Am. J. Hum. Genet. 55:1202–1208, 1994

A Stable Acentric Marker Chromosome: Possible Existence of an Intercalary Ancient Centromere at Distal 8p

Hirofumi Ohashi,[1] Keiko Wakui,[2] Kiyoshi Ogawa,[3] Tetsuroh Okano,[4] Norio Niikawa,[5] and Yoshimitsu Fukushima[1]

[1]Division of Medical Genetics, Departments of [2]Clinical Laboratory and [3]Cardiology, Saitama Children's Medical Center, Iwatsuki, Japan; [4]Hygienic Science Research and Development Center, Kitazato University, Tokyo; and [5]Department of Human Genetics, Nagasaki University School of Medicine, Nagasaki

Summary

A centromere is considered to be an essential chromosomal component where microtubule-kinetochore interaction occurs to segregate sister chromatids faithfully and acentric chromosomes are unstable and lost through cell divisions. We report a novel marker chromosome that was acentric but stable through cell divisions. The patient was a 2-year-old girl with mental retardation, patent ductus arteriosus, and mild dysmorphic features. G-banded chromosome analysis revealed that an additional small marker chromosome was observed in all 100 cells examined. By the reverse-chromosome-painting method, the marker was found to originate from the distal region of 8p, and a subsequent two-color FISH analysis with cosmid probes around the region revealed that the marker was an inverted duplication interpreted as 8pter→p23.1::p23.1→8pter. No centromeric region was involved in the marker. By FISH, no α-satellite sequence was detected on the marker, while a telomere sequence was detected at each end. Anti-kinetochore immunostaining, using a serum from a patient with CREST (calcinosis, Raynaud syndrome, esophageal dismotility, sclerodactyly, and telangiectasia) syndrome, showed a pair of signals on the marker, which indicated that a functional kinetochore was present on the marker. The analysis of this patient might suggest the possibility that an ancient centromere sequence exists at distal 8p (8p23.1-pter) and was activated through the chromosome rearrangement in the patient.

Introduction

A functional mammalian chromosome is considered to have at least three components essential for stable replication and segregation: a centromere, two telomeres, and replication origins. The centromere is the cytologically defined chromosomal region at which sister chromatids are held together after DNA replication, and kinetochore proteins are formed to interact with spindle fibers to segregate the sister chromatids faithfully into daughter cells. Acentric chromosomes are considered to be passively transported into daughter cells unable to attach to a mitotic spindle and become eliminated through cell divisions.

A double minute chromosome (DM), occasionally found in neuroblastoma and some other tumor cells, is an exceptional example of acentric chromosomes that persist in multiple cell divisions. It is known that neither the functional centromere nor the telomere exists in DMs (Haaf and Schmid 1988; Lin et al. 1990). However, DMs show highly numerical heterogeneity among cells, a phenomenon that indicates anomalous mitotic segregation (Levan et al. 1981). Therefore, they are not truly stable.

Functions/structures of the centromere/kinetochore have been investigated mainly through the following two tools: (1) an α-satellite that is the predominant and best-characterized centromeric DNA on the basis of a 171-bp monomeric unit and arranged in a highly tandemly repeated array and (2) centromeric proteins (CENPs) that were identified by antibodies from patients with the autoimmune disease called "scleroderma CREST" (calcinosis, Raynaud syndrome, esophageal dismotility, sclerodactyly, and telangiectasia) (for references, see Bloom 1993). Not much has been known until now about the molecular basis of the centromere/kinetochore function, but the α-satellite is considered to be one of the critical DNA sequences for centromere activity, on the basis of the observations that it is present on all human centromeres and contains binding sites for the CENP-B (Masumoto et al. 1989). Currently, one of the main interests in the study of the centromere is the identification of the actual sequence that is responsible for chromosome segregation.

We report here a novel acentric marker chromosome derived from distal 8p found in a girl with mild dysmorphic features. It involved no detectable α-satellite sequence by FISH, but it was mitotically stable.

Case, Material, and Methods

Case Report

A.S.(4-1221-6), a 2-year-old girl, was born after a 35-wk gestation to a 35-year-old mother and 30-year-old father,

Received June 10, 1994; accepted for publication July 26, 1994.

Address for correspondence and reprints: Dr. Hirofumi Ohashi, Division of Medical Genetics, Saitama Children's Medical Center, 2100 Magome, Iwatsuki, Saitama 339, Japan.

0002-9297/94/5506-0018$02.00

both healthy and unrelated. The pregnancy was uneventful except for premature delivery due to premature rupture of the embryonic membrane. Her birth weight was 2,035 g (−0.7 SD). There was no family history of malformation. At 2 wk after birth, she was noticed to have a heart murmur. She was diagnosed, by echocardiography and cardiac catheterization, as having patent ductus arteriosus (PDA) associated with pulmonary hypertension. PDA was successfully surgically ligated at age 16 mo. She required hospital treatments three times because of upper/lower respiratory infections associated with wheezing. At age 2¼ years, her height was 86.0 cm (−0.4 SD), weight 11.72 kg (−0.52 SD), and head circumference 50.2 cm (+1.6 SD). She showed a broad forehead, but she looked nondysmorphic otherwise. Her developmental quotient at age 2 years was 73.

Cytogenetic Studies

Metaphase and prometaphase chromosomes were obtained from phytohemagglutinin (PHA)–stimulated peripheral blood lymphocytes (PBL) by using a method described by Ikeuchi (1984) and were analyzed using G-banding and C-banding techniques by standard protocols.

Microdissection and PCR Amplification of Marker Chromosome

Procedures essentially followed the method described elsewhere (Deng et al. 1992; Ohta et al. 1993). In brief, metaphase chromosomes for microdissection were collected from short-term cultures of PHA-stimulated PBL separated using a 2% dextran solution. Following treatment with a hypotonic solution consisting of 0.2% NaCl and 0.2% KCl, cells were fixed with 100% methanol and stored at −20°C until used. A fixed-cell suspension was mixed with acetic acid and then immediately was spread onto a clean glass slide. Non-aged chromosomes were GTG-banded with 0.025% trypsin and stained with 3% Giemsa. A marker chromosome was microdissected with a fine glass needle under an inverted microscope. Thirty pieces of dissected chromosomal materials were transferred into a collection chamber and were covered with paraffin oil. Further steps, including proteinase K digestion, DNA extraction, *Sau*3AI digestion, DNA ligation to a linker/primer set, and PCR, were carried out as described elsewhere (Hirota et al. 1992; Jinno et al. 1992).

Chromosome Preparations for FISH

To obtain G-banded chromosomes after FISH, cultures of PHA-stimulated PBL were synchronized by adding excess BrdUrd (200 μg/ml) for 18 h, followed by thymidine (0.3 μg/ml) release for 6.5 h, and were harvested as usual.

FISH

One-fiftieth volume of the total PCR product from the dissected marker chromosome was used as a probe pool after labeling it with biotin-16-dUTP by a second round PCR as described elsewhere (Ohta et al. 1993). After addition of 10 μg sonicated herring DNA, and 10 μg human Cot-1 DNA (catalog no. 5279SA, GIBCO BRL) as a competitor into a 1/20 volume of the second PCR product, the DNA mixture was ethanol precipitated, was resuspended in 10 μl formamide, was denatured at 75°C for 10 min, and was mixed with a hybridization solution consisting of 20 mg BSA/ml , 10 × SSC, and 50% dextran sulfate in a volume ratio of 1:2:2. The hybridization mixture was put onto denatured chromosomes; overnight hybridization was performed at 37°C; and the slides were washed in 50% formamide and 2 × SSC at 37°C, then in 2 × SSC and 1 × SSC, and finally in 4 × SSC at room temperature, each for 15 min. The slides were incubated with 100 μl fluorescence isothiocyanate (FITC)–conjugated avidin (1 μg in 500 μl, 4 × SSC, and 1% BSA) at 37°C for 45 min, and rinsed in 4 × SSC, 4 × SSC containing 0.1% Triton-X, and then in 4 × SSC. After counterstaining with 1 μg propidium iodide/ml, the slides were mounted with an antifading buffer. Photomicroscopy was performed under a fluorescence microscope equipped with a B-2A filter (Nikon). Post-FISH G-banding was performed by washing the slides in 2 × SSC at room temperature for 10 min, staining with Hoechst 33258, exposing them to UV light, and subsequent staining with 3% Giemsa.

Two-color FISH using two cosmid probes, cCI8-1 (8p23.3) and cCI8-1198 (8p22 or 23.1), was performed according to the method of Inazawa et al. (1994). The cCI8-1 and 1198 probes were labeled with digoxigenin and biotin-16-dUTP, respectively, each by nick translation, and precipitated with sonicated salmon sperm DNA and *Escherichia coli* tRNA, and then they were mixed in a weight ratio of 7:3 together with 50-fold sonicated human placental DNA as a competitor. The probes were hybridized in situ to the patient's chromosomes at 37°C for 18 h and then were detected by anti-digoxigenin rhodamine and avidin-FITC. The slide was counterstained with 4,6-diamidino-2-phenylindole-dihydrochloride and was mounted in an antifade solution. Metaphase chromosomes were identified through a Nikon UV-2A filter, and then two-color signals were visualized simultaneously through a double-bandpass filter (Omega Optical). The photomicrographs were taken with Ektachrome 100HC film (Kodak) exposed at 400 ASA. FISH using α-satellite DNA (Oncor) either for all human centromeres or specific for chromosome 8 was carried out according to the manufacturer's recommendation. A telomere probe for all human chromosomes (Oncor) was also used for FISH.

Anti-kinetochore-Antibody Immunostaining

The serum of a patient with CREST variety of scleroderma was used for indirect immunofluorescence staining of kinetochore protein. The procedure essentially followed the method described by Merry et al. (1985). Meta-

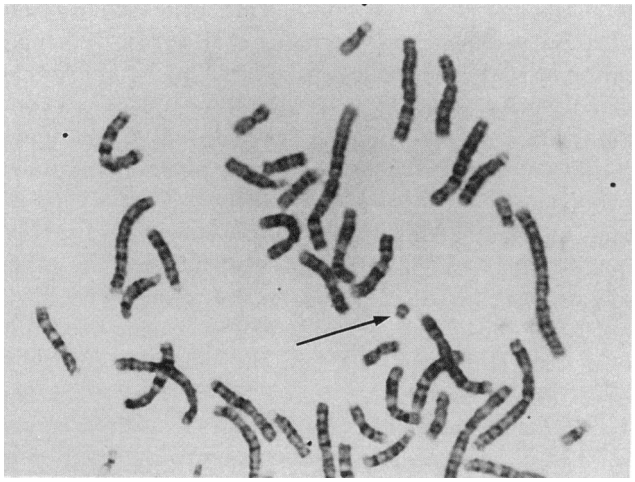

Figure 1 G-banded chromosomes of the patient. Arrow indicates an additional marker chromosome.

phase chromosomes were collected from an Epstein-Barr virus–transformed lymphoblastoid cell line of the patient, which was established as described elsewhere (Fukushima et al. 1992). Following treatment with a tris-buffered hypotonic solution, the cells in aqueous suspension were centrifuged onto a glass slide by using Cytospin 2 (Shandon) and fixed in chilled 80% ethanol. The cells were then soaked with the antiserum diluted 1:50 in PBS and incubated for 30 min in a moist chamber at 37°C. After two washes in PBS the slide was incubated with FITC-conjugated sheep anti-human immunoglobulin for 30 min at 37°C. After two washes with PBS the slide was counterstained with ethidium bromide and was mounted in glycerol/PBS. After observation under a fluorescence microscopy, the slide was subsequently stained by 4% Giemsa for the identification of the marker chromosome.

Results

Cytogenetic Analyses of the Marker Chromosome

G-banded chromosomes of PHA-stimulated PBL revealed a 47,XX,+mar karyotype. The marker was about one-third of a G-group chromosome in size, and it appeared in all 100 cells examined. At a 550–800-band level of resolution, the marker consisted of two terminal G-positive bands and a middle G-negative band and showed no apparent primary constriction (fig. 1). C-banding analysis revealed that the marker was C-negative (data not shown). The parents were cytogenetically normal.

Identification of the Origin of the Marker Chromosome

A probe pool was made from 30 pieces of the entire marker chromosomes of the patient by using chromosome microdissection and PCR amplification. FISH with this probe on metaphase chromosomes of a karyotypically normal individual painted the distal third or half region of 8p (fig. 2*a* and *b*). FISH with the same probe onto the patient's chromosomes painted the marker chromosome entirely, as well as the normal distal 8p regions (fig. 2*c*), indicating that the probe pool was successfully generated from the entire region of the marker chromosome. Thus, we concluded that the marker originated from distal 8p, involving no centromeric region.

Characterization of the Structure of the Marker Chromosome

By FISH, neither α-satellite probe for all human centromeres nor α-satellite probe specific for chromosome 8 hybridized the marker (fig. 2*d*), while a telomere-specific sequence was detected at each end (fig. 2*e*). Two-color FISH using cosmid probes cCI8-1 (8p23.3) and cCI8-1198 (8p22 or 23.1) showed an 8-1198 signal sandwiched between two 8-1 signals on the marker (fig. 2*f* and *g*), indicating that the marker was an inverted duplication interpreted as 8pter→p22or23.1::p22or23.1→8pter. Judging from the G-banding pattern of the marker (a middle G-negative band between two terminal G-positive bands), we concluded that her karyotype was 47,XX,+der(8)(pter→p23.1::p23.1→pter) de novo and that she was tetrasomic for its region (fig. 3).

Detection of Kinetochore Protein

Anti-kinetochore immunostaining using a serum from a patient with CREST syndrome revealed a pair of fluorescent signals on the marker. It appeared to be of almost the same intensity as those of other chromosomes, while it was about one-third of the others in size (fig. 4). The precise location of the signals in the marker could not be determined. Although this anti-kinetochore serum was not characterized in detail for its specificity to CENPs, this result indicated that at least some functional kinetochore proteins were generated on the marker.

Discussion

A stable supernumerary small marker chromosome was found in a patient with mild dysmorphic features. The origin of the marker was successfully identified to be around the distal region of 8p by using the technique that involves microdissection of a defined chromosomal region (the entire marker chromosome in this case), generation of a FISH probe pool from the dissected chromosomal DNA by PCR, and FISH (Ohta et al. 1993). Further, two-color FISH using two cosmid probes both assigned to the distal 8p region revealed that the marker was an inverted duplication interpreted as 8pter→p23.1::p23.1→8pter and that the patient was tetrasomic for its region. Although there has been no reported case of tetrasomy for the same 8p23.1-pter region as in our patient, some manifestations in the patient were compatible with those previously de-

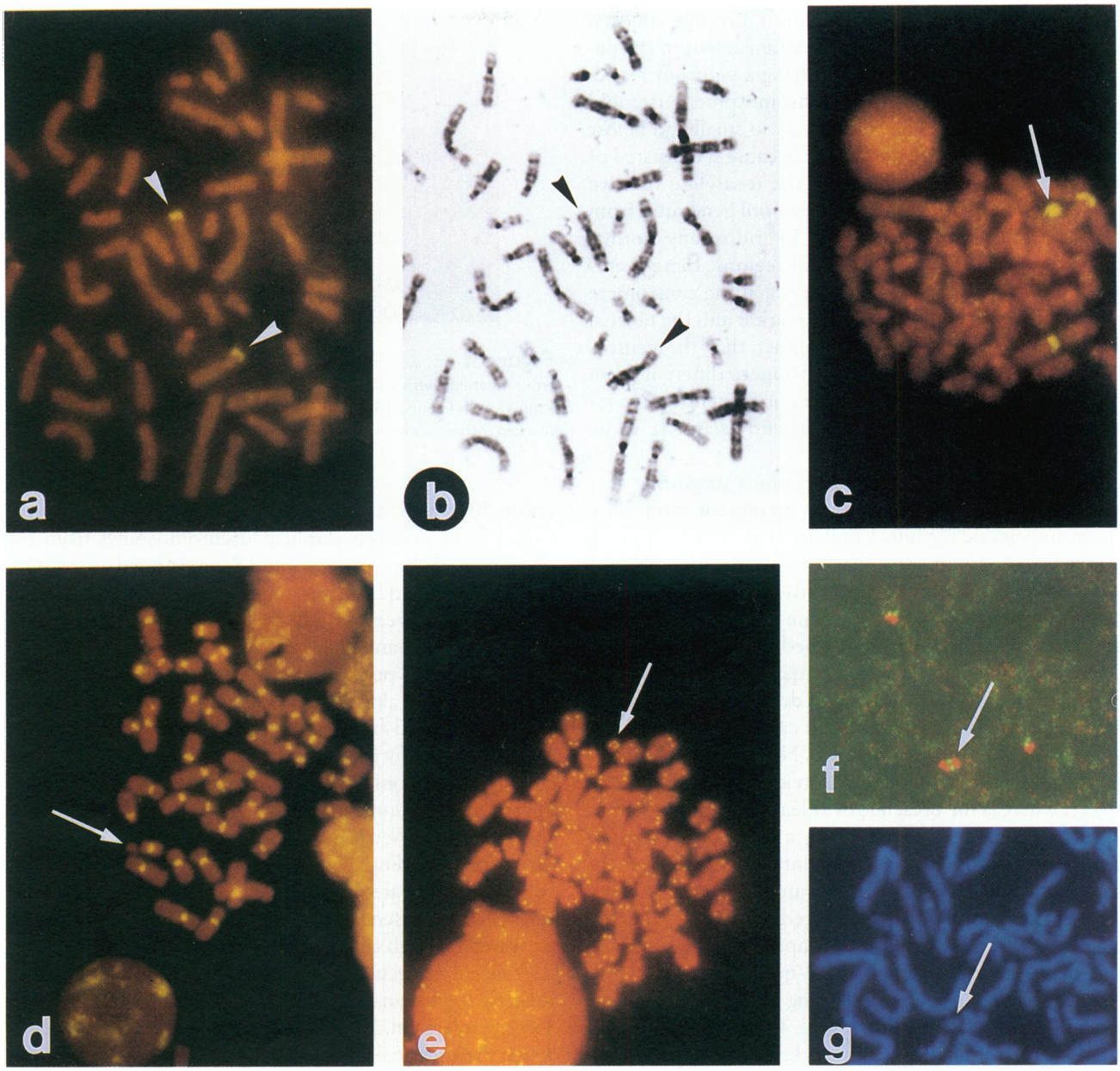

Figure 2 FISH analyses on normal metaphase chromosomes (*a* and *b*) and on patient's chromosomes (*c–g*), using various DNA probes. *a*, FISH using a probe pool derived from the marker chromosome of the patient on a normal metaphase. *b*, Same chromosomes subsequently G-banded. *c*, Same probe hybridized to the patient's metaphase chromosomes. *d*, α-Satellite probe for all human centromeres. *e*, Telomere-specific probe for all human chromosomes. *f*, Two-color FISH using cosmid probes cCI8-1 assigned to 8p23.3 (rhodamin-red) and cCI-1198 initially assigned to 8p22 or 23.1 (FITC-green). *g*, Same chromosomes counterstained with DAPI. Arrowheads indicate the painted chromosomal domains, and arrows indicate the marker chromosomes.

scribed in mosaic tetrasomy for the entire 8p, including a congenital heart defect, a broad forehead, and mental retardation (Schrander-Stumpel et al. 1994).

It was unusual that no centromeric region was involved in the marker, because acentric chromosomes, as mentioned earlier, are generally believed to be mitotically unstable and lost during cell divisions. FISH using an α-satellite probe either for all human chromosomes or specific for chromosome 8 failed to detect a signal on the marker. However, anti-kinetochore staining using the serum from a CREST patient revealed a pair of fluorescent spots on the marker, indicating that a functional kinetochore protein was present on the marker. Thus, we assumed that an ancient centromere sequence exists at distal 8p (8p23.1-pter) and was activated through the chromosome rearrangement in the patient.

An alternative possible explanation for this unusual marker is that the chromosomal rearrangement in the patient is a more complex one, involving a centromeric region of chromosome 8 or other chromosome, but could not be detected, either because it was so small and below our detection sensitivity of FISH or because it consisted of centromeric sequences other than the α-satellite that we used. Since the FISH with the probe pool generated from the marker chromosome did not hybridize any normal centromeres or other chromosomal regions, there seems to be a lack of major homology between the centromere that is assumed to be present in the marker and the normal centromeres. This might suggest either that the centromere of the marker is a novel centromere different from normal centromeres or else that the critical centromere region is small, and FISH is not sufficiently sensitive to be able to detect this region.

Recently, there have been observations suggesting that some centromere sequences might be present intracalary at noncentromeric regions. Voullaire et al. (1993) reported an unusual supernumerary marker chromosome derived from chromosome 10 in a boy with mild developmental delay. A chromosome 10 homologue was broken at p12.1 and q23.2 and subsequently rejoined, producing two derivative chromosomes: r(10)(p12.1q23.2) and der(10)(pter→p12.2::q23.3→qter). The later der(10) was apparently lacking a centromeric region, but it carried a primary constriction and was mitotically stable. No detectable α-satellite, satellite III, or CENP-B protein was found in the marker, whereas the presence of at least some kinetochore proteins was demonstrated using a CREST antiserum. They hypothesized that a latent intercalary centromere was present at 10q25 and was activated through the chromosome rearrangement that occurred in the patient. Magnani et al. (1993) reported a stable supernumerary chromosome that was derived from 14q32-qter confirmed by the chromosome-painting method using a chromosome 14–specific probe pool. Blennow et al. (1994) reported two patients who both had an extra marker chromosome present in 70%–80% of their lymphocytes. The markers were found to be inversely duplicated chromosomes from the distal part of the chromosome 15, interpreted as inv dup(15)(qter→q23::q23→qter) and inv dup(15)(qter→q24::q24→qter), respectively. Both markers included no centromeric region, and no α-satellite DNA could be detected at the site of the primary constriction. Baldini et al. (1993) reported that an alphoid DNA sequence that had been isolated and cloned from a human chromosome 21–specific plasmid library by using vector-CENP-B box PCR hybridized in situ not only to every centromere but also to noncentromeric regions at 2q21 and 9q13. They concluded that alphoid DNA sequences were present at 2q21 and 9q13. These findings, together with the present case, seem to strongly indicate that intercalary ancient centromere sequences are present in the human genome and could be activated through chromosome rearrangements.

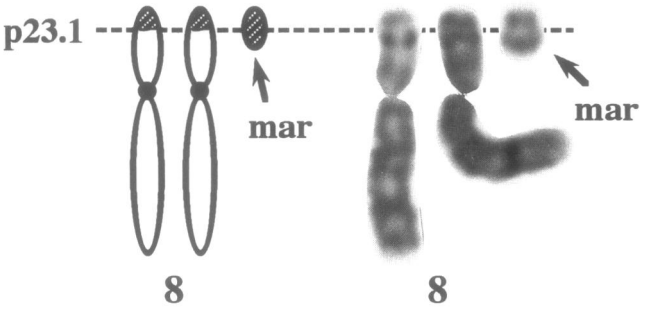

Figure 3 Schematic representation of the marker structure (*left*), together with a G-banded partial karyotype of the chromosome 8 pair and the marker chromosome (*right*). Shaded areas indicate the 8p23.1-pter region that was inversely duplicated in the marker.

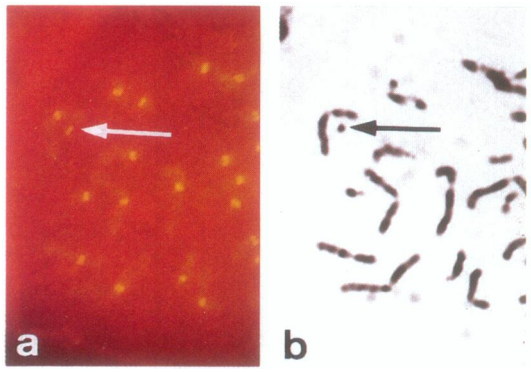

Figure 4 *a*, Anti-kinetochore immunostaining using the serum from a patient with CREST syndrome. *b*, Same metaphase sequentially stained with Giemsa for the marker identification. Arrows indicate the marker chromosomes.

A recent molecular cytogenetic technique has helped to identify the origins of marker chromosomes (Callen et al. 1992; Crolla et al. 1992; Rauch et al. 1992; Blennow et al. 1993; Plattner et al. 1993). The strategies in most previous studies were to identify it by FISH using chromosome-specific centromeric satellite probes (usually α-satellite). However, several marker chromosomes have been reported to be unidentifiable by FISH using various centromeric satellite probes (Callen et al. 1992; Crolla et al. 1992; Rauch et al. 1992). It might be possible that some of them arose from a noncentromeric region, as was the case in our patient. Thus, it is important to be aware of the possible existence of such a marker chromosome that cannot be identified by centromeric satellite probes. To identify the origins of these marker chromosomes, the "reverse chromosome painting" technique, termed by Carter et al. (1992)—in which the probe is generated from the aberrant chromosome itself and is applied to metaphase spreads of normal subjects by using FISH to know the origin of the marker by observing the painted chromosome region—is

most useful. To isolate a marker chromosome, the following two techniques are currently available: (1) chromosome microdissection (Guan et al. 1993; Ohta et al. 1993), which we employed in the present case, and (2) flow sorting (Blennow et al. 1992; Carter et al. 1992), both followed by PCR amplification. In view of the purity of the generated DNA probe pool and the capability of making a probe from even a small marker chromosome, the former technique might have some advantage.

In conclusion, this case may suggest that an ancient centromere sequence exists at distal 8p (8p23.1-pter) and was activated through the chromosome rearrangement. Further investigation of this marker chromosome at a molecular level would be very valuable for identifying the actual sequence that is responsible for chromosome segregation.

Acknowledgments

We thank Dr. Yusuke Nakamura for providing the cosmid probes cCI8-1 and 1198. We are also indebted to Dr. Joji Inazawa for his expert technical advice in two-color FISH.

References

Baldini A, Ried T, Shridhar V, Ogura K, D'Aiuto L, Rocchi M, Ward DC (1993) An alphoid DNA sequence conserved in all human and great ape chromosomes: evidence for ancient centromeric sequences at human chromosomal regions 2q21 and 9q13. Hum Genet 90:577–583

Blennow E, Anneren G, Bui TH, Berggren E, Asadi E, Nordenskjöld M (1993) Characterization of supernumerary ring marker chromosomes by fluorescence in situ hybridization (FISH). Am J Hum Genet 53:433–442

Blennow E, Telenius H, de Vos D, Larsson C, Henriksson P, Johansson Ö, Carter NP, et al (1994) Tetrasomy 15q: two marker chromosomes with no detectable alpha-satellite DNA. Am J Hum Genet 54:877–883

Blennow E, Telenius H, Larsson C, de Vos D, Bajalica S, Ponder BAJ, Nordenskjöld M (1992) Complete characterization of a large marker chromosome by reverse and forward chromosome painting. Hum Genet 90:371–374

Bloom K (1993) The centromere frontier: kinetochore components, microtubule-based motility, and the CEN-value paradox. Cell 73:621–624

Callen DF, Eyre H, Yip MY, Freemantle J, Haan EA (1992) Molecular cytogenetic and clinical studies of 42 patients with marker chromosomes. Am J Med Genet 43:709–715

Carter NP, Ferguson-Smith MA, Perryman MT, Telenius H, Pelmear AH, Leversha MA, Glancy MT, et al (1992) Reverse chromosome painting: a method for the rapid analysis of aberrant chromosomes in clinical cytogenetics. J Med Genet 29: 299–307

Crolla JA, Dennis NR, Jacobs PA (1992) A non-isotopic *in situ* hybridisation study of the chromosomal origin of 15 supernumerary marker chromosomes in man. J Med Genet 29:699–703

Deng H-X, Yoshiura K, Dirks RW, Harada N, Hirota T, Tsukamoto K, Jinno Y, et al (1992) Chromosome-band-specific painting: chromosome in situ suppression hybridization using PCR products from a microdissected chromosome band as a probe pool. Hum Genet 89:13–17

Fukushima Y, Ohashi H, Wakui K, Nishida T, Oh-ishi T (1992) A rapid method for starting a culture for the establishment of Epstein-Barr virus transformed human lymphoblastoid cell lines. Jpn J Hum Genet 37:149–150

Guan X-Y, Trent JM, Meltzer PS (1993) Generation of band-specific painting probes from a single microdissected chromosome. Hum Mol Genet 2:1117–1121

Haaf T, Schmid M (1988) Analysis of double minutes and double minute-like chromatin in human and murine tumor cells using antikinetochore antibodies. Cancer Genet Cytogenet 30:73–82

Hirota T, Tsukamoto K, Deng H-X, Yoshiura K, Ohta T, Tohma T, Kibe T, et al (1992) Microdissection of human chromosomal regions 8q23.3-q24.11 and 2q33-qter: construction of DNA libraries and isolation of their clones. Genomics 13:349–354

Ikeuchi T (1984) Inhibitory effect of ethidium bromide on mitotic chromosome condensation and its application to high-resolution chromosome banding. Cytogenet Cell Genet 38: 56–61

Inazawa J, Ariyama T, Tokino T, Tanigami A, Nakamura Y, Abe T (1994) High resolution ordering of DNA markers by multicolor fluorescent in situ hybridization of prophase chromosomes. Cytogenet Cell Genet 65:130–135

Jinno Y, Harada N, Yoshiura K, Ohta T, Tohma T, Hirota T, Tsukamoto K, et al (1992) A simple and efficient amplification method of DNA with unknown sequences and its application to the microdissection/microcloning. J Biochem 112:75–80

Levan A, Levan G, Mandahl N (1981) Double minutes and C-bandless chromosomes in a mouse tumor. In: Arrighi FE, Rao PN, Stubblefield E (eds) Genes, chromosomes, and neoplasia. Raven, New York, pp 223-251

Lin CC, Meyne J, Sasi R, Moyzis RK (1990) Apparent lack of telomere sequences on double minute chromosomes. Cancer Genet Cytogenet 48:271–274

Magnani I, Sacchi N, Darfler M, Nisson PE, Tornaghi R, Fuhrman-Conti AM (1993) Identification of the chromosome 14 origin of a C-negative marker associated with a 14q32 deletion by chromosome painting. Clin Genet 43:180–185

Masumoto H, Masukata H, Muro Y, Nozaki N, Okazaki T (1989) A human centromere antigen (CENP-B) interacts with a short specific sequence in alphoid DNA, a human centromeric satellites. J Cell Biol 109:1963–1973

Merry DE, Pathak S, Hsu TC, Brinkley BR (1985) Anti-kinetochore antibodies: use as probes for inactive centromeres. Am J Hum Genet 37:425–430

Ohta T, Tohma T, Seojima H, Fukushima Y, Nagai T, Yoshiura K, Jinno Y, et al (1993) The origin of cytologically unidentifiable chromosome abnormalities: six cases ascertained by targeted chromosome-band painting. Hum Genet 92:1–5

Plattner R, Heerema NA, Yurov YB, Palmer CG (1993) Efficient identification of marker chromosomes in 27 patients by stepwise hybridization with alpha-satellite DNA probes. Hum Genet 91:131–140

Rauch A, Pfeiffer RA, Trautmann U, Liehr T, Rott HD, Ulmer

RA (1992) A study of ten small supernumerary (marker) chromosomes identified by fluorescence *in situ* hybridisation (FISH). Clin Genet 42:84–90

Schrander-Stumpel CTRM, Govaerts LCP, Engelen JJM, van der Blij-Philipsen M, Borghgraef M, Loots WJG, Peters JJM, et al (1994) Mosaic tetrasomy 8p in two patients: clinical data and review of the literature. Am J Med Genet 50: 377–380

Voullaire LE, Slater HR, Petrovic V, Choo KHA (1993) A functional marker centromere with no detectable alpha-satellite, satellite III, or CENP-B protein: activation of a latent centromere? Am J Hum Genet 52:1153–1163