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Brief Communications

A Stable Human Dicentric Chromosome, t dic (12;14)(p13;p13) including an Intercalary Satellite Region between Centromeres

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The satellite regions of all five pairs of acrocentric chromosomes in man function as nucleolus organizers [1] and are the site of rDNA loci [2]. Although the acrocentric chromosomes are those most commonly involved in translocations, there has so far been no clear demonstration in man of either a centric fusion or a reciprocal translocation with satellite material in other than the terminal position of a chromosome. According to the classical idea that translocations cannot involve unbroken chromosome ends [3], such intercalary satellites would have to be preceded by a break in the satellite region itself, with loss or translocation of a telomere.

A dicentric chromosome has rarely been described as a consistent feature of the chromosome complement of any individual. This is not unexpected, since such chromosomes are known to be unstable; their potential to be pulled apart in opposite directions during mitosis or meiosis leads to chromosome breakage, rearrangements, and loss [4]. A number of dicentric Y chromosomes have been described, usually in a mosaic with an XO cell line also present [5–7]. Such chromosomes have the two presumed centromeric constrictions very close together and are essentially isochromosomes, usually for the long arm. The reason for their relative stability has not been investigated. The use of the new chromosome banding techniques may reveal an unexpectedly large number of chromosomes incorporating two centromeric regions. Neibuhr [8] has recently reported that some Robertsonian D/D translocations may be dicentric, and Disteche et al. [9] have shown two centromeric regions (one nonfunctional) in an X/X translocation.

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We will describe here a translocation chromosome, found in a girl with gonadal dysgenesis, which we believe to be the first demonstration in man of intercalary satellite material containing rDNA. This chromosome also appears to be a stable autosome which contains the centromeric regions of two chromosomes.

CASE REPORT

This 17-year, 9-month female was evaluated because of primary amenorrhea and the lack of normal breast development. Her past history was negative, with normal growth and development and satisfactory performance in school. There is no history of familial disease. The mother had her menarche at 12 years of age, and a female sibling had her menarche at 13 years of age.

Physical examination revealed a pleasant and alert teenager who appeared her stated age. Her height was 62 inches and her weight was 88 lb. No breast development was noted, and there was only early axillary and pubic hair development. Bimanual examination revealed a juvenile uterus.

Skull X-ray was normal. Determinations of 17-ketosteroids, 17-OH steroids, and pregnanediol- and follicle-stimulating hormone were all normal. Complete blood group typing revealed no unanticipated results. Both parents and the patient were Xg^a+.

Exploratory laparatomy revealed a juvenile uterus. There were intact fallopian tubes and fimbriae, but the gonads were streaks of fibrous tissue. Histologic sections of the gonads revealed fibrous stroma with no ovarian elements.

CYTOLOGICAL STUDIES

Karyotype analysis was first carried out on this patient before banding techniques were available. All cells from a peripheral blood culture had 45 chromosomes, with a missing D and C group chromosome and an abnormal large submetacentric chromosome with a prominent secondary constriction. A similar karyotype was found in fibroblast cultures grown from a biopsy of a streak gonad and from skin. There was a normal-sized sex chromatin body in a buccal smear. Because of the phenotype of gonadal dysgenesis in the patient, it was postulated that the abnormal chromosome represented an X/D translocation, with some loss of X short arm material.

However, when trypsin-Giemsa banding was used, the translocation was found to involve chromosomes 12 and 14. There was no visible loss of chromosome 12 material; appended to the short arm of chromosome 12 was an additional dark band, followed by a large nonstaining area, and then apparently all of chromosome 14 (fig. 1). Both X chromosomes appeared normal, and DNA replication studies using tritiated thymidine and autoradiography showed one of the X chromosomes to be late replicating.

Quinacrine fluorescent staining gave the added information that the extra band proximal to the secondary constriction of the translocation chromosome was brilliantly fluorescent, resembling the material found as a polymorphic trait in some satellited chromosomes (fig. *2a*). Karyotypes of the parents were both normal; one chromosome 14 of the father had brilliant satellites, and there were no brilliant polymorphic regions in any other acrocentric chromosome (fig. *2b*).

In situ hybridization using tritium labeled rRNA [2] showed significant labeling of the translocated chromosome in the region of the secondary constriction (fig. 3). Configurations suggesting satellite associations between other acrocentric chromo-

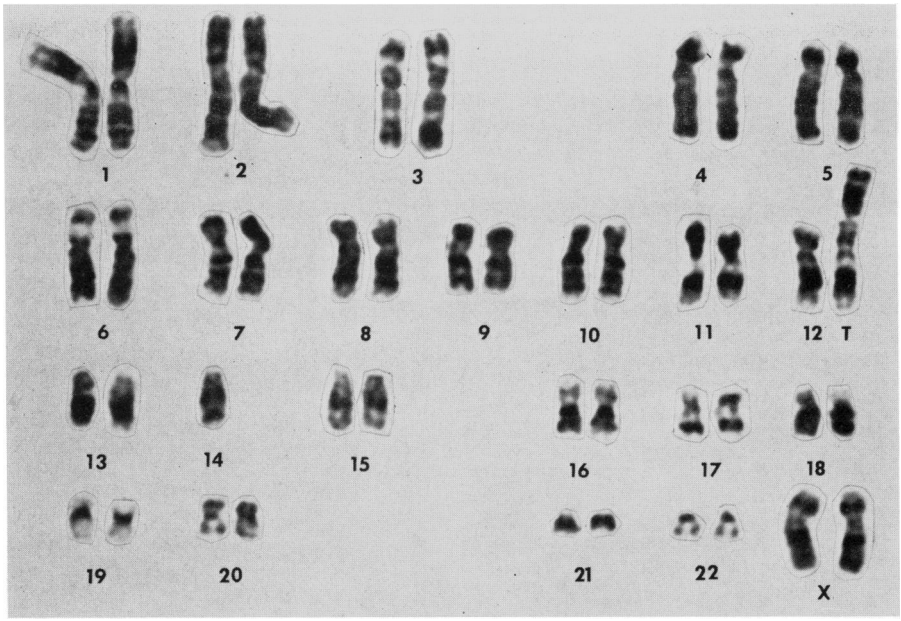

FIG. 1.—Karyotype of trypsin-Giemsa banded chromosomes showing 12/14 translocation and two normal X chromosomes.

somes and this region were seen, and labeled connecting strands were sometimes present between the constriction and other nearby satellited chromosomes [10].

The translocation chromosome in this patient can most simply be interpreted as a tandem union between chromosome 12 and the paternal chromosome 14 which had quinacrine-brilliant satellites, t dic (12;14)(p13;p13) [11]. Both centromeric

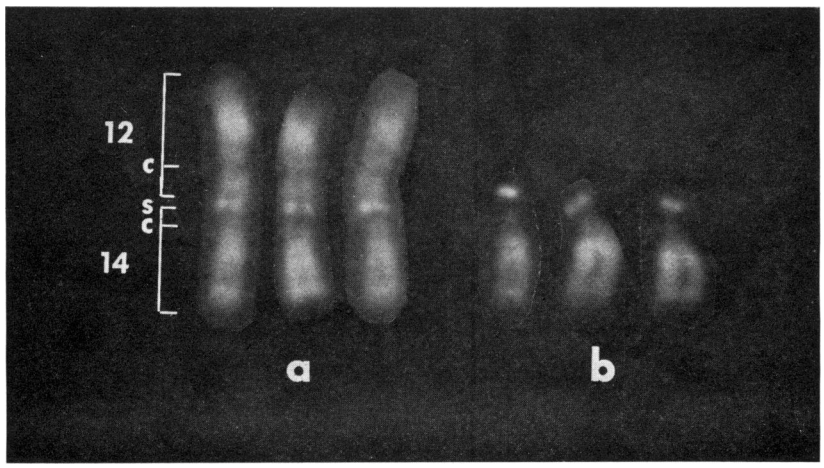

FIG. 2.—*a*, The 12/14 translocation chromosome from three cells, with quinacrine fluorescent staining; *c* = position of centromeres, *s* = position of satellite region of chromosome 14. *b*, Quinacrine fluorescent staining of chromosome 14 with brilliant satellites from three cells of the father.

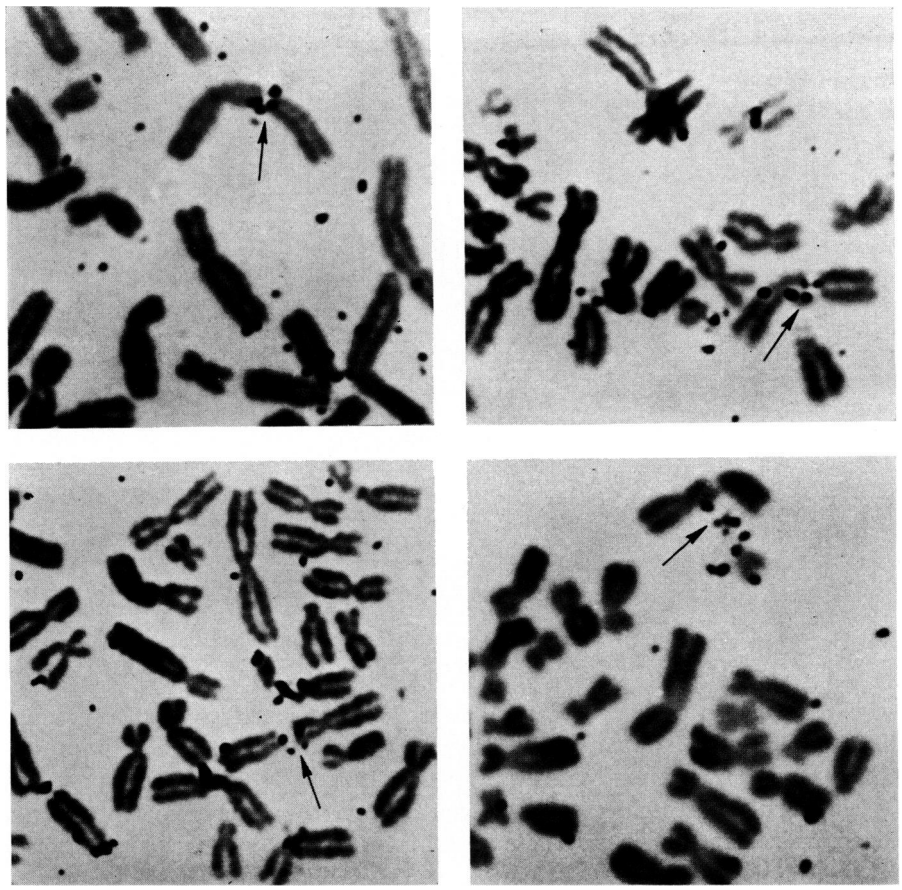

FIG. 3.—Portions of four metaphase cells from the patient after in situ hybridization with labeled rRNA. Arrows indicate labeling at the intercalary satellite region of the translocation chromosome.

regions, the satellite material, and the rDNA loci are retained. Loss of a small amount of material from the ends of the chromosomes cannot be demonstrated with present techniques.

In some cells, particularly those with less contracted chromosomes, a constriction of the translocation chromosome at the 14 centromeric region and faintly staining short arm material could be seen. In more contracted chromosomes, the chromatids were usually separated at the 14 centromeric region, while remaining held together at the 12 centromere (fig. 4).

DISCUSSION

Although interstitial deletion of the 14 centromeric material cannot be ruled out, cytological evidence supports the hypothesis that the 12/14 translocation chromosome contains the centromeric material of both chromosomes. Studies of

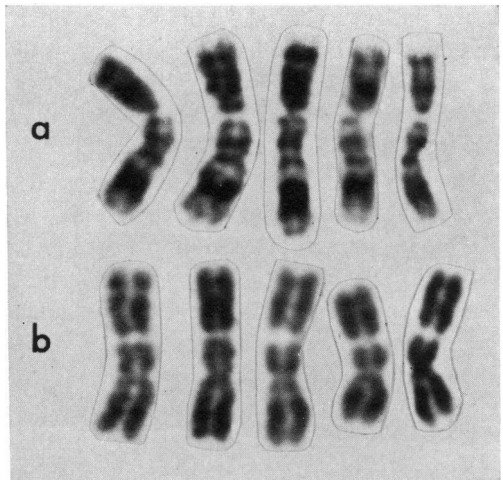

FIG. 4.—*a*, Translocation chromosomes showing a constriction at the centromeric regions of both chromosomes 14 and 12. *b*, Translocation chromosomes with chromatids separated at the 14 centromeric regions, but still held together at the 12 centromere.

anaphase might have added information on this subject, but unfortunately these have not yet been possible. There is no evidence, however, of instability of this chromosome at mitosis, judging from its consistent presence and morphology in all the cells examined from three different tissues.

There are two analyses of the behavior of stable dicentric chromosomes in the cytologic literature. Sears and Camara [12] described a dicentric chromosome in wheat, *Triticum aestivum,* which segregated regularly at the second meiotic division and at mitosis. The stability was attributed to the dominance of one centromere over the other, although the "weaker" centromere could function normally when the other centromere was absent. Hair [13] found a stable derivative of an originally unstable dicentric isochromosome in *Agropyron scabrum.* He believed the stability was due to the closeness of the two centromeres in the derivative. In the translocation described here, the separation of the two chromatids at the region of the 14 centromere in more contracted metaphase chromosomes suggests that this centromere was not functioning normally. Such premature separation might lead to dominance over the 12 centromere or might simply indicate lack of true centromeric activity.

The intercalary satellite material of the translocation chromosome seems to function as a nucleolus organizer, as shown by the occurrence of satellite associations and the rDNA connectives with acrocentric chromosomes. Thus, a terminal location of the rDNA is presumably not necessary for transcription. It is remarkable, then, that in man the five chromosomal locations of the rDNA are all in morphologically similar regions on the ends of acrocentric chromosomes.

It is difficult to relate the patient's phenotype ("pure" gonadal dysgenesis) to the chromosome abnormality we have demonstrated. The association may be fortuitous, and another unrelated X chromosome abnormality may be present in some

tissues to account for the patient's clinical features. A complex translocation somehow involving the X chromosome cannot be ruled out with the techniques available, but the evidence is against this. It is also possible that loss of genetic material from chromosome 12 or 14 can produce the phenotype of gonadal dysgenesis without the X chromosome being involved.

Ferguson-Smith (personal communication) has suggested that some "centric fusion" chromosomes might arise due to pairing in meiosis of nonhomologous chromosomes at the rDNA regions, with crossing over leading to the new chromosome, which would be dicentric. Neibuhr [14] has described a 13/14 translocation which has a pronounced secondary constriction next to a quinacrine-brilliant band. Parental karyotypes are not presented, so that one cannot decide whether the polymorphic brilliant region is derived from the short arm of chromosome 13 or the satellites of chromosome 14. Our demonstration of a translocation involving a satellited chromosome which has retained its centromere and satellites makes it very plausible that some translocations of the Robertsonian type may indeed retain the satellites of one (or both) of the chromosomes involved and may be dicentric.

SUMMARY

A stable dicentric chromosome t dic (12;14)(p13;p13) was found in all studied cells from peripheral blood and fibroblast cultures of a girl with gonadal dysgenesis. Both X chromosomes appeared normal. Between centromeres, the translocation chromosome contained a quinacrine-brilliant intercalary satellite region which was shown by in situ hybridization to contain rDNA. A paternal origin for the translocation was shown by the presence of brilliant satellites on a chromosome 14 from the father. Premature separation of the chromatids occurred at the 14 centromeric region of the translocation chromosome.

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