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Tandem Duplication dup(X)(q13q22) in a Male Proband Inherited from the Mother Showing Mosaicism of X-Inactivation

P. Steinbach[1*], W. Horstmann[2], and W. Scholz[1]

[1] Arbeitsgruppe Humancytogenetik und Klinische Genetik, Ruhr-Universität Bochum, Federal Republic of Germany
[2] Vestische Kinderklinik Datteln, Federal Republic of Germany

Summary. An aberrant X chromosome containing extra material in the long arm was observed in a psychomotoric retarded boy and his healthy, short-statured mother. The proband showed generalized muscular hypotony, growth retardation, and somatic anomalies including hypoplastic genitalia and cryptorchism.

Chromosomal banding techniques suggested a tandem duplication of the segment Xq13→Xq22.

In the mother the vast majority of lymphocytes showed late replication of the aberrant X chromosome. Some of her cells, however, contained an apparently active aberrant X. Both the early- and late-replicating aberrant X exhibited late replication patterns very similar to those described for normal X chromosomes in lymphocytes. Asynchrony of DNA replication among the two segments Xq13→Xq22 in the dup(X) was never observed.

We consider that the clinical picture of the proband is caused by an excess of active X material.

Introduction

According to Lyon's hypothesis all X material in excess of one X chromosome is usually inactivated at an early stage of development in somatic cells of humans. As a result of this inactivation, whose purpose most probably is to maintain a balanced genetic constitution in the cells, late labelling of X chromosomal material occurs.

Structural aberrations of the X chromosome gave a variety of inactivation patterns which are of particular interest because of the role they may play with respect to clinical anomalies. Recently, DNA replication patterns have been found in cultured lymphocytes which were suggestive for an excess of active X chromosomal material (Laurent et al., 1975; Hagemeijer et al., 1977; Bühler, 1977; Bühler et al., 1977; Zabel et al., 1978).

We report here further cases of partial disomy for probably active X in a female and her son showing a hitherto undescribed duplication in the long arm of an X chromosome.

Case Report

Proband

The proband, a Turkish boy, was first seen at the age of 7 weeks when admitted to the hospital because of feeding problems and obstipation. He is the first child born to a 23-years of age primigravida and a 40-year-old father. After apparently uneventful pregnancy the proband was born at home. Birth weight was about 2850 g, length 49–50 cm (Fig. 1).

At admission length was 53 cm, weight 3100 g, and head circumference 36 cm. The following anomalies were noted: Flat eyebrows, flat nose bridge, low-set ears; short fingers, low-set thumbs, bilateral clinodactyly V, bilateral simian crease; edema on the dorsae of the feet, low-set right third toe; hypoplastic genitalia, small testes palpable in the inguinal canal; generalized muscular hypotony, legs and arms held in a frog-like position; athetosic motor restlessness.

X-rays showed a scoliosis in the transitional zone from thoracic to lumbar vertebrae. Skull, thorax, and extremities were unremarkable. At the age of 6 months bone age corresponded to 3 months.

ECG was normal on several occasions. The EEG was poorly differentiated. Laboratory screening including TSH, T_4, and 17-ketosteroids gave normal results, as did urinalysis. Abdominal palpation revealed no organomegaly. During an ophthalmologic examination no anomalies were found.

Course. Although the proband was able to swallow, feeding was rather difficult and frequently required the use of a stomache

* *Present address:* Universität Ulm, Abt. Klinische Genetik, Prittwitzstraße 6, D-7900 Ulm/Donau, FRG

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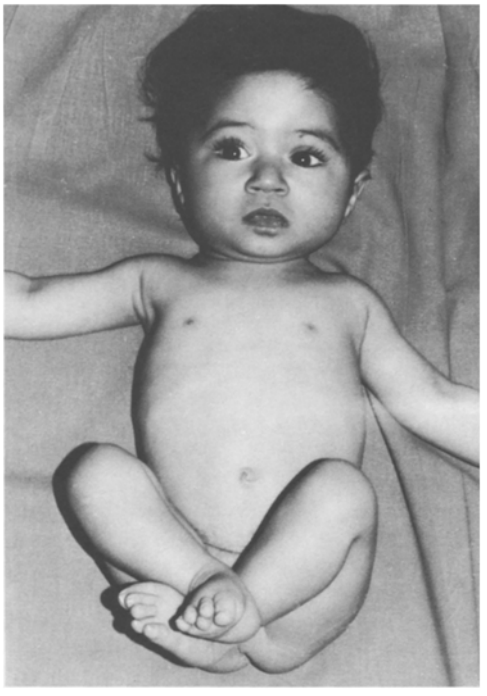

Fig. 1. The proband at the age of 6.5 months

tube. Growth retardation became evident. At the age of 6.5 months weight was 4620 g, length 62 cm, head circumference 40 cm (all below the third percentile). Proprioception was sluggish and head control was poor. Grasping reflexes were absent from both hands but present on the feet. Visual fixation was normal. There was no protective extension and only little active grasping. He was still unable to turn around from the dorsal into the prone position and still retracted both shoulders.

Mother

The proband's mother was 23-years of age when examined. Her height was 148.8 cm, weight 50 kg. Besides bilateral clinodactyly V no somatic anomalies were noted. Secondary sexual characteristics were normally developed. Menarche was reported for 17 years of age.

Dermatoglyphic Findings

An attempt at dermatoglyphic analysis was not completely successful because the patterns of the child were fine and poorly developed. The proband's fingertips showed nine whorls and one ulnar loop (left thumb). The finger ridge counts were all above 12.

The digital patterns of the mother showed eight whorls and two ulnar loops (second and third right fingers). TRC was 162. The distal triradius c was found absent from the left palm, the right showed an ulnar displaced b. All these features are usually observed in Turner's syndrome (Loeffler, 1969). The a–b ridge counts and the atd angles were, however, within normal limits.

The digital patterns of the father exhibited two arches (second fingers), three ulnar loops (third fingers and left 5th finger), and 5 whorls. The whorls and one of the ulnar loops showed ridge counts above 12.

Cytogenetic Findings

Chromosomal analysis was carried out on peripheral lymphocytes. Slides were conventionally stained with aceto-orcein. G-banding was performed according to Sumner et al. (1971). C-bands were visualized by the technique of Sumner (1972). To allow a study of DNA replication patterns the T-pulse protocol (Latt et al., 1976) was used:

Cells were grown 42 h in Ham's F10 medium containing 20% fetal bovine serum (FBS), 1.5% phytohemagglutinin solution (Gibco), 10^{-4} *M* 5-bromodeoxyuridine (BrdU), 4×10^{-7} *M* 5-fluorodeoxyuridine (FdU), and 6×10^{-6} *M* uridine (U). Seven hours before harvest this medium was replaced by Ham's F10 medium containing 20% FBS and 1.2×10^{-5} *M* thymidine (T).

BrdU containing cells were stained with bisbenzimide (Serva), exposed to light, and finally stained with Giemsa (Perry and Wolff, 1974).

Proband

Analysis of orcein-stained metaphase revealed a male karyotype containing 46 chromosome in all cells examined. G-banding showed extra material in the long arm of the X chromosome (Fig. 2a). C-banding gave no evidence for a second centromeric region in this aberrant chromosome. The rest of the karyotype was normal.

Mother

Buccal smear X-chromatin of the proband's mother showed a female pattern. Several of her Barr bodies appeared to be enlarged. Chromosome analysis revealed an aberrant X chromosome in all cells examined. This was identical to the X in her son (Fig. 2c). There was no evidence of either a reciprocal translocation or a chromosome mosaic which would include a 45,X cell line.

DNA Replication Patterns

The T-pulse protocol allowed clear identification of both X chromosomes in every metaphase showing the typical late replication pattern (Grzeschik et al., 1975). The aberrant X chromosome exhibited an additional dark replication band which corresponded to the additional dark G-band (Fig. 2d). A total of 123 meta-

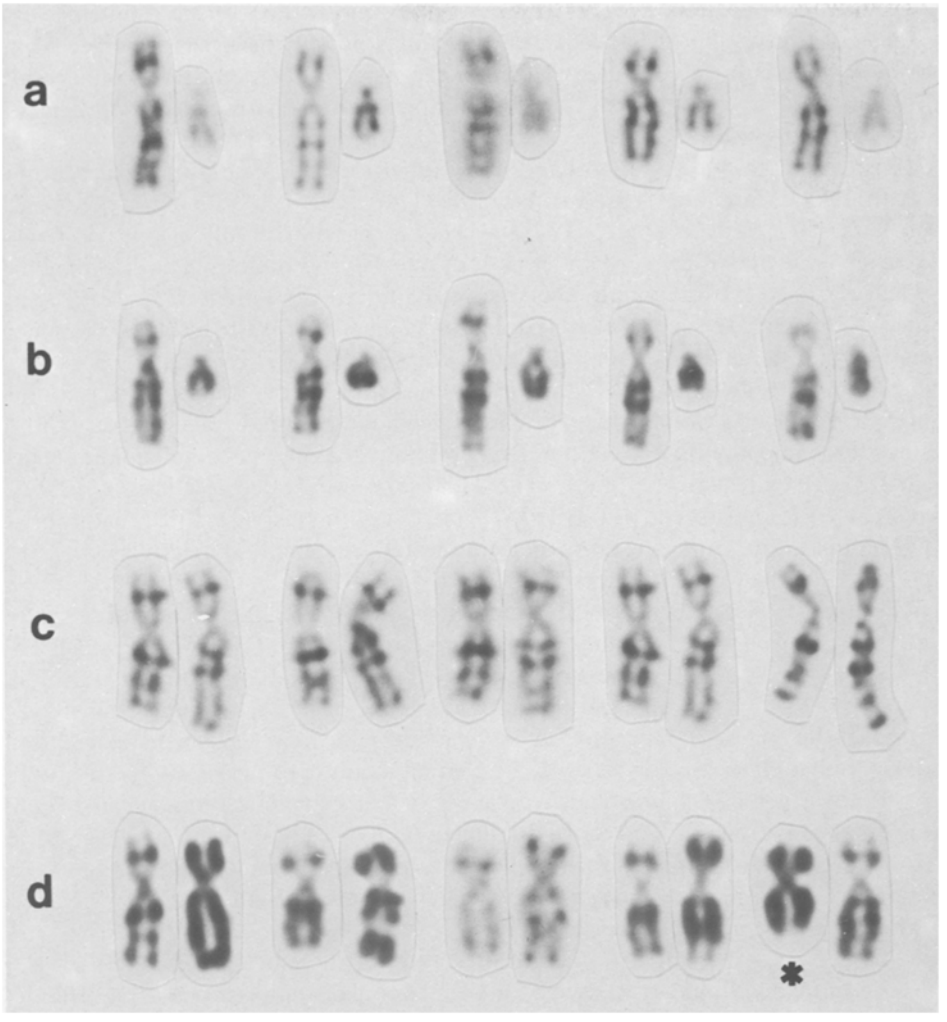

Fig. 2a—d. Gonosomes of the proband (**a**, **b**) and his mother (**c**, **d**): G-banding pattern (**a**, **c**); late replication pattern (**b**, **d**). Late replicated normal X

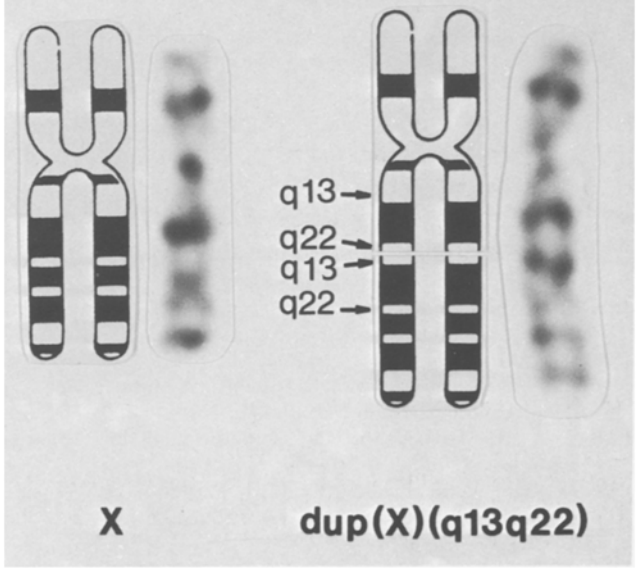

Fig. 3. Interpretation of the aberration as duplication dup(X)(q13q22). G-banding pattern

phases were analyzed in the mother. The aberrant X chromosome was found late replicated in 116 cells (94.3%). Five cells (4.1%) showed late replication of the normal X chromosome while the aberrant X was replicated earlier. In two cells (1.6%) none of the X chromosomes could be interpreted as late replicated.

In the proband there was no evidence for a late labelled segment in the aberrant X chromosome (Fig. 2b).

In the late-replicating aberrant X (found only in the mother) and in the early-replicating aberrant X (found in the proband and in the mother) the band q21 and the additional dark band were always found among the last dark bands to replicate: The additional dark band and band q21 replicate almost simultaneously.

Discussion

The structurally abnormal X chromosome of our proband and his mother exhibit a G-banding pattern

can be explained as the result of a tandem duplication of the segment Xq13→Xq22 (Fig. 3). An insertional X/autosome translocation is, however, not completely ruled out by this pattern (Sparkes et al., 1977).

X/X translocations generally show late replication when examined in postnatal life (Distèche et al., 1972; Therman and Pätau, 1974; Kim et al., 1974; Laurent et al., 1975; Latt et al., 1976; Sillesen et al., 1976; Sinha et al., 1976; Daly et al., 1977; Hagemeijer et al., 1977; Schwanitz et al., 1977; De la Chapelle et al., 1978; Dewald et al., 1978). Karyotypes with unbalanced X/autosome translocations containing a second normal X exhibit a diversity of late labelling patterns. The abnormal X is frequently found late replicated but mosaicism of inactivation seems not to be uncommon (Buckton et al., 1971; Opitz et al., 1973; Crandall et al., 1974; Jenkins et al., 1974; Laurent et al., 1975; Leisti et al., 1975; Hagemeijer et al., 1977; Mattei et al., 1978; Zabel et al., 1978). Spread of inactivation from the translocated X into the autosome fragment may occur (Engel et al., 1971; Jenkins et al., 1974; Leisti et al., 1975; Gaál and László, 1977; Mattei et al., 1978; Zabel et al., 1978).

The mother of our proband showed late replication of the aberrant X in the vast majority of lymphocytes. This is in good agreement with the hypothesis of a dup(X). Late replication of the normal X in a small number of cells probably results from initial random inactivation with subsequent selection among different cell populations, the cells with an active aberrant X being at a disadvantage.

Late labelling patterns suggesting more than one active X chromosome have previously been described in cultured lymphocytes [Laurent et al., 1975 (Case 1); Hagemeijer et al., 1977 (Case 2); Bühler, 1977 (Case 1); Bühler et al., 1977; Zabel et al., 1978 (Case 3)]. BrdU incorporation studies on cultured fibroblasts combined with biochemical analysis of X chromosomal mediated enzymes are in progress (W. Vogel, personal communication, concerning the case of Bühler et al., 1977). It could, however, not yet be confirmed by biochemistry that fibroblasts showing excess material of early-replicated X do exhibit an excess dose of X chromosomal gene activity. The preliminary results suggest that perhaps most of such 'abnormal' X replication patterns may represent technical artifacts.

The aberrant X chromosome of our patients exhibit replication patterns which are very similar to corresponding normal X chromosomes in lymphocytes (Latt, 1974; Grzeschik et al., 1975; Willard and Latt, 1976; Willard, 1977; Dewald et al., 1978). Termination of DNA replication is almost synchronous among band Xq21 and the additional dark band in the aberrant X of both patients. Such findings are very uncommon to the hitherto studied X/autosome translocations in man.

An insertional translocation of an autosome segment into the X has not yet been clearly detected in man. It can be expected that this insertion would result in a functional trisomy for a distinct autosome segment (Cattanach, 1974). The health of the mother of our proband does not agree with such a type of chromosome aberration.

These aspects together with all existing cytologic data let us consider that the additional dark G band in the aberrant X of our patients actually is a second band q21, and that this aberrant X is the result of a tandem duplication of the segment Xq13→Xq22.

An aberrant X exhibiting two bands p21 was found in a 16-year old mentally retarded male with very short stature (K. Brøndum Nielsen, personal communication). The karyotype was interpreted as 46,Y,dup (X)(p113p221)mat. As in our case BrdU incorporation studies indicated 'preferential' inactivation of the aberrant X in the mother.

The clinical picture of our proband is most probably caused by a chromosomal aberration which resulted in excess of active X material. Minor congenital malformations, a more or less severe muscular hypotony, an only moderate prenatal growth retardation followed by postnatal failure to thrive, and retardation of growth and psychomotor development have been reported from previous cases showing evidence of a functional disomy for active X material (Laurent et al., 1975; Hagemeijer et al., 1977; Bühler et al., 1977; Zabel et al., 1978).

The short stature of the mother could be likewise caused by this functional disomy found in a few cells, or by inactivation of gene loci in the aberrant X which are normally active in both X chromosomes. This would be equivalent to partial monosomy X in a majority of cells.

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