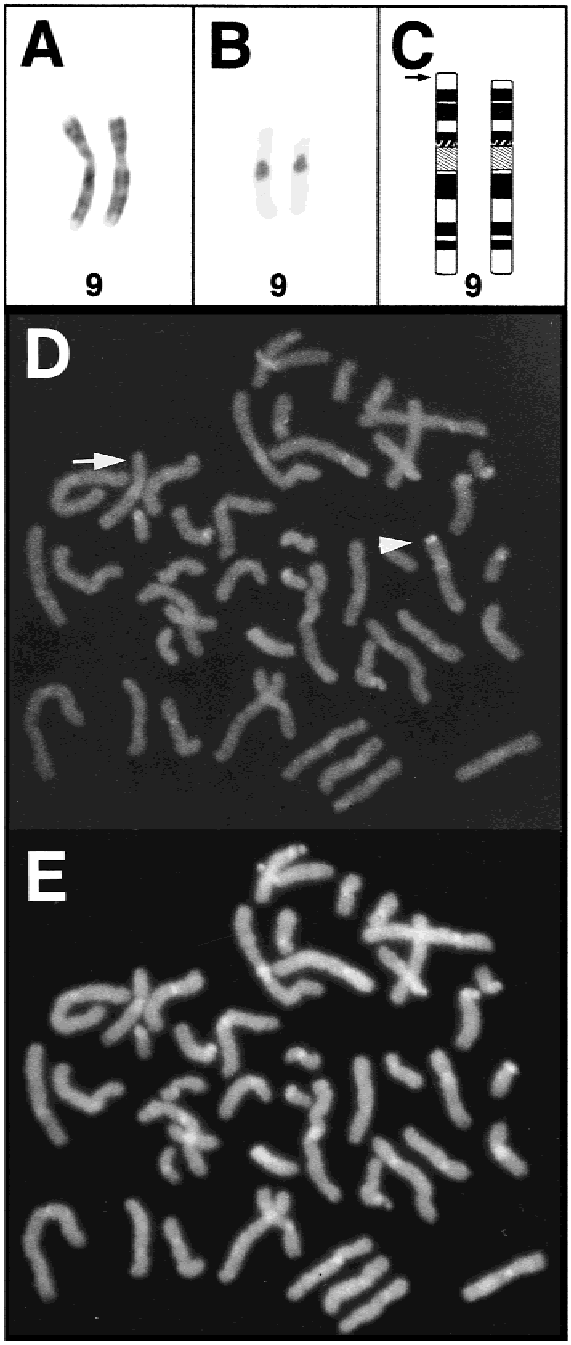

Fig. 2. **A:** GTG-banded pair of chromosome 9s. **B:** CBG-banded chromosome 9s . **C:** Corresponding ideogram. The abnormal chromosome 9 is on the left, and 9p24 is indicated by the arrow. **D:** FISH with YAC 765H2. The normal chromosome 9 shows hybridization (arrowhead), while the abnormal one does not (arrow). **E:** Corresponding DAPI image.

labeling or they were labeled in their entirety by nick translation with biotin-16-dUTP or digoxigenin-11-dUTP [Knoll and Lichter, 1994]. Biotin was detected with avidin-fluorescein isothiocyanate (FITC) and digoxigenin with anti-digoxigenin-rhodamine. Chromosomes were counterstained with 4,6 diamidino-2-phenylindole (DAPI; 0.1 g/ml) and/or propidium iodide (PI; 0.4 g/ml). Hybridizations were observed with an epifluorescence microscope equipped with a dual band pass filter set (FITC/ Texas Red; Omega Optical, Inc., Brattleboro, VT) for detection of FITC, rhodamine, Spectrum Green, and PI. A standard single band pass filter (Carl-Zeiss, Inc., Thornwood, NY) was used to view the DAPI counterstain. At least 10 metaphase cells were examined for each hybridization. Cells were photographed in color (Kodak Ektar 1000) using a 35-mm camera and converted to black and white.

Each whole chromosome probe gave different coverage along chromosome 9. WCP-9 did not hybridize to the entire 9p24 and Coatasome 9 did not hybridize to the sub-band 9p24.3, in either the normal or the add (9)(p24) chromosome; thus, they were not useful in identifying or excluding the additional material as derived from that region. Hybridization with YAC 765H2, the most distal probe tested, showed a deletion in the propositus and his phenotypically normal mother in the add (9)(p24) chromosome (Fig. 2D). YACs 783G1 and 727D12 remained intact (not shown). They hybridized to both chomosome 9s with equal intensity in both individuals and thus a duplication at D9S1813 or D9S54 was not evident. YACs 783G1 and YAC 727D12 are chimeric and also mapped to 4q and 3p/3q, respectively. These data show that the add (9)(p24) chromosome involves not only an addition of chromatin but also a loss of material.

DISCUSSION

This report describes a rearrangement of 9p24.3 that involves the presence of additional material distal of D9S1813 and a deletion at D9S1858 in a child with congenital anomalies and in his normal mother. Routine GTG-banding showed the presence of additional light staining chromatin at 9p24. FISH demonstrated a deletion in 9p24.3, delimited a potential duplication of this band between D9S1858 and D9S1813 and excluded D9S54 which maps to 9p24.2. Chromosome 9p24.3 did not hybridize with either commercially available whole chromosome 9 probe and the origin of the additional material remains unknown. GTG-banding and FISH did not exclude a terminal duplication of 9p24.3. Phenotypically, the child did not have signs of 9p- syndrome. These findings are consistent with our placement of the aberration at 9p24.3 and those of Wagstaff and Hemann [1995] that place the critical region of the 9p- syndrome in 9p22-p23. In addition, sex reversal was not present in our patient and therefore the region encompassing D9S1858 is an unlikely location for a potential sex reversal gene [Flejter et al., 1996], though variable expressivity of this anomaly in 9p deletions has been described [Magenis et al., 1990]. The patient's manifestations were not consistent with those described in partial 9p trisomy [Young et al., 1982; Wilson et al., 1985] and no duplications of the loci examined were detected.

The relationship of the cytogenetic and clinical findings is unclear. The nonspecific clinical findings in this child may be unrelated to the chromosomal finding, especially since the same rearrangement is present in his phenotypically normal mother. This rearrangement may represent a rare variation within the normal population as described for other chromosomal regions [Barber, 1994; Lin et al., 1994; Zaslav et al.,1993; Jalal et al., 1990]. Alternatively, the chromosomal findings may play a role in the patient's phenotype and the discrepancy between mother and child could arise in several ways. These include: (1) the chromosome 9s may be different in mother and child but not detectable with the methods used; (2) a recessive gene could be unmasked on the paternally derived chromosome 9 in the propositus; and/or (3) this region may contain imprinted genes with differential expression dependent on parent of origin. The rearrangement is of maternal origin in the propositus and of unknown origin in the mother. Further characterization of the rearrangement in the mother and child plus chromosomal studies in maternal grandparents may help to distinguish these alternatives. Microdissection of 9p24.3 [Ohta et al., 1993] may be useful in determining the origin of the duplicated material, as FISH with whole chromosome 9 probes was not. The findings in this study illustrate three important points: (1) the current limitations of FISH especially when using whole chromosome probes and examining small terminal rearrangements; (2) the necessity of performing parental chromosome studies before attributing clinical findings to a particular chromosomal finding; and (3) the telomeric loss of a single copy of D9S1858 can be associated with a normal phenotype.

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