American Journal of Medical Genetics 71:436–442 (1997)

An Apparently Acentric Marker Chromosome Originating From 9p With a Functional Centromere Without Detectable Alpha and Beta Satellite Sequences

G.H. Vance,[1]* C.A. Curtis,[1] N.A. Heerema,[1] S. Schwartz,[2] and C.G. Palmer[1]

[1]Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis
[2]Center for Human Genetics, Cleveland, Ohio

Recently, we studied a patient with minor abnormalities and an apparently acentric marker chromosome who carried a deleted chromosome 9 and a marker chromosome in addition to a normal chromosome 9. The marker was stable in mitosis but lacked a primary constriction. The origin of the marker was established by fluorescent in situ hybridization (FISH) using a chromosome 9 painting probe. Hybridization of unique sequence 9p probes localized the breakpoint proximal to 9p13. Additional FISH studies with all-human centromere alpha satellite, chromosome 9 classical satellite, and beta satellite probes showed no visible evidence of these sequences on the marker [Curtis et al.: Am J Hum Genet 57:A111, 1995]. Studies using centromere proteins (CENP-B, CENP-C, and CENP-E) were performed and demonstrated the presence of centromere proteins. These studies and the patient's clinical findings are reported here. Am. J. Med. Genet. 71:436–442, 1997. **© 1997 Wiley-Liss, Inc.**

KEY WORDS: marker chromosome; fluorescent in situ hybridization; chromosome 9; centromere proteins

INTRODUCTION

In the past few years, marker chromosomes have been investigated by a variety of methods. Fluorescent in situ hybridization (FISH) studies with alpha satellite probes [Callen et al., 1992; Crolla et al., 1992; Plattner et al., 1991, 1993] have identified the centromeres of these chromosomes. Flow sorting [Blennow et al., 1992] or microdissection [Viersbach et al., 1994; Muller-Navia et al., 1995] coupled with polymerase chain reaction (PCR) amplification and FISH have permitted the identification of the genetic composition of others. Markers lacking cytogenetically detectable alpha satellite DNA were reported early in these investigations and reports of similar markers have continued. Some of the acentric markers have been investigated with FISH using other satellite probes and a few have been studied by immunologic techniques for the presence of centromere proteins (Table I). We report here the use of FISH and immunofluorescent staining of centromere proteins for the identification of a marker originating from chromosome 9p.

CLINICAL REPORT

The patient, B.S. (Family no. 88681), was born to a 22-year-old black woman at 35 weeks of gestation. Her birth weight was 2.53 kg (50th–75th centile for gestational age) and length was 47 cm (10th centile). The patient had a prior history of feeding difficulties and dehydration requiring hospitalization. Results of metabolic screen of urine were normal.

On examination at age 15 months (Fig. 1), her height, weight, and OFC were all below the 5th centile. She had brachycephaly, plagiocephaly, sloping forehead and bitemporal narrowing, epicanthal folds, hypertelorism, apparently low-set, cupped and posteriorly angulated ears, a broad nasal bridge, prominent nasal tip with anteverted nares, and a large mouth with down-turned corners of the upper lip. There was hyperextension of the fingers with fifth finger clinodactyly, brachydactyly, and nail hypoplasia. Bilateral single palmar creases, hyperreflexia, and diastasis recti were also observed. Radiologic studies of the hands demonstrated symmetric abnormalities of both hands, with short, broad first metacarpals, and short fifth middle and distal phalanges with clinodactyly, characteristic of trisomy 9p [Schinzel, 1979]. A head CT scan showed mild ventriculomegaly. She was devel-

*Correspondence to: Gail H. Vance, Department of Medical and Molecular Genetics, Indiana University School of Medicine, Medical Research and Library Building, 130, 975 W. Walnut Street, Indianapolis, IN 46202-5251.

Received 18 September 1996; Accepted 4 February 1997

TABLE I. Markers Lacking Alpha Satellite DNA*

Chromosome	C band	FISH		Centromeric proteins		Reference
mar 19 (case 11)	NR	Alpha	Neg			Crolla et al. [1992]
		SAT II/SAT III	Neg			
47,XX,+mar (case 15)	NR	Alpha	Neg			Crolla et al. [1992]
		SAT II/SAT III				
47,XY,+mar/46,XY (case 20)	NR	Alpha SAT	Neg			Callen et al. [1992]
		RR216	Neg			
del 10q25	Neg	Alpha SAT	Neg	CENP-B	Neg	Voullaire et al. [1993]
		SAT III	Neg	CREST	Pos/Neg	
inv dup 15q23	Neg	Alpha SAT	Neg			Blennow et al. [1994]
inv dup 15q24		SAT III	Neg			
i(13q) (1 case)	NR	Alpha SAT	Neg			Schwartz et al. [1994]
		(4 cases)				
i(15q) (3 cases)	NR	SAT III	Neg			
		(3 of 4 cases)				
inv dup 8 (pter → p23.1)	Neg	Alpha SAT	Neg	CREST	Pos	Ohashi et al. [1995]
		All human alpha SAT	Neg			
dup 9 (pter → 13)	Neg	All human centromere	Neg	CENP-B	Neg	This case
		Classical SAT (9)	Neg	CENP-C,E	Pos	
		Beta SAT (9)	Neg			

*NR, not reported; Neg, negative; Pos, positive; SAT, satellite.

opmentally delayed. At 15 months, she held a bottle independently, rolled from front to back, transferred objects from hand to hand, and scooted on her abdomen.

A 19-year-old maternal aunt had Down syndrome and a 21-year-old maternal uncle had seizures and an attention deficit disorder. The patient's mother has rheumatoid arthritis.

MATERIALS AND METHODS

Peripheral blood was collected from the patient and her mother. Paternal blood was not available. Peripheral blood cultures and a lymphoblastoid cell line were prepared and analyzed using standard cytogenetic procedures. Metaphases were analyzed with GTG-banding at the 600 band level. CBG-banding (C-banding with BaOH) was performed.

FISH analysis included use of a chromosome 9 "coatasome" painting probe, an all-human centromere alpha satellite probe and chromosome 9 classical satellite and beta satellite probes (ONCOR). The biotin-labeled chromosome 9 classical satellite probe (D9Z1) and chromosome 9 beta satellite probe (D9Z5) were detected with avidin-FITC and a propidium iodine counterstain. All other probes were labeled with digoxigenin and detected with anti-digoxigenin-FITC and counterstained with propidium iodide according to the vendor's protocol. Probe Cos 4, a 41.6 kb cosmid which maps to 9p13, was kindly provided by Dr. Christina Brahe [Brahe et al., 1991, and personal communication]. The second cosmid probe, ICRFc100D11145, which also maps to 9p13, was obtained from the Max-Planck-Institut Reference Library [Lehrach et al., 1990]. Probe DNA was nick-translated with digoxigenin-11-dUTP/dTTP, purified through a sephadex column, and detected with anti-digoxigenin FITC with a propidium iodine counterstain. Photomicroscopy was performed using a Leitz Aristoplan fluorescence microscope.

Polyclonal antibodies to CENP-B and CENP-C were obtained from Dr. William Earnshaw (Johns Hopkins University, Baltimore, MD) and Dr. Huntington Willard (Case Western Reserve University Cleveland, OH) [Cooke et al., 1990; Saitoh et al., 1992]. Monoclonal antibodies to CENP-E were provided by Dr. Tim Yen (Philadelphia, PA) and Dr. Willard [Yen et al., 1991, 1992]. A modification of the technique of Page et al. [1995] and Earnshaw et al. [1989] was used for the detection of CENP-B, CENP-C, and CENP-E [Sullivan and Schwartz, 1995].

CENP antibodies were detected with secondary antibodies: fluoresceinated goat antirabbit (for CENP-B and-C antibodies) and rhodamine-conjugated goat antimouse (for CENP-E antibodies). Digital images were captured using a Zeiss epifluorescent microscope (Ax-

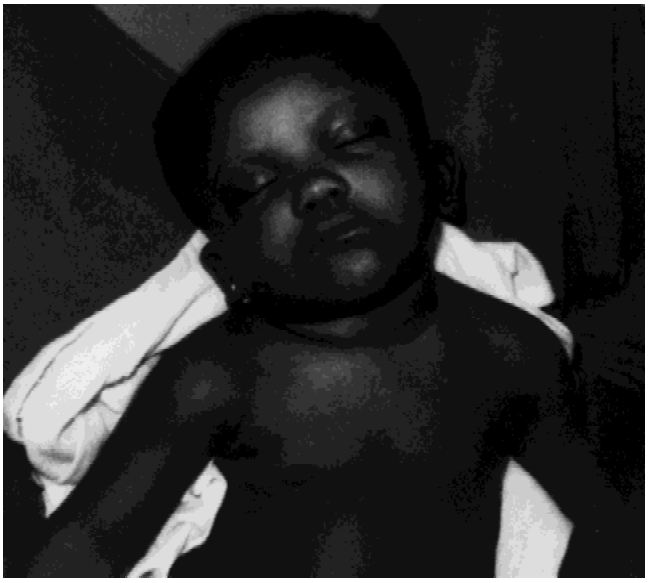

Fig. 1. Patient at age 15 months.

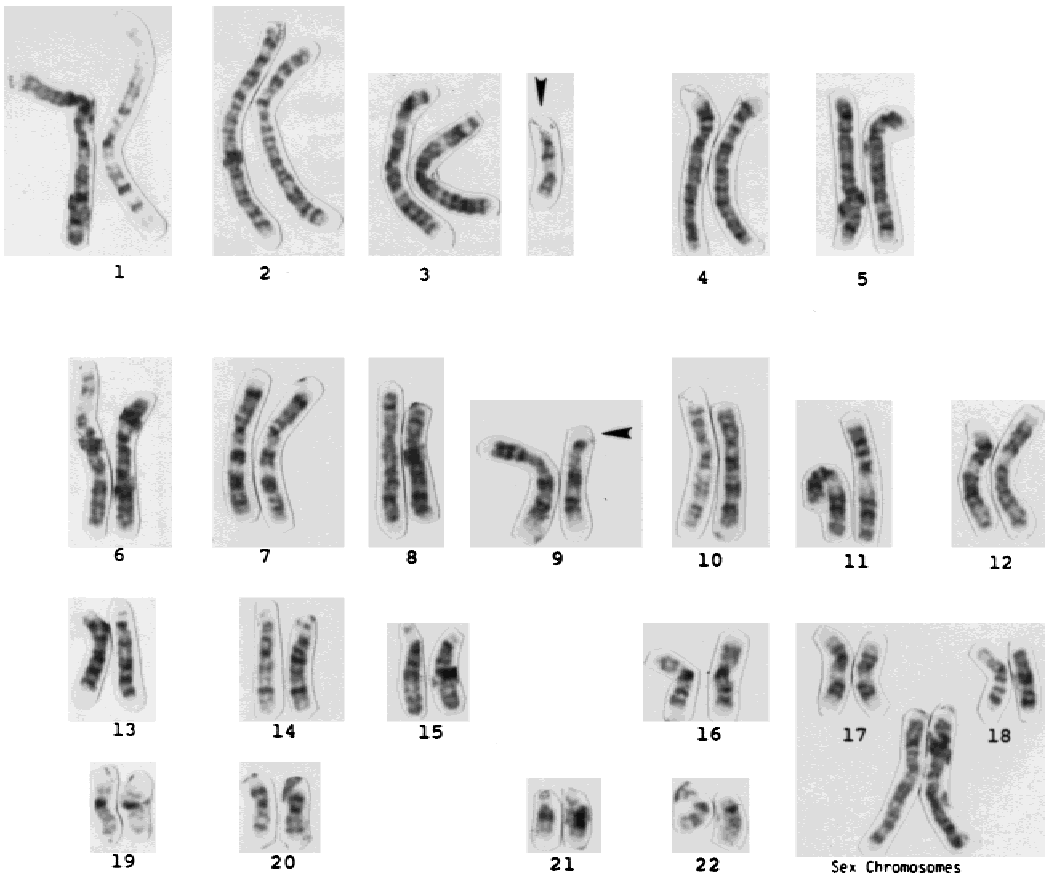

Fig. 2. GTG-banded karyotype from the patient. Arrows indicate the marker and the deleted chromosome 9.

ioplan) equipped with a cooled CCD camera (Photometrics).

RESULTS

Cytogenetic analysis of peripheral blood lymphocytes showed 47 chromosomes in 49 of 50 GTG-banded blood cells and in 16 of 16 CBG-banded cells. Analysis of 50 metaphases from the lymphoblastoid cell line demonstrated the marker in each cell. GTG banding showed a normal chromosome 9, a deleted chromosome 9 missing the segment distal to p12, and a marker chromosome (Fig. 2). The symmetrical banding pattern of the marker suggested an isochromosome derived from 9p, but the marker appeared to lack a primary constriction and was C-band negative (Fig. 3). The mother's chromosomes were normal, 46,XX, and her chromosome 9 heteromorphism was neither inverted nor large.

The chromosome 9-specific painting probe hybridized to the marker, the deleted 9, and the normal chromosome 9 (Fig. 4a). To confirm that the marker was derived from 9p, two cosmids previously mapped to 9p13, Cos 4 [Brahe et al., 1991] and ICRFC100D11145 [Lehrach et al., 1990] were hybridized to metaphase chromosomes. Neither hybridized to the deleted 9, both hybridized to the normal 9, and each produced double signals on the marker (Fig. 4b, c). These results confirmed the marker origin as 9p and the breakpoint on chromosome 9 as proximal to 9p13. Thus, the nomenclature was written as 47,XX,del(9)(p12),+dup(9)(pter→p12::p12→pter).

The chromosome 9 classical satellite and the beta satellite probes did not hybridize with the marker chromosome but did hybridize to the patient's deleted and normal 9 chromosomes (Fig. 4d, e). An "all human" centromere probe hybridized to all chromosomes except the marker, indicating the absence of alpha satellite sequences (Fig. 4f). These findings suggested that this marker, originating from chromosome 9, which lacked a primary constriction, contained neither alpha nor beta satellite DNA, yet was regularly transmitted in mitosis. Immunological studies for centromere proteins were negative for CENP-B but positive for CENP-C and E, suggesting a functional centromere region.

DISCUSSION

The marker chromosome described in this report originated from the breakage of chromosome 9 at p12 and resulted in a deleted chromosome 9 and duplication of 9p. The duplicated 9p lacks a primary constriction and detectable alpha and beta satellite DNA.

A number of previous studies have described marker chromosomes by cytogenetic and, more recently, by molecular methods. The clinical effects have been quite variable, depending on the particular chromosome involved and the amount of euchromatin contained in the marker. Initial molecular studies of such markers used

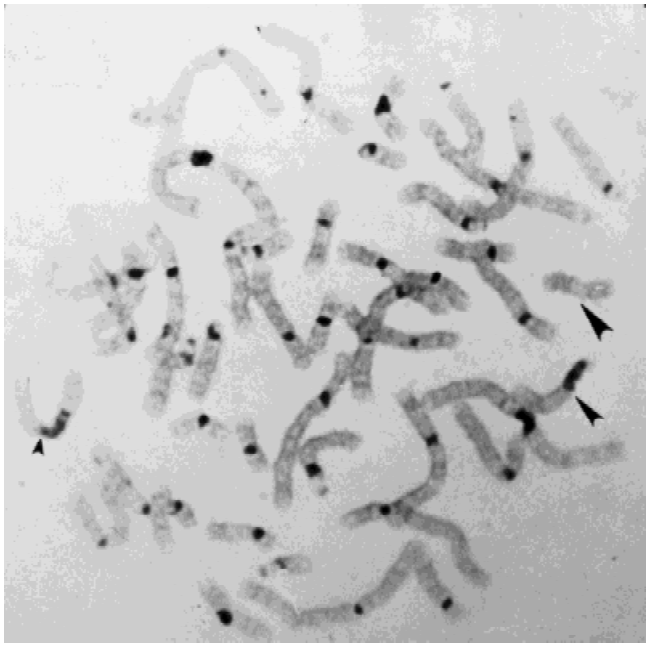

Fig. 3. C-banded metaphase. Small arrow points to the normal 9; medium arrow points to the deleted 9; large arrow to the marker chromosome.

panels of alpha satellite probes to identify the chromosomes of origin. Occasional markers failed to hybridize with any of the probes (Table I). Callen et al. [1992] described a 5-year-old patient with a normal IQ (range 90–109) whose marker chromosome did not hybridize with a panel of DNA probes specific for the pericentric repeats of individual chromosomes or with a probe (RR216) which hybridized to all alphoid sequences. Subsequently, Crolla et al. [1992] reported two cases of such acentrics among 15 supernumerary markers identified in 15 patients. In one case, the marker occurred in mosaic form in a patient having four distinguishable cell lines. Forty percent of the cells were normal (46,XY); 27% contained a small centric marker with a single chromosome 19 centromere signal; and 13% contained a dicentric marker with two chromosome 19 centromere signals. In the remaining 20% of the patient's cells, the mar(19) was replaced by a smaller marker which failed to hybridize with any of the 26 probes of an alphoid library which included DNA from all chromosomes. In the second case of Crolla et al. [1992], a small marker chromosome, detected in amniocytes, failed to hybridize with any of the library probes. The pregnancy was terminated and an autopsy report described an apparently normal fetus.

Magnani et al. [1993] reported a marker chromosome similar to the one described in this paper in a child with developmental delay. In their case, deletion of chromosome 14 at q32.1 occurred along with an acentric marker which was C-band and AgNOR negative. The marker was stable in leukocytes and for at least six months of continual cultivation in a lymphoblastoid cell line. The marker was identified as originating from chromosome 14 using ALU-PCR products generated from a human–mouse somatic cell hybrid containing human chromosome 14. Alphoid probes were not used and the authors hypothesized a complex rearrangement in which the marker acquired a functional centromere.

Two clinically abnormal patients had extra acentric marker chromosomes which were identified as inverted duplications of distal 15q [Blennow et al., 1994]. Libraries constructed from these markers, isolated by flow sorting and subsequent PCR amplification, were used to identify the marker chromosomes by reverse chromosome painting. In both cases, the absence of detectable centromere sequences was confirmed by hybridization with an all-human satellite probe. While each of these markers had a primary constriction, they were mosaic in the peripheral blood and totally disappeared from lymphoblastoid cell lines grown over a period of time, indicating some mitotic dysfunction of the marker.

Alpha satellite DNA appears to be a necessary component of centromere function [Haaf et al., 1992]. Indirect evidence for the presence of functional centromere regions has come from immunofluorescence studies using anticentromere antibodies derived from the sera of human patients with the CREST (calcinosis, Raynaud syndrome, esophageal dismotility, sclerodactyly, and telangiectasia) syndrome of scleroderma. These sera react with centromere proteins, which are associated with centromere DNA and represent components of the kinetochore [Bloom, 1993]. Voullaire et al. [1993] used not only panels of alpha satellite probes and a classical satellite III probe to study an acentric marker derived from chromosome 10, but also performed immunofluorescence studies with antibodies to centromere proteins. The C-band negative marker they described had neither detectable alpha satellite nor satellite III, suggesting absence of a centromere. However, when centromere proteins were studied, CENP-B was absent but CREST antiserum reacted positively, suggesting the presence of centromere proteins. The regular transmission of the marker indicated the presence of a functional centromere.

Similarly, Ohashi et al. [1994] reported a stable acentric marker identified as an inv dup (8) (pter→p23.1::p23.1→pter). The marker was studied by microdissection, PCR amplification, reverse chromosome painting, and cosmid probes localized to 8p23.3 and 8p22 or 23.1. In situ hybridization with alpha satellite and telomere probes was also performed. Despite the absence of detectable alpha satellite sequences, reaction with CREST antiserum was positive.

Other apparently acentric supernumerary markers derived from chromosomes 10, 11, 13, and 15 and lacking both chromosome-specific alpha satellite DNA and pericentromeric satellite DNA have been shown to contain both CENP-C and CENP-E, but not CENP-B [Schwartz et al., 1994, and in preparation]. The abnormality reported in our paper fits into one group of acentric chromosomes delimited by these authors.

Various suggestions have been made to account for the mitotic stability of acentric marker chromosomes. Dutrillaux et al. [1979, 1987] found frequent breakage at sites in human chromosomes that corresponded to positions of centromeres in primate genomes (e.g., 2q22). Thus, Ohashi et al. [1994] suggested an evolu-

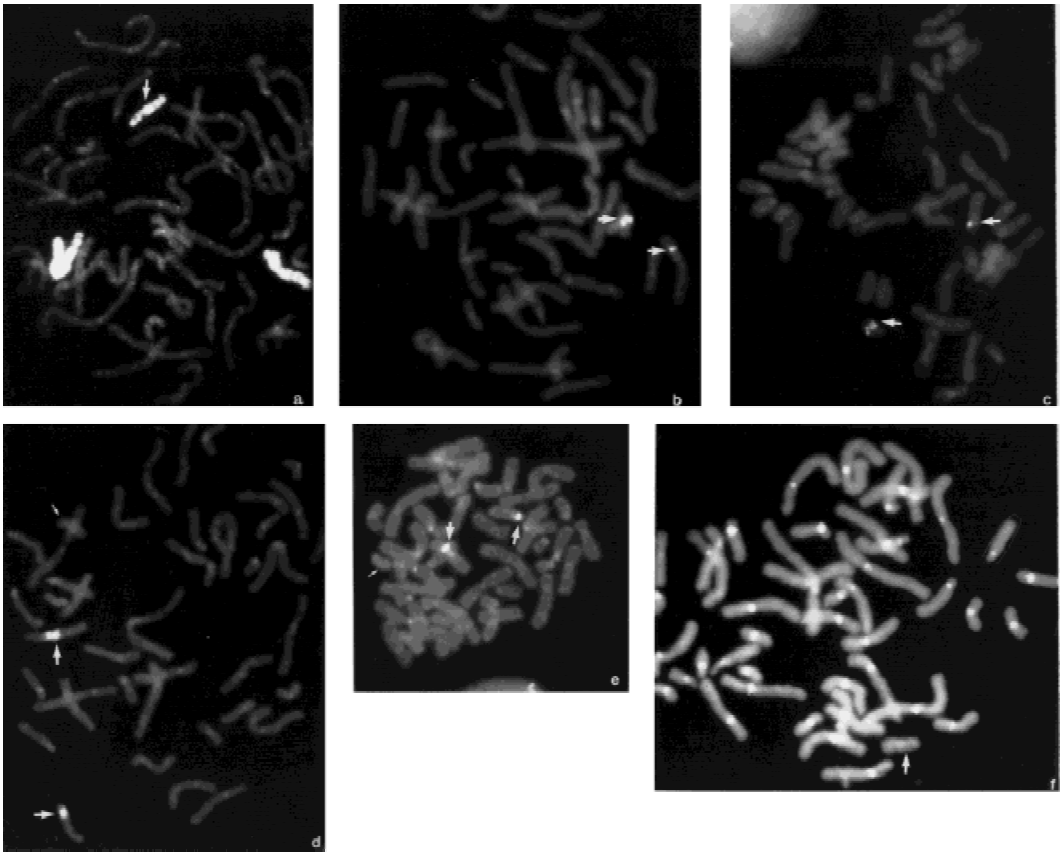

Fig. 4. **a:** Hybridization of the chromosome 9 "coatasome" painting probe to the normal 9, deleted 9, and marker chromosome (arrow). **b:** Metaphase following hybridization with probe Cos 4. Arrows point to double signals on the marker and a single signal on the normal 9. No signal is observed on the deleted 9. **c:** Metaphase following FISH with cosmid ICRFC100D11145 showing identical findings as b. **d:** FISH with a classical satellite probe specific for the pericentric heterochromatin of chromosome 9. The del 9 and normal 9 (large arrows) demonstrate signal, but none is seen on the marker chromosome (small arrow). **e:** Signals representing hybridization of the chromosome 9 beta satellite probe are seen on the normal and deleted 9 chromosomes (large arrows). A signal is not observed on the marker chromosome (small arrow). Some background hybridization to acrocentric chromosomes is also observed. **f:** Metaphase following hybridization of the all human chromosome centromere probe. All chromosomes demonstrate signal except the marker (arrow).

tionarily ancient centromere may be reactivated in acentric markers. Voullaire et al. [1993] propose activation of a latent intercalary centromere, citing as evidence the detection of weakly hybridizing areas of alpha satellite DNA in human chromosome 2q21 and 9q13 [Aleixandre et al., 1987; Baldini et al., 1991]. Brown and Tyler-Smith [1995] consider the possibility of transposition of a functional centromeric sequence.

TABLE II. Mosaicism and Clinical Abnormalities in Patients With Acentric Markers

Cytogenetic findings	Clinical abnormalities	Reference
47,XY,+mar1(19)/47,XY,+mar2(19)	Developmental delay	Crolla et al. [1992]
47,XY,+mar3(19)/46,XY (case 11)	Congenital abnormalities	
47,XX,+mar(?origin) (case 15)	Terminated pregnancy, normal exam on necropsy	Crolla et al. [1992]
47,XX,+mar/46,XY	Normal	Callen et al. [1992]
48,XY,−10,+r del(10)(p12.2 → q23.3),+mar,del(10) (pter → 12.2::q23.3 → qter),+bisatellited mar	Developmental delay, small, no congenital abnormalities	Voullaire et al. [1993]
47,XY,+inv dup(15)(qter → q23::q23 →qter)/46,XY	Congenital abnormalities Delayed development	Blennow et al. [1994]
47,XX,+inv dup(15)(qter → q24::q24 → qter)/46,XX	Minor anomalies Developmental delay	Blennow et al. [1994]
47,XX,+inv dup(8)(pter → p23.1::p23.1 → pter)	Heart defect No congenital anomalies Developmental delay	Ohashi et al. [1994]
47,XX,del(9)(p12) +dup(9)(pter → 13::p13 → pter)	Congenital anomalies Developmental delay	This case and Curtis et al. [1995]

TABLE III. Comparison of Clinical Findings

Craniofacial	Monosomy 9p	Trisomy 9p	Present case
Head	Trigonocephaly	Brachycephaly	Plagiocephaly/ brachycephaly
		Bitemporal narrowing	Bitemporal narrowing
Forehead	Prominent	Flat	Frontal bossing/sloping forehead
Eyes	Upslanting palpebral fissures	Downslanting palpebral fissures	Slight upslanted palpebral fissures
	Exopthalmos	Endopthalmos	Normal
	Epicanthal folds	Epicanthal folds	Epicanthal folds
	Hypertelorsm	Hypertelorsm	Hypertelorism
Ears	Low-set/malformed	Low-set/malformed large, protruding	Low-set, large, protruding
Nose	Flat nasal bridge antiverted nares	Broad nasal bridge, fleshy tip, antiverted nares	Fleshy tip and antiverted nares
Mouth	Small	Large, downturned corners of mouth	Downturned corners of mouth
Skeletal	Long fingers	Hyperextensive, short, hypoplastic phalanges	Hyperextensible fingers
			Brachydactyly
	Proximally-placed thumbs		Clinodactyly/short 5th finger
	Hyperconvex nails	Hypoplastic nails	Hypoplastic nails
Dermatoglyphics	Simian creases	Simian creases	Simian creases
Central nervous system	Psychomotor retardation	Psychomotor retardation	Global developmental delay

They also point out that activation of an additional centromere sequence might result in a functional dicentric chromosome which could break or misdivide to produce two fragments, each with a functional centromere.

Centromere activation is not unknown in other organisms [Brown and Tyler-Smith, 1995]. It was induced experimentally in segmented chromosomes of *Schizosaccharomyces pombe* [Steiner and Clarke, 1994]. The classic example of centromere activation is the functional neocentromere formation from a heterochromatic knob of chromosome 10 in corn [Rhoades, 1978]. This evidence, together with alpha satellite hybridization at 9q13 [Aleixandre et al., 1987; Baldini et al., 1991], suggest that additional centromere activity may be located in or near the heterochromatin of chromosome 9.

The marker chromosome described in this report most likely resulted from the breakage of one chromosome 9 at p12 producing a deleted 9 and an acentric marker. The rearrangement could have involved sister chromatids of 9p. However, breakage of chromosome 9 appears to occur most frequently in the 9q1 region [Mamuris et al., 1991]. Thus, it is possible that breakage could have occurred in the short arm of a chromosome 9, bearing an inversion of the 9qh heterochromatic segment. Perhaps the father of our patient had one chromosome 9 with such an inversion. In this case, the deleted chromosome 9 bearing a centromere and an acentric fragment would result. Duplication of the fragment and activation of quiescent centromere sequences might have occurred at the next DNA replication to produce the marker chromosome.

Previously reported patients with acentric marker chromosomes have been either normal or developmentally delayed with or without minor abnormalities. Cytogenetically they may have mosaicism for a normal cell line and the marker, or even in rare instances [Voullaire et al., 1993; Ohashi et al., 1994; this report] have a stable marker detected in all cells analyzed (Table II).

Trisomy of chromosome 9p is a well-described syndrome [Centerwall et al., 1975; Schnizel, 1979]. Common clinical findings present in the syndrome and identified in this patient include brachycephaly, hypertelorism, broad nasal bridge with a bulbous tip, cupped ears, short fingers and toes with hypoplastic nails, and developmental delay. The patient's overall phenotype, especially the facial and skeletal findings, are representative of the phenotype of the trisomy 9p syndrome. Comparison of the abnormalities reported for monosomy and trisomy 9p [Funderburk et al., 1979; Hernandez et al., 1979; Huret et al., 1988] with those found in this case (Table III) support the absence of mosaicism for a cell line with monosomy 9p. In addition, this patient lacked skin pigmentation abnormalities often seen in individuals with mosaic karyotypes [Thomas et al., 1989]. Thus, the cytogenetic and immunological studies and the clinical findings indicate regular segregation of the duplicated 9p, even though alpha satellite sequences could not be demonstrated.

ACKNOWLEDGMENTS

The authors acknowledge the technical assistance and expertise of Jennifer Verbrugge, Ellen Shaw, and Theresa Depinet.

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