The inheritance of fragile sites: apparent absence of fra(2)(q13) in the parents of three unrelated probands

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SUMMARY We describe the inherited folate sensitive fragile site, fra(2)(q13), in three unrelated mentally retarded children, two of them with different forms of epilepsy. Fra(2)(q13) was detected in one healthy sib of one of the probands. Except for one cell in one of the fathers fra(2)(q13) could not be detected in any of the six parents, who were repeatedly studied using methods known to induce fragile sites of this type. These findings suggest that fra(2)(q13) is not associated with the clinical features of our patients and can be transmitted by persons not expressing it. The expression of fra(2)(q13) may be age dependent.

A fragile site is a non-staining gap which is always in the same place in the chromosome. The gap is of variable width and usually involves both chromatids.¹⁻³ Fragile sites are classified into four main categories^{4,5}: folate sensitive, distamycin A inducible, BrdU inducible, and common fragile sites. Unlike the others, the latter are not necessarily heritable. The heritable fragile sites have a tendency to produce acentric chromosome fragments, triradial figures, and chromosome deletions at the site of the fragility.¹

Like other chromosomal polymorphisms the heritable fragile sites are transmitted in a dominant fashion.² Half of the offspring of a carrier will receive the chromosome with the fragile site. New mutations have not been convincingly documented.² In other words, whenever a fragile site is observed in a subject, one parent is also found to have it if adequately studied. Exceptions to this rule are discussed below. This report describes three probands with fra(2)(q13) in whose parents the fragile site was not demonstrable (except for one cell in one father). A sib of one of the probands had the fragile site, indicating that in at least one of the families it must have been transmitted by a parent not expressing it. We discuss these findings with special reference to the clinical features that prompted the investigation of the probands.

Received for publication 19 November 1985.
Revised version accepted for publication 22 September 1986.

Methods

Chromosome analyses were performed on mitoses obtained from lymphocyte cultures (0.5 ml whole blood in 8 ml medium). Four different types of culture media and methods were used, as follows: (1) Medium 199 (IX) with 5% fetal calf serum (Gibco, USA). (2) Medium F10-FA (Gibco) with 5% fetal calf serum. These cultures were incubated for 96 hours. The two other methods used RPMI-1640 (Gibco) with 20% fetal calf serum incubated for 72 hours, after which (3) methotrexate (MTX, Lederle, USA, 1 mg per culture) or (4) 5-fluoro-2-deoxyuridine (FudR, Sigma, USA, 1 µg per culture) was added and the incubation continued for 24 hours. All media contained 50 U/ml penicillin, 50 µg/ml streptomycin, 3.75 µg/ml L-glutamine (Gibco), and 3.75 µg/ml leucoagglutinin (Pharmacia, Finland). Colemid (Gibco), at a concentration of 0.125 µg/ml, was present for the last hour of incubation. The cells were treated with hypotonic solution, fixed, and stained using conventional methods. Trypsin was used to obtain G banding.

Patients

Proband 1 is a 12 year old girl with mild mental retardation and epilepsy. She is the oldest of three children. Her parents and two younger sisters are healthy. A paternal aunt has grand mal epilepsy.

The pregnancy was uncomplicated. She was a term baby weighing 3560 g with an Apgar score of 10 in spite of prolonged labour. Her developmental milestones were delayed from the beginning. She walked without support at the age of two years and at the

age of 12 her developmental age was 10 years. She has no dysmorphic features. She began to have grand mal seizures at the age of five and a half years. The EEG showed a focal disturbance in the left parietal area together with general slowing. A later

TABLE 1 Cytogenetic findings.

	Year of birth	Month and year of study	Medium	No of cells analysed	Cells with fra(2)(q13)		No of cells with other fragile sites and gaps
					No	%	
Family 1							
Proband	1974	3/84	199	60	11	18	14
		9/84	F10-FA	61	2	3	1
		3/85	199	60	15	25	9
		2/86	199	60	9	15	8
		2/86	RPMI+FUdR	61	14	23	10
		2/86	RPMI+MTX	60	26	43	20
Mother	1956	7/84	199	30	0	0	1
		9/84	F10-FA	54	0	0	1
		3/85	199	151	0	0	48
		2/86	199	57	0	0	1
		2/86	RPMI+FUdR	60	0	0	26
		2/86	RPMI+MTX	60	0	0	28
Father	1951	7/84	199	60	0	0	0
		9/84	F10-FA	60	0	0	6
		3/85	199	153	0	0	38
		2/86	199	60	0	0	9
		2/86	RPMI+FUdR	60	0	0	18
		2/86	RPMI+MTX	61	0	0	16
Sister	1981	3/85	199	64	0	0	6
		2/86	199	60	0	0	5
		2/86	RPMI+FUdR	60	0	0	8
		2/86	RPMI+MTX	52	0	0	10
Sister	1984	3/85	199	61	12	20	10
		2/86	199	10	2	20	1
		2/86	RPMI+FUdR	60	19	32	11
		2/86	RPMI+MTX	60	10	17	17
Family 2							
Proband	1977	7/84	199	52	4	7	6
		9/84	F10-FA	60	8	13	11
		1/85	F10-FA	60	1	2	4
		5/86	199	60	2	3	2
		5/86	RPMI+FUdR	60	5	8	6
		5/86	RPMI+MTX	61	7	11	8
Mother	1951	9/84	F10-FA	62	0	0	18
		1/85	F10-FA	82	0	0	5
		5/86	199	60	0	0	11
		5/86	RPMI+FUdR	60	0	0	19
		5/86	RPMI+MTX	60	0	0	18
		5/86	RPMI+MTX	60	0	0	18
Father	1952	9/84	F10-FA	60	0	0	18
		1/85	F10-FA	60	0	0	9
		5/86	199	14	0	0	1
		5/86	RPMI+FUdR	60	0	0	7
		5/86	RPMI+MTX	60	1	2	6
		5/86	RPMI+MTX	60	1	2	6
Family 3							
Proband	1980	5/85	199	25	7	28	6
		2/86	199	17	6	35	0
		2/86	RPMI+FUdR	60	6	10	7
		2/86	RPMI+MTX	48	6	13	10
Mother	1952	7/85	199	60	0	0	18
		2/86	199	17	0	0	1
		2/86	RPMI+FUdR	36	0	0	13
		2/86	RPMI+MTX	17	0	0	6
Father	1945	7/85	199	60	0	0	14
		2/86	199	60	0	0	9
		2/86	RPMI+FUdR	10	0	0	3
		2/86	RPMI+MTX	42	0	0	10

FIG 1 Chromosomes 2 from four cells from (top line) proband 1 (G banding); (second line) younger sister of proband 1 (unbanded); (third line) proband 2 (G banding); (bottom line) proband 3 (unbanded). A *fra*(2)(q13) can be seen in the left hand homologue of each pair.

EEG showed only a general disturbance. Slightly enlarged lateral ventricles without focal lesions were seen in a CT scan of the brain. She was first treated with carbamazepine, but after eczema appeared the medication was changed to phenytoin. Because of urinary infections she has also been treated with trimethoprim and nitrofurantoin.

Proband 2 is a nine year old boy with Lennox syndrome. He is the only child of non-consanguineous, healthy parents. There are no relatives with epilepsy or mental retardation. He was born at term after an uncomplicated pregnancy. He weighed 3350 g and Apgar score was 8. His early development was considered normal, although he learned to walk at the age of 15 months. During the first year he occasionally became cyanotic after vigorous crying. He began to have drop attacks at the age of two and a half years. The EEG showed a petit mal variant disturbance. A CT scan of the brain was normal. He continues to have daily seizures and is moderately mentally retarded. Trials with valproic acid, ethosuximide, clonazepam, and carbamazepine have been unsuccessful in controlling the seizures.

Proband 3 is a six year old boy with mild psychomotor retardation. He is the only child of non-consanguineous, healthy parents. During the last two months of the pregnancy the mother took nitrofurantoin because of a urinary infection. The proband was born at 41 weeks' gestation weighing 3330 g. Cephalhaematoma developed during delivery. At the age of one and a half years he was noticed to fall often and at two he could not say any words. At the age of four and a half years his speech development was considered to be delayed by one year. The EEG showed slight instability but he has had no seizures.

Results

The cytogenetic findings are summarised in table 1. The year of birth of each subject is also shown.

Examples of the fragile site are shown in fig 1. As can be seen, the probands had varying proportions of cells positive for *fra(2)(q13)* in every culture performed. While proband 2 appeared to have lower proportions of *fra(2)* positive mitoses (2 to 13%) than probands 1 and 3 (3 to 43%), there was no obvious consistency regarding which type of medium or method produced the highest or lowest proportion of positive cells. The younger sister of proband 1 had 17 to 32% cells positive for the fragile site. However, all six parents were consistently negative, except for one metaphase in the father of proband 2 (see below). The older sister of proband 1 was also negative. Table 1 shows the number of

FIG 2 The only cell from the father of proband 2 showing *fra(2)(q13)* (arrow).

TABLE 2 Blood group findings in families 1 and 2.

	ABO	MN	Ss	Rh	K	Fy ^a	Fy ^b	Xg ^a	I2E7	Co ^b	P _I	Lu ^a	Jk ^a	Jk ^b
Family 1														
Proband	O	MN	++	R ₁ R ₁	—	—	+	—	—	—	—	—	—	+
Mother	A	M	++	R ₁ r	—	—	+	—	—	—	—	—	+	+
Father	B	MN	++	R ₁ R ₂	—	+	+	—	+	—	—	—	—	+
Sister 1	AB	MN	++	R ₁ R ₁	—	+	+	+	—	—	—	—	—	+
Sister 2	A ₁	M	++	R ₁ r	—	—	+	—	—	—	—	—	—	+
Family 2														
Proband	A ₁			R ₁ r	—			+		—			+	+
Mother	A ₁			R ₁ R ₁	—			+		—			+	+
Father	O			R ₂ r	—			+		—			—	+

mitoses in which other fragile sites and gaps occurred in these cultures. In as much as these figures reflect the levels at which fragile sites in general were induced in the cultures, it appears that the cultures were appropriate in this respect. Banding showed that in each of the three probands the fragile site was in 2q13 rather than in 2q11 (fig 1).

One metaphase from the father of proband 2 deserves special comment. In the methotrexate culture from this subject, one metaphase did show a fra(2)(q13). Even though the chromosomes were not banded, and even though the fragility did not necessarily appear to involve both chromatids (fig 2), our interpretation is that this represents fra(2)(q13).

Blood group studies (table 2) were compatible with paternity in families 1 and 2. In family 3 blood group studies were not performed but the size of the Y chromosome was the same in father and son, which is compatible with paternity.

Discussion

The indication for chromosome study using folic acid deficient medium was mental retardation in all three patients. The results initially raised the question of whether the observed de novo fragile site might be aetiologically related to the clinical abnormalities. Lejeune *et al*⁶ described a mother and daughter, both with borderline intelligence, with an unstable secondary constriction in the long arm of chromosome 2. Ferguson-Smith⁷ described a family with fragility of chromosome 2 in three generations. The fra(2) was found in three subjects with hyperlipoproteinaemia and in two normal subjects. None of the persons was mentally retarded. Later Williams and Howell⁸ described a mentally retarded patient with a secondary constriction in the proximal part of the long arm of chromosome 2 that might have been a fra(2)(q13). No fragility was found in either parent or a sister of the patient. Annerén and Gustavson⁹ also described a fragile site in the proximal part of the long arm of chromosome 2 in four probands with different clinical features: a boy with cardiomyopathy, a girl with malformations of the central nervous system (neither set of parents had abnormalities of chromosome 2), one mentally retarded boy (whose father had no fra(2); the mother was not examined), and a mentally retarded girl whose healthy mother had fra(2q).

Two different fragile sites very close to each other occur in chromosome 2, fra(2)(q11) and fra(2)(q13). Both are rare, folate sensitive fragile sites according to the classification of Sutherland and Hecht.¹⁰ In the papers quoted above,⁶⁻⁹ the precise localisation of the fragile site was not stated, so it is not known

which of the two areas was involved in each case. Gilgenkrantz *et al*¹¹ identified a fra(2)(q11) in a girl with familial lymphohistiocytosis. The fragile site of our probands was in 2q13, clearly more distal than the 2q11 fragile site described by Gilgenkrantz *et al*.¹¹

The fact that one healthy sister of our proband showed fra(2)(q13) suggests two interpretations. Firstly, one of the parents must have transmitted the fragile site to both children. This indicates that persons not expressing the fra(2)(q13) can transmit it and raises the possibility that the expression of fra(2)(q13) is age dependent. In this respect, the fra(2)(q13) behaves in a fashion similar to fra(X)(q27).¹² If this hypothesis could be confirmed by the study of more families with fra(2)(q13) and perhaps other autosomal fragile sites, it would help remove uncertainties as to the regular inheritance of these chromosomes. Secondly, our findings suggest that the fra(2)(q13) is unrelated to the developmental disorders of our probands. This hypothesis could be tested by clinical and cytogenetic studies of other families in which fra(2)(q13) segregates. To the best of our knowledge an association between abnormal clinical features and autosomal fragile sites has not yet been proven, even though it has been discussed.^{2, 13}

We thank Dr Patricia Tippett of the MRC Blood Group Unit, London, for the blood group testing. This study was supported by grants from the Sigrid Jusélius Foundation, the Finska Läkarsällskapet and the Academy of Finland. Part of the study was performed at the Folkhälsan Institute of Genetics.

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