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Short Communication

CHROMOSOME ABNORMALITIES AND RARE FRAGILE SITES DETECTED IN AZOOSPERMIA PATIENTS

Naohiko SEKI,[1,2] Hidenori SUMIYA,[3] Jun SHIMAZAKI,[3] Yoshiro TOYAMA,[2] Ei-ichi TAKAHASHI,[1] Motoi MURATA,[4] and Tada-aki HORI[1]

[1]*Division of Genetics, National Institute of Radiological Sciences, Inage-ku, Chiba 263, Japan*
[2]*Departments of Anatomy and* [3]*Urology, School of Medicine, Chiba University, Chuo-ku, Chiba 260, Japan*
[4]*Division of Epidemiology, Chiba Cancer Center Research Institute, Chuo-ku, Chiba 260, Japan*

Summary We have examined constitutional chromosome abnormalities and fragile sites in 40 patients with azoospermia. Chromosome abnormalities were found in four cases. Three cases showed a deletion of the long arm of the Y chromosome 46,X,del(Yq) and the other case had a ring of G group chromosome 46,XY,r(G). In a rare fragile sites test, four fragile site carriers were detected and three rare autosomal fragile sites were identified; fra(8)(q24.1), fra(11)(p15.1), and fra(17)(p12). The expression of these fragile sites were induced specifically by AT-specific DNA ligands, such as distamycin A and Hoechst 33258. In addition, one patient was found to be the case of double ascertainment of fragile sites, fra(8)(q24.1) and fra(17)(p12). The overall frequency of distamycin A-inducible fragile sites in azoospermia patients appeared to be higher than those reported for Japanese healthy subjects and cancer patients. However, no significant relation among fragile sites, clinical and histological findings has been detected so far.

Key Words azoospermia, chromosome abnormality, rare fragile site

INTRODUCTION

Cytogenetic studies on infertile men have demonstrated that the frequency of chromosome abnormalities in patient group is definitely higher than that in

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normal subjects. More than 10% of the azoospermic and severe oligozoospermic males had abnormal chromosomes (see review De Braekeleer and Dao, 1991). Chromosome abnormalities in infertile men were detected mainly in the sex chromosomes. Further studies on Y chromosome abnormalities have indicated that the defects in spermatogenesis are associated with deletion of the Y chromosome (see reviews Davis, 1981; Bühler, 1985).

The rare heritable fragile sites on human chromosomes can be classified into three groups according to the culture conditions which elicit expression of fragile sites; folate sensitive, distamycin A-inducible and BrdU-requiring (Berger *et al.*, 1985). At present, 25 rare fragile sites have been identified on human chromosomes (Sutherland and Ledbetter, 1989).

Although the fragile site on the X chromosome, fra(X)(q27.3) is associated with a form of mental retardation, the clinical significance of the other autosomal fragile sites have not been elucidated yet. The autosomal rare fragile sites are frequently detected in certain patient groups referred to a cytogenetic test in some reasons, and thus, it has been suggested that the fragile sites may act as predisposing factors for human chromosome instability and may be associated with a variety of clinical manifestations including cancers (Yunis, 1983; Sutherland and Hecht, 1985; Shabtai *et al.*, 1985; LeBeau, 1986).

Population cytogenetic screenings of rare fragile sites have been carried out in the Japanese healthy subjects and cancer patients (Takahashi *et al.*, 1988a; Murata *et al.*, 1988; Hori *et al.*, 1988a). Out of 25 rare autosomal fragile sites defined in HGM10, the following nine were identified in the Japanese populations: folate-sensitive fra(2)(q11), fra(11)(q13) and fra(11)(q23), distamycin A-inducible fra(8)(q24.1), fra(11)(p15.1), fra(16)(p12), fra(16)(q22) and fra(17)(p12), and BrdU-requiring fra(10)(q25). Three distamycin A-inducible fragile sites located on 8q24.1, 11p15.1 and 16p12 were found only in Japanese population (Takahashi *et al.*, 1988a, b,c; Hori *et al.*, 1988a,b; Ochi *et al.*, 1988; Sutherland and Ledbetter, 1989).

In the present study, we investigated chromosome abnormalities and fragile sites in 40 azoospermic patients in order to explore a possible association between chromosomal defects and spermatogenesis.

MATERIALS AND METHODS

Forty patients with azoospermia were investigated in the present study. All patients were referred to Chiba University Hospital, because of fertility problem. We examined testis volume, semen and serum hormones. When no sperm was detected in ejaculated seminal fluid, the patient was judged as azoospermia. If the patient agreed with biopsy test, testicular biopsies were taken and histological preparations were made. The defects in spermatogenesis were evaluated by Johnsen's score count (Johnsen, 1970). All patients did not exhibit any other recognizable phenotypic abnormalities except for azoospermia.

Cytogenetic analysis was done on the chromosome preparations from cultures of peripheral blood lymphocytes, by using G- and C-banding methods. For the assay of rare fragile sites, the following method originally described by Takahashi *et al.* (1988a) was applied. For folate-sensitive fragile sites, we used folic acid- and thymidine-deficient Ham's F10 medium (M-F10, Gibco) supplemented with 5% fetal bovine serum (FBS, Difco) and 2% phytohemagglutinin (PHA, Wellcome) and the cultures were incubated for 72 hr. For distamycin A-inducible fragile sites, the cultures were treated with distamycin A (Sigma, 50 μg/ml) or Hoechst 33258 (Wako, 25 μg/ml) during final 24 hr in an RPMI 1640 medium (Nissui) supplemented with 10% FBS and 2% PHA. For the BrdU-requiring fragile sites, the same culture medium was used and treated with BrdU (Sigma, 7 μg/ml) for the final 24 hr. More than fifty Giemsa-stained metaphases were examined on each assay. If more than 4% of metaphases expressed fragility at the same chromosomal locations, it was recognized as a rare fragile site and trypsin G-banding method was used for the detailed analysis of the location.

RESULTS AND DISCUSSION

We have examined 40 patients with azoospermia for constitutional chromosome abnormalities and fragile sites. The cytogenetic and testicular biopsy findings are summarized in Table 1. Three patients (1, 22, and 30) were found to carry a deletion in the long arm of the Y chromosome. In every case, all 50 cells examined showed identical abnormality and no 45,X cell was detected. Examples of C-banded metaphase chromosomes are shown in Fig. 1, a and b. Patient 1 had a dot-like chromosome which probably retained centromere region and SRY (sex determining region of Y) gene. The heterochromatic region of the long arm of the Y chromosome appeared to be deleted.

Many patients with azoospermia have been shown to exhibit Y chromosome abnormalities including the deletion of the long arm. Several cases were reported a deletion of the long arm of the Y chromosome in azoospermic patients in Japan (Sasagawa *et al.*, 1985; Hori *et al.*, 1987; Hazama *et al.*, 1988; Baba *et al.*, 1989). At least three major genes, such as azoospermia, growth control Y and amelogenin-like, have been mapped on the proximal part of long arm of the Y chromosome (Weissenbach *et al.*, 1989). It has been suggested that the azoospermia factor might locate at the Yq distal euchromatic/heterochromatic interface, since the patients showed no other clinical abnormality (see reviews Davis, 1981; Bühler, 1985). Our findings seem to support this hypothesis, although further studies are needed by using both cytogenetic and molecular techniques to define the deleted region and relate it to the azoospermia. Recently, Nakahori *et al.* (1991) have isolated the Y-specific DNA fragments and mapped them on the Y chromosome. They suggested that amelogenin-like sequence, previously mapped on the long arm, is located on the short arm of the Y chromosome. These Y-specific probes may

Table 1. The cytogenetic and testicular biopsy findings on 40 azoospermia patients.

Patient No.	Date of birth	Johnson's score in testicular biopsy		Chromosome constitution	Fragile site detected	Expression frequency (%) induced by distamycin A / Hoechst 33258
		Right	Left			
1	1949-04-01	2.8	2.1	46,X,del(Yq)		
2	1952-12-10	7.0	5.0	46,XY		
3	1953-04-02	3.9	4.5	46,XY,r(G)		
4	1951-05-25	2.0	2.0	46,XY		
5	1962-01-02	7.1	2.0	46,XY		
6	1960-01-31	6.6	7.0	46,XY		
7	1953-04-07			46,XY		
8	1954-08-11			46,XY		
9	1953-07-10			46,XY		
10	1955-08-21	2.8	2.8	46,XY	Fra(8)(q24.1)	10 / 22
					Fra(17)(p12)	24 / 32
11	1956-04-25	7.1	7.2	46,XY	Fra(17)(p12)	8 / 12
12	1952-07-27	4.1	1.6	46,XY		
13	1950-03-14			46,XY		
14	1958-06-21	3.7	6.1	46,XY		
15	1947-08-18	2.0	2.0	46,XY		
16	1950-06-05			46,XY		
17	1955-11-27	2.2	2.2	46,XY		
18	1954-10-10	7.0	7.0	46,XY	Fra(17)(p12)	10 / 16
19	1954-05-16	2.2	2.3	46,XY		
20	1949-04-03	9.1	9.2	46,XY		
21	1958-08-28	2.2	2.2	46,XY		
22	1964-09-23	3.3	1.6	46,X,del(Yq)		
23	1949-09-12	2.2	2.3	46,XY		
24	1956-04-21	3.6	3.7	46,XY		
25	1954-10-22			46,XY		
26	1963-10-16	2.1	2.1	46,XY		
27	1950-07-07			46,XY	Fra(11)(p15.1)	18 / 22
28	1938-04-22	6.9	7.0	46,XY		
29	1957-08-19	9.0	9.1	46,XY		
30	1953-03-13	2.8	1.7	46,X,del(Yq)		
31	1938-10-20	8.4	8.4	46,XY		
32	1950-09-08			46,XY		
33	1962-01-31	7.6	7.9	46,XY		
34	1957-08-15			46,XY		
35	1956-01-16	2.2	2.2	46,XY		
36	1958-05-21	7.4	7.9	46,XY		
37	1970-07-01		3.9	46,XY		
38	1952-11-30			46,XY		
39	1954-11-02			46,XY		
40	1947-12-30	1.2	1.4	46,XY		

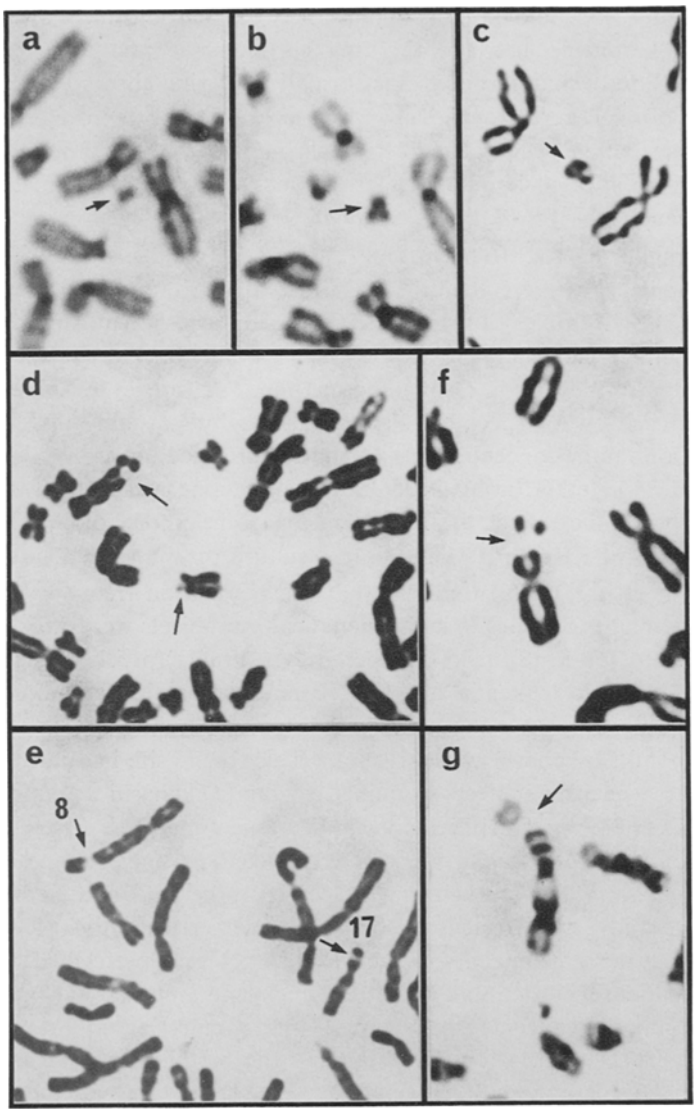

Fig. 1. Partial metaphase showing chromosomal abnormalities (a–c) and the expression of fragile sites (d–g). a, patient 1 with 46,X,del(Yq); b, patient 27 with 46,X,-del(Yq); c, patient 3 with 46,XY,r(G); d and e, patient 10 with a double ascertainment of fra(8)(q24.1) and fra(17)(p12); f and g, patient 27 with fra(11)(p15.1). e and g, G-banded chromosomes. Arrows indicated the del(Yq) in a and b, r(G) in c and fragile site expression in d to g.

be useful to analyze structurally abnormal Y chromosome.

We have also found a patient with a ring chromosome of the G group, as shown in Fig. 1c. We did not performed detail analysis of this patient's chromosome

in some reasons, so could not distinguish between chromosome 21 and 22. Although the relationship between the ring chromosome and azoospermia is not known, the failure of pairing due to the formation of ring chromosome might result in an arrest of the spermatogenesis. McIlree (1966) has reported a similar case of azoospermia patient who carried a ring chromosome 21 or 22 in which a failure of pairing of the affected homologues at diakinesis led to the complete breakdown of spermatogenesis.

In the fragile site test, four carriers (Patients 10, 11, 18, and 27) were detected and their fragile sites were identified as fra(8)(q24.1), fra(11)(p15.1) and fra(17)-(p12) (Table 1). Examples of fragile site expression are shown in Fig. 1, d–g. The fragile sites in all carriers were expressed in a heterozygous fashion. A detail analysis was performed using G-banding method. As shown in Table 1, all fragile sites were induced by AT-specific DNA ligands, such as distamycin A and Hoechst 33258. The optimum concentrations of the culture medium were 50 μg/ml (distamycin A) and 25 μg/ml (Hoechst 33258) and higher concentrations of these inducers reduced mitotic index and destroyed chromosome morphology. No fragility were detected in M-F10, BrdU and control culture medium (data not shown).

Takahashi *et al.* (1988a) reported that the population frequency of the distamycin A-inducible fragile sites in a Japanese healthy subjects were 3.08% for fra(17)-(p12) and 0.71% for fra(8)(q24.1), respectively. Fra(11)(p15.1) was found in two acute nonlymphocytic leukemia (ANLL) patients whose leukemia cells contained a specific chromosome rearrangement with a corresponding breakpoint in 11p15 [t(7:11)(p13:p15)] (Takahashi *et al.*, 1988c). This fragile site had not been detected in 845 Japanese healthy subjects examined so far (Takahashi *et al.*, 1988a). The fragile sites at 8q24.1 and 11p15.1 appear to be new members of a distamycin A-inducible group. These fragile sites were detected in only Japanese population (Takahashi *et al.*, 1988b, c; Hori *et al.*, 1988a; Sutherland and Ledbetter, 1989). In the present study, we detected one fra(11)(p15.1) carrier among 40 azoospermia patients, though pedigree analysis of the case has not been performed. The clinical chart of this patient recorded that his father, uncle and aunt had died of lung cancer, leukemia and uterine cancer, respectively. Thus, it might be important to examine the nature of fra(11)(p15.1) for understanding the biological significance of its involvement in azoospermia and oncogenesis.

One of four carriers (Patient 10) had a fragile sites at both 8q24.1 and 17p12 (Fig. 1, d and e). The expression frequencies of the double ascertainment of fragile sites in this patient were determined at two different times and were not significantly different (data not shown). The double ascertainment of fragile sites is very infrequent (Sutherland and Hecht, 1985). The biological and clinical significance of double ascertainment have not been clearly understood. As discussed by Sutherland and Hecht (1985), a transposition model, in which DNAs amplified at fragile site are integrated into other chromosomal regions, may provide an explanation for double ascertainment of fragile sites.

Although the present results on constitutional chromosome abnormalities confirmed the previously reported findings in azoospermia patients, the involvement of fragile sites in azoospermia was not proved, since no clear association among autosomal rare fragile sites, clinical and histopathological findings were observed. However, we consider that the following findings would be more than a chance occurrence: 1) we found four fragile site carriers among 40 patients. Although the number of patients examined was small, the overall frequency of distamycin A-inducible fragile sites (12.5%, 5/40) appeared to be much higher than those reported for healthy subject (5.2%, 44/845), patients with hematological disorders (5.0%, 12/239) and solid tumors (6.1%, 8/131); 2) one of the four carriers had double ascertainment of fragile sites, which is a very rare event in general population; and 3) a very rare fragile site fra(11)(p15.1) was detected in this screening.

To assess the biological significance of rare fragile site on azoospermia, further intensive population studies are required. Especially, molecular cloning of autosomal rare fragile site, as has been done in fra(X)(q27.3) (Verkerk *et al.*, 1991; Kremer *et al.*, 1991) is an essential step not only for understanding of fragile site itself, but also for chromosomal instability of human genome.

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