

# Deletion/inversion in the X-chromosome and increased telomeric associations in a female with primary amenorrhea

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## ABSTRACT

We describe a new case of a partial interstitial deletion and inversion of the long arm of the X-chromosome associated with a high incidence of telomeric associations in an 18-year old female who showed underdeveloped secondary sex characteristics, including small breasts and primary amenorrhea. Her karyotype was considered to be 46,X,del(Xq13 → q22)inv(X)(q23-q27). The buccal mucosal cells showed absence of a typical Barr body, and the 5'-bromo-2-deoxyuridine incorporation studies revealed that neither the normal X- nor the abnormal X-chromosome was late replicating. The case is being presented for its extreme rarity.

## INTRODUCTION

The two X-chromosomes present in females and the single one in the male causes an imbalance in the genetic physiology between the two sexes. As demonstrated by Lyon (1961), one X-chromosome in somatic cells of the mammalian female is rendered inactive to permit equivalency of gene dosages with the male cells, in which only one active X-chromosome is present. In general, the inactivation that occurs in early embryonic development is random in different cell types. The study of inactivation in structurally altered X-chromosomes, such as in deletions, unbalanced X-autosome translocations, and iso-dicentric formations, suggests the existence of a single inactivation center in

the proximal region of the Xq, between bands q11 and q21 (Therman, 1983).

For normal development in females, two X-chromosomes are essential during early embryonic life. Any abnormality of the X-chromosome (structural or numerical) present at this early stage may be expressed later in abnormal phenotypes, such as Turner syndrome, ovarian dysgenesis, and other conditions (Jacobs *et al.*, 1961; Ferguson-Smith, 1965; Polani, 1969; Forabosco and Dallapiccola, 1974). Numerical and structural abnormalities of the X-chromosomes also frequently contribute to abnormal pathology and cancer development. A number of studies have shown that genetic determinants controlling the function of oocytes are present on both the long (q) and the short (p) arms of the X-chromosome. Functional ovarian tissue is present more often in females with deletions of Xp than in those with Xq deletions (Goldman *et al.*, 1982). These authors also presented evidence that deletion of the pericentromeric region of the X leads to primary amenorrhea in some females.

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## CASE REPORT

An 18-year old phenotypic female with the complaint of primary amenorrhea (Figure 1) was referred for cytogenetic analysis. She was the fourth child born to nonconsanguineous parents. The mother had an uneventful pregnancy. The father was 37 years old and the mother was 32 at the time of delivery. Clinical examination revealed: height, 162 cm, weight, 47 kg, and arm span, 161 cm. She had underdeveloped secondary sex characteristics, including scanty axillary and pubic hair, underdeveloped breasts, but had normal labia majora and clitoris. There was an increased distance between her big toe and the second toe on both feet. Diagnostic laparoscopy revealed a hypoplastic uterus (3 x 1 cm), small streak ovaries, and normal fallopian tubes. The patient had withdrawal bleeding in response to estrogen and progesterone treatment. Endocrine findings revealed a slightly elevated level of serum follicle-stimulating hormone (24 Milli international unit/ml) with normal serum luteinizing hor-



Figure 1 - Appearance of the proband at the time when peripheral blood was analyzed. External features show underdeveloped secondary sexual characteristics.

none (18 Milli international unit/ml) and serum prolactin (10 ng/ml) levels.

The karyotypes of her parents, two brothers, and one sister, who were phenotypically normal, were also studied.

## MATERIAL AND METHODS

Cytogenetic investigations of the proband and her family members were carried out by routine phytohemagglutinin (PHA)-stimulated 72-h peripheral blood cultures. Standard GTG-, QFQ-, and CGB-banding techniques were used for the identification of the chromosomes (Pathak, 1976). A total of 200 metaphase plates were analyzed from each sample. The human telomeric DNA probe utilized for the fluorescence *in situ* hybridization (FISH) study was from Oncor, Inc. (Gaithersburg, MD). The hybridization and detection protocols followed the Oncor instructions.

For late-replicating X-chromosome studies, 5-bromo-2-deoxyuridine (BrdU) was added to the proband's lymphocyte culture at a final concentration of 60 µg/ml 5-6 h prior to harvesting. Cytogenetic preparations were stained with Giemsa to evaluate the pattern of X-chromosome inactivation.

Buccal mucosal cells were scraped, stained with aceto-orcein (2%), and analyzed for the presence of X-chromatin.

## RESULTS

Cytogenetics revealed a 46,X,del(X)(q13 → q22)inv(X)(q23-q27) chromosomal constitution. A typical G-banded karyotype from the proband is shown in Figure 2A. At first glance the rearranged X chromosome looked as if it were an i(Xp). Close examination of the altered X from a large number of metaphases revealed that: a) this chromosome was present in every metaphase spread, b) this chromosome showed signals on both telomeric ends (Figure 2B), and c) this was the result of two events. First, there was an interstitial deletion in the long arm of one X-chromosome between q13 and q22 regions, followed by restitution and a paracentric inversion in the same arm between bands q22 and q27, as diagrammatically shown in Figure 3. Because of these two events (deletion/inversion) the derivative X-chromosome is shorter than the normal X-chromosome, and has two prominent bands in the lower arm. The p arm of this altered X had a normal G-banding pattern. No other chromosome showed any structural or numerical abnormality. Mosaicism was ruled out in this patient after scanning 200 G-banded metaphase spreads.

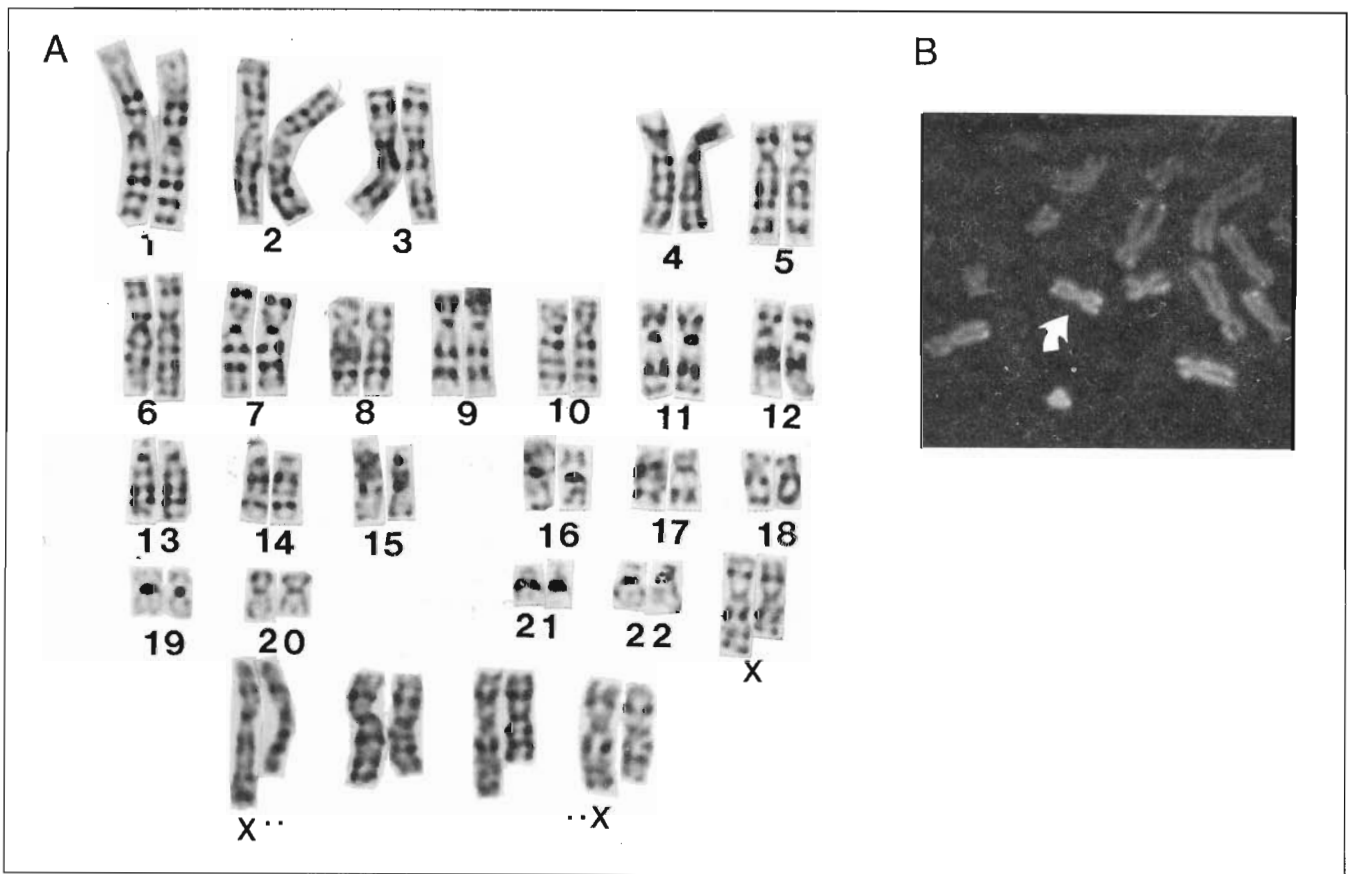


Figure 2 - (A) A G-banded karyotype of the patient showing an altered X-chromosome. All autosomes and the other X-chromosome appear normal in their banding patterns. Two X-chromosomes (one normal and the other altered) from four additional metaphase spreads are shown on the bottom row. (B) FISH preparation of a partial metaphase plate showing telomeric signals on both ends of the altered X-chromosome (arrow).

All family members whose lymphocytes were analyzed showed normal 46,XX or 46,XY chromosome constitutions.

### X-Chromatin and late replication analyses

Barr body (X-chromatin) was analyzed in 300 interphase nuclei prepared from buccal mucosal cells of the proband. Two interphase cells showed a possible presence of X-chromatin. The size of the X-chromatin appeared to be normal, indicating that it was probably formed by the intact X-chromosome.

Approximately 100 metaphase spreads from the BrdU-incorporated experiments were analyzed for the late-replication pattern of the X-chromosome. We did not find any cells with a late-replication pattern in either the normal or the altered X-chromosome (data not shown).

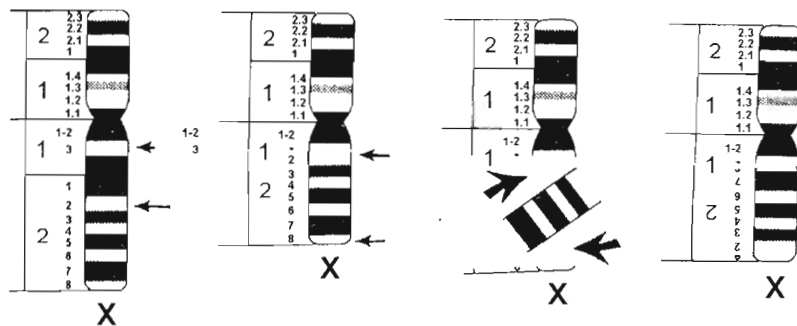


Figure 3 - Diagrammatic pathways showing the formation of an altered X-chromosome.

### Telomeric associations

Telomeric associations (TAs) involving mostly single chromatids were present in 80 of 98 metaphase spreads examined. Typical examples of selected TAs in the lymphocytic metaphases of the patient are shown in Figure 4. Practically every chromosome, including the altered X-chromosome, was a participant in such associations (Figure 4B and C). The altered X-chromosome was involved in TAs with the same frequency as the normal X. Mostly two, but sometimes three, chromo-

somes were also involved in telomeric associations (Figure 4A). Telomeric association involving both sister chromatids within or between chromosomes was a rare phenomenon. The frequency of associations for each pair of chromosomes is shown in Table I. There were from 0 to 6 associations between metaphases, with a mean value of 2.4 telomeric associations per cell. A large majority of cells showed one or two such associations; cells with more than five associations were rarely present (Table II). Not a single cell showed a complete ring configuration.

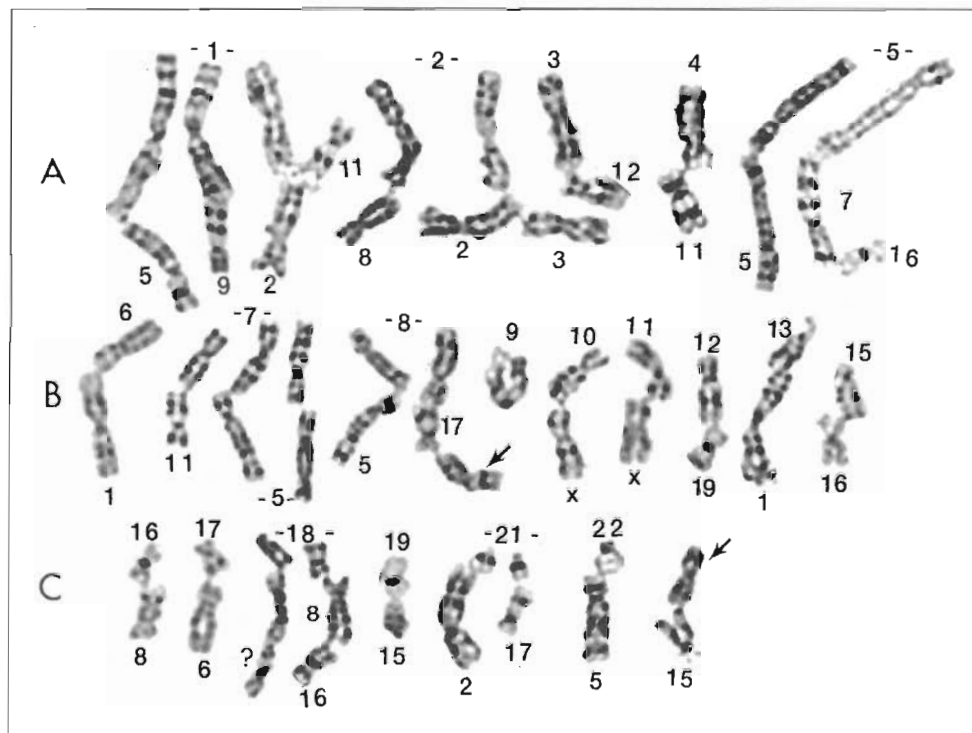


Figure 4 - Examples of telomeric associations present in the lymphocyte cultures of our patient. Most chromosomes are identified by their characteristic banding patterns, including the altered X-chromosome (arrows).

## DISCUSSION

Genes essential for normal ovarian development are located on both arms of the X-chromosome (Fitch *et al.*, 1982). An abnormality in the number or structure of the X-chromosome results in a disturbance in the normal process of translation of genetic sex and the final determination of phenotypic sex (Jost, 1959-1960; Jost, 1972). Women with an Xq deletion, ascertained because of primary or secondary amenorrhea, show gonadal dysgenesis. However, with few exceptions, the stature of these individuals is normal (Ferguson-Smith, 1965; Therman, 1983). Therman (1983) proposed that the Xq13 → Xq27 region also has to be intact in both the X-chromosomes to allow normal ovarian and female sexual development. Studies on the deletion of the X-chromosome have revealed that in most of these cases the breaks occur within this critical Xq13 → Xq27 region (Summit *et al.*, 1978). Also, in the present case the deletion observed, Xq13 → Xq22, involved the critical region of the X-chromosome, along with an inversion (Figures 2 and 3).

There have been several reports of deletions at various sites of the long arm of the X-chromosome, with different clinical manifestations. These included del(X)(q22) (Ruthner *et al.*, 1979), del(X)(q26)-(3 cases) (Fitch *et al.*, 1982), del(X)(q23) (Mijin *et al.*, 1982), del(X)(q21) associated with gonadoblastoma (Seki *et al.*, 1981), and (del)(Xq22) (Wyss *et al.*, 1982). In all these reported cases, patients showed menstrual irregulari-

Table I - Involvement of chromosomes in telomeric associations (TAs).

Chromosome number	No. of times involved in TAs
1	14 (4.46)
2	15 (4.78)
3	14 (4.46)
4	10 (3.18)
5	24 (7.64)
6	11 (3.50)
7	18 (5.73)
8	11 (3.50)
9	15 (4.78)
10	18 (5.73)
11	14 (4.46)
12	12 (3.82)
13	11 (3.50)
14	16 (5.09)
15	12 (3.82)
16	13 (4.14)
17	8 (2.55)
18	9 (2.87)
19	23 (7.32)
20	9 (2.87)
21	14 (4.46)
22	8 (2.55)
X	8 (2.55)
del(X)	7 (2.23)

Numbers in parentheses represent percentage (%) of individual chromosomes involved in TAs.

ties, but none of them had typical features of Turner syndrome.

**Table II** - Frequency distribution of telomeric associations (TAs) in different cells.

No. of TAs/cell	No. of cells with TAs
0	9
1	13
2	17
3	12
4	8
5	4
6	2

Mean association per cell: 2.4 (65 total cells).

Goldman *et al.*, (1982) reviewed 21 cases of Xq deletions and reported three patients with Xq deletions and amenorrhea, including one with an interstitial deletion at Xq21 → q26 and another with del(X)(q13). These authors also suggested that, in general, long arm deletions are compatible with a relatively normal phenotype, except for amenorrhea. Wyss *et al.* (1982) reviewed 28 cases of Xq deletions and reported a case of secondary amenorrhea with del(X)(q22). From these observations they concluded that large Xq deletions with a break point at or proximal to Xq21 led to gonadal dysgenesis, and half of these patients showed Turner syndrome stigmata. Terminal deletion of Xq with the break point at or distal to Xq22, however, is generally associated with pure gonadal dysgenesis. A del(X)(q13 → q26) was also reported by de la Chapelle and associates (1975) in a patient with normal gonadal function but features of Turner syndrome. However, in the present case, the deletion was in the region Xq13 → q22, and the patient did not show Turner syndrome stigmata but had gonadal dysgenesis.

In most reported cases, the proximal part of the Xq (along with its inactivation center) was left intact, and the abnormal X-chromosome was inactivated (Therman *et al.*, 1974; de la Chapelle *et al.*, 1975; Ruthner *et al.*, 1979; Mattei *et al.*, 1981; Goldman *et al.*, 1982; Fitch *et al.*, 1982; Wyss *et al.*, 1982). Therman *et al.* (1979) proposed that the X-inactivation center was in the Q-dark region and adjoining bright region of the proximal part of Xq (q13 → q21). A chromosome lacking this segment would result in more than one active X-chromosome in each cell. Some investigators (Therman and Patau, 1974; Therman *et al.*, 1974 and 1979) questioned the possibility that a live individual could have an X-chromosome so altered that it contained no inactivation center. However, in the present case, the abnormality involved the proximal region of the long arm of X-chromosome (Xq13 → q22) and inv(X)(q23-q27), probably deleting/altering genes in the X-inactivating region, as evident from the absence of the Barr body in buccal mucosal cells and also by the

observation that the X-chromosomes were not late replicating.

The proband showed a large proportion of metaphases (56/65) (Table II) with telomeric associations involving practically every chromosome (Table I). Telomeric associations have been reported in leukemic cells (Fitzgerald and Morris, 1984; Morgan *et al.*, 1986; Howell *et al.*, 1993), in solid tumors (Dewald *et al.*, 1987; Kovacs *et al.*, 1987; Pathak, *et al.*, 1988), in cells undergoing apoptosis (Pathak *et al.*, 1994a,b), and in spontaneously regressing swine melanomas (our unpublished data). Telomeric associations occur due to the loss of terminal DNA sequences forming the telomeres (Harley, 1991; Counter *et al.*, 1992). Dhaliwal *et al.* (1994) reported a high frequency of telomeric associations in lymphocytic metaphases of a father whose twin daughters presented with multiple congenital neoplasia. Seki *et al.* (1981) reported a bilateral gonadoblastoma in a female with del(X)(q21) and gonadal dysgenesis. The structural abnormality of the X-chromosome, gonadal dysgenesis, and a high incidence of TAs in the metaphase preparations of our proband could increase her predisposition to certain types of cancer. The patient has been counselled and is being followed up for any new development that might lead to neoplastic transformation.

A systematic and careful cytogenetic and molecular analyses of the X-chromosome deletions are required to define the role of genes on the X-chromosome in the development and maintenance of ovarian function. Such studies may also shed light on the possible involvement of X-chromosome deletions in the predisposition to certain neoplasias.

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## RESUMO

Um novo caso de supressão e inversão intersticial parcial do braço longo do cromossomo X em combinação com uma alta incidência de associações teloméricas foi mostrado em uma jovem do sexo feminino de 18 anos com características sexuais secundárias subdesenvolvidas, incluindo

seios pequenos e amenorréia primária. Seu cariótipo foi considerado 46,X,del(Xq13 → q22)inv(X)(q23-q27). As células mucosas bucais apresentaram ausência de um típico corpúsculo de Barr e os estudos de incorporação de 5'-bromo-2-deoxiuridina revelaram que nem o cromossomo X normal nem o anormal tinham replicação tardia. Este caso foi apresentado por sua extrema raridade.

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