

Chromosome 13q Neocentromeres: Molecular Cytogenetic Characterization of Three Additional Cases and Clinical Spectrum

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We report three new cases of chromosome 13 derived marker chromosomes, found in unrelated patients with dysmorphisms and/or developmental delay. Molecular cytogenetic analysis was performed using fluorescence in situ hybridization (FISH) with chromosome-specific painting probes, alpha satellite probes, and physically mapped probes from chromosome 13q, as well as comparative genomic hybridization (CGH). This analysis demonstrated that these markers consisted of inversion duplications of distal portions of chromosome 13q that have separated from the endogenous chromosome 13 centromere and contain no detectable alpha satellite DNA. The presence of a functional neocentromere on these marker chromosomes was confirmed by immunofluorescence with antibodies to centromere protein-C (CENP-C). The cytogenetic location of a neocentromere in band 13q32 was confirmed by simultaneous FISH with physically mapped YACs from 13q32 and immunofluorescence with anti-CENP-C. The addition of these three new cases brings

the total number of described inv dup 13q neocentric chromosomes to 11, representing 21% (11/52) of the current overall total of 52 described cases of human neocentric chromosomes. This higher than expected frequency suggests that chromosome 13q may have an increased propensity for neocentromere formation. The clinical spectrum of all 11 cases is presented, representing a unique collection of polysomy for different portions of chromosome 13q without aneuploidies for additional chromosomal regions. The complexity and variability of the phenotypes seen in these patients does not support a simple reductionist view of phenotype/genotype correlation with polysomy for certain chromosomal regions.

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INTRODUCTION

The centromere of mammalian chromosomes is responsible for the proper segregation of chromosomes to daughter cells during mitosis and meiosis. Alpha satellite DNA is found at every normal endogenous centromere, and transfection of alpha satellite DNA into human HT1080 cells can result in the de novo formation of centromeres [Harrington et al., 1997; Ikeno et al., 1998]. However, the discovery of neocentromeres on mitotically stable rearranged chromosomal fragments that do not contain alpha satellite DNA suggests that centromere formation may not be strictly

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dependent on primary DNA sequence [Voullaire et al., 1993; Karpen and Allshire, 1997]. Neocentromeres assemble fully functional kinetochores on low or single copy genomic DNA, and thus provide an opportunity to examine the role of DNA sequence in centromere formation in the absence of the large amounts of repetitive DNA that has hindered similar analyses of normal centromeres [Lo et al., 2001a]. Neocentromeres provide mitotic stability to chromosome fragments that have separated from endogenous centromeres and would otherwise be acentric and rapidly lost, usually resulting in partial aneuploidy for chromosomal regions. Here we report three independent cases of neocentromere formation leading to the mitotic stability of inversion duplications of distal portions of human chromosome 13q. This report adds three additional cases to the eight previously reported inv dup13q neocentric chromosomes [Warburton et al., 2000], supporting the conclusion that the long arm of chromosome 13 may have an increased propensity for neocentromere formation.

MATERIALS AND METHODS

Clinical Reports

Case 1. Patient B.C. A female was delivered at term, but was small for gestational age (birth weight = 5.68 lbs, < 3rd centile, occipitofrontal circumference (OFC) of 12.8 in < 2nd centile). Her growth remained poor for the first two years of life, but at four years, her length and OFC were on the 50th centile and weight was greater than the 97th centile. Her unexplained weight gain and mild phenotypic abnormalities lead her local doctor to perform a karyotype analysis. She had few health problems in childhood. At birth she was noted to have mild hip dysplasia, which was managed in a Pavlik harness. She had some mild delay in her early milestones, walking at 17 months and talking at 20 months. At the time of her genetics review she had mild impairment of her fine motor skills, but normal language and gross motor skills and no evidence of intellectual disability. At four years, she was noted to have a mild convergent strabismus. On examination she had a subtle phenotype with bitemporal narrowing, hypotelorism, and persistent nevus flammeus on the nasal bridge. She had a small mouth with tented upper lip and an extra lower incisor. There was fifth finger clinodactyly, and 2/3 syndactyly of the feet. GTL banding showed a small metacentric marker with a symmetrical banding pattern present in 54% of lymphocytes. The parents had normal karyotypes.

Case 2. Patient J.M. A 12-year-old male was referred for karyotyping because of intellectual handicap and multiple dysmorphisms. The blood karyotype showed mosaicism with a SMC present in 14% (7/50) of lymphocytes. At two years, he had a repair for undescended testes and he had been epileptic since that age. At 20 years, he was reassessed and he was seen to be severely retarded and dysmorphic. He had short stature (< 3rd centile), thoracic scoliosis, short broad feet with left talipes equinovarus, finger contractures, hypoplastic interphalangeal creases, single transverse

palmar creases, and ulnar deviation of the wrists. His facial dysmorphism included frontal balding, a prominent nose, prognathism with crowded teeth, bilateral anterior segment dysgenesis of the eyes, and iris synchia. At this time, a fibroblast culture was performed and a large SMC with several prominent GTL bands was detected in 20% of cells. The blood karyotype was repeated and the same marker was seen in 14% of cells.

Case 3. Patient M.G. A six-month-old Caucasian boy was referred because of dysmorphic features. He was delivered one week before term and weighed 7.14 pounds (50th centile) and his length was 20.5 inches (< 50th centile). At birth he had moderate jaundice and hypoglycemia. He had marked nevus flammeus on the left side of the scalp and occipital area. There was an overlying occiput that was asymmetrical. The ears were low-set and showed abnormal ear lobe. Eyes were widely spaced with a cyst in the right eye and cataract in the left. He has since undergone surgery to remove the cyst. His skin showed unusual striations following more or less the skin lines in the back. His development was slightly delayed. At age six months, his weight was 17.5 pounds and length was 26.5 inches (both at 50th centile). His OFC was 17.32 in (50th centile). Chromosome analysis from peripheral blood showed two small marker chromosomes in approximately 12% (3/25) of the cells. The marker chromosomes appeared identical in morphology and were of the size of a G-group chromosome. Special staining techniques, such as CBG and AgNOR, were negative. Fluorescence in situ hybridization (FISH) studies with various alpha satellite chromosome probes for the centromeres, including the chromosomes 13 and 21, were unsuccessful in identifying the origin of the marker chromosomes.

M.G. was re-evaluated on two more occasions, at age 21 months and seven years, respectively. At age 21 months, his growth parameters were normal with height and weight at 25th centile and OFC at 30th centile. In addition to the abnormalities noted at birth, he had developed a seizure disorder. His computed tomography (CT) scan showed an irregular shape of the skull and his electroencephalograph was abnormal. He was on phenobarbital, and according to his parents, showed improvement in motor skills and did not have any seizures. At age seven years, his height was 47 inches (< 50th centile) and his weight was 59 pounds (> 75th centile), and he showed severe developmental retardation. He continued to show the hypopigmented areas on his neck, back, and face, and had a nevus flammeus on the forehead,iltrum and nape of the neck, scalp, and back. Other dysmorphic features present included hypertelorism, bushy eyebrows, ptosis of the right eyelid, and anteverted nares. He had a vagal nerve stimulator implanted in April 2000 to control his seizures. A repeat chromosome analysis from peripheral blood in April 2000 showed 26% of the cells with the two markers.

Molecular Cytogenetic Analysis

FISH and immunofluorescence were performed essentially as described [Alonso et al., in press]. FISH

with commercially available probes, e.g., WCP 13 (Vysis, Downer's Grove, IL), CEP 13/21 (Vysis), LSI RB1 (Vysis), Tel 13q (Vysis), and D13S319/D13S25 (with 13qter sub-telomeric probe control) (Cytocell, Adderbury, Banbury, Oxfordshire, UK), "all centromeres" (pancentromeric), and "all telomeres" (Oncor, Bethesda, MD) was performed according to the manufacturers' directions. Cosmids from chromosome 13q and their map positions were derived from the chromosome 13 physical map available from the Columbia Genome Center (<http://genome1.ccc.columbia.edu/~genome>) [Cayanis et al., 1998]. YACs from chromosome 13q were obtained from Research Genetics (Invitrogen, Huntsville, AL), based on Genethon genetic linkage map of the human genome (<http://www-genome.wi.mit.edu>) [Dib et al., 1996]. DNA used for FISH probes was labeled with biotin or digoxigenin and detected with fluor-conjugated streptavidin or anti-digoxigenin using standard protocols [Alonso et al., 2001].

Immunofluorescence with antibodies to centromere protein-C (CENP-C) were performed on 3:1 methanol:acetic acid fixed chromosomes from primary lymphocytes or Epstein Barr virus transformed lymphoblasts. Rabbit anti-CENP-C was detected with goat anti-rabbit IgG. Chromosomes were counterstained with DAPI (blue). Photomicrographs were obtained on a Nikon Eclipse 800 fluorescent/phase microscope equipped with a Sony DKC 5000 camera. Each image was collected separately using single pass filter sets (Chroma, Brattleboro, VT), and separate RGB color channels were merged using Adobe Photoshop. Some FISH images were viewed with a Zeiss Axioscope utilizing digital capture and analysis on a Cytovision™ Chromofluor workstation (Applied Imaging Corp, Santa Clara, CA).

Comparative genomic hybridization (CGH) analysis was performed as described [Levy et al., 1998]. CGH images were captured with an ImagePoint cooled CCD video camera (Photometrics, Tucson, AZ) through a Labophot-2A fluorescence microscope (Nikon, Melville, NY). Chromosome identification and karyotyping was facilitated by counterstaining with 0.5 µg/ml DAPI in Vectashield (Vector Laboratories, Burlingame, CA). An upper threshold of > 1.20 was used to define a gain of chromosomal material, while a lower threshold of < 0.80 was used to interpret a loss (deletion) of chromosomal material. Gains and losses were only considered relevant if the 95% confidence intervals (data not shown), derived from the combination of each single chromosome profile, met the threshold criteria. Digital image analysis was performed using a Cytovision™ Probe system and CGH software.

RESULTS

Molecular Cytogenetic Analysis

Three unrelated patients were referred for cytogenetic analysis due to dysmorphism and/or developmental delay. Chromosome analysis of cultured lymphocytes by GTL or GTG banding showed that patient M.G. had two small (G group size) supernumerary chromosomes in ~26% of metaphases, patient B.C. had one small metacentric supernumerary chromosome in 54% of

metaphases, and patient J.M. had a large metacentric supernumerary chromosome in 14% of metaphases. Each of these marker chromosomes was observed to be C-band negative and to show a symmetrical G-banding pattern (Fig. 1A). A WCP array (Cytocell) was used for patient B.C. and M-FISH was used for patient M.G. in order to identify the chromosome 13 origin of the smaller markers. The symmetrical distinct GTL banding pattern of the larger marker from patient J.M. was used to deduce the chromosome 13 origin. The chromosome origin of all three marker chromosomes was subsequently confirmed by FISH with WCP13 (Vysis) (Fig. 2).

Based on the negative C banding and symmetrical GTL or GTG banding morphology of these marker chromosomes (Fig. 1A), they were suspected to be anaphoid inverted duplications with neocentromeres. The absence of detectable alpha satellite DNA on each of these markers was confirmed by the negative FISH signal with chromosome 13 specific alpha satellite DNA (shown for patient M.G. in Fig. 2A) and/or a pancentromeric alpha satellite DNA probe (shown for patient B.C. in Fig. 2B). The all-telomeres probe (Vysis) or the 13qter (subtelomeric) probe (Cytocell) were used to show that there were conventional telomeres on each end of the markers despite the absence of a conventional centromere (data not shown). The presence of a functional kinetochore on these chromosomes was confirmed by immunofluorescence with antibodies to CENP-C (shown for patient J.M. and M.G. in Fig. 2C and D, respectively).

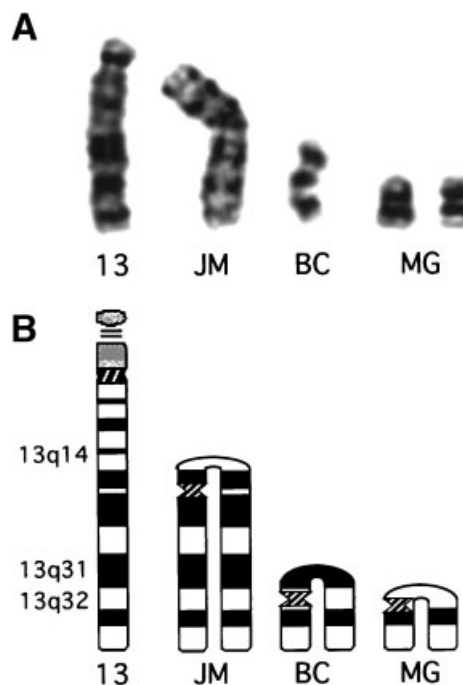


Fig. 1. Ideograms and GTL banded inv dup 13q chromosomes. **A:** GTL (M.G.) and GTG (J.M., B.C.) banded marker chromosome(s) seen in the karyotype of each patient, relative to a normal chromosome 13 (from patient M.G.) for comparison. **B:** Ideograms of marker chromosomes, which were shown by molecular cytogenetic analysis to be inverted duplication 13q chromosomes, indicating the position of inversion breakpoint relative to a normal chromosome 13, and the position of the neocentromere.

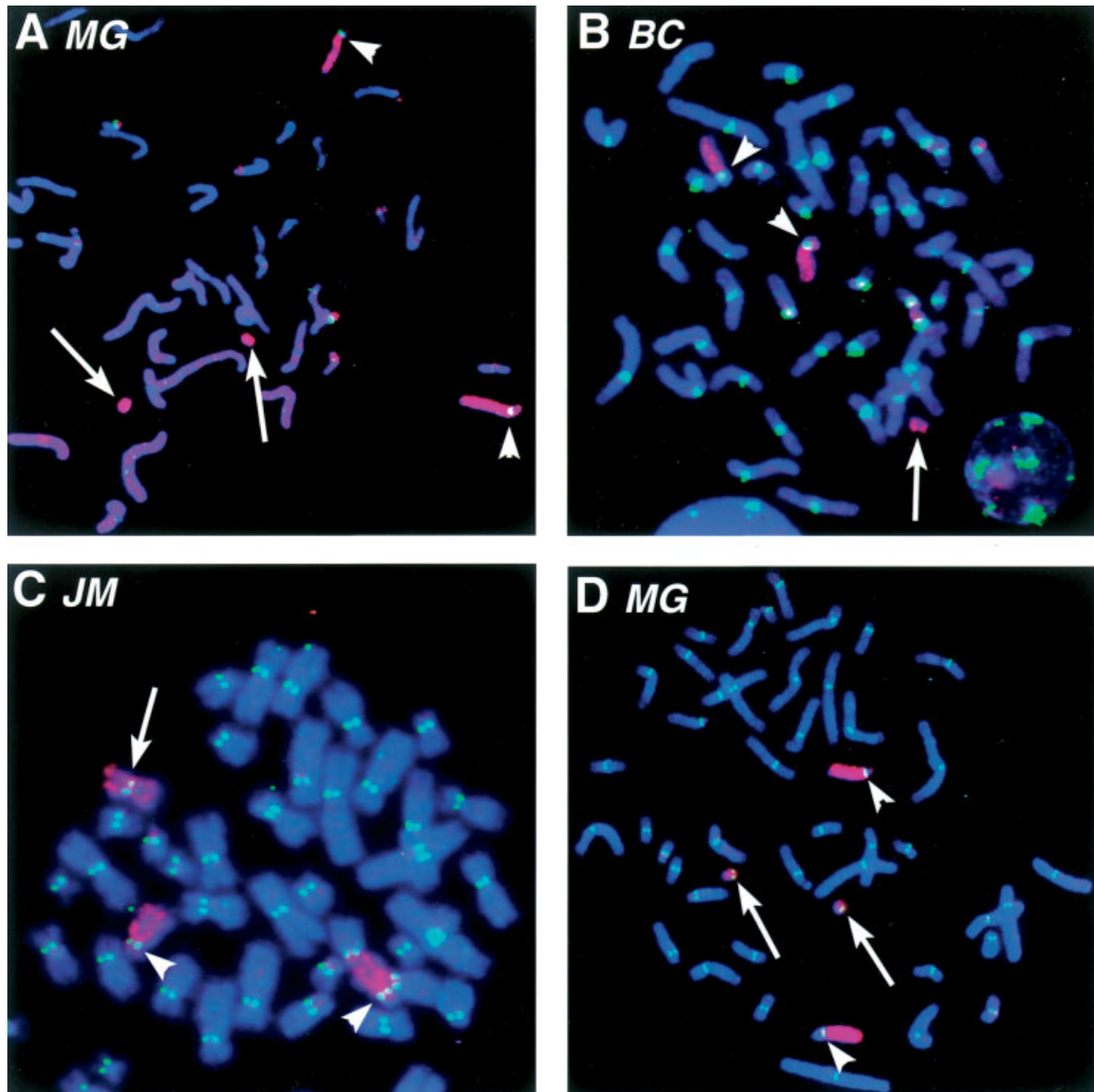


Fig. 2. Molecular cytogenetic analysis of marker chromosomes. **A:** Metaphase from patient M.G. Chromosome 13/21 specific alpha satellite DNA (green) is not found on the two marker chromosomes (white arrows), but is found on the normal chromosomes 13 (white arrow heads), as well as the two chromosomes 21. **B:** Metaphase from patient B.C. The all alpha satellite DNA FISH probe (green) is not found on the marker chromosome (white arrow), but is found at every centromere, including the normal chromosomes 13 (white arrow heads). **C:** Metaphase from patient J.M. CENP-C (green) is found at every centromere, including the marker chromosome (white arrow) and the normal chromosomes 13 (white arrow heads). **D:** Metaphase from patient M.G. CENP-C (green) is found at every centromere, including the marker chromosomes (white arrows) and the normal chromosomes 13 (white arrow heads). A–D: Marker chromosome(s) and chromosomes 13 identified with whole chromosome paint (wcp) 13 (red). Chromosomes counterstained with DAPI (blue).

The positional origin of these chromosomes was further investigated by using CGH (Fig. 3A and B), where patient and normal reference genomic DNA are differentially labeled and hybridized to normal metaphase chromosomes [Levy et al., 1998, 2000]. For the markers from both B.C. and M.G., CGH showed the origin to be from the distal end of chromosome 13q (Fig. 3A and B). CGH was not possible for the marker in J.M. due to its presence in a low percentage of cells, but hybridization of the specific 13qter (subtelomeric probe)(Cytocell) to both ends of the marker in patient J.M. confirmed its

assignment as an inversion duplication derived from chromosome 13q (Fig. 3C). The inverted duplication in M.G. was confirmed by hybridization of the specific 13qtel probe (Vysis) to both ends of the marker chromosome (Fig. 3D). The inverted duplication in B.C. was confirmed using YAC probes from 13q32 (e.g., Fig. 4B), which hybridized to both ends of the chromosome on either side of the central breakpoint.

The position of the breakpoint in these chromosomes was confirmed by FISH with physically mapped cosmids from 13q [Cayanis et al., 1998]. The presence

of cosmid 109d12 (STS D131723) and absence of cosmid 176a11 (STS D13S1582) on the marker chromosome from patient M.G. (data not shown) confirmed the position of the breakpoint to within proximal 13q32, slightly distal to the breakpoint in the previously published inv dup13qter → 13q32 cell line 13f [Warburton et al., 2000]. (The ordered positions within chromosome 13q of the cosmids used in this report and in Warburton et al. [2000] are obtained from the physical map of chromosome 13 [Cayanis et al., 1998]). The presence of cosmid 140f10 (STS D13S1642) and absence of cosmid 120h9 (STS D13S1640) on the marker chromosome from patient B.C. (data not shown) confirmed the position of the breakpoint to within 13q31 [Cayanis et al., 1998], slightly distal to the breakpoint in the previously published inv dup13qter → 13q31 cell line 13e. The absence of both the Rb1 locus (data not shown) and D13S319/D13S25 probe (at 13q14.3) on the marker from patient J.M. (Fig. 3C), combined with the GTL banding profile of this chromosome (Fig. 1), confirmed that the breakpoint is in distal 13q14.3, distal to the breakpoint in the previously published inv dup13qter → 13q14 cell line 13a [Warburton et al., 2000; Morrissette et al., 2001].

Thus, these molecular cytogenetic analyses demonstrate that the marker chromosome in patient M.G. is an inv dup(13)(qter → q32::q32 → qter), in patient B.C. is an inv dup(13)(qter → q31.3::q31.3 → qter), and in patient J.M. is an inv dup(13)(qter → q14.3::q14.3 → qter) (see Fig. 1B).

Localization of Chromosome 13q Neocentromeres

In order to confirm the position of the neocentromere in the marker from patient B.C., FISH with physically mapped YACs from 13q32 and simultaneous immunofluorescence with antibodies to CENP-C was performed on an EBV-transformed lymphoblast line. FISH using YAC 805f7, which is found in proximal 13q32 [Dib et al., 1996; Cayanis et al., 1998], showed a single large signal that is very near the inversion breakpoint, and has therefore not resolved sufficiently to see the individual duplicated arms (Fig. 4A). CENP-C did not colocalize with the YAC 805f7 FISH signal, but instead was slightly telomeric to the inversion breakpoint on one of the duplicated arms (Fig. 4A). In contrast, FISH using YAC 950d10, which is found in distal 13q32, showed

two well separated signals on either duplicated arm (Fig. 4B). CENP-C did not colocalize with the YAC 950d10, but instead was slightly proximal (towards the inversion breakpoint) to one of the FISH signals. These results demonstrate that the neocentromere in the marker from patient B.C. does not colocalize with the inversion breakpoint, but instead is between YACs 805f7 and 950d10 in 13q32.

In patient M.G., the position of the neocentromere was determined by molecular cytogenetic analysis, which was limited by available patient material and the high degree of mosaicism. Analysis of the marker chromosome in patient M.G. (Fig. 3D) showed that immunofluorescence signals with antibodies to CENP-C are proximal to FISH signals with 13q telomere probes. The CENP-C signals on the marker from patient M.G. are consistently off-center and distal to the inversion breakpoint in proximal 13q32 (Figs. 1D and 2C), suggesting that the neocentromere is in distal 13q32. The position of the neocentromere in the marker from J.M., as judged by the position of the constriction on GTL banded chromosomes (Fig. 1A) and immunofluorescence with CENP-C (Fig. 2C), is also off-center and not colocalized with the inversion breakpoint, but instead appears to be within band 13q21.

Clinical Spectrum of 13q Neocentromere Patients

The detailed clinical features of the eight other patients with 13q neocentromeres reported in Warburton et al. [2000], with the three additional cases reported here, bring the total to 11 neocentromere-containing inv dup 13q chromosomes. This unique set of patients permit analysis of the clinical manifestations of pure polysomy for different portions of chromosome 13q, without any contribution from aneuploidy for any other chromosomes, as is usually the case with chromosomal deletions or translocations [Tharapel et al., 1986]. These patients can be grouped into a more proximal (q14/q21 → qter, $N=5$, trisomy or mosaic tetrasomy) and more distal (q31/q32 → qter, $N=6$, mosaic tetrasomy and/or hexasomy) imbalances (Table I). Several specific clinical features were observed within the more distal group (q31/32 → qter), where five of six cases exhibit hemangiomas, five of six have seizures, two of six have coloboma, and two of six have microphthalmia, all of which are seen in greater than 50% of trisomy 13

Fig. 3. Analysis of the chromosomal position of marker chromosomes. **A:** CGH analysis of the patient M.G. Partial ideograms and CGH profiles showing a gain on chromosome 13q from 13qter to 13q32. **B:** CGH analysis of the patient B.C. Partial ideograms and CGH profiles showing a gain on chromosome 13q from 13qter to 13q31. **A** and **B:** The lines in the CGH profiles represent ratios of (from left to right) 0.5, 0.75, 1.0, 1.25, and 1.5. A ratio of 1.0 represents the balanced state of the chromosomal copy number. CGH results are displayed with representative chromosome profiles; all other chromosomes had normal CGH profiles. **C:** The D13S319/D13S25 probe (red) from 13q14 does not hybridize to the inv dup13q14 chromosome in patient J.M. (white arrow), but does hybridize to the normal chromosomes 13 (white arrow heads). The 13q subtelomeric probe (green) hybridizes to both ends of the inv dup 13q14 chromosome, as well as the normal chromosome 13q. **D:** The tel13q FISH probe (red) hybridizes to both ends of the marker chromosomes from patient M.G. (white arrows), as well as to one end of the normal chromosomes 13 in the cell (white arrow heads). CENP-C (green) localizes near one end of the invdup13q32 chromosome between the tel13q FISH probes. The tel13q probe is observed to cross hybridize to several additional chromosomal locations.

Fig. 4. Localization of the 13q32 neocentromere in patient B.C. **A:** YAC 805f7 (red), which maps to proximal 13q32, hybridizes as a single signal near the inversion breakpoint in the marker chromosome (white arrow). CENP-C (green) localizes distal to the inversion breakpoint in one of the duplicated arms. Normal chromosomes 13 indicated by white arrow heads. **B:** YAC 950d10 (red), which maps to distal 13q32, hybridizes to both ends of the marker chromosome in each duplicated arm (white arrow). CENP-C (green) localizes proximal to YAC 950d10 in one of the duplicated chromosome arms. Normal chromosomes 13 indicated by white arrow heads.

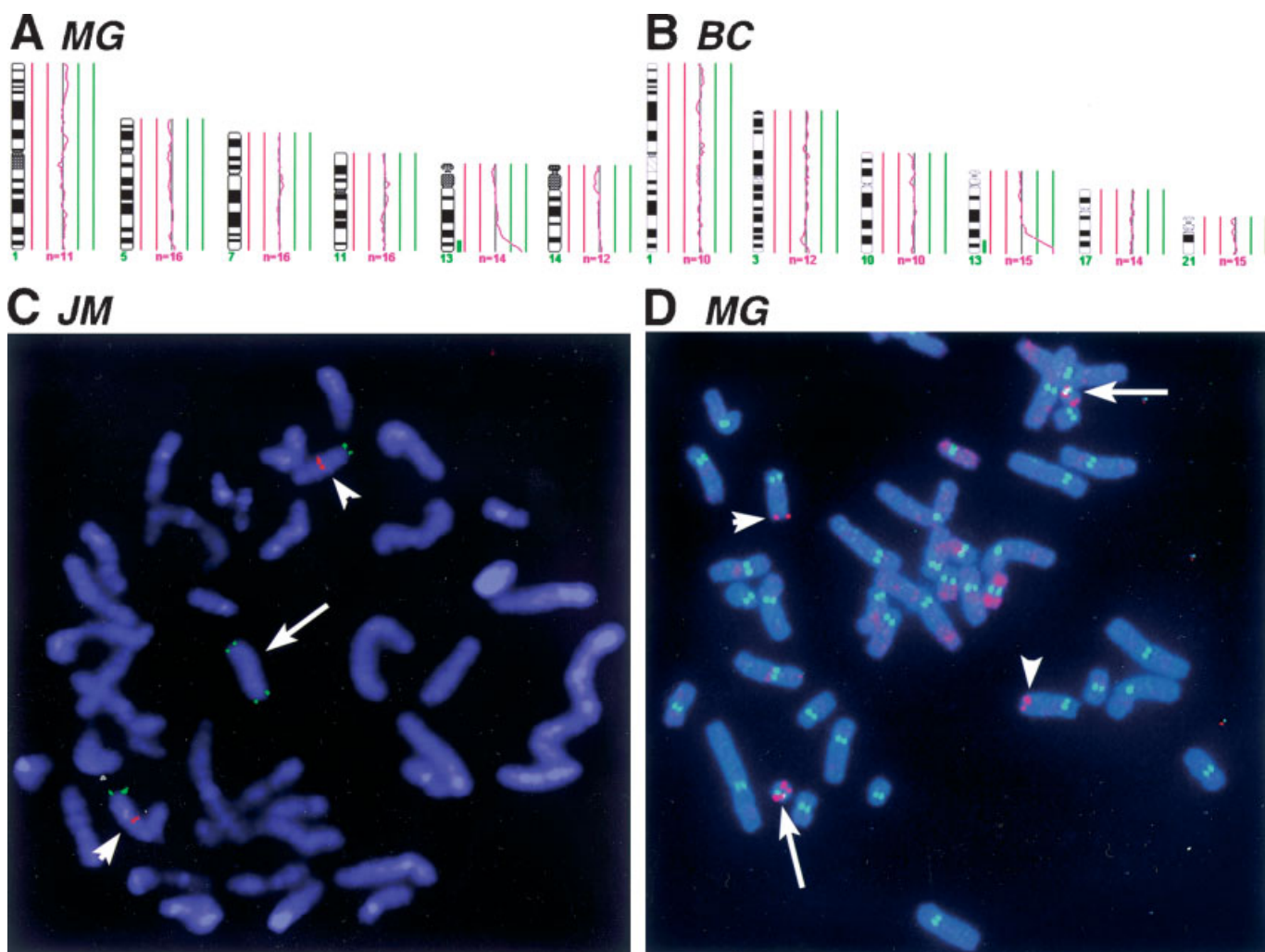


Fig. 3.

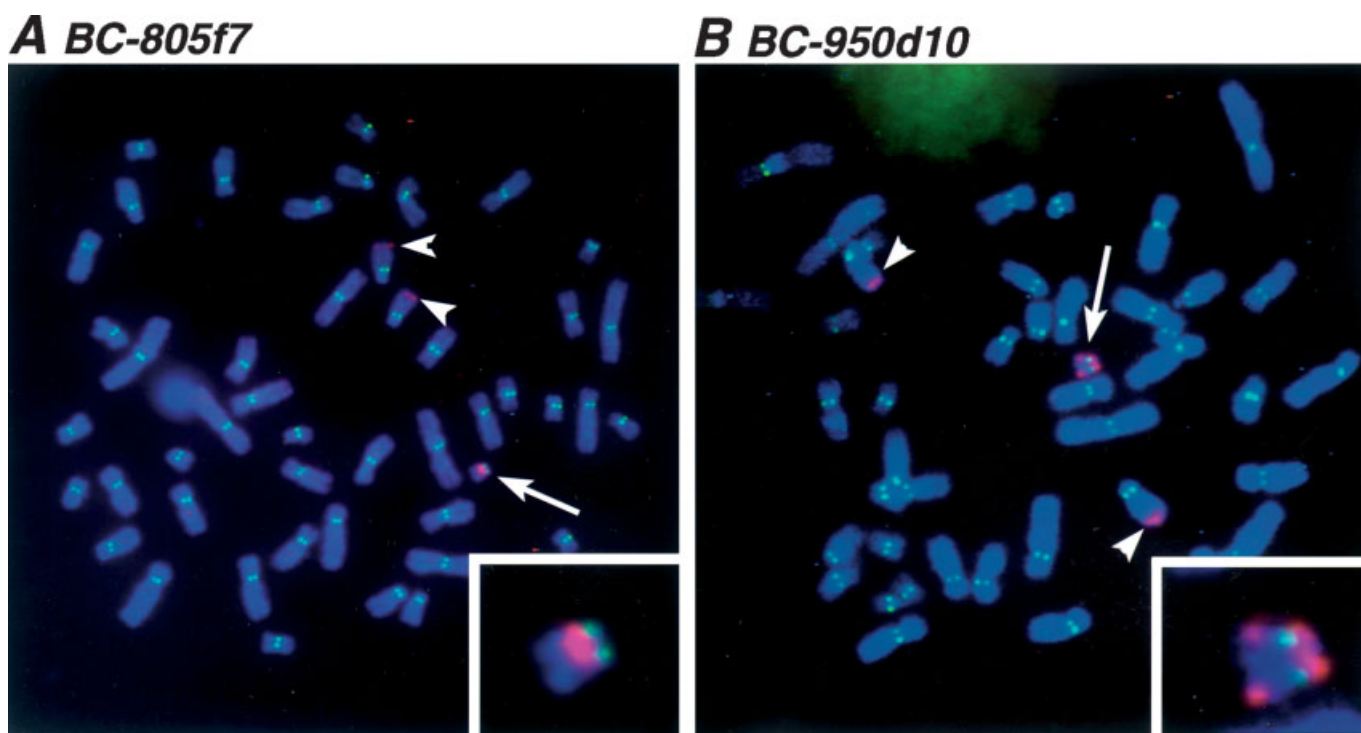


Fig. 4.

[Chu et al., 1994]. In contrast, the patients with more proximal 13q14/q21 → qter imbalances (which encompasses the smaller region 13q32 → qter) also exhibit hemangioma (two of five), microphthalmia (one of five), and seizures (one of five), but no colobomata was observed. In addition, polydactyly was observed in two of five of the more proximal imbalances, but none of the more distal imbalances, suggesting an association with proximal 13q. Notably, holoprosencephaly was not seen in any patient, despite its appearance in greater than 50% of trisomy 13, suggesting its association with the regions proximal to 13q14. Given that only a fraction of patients with a particular chromosomal imbalance may present specific associated clinical features, the presence of certain specific features in a significant proportion of the patients strongly associates them with distal chromosome 13q (Table I).

DISCUSSION

We report three independent cases of dysmorphic patients that contain a supernumerary inversion duplication of a portion of chromosome 13q with a neocentromere. These cases add three additional examples to the eight previously reported inv dup13q neocentromere-containing chromosomes [Rivera et al., 1999; Warburton et al., 2000; Morrissette et al., 2001]. Since a comprehensive list of 40 neocentromeres was published by Warburton et al. [2000], several additional neocentric chromosomes have been reported (Table II) [Assumpção et al., 2000; Barbi et al., 2000; Gimelli et al., 2000; Higgins et al., 2000; Levy et al., 2000; Rowe et al., 2000; Voullaire et al., 2001]. Only neocentromere-containing chromosomes for which the chromosome and position of origin have been identified are included in these surveys; several additional anaphoid supernumerary marker chromosomes have been identified but not well characterized [Crolla, 1998]. Thus, out of the current total 52 cases of neocentromeres reported, eleven (21%) have formed on chromosome 13q.

While the relatively high proportion of chromosome 13q neocentromeres might be attributed to ascertainment bias due to the viability of trisomy for chromosome 13, several of the 11 cases had relatively minor phenotypes and/or were highly mosaic. If all chromosome regions had an equal propensity to form neocentromeres and the determining factor was survival to recognizable pregnancy or fetal survival, then neocentromeres would be expected to be seen at comparable frequencies on other chromosomes that are well tolerated in trisomy, such as chromosome 21, which has had only a single reported neocentromere [Barbi et al., 2000]; chromosome 18, with no reported neocentromeres; and the X chromosome, also with a single reported case [Kaiser-Rogers et al., 1995]. Thus, the higher than expected frequency of observed neocentromeres on chromosome 13q suggests that this region may have a bonafide increased propensity for neocentromere formation [Warburton et al., 2000].

The basis of this increased propensity for neocentromere formation remains unknown, but may be due to some sequence or structural property of 13q. Certain

other chromosomal regions demonstrate a higher than expected number of observed neocentromeres, such as 15q ($N=8$) and 3q ($N=6$). The positions of neocentromeres throughout the genome and at these putative "neocentromere hotspots" does not appear to be biased towards any cytogenetic characteristic, such as light or dark G banding or increased frequency of chromosomal rearrangements or marker formation in these regions. In contrast, the four independent cytologically identical inv dup 8(pter → p23::p23 → pter) neocentric markers that have been described [Ohashi et al., 1994; Voullaire et al., 2001] (Warburton and Papenhausen, unpublished) may at least be partially attributed to the apparently high frequency of chromosomal rearrangements at 8p23 [Voullaire et al., 2001].

The 11 cases of 13q neocentromeres display a wide range of mosaicism, ranging from 100% trisomy for 13q14 → qter to 26% hexasomy for 13q32 → qter (Table I). The karyotypes of the two cases of trisomy for a portion of 13q (cases 13a and 13b, Table I) include a chromosome 13 with a terminal deletion that appears to be complementary to the inverted duplication, thus providing a strong selection for retention of the neocentric marker in these cases [Warburton et al., 2000]. The other neocentric markers are present in lymphatic tissue, ranging from 100% to as low as 14% (Table I), leading to suggestions that this mosaicism reflects a dysfunction of some neocentromeres. However, centric marker chromosomes also frequently display wide ranging degrees of mosaicism [Crolla, 1998], suggesting an inherent instability of marker chromosomes in general during early development, perhaps due to slight selection/growth advantage for karyotypically normal cells and/or lack of selection against loss of the marker chromosome. Recently, a lymphoblastoid line originally mosaic for an invdup8p23 neocentric chromosome was subcloned to 100% presence, demonstrating that the neocentromere was fully functional [Voullaire et al., 2001]. Thus, current data do not support a role for mitotic infidelity of the neocentromere in the observation of mosaicism for these marker chromosomes.

Phenotype/Genotype Correlation

The complexity and variability of the phenotypes seen in the set of 11 patients with polysomy for a portion of chromosome 13q clearly does not support a simple reductionist view of phenotype/genotype correlation with polysomy for certain chromosomal regions. The difficulty in correlating particular clinical features with specific chromosomal imbalances has been recognized for many years, with some collective features (e.g., facial and/or musculo-skeletal dysmorphisms) found associated with many different chromosome abnormalities [Daniel, 1979; Shapiro, 1989]. As early as 1968, Taylor pointed out that patients with trisomy 18 and trisomy 13 share 50% of clinical features [Taylor, 1968]. Daniel et al. [1989] has shown that the viability of fetuses with chromosome imbalances arising from reciprocal translocations is primarily related to the size of the imbalance rather than to its chromosomal origin. Furthermore, the wide range of mosaicism and

TABLE I. Clinical Features Seen in Reported Cases of invdup13q Chromosomes With Neocentromers

Case ^a Imbalance (% cells)	13a Trisomy q14 → qter (100%)	JM Tetrasomy q14 → qter (14%)	13b Trisomy q21 → qter (100%)	13c Tetrasomy q21 → qter (49%)	13d Tetrasomy q21 → qter (88%)	13e Tetrasomy q31 → qter (60%)	BC Tetrasomy q31 → qter (54%)	13f Tetrasomy q32 → qter (98%)	13g Tetrasomy q32 → qter (100%)	13h Tetrasomy q32 → qter (74%)	MG Hexasomy q32 → qter (26%)
Hemangioma	+	-	+	-	-	-	+	+	+	+	+
Seizures	-	+	-	-	-	+	-	+	+	+	+
Micro-ophthalmia	-	-	+	-	-	-	-	+	-	+	-
Polydactyly	-	-	+	-	+	-	-	-	-	-	-
Colobomata	-	-	-	-	-	-	-	-	+	+	-

^aCase numbers other than those presently reported are referred to in Warburton et al. [2000].

TABLE II. Updated Survey of Neocentromere-Containing Marker Chromosomes (Since Warburton et al. [2000])

Chromosome	Abnormal chromosomes ^a	Rearrangement ^b	Complement ^c	% Mosaicism ^d	Reference
1	del(pter → q23;q32 → qter), + ring (q23 → neocen → q32)	Para del	Balanced	100% amnio	Higgins et al. [2000] ^e
3	+ inv dup (qter → q21.2::q21.2 → neocen → qter)	inv dup	Tetrasomy q21.2 → qter	87% fib, 0% lym	Gimelli et al. [2000]
8	+ Inv dup (pter → p23.1::p23.1 → neocen → pter)	inv dup	Tetrasomy pter → p23	44% lym	Voullaire et al. [2001]
8	+ inv dup (pter → p23.1::p23.1 → neocen → pter)	inv dup	Tetrasomy pter → p23	60% lym	Voullaire et al. [2001]
8	+ inv dup (pter → p23.1::p23.1 → neocen → pter)	inv dup	Tetrasomy pter → p23	100% lym	Unpublished ^f
10	+ inv dup (pter → p15::p15 → pter)	inv dup	Tetrasomy pter → p15	100% amnio	Levy et al. [2000]
13	+ inv dup (qter → q14::q14 → q21 neocen → qter)	inv dup	Tetrasomy 14 → qter	14% lym	This report
13	+ inv dup (qter → q31::q31 → q32 neocen → qter)	inv dup	Tetrasomy q31 → qter	56% lym	This report
13	+ inv dup (qter → q32::q32 → neocen → qter)	inv dup	Hexasomy q32 → qter	26% lym	This report
15	+ inv dup (qter → q25::q25 → neocen → qter)	inv dup	Tetrasomy q25 → qter	100% lym	Rowe et al. [2000]
21	+ inv dup (qter → q21.1::q21.1 → neocen → qter)	inv dup	Trisomy q21.1 → qter ^g	100%	Barbi et al. [2000]
Y	Inversion Y (qter → q11::q11 → qter)	inv dup	Del (Y)	100%	Assumpcao et al. [2000] ^e

^aAll deletions and rearrangements written inclusive of chromosomal material present.

^bPara del-paracentric deletion, inv dup-inverted duplication.

^cChromosome complement approximated cytogenetically and does not consider possible microdeletions or insertions.

^dMosaicism for neocentromere containing chromosome, % mosaicism for fibroblasts (fib), lymphocytes (lym), amniocytes (amnio) given when ascertained.

^eASHG annual meeting abstract.

^fWarburton and Papenhausen unpublished.

^gAs one of the chromosome 21s was also missing, the patient was additionally monosomic for 21pter → q21.1.

exact chromosome imbalances seen for the 11 described cases of 13q marker chromosomes (Table I) must account to some extent for the wide variability in observed phenotypes. Patients with tetrasomies or trisomies for the same region can have quite different phenotypes, e.g., tetrasomy 18p is more severe than the corresponding trisomy [Callen et al., 1990; Yu et al., 1993]. A further complication may be the presence of the neocentromere in these chromosomes, where the formation of a kinetochore may result in chromatin modifications that could influence gene expression over a chromosomal region of several Mbp [Levy et al., 2000].

Neocentromere Structure

At least three distinct regions in 13q have been shown to contain neocentromeres, 13q21 ($N = 3$, including the marker from J.M.), 13q31 ($N = 1$), and 13q32 ($N = 7$, including the marker from B.C. and M.G.). The DNA sequence underlying several independent neocentromeres from chromosomal locations such as 10q25 [Lo et al., 2001a], 20p12 [Lo et al., 2001b], and 9p [Satinover et al., 2001] have been isolated to regions of several hundred kilobases (kb). Sequence analysis of these regions revealed no obvious common sequence or structural characteristics, with the exception of an increase in AT richness, which was also noted for alpha satellite DNA sequences at normal human centromeres.

Neocentromeres appear to form fully assembled kinetochores with the full complement of functional kinetochore proteins [Saffery et al., 2000; Warburton, 2001]. The neocentromeres in all three of the present cases appear to be displaced to one side of an otherwise symmetrical inv dup chromosome, with homologous sequences in the other half of the inv dup, as well as on the normal chromosomes 13 not expressing centromeric activity. Thus, neocentromeres support an epigenetic mechanism of centromere formation that is not dependent on underlying DNA sequence [Karpen and Allshire, 1997]. Nonetheless, the unique collection of 11 independent 13q neocentromere-containing chromosomes represents the opportunity to examine and compare DNA sequences underlying neocentromeres from the same chromosomal "hotspot", e.g., band 13q32. Multiple 13q32 neocentromeres might be forming on the same underlying several hundred kb of DNA, which would strongly support a role for that particular genomic sequence in neocentromere formation. Alternatively, 13q32 neocentromeres may be distributed over the several Mbp encompassing the cytological chromosome band, suggesting that more global aspects of chromosome structure are important for neocentromere formation. With the development of chromatin immunoprecipitation techniques to isolate neocentromeric DNA [Lo et al., 2001a,b], analysis of 13q neocentromeres may yield important insights into the epigenetic process of human centromere formation.

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