Am. J. Hum. Genet. 50:76–83, 1992

Aphidicolin-inducible Common Fragile-Site Expression: Results from a Population Survey of Twins

M. J. F. Austin,* J. M. Collins,† L. A. Corey,* W. E. Nance,* M. C. Neale,* R. M. Schieken,‡ and J. A. Brown*

Departments of *Human Genetics, †Biochemistry and Molecular Biophysics, and ‡Pediatrics, Virginia Commonwealth University, Medical College of Virginia, Richmond

Summary

Common chromosomal fragile sites appear to be ubiquitous in humans and other mammals, and, although the molecular basis and function of these sites remain an enigma, it has been speculated that they may be a cytogenetic expression of gene activity. A population survey of 28 twin pairs was conducted to assess the heritability of common fragile-site expression. Our data yielded a heritability estimate of .88 for total site expression, suggesting that these sites may result from some common process that is under relatively stringent genetic control. An analysis of the expression of individual autosomal sites revealed that expression on both homologues in the same cell occurred more frequently than expected.

Introduction

Fragile sites that are present in less than 2.5% of the population are considered to be rare, while those present at higher frequencies have been designated as common sites (Berger et al. 1985). Both rare and common sites are expressed in culture under conditions which inhibit DNA synthesis (reviewed by Hecht et al. 1988) and have been subclassified on the basis of their mode of induction in culture (e.g., folate-sensitive, aphidicolin-inducible, BrdUrd-inducible, etc.). Rare sites have been observed only in the heterozygous state, whereas common sites are often expressed on both homologues in the same cell. Both Yunis et al. (1987) and Hecht (1988) have speculated that the aphidicolin-inducible common fragile sites may correspond with active gene regions.

Common sites have also been induced in the gorilla, chimpanzee, mouse, laboratory rat, and dog (Yunis and Soreng 1984; Djalai et al. 1987; Robinson and Elder 1987; Stone et al. 1988). Their evolutionary significance has been documented by studies showing that more than half of the breakpoints in primate chromosome evolution are at or near fragile sites (Miro et al. 1987) and that the occurrence of common sites within gene linkage groups appears to be conserved between mouse and man (Djalai et al. 1987).

Though normal variation in site expression has been addressed by some researchers (Craig-Holmes et al. 1987; Roa et al. 1988*a*, 1988*b*), sample sizes have not been adequate to reliably assess individual variation, and no effort has been made to determine the heritability of site expression. We therefore conducted a study of aphidicolin-induced fragile sites in twins, to assess both their individual variation and heritability. The use of identical and fraternal twins provides an opportunity to measure not only the contribution of genetic factors but the types (random and shared) of environmental effects that act on fragile-site expression.

Material and Methods

Heparinized blood samples were obtained from 15 MZ and 13 like-sexed DZ twin pairs. The twins ranged in age from 11 to 16 years, with a mean of 14 years for both groups. Blood from both members of a pair was processed on the same day. Three PHA-stimulated lymphocyte cultures were established from each individual by using 0.5 ml of whole blood in

Received February 26, 1991; revision received July 26, 1991.

Address for correspondence and reprints: M. J. Finley Austin, Box 230, MCV Station, Richmond, VA 23298.

0002-9297/92/5001-0009$02.00

RPMI-1640 medium supplemented with 10% FBS and 0.8% L-glutamine. After 70 h of culture (26 h prior to harvest) 0.2 μM aphidicolin was added to duplicate samples, to induce fragile-site expression. An equal volume of dimethylsulfoxide, the aphidicolin vehicle, was added to the third culture as a control. Standard cytogenetic techniques were used for harvests and slide preparation.

Fifty GTG-banded complete metaphase spreads were scored for all types of aberrations from each test culture and control from each twin. All chromosomal aberrations were recorded and localized according to International System for Human Cytogenetic Nomenclature (ISCN 1985). The occurrence of any type of aberration was counted as a single event at the band(s) involved.

Results

Descriptive Statistics

A preliminary comparison showed no differences in the frequency or location of previously reported fragile sites in the duplicate cultures, so the data were combined for subsequent analysis.

A total of 20,327 aberrations were recorded in 5,600 treated metaphases from the 28 twin pairs. If a haploid karyotype composed of 400 equally sized bands is assumed, an average of 25 aberrations would be expected in each band (20,327/800 = 25.4; 800 bands were used, since aberrations seen in homologous bands in a cell were counted as two events). If one assumes that the aberrations occurred according to a Poisson distribution, of the 272 bands where one or more aberrations were recorded, 54 were found to have an excess number of aberrations ($P < .01$, with more than 40 aberrations) (Steel and Torrie 1980). These 54 locations were designated fragile sites (table 1).

The 18,627 aberrations seen at these fragile sites accounted for 92% of the total. The site at 3p14.2 was expressed most frequently: 4,812 aberrations were observed, or 26% of the total. The next nine most frequent sites accounted for an additional 37%.

Forty-two of the 54 sites identified in the present study appear to be the same as those listed in the HGM 9 report (Sutherland and Mattei 1987) as aphidicolin-inducible common fragile sites. Twenty-four of the 42 previously reported sites are confirmed, while the remainder are provisional. Six of the confirmed sites were not identified as fragile sites in our sample. One region, 5q15, found in the present study to be induced by aphidicolin is reported as a common BrdUrd-inducible site with tentative status in HGM 9 (Sutherland and Mattei 1987). Eleven "new" sites were found in the present study (table 1). Several of these sites, however, have been detected by other laboratories with either aphidicolin induction or FUdR induction (Craig-Holmes et al. 1987; Hecht et al. 1988; Roa et al. 1988*b*).

A total of 101 aberrations were seen in the 2,800 control metaphases. Of these, 43 occurred at 24 of the 54 fragile sites (table 2). If spontaneous aberrations were to occur randomly, only 13 aberrations would be expected to have occurred at the 54 sites. This clustering of spontaneous aberrations is highly significant ($\chi^2 = 60.0$, df = 1, $P << .005$).

Genetic Analysis

For the genetic analysis, four summary statistics were calculated: the number of aberrations occurring at all of the fragile sites, as well as the 10, 5, and 3 most frequently expressed sites. These variables are referred to as Totfra, Top10, Top5 and Top3, respectively. All four variables were normally distributed within the study population and within the zygosity groups taken separately.

A simple additive genetic (h), random environmental (e), and common environmental (c) model was assumed in which the phenotype (P) would result from the additive effects of these three components of variance as shown in $\mathrm{Var_P} = h^2 + e^2 + c^2$, with covariances of MZ and DZ twin pairs predicted to be $\mathrm{Cov_{MZ}} = h^2 + c^2$ and $\mathrm{Cov_{DZ}} = \frac{1}{2}h^2 + c^2$, where h^2, e^2, and c^2 are the variance components reflecting the h, e, and c effects, respectively. We elected to omit dominance from the full model, because of the small number of twin pairs in our sample.

The model-fitting program LISREL VI (Jöreskog and Sörbom 1986) was used to fit the alternative models (Health et al. 1989). The LISREL VI program provides maximum likelihood estimates of the unknown parameters, as well as a χ^2 goodness-of-fit statistic for each model. Variance-covariance matrices were computed for the two zygosity groups, on Totfra, Top10 Top5, and Top3 and on the sites at 3p14.2, 16q23.2, and Xp22.31 taken individually (data available on request from M.J.F.A.). Four alternative models—(1) only e effects, (2) h and e effects (h,e), (3) c and e effects (c,e), and (4) the full model, i.e., h, c, e, effects (h,e,c)—were tested to determine which best fit the data.

Table 1

Band Locations Found to Contain Fragile Sites

Chromosome Band	No. of Aberrations Observed
1p36.2	58
1p32	321
1p31.2	266
1p22	550
1q25.1	59
1q44.1	386
2p24.2	177
2p16.2	250
2p15[a]	79
2q21.3	46
2q32.12	584
2q33	89
2q37.3	83
3p26[a]	64
3p24.2	190
3p14.2	4,812
3p14.1[a]	92
3q13.2[a]	145
3q26.2[a]	46
3q27	94
4p16.1	45
4q23[a]	68
4q31.1	144
5p14[a]	49
5q15	150
6p25	186
6q16[a]	85
6q21	41
6q22[a]	57
6q26	1,000
7p22	108
7p13	177
7q11.2[a]	52
7q21.2	84
7q31.2	550
7q32.3	552
8q22.1	134
9q32	167
10q22.1	167
10q26.1	100
11p15.1	62
11p14.2	190
11q14.2	295
12q21.3	48
13q13.2	219
13q34[a]	46
14q24.11	437
16q22.1	156
16q23.2	2,690
18q12.2	183
20p12.2	43
22q12.2	259
Xp22.31	1,114
Xq22.1	575
Total	18,627

[a] Fragile site detected in the present study and reported by others but that were not listed in HGM 9 (Sutherland and Mattei 1987).

On the basis of parameter estimates generated by the model fitting, the heritability (h_P^2) can be estimated as a proportion of the total variation, such that $h_P^2 = h^2/(h^2 + e^2 + c^2)$.

The results of the model fitting are given in table 3. For Totfra, Top10, and Top3, both the *h,e* and the *h,e,c* models gave a good fit to the data (the *P* values are nonsignificant; thus the models are not rejected by the data, whereas the significant *P* values for the purely environmental models indicate a poor fit). Comparison of the χ^2 values (reviewed by Neale et al. 1989) for the *h,e* and the *h,c,e* models, however, showed no significant improvement in the goodness of fit of the *h,e,c* model. The data therefore provide no evidence that common environmental effects have a significant influence on these variables. The parameter estimates derived from the *h,e* model yielded h_P^2 estimates of .88, .92, and .91 for Totfra, Top10, and Top3, respectively.

All of the models gave a poor fit to the data from Top5. However, the data deviated least from the expectations under the *h,e* model and the *h,e,c* model.

When the three most frequent sites were considered individually, the *h,e*, *c,e*, and *h,e,c* models all provided a good fit to the data. In the case of 16q23.2, a comparison of the χ^2 values indicated that the *h,e,c* model gave a significantly better fit to the data than did the *c,e* model.

To assess not only the goodness of fit of models but also the simplicity of the model that best represents the data, Akaike's information criterion (AIC) was used (Akaike 1970, 1987). This is calculated as $\chi^2 - 2(DF)$. A negative value of AIC is indicative of an improvement over a fully saturated model, and the

Table 2

Spontaneous Aberrations Occurring at Fragile Sites

Chromosome Band	No. of Aberrations Observed
1p32	1
1p31.2	2
2p24.2	3
2q21.3	2
3p24.2	2
3p14.2	4
3q13.2	1
4q23	1
4q31.1	1
5p14	1
6p25	2
6q16	1
6q26	4
7p13	1
7q21.2	1
7q32.3	1
8q22.1	1
10q26.13	1
11p14.2	2
12q21.32	1
13q13.2	2
22q12.2	3
Xp22.31	4
Xq22.1	1
Total	43

model with the lowest AIC is typically the preferred model. Application of AIC in this situation indicated that the *h,e* model provided the best explanation for the data from 16q23.2. Under this model, the heritability was estimated to be .84.

For both Xp22.31 and 3p14.2, a comparison of the AIC values of the three models under consideration indicated that the *h,e* model is preferred. From this, the H_P^2 values were estimated as .85 and .76, respectively.

Analysis of Homozygous Site Expression

A further analysis of the twin data was conducted to determine whether the expression of sites at homologous loci was independent. If site expression is equally likely to occur on either homologue, homozygous site expression would be a simple function of the probability of a site being expressed twice in the same cell, with the probability of site expression being derived from the total expression frequency.

The total number of cells analyzed would be comprised of the number of cells where a site is observed to express on both homologues (BB), plus the number of cells where a single expression is observed (Bb), and the remainder of cells where no expression of the site is observed (bb). Thus, total cells = BB + Bb + bb. According to the hypothesis of independent expression, the number of cells expected in each category (BB, Bb, and bb) would be $p^2 + 2pq + q^2$, where p is the probability of site expression, and $q = 1 - p$, the probability of no site expression. The value of p can be estimated $[2(BB) + Bb]/N$, where N is the total number of cells analyzed. If this model is correct, then the predicted numbers of BB, Bb, and bb cells are p^2, $2pq$, and q^2, respectively. The value of p was estimated for eight autosomal sites, and the expected numbers of cells were calculated for each category of site expression. A comparison of the expected and observed values revealed that, for all of the sites, the observed number of cells differed significantly from the expected number (table 4). Furthermore, the deviation from expected was always in the same direction: more cells than expected were observed to express sites on both homologues or not at all, and fewer cells than expected were observed to express sites on only one member of a homologous pair.

Discussion

In general, most of the fragile sites detected in the present survey had been reported elsewhere. Though six confirmed sites were not detected in the present study, several other laboratories have failed to detect many of these sites in their surveys of common fragile-site induction (Craig-Holmes et al. 1987; Green et al. 1988; Hecht et al. 1988). Some differences in the frequency and distribution of sites may reflect true population variation. Sample size clearly affects the ability to detect sites. In a survey of this magnitude, the actual existence of the sites detected is reasonably certain. Some sites, however, may have been masked or missed.

In agreement with previous reports, we found the site at 3p14.2 to be expressed most frequently, followed by 16q23.2. After these two sites, previously published reports vary as to the rank order of sites (Glover et al. 1984; Kuwano et al. 1988; Rao et al. 1988*a*, 1988*b*). Even though the rank order differs, the most frequently expressed sites in the present study (i.e., Xp22.31, 6q26, and 7q32.3) were also highly

Table 3

Results of Model Fitting

Variable and Model[a]	Goodness of Fit			Parameter Estimate[b]		
	χ^2	df	P	*h*	*e*	*c*
Totfra:						
e	29.5	5	.0	. . .	77.4	. . .
h,*e*[c]	6.7	4	.15	73.3 (88)	26.9 (12)	. . .
c,*e*	10.3	4	.04	. . .	40.8 (28)	65.9 (72)
h,*c*,*e*	5.7	3	.12	58.9 (55)	27.5 (12)	45.8 (33)
Top10:						
e	35.1	5	.0	. . .	46.8	. . .
h,*e*[c]	7.1	4	.13	45.8 (92)	13.2 (8)	. . .
c,*e*	15.4	4	.00	. . .	24.4 (27)	40.2 (73)
h,*c*,*e*	6.6	3	.08	39.8 (67)	13.3 (8)	24.4 (25)
Top5:						
e	52.4	5	.0	. . .	32.2	. . .
h,*e*	10.2	4	.04	31.4 (97)	5.9 (3)	. . .
c,*e*	23.4	4	.0	. . .	13.7 (18)	29.3 (82)
h,*c*,*e*	8.6	3	.03	24.7 (55)	5.9 (4)	21.3 (41)
Top3:						
e	35.7	5	.0	. . .	26.5	. . .
h,*e*[c]	6.8	4	.15	24.7 (91)	7.9 (9)	. . .
c,*e*	9.5	4	.05	. . .	11.9 (20)	23.6 (80)
h,*c*,*e*	4.9	3	.18	8.2 (10)	17.6 (43)	18.5 (47)
Fragile site at 3p14.2:						
e	19.3	5	.0	. . .	13.3	. . .
h,*e*	2.9	4	.58	11.2 (76)	6.4 (24)	. . .
c,*e*	3.8	4	.44	. . .	7.6 (33)	10.9 (67)
h,*c*,*e*	2.5	3	.48	8.6 (43)	7.4 (32)	5.7 (25)
Fragile site at 16q23.2:						
e	20.6	5	.0	. . .	14.1	. . .
h,*e*	.7	4	.96	12.9 (84)	5.5 (16)	. . .
c,*e*	4.4	4	.35	. . .	7.9 (32)	11.6 (68)
h,*c*,*e*	.4	3	.94	11.4 (60)	6.6 (20)	6.4 (20)
Fragile site at Xp22.31:						
e	27.3	5	.0	. . .	9.1	. . .
h,*e*	4.6	4	.33	8.2 (85)	3.4 (15)	. . .
c,*e*	4.7	4	.32	. . .	4.4 (24)	7.9 (76)
h,*c*,*e*	2.7	3	.43	5.9 (35)	3.5 (15)	6.4 (50)

[a] *e* = random environmental effects; *c* = common environmental effects; and *h* = additive genetic effects.

[b] Numbers in parentheses are proportion of variance.

[c] Most parsimonious explanation of data.

expressed in other studies. The general hierarchy of site expression appears, therefore, to be rather consistent between laboratories.

Hecht et al. (1988) reported that bands that displayed two or more spontaneous aberrations in untreated cells tended to correspond with highly expressed common fragile sites. Our findings in the control cultures corroborate their observations. The clustering of spontaneous aberrations at fragile sites may indicate that these regions are physiologically active at some point in S phase but that, in the absence of an exogenous block to DNA synthesis, aberrations typically either do not occur at these "expressed" regions or are not maintained into metaphase.

One of the 11 new sites detected in the present study was unequivocally localized to a dark G-band, and 6

Table 4

Comparison of Expected and Observed Homozygous and Heterozygous Site Expressions

Site	Expected (observed)			χ^2	df	P
	BB	Bb	bb			
3p14	1,034.15 (1,161)	2,744.70 (2,491)	1,821.15 (1,948)	47.85	1	<.005
16q23	323.53 (533)	2,044.96 (1,626)	3,231.52 (3,441)	235.05	1	<.005
6q26	45.54 (74)	918.92 (826)	4,635.54 (4,664)	21.49	1	<.005
2q32	15.54 (32)	558.92 (526)	5,025.54 (5,042)	19.43	1	<.005
7q32	13.60 (40)	524.79 (472)	5,061.60 (5,088)	56.67	1	<.005
7q31	13.60 (46)	524.79 (460)	5,061.60 (5,094)	85.37	1	<.005
1p22	13.60 (52)	524.79 (448)	5,061.60 (5,100)	119.91	1	<.005
14q24	8.53 (28)	419.95 (381)	5,171.53 (5,191)	48.17	1	<.005

were localized to light G-bands. Overall, our findings confirmed the clustering of aphidicolin-inducible sites in light G-bands, as reported by Hecht (1988).

Previously published reports (Glover et al. 1984, 1986; Kuwano et al. 1988; Rao et al. 1988*b*) suggest that there is substantial individual variation in the frequency of fragile-site expression. In a pilot study of unrelated individuals (data not shown), we observed significant individual variation in site expression, which prompted the present study.

Many conceivable factors could account for the individual differences in fragile sites. Individual differences could arise from genetic variation in the metabolism of aphidicolin as well as from polymorphic variation in the sensitivities of polymerases alpha and delta to the drug. Differences may also exist in individuals' abilities to repair induced sites. A threefold differences in UV-induced postreplication repair synthesis has been observed in normal subjects (Setlow 1983); mechanisms analogous to these may effect fragile sites. Last, the DNA sequence at site regions may influence induction. Polymorphic variation at the nucleotide level could contribute to the observed variation.

Model fitting of the data was performed to measure the contribution of genetic and environmental factors to the observed variation in fragile sites. The *h,e* models provided a good fit to the summed frequencies, and there was no significant improvement when *c* effects were included. The shared culture environment of the twins' cells did, however, appear to affect the observed correlations. Though the model fitting was not significantly influenced by this, it indicates that day effects should be considered when designing studies to compare expression frequencies between phenotypically divergent groups.

The methods used to determine the best model are not without limitations. The χ^2 is sensitive to sample size, and the AIC is a reflection of a comparison of the complexity of a model to the goodness of fit—it is not a probability of one model providing a better fit as compared with another.

If one keeps in mind the limitations of the model testing, it appears that the *h,e* model best explains the variation observed in total fragile-site expression. If one assumes that the *h,e* model is correct, then h_P^2 is estimated to be between .88 and .92 for summed site expression. This high h_P^2 value indicates that genetic factors are a major contributor to variation in total site expression. This is not inconceivable, since the processes of replication and repair which appear to be involved in the production of sites must, of necessity, be under stringent genetic control. If the DNA sequences at fragile sites influence expression frequency, this alone would contribute significantly to the h_P^2. The high h_P^2 value, however, does not mean that environmental factors cannot significantly alter variation in fragile-site expression.

For the pooled fragile-site frequencies, our data indicate a high degree of genetic determination, but, for individual sites, environmental effects assume a greater importance. These findings suggest that a general genetic factor may influence breaks at all sites. The effects of the genetic factor would be reinforced by summing individual break frequencies, in contrast to the expectation for either random environmental or independent genetic effects.

Our study provides further support for the sugges-

tion of Yunis et al. (1987) and Hecht (1988) that aphidicolin-inducible sites are preferentially found in chromosomal regions containing active genes. The "new" sites found in our study tended to occur in light G-bands, which are thought to correspond with active gene regions (Sumner 1981; Holmquist et al. 1982). Furthermore, on the basis of the replication patterns and gene activity properties of the X chromosomes, the observed pattern of site expression at Xp22.31 and Xq22.1 fits with what one would predict for the X chromosomes if sites do correspond to active genes. An active gene cluster, localized to Xp22.3, has been demonstrated on the inactivated X chromosome (reviewed by Mandel et al. 1989). Activity in this region would explain (1) why the site at Xp22 is expressed twice as often in females as in males, while Xq22 expression is the same in both sexes and (2) why homozygous expression at Xp22 is frequently seen in females, while it normally does not occur at Xq22 (Austin 1991).

The greater than expected homozygous expression of sites fits with a model that assumes that sites are induced at active gene regions. If a site was expressed or not expressed on one homologue, there was a tendency for that site to also be expressed or not expressed on the other homologue in the same cell. This strongly argues against independent site expression at homologous loci. Unless they are imprinted, both alleles of autosomal genes are assumed to express; if fragile-site expression is related to gene activity, greater homozygous expression would be expected. Heterozygous site expression may simply reflect incomplete repair of induced sites before the cell reaches metaphase.

The conservation of fragile sites across species, the similarity in modes of induction, the clustering of spontaneous aberrations at fragile sites, and the strong genetic influence on site expression observed in the present study suggest that fragile sites may reflect some biologic process related to gene activity and chromosome replication. On the basis of how and when the sites are induced to express as aberrations, they can be viewed as areas of the chromosomes that are particularly sensitive to the inhibition of DNA synthesis. The different classes of common fragile sites, classes that are based on the mode of induction, may reflect a common cellular process operating in divergent genomic regions.

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