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Heritable Fragile Sites on Human Chromosomes. VIII. Preliminary Population Cytogenetic Data on the Folic-Acid-Sensitive Fragile Sites

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SUMMARY

The incidence of the autosomal folic-acid-sensitive fragile sites in 524 institutionalized retardates (.0095) was found to be significantly higher than in 1,019 unselected neonates (.00098), suggesting that heterozygosity for these fragile sites may not be as harmless as previously thought. When one of the parents of an index case was found to carry the fragile site, that parent was always the mother. The fragile site at Xq27 was not found among the neonates studied, but was present in 1.6% of the institutionalized retarded males examined; if this fragile site occurs in normal males, then it does so rarely. Further cytogenetic studies of fragile sites are required on both normal and abnormal populations.

INTRODUCTION

There are now nine known folic-acid-sensitive fragile sites on the human karyotype [1, 2]. The frequencies of these in normal and abnormal populations are unknown. Chromosomal surveys of randomly selected neonates that have established the frequencies of other constitutional chromosomal anomalies have not provided data on fragile sites because they were mostly carried out using unsuitable culture medium and were based upon the examination of small numbers (usually two) of cells. The only such survey to record a fragile site [3] was a two-cell study that found one child with a fragile site on C group chromosome among 3,543 phenotypically normal infants studied. This was almost certainly an incomplete detection of the fragile sites in that population.

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Three groups within the South Australian population have been examined for folic-acid-sensitive fragile sites. These were unselected neonates, patients referred for diagnostic chromosome study, and institutionalized retardates.

MATERIALS AND METHODS

Neonates

Cord blood was collected by the nursing staff from the first 20 infants born after 8 P.M. Sunday each week in an obstetric hospital in which there were approximately 3,000 deliveries per year. As these criteria were not rigorously adhered to, some minor selection may have been exercised. Cultures were established in MEM-FA supplemented with 5% fetal bovine serum as described [4, 5], and harvested after 72 hrs using standard methods. Two unbanded cells were fully analyzed microscopically, and a further 48 examined for fragile sites.

Patients

Since late 1976, most patients referred for diagnostic chromosome study have had their lymphocytes cultured in medium suitable for the expression of fragile sites. Initially, this was medium 199, but more recently MEM-FA has been used. Venous blood was studied in the majority of cases, but in neonates and small children, capillary blood collected by heel or finger prick was used. Thirty unbanded metaphases per person were examined microscopically, a further two or three G-banded metaphases were also examined, and a banded karyotype prepared. In retarded males suspected clinically of having the fra(X)(q27), a further 20 metaphases were examined for fragile sites. The patients were not all retarded, but covered the spectrum seen in a general cytogenetics unit (e.g., retardates, couples with reproductive problems, and dysmorphic children). When families were studied, only the index case of any family is shown in table 1.

TABLE 1

FOLIC-ACID-SENSITIVE FRAGILE SITES IN THE GROUPS STUDIED

GROUP	NO. STUDIED	FRAGILE SITES	
		Xq27	Autosomal
Neonates:			
Males	522	0	0
Females	497	0	1
Patients	2,237	7	10
Minda Home:			
Males	298	5	1
Strathmont:			
Males IQ > 36	98	2	2
Other males	6	0	0
Females	35	0	0
Ru Rua:			
Males	42	0	2
Females	45	0	0

Retardates

All the male residents with otherwise normal chromosomes from Minda Home, a residential institution for the retarded, were studied by capillary blood culture in the first instance. If a fragile site was detected or suspected, then a repeat culture from venous blood was performed. All residents of Ru Rua, an institution for the totally dependent mentally retarded, with otherwise normal karyotypes, were studied from venous blood. Strathmont, a residential institution for the retarded that has been previously studied for major chromosome anomalies [6] was restudied in part. Initially, all males with an otherwise normal karyotype and I.Q.s greater than 36 were studied from venous blood; thereafter, both males and females with otherwise normal karyotypes who were venipunctured for other reasons were studied. Institutional residents' lymphocytes were cultured in MEM-FA, and at least 50 unbanded metaphases per person were examined for fragile sites.

RESULTS

The number of individuals in each of the populations studied and the fragile sites found are shown in table 1. The numbers in the patient and neonate groups are those in which the requisite number of metaphases could be scored and not the total number studied. The incidence of the autosomal fragile sites in the retardates is significantly greater than that in the neonates ($\chi^2_1 = 3.87$, $P < .05$) but not different from that in the patient group ($\chi^2_1 = 1.6$, not significant). Similarly, the difference between the retardate and patient groups is not significant ($\chi^2_1 = 1.19$, not significant).

Details of the 16 propositi with the autosomal folic-acid-sensitive fragile sites are shown in table 2. The most common fragile site seen in this group was that at 10q23, which accounted for seven ascertainments. All the other fragile sites detected have been documented previously except for the one at 9q31. This showed the typical behavior of a folic-acid-sensitive fragile site with regard to response to culture medium composition, although medium pH was not studied. Cytogenetically, the appearance was typical, showing chromosome and chromatid breaks at 9q31 and, more importantly for confirmation as a fragile site, triradial figures and deletions [2].

Family studies showed the mothers of the index cases to have the fragile site in all but two instances when it could not be detected in either parent. In these two instances, genetic marker studies showed a high probability of correct paternity. In a further two cases, family studies could not be carried out.

All the index cases with fra(X)(q27) were retarded males, with one exception in the patient group being a borderline retarded female. Family studies of a number of these have been reported [7]; in most cases, the fra(X)(q27) was found in other family members, although in three instances, extensive studies suggested that the index case may have been the result of a new mutation. One of the males with fra(X)(q27) also had fra(16)(q22), but family studies in this instance were not achieved (subject B [5]).

DISCUSSION

The detection of fragile sites is still not without difficulties; hence the data presented is for a minimum incidence of fragile sites. Even using the best-known

TABLE 2

DATA ON PROPOSITI WITH FOLIC-ACID-SENSITIVE AUTOSOMAL FRAGILE SITES

Patient	Group	Age	fra	Sex	Maximum frequency of lesions at site (%)	Parental origin	Maximum frequency of lesions at site in parent	Clinical status
559/80	Neonate	Newborn	10q23	F	7	Maternal	34	Normal neonate
797/79	Patient	8 yrs	2q1	M	56	Maternal	6	Benign ovarian teratoma
463/80	Patient	1 yr	10q23	M	14	Maternal	14	Retarded
852/77	Patient	7 yrs	16p12	M	82	Maternal	72	Family D [10], leukemia
485/80	Patient	2 yrs	12q13	F	16	Maternal	30	Epidermolysis bullosa [1]
302/80	Patient	12 yrs	10q23	F	28	Not done	...	Short stature
833/80	Patient	20 yrs	11q13	F	26	Maternal	12	Multiple miscarriages
169/80	Patient	2 yrs	10q23	M	40	Maternal	20	Failure to thrive, ? retarded
91/81	Patient	11 yrs	9q31	M	21	? Mutant	0	Gynecomastia
208/81	Patient	6 days	10q23	M	28	Maternal	2	Severe hypospadias
185/81*	Patient	14 yrs	10q23	F	24	Maternal	19	Mildly retarded
591/77	Minda	20 yrs	20p11	M	46	Not done	...	Retardate
360/72	Strathmont	12 yrs	2q1	M	78	Maternal	42	Family F [10], retardate
558/72	Strathmont	31 yrs	10q23	M	86	Maternal	45	Family Ay [10], retardate
505/73	Ru Rua	6 yrs	20p11	M	66	Maternal	2	Family Mi [10], retardate
587/73	Ru Rua	19 yrs	11q13	M	36	? Mutant	0	Totally dependent retardate

* Also has the BrdU-requiring fragile site at 10q25 on the same chromosome.

conditions for lymphocyte culture, the site at Xq27 is not detectable in some females who are obligate carriers of it and is seen in a very low percentage of metaphases in some males [7]. The section of this work carried out using capillary blood is probably less reliable than that carried out using venous blood. One of the males in Minda Home had the fra(X)(q27) in only 2% of cells from capillary blood but in 10% from venous blood. A possible reason for this difference is the observation that microbial contamination of lymphocyte cultures renders them useless for fragile-site detection, even if adequate metaphases are present to permit karyotyping; such low-grade contamination is more common in capillary blood than in venous blood cultures.

Demonstration of the autosomal fragile sites can also be a problem in some families, and presumably in some individuals. Table 2 shows that the fragile site was detected in less than 5% of metaphases in some family members. Two of the autosomal fragile sites detected seemed to be new mutants in that they were not detected in either of the parents of the index cases. However, a better explanation may be that these apparent new mutants were familial cases but that the fragile sites were not detected in the carrier parents; it is not possible to be certain about this. If a new mutant fragile site is harmless, then it is most unlikely that two such rare events would be detected in this small series. There is often morbidity associated with new mutant translocations [8]; perhaps the same could hold for new mutant fragile sites, in which case their detection in abnormal individuals would not be unexpected. Three of the retarded males with fra(X)(q27) appear to be new mutants; indeed, if the reproductive fitness of these males is zero (and the mutation rates in the two sexes are equal), then one-third of new cases would be expected to be mutants [9].

The finding of a higher incidence of autosomal fragile sites in retardates than in neonates was unexpected since it had been thought that heterozygosity for the autosomal fragile sites was without phenotypic effect [10]. The findings from the present study require close examination. The significance may be due to chance or possibly to bias. Only one fragile site was found in the neonates; if one more had been found in this group, then the significance would disappear. Since there may have been some minor selection in the neonates studied, it could be argued that if heterozygosity for the fragile sites was deleterious then such infants may not have been studied. This is possible but unlikely as all infants born in the hospital during the period of the survey who had clinical indications for chromosome studies were scored for fragile sites and none were found. Further bias comes from the fact that some of the retardates were known to have fragile sites prior to the survey; indeed, it was the availability of such individuals that first stimulated the author's interest in fragile sites some years ago. The possibility that expression of fragile sites in cord blood lymphocytes is different from that in lymphocytes of older individuals could also account for these results. There is no evidence available on this point; however, there is usually no special difficulty in demonstrating fragile sites in young children; indeed, the fragile site at Xq27 becomes increasingly difficult to detect with advancing age in females [7]. This work needs to be repeated on other

retarded populations, a more rigorously selected group of neonates, and a group of age-matched controls for the retardates.

If the difference in incidence of fragile sites in retardates and normal individuals is confirmed, the mechanism of the effect is worth considering. Williams and Howell [11] suggested that breakage of the fragile sites at critical stages of development could lead to monosomic cell lines that might persist and have a deleterious effect either genetically or through poor viability. In individuals with fragile sites, 5%–10% of metaphases expressing the fragile site are aneuploid as a result of breakage at this site. If this breakage occurs in vivo, even to a lesser extent, it could explain why some but not all carriers of fragile sites are phenotypically abnormal and why there is no consistent pattern to the abnormalities.

In all instances in which family studies yielded a parent of an index case with the autosomal fragile site, this parent was the mother. While the probability of this occurring by chance is small, it is difficult to attribute any biological significance to it since males readily transmit fragile sites. Could it be that if heterozygosity for the autosomal recessive fragile sites can be deleterious then this deleterious effect is more likely to occur if the fragile site is inherited from the mother?

In the retarded populations in Minda and Strathmont, 1.7% of males had the fra(X)(q27). In these institutions, about 12%–15% of the males had Down syndrome [6, 12]. Most of the males with fra(X)(q27) function at about the same intellectual level as Down syndrome patients. Hence, if the calculation of Herbst and Miller [13] of an incidence of fra(X)(q27) in males of .92 per 1,000 is correct, then almost the same number of fra(X)(q27) males as Down syndrome males would have been expected, especially since there is no evidence of the increased infant mortality in fra(X)(q27) males that is seen in Down syndrome. Even with the limitations of technique in detecting fra(X)(q27), there is no doubt that in the institutions studied there are many more Down syndrome individuals than individuals with fra(X)(q27). It would appear that the estimate of Herbst and Miller [13] may be a very considerable overestimate. Not enough retarded females have been studied to allow comment upon the finding [14] that 7% of mildly retarded females without physical stigmata have the fra(X)(q27).

This preliminary study of the population cytogenetics of fragile sites has asked more questions than it has answered. The incidence of the folic-acid-sensitive fragile sites in the general population remains unknown. The possibility that these fragile sites might be deleterious in some heterozygotes, particularly if inherited maternally, has been raised. The fragile site at Xq27 was not detected among the neonates studied; however, all that can be concluded from this is that it is much less common in such a population than it is in retarded populations and that if it does occur in normal males [15], then it does so rarely. The fra(X)(q27) was recognized in about 2% of institutionalized retarded males with otherwise normal karyotypes. It would seem that its incidence is considerably less than that estimated by Herbst and Miller of .92/1,000 males [13]. Further population cytogenetic studies of normal and retarded populations are required to help elucidate the biological significance of fragile sites.

ADDENDUM

Further studies on the family of 587/73 (table 2) have indicated that the subject's fragile site is familial since it was found in his siblings; hence, it was undetected in an obligate carrier parent. Another male with fra(X)(q27) was identified in Minda Home (total now six) after his mildly retarded sister was independently ascertained to have the fragile X. This male had been studied by capillary blood culture when the institution was surveyed but was not identified at that time.

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