Am. J. Hum. Genet. 54:877–883, 1994

Tetrasomy 15q: Two Marker Chromosomes with No Detectable Alpha-Satellite DNA

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Summary

Two patients with specific and similar phenotypes were both found to have an unusual marker chromosome present in 70%–80% of their lymphocytes at routine cytogenetic examination. The marker chromosomes were isolated by flow sorting and were amplified by degenerate oligonucleotide-primed PCR. These libraries and a cosmid probe located at 15q26 were used to characterize the marker chromosomes by FISH. Both marker chromosomes were found to consist of duplicated chromosome material from the distal part of chromosome 15q and were identified as inv dup(15) (qter→q23::q23→qter) and inv dup(15) (qter→q24::q24→qter), respectively. Hence, the markers did not include any known centromere region, and no alpha-satellite DNA could be detected at the site of the primary constriction. Tetrasomy 15q may be a new syndrome, associated with a specific type of marker chromosome. In addition, further analyses of this type of marker chromosome might give new insight into the structure and function of the mammalian centromere.

Introduction

Small supernumerary chromosomes are also known as marker chromosomes or extra structurally abnormal chromosomes (Hook and Cross 1987). Accordingly, patients possessing this rearrangement have 46 normal chromosomes and an extra unidentified chromosome. The symptoms associated with such a chromosome depend on the genetic content in each separate case. If the marker chromosome consists purely of heterochromatic material, it will have no effect on the phenotype, whereas euchromatic content will give symptoms caused by the specific trisomy or tetrasomy. Many marker chromosomes are left unidentified by regular chromosome banding. This fact often creates serious problems in counseling situations, especially when a de novo marker chromosome is found during prenatal testing. The risk of abnormality will then have to be based on large studies, giving overall and unspecific risk figures (Warburton 1991).

During recent years, progress has been made by the introduction of FISH as a method to identify marker chromosomes. In >70 published cases the centromeric origin has been established by using centromere-specific probes (Callen et al. 1992; Crolla et al. 1992; Rauch et al. 1992; Blennow et al. 1993). Unfortunately, some marker chromosomes fail to show labeling with these probes (Callen et al. 1992; Crolla et al. 1992), and, furthermore, some of the marker chromosomes are complex, consisting of material from more than one chromosome (Blennow et al. 1992). This calls for further, more detailed examination.

We report here on two patients with similar phenotypes, possessing extra, metacentric marker chromosomes. The extra chromosomes were isolated by flow sorting and were amplified by degenerate oligonucleotide-primed (DOP)-PCR. The constructed libraries were used to outline the abnormal chromosomes by FISH and "reverse chromosome painting" (Blennow et al. 1992; Carter et al. 1992) and were found to contain the distal part of 15q in both cases. The lack of a cen-

Received December 6, 1993; accepted for publication January 24, 1994.

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0002-9297/94/5405-0018$02.00

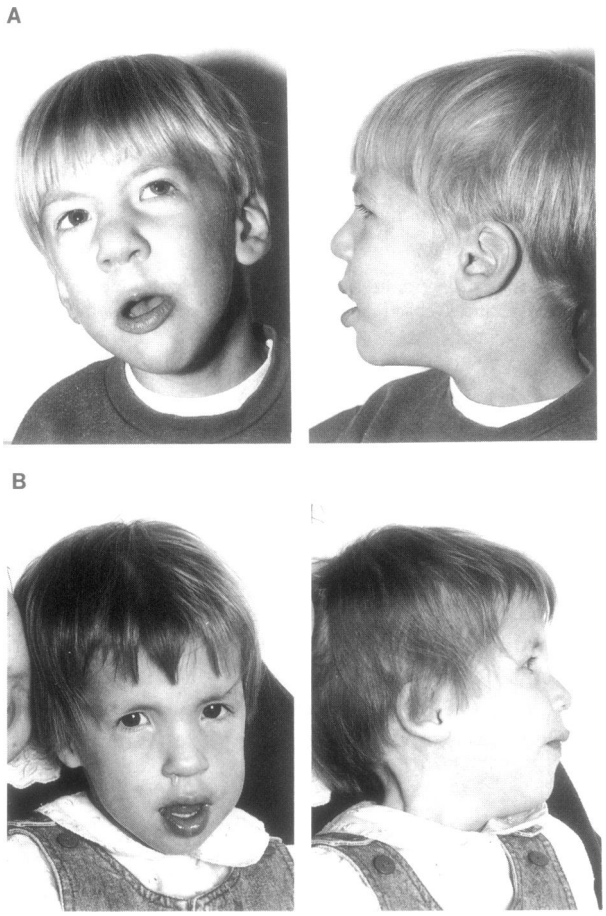

Figure 1 Photographs of patients: case A (*top*) and case B (*bottom*).

tromere signal was confirmed by hybridization with an all-human alpha-satellite probe.

Patients, Material, and Methods

Patients

Case A.—This boy was born as the third child of a 32-year-old woman and a 28-year-old man. The older siblings are normal. The pregnancy was uneventful, with delivery at term. The birth weight was 3,370 g, and the birth length was 51 cm. The child had a peculiar appearance, with antimongoloid slant of palpebral fissures, epicanthal folds, low-set ears, a bulbous nose with a broad nasal root, retrognathia, down-turned corners of the mouth, a narrow high-arched palate, long philtrum, arachnodactyly of fingers and toes, and joint contractures of the hips. The head and thorax were asymmetric, with thoraco-lumbar kyphosis. Chromosome analysis revealed the karyotype 46,XY/47,XY,+mar, with a metacentric marker chromosome in the size of the F-group in 70% of the lymphocytes and in 11% of the fibroblasts. The psychomotor development has been delayed, and he has a moderate-to-severe mental retardation. Linear growth has been rapid. Body height at 2 years of age was 98 cm (98th percentile). He has a nasal stenosis and chronic rhinorrhea and is very sensitive to light, because of achromatopsia. A bilateral sensorineural hearing loss has been found. Photographs of the patient are presented in figure 1.

Case B.—This girl was born as the second child of a 30-year-old woman and a 34-year-old man. An older brother and a younger sister are healthy. The patient was delivered at term by cesarean section, because of breech presentation. The birth weight was 4,510 g, and the birth length was 55 cm. Shortly after birth, some dysmorphic features were noted: low-set dysplastic ears, micrognathia, high-arched palate, antimongoloid slant of palpebral fissures, epicanthal folds, bulbous nose, long philtrum, down-turned corners of the mouth, ulnar-deviated hands, and arachnodactyly of fingers and toes. The head, face, and thorax were asymmetric, with scoliosis, and there were joint contractures involving the hips. Bilateral inguinal hernia was corrected at 2 mo of age. Investigation with ultrasound and computed-tomography scan showed moderate hydrocephalus. No other cerebral malformations were found. A bilateral sensorineural hearing loss has been noted. Chromosome analysis revealed an extra metacentric chromosome in 80% of the lymphocyte metaphases, and i(18p)-syndrome was initially suspected. The psychomotor development has been delayed. She started to walk at 3 years of age and has a moderate mental retardation. Postnatal growth has been above the 95th percentile. Photographs of the patient are presented in figure 1.

Cytogenetic Studies

Metaphase slides were prepared from lymphocyte and fibroblast cultures. QFQ- and C-banding were performed using standard procedures.

Probes

Epstein-Barr virus–transformed lymphoblastoid cell lines were established from both patients. A marker chromosome–specific library from each patient was created by flow sorting of the marker chromosome, followed by DOP-PCR amplification and biotinylation as described by Blennow et al. (1992).

Centromere-specific probes from all human chromo-

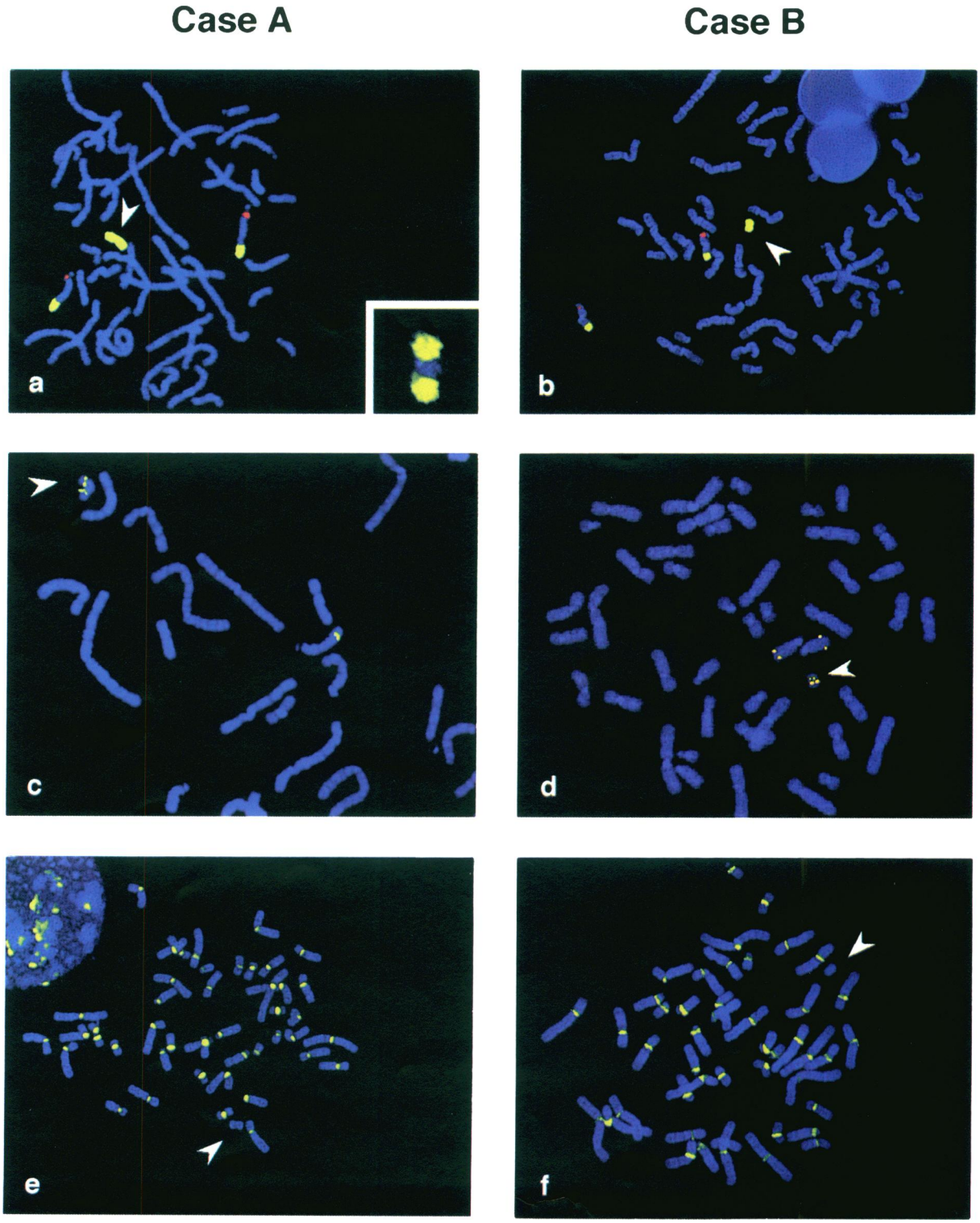

Figure 2 Partial (*a–d*) and complete (*e* and *f*) metaphases from patients A and B. The marker chromosome–specific libraries of cases A (*a*) and B (*b*) were used for reverse painting on the patient's own metaphases. The centromere region of chromosome 15 was labeled in red, using the pericentromere-specific probe pHSr. The marker chromosome–specific library of case B was also hybridized to the metaphases of case A (*a, inset*), but only the distal parts of the marker chromosome were labeled (*painted*). The marker chromosomes showed two signals, one on each chromosome arm, when the cosmid probe cAGG2-10 was used, hybridizing to 15q26 (*c* and *d*), but did not show any labeling (*arrow*) when a pancentromeric alpha-satellite probe was used (*e* and *f*).

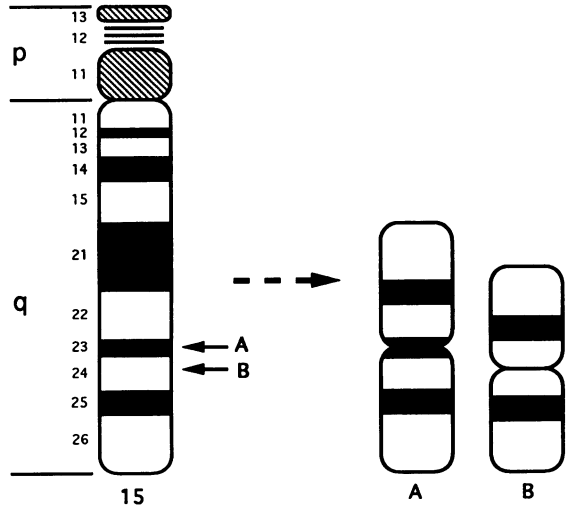

Figure 3 *Left,* Ideogram of breakpoints on chromosome 15q. *Right,* Resulting ideograms of the inv dup(15)(qter→q23::q23→qter) (case A) and the inv dup(15)(qter→q24::q24→qter) (case B).

somes were labeled with fluoro-red-dUTP (Amersham) or biotin-16-dUTP (Boehringer-Mannheim) by nick-translation. A complete list of the probes and their sources has been published previously (Blennow et al. 1993). The all human alpha-satellite probe and the all-human telomere probe were purchased from Oncor (Gaithersburg, MD).

Isolation of Genomic Clones

The 1.9-kb insert of a cDNA probe (pAGG2) for the human aggrecan gene, previously assigned to chromosomal region 15q26 (Korenberg et al. 1993), was used to screen 6×10^5 colonies of a human cosmid library prepared from placental DNA (Evans et al. 1989). Cosmid DNA was isolated from purified positive clones by a minilysate procedure (Berger and Kimmel 1987) and was verified by Southern blot analysis after *Eco*RI cleavage. The chromosome 15 origin of the identified cosmid clone cAGG2-10 was confirmed by FISH analysis on normal human metaphase chromosomes.

FISH

The chromosome slides were prepared from lymphocyte cultures as described elsewhere (Blennow et al. 1992). The marker chromosome–specific libraries and the cosmid were hybridized in 50% formamide, 2 × SSC, 50 mM phosphate buffer pH 7.0 at a probe concentration of 3–4 ng/μl. In addition, 2–3 μg Cot-1 DNA (BRL) was added to the probe mixture. After denaturation at 75°C for 5 min, the probe mixture was left to prehybridize at 37°C for 1 h. Hybridization was performed in a moist chamber at 37°C overnight. The slides were then washed three times for 5 min in 50% formamide, 2 × SSC at 42°C and twice in 2 × SSC at 42°C (the cosmid was washed three times in 0.1 × SSC at 60°C).

The centromere-specific probes were hybridized as described elsewhere (Blennow et al. 1993). The all-human alpha-satellite probe and the all-human telomere probe were hybridized according to the manufacturer's instructions.

After the washing, all slides were left in BT-buffer (0.1 M $NaHCO_3$ pH 8, 0.05% Tween 20) for 30 min. Probe detection and signal amplification were performed by applying two alternating layers of fluorescein-avidin DCS (Vector Lab) and biotinylated anti-avidin antibodies (Vector Lab) (Pinkel et al. 1986). After dehydration, the slides were mounted in glycerol containing 2.3% DABCO (1,4-diazabicyclo-(2,2,2) octane) as antifade and DAPI (4,6,-diamino-2-phenyl-indole) at 0.5 μg/ml as counterstain of the chromosomes.

Table 1

Main Symptoms of the Present Cases, Compared with Those Found in Trisomy 15q

	Case A	Case B	Trisomy 15q[a]
Antimongoloid slant	+	+	+
Low-set ears	+	+	+
Bulbous nose	+	+	+
Nasal stenosis	+	−	−
Long philtrum	+	+	+
Down-turned corners of the mouth	+	+	+
Midline crease in lower lip	−	−	+
High-arched palate	+	+	+
Micro- or retrognathia	+	+	+
Short neck	−	−	+
Asymmetry of the head	+	+	+
Sloping forehead	−	−	+
Asymmetry of the thorax	+	+	+
Kyphosis/scoliosis	+	+	+
Arachnodactyly	+	+	+
Genital anomalies	−	−	+
Cardiac defects	−	−	+
Joint defects	+	+	+
Achromatopsia	+	−	−
Sensorineural hearing loss	+	+	−
Mental retardation	+	+	+
Increased postnatal growth	+	+	−

[a] Reported in >60% of the patients with trisomy 15q.

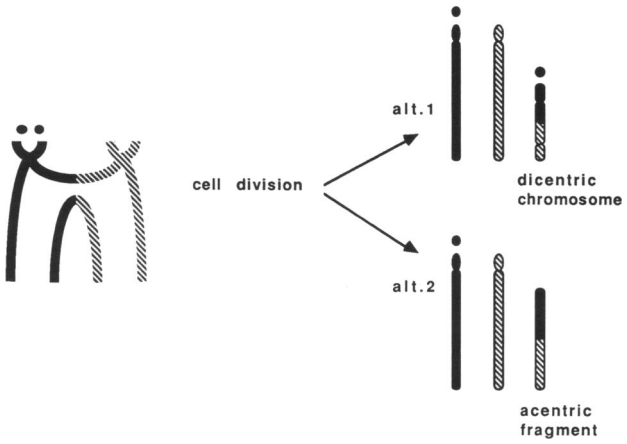

Figure 4 Formation of an inv dup(15)(pter→q11-13::q11-13→pter), which includes the formation of an acentric fragment that is presumed to be lost when the cell divides.

Digital Imaging Microscopy

The signal was visualized using a Zeiss Axiophot fluorescence microscope equipped with a cooled CCD-camera (Photometrics Nu 200/CH 250) controlled by a Macintosh Quadra 950 computer. Gray-scale images were captured, pseudocolored, and merged using the SmartCapture software (Digital Scientific, Cambridge).

Results

In both cases, cytogenetic investigation including QFQ- and C-banding revealed a supernumerary, metacentric marker chromosome with no visible C-band, in varying proportions of the cells (see Patients, Material, and Methods). The banding pattern on the marker chromosomes was unspecific and could not be referred to any defined chromosome region. In case A, the primary constriction of the marker was less pronounced compared with that in the other chromosomes, and the size of the marker chromosome was comparable with that of the F-group. In case B the marker chromosome was smaller, in the size of the G-group, and the primary constriction appeared to be normal. When grown for a period of time as lymphoblastoid cell lines, the marker chromosomes were lost in the culture.

FISH using centromere-specific probes from all of the human chromosomes gave no positive signal on the marker chromosomes. In order to identify the origin, marker chromosome–specific libraries were constructed by flow sorting and DOP-PCR (see Patients, Material, and Methods). "Reverse painting," using the marker chromosome–specific libraries on the patient's slides, revealed the exact origin of the marker chromosomes. In both cases the library labeled the distal part of chromosome 15q (fig. 2*a* and *b*). Posthybridization QFQ-banding of the same metaphases gave the breakpoint 15q23 in case A and gave the breakpoint 15q24 in case B. No centromere or centromeric region was labeled, even after omission of prehybridization with Cot-1 DNA.

The cytogenetic appearance and the hybridization pattern suggested that the marker chromosomes were inverted duplications. The flow-sorted chromosome-specific library of case B was hybridized to the metaphases of case A (fig. 2*a*, *inset*). The library labeled the distal parts, but not the centromeric region of the marker chromosome of case A. The cosmid probe cAGG2-10, hybridizing to chromosome 15q26, was used to confirm the duplication. As expected, the cosmid labeled both arms of the marker chromosomes (fig. 2*c* and *d*), thereby demonstrating tetrasomy for this gene.

The marker chromosomes did not show any labeling when a pancentromeric alpha-satellite probe (fig. 2*e* and *f*) was used, nor when the satellite III probe pHSr (fig. 2*a* and *b*), specific for the pericentromeric region of chromosome 15, was used. Telomere sequences could be found at both ends of the marker chromosomes (data not shown).

When these data are taken together, the correct karyotype of case A is 46,XY/47,XY,inv dup(15)(qter→q23::q23→qter) de novo, and that of case B is 46,XX/47,XX,inv dup(15)(qter→q24::q24→qter) de novo. The corresponding ideograms are shown in figure 3.

Discussion

The marker chromosomes reported here have both been shown to consist of duplicated chromosome material from the terminal part of chromosome 15. The patients, who share a similar phenotype, are thus both mosaic for pure tetrasomy 15q, which, to our knowledge, never has been described before. However, trisomy of the distal part of chromosome 15q has been reported in >30 cases (Fujimoto et al. 1974; Gregoire et al. 1981; Kristoffersson and Bergwall 1984; Goldstein et al. 1987; Kristoffersson et al. 1987; Lacro et al. 1987; Fryns et al. 1988). All but two were caused by unbalanced reciprocal translocations. Therefore, in each case, the trisomy was accompanied by monosomy of another chromosome. Pure trisomy 15q was reported in a patient with a recombination aneusomy, caused by

a pericentric inversion in the mother (Coco and Penchaszadeh 1978), and in another case with a tandem duplication of 15(q21→qter) (Yip et al. 1982). On the basis of observations made in all these cases, a trisomy 15q syndrome has been postulated. The most common features are facial asymmetry, prominent bulbous nose, narrow palpebral fissures with antimongoloid slants, low-set ears, long upper lip, pronounced philtrum, down-turned corners of the mouth, midline crease in lower lip, micrognathia, short neck, arachnodactyly, joint defects, cardiac defects, genital anomalies, and mental retardation. The two cases reported here have many features in common and resemble those with trisomy 15q. In table 1, the most significant symptoms are presented and compared with the features of trisomy 15q.

No alpha-satellite or satellite III material could be detected at the centromeric regions of the marker chromosomes. This means that these sequences are either absent or at a copy number below the sensitivity of FISH. Nonetheless, the marker chromosomes do have a primary constriction and are capable of replication and segregation, even though some dysfunction must be suspected. First, the patients are mosaic for the marker chromosome, making it likely that the marker sometimes has been lost during cell division. Second, in both cases, the marker chromosome totally disappears when grown for a period of time in a rapidly dividing lymphoblastoid cell line.

There is still much confusion concerning the understanding of the structure and function of the mammalian centromere. It has been identified cytogenetically as the site of the primary constriction, visible in metaphase. The centromere is essential for proper segregation of the sister chromatids during anaphase, and malfunction will result in chromosome loss. All the key components for a functional centromere have not been fully established. The centromere consists of highly repeated satellite DNA, to which the kinetochore is attached (Pluta et al. 1990; Willard 1990). The best-characterized is the alpha-satellite, containing a 171-bp motif, present on all human centromeres and therefore likely to form an essential part of a functional centromere. Alpha-satellite DNA has also been found to interact with one of the centromere proteins (CENP-B), which has been localized to the centromeric heterochromatin underlying the kinetochore. However, today the true importance and function of alpha-satellite DNA is still obscure.

Marker chromosomes without alpha-satellite labeling have been reported on at least two occasions (Callen et al. 1992; Crolla et al. 1992). In addition, Voullaire et al. (1993) have described a mitotically stable, unusual marker chromosome with a functional centromere but without alpha-satellite and satellite III DNA at the primary constriction site. CENP-B protein could not be demonstrated, and activation of a latent intercalary centromere was suggested as the mechanism of formation. In the two cases reported here, this explanation would seem unlikely, since it requires the presence of latent intercalary centromeres at both 15q23 and 15q24.

Chromosome 15 is by far the chromosome most often involved in the formation of marker chromosomes. Of all marker chromosomes reported, ≥50% are identified as inv dup(15)(pter→q11-13::q11-13→pter), a structurally but not functionally dicentric marker chromosome (Buckton et al. 1985). Many theories have been postulated concerning the formation of dicentric isochromosomes, including (1) a non–sister-chromatid exchange followed by a second-division nondisjunction (Wisniewski et al. 1979); (2) errors in replication, caused by parental inversion heterozygosity; (3) translocation or (4) U-type exchange (Schreck et al. 1977); or (5) mitotic errors originating from ligation error in a replication fork during S-phase, followed by a new replication (Wik Sjöstedt et al. 1989). In addition to the inv dup(15)(pter→q11-13::q11-13→pter), these theories usually include the formation of an acentric fragment (fig. 4), which, because of the lack of a functional centromere, is assumed to be lost. One may speculate that this acentric fragment could somehow be retained, if it acquired or activated sequences that would function as a new centromere, either by new formation or perhaps by the donation of centromere sequences from another chromosome. Both cases described here have inverted duplications that include only the distal part of 15q. The primary constriction does not seem to include alpha-satellite DNA, which is normally present at every human centromere. This points to the creation of a new centromere, with very little or no alpha-satellite content. A similar marker chromosome with a tandem duplication, 13q32→13qter, recently has been described elsewhere (Zinn et al. 1993). The formation of this type of marker chromosome may have been overlooked in the past, because of difficulties in resolving the rearrangements. The isolated marker chromosome–specific libraries will be useful in the identification of new cases, and, in addition, the libraries may be used to identify new DNA markers from the distal part of chromosome 15. This type of marker chromosome may also be used to delineate the key components of the functioning centromere.

Acknowledgments

We thank Dr. Peter Roughley for supplying the plasmid clone pAGG2. This work was supported by grants from the Swedish Medical Research Council, the Axel and Margaret Ax:son Johnsons Fund, and Magnus Bergvalls Stiftelse.

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