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Cytogenetics of Autosomal Fragile Sites: A Basque Population Study

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ABSTRACT Population cytogenetic data on autosomal fragile sites show differences among different ethnic groups. The Basques are an ancient population; their origin is not exactly known and many studies using several traits have shown peculiarities in the Basques. This is the first study about the incidence of autosomal fragile sites in a healthy Basque sample. The results show interindividual variability, no sex differences at a global level, but differences for some fragile sites. Compared with other populations, a higher incidence of rare autosomal fragile sites has been demonstrated (8%).

"Fragile sites (FS) on chromosomes are points at which the chromosome is liable to break. The expression of all known fragile sites is dependent on specific chemical agents or on the modification of normal tissue culture conditions" (Sutherland and Hecht, 1985). The first fragile site was described by Debakan (1965), though the term was defined for the first time in 1970 (Margulis et al., 1970). In the late 1970s, the study regained importance because of the discovery of a FS on the distal zone of the X chromosome (the Xq27.3) that was somehow associated with mental retardation (Giraud et al., 1976; Harvey et al., 1977). Presently, according to Human Gene Mapping Project (McAlpine et al., 1990), there are 114 different FS.

There are two criteria for their classification: the frequency and the means of induction. According to the first criterion, they are classified as common fragile sites (CFS) when they can be observed in virtually every individual and as rare fragile sites (RFS) when they are observed in a relatively small percentage of the population (Hecht et al., 1990). According to the second criterion, there are several groups based on the culture conditions in which they are expressed. Hence, CFS can be divided into aphidicolin inducible, 5-azacitidine inducible, and BrdU inducible; RFS can be classified as folate sensitive, distamicine A inducible, and BrdU inducible (Sutherland and Hecht, 1985; Sutherland, 1991).

Despite the studies that have taken place to date, only one rare fragile site at Xq27.3 is known to be associated with a specific clinical entity, the fragile X syndrome (Hagerman, 1992; Fisch, 1993; Tarleton and Saul, 1993). The biological and medical meaning of the remainder of FS is unclear and controversial (Sutherland et al., 1985; Le Beau, 1986; Laird et al., 1987; Kähkönen et al., 1989; Chudley et al., 1990; Jordan et al., 1990; Rassool et al., 1992).

Recently, the molecular basis of fragility in the fragile X syndrome has been described (Kremer et al., 1991; Verkerk et al., 1991). Individuals showing the rare folate sensitive fragile site carry a variable number of CGG repetitions that interfere with gene expression.

According to Sutherland and Hecht (1985), "population cytogenetics of fragile sites present a major area in need of exploration. The resultant data will be of both theoretical and practical values in Human Biology." Although many population cytogenetic studies have been carried out, very little information about fragile sites has been provided. In addition, prior studies have generally included patients or mentally retarded individuals, and the amount of cytogenetic data on FS in normal individuals is very small (Quack et al., 1978; Guichaoua et al.,

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1982; Sutherland, 1982a, 1985; Kähkönen et al., 1986, 1989; Petit et al., 1986; Fryns and Petit, 1987; Webb et al., 1987; Chudley et al., 1990). Also, in relation to the racial distribution, the fragile X site has been the most analyzed (Sutherland and Hecht, 1985).

The Basques are an ancient population now living in the west of the Pyrenees Mountains. The origin of the Basques is not exactly known. Many studies, using different traits, have shown peculiarities compared with other European populations (Cavalli-Sforza and Piazza, 1993). Dermatoglyphic research of the Basques has shown peculiarities and also differences between this population and others (Arrieta et al., 1987a,b, 1990, 1991, 1992).

In previous work (unpublished data), the incidence of FS in an autistic Basque sample was analyzed. There are no previous studies in a healthy Basque population. In this first study, the spontaneous expression of autosomal fragile sites (AFS) and expression in folic acid–deficient culture conditions are analyzed. The study takes both sexes into account separately.

MATERIALS AND METHODS

Sample

The sample is 50 healthy unrelated individuals, 25 women and 25 men, of Basque origin. Age is very homogeneous (mean age was 25.5 years), which does not permit analysis of the influence of age. Basque origin was tested by surnames and birthplace of grandparents and great-grandparents; at least the first eight surnames had to be Basque.

Cytogenetic analysis

Chromosomal analysis was performed on cultured lymphocytes from peripheral blood. Spontaneous expression of fragile sites was examined in RPMI-1640 medium (R-1640) without any inducing agent. TC-199 (a folic acid–deficient medium) and RPMI-1640 with methotrexate (R+MTX) was used to induce the expression of FS under conditions of folate deprivation. Methotrexate, an inhibitor of the hydrofolate reductase, was added 24 hours before harvesting.

After 72 hours of culture in R-1640 and TC-199, and after 96 hours of culture in R-MTX, chromosomal preparations were made by standard procedures following colcemid treatment. Approximately 100 randomly selected metaphases were analyzed per individual and culture medium.

The chromosomes were stained with Giemsa for the location of chromosomal lesions (CL). The lesionated metaphases were sequentially GTG banded to localize the lesion sites. Since the aberrations were almost exclusively gaps and breaks on chromatids and chromosomes, they were assigned as a single event on a low definition haploid karyotype of 400 bands. In this study, other possible forms of FS expression were not taken into account. For classification of lesions the International System for Human Cytogenetic Nomenclature (ISCN, 1985) was used. To determine the degree of coincidence between CL and AFS, only AFS accepted by the Human Gene Mapping Project have been considered (McAlpine et al., 1990).

Statistics

One-way and two-way analysis of variance (ANOVA) and Student t-tests were used. The procedure of Mariani (1989), which is based on the Poisson distribution, was followed to appreciate, on one hand, which AFS are significantly expressed in the population and, on the other, whether the lesions not coincident with known AFS are random events or not. The X^2 contingency test was used to evaluate the different expression of AFS with regard to the sex and medium.

RESULTS

Extent of chromosomal lesions

Some gaps and breaks appeared in chromosomal bands not considered as AFS. The term *chromosomal lesions* (CL) was used for these whether or not there are loci accepted as FS. The frequency of CL in the total sample (Table 1) was 371 in R-1640 (mean ± standard deviation of CL per individual per cell = 0.073 ± 0.046), 929 in TC-199 (mean ± standard deviation of CL per individual per cell 0.18 ± 0.116), and 2,531 in R+MTX (mean ± standard deviation of CL per individual per cell = 0.506 ± 0.440).

The incidence of CL differed among the individuals in the three culture mediums. The one-way ANOVA results showed that the interindividual variability was significant ($P < 0.01$). The frequency of gaps and breaks in bands classified as AFS was 362 in R-1640 (mean ± standard deviation of AFS per individual per cell = 0.071 ± 0.045), 879 in TC-199 (mean ± standard deviation of AFS per individual per cell = 0.170 ±

TABLE 1. Frequency of CL and AFS in the three culture mediums

Medium	Cells analyzed	Total CL (%)	CL Mean ± SD	Total AFS (%)	AFS Mean ± SD
RPMI 1640	5,050	7.35	0.073 ± 0.046	7.17	0.071 ± 0.045
TC-199	5,150	18.04	0.180 ± 0.116	17.07	0.170 ± 0.106
RPMI + MTX	4,980	50.82	0.506 ± 0.440	48.71	0.485 ± 0.430

0.106), and 2,426 in R-MTX (mean ± standard deviation of AFS per cell = 0.485 ± 0.430). The coincidence among FS and CL was 97.57% in R-1640, 94.62% in TC-199, and 95.85% in R+MTX.

A two-way ANOVA with the culture medium and sex was done to test for significant differences in the incidence of CL. The ANOVA revealed that the differences due to the culture mediums were significant ($P < 0.001$); however, the contribution of sex was not significant ($P > 0.05$). Because of these results, the data were not divided by sex in Table 1.

Differences between R-1640 and TC-199, the TC-199 and R+MTX, and R-1640 and R+MTX were significant ($P < 0.001$). Although the differences are significant, it is obvious that the incidence of CL in the medium with methotrexate was the most important.

Location of chromosomal lesions

Overall, 92 different chromosomal regions with lesions in the three mediums were detected. Of these, 23 do not correspond to any AFS shown in Human Gene Mapping Project, neither confirmed, provisional, nor tentative (McAlpine et al., 1990). Of the 69 other chromosomal regions considered as AFS, 6 were ARFS (autosomal rare fragile sites) and 63 were ACFS (autosomal common fragile sites).

The average number of CL per band was 0.93 (371/400) in R-1640, 2.32 (929/400) in TC-199 and 6.33 (2531/400) in R+MTX. Accordingly, in this sample any band with eight or more lesions in R-1640, with 11 or more lesions in TC-199, and with 22 or more lesions in R+MTX were considered as a significantly expressed fragile site.

Table 2 shows the chromosomal location of CL not described as AFS in each medium. The number of chromosomal lesions not coincident with AFS is quite superior in R+MTX, which is also the medium where more AFS are expressed. According to the Poisson distribution, the number of lesions in each band does not appear with sufficient frequency to be considered as a nonrandom event.

From the 63 CL described as ACFS, only the chromosomal location of the 23 ACFS significantly expressed in the population is reported (Table 3). The 3p14 is the most frequently expressed FS in the three mediums, followed by the 6q21 in R-1640 and the 16q23 in TC-199 and R+MTX. The results of the X^2 test show no significant differences between the sexes in the three mediums ($P > 0.05$); however, there are sex differences for some ACFS. Sex differences have been shown for the 1q21 ($P < 0.05$), the 2q21, 2q32, 6p25 ($P < 0.001$), and the 10q26 ($P < 0.05$) fragile sites in R+MTX.

Table 4 shows the chromosomal location of CL described as ARFS in each medium. The ARFS number is higher in the inducing mediums, and among them, the medium with methotrexate induces the expression of a higher number of bands. The X^2 results show that the variation between sexes is not significant in the three mediums ($P > 0.05$). The X^2 tests for each medium and for each fragile site are also not significant.

According to the results based on the Poisson distribution, the ARFS significantly expressed in this sample are the 2q22.3 in R-1640, and the 2q22.3, 10q24.2, 11q23.3, and 12q24.13 in TC-199 and R+MTX. However, the 8q22.3 and 9p21 ARFS are expressed in a low frequency in this population.

Comparison with other populations

Table 5 summarizes published studies of ACFS frequency in healthy populations. The average number of gaps and breaks per cell was 0.06 in a Canadian population (Chudley et al., 1990), 0.17 in a Finnish population (Kähkönen, 1988), and 0.15 in the present sample. In this population, 13 ACFS from the 63 were significantly expressed in TC-199 medium; in the Finnish sample (Kähkönen 1988), 30 ACFS of 52 were expressed in TC-199. This number is much larger than that observed in the Basque sample. On the other hand, Chudley et al. (1990) noted the

TABLE 2. Frequency of CL not included as AFS in Human Gene Mapping 10.5 (1990)

	R-1640					TC-199						R + MTX					
Bands	MCL	FCL	TI	CLm	CLf	TCL	Bands	MCL	FCL	TI	CLm	CLf	TCL	Bands	MCL	FCL	TI	CLm	CLf	TCL
4p12	2	—	4	5	—	1.35	2q36	1	1	4	3	3	0.65	1p34	—	1	2	—	6	0.24
							5q12	—	2	4	—	7	0.75	1q24	2	1	6	7	1	0.32
							6q23	1	2	6	2	5	0.75	2p21	—	1	2	—	5	0.18
							8q12	3	1	8	6	1	0.75	2q34	3	1	8	8	1	0.36
							11p12	2	1	6	5	3	0.86	3p25	1	1	4	3	6	0.36
							13q22	2	2	8	4	2	0.65	3p21	1	—	2	3	—	0.12
							17q21	2	1	6	5	4	0.97	4q22	2	1	6	4	5	0.36
														4q33	2	—	4	9	—	0.36
														5q34	1	1	4	2	2	0.16
														6p21	1	2	6	4	3	0.28
														6q24	1	3	8	1	8	0.36
														8p22	—	2	4	—	6	0.24
														13q31	1	1	4	1	4	0.18
														16q12	1	3	8	1	6	0.28
														17q25	2	1	6	5	4	0.36

MCL, number of males with CL; FCL, number of females with CL; TI, total individuals (%); CLm, number of CL in males; CLf, number of CL in females; TCL, total CL (%); TCL = (CLm + CLf)/CL frequency in the whole sample per medium (see Results).

appearance of 26 ACFS; it is not known whether this is the frequency of each ACFS and whether the 26 ACFS are those that have been expressed significantly in the population. The published data on the incidence of ARFS in several healthy populations (Table 6) vary greatly, from 8% in the Basques in TC-199, to 0.14% in Australians (Sutherland, 1982a, 1985). The Basque show the highest frequency, while the Belgians have a similar value (Fryns and Petit, 1987).

DISCUSSION

In this study of the expression of AFS in a healthy Basque sample, the mean frequency of CL varies according to the medium. The lower expression appears in the non-inducing medium, and of the two inducing mediums, R+MTX shows the highest expression levels, and this happens not only in relation to the ARFS but also to the ACFS. Green et al. (1988) showed that low levels of folic acid increased the CL expression. Most of the CL have appeared in bands included as AFS in the Human Gene Mapping Project (McAlpine et al., 1990). These results are similar to the previous studies (Kähkönen, 1988; Fuster et al., 1989). Following Mariani (1989), CL not considered as AFS are random lesions in the Basque sample. The incidence of CL varies significantly among individuals. Some authors have also pointed out that the CL expression can vary greatly among individuals, although the inducing mediums have not always been the same (Craig-Holmes et al., 1987; Fuster et al., 1989; Kähkönen, 1988; Rao et al., 1988).

Sex does not appear to have a significant effect on AFS expression at a global level. Previous studies have also shown no sex influence on FS expression (Chudley et al., 1990; Smeets and Merkx, 1990; Tedeschi et al., 1992). The most comparable results with the present study are those of Smeets and Merkx (1990), since they analyzed a healthy sample and also one of the mediums used in this study. However, Tedeschi et al. (1992) used aphidicolin as inducing agent. The comparison with the data of Chudley et al. (1990) has to be interpreted with care since their sample was composed of newborns and they included FUdR as an inducing agent.

Despite the fact that no global sex differences were observed, the different frequency of some ACFS in relation to sex should be noted. The 1q21, 2q21, 2q32, and 10q26 are significantly more expressed in males, and

TABLE 3. Frequency of CL included as ACFS in Human Gene Mapping 10.5 (1990)

R-1640							TC-199							R + MTX						
Bands	MCL	FCL	TI	CLm	CLf	TCL	Bands	MCL	FCL	TI	CLm	CLf	TCL	Bands	MCL	FCL	TI	CLm	CLf	TCL
3p14.2	10	12	44	29	34	11.59	1p36	2	3	10	8	10	1.94	1p22	10	11	42	27	25	2.05
5q15	1	1	4	5	4	2.43	1p22	4	5	18	13	16	3.12	1q21	12	7	38	29	14	1.70
6q21	3	4	14	13	16	7.82	1q21	7	6	26	19	13	3.44	1q25.1	7	6	26	27	23	1.98
7q22	1	2	6	6	8	3.77	1q25.1	2	1	6	9	6	1.61	2q21.3	19	8	54	40	17	2.25
13q21	2	3	10	11	13	6.47	2q21.3	6	5	22	19	11	3.23	2q32.1	10	6	32	25	10	1.38
18q21.3	1	3	8	9	11	5.39	2q32.1	6	4	20	19	16	3.77	3p14.2	21	20	82	60	56	4.58
							3p14.2	15	17	64	46	56	10.99	3p24.2	6	7	26	10	17	1.07
							5q15	2	1	6	7	6	1.40	4q31.1	13	10	46	19	11	1.19
							5q21	5	6	20	21	27	5.17	5q15	11	12	16	17	24	1.62
							5q31.1	6	7	26	31	36	7.21	5q21	8	8	32	12	15	1.07
							6q21	7	5	24	28	26	5.81	5q31.1	12	12	48	30	37	2.65
							14q24.1	4	2	12	9	5	1.51	6p25.1	10	14	48	13	39	2.05
							16q23.2	13	13	52	35	37	7.75	6q21	11	10	42	29	26	2.17
														7q32.3	5	4	18	17	14	1.22
														10q26	17	13	60	32	18	1.98
														14q24.1	6	5	22	14	10	0.95
														16q23.2	18	16	68	40	31	2.80
														18q21.3	7	7	28	12	14	1.03

MCL, number of males with CL; FCL, number of females with CL; TI, total individuals (%); CLm, number of CL in males; CLf, number of CL in females; TCL, total CL (%); TCL – $\frac{(CLm + CLf)}{\text{CL frequency in the whole sample per medium (see Results)}}$.

TABLE 4. Frequency of CL included as ARFS in Human Gene Mapping 10.5 (1990)

	R-1640					TC-199					R + MTX				
Bands	MCL	FCL	TI	CLm	CLf	TCL	Bands	MCL	FCL	TI	CLm	CLf	TCL	Bands	MCL	FCL	TI	CLm	CLf	TCL
2q22.3	1	—	2	11	—	2.96	2q22.3	1	—	2	20	—	2.15	2q22.3	1	1	4	31	7	1.50
							10q24.2	—	1	2	—	35	3.77	8q22.3	1	—	2	6	—	0.24
							11q23.3	1	—	2	18	—	1.94	9p21	—	1	2	—	17	0.67
							12q24.13	1	—	2	18	—	1.94	10q24.2	1	2	6	12	44	2.21
														11q23.3	1	—	2	22	—	0.87
														12q24.13	1	—	2	34	—	1.34

MCL, number of males with CL; FCL, number of females with CL; TI, total individuals (%); CLm, number of CL in males; CLf, number of CL in females; TCL, total CL (%); TCL = CL frequency in the whole sample per medium (see Results).

(CLm + CLf)

the 6p25 is significantly more expressed in females when methotrexate is added. Tedeschi et al. (1992) also found a sex influence on the expression of some FS.

Comparison with other populations

Most of the gaps and breaks have been located in bands considered to be ACFS. It is not easy to evaluate the present results in relation to other populations because the data about ACFS in healthy populations are scarce. The culture conditions in the study of Kähkönen (1988) and in this study are very similar (peripheral blood lymphocytes and TC 199). This fact could explain the similarities. The differences in frequencies with the Canadian population could be due not only to the culture medium and sample, but also to the fact that single chromatid breaks were not recorded by Chudley et al. (1990).

The ACFS in band 3p14 has been the most expressed site in the three culture mediums. According to Smeets and Merkx (1990), when the ACFS expression is induced by adding break-inducing agents (methotrexate and low levels of folic acid in our study), the relative expression of gaps and breaks at 3p14 decrease apparently in favor of the other less frequent ACFS. The data of Chudley et al. (1990) and Kähkönen (1988) show that in the Canadian and Finnish populations, the 3p14, 6q26, and 16q23 are the most commonly expressed ACFS in TC-199. In the same culture medium, 3p14, 15q31, and 16q23 are the most commonly expressed ACFS in the Basque.

Six folate-sensitive ARFS appeared in the Basque sample: 8q22.3, 9p21, 10q24.2, 11q23.3, and 12q24.13 under folate deprivation conditions and 2q22.3 not only under folate deprivation conditions but also under no deprivation conditions. Takahashi et al. (1988) found spontaneous expression of ARFS as well. From these ARFS, four have been significantly expressed in the folate-deficient culture mediums, (2q22.3, 10q24.2, 11q23.3, 12q24.13). The overall frequency among was 4/50 in TC-199 and 9/50 in R+MTX.

The difference between the Basque and other samples in ARFS can be due to study methods. In Sutherland (1982a, 1985), newborns were analyzed and additional inducing agents were not used. However, Chudley et al. (1990) utilized FUdR that should overcome the effects of higher folic acid levels in newborns; this could explain the higher

TABLE 5. Number of gaps and breaks in bands described as ACFC: Population studies

Population	Individuals analyzed	Cells analyzed	Gaps & breaks	Average G & b[1]	Reference
Canadian	790	37,288	2,320	0.06	Chudley et al. (1990)
Finnish	85	4,250	710	0.17	Kähkönen (1988)
Basque	50	5,150	788	0.15	Present study

[1]Average G & b indicates the average number of gaps and breaks per cell.

TABLE 6. Autosomal rare folate-sensitive fragile sites: Population studies

Population	Fragile sites	Incidence (%)	References
Australian	8q22,10q23,11q13,12q13 (2)	5/3458 (0.14)	Sutherland (1982, 1985)
Belgian	2q11 (6), 8q22, 10q23 (6)	13/200 (6.50)	Fryns and Petit (1987)
Canadian	2q11, 9p13 (2)	3/790 (0.38)	Chudley et al. (1990)
Finnish	9p21, 12q13	2/180 (1.11)	Kähkonen et al. (1989)
Japanese	2q11, 11q13 (2), 11q23, 17p12	5/1022 (0.49)	Takahashi et al. (1988)
Basque	2q22, 10q24, 11q23, 12q24	4/50 (8.00)	Present study

incidence in this study in relation to the observations of Sutherland (1982a, 1985). Fryns and Petit (1987) analyzed normal females from fra-X families. These females were at risk fra-X carriers, and as such they could have had a higher incidence of ARFS; this could explain the high frequency (6.50%). The studies of Takahashi et al. (1988) and Kähkönen et al. (1989) are the most comparable to the present study; however, there is a large difference in results. This could suggest that the differences among populations are not only due to different study methods.

There is no difference in the frequency of ACFS between the Basque and Finnish samples. However, there are differences in the chromosomal location of the ACFS and in the numbers of ACFS that are significantly expressed (13 in the Basques and 30 in the Finns). This suggests population differences.

According to Glover et al. (1994), any condition or agent that has an inhibitory effect on DNA replication or DNA repair via polymerase α will induce these fragile sites to some degree. For Laird et al. (1987), incomplete DNA replication and chromatin condensation caused by late replication may result in such fragility. But, it is not known at present whether these fragile sites are due to that alterations or other mechanisms. In any case, a genetic basis in ACFS expression, which could show differences among populations, may be possible.

The common fragile sites are probably without direct phenotypic effect. However, since these fragile sites have only recently been subjected to study, any definitive statement must await more data on frequencies in populations. Adequate data on the frequency of fragile sites in different abnormal groups and also in different ethnic groups are needed.

The fragile site at 11q23 is the only site of the four folate-sensitive ARFS that appears not only in the Basque but also in the Japanese (Takahashi et al., 1988). The incidence in the two populations is different: 1 in 50 vs. 1 in 1,022, respectively. This may reflect a true ethnic difference at this level. According to the classification of Hecht (1986), FS at 11q23 is a polymorphic fragile site in the Basques. Another polymorphic fragile site previously described is 10q25 in an Australian Caucasian population (Sutherland, 1982b). FS at 11q23 is present in about 1 in 50 and FS at 10q25 in 1 in 40, respectively. The frequencies may suggest that the two populations are deviant, as has been also demonstrated in relation to other genetic markers (Cavalli-Sforza and Piazza, 1993). Although the biological significance of these polymorphisms remains unknown, it would be interesting to know if the frequency of FS differs between populations.

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