Jpn. J. Human Genet. **31**, 21–26, 1986

THE LOSS OF CENTROMERIC HETEROCHROMATIN FROM AN INACTIVATED CENTROMERE OF A DICENTRIC CHROMOSOME

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Summary In most cases with a constitutional dicentric chromosome, one of the two centromeres on each dicentric chromosome is inactivated. This is associated with the loss of Cd-positive structure although both centromeres remain C positive.

In this paper, a case of dicentric chromosome is described in which the inactivation of a centromere is, to our surprise, associated with the loss of C-positive heterochromatin.

INTRODUCTION

In 1974, Eiberg described a technique called Cd banding which stains two small dark spots on both sides of a centromere (Eiberg, 1974). Later, Nakagome *et al.* (1976) described a patient in whom both active and inactive centromeres of each dicentric chromosome were positive by C banding but only the active centromere was positive by Cd banding. The loss of Cd-positive structure appears to be a general phenomenon which is associated with the inactivation of a centromere although it remains C positive (Nakagome *et al.*, 1976, 1980, 1984; Daniel, 1979; Maraschio *et al.*, 1980).

In the present study a constitutional case of a dicentric chromosome is described in which both Cd-positive structure and a block of C-positive centromeric heterochromatin are missing from an inactivated centromere on each dicentric chromosome.

Received October, 19, 1985

MATERIALS AND METHODS

We observed a total of nine patients with constitutional dicentric chromosomes. In each of them, one of the two centromeres was inactivated and the dicentrics assumed a monocentric appearance forming a pseudo-dicentric chromosome. Results of G- and Cd-band studies of these cases were described earlier (Nakagome *et al.*, 1976, 1980, 1983, 1984; Abe *et al.*, 1977; Matsubara *et al.*, 1981).

In all nine cases, only one Cd-positive site was detected on each "dicentric" chromosome and it corresponded to the active centromere. C-band studies were carried out in five cases (Nakagome *et al.*, 1976, 1980; Abe *et al.*, 1977; Matsubara *et al.*, 1981). Two C-positive sites were detected on each dicentric chromosome and both active and inactive centromeres were C positive in all five cases.

In the present study, the remaining four cases were examined: karyotypes were 46,X,psu dic(X)(p22.3), (case 2422); 46,X,psu dic(X)(q22), (case 2430); 46,X,psu dic(X)(q27), (case 2444); and 47,XX,+psu dic(15)(q12 or 13), (case 2493). They were studied by both C-banding (Sumner, 1972) and distamycin-DAPI staining (abb. dD, Schweizer *et al.*, 1978) followed by identification of chromosomes with DAPI (4'-6-diamidino-2-phenylindole) (abb. dD/D). All observations were based on photographic prints and at least 20 (C) or 10 (dD/D) mitotic spreads were examined.

Data from both parents of case 2444 were also available.

RESULTS

In two cases, two C-positive sites were detected on each psu dic chromosome; they corresponded to the active and the inactive centromeres (2422 and 2493); the results were in agreement with the five previous cases. To our surprise, in cases 2430 and 2444, only the active centromere was C-band positive (Figs. 1 and 2). To avoid possible technical variables in the process of C-banding, dD/D staining was also carried out. Again, only the active centromere was stained (Fig. 1). As mentioned earlier, only the active centromere was Cd positive in each dicentric chromosome in all of the present cases. Thus, in these two cases each psu dic chromosome showed positive reaction at the site of the active centromere with all three techniques, C, Cd and dD/D. On the other hand, the inactive centromere was not positive on any of the three methods.

All three X chromosomes of the parents of case 2444 (one X from the father and two from the mother) showed bright fluorescence at their centromeres by the dD staining. They were individually identifiable based on DAPI staining (dD/D). Every C-group chromosome had a distinct C- and Cd-positive spot at its centromere in both of the parents. Thus, all the three X chromosomes of the parents were

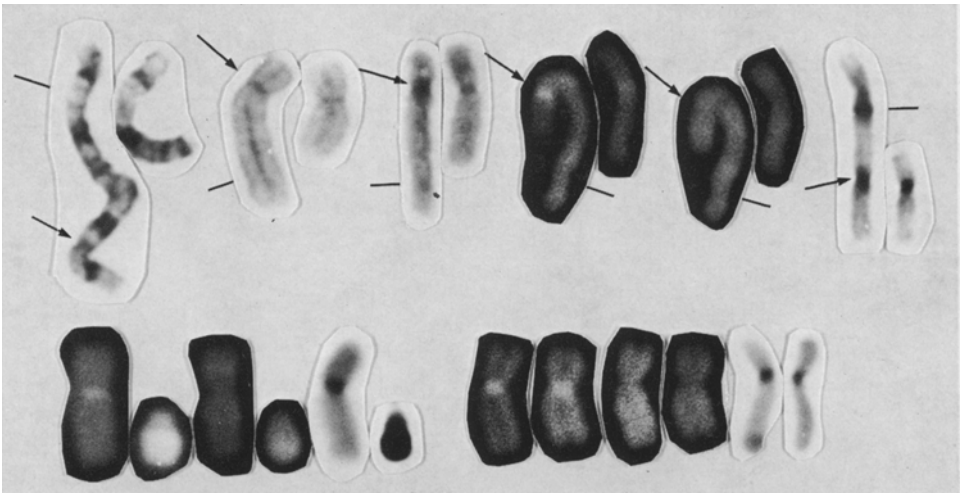

Fig. 1. X chromosomes of case 2444 and her parents. Top row, from left: G, Cd, C, distamycin-DAPI (dD), DAPI stained X chromosomes of case 2444 and a C-stained chromosome pair of case 2422. DAPI staining was made after destaining of dD stained chromosomes. Within a pair, a dicentric X is placed on the left. In either of C and Cd stained preparations, a normal X is not individually identifiable. Thus, a presumed X based on the size and shape is shown. It is certain, though, that each X shows positive staining, as all the C group chromosomes were positive by both procedures. Only one of two centromeres of a dicentric chromosome showed a positive spot (arrow). The other was negative (short bar) in C, Cd- and dD stained preparations. Case 2422 has a psu dic(X)(qter→cen→p22.3::p22.3→qter) constitution and is included for comparison. Both active and inactive centromeres are C positive. Bottom row, from left: X and Y chromosomes of the father and two X chromosomes of the mother, dD-, DAPI-, and C-staining. All 3 X chromosomes are both dD and C positive.

tacitly C and Cd positive, although they were not individually identified. The parents of case 2430 were not available for C or dD/D study.

DISCUSSION

Except for some of the cases of "dicentric" Robertsonian translocation in which both centromeres were in very close proximity, in most, if not all, constitutional cases of dicentric chromosomes, one of the two centromeres on each dicentric chromosome is inactivated and assumes a monocentric appearance. In at least 21 of them, the loss of Cd-positive structure from an inactivated centromere has been established (Nakagome *et al.*, 1976, 1980, 1984; Daniel, 1979; Maraschio *et al.*, 1981; Romain *et al.*, 1982; Zuffardi *et al.*, 1982). In aged individuals, a chromosome in a mitotic spread may assume a parallel-bar appearance in a small number

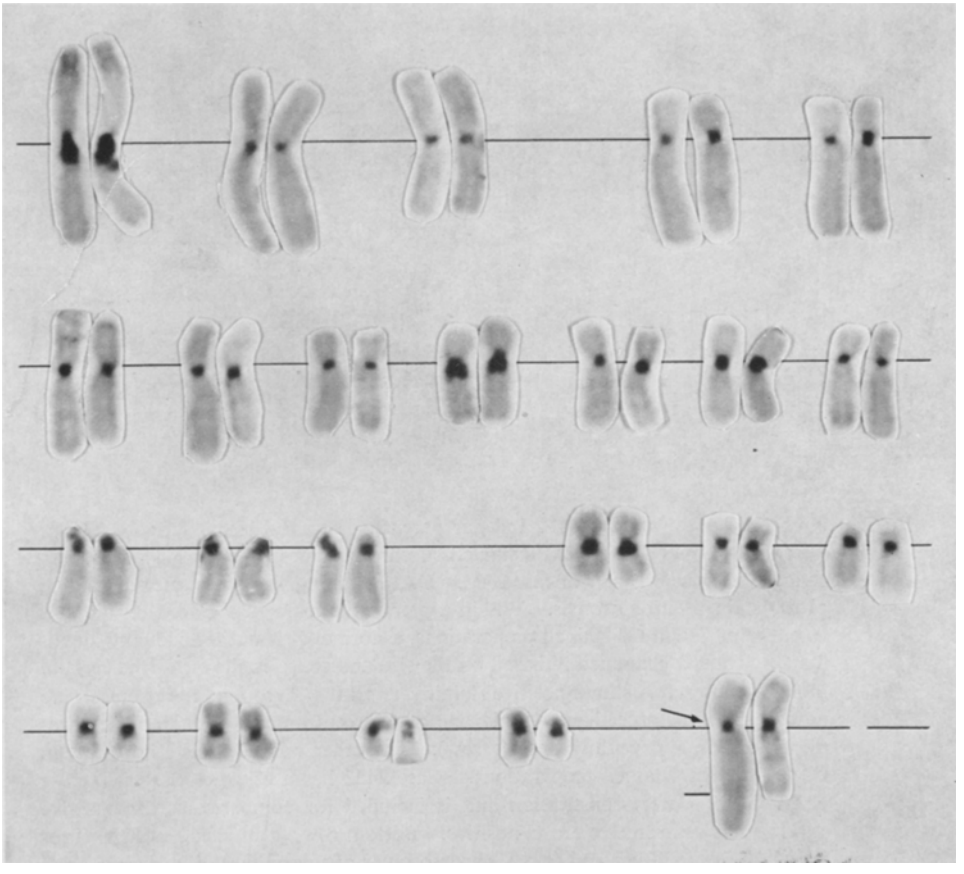

Fig. 2. C-stained karyotype of case 2430. The dicentric X has only one C-positive spot (arrow). The inactivated centromere is also marked (short bar). Note that every chromosome has a C-positive spot at its centromere.

of cells due to probable inactivation of the centromere (Fitzgerald *et al.*, 1975). It was associated with the loss of Cd-positive structure (Nakagome *et al.*, 1984). Thus, the loss of Cd-positive structure might be a general phenomenon in an inactivated centromere. The loss of C-positive structure has not been detected in any of the inactivated centromeres.

All the three X chromosomes of the parents of case 2444 were Cd, C and dD/D positive. Whatever the mechanism of dicentric formation is, neither of the parents has an X chromosome which can contribute a C-, Cd- and dD-negative centromere to the dicentric (psu dic) X chromosome of the propositus. In this particular case, the inactivated centromere of the dicentric chromosome presumably lost a block of C- and dD-positive heterochromatin in addition to Cd-positive material.

C-positive material in the secondary constriction of chromosome nos. 1, 9 or

16 or on either the satellite or the short arm of an acrocentric may not be as stable (Ferguson-Smith, 1974; Craig-Holmes and Shaw, 1975; Nakagome *et al.*, 1977) as is generally believed. However, the present case appears to represent the first example of the loss of C-positive material from the centromere region of a chromosome.

The parents of case 2430 were not available for C-banding but it is theoretically possible to assume that one of the three X chromosomes of the parents was C negative and was the source of the C-negative centromere of the dicentric chromosome. It then lost its function and Cd-positive structure. Although the presence of a C-negative X chromosome appears to be unusual, it is not possible to rule it out with certainty.

Acknowledgments Cases 2444 and 2430 were kindly made available by Dr. H. Fujita, Department of Child Health, Osaka City University and Mr. M. Maruyama, St. Mary's Hospital, Kurume, respectively.

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