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Identification of an Unusual Marker Chromosome by Spectral Karyotyping

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We ascertained a newborn girl with multiple congenital anomalies including severe hypotonia, cardiovascular defects, hearing loss, central nervous system anomalies, and facial anomalies. The infant died at 12 days. Cytogenetic analysis showed a de novo supernumerary marker chromosome. Fluorescence in situ hybridization (FISH) with a combination of chromosome specific alpha-satellite probes and an all-human centromere probe failed to show hybridization to the marker, indicating that the marker chromosome lacked detectable alpha satellite sequences. Spectral karyotyping (SKY) was performed and showed that the marker was chromosome 15 in origin. This was confirmed by FISH with a 15q specific subtelomeric probe, which showed hybridization to both ends of the marker chromosome. Based on FISH information and G-banding pattern, the marker was determined to be an inverted duplication of 15q25-qter, leading to partial tetrasomy for chromosome 15. Although the marker chromosome lacked detectable centromeric alpha-satellite sequences, it seemed to have a functional centromere as it is mitotically stable. This observation is consistent with previous studies on acentric marker chromosomes, which suggested that the DNA sequence at the breakpoint could function similarly to alpha-satellite sequences once activated through marker formation. Am. J. Med. Genet. 80:368–372, 1998. © 1998 Wiley-Liss, Inc.

KEY WORDS: marker chromosome; alpha-satellite sequence; centromere; fluorescence in situ hybridization; spectral karyotyping; tetrasomy of chromosome 15

INTRODUCTION

Supernumerary marker chromosomes occur approximately 0.1 to 0.3/1000 livebirths [Friedrich and Nielson, 1974; Buckton et al., 1985]. With the development of FISH technology, the chromosomal origin of the marker chromosomes can be determined by performing hybridizations with chromosome specific centromere or painting probes. However, to identify the chromosome origin of the marker, multiple hybridization with probes for each human chromosome may be needed. A new technology, spectral karyotyping (SKY) has been recently developed [Schröck et al., 1996]. SKY allows detection of all 24 human chromosomes in one hybridization and has been found to be very useful in characterizing chromosome aberrations in cancer and clinical cytogenetics [Veldman et al., 1997; Schröck et al., 1997].

We report on a case in which a supernumerary marker chromosome was found in a newborn girl with multiple congenital anomalies. The marker lacked of detectable alpha-satellite sequences and was identified as chromosome 15 in origin by SKY. We discuss the phenotype associated with partial tetrasomy of chromosome 15 caused by the marker chromosome.

CLINICAL REPORT

The index-case, a newborn girl, was born at term to a G1 mother. Birth weight and length were appropriate to the gestational age. Her nasal tip was flattened and broad. The ear helices were short and protruding with a flattened superior helix obliterating the antihelical groove. The left zygomatic arch was prominent contributing to facial asymmetry. The upper lip was thin with

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a long philtrum. The labia majora was hypoplastic. The skeletal findings included incomplete hip abduction, decreased range of motion of fingers, and absent flexion creases of the proximal interphalangeal joints. She was markedly hypotonic. Cyanosis lead to the discovery of aortic arch hypoplasia, severe coarctation of the aorta distal to the takeoff of the left subclavian artery, a bicuspid hypoplastic aortic valve, dysplastic mitral valve, and a moderate ventricular septal defect. Magnetic resonance imaging of the brain demonstrated a Dandy-Walker variant with absence of the inferior portion of the cerebellar vermis and small cerebellar hemispheres. The cavity of the cerebrospinal fluid was continuous with a roofless fourth ventricle. The lateral ventricles were moderately enlarged, and the third ventricle was mildly enlarged. There was a generalized delayed development of the cerebral gyral pattern with incomplete operculation. The BAER (brainstem evoked response) showed severe hearing loss. The infant died at 12 days of age, and the parents declined autopsy.

CYTOGENETIC AND FISH STUDIES

A peripheral blood specimen was collected at age 2 days. Lymphocyte culture, GPG, and CBG banding were performed using standard methods.

Fluorescence in situ hybridization (FISH) with chromosome-specific alpha-satellite probes was performed with a modification of the cross-hybridization protocol of Plattner et al. [1993]. Probes for the centromeres of chromosomes 17(D17Z1), 18(D18Z1), 13/21(D13Z1/D21Z1), and 1,5,19(D1Z7/D5Z2/D19Z3) were hybridized under standard conditions, followed by a less stringent posthybridization wash in 2 × SSC for 5 min at room temperature. The less stringent 2 × SSC wash allows for cross-hybridization to other chromosomes. The intensity and pattern of cross-hybridization to the marker chromosome narrow the choices for a positive identification, which is confirmed with a subsequent hybridization and washes performed at the manufacturer recommended conditions (higher stringency).

As the marker failed to hybridize to any of the probes, FISH with an all human centromere probe (Oncor, Gaithersburg, MD) was performed. SKY was performed, as described by Schröck et al. [1996], to identify the marker chromosome. FISH with a chromosome 15q-specific subtelomeric probe [the National Institutes of Health and Molecular Genetics Institute Collaboration, 1996] was also performed using standard protocol.

RESULTS

Cytogenetic analysis demonstrated a 47,XX,+mar karyotype in all 20 cells examined. The marker appeared to be metacentric with a primary constriction, and the banding pattern did not suggest a chromosomal origin (Fig. 1). CBG-banding showed negative staining on the marker chromosome. Parental blood chromosome analysis did not show this marker chromosome. In an attempt to identify the marker, FISH was performed with a combination of chromosome specific alpha-satellite sequence probes. When the marker failed to hybridize to any of the probes, an all human centromere probe was used. In this case, no hybridization was seen. The lack of hybridization signals suggested that the marker chromosome is devoid of detectable alpha-satellite sequences.

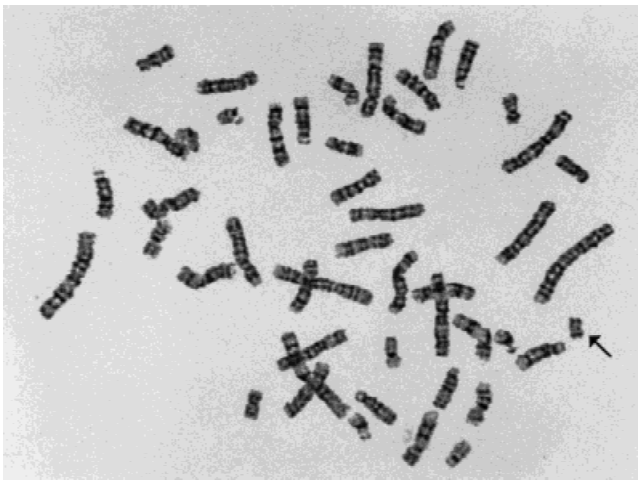

Fig. 1. G-banding metaphase spread. The marker chromosome is indicated by an arrow.

In order to determine the origin of the marker chromosome, SKY was performed and led to the identification of the marker chromosome as chromosome 15 in origin (Fig. 2). Subsequent FISH analysis was performed with a 15q subtelomeric probe and showed hybridization to both ends of the marker chromosome (Fig. 3), thus confirming the results from SKY. The chromosome 15 specific centromeric probe (D15Z) failed to hybridize to the marker chromosome. Based on the FISH and SKY results, a reinterpretation of the G-banding pattern suggested the marker to be an inverted duplication of 15q25-qter.

DISCUSSION

Although tetrasomy of proximal chromosome 15q is commonly seen in the inv dup(15) (psu dic(15)) cases, tetrasomy of the distal region of chromosome 15q is rare. Clinical findings of mosaic tetrasomy of distal 15q were reported previously in three cases by Blennow et al. [1994] and Van den Enden et al. [1996]. Similar to the present case, the tetrasomy was because of a marker chromosome that was composed of inverted duplication of distal chromosome 15q. A summary and comparison of the anomalies of this patient and the three reported cases with mosaic tetrasomy of distal 15q are listed in Table I. Compared with the two cases reported by Blennow et al., the facial anomalies of the present patient seemed to be less prominent, possibly because of young age. However, the systemic defects observed in our patient were far more severe, with the involvement of cardiovascular and central nervous systems. This patient died at age 12 days, whereas these two patients survived. The severity of the condition of our patient is more like that of the case reported by Van den Enden et al. The difference in severity may be explained by the differential distribution of the mosaicism in the reported cases.

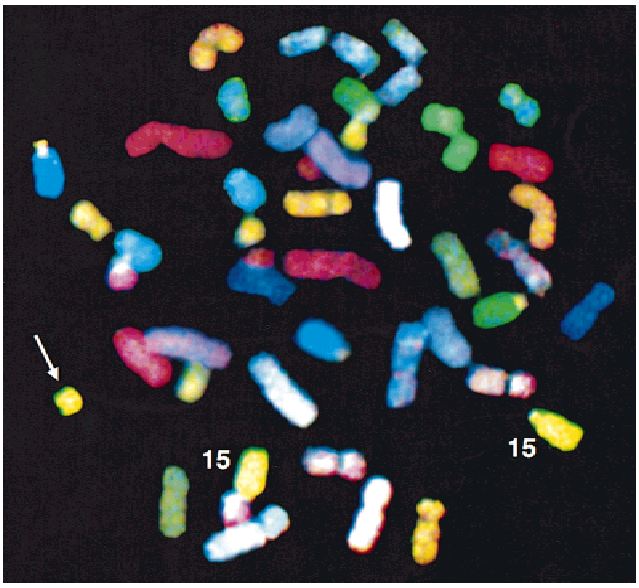

Fig. 2. Spectral karyotyping. The marker chromosome is showed with an arrow, and two normal chromosomes 15 are also indicated. The metaphase spread is shown in display color by assigning red, green, and blue colors to specific spectral ranges to convert the emission spectra of the painting probes for visualization. Spectra-based classification using a SKY software led to definitive chromosome identification.

Centromeres of human chromosomes are known to contain a large copy number of alpha-satellite sequences. Studies have shown that these sequences play an essential role in centromere function [Haaf et al., 1992; Tyler-Smith et al., 1993; Larian et al., 1994; Harrington et al., 1997]. However, the marker chromosome in our patient is stable, although without any apparent alpha-satellite sequences. In fact, marker chromosomes lacking a detectable centromere have been reported previously [Callen et al., 1991, 1992; Crolla et al., 1992; Blennow et al., 1994; Daniel et al., 1994; Gravholt and Friedrich, 1995; Sacchi et al., 1996; Van den Enden et al., 1996; du Sart et al., 1997; Depinet et al., 1997]. The mechanism for the stability of these marker chromosomes has been hypothesized to be through activation of latent centromeric sequences. Sacchi et al. [1996] reported on an inverted duplication of the long arm of chromosome 14 without detectable alpha-satellite sequences and demonstrated the presence of a very low copy number of DNA sequences that shared homology to the centromeric sequence of chromosome 18 (D18Z1). du Sart et al. [1997] reported a chromosome 10-derived marker chromosome devoid of alpha-satellite repeats and showed that a latent, non-alphoid DNA sequence from chromosome band 10q25 could function as a neocentromere that bound anticentromere antibodies. Depinet et al. [1997] studied a series of marker chromosomes lacking detectable alpha-satellite sequences and demonstrated centromere activity in all cases. These studies suggest that other regions in human chromosomes contain latent DNA sequences that can potentially function similarly to the alpha-satellite sequences and may be activated through chromosome rearrangements. Evidence for the existence of neocentromere activity was also observed in *Drosophila* [Williams et al., 1998]. Additional investigation of these neocentromeres would further define the functional units of the human centromeres and may lead to progress in the development of human artificial chromosomes.

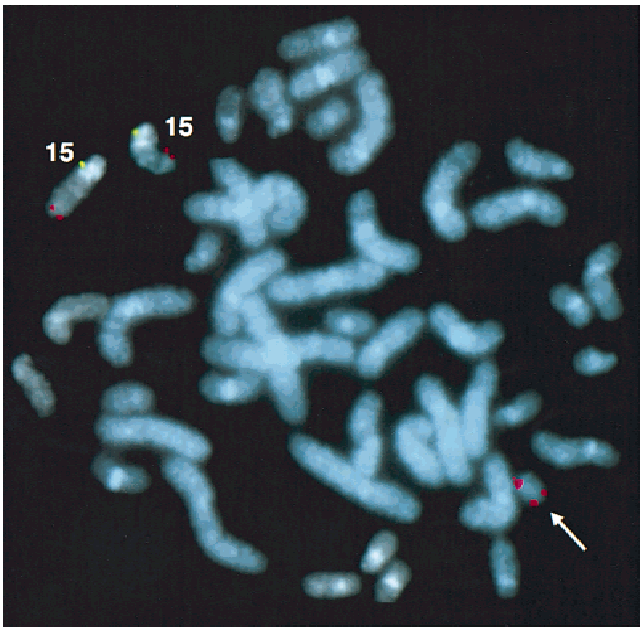

Fig. 3. Characterization of the marker chromosome with a 15q-specific telomeric probe. A chromosome 15 centromeric probe (D15Z), appearing as green (FITC) signals, hybridized to the centromere of both normal chromosome 15 but not to the marker chromosome. A chromosome 15q-specific telomeric probe, appearing as red (spectrum orange) signals, hybridized to the normal chromosome 15s as well as both ends of the marker, confirming that the marker chromosome is chromosome 15 in origin and consistent with an inverted duplication of distal 15q.

TABLE I. Phenotype Comparison Between the Previous and the Present Studies

Clinical findings	Blennow et al. [1994]	Van den Enden et al. [1996]	Present study
Antimongoloid slant	+	–	–
Bulbous nose	+	+	–
Broad nasal bridge	–	+	+
Long philtrum	+	+	+
High-arched palate	+	?	–
Micrognathia	+	+	–
Asymmetry of the head	+	+	+
Heart defects	–	+	+
CNS defects	–	–	+
Joint defects	+	+	+
Sensorineural hearing loss	+	?	+

Dicentric inverted duplication of chromosome 15 (psu dic(15)) is the most common supernumerary marker chromosome found in humans. The mechanism for the formation of the dicentric inv dup(15) is thought to be through a U-type exchange between nonsister chromatids in meiosis I, with the loss of the acentric fragment resulting from this exchange [Van Dyke et al., 1997; Schreck et al., 1977]. Blennow et al. [1994] speculated that the acentric fragment may sometimes be retained and lead to the formation of the acentric

marker chromosome described here. Indeed, when the breakpoint is on the distal end of chromosome 15q, the cells with the dicentric inv dup(15) will have tetrasomy of most of the long arm of chromosome 15 and are unlikely to be viable. However, if the rearrangement activates a latent centromere sequence, the acentric fragment could be retained and be relatively stable in mitosis. Depinet et al. [1997] studied three patients with the acentric chromosome 15 markers with DNA polymorphism and found that the markers were all paternal in origin. Two of the three cases were likely to be derived from a mitotic error, and one case was probably the consequence of a meiotic error. Therefore, at least two mechanisms (both meiotic and mitotic errors) might lead to the formation of this acentric inv dup(15). Chromosome rearrangements involving chromosome 15 are relatively common. The reason for this relatively high frequency is thought to be caused by the presence of low copy DNA repeats in different breakpoint regions that are prone to chromosome breakage [Kuwano et al., 1992; Christian et al., 1995; Huang et al., 1997; Wanstrat et al., 1998]. It would be interesting to investigate, once these repeats are isolated, whether some of them can function similarly to the alpha-satellite sequences when activated.

Last, we used SKY to identify a small marker chromosome in this study. SKY has been shown to be a powerful new technique in characterizing chromosome rearrangement in clinical and cancer cytogenetics [Schröck et al., 1996, 1997; Veldman et al., 1997]. It is especially useful in this case as the marker chromosome is devoid of the alpha-satellite sequences and our routine FISH protocol for characterizing marker chromosome using centromeric probes failed to yield signals. The sensitivity of SKY was explored previously by using cell lines with translocations of known sizes. We could establish that at chromosomes with approximately 600-band resolution the detection limit for translocations was in the range of 600 kbp to 1.2 Mbp [Schröck et al., 1996]. In other studies, SKY was shown to be sensitive enough to characterize the chromosomal origin of double minute chromosomes [Knutsen et al., 1997] as well as the origin of small markers [Schröck et al., 1997]. In general, the resolution of SKY is in the same range as any other technique using chromosome painting probes and depends on the quality of the chromosome-painting probes and the condensation of the chromosome preparations. It is clear that the search for marker chromosomes can also be conducted using iterative hybridization with chromosome-specific painting probes. However, the power of SKY is that it serves, like G-banding with which it can be combined [Schröck et al., 1997], as a screening test for fast and one-step identification of chromosomal aberrations. This is, unarguably, of great reassurance in the diagnostic evaluation of chromosomal aberrations in the clinical cytogenetic laboratories.

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