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COMMON FRAGILE SITES INDUCED BY FOLATE DEPRIVATION, BRDU AND APHIDICOLIN: THEIR FREQUENCY AND DISTRIBUTION IN JAPANESE INDIVIDUALS

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Summary The frequencies and distribution of common fragile sites in normal Japanese individuals were studied using lymphocyte chromosome preparations from cultures under folate deprivation, 5-bromodeoxyuridine (BrdU) or aphidicolin treatment. Defining the 4% level of total breaks as a cut-off point, 13 folate-sensitive, 20 BrdU-induced and 8 aphidicolin-induced sites were identified in one or more individuals studied. After eliminating overlapping, 28 fragile sites were identified. Of these, 23 have been reported, while the other five have not been described. Of the latter, those at 4q34 and 6q22 were each found in only one individual. The site at 3q26.2 has not been reported but one at 3q27 has been identified. Excluding these three sites as inconclusive, there remain two sites hitherto undescribed: 1) a folate-sensitive site at 17q21, found in two of eight individuals studied, and 2) a BrdU-requiring site at 13q31, found in five of eight individuals.

INTRODUCTION

Chromosomal fragile sites are now classified into three groups based on their frequencies in the general population (Hecht, 1986): rare, present in less than 1% of the general population; polymorphic, 1–50%; and common, over 50%. While we agree with the classification, the frequencies of fragile sites vary considerably depending on the means of their induction. We will thus use the term "common" to include both polymorphic and common fragile sites.

Common fragile sites (c-fra) are inducible at low frequencies under culture conditions that induce thymidylate stress (folate and thymidine deprivation, methotrexate or fluorodeoxyuridine treatment), and by 5-bromodeoxyuridine (BrdU) or

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5-azacytidine treatment, while they are induced at high frequencies by treatment with aphidicolin, aphidicolin plus ethanol, caffeine, or by a combination of these with other conditions (*cf.* Sutherland and Hecht, 1985; Kuwano and Kajii, 1987, for references).

Reports on c-fra sites in Caucasians have included those induced by folic acid deprivation (Sutherland, 1979; Yunis and Soreng, 1984; Marlhens *et al.*, 1986), by treatment with methotrexate (Barbi *et al.*, 1984), fluorodeoxyuridine (Barbi *et al.*, 1984; Daniel *et al.*, 1984; Yunis and Soreng, 1984; Rao *et al.*, 1988), 5-bromodeoxyuridine (Sutherland *et al.*, 1985), 5-azacytidine (Sutherland *et al.*, 1985), caffeine (Yunis and Soreng, 1984), or aphidicolin (Glover, 1984; Craig-Holmes *et al.*, 1987; Rao *et al.*, 1988). Little has been known of their individual or racial difference.

This report will deal with c-fra sites in Japanese individuals induced by folate deprivation, BrdU and aphidicolin.

MATERIALS AND METHODS

Heparinized whole blood samples from healthy Japanese individuals were cultured for 4 days in Eagle's minimum essential medium (MEM) containing 5% fetal calf serum, 4% phytohemagglutinin, penicillin and streptomycin.

In one group, blood samples from eight healthy individuals, four males and four females, ranging in age from 7 to 31 years (Subjects 1–8), were cultured in MEM without folic acid or thymidine. Additional samples from the same individ-

Table 1. Individuals studied.

No.	Sex	Age (yr.)	Occasional alcohol drinking	Breaks under routine culture condition [a]
1	F	28	+	2
2	F	26	+	6
3	F	24	+	5
4	M	23	+	5
5	F	7	−	7
6	M	31	+	7
7	M	8	−	5
8	M	8	−	6
9	F	22	+	4
10	F	23	+	2
11	M	24	+	2
12	F	24	−	2
13	F	34	+	4
14	F	26	−	4
15	M	27	−	2

[a] Total number of breaks in 100 peripheral blood lymphocytes short term cultured under a usual condition.

uals were cultured in MEM (containing 1.0 mg/liter folic acid) and BrdU was added during the final 6 hr at a final concentration of 20 mg/liter. In another group, cultures from seven healthy individuals, 2 males and 5 females aged 22 to 34 years (Subjects 9–15), were set up using MEM. Aphidicolin, 0.2 μM dissolved in 0.02% dimethylsulfoxide, was added during the final 26 hr.

Colcemid was added to both of the above groups 2 hr prior to harvest, and conventional, air-dried chromosome slides were prepared and Giemsa stained. A total of 200 metaphases were scored for gaps and breaks in slides from cultures with folate deprivation and BrdU, and 100 metaphases in those with aphidicolin. The chromosomes were then destained, trypsin-G banded, and their band localization was photographically determined.

The 15 subjects in this study included 6 males and 9 females, ranging in age from 6 to 34 years (Table 1). None of them smoked, nine took alcoholic beverages only on social occasions, and none had virus infections, was exposed to X-ray, or took drugs during a two-week-period preceding the sampling. Their peripheral blood lymphocytes cultured for 4 days in MEM with 10% fetal calf serum yielded 2 to 7 breaks per 100 cells screened.

RESULTS

Folate-sensitive c-fra

A total of 536 breaks were observed among 1,600 cells scored (Table 2). The rates of total breaks in 100 cells ranged from 16 to 45.5 among the eight subjects studied, with a mean of 33.5. There were 13 sites that occurred with 4% or more of total breaks in one or more subjects, accounting for 62.9% of total fragile sites observed. Of these, seven were present at $\geq$4% in only one individual. The remaining six sites were seen at $\geq$4% in two or more individuals. The most common fragile site was 3p14 with 27.4% of the total breaks. This site was seen in all eight subjects. Another c-fra site seen in all subjects was 6q26 with a frequency of 10.4% of total breaks. Other sites of breakage observed in decreasing order of frequency were: 16q23 (5.6%), 1p31 (5.2%), 3q26.2 (3.7%), 17q21 (1.7%).

BrdU-sensitive c-fra

A total of 320 breaks were observed among 1,600 cells from cultures with BrdU (Table 3). Breaks per 100 cells ranged from 15 to 23.5 among the eight individuals studied, with a mean of 20. There were 20 sites that occurred with $\geq$4% of total breaks in one or more subjects. They accounted for 58.1% of the total breaks. Of these, 10 were present at $\geq$4% in only one individual. The most common site was 5p14 with 9.7% of the total breaks seen in all subjects. The second common fragile site was 3p14 (8.8%), followed by 9p13 (6.6%), 13q31 (4.4%), 16q23 (3.1%), 3q26.2 (2.5%), 16q22 (2.5%), 5q31 (2.2%), and 8q24 (2.2%).

Table 2. Analysis of fragile sites induced by folate-free medium, values based on 200 metaphases each.

Subject	1	2	3	4	5	6	7	8	Total breaks	Total individuals
Total breaks	32	80	60	73	91	73	53	74	536	8
Fragile sites										
1p31				4 (5.5)	12(13.2)	4 (5.5)			28 (5.2)[b]	3 (37.5)[c]
1q25	2 (6.3)[a]								5 (0.9)	1 (12.5)
2p24							3 (5.7)		8 (1.5)	1 (12.5)
3p14	25(78.1)	16(20)	16(26.7)	20(27.4)	17(18.7)	15(20.5)	11(20.8)	27(36.5)	147(27.4)	8(100)
3q26.2				8(11)	4(4.4)	3 (4.1)			20 (3.7)	3 (37.5)
4q31						3 (4.1)			5 (0.9)	1 (12.5)
5p14	3 (9.4)								8 (1.5)	1 (12.5)
6q26	3 (9.4)	9(11.3)	8(13.3)	5 (6.8)	13(14.3)	6 (8.2)	4 (7.5)	8(10.8)	56(10.4)	8(100)
7q22						3 (4.1)			6 (1.1)	1 (12.5)
7q31			3(5)						7 (1.3)	1 (12.5)
16q22		4(5)							8 (1.5)	1 (12.5)
16q23	4(12.5)	5(6.3)	3(5)	3 (4.1)	6(6.6)			6 (8.1)	30 (5.6)	6 (75)
17q21							3 (5.7)	4 (5.4)	9 (1.7)	2 (25)
13[d]									337(62.9)	

[a] Numbers in parenthesis represent % of total breaks in the individual. [b] Numbers in parenthesis represent % of total breaks in all individuals. [c] Numbers in parenthesis represent % of individuals. [d] Total number of fragile sites.

Table 3. Analysis of fragile sites induced by BrdU, values based on 200 metaphases each.

Subject	1	2	3	4	5	6	7	8	Total breaks	Total individuals
Total breaks	30	44	47	47	36	38	34	44	320	8
Fragile sites										
1p36			2 (4.3)						3 (0.9)	1 (12.5)
1p31			2 (4.3)						6 (1.9)	1 (12.5)
3p14		5(11.4)		2 (4.3)	4(11.1)	2(5.3)	8(23.5)	7(15.9)	28 (8.8)	6 (75)
3q26.2						2(5.3)	4(11.8)		8 (2.5)	2 (25)
4q31								2 (4.5)	6 (1.9)	1 (12.5)
4q34				3 (8.5)					5 (1.6)	1 (12.5)
5p14	6(5)	4 (9.1)	7(14.9)	3 (8.5)	3 (8.3)	3(7.9)	3 (8.8)	2 (4.5)	31 (9.7)	8(100)
5q31			2 (4.3)			2(5.3)			7 (2.2)	2 (25)
6q22					2 (5.6)				2 (0.6)	1 (12.5)
6q26			2 (4.3)			2(5.3)		5(11.4)	12 (3.8)	3 (37.5)
7q31				2 (4.3)					4 (1.3)	1 (12.5)
7q32				2 (4.3)					4 (1.3)	1 (12.5)
8q24				2 (4.3)		2(5.3)		2 (4.5)	7 (2.2)	3 (37.5)
9p13		7(15.9)	3 (6.4)	2 (4.3)	5(13.9)			2 (4.5)	21 (6.6)	5 (62.5)
10q23				2 (4.3)					2 (0.6)	1 (12.5)
12p12					2 (5.6)				3 (0.9)	1 (12.5)
12q24				5(10.6)					5 (1.6)	1 (12.5)
13q31	2 (6.7)		4 (8.5)	3 (8.5)	2 (5.6)	2(5.3)			14 (4.4)	5 (62.5)
16q22			3 (6.4)		2 (5.6)				8 (2.5)	2 (25)
16q23			4 (8.5)			2(5.3)		2 (4.5)	10 (3.1)	3 (37.5)
20									186(58.1)	

Table 4. Analysis of fragile sites induced by aphidicolin, values based on 100 metaphases each.

Subject	9	10	11	12	13	14	15	Total breaks	Total individuals
Total breaks	201	296	434	274	288	290	275	2, 058	7
Fragile sites									
2p24	10 (5)	12 (4. 1)						45 (2. 2)	2 (28. 6)
2q32		16 (5. 4)						66 (3. 2)	1 (14. 3)
3p14	38 (14. 9)	48 (16. 2)	96 (22. 1)	40 (14. 6)	39 (13. 5)	40 (13. 8)	50 (18. 1)	351 (17. 1)	7 (100)
6q26	14 (7)							56 (2. 7)	1 (14. 3)
7q32	10 (5)	22 (7. 4)		19 (6. 9)	15 (5. 2)		20 (7. 3)	111 (5. 4)	5 (71. 4)
14q24	9 (4. 5)	13 (4. 4)		22 (8)			13 (4. 7)	84 (4. 1)	4 (57. 1)
16q23	30 (15)	46 (15. 5)	64 (14. 7)	39 (14. 29)	21 (7. 3)	22 (7. 6)	45 (16. 4)	267 (13)	7 (100)
Xp22	15 (7. 5)	19 (6. 4)		18 (6. 6)	43 (14. 9)	30 (10. 39)	11 (4)	147 (7. 1)	6 (85. 7)
8								1, 127 (54. 7)	

Aphidicolin-sensitive c-fra

Among the three treatments, cells treated with aphidicolin showed the highest frequency of fragile sites (Table 4). A total of 2,058 breaks were observed among 700 cells scored, with a mean of 294 breaks per 100 cells. There were 8 sites that occurred with $\geq 4\%$ of total breaks in one or more individuals, and accounted for 54.8% of the total breaks. Of these sites, two were present in only one individual. The most common sites was 3p14 with 17.1% of the total breaks, followed by 16q23 (13.0%), Xp22 (7.1%), 7q32 (5.4%), 14q24 (4.1%), 6q26 (2.7%), and 2p24 (2.2%).

DISCUSSION

Most of the previous reports on the frequency of a fragile site have been based on the total number of cells that express a fragile site. Hecht and Sutherland (1984) recommended that two or more cells with the same fragile site are necessary to classify the individual as positive for that fragile site. A 4% cut-off frequency has been suggested for determining the presence of fragile X (Jacobs *et al.*, 1980). These criteria are acceptable under conditions where the frequency of background fragile sites that are simultaneously expressed in the cells is low. This applies to the common fragile sites induced by folate deprivation or BrdU. The criteria, however, are not acceptable under conditions where the frequency of fragile sites is high, as is the case with aphidicolin or caffeine treatment. Thus, we estimated the frequency of fragile sites in relation to total breaks, as suggested by Rao *et al.* (1988).

Using the 4% level of total breaks as a criterion, 13 folate-sensitive sites, 20 BrdU-induced sites, and 8 aphidicolin-induced sites were identified in one or more

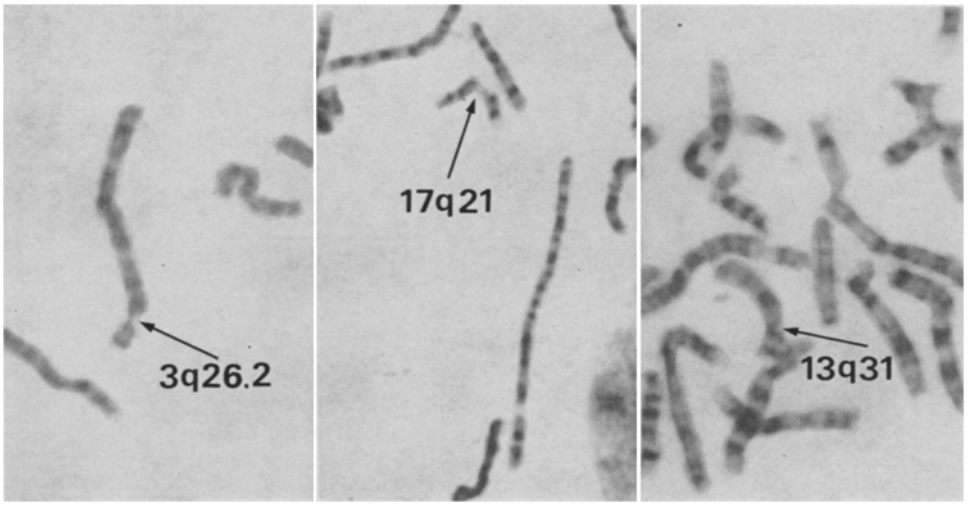

Fig. 1. Fragile sites at 3q26.2 (left) and 17q21 (center), both induced by folic acid deprivation, and 13q31 induced by BrdU.

Table 5. Fragile site expression upon the three treatments.

Sites	No. individuals identified	References [a]
Unique to folate deficiency		
1q25	1	5, 6, 8
7q22	1	5, 8, 10, 7q21 by 6, 9
7q31	1	6, 8, 9
17q21	**2**	Not described
Unique to BrdU		
1p36	1	5
4q34	**1**	Not described
5q31	2	5, 6, 8
6q22	**1**	Not described, 6q21 by 8
7q31	1	6
8q24	3	6, 8, 10
9p21	5	4, 7
10q23	1	1, 4, 10q22 by 10
12p12	1	8
12q24	1	7, 12q23 by 8
13q31	**5**	Not described
Unique to aphidicolin		
2q32	1	6, 2q31 by 5, 8, 10
14q24	4	5, 6, 8, 10, 14q23 by 2
Xp22	6	2, 5, 6, 8, 9, 10
Common to folate deficiency and BrdU		
1p31		3, 6, 10, 1p32 by 5, 8
3q26.2		Not described, 3q27 by 5, 6, 8
4q31		6, 8
5p14		8, 10, 5p13 by 7
16q22		1, 4, 6, 8, 10
Common to folate deficiency and aphidicolin		
2p24		6, 9, 2p23 by 8
Common to BrdU and aphidicolin		
7q32		2, 5, 6, 8, 10
Common to all treatments		
3p14		2, 3, 4, 5, 6, 8, 9, 10
6q26		3, 4, 5, 6, 8, 10
16q23		2, 3, 4, 5, 6, 8, 9, 10

[a] 1, Sutherland (1979); 2, Barbi *et al.* (1984); 3, Daniel *et al.* (1984); 4, de la Chapelle and Berger (1984); 5, Glover (1984); 6, Yunis and Soreng (1984); 7, Sutherland *et al.* (1985); 8, Craig-Holmes *et al.* (1987); 9, Marlhens *et al.* (1986); 10, Rao *et al.* (1988).

individuals in our study. After eliminating overlapping, a total of 28 sites were identified (Table 5). Of these, 23 (82%) have been reported previously while the remaining five sites, 3q26.2, 4q34, 6q22, 13q31 and 17q21, have not been described (Fig. 1). Of the latter five, those at 4q34 and 6q22, both BrdU induced, were each found in only one individual, and thus need further confirmation. The site at 3q26.2, both folate sensitive and BrdU induced, has not been described, but a site at the adjoining band 3q27 has been reported (Glover, 1984; Yunis and Soreng, 1984; Craig-Holmes *et al.*, 1987). The question of whether or not they are identical remains to be answered. This leaves two sites previously unreported: 1) a folate sensitive site at 17q21, identified in two of eight individuals studied, and 2) a BrdU-requiring site at 13q31, found in five of eight individuals.

Takahashi *et al.* (1988), in a recent survey of rare fragile sites in Japanese blood donors, reported two new fragile sites: 1) a folate-sensitive site at 17p12, and 2) a distamycin A-inducible site at 8q24.1. Since rare fragile sites may result from mutations at common fragile sites, it would be worthwhile to check for their presence among common fragile sites. Neither of these sites, however, was detected in our study.

In conclusion, most of the fragile sites found in our study were common with those reported in various Caucasian populations. Some sites, notably a folate-sensitive site at 17q21 and a BrdU-requiring site at 13q31, have not been described in Caucasian populations. It remains to be seen whether these sites represent racial or population difference.

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