Alternate centromere inactivation in a pseudodicentric (15;20)(pter;pter) associated with a progressive neurological disorder

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SUMMARY A 13 year old male with a severe progressive neurological disorder was found to have a pseudodicentric chromosome resulting from a telomeric fusion 15p;20p. In lymphocytes, the centromeric constriction of the abnormal chromosome was always that of the chromosome 20, while in fibroblasts both centromeres were alternately constricted. Cd staining was positive only at the active centromere, but a weak anticentromere immunofluorescence was present at the inactive one. We suggest that centromere inactivation results from a modified conformation of the functional DNA sequences preventing normal binding to centromere specific proteins. We also postulate that the patient's disorder, reminiscent of a spongy glioneuronal dystrophy as seen in Alper's and Creutzfeldt-Jakob diseases, may be secondary to the presence of the pathogenic isoform of the prion protein encoded by a gene mapped to 20p12→pter.

The concept of a non-functional or weak centromere was first advanced to explain the behaviour of a dicentric wheat chromosome during cell division.¹ The term centromere inactivation was introduced later to account for comparable observations in man² and is currently used in mammalian cytogenetics. Inactive centromeres behave as inert structures during cell division possibly as a consequence of ineffective spindle attachment. This functional inability is consistently correlated with negative Cd staining^{3,4} and, in most cases, with the absence of constriction in mid-metaphase, while the centromere associated heterochromatin usually retains its staining properties. In most heterodicentric chromosomes, the inactivation affects only one centromere in an apparently random manner.^{5,6} We report on a heterodicentric chromosome exhibiting alternate centromere inactivation.

Case report

The patient was born on 6.4.76 to a 35 year old, G3P1A1 mother and an unrelated 42 year old father. Both parents and the only sib are healthy.

The pregnancy was complicated by maternal hypoglycaemia in the sixth month. Delivery took place in the 38th week with cephalic presentation; birth weight was 2750 g. Because of severe perinatal hypoxia, the infant was kept in an incubator for 20 days. At three months of age, he had a generalised febrile convulsion, which recurred one year later. Psychomotor development was reportedly normal until three years when he suffered from a severe encephalitis-like illness with loss of consciousness for 10 days; afterwards, he was no longer able to walk and talk and showed progressive intellectual impairment. At that time, an EEG disclosed a disorganised pattern with diffuse paroxysms and mixtures of spikes and sharp waves. CT scan showed slight enlargement of the cerebral fissures and ventricular system. Other examinations, such as muscle biopsy, plasma chromatography, lysosomal enzyme determinations, and skeletal radiographs, yielded normal results. At 10 years, a fundoscopy showed pale papillae, some pigmentary changes, and incipient capsular lens opacification. At 13 years, the patient was confined to a supine position and had axial hypotonia but hypertonic tetraparesis and increased deep tendon reflexes; Babinsky sign was negative. He was unable to communicate and was incontinent. Hearing and vision were apparently preserved. Facial features

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FIG 1 The pseudodicentric 15;20. (a) High resolution G banding (the normal 15 is upside down). (b, c) The active No 15 centromere as identified by C and DA-DAPI banding. (d, e, f) The active No 20 centromere as identified by Cd, C, and DA-DAPI banding. Also note the Ag-positive NOR in the abnormal chromosome.

were somewhat coarse and the tongue was protruding. A slower activity was observed in repeated EEGs.

Material and methods

Lymphocyte and skin fibroblast cultures were prepared and processed by standard techniques. Chromosome preparations were processed for Q, G, C, NOR, DA-DAPI, and high resolution⁷ banding techniques. The centromeres were further characterised by the Cd⁸ and anticentromere immunofluorescence⁹ staining techniques. Parental chromosomes were studied in blood lymphocytes with Q and NOR banding.

TABLE Patterns of active centromeres in lymphocytes and fibroblasts identified by C or DA-DAPI banding.

	Active No 15	Active No 20
Lymphocytes	0	144 (100%)
Fibroblasts 2n*	6 (7.9%)	70 (92.1%)
Fibroblasts 4n	4 (12.5%)	28 (87.5%)

*In one additional cell the chromosome appeared dicentric.

Results

The patient had 45 chromosomes with an abnormal chromosome resulting from an end to end fusion of 15p;20p. This chromosome had only one primary constriction, which, in lymphocytes, always corresponded to the No 20 centromere, whereas in fibroblasts both centromeres were alternately constricted (fig 1, table). In one fibroblast, the chromosome appeared as a true dicentric. The heterochromatin associated with either the suppressed or active centromeres was C and DA-DAPI positive. The inactive No 15 centromere was Cd negative in lymphocytes and exhibited a weak fluorescence after antikinetochore staining in fibroblasts (fig 2). The smaller number of cells with the opposite inactivation pattern prevented a similar characterisation of the suppressed No 20 centromere. The intercalary NOR was Ag positive in all of the 28 lymphocytes scored. The patient also had a maternally inherited inv(9) and thus his karyotype is 45,XY,inv(9)(p11q13)mat,-15,-20,+psu dic (15;20)(pter;pter).

FIG 2 A metaphase plate after anticentromere immunofluorescence (left) and DA-DAPI staining (right). Note the weak fluorescence at the inactive No 15 centromere.

At the first and second passages 64 of 252 fibroblast metaphases (25.4%) were tetraploid, but at the seventh passage only three of 31 cells were. In 32 C banded tetraploid cells, both abnormal chromosomes showed the same centromere inactivation pattern.

Parental karyotypes had no informative chromosome 15 polymorphisms.

Discussion

This abnormal chromosome appears to result from a telomeric fusion without visible material loss. It is indeed a pseudodicentric with two centromere inactivation patterns, the No 20 centromere being predominantly active. We know of four other heterodicentrics with alternate centromere inactivation,¹⁰⁻¹³ but whether such functional mosaicism results from successive inactivation-reversion processes or long enough persistence of a functionally dicentric chromosome to produce the distinct cell lines is still uncertain.^{14 15} Although an irreversible inactivation has been documented in one instance,¹⁶ the finding in the same cell of pseudodicentrics with different centromere inactivation patterns^{12 17} indicates that a reversible process can also exist. Our case differs from the previous ones in the apparently tissue limited differential centromere activity, but whether this is the result of sampling error or represents a genuine difference of potential significance remains open to question.

As to the mechanism of centromere inactivation, a functional modification^{2 18} or a deletion^{11 15 19} of critical DNA sequences have been proposed. In fact, a centromeric deletion was shown in a cell line of a patient with a terminal rearrangement 13p;20q.¹⁵ Nevertheless, the following points are in favour of a functional modification. (1) The common finding that an inactive centromere is C band positive suggests that deletions detectable by light microscopy do not usually occur. (2) It is hard to conceive the concurrence of similar or identical deletions, especially if secondary to mechanical forces,¹⁵ in heterodicentric and multicentric chromosomes with inactivation of two or more centromeres.^{10-12 20} (3) The diverse amounts and presumably classes of centromeric antigens detected by specific antibodies on inactive centromeres^{9 21 22} could reflect different degrees of the inactivation phenomenon, as suggested by T C Hsu to Earnshaw and Migeon.²¹ (4) A conspicuous cell to cell intraindividual variability of Y isodicentrics, ranging from a chromosome with two active centromeres to a monocentric element,^{17 23 24} has been noted.

Centromeric constituents of eukaryotic chromosomes have been conserved during evolution.^{25 26} By analogy with the well known centromeric DNA sequences of yeast, there may be repeats of a prototypical unit essential for spindle attachment in higher organisms.²⁷ In addition, satellite DNA seems to play a crucial role in the pairing of sister

chromatids at the primary constriction.²⁸⁻³⁰ Both these functions are mediated by specific DNA-protein interactions. Among the several centromeric proteins, the CENP-B antigen would be responsible for the open conformation of the centromeric chromatin,³¹ whereas a mitosis specific polypeptide seems to participate in the anchorage of the kinetochore to functional DNA domains,³² and still another protein subset would be involved in centromere constriction, probably via its association with alphoid or other satellite DNA sequences.³³⁻³⁴ Taken together, these data suggest that centromere inactivation may result from a modified conformation of the critical DNA sequence preventing normal binding to proteins, but how this is accomplished is as yet unclear. The disappearance of the constriction and the eventual negativity to C banding³⁵ are likely to be a consequence of this inactivation process. Thus, there are at least three distinct but not mutually exclusive mechanisms accounting for the stability of dicentric or multicentric chromosomes. These are centromere inactivation, centromere deletion, and coordinated segregation of two (close) active centromeres.³⁶⁻³⁷ The premature separation of supernumerary centromeres, previously proposed as a stabilising device,²⁰⁻³⁸ should then be regarded only as an inherent manifestation of the inactivation process.

The NOR activity in the present case seems unaffected by the suppression of the acrocentric's centromere and so conforms to previous similar cases.⁵⁻¹⁵ On the other hand, the significant loss of NOR activity observed in a 9;13 pseudodicentric when both centromeres were constricted suggests that the control of these phenomena is related.¹³

Our patient, as was found in the case of Vianna-Morgante and Rosenberg,¹⁵ had a higher proportion of tetraploid fibroblasts. The cause and significance of this finding remains obscure.

Although a non-genetic aetiology of our patient's neurological disorder cannot be excluded, the clinical course is reminiscent of a spongy glioneuronal dystrophy as seen in Alper's and Creutzfeldt-Jakob diseases.³⁹ The gene coding for both the cellular and the scrapie isoforms of the prion protein associated with Creutzfeldt-Jakob disease has been mapped to 20p12→pter.⁴⁰ The chromosome rearrangement in our patient could have resulted in a disruption of the gene function and thus in the actual phenotype. For instance, it could be that the adjacent active NOR determines an overproduction of the cellular isoform which in turn supersedes the control mechanisms and, via adequate post-translational events,⁴¹ ultimately results in the presence of the pathogenic scrapie isoform.

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Note added in proof

Prusiner *et al* (Nature 1989;338:342-5) reported that the prion protein gene on the short arm of human chromosome 20 might be abnormal in the apparently autosomal dominant Gerstmann-Straussler syndrome.

References

- Sears ER, Câmara A. A transmissible dicentric chromosome. *Genetics* 1952;37:125-35.
- Therman E, Sarto GE, Patau K. Apparently isodicentric but functionally monocentric X chromosome in man. *Am J Hum Genet* 1974;26:83-92.
- Daniel A. Single Cd band in dicentric translocations with one suppressed centromere. *Hum Genet* 1979;48:85-92.
- Maraschio P, Zuffardi O, Lo Curto F. Cd bands and centromeric function in dicentric chromosomes. *Hum Genet* 1980;54:265-7.
- Howard PJ, Berry AC. Familial transmission of a non-Robertsonian translocation dicentric. *Clin Genet* 1986;29:246-50.
- Dewald GW. Translocation dicentric chromosomes among human autosomes and centromeric suppression. In: Daniel A, ed. *The cytogenetics of mammalian autosomal rearrangements*. New York: Alan R Liss, 1988:533-50.
- Dutrillaux B, Viegas-Pequignot E. High resolution R- and G-banding on the same preparations. *Hum Genet* 1981;57:93-5.
- Denton TE, Brooke WR, Howell WM. A technique for the simultaneous staining of both nuclear organizer regions and kinetochores of human chromosomes with silver. *Stain Technol* 1977;52:311-3.
- Peretti D, Maraschio P, Lambiasi S, Lo Curto F, Zuffardi O. Indirect immunofluorescence of inactive centromeres as indicator of centromeric function. *Hum Genet* 1986;73:12-6.
- Niebuhr E. A 45,XX,5-,13-,dic+ karyotype in a case of cri-du-chat syndrome. *Cytogenetics* 1972;11:165-77.
- Dewald GW, Boros SJ, Conroy MM, Dahl RJ, Spurbeck JL, Vitek HA. A t(5;15)(p13;p11) chromosome showing variation for constriction in the centromeric regions in a patient with the cri du chat syndrome. *Cytogenet Cell Genet* 1979;24:15-26.
- Ing PS, Smith SD. Cytogenetic studies of a patient with mosaicism of isochromosome 13q and a dicentric (Y;13) translocation showing differential centromere activity. *Clin Genet* 1983;24:194-9.
- Daniel A, Ekblom L, Philips S, Fitzgerald JM, Opitz JM. NOR activity and centromere suppression related in a de novo fusion t(9;13)(p22;p13) chromosome in a child with del(9p) syndrome. *Am J Med Genet* 1985;22:577-84.
- Zuffardi O, Tiepolo L. Latent centromeres and chromosome function in man. *Clin Genet* 1981;19:547.
- Vianna-Morgante AM, Rosenberg C. Deletion of the centromere as a mechanism for achieving stability of a dicentric chromosome. *Cytogenet Cell Genet* 1986;42:119-22.
- Drets ME, Therman E. Human telomeric 6;19 translocation chromosome with a tendency to break at the fusion point. *Chromosoma* 1983;88:139-44.
- Schmid W, D'Apuzzo VD. Centromere inactivation in a case of Turner variant with two dicentric iso-long arm Y chromosomes. *Hum Genet* 1978;41:217-23.
- Hsu TC, Pathak S, Chen TR. The possibility of latent

- centromeres and a proposed nomenclature system for total and whole arm translocations. *Cytogenet Cell Genet* 1975;15:41-9.
- ¹⁹ Distèche C, Hagemijer A, Frederic J, Progneaux D. An abnormal large human chromosome identified as an end-to-end fusion of two Xs by combined results of the new banding techniques and microdensitometry. *Clin Genet* 1972;3:388-95.
 - ²⁰ Vig BK. Sequence of centromere separation: orderly segregation of multicentric chromosomes in mouse L-cells. *Chromosoma* 1984;90:39-45.
 - ²¹ Earnshaw WC, Migeon BR. Three related centromere proteins are absent from the inactive centromere of a stable isodicentric chromosome. *Chromosoma* 1985;92:290-6.
 - ²² Merry DE, Pathak S, Hsu TC, Brinkley BR. Antikinetochore antibodies: use as probes for inactive centromeres. *Am J Hum Genet* 1985;37:425-30.
 - ²³ Angell R, Gianelli F, Polani PE. Three dicentric Y chromosomes. *Ann Hum Genet* 1970;34:39-50.
 - ²⁴ Daniel A, Lyons N, Casey JH, Gras L. Two dicentric Y isochromosomes, one without the Yqh heterochromatic segment. Review of the Y isochromosome. *Hum Genet* 1980;54:31-9.
 - ²⁵ Cox JV, Olmsted JB. Kinetochore antigens: complexity, synthesis, and evolutionary conservation. In: Borisy GG, Cleveland DW, Murphy DB, eds. *Molecular biology of the cytoskeleton*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1984:71-7.
 - ²⁶ Brinkley BR, Tousson A, Valdivia MM. The kinetochore of mammalian chromosomes: structure and function in normal mitosis and aneuploidy. In: Dellarco VL, Voytek PE, Hol-laender A, eds. *Aneuploidy: etiology and mechanism*. New York: Plenum Press, 1985:243-67.
 - ²⁷ Hieter P, Pridmore D, Hegemann JH, Thomas M, Davies RW, Philippsen P. Functional selection and analysis of yeast centromeric DNA. *Cell* 1985;42:913-21.
 - ²⁸ German J. Robert's syndrome. I. Cytological evidence for a disturbance in chromatid pairing. *Clin Genet* 1979;16:441-7.
 - ²⁹ Vig BK, Zinkowski RP. Sequence of centromere separation: influence of pericentromeric heterochromatin (repetitive DNA) in *Mus*. *Genetica* 1985;67:153-9.
 - ³⁰ Lica LM, Narayanswami S, Hamkalo BA. Mouse satellite DNA, centromere structure, and sister chromatid pairing. *J Cell Biol* 1986;103:1145-51.
 - ³¹ Earnshaw WC, Sullivan KF, Machlin PS, et al. Molecular cloning of cDNA for CENP-B, the major human centromere autoantigen. *J Cell Biol* 1987;104:817-29.
 - ³² Hadlaczký G, Praznovszky T, Rasko I, Kereso J. Centromere proteins. I. Mitosis specific centromere antigen recognized by anti-centromere autoantibodies. *Chromosoma* 1989;97:282-8.
 - ³³ Alexandre C, Miller DA, Mitchell AR, et al. p82H identifies sequences at every human centromere. *Hum Genet* 1987;77:46-50.
 - ³⁴ Rattner JB, Kingwell BG, Fritzler MJ. Detection of distinct structural domain within the primary constriction using autoantibodies. *Chromosoma* 1988;96:360-7.
 - ³⁵ Nakagome Y, Nakahari Y, Mitani K, Matsumoto M. The loss of centromeric heterochromatin from an inactivated centromere of a dicentric chromosome. *Jpn J Hum Genet* 1986;31:21-6.
 - ³⁶ Hair JB. The origin of new chromosomes in *Agropyron*. *Heredity* 1953;6(suppl):215-33.
 - ³⁷ Ying KL, Ives EJ. Mitotic behaviour of a human dicentric Y chromosome. *Cytogenetics* 1971;10:208-18.
 - ³⁸ Vig BK, Zinkowski RP. Sequence of centromere separation: a mechanism for orderly separation of dicentrics. *Cancer Genet Cytogenet* 1986;22:347-59.
 - ³⁹ Crompton MR. Alper's disease—a variant of Creutzfeldt-Jakob disease and subacute spongiform encephalopathy? *Acta Neuropathol (Berl)* 1968;10:99-104.
 - ⁴⁰ Sparkes RS, Simon M, Cohn VH, et al. Assignment of the human and mouse prion protein genes to homologous chromosomes. *Proc Natl Acad Sci USA* 1986;83:7358-62.
 - ⁴¹ Basler K, Oesch B, Scott M, et al. Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. *Cell* 1986;46:417-28.

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