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DNA REPLICATION STUDY IN A FEMALE INFANT WITH A KARYOTYPE OF 45,X/46,X, psu dic(X) (p22::p22) AND REVIEW OF THE LITERATURE

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Summary A newborn female with anal atresia, duodenal perforation and mild Turner's stigmata was found to have a karyotype of 45,X/46,X, psu dic(X)(qter→p22::p22→qter) in her lymphocytes. The rearranged chromosome had only one primary constriction and always demonstrated late replication. The constitutive heterochromatin at the pseudocentromeric region was C-band positive but Cd-band negative. Analysis of the DNA replication sequence in initiation by B-pulse methods in lymphocytes revealed a frequent asymmetric pattern and more than two types of sequence at both long arms of psu dic(X). The pattern of DNA replication did not relate to the position of the active centromere. The existence of replication variants by B-pulse method may merely indicates the occasional asynchronous start of segments in the distal long arm of X chromosome.

INTRODUCTION

Iso pseudodicentric X chromosomes are of special interest because they provide an opportunity to study the replication behavior of duplicate copies of the same chromosome in each cell with least risk of technical artefacts. We describe here a female patient with a karyotype of 45,X/46,X, psu dic(X)(p22::p22) and present the results of various cytogenetic studies, especially on the DNA replication sequence in the late replicating psu dic(X) chromosome. The following problems are also discussed: 1) the Cd-band and function of the centromere, 2) asymmetric or symmetric DNA

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replication patterns, 3) relation of the inactive centromere to the replication pattern, and 4) the origin of the variation in DNA replication sequence.

MATERIALS AND METHODS

Case report. The patient, M.S., JMSH 254821, was the second child of a 27-year-old mother and a 29-year-old father. Both parents and her elder brother were healthy. The parents were not related. Pregnancy was not complicated. The girl was born by breech delivery after 39 weeks gestation. Hir birth weight was 2,230 g, length 45.0 cm and head circumference 30.0 cm. On the first day of life, she showed abdominal distension and feeding difficulty. Immediately after diagnosis of imperforate anus and duodenal perforation, an operation was performed. At the age of 11 months, she was −4.0 SD in height and −4.0 SD in weight. She had simple prominent ears, a long philtrum, narrow palate, strabismus, single palmar flexion crease and short metacarpal of the left fourth finger. Psychomotor development was delayed and her DQ was estimated at about 54.

A dermatoglyphic investigation revealed bilateral high axial triradius (t′) and hypothenar pattern.

Results of routine chemical and hematological studies were normal. The Xg^a blood type of the patient and her parents were all Xg^{a+}.

Cytogenetic studies. Chromosome preparations were obtained from short term blood cultures. G-banding by the GTG technique was employed for chromosome identification. C-banding of chromosomes was carried out by Sumner's technique (Sumner, 1972), and Cd-staining by Eiberg's technique (Eiberg, 1974). A DNA replication study was performed following the BrdU-Hoechst 33258 Giemsa method of Perry and Wolff (1974) with modifications: BrdU was added to short-term lymphocytes cultures at 5, 6, 7, and 8 hr before harvesting at a concentration of 100 μg/ml (B-pulse). Slides were stained with Hoechst 33258 (50 μg/ml) for 15 min, washed with water, and air dried. The slides were then mounted with Sørensen phosphate buffer (pH 6.8) and exposed to a 60 W white lamp at a distance of 15–20 cm (60°C) for 60 to 120 min. The slides were washed with water and stained with 3% Giemsa solution for 10 min, rinsed with water and air dried.

A sex chromatin study was performed on buccal mucosal smears after calbolfuchsin staining.

RESULTS

Routine chromosome analysis was carried out on 65 cells from the blood cultures. A cell line having 45 chromosomes revealed one of the X chromosomes to be missing. In another cell line with 46 chromosomes, an X chromosome was replaced by a large submetacentric chromosome resembling a No. 2 chromosome. The abnormal chromosome had two C-staining regions (Fig. 1A) and only one pair

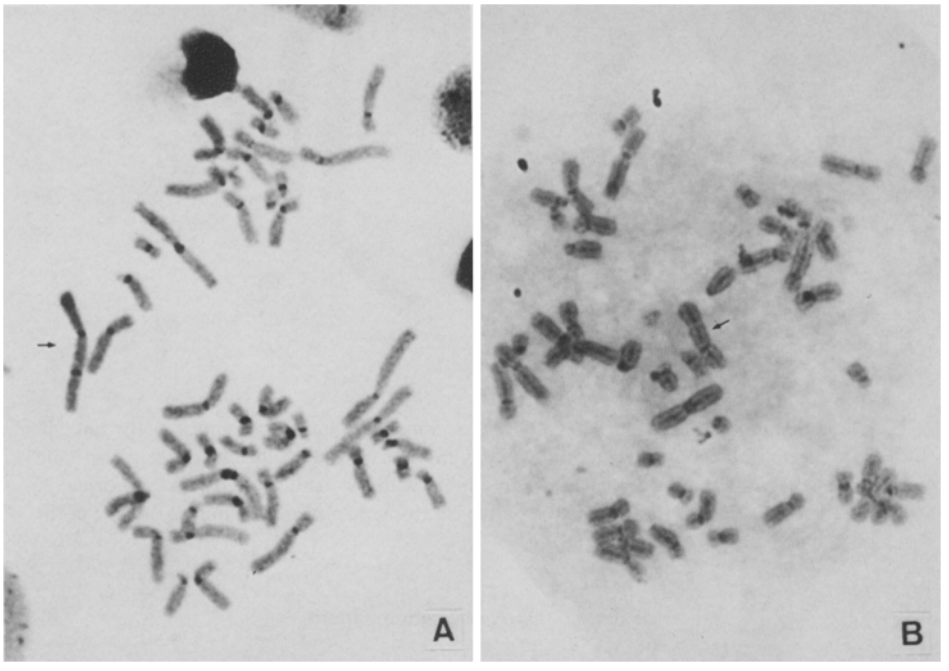

Fig. 1. C-band (A) and Cd-staining (B) pattern of chromosomes including psu dic(X)s in the patient. Arrows indicate the rearranged X chromosomes.

of centromeric dots produced by Cd-banding methods (Fig. 1B). The G-banding technique showed that this abnormal large chromosome was made up of two X chromosomes fused by their short arms. The existence of bipartite or large X chromatin in buccal smear cells (15%) also suggested this kind of chromosome rearrangement. The break points of the two fused Xs were in the short arms p22. The patient thus had a presumptive karyotype of 45,X/46,X, psu dic(X)(qter→p22::p22→qter) in her lymphocytes (Fig. 2). The ratio of 46,X, psu dic(X) cell line was 17%. The karyotypes of her mother and her father were normal.

Replication studies on 40 cells from the peripheral blood cultures demonstrated that the rearranged chromosome was always preferentially inactivated with respect to the normal X chromosome.

An asymmetric DNA replication pattern was observed by the B-pulse method in 25 out of 40 analyzed karyotypes. Among these 25 cells, the long arm portion of the abnormal X was always asymmetric. An asymmetric pattern in the area of near the centromere was observed in 3 out of 25 cells (Tabel 1). The DNA replication analysis revealed that the side of the active centromere always replicated earlier than the other in the area of near the centromere (3/3). At the long arms of the abnormal X, however, 17 out of 25 replicated earlier at the side of the active cen-

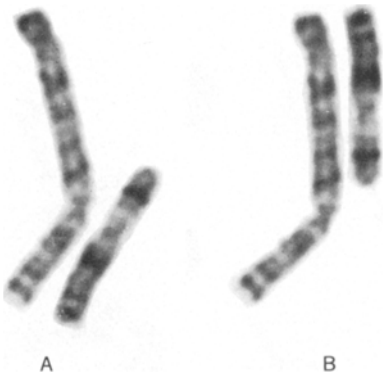

Fig. 2. G-banded chromosome X pair from a clone with 46 chromosomes in the patient. The chromosome placed on the left is the rearranged X chromosome. The band pattern of the abnormal X is compatible with the band pattern of the normal X chromosome at the side of the active centromeric moiety (A), and at the side of the inactive centromeric one (B).

Table 1. DNA replication pattern.

Symmetric			15
Asymmetric			25
near centromere	symmetric	22	
	asymmetric	3	
long arm portion		25	
Total			40

Table 2. Side of earlier replication.

	Near centromere	Long arm portion
Cen.[a]	3	17
Cen.[i]	0	8
Total	3	25

Cen.[a] and Cen.[i] indicate the moiety of active and inactive centromere, respectively.

tromere and 8 out of 25 replicated earlier at the side of the inactive centromere (Table 2).

The results of analysis of the DNA replication sequence by the B-pulse method in 25 cells with an asymmetric DNA replication pattern are shown in Fig. 3, A–E. Three types of DNA replication initiation sequence were observed in the long arms of the abnormal X chromosome: 1) distal to proximal q (Fig. 3A), 2) proximal to

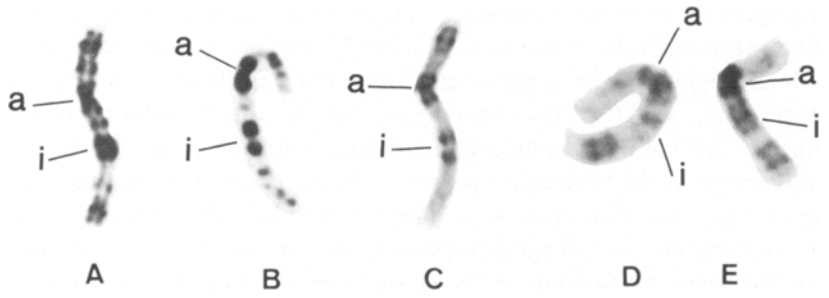

Fig. 3. DNA replication sequence by the B-pulse method. Three types were noted: distal to proximal q (A), proximal to distal q (B), and an unknown type (C). The frequencies of A, B, and C were 7, 12, and 6 among 25 karyotypes, respectively. Two cells revealed a peculiar DNA replication timing (D, E). a, active centromere; i, inactive centromere.

distal q (Fig. 3B), and 3) an unknown type (Fig. 3C). In Fig. 3, A and B, the DNA replication patterns differ from each other at both ends of the long arms but the DNA replication sequences are not inconsistent with each other. On the other hand, in the case of Fig. 3C, the DNA replication sequence could not be decided exactly because one side of the long arms showed no definite bands, even though the centric moiety exhibited a distal to proximal q type replication pattern. In most of the inactive abnormal X chromosomes, Xp11 and Xp13 were the initiating bands, p22, q22, q24, q26, q28 were the next and G-bands were the last. In 2 out of 6 cells, a peculiar DNA replication timing was observed. As shown in Fig. 3, D and E, distal segments in the long arm at the acentric moiety had already completed replication as early as the near centromere, in spite of the centric moiety showing no definite bands at the other side.

DISCUSSION

Cd band and function of the centromere. In our patient, the abnormal psu dic(X) chromosome showed two C-banded heterochromatins, but only one active centromere with primary constriction had Cd-dots. These findings are compatible with the idea that the Cd-band is related to active centromeric function (Daniel, 1979; Maraschio *et al.*, 1980a; Lambiase *et al.*, 1984).

Asymmetric DNA replication pattern. Asymmetry of the DNA replication pattern of both arms of psu dic(X) attached by their short arms has always been observed by B-pulse methods in spite of being of variable degree. For example, Dewald *et al.* (1978) reported that about 70% of cells showed an asymmetric DNA replication pattern at both arms, Maraschio *et al.* (1980b) around 36%, Mutchinik *et al.* (1981) 29%, Yu *et al.* (1982) 66% (by B-pulse, fibroblasts), and Rivera *et al.* (1984) 10%, respectively. In our patient, asymmetric pattern was observed in 62.5%

of informative cells by B-pulse methods. On the other hand, a symmetric replication pattern has been reported in the cases with dic(X) attached by their long arm having various break points in the range of q21 to q23 (Chapelle *et al.*, 1978; Yu *et al.*, 1980; Petit *et al.*, 1982). This phenomenon may be due to deletion of the distal long arm (q25–q27) in these cases, which constitutes a major factor for the formation of an asymmetric DNA replication pattern. In the cases of Mirzayants and Baranovskaya (1978), and Robertson *et al.* (1982) which possessed break points in q27 and qter respectively, an asymmetric pattern was observed. It can be concluded therefore that some fluctuations in the replication pattern in initiation are not unusual, and the onset of replication is separately determined in the two parts of the abnormal chromosome within a cell.

Pelliccia *et al.* (1984) stressed that the long arm of the acentric moiety was a consistently later replicator than the centric one. However in other case reports (Maraschio *et al.*, 1980; Yu *et al.*, 1982) such a strict relationship was not mentioned. In our case, as regards the centromeric region, the acentric moiety always replicate late in all of three informative cells. However at the portion of the long arms, eight out of 25 cells showed earlier replication at the side of the inactive centromere. In a case report of qter fusion, Robertson *et al.* (1982) stated that the later replicating portion appeared to be unrelated to the position of functioning of the centromere. It may be that, at least for the distal half of the long arm, the relationship of the later replicating and the acentric moiety is not a strict one, even though such a tendency was noticed in a case of Sarto and Therman (1980).

The origin of the variation in DNA replication sequence. According to Willard (1977) and Schmidt *et al.* (1982), more than two types of replication variants of the inactive X chromosome occur in human lymphocytes as regards both initiation and termination of the process. Schmidt *et al.* (1982) observed a type showing earlier replicating bands at the distal long arm and an alternative one involving the proximal long arm, and these had almost the same frequency of around 50% respectively. In our study, a similar phenomenon was observed at both arms of the abnormal inactive X chromosome by the B-pulse method. The frequency of the DNA replication sequence of distal to proximal q was 42% (13/31) and of proximal to distal q was 58%. The sequence was variable among different cells, but almost stable within each cell. In two cells, the timing of replication in the distal long arm at the side of the active centromere was markedly delayed, but we failed to find example of complete dissociation of the DNA replication sequence at both arms. These results seem to mean that at least two segments of late replicating Xq are controlled separately at the cell level and compatible with the speculation that the variation in replication patterns is correlated with the tissue specificity (McCaw and Latt, 1977; Willard, 1977). On the other hand, Latt *et al.* (1981) indicated that the cellular origin may have a strong but not absolute influence on the DNA replication sequence of inactive X chromosome. But these conclusions were made mainly by T-pulse method. Almost the same frequency of the replication variants of the

inactive X chromosomes by B-pulse method in our patient and the results of study by Schmidt *et al.* (1982) are incompatible with the tissue specific theory. Furthermore, Kim *et al.* (1975) observed some asynchrony in initiation of replication between homologous autosomes and variable pattern in the distal long arm segments of early replicating X chromosomes in normal female lymphocytes. The existence of replication variants by B-pulse method may thus merely indicates the occasional asynchronous start of segments in the distal long arm of early replicating X chromosomes and not relates to the theory of multiple inactive centers on inactive X chromosome (Nakagome, 1982).

It is interesting to know whether the fluctuations of the replication timing or sequence in multiple X individuals have clinical effects such as mental retardation and bone abnormalities or not. Three early replicating segments escaping inactivation in human X chromosome involving Xp22.13, Xp22.3 and Xq13.1 or Xq11 have been suggested (Schempp and Meer, 1983; Camargo and Cervenka, 1984). Although the segments Xq22, 24, 26, 28 are not involved in these early replicators, some summation effect in multiple X individuals may be possible. On the other hand, according to Lykkesfeldt *et al.* (1984) even in the case of steroid sulfatase (STS) loci on inactive X chromosome (assigned to Xp22), it is estimated that it expresses only 45% of the active X chromosome. They indicated that the STS activities in a 47,XXX individual and in a 49,XXXXY individual were in the female range, which also supports the conclusion of a reduced expression of STS loci in heterocyclic inactive X chromosomes. Camargo and Cervenka (1982) suggested that the entire late X initiates replication after it has completed the autosomal R-bands with minimal or no overlap. Fluctuation of DNA replication in inactive X chromosomes may thus not be a major factor for abnormal phenotypes in multiple X syndromes.

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