Fragile X trait in a large kindred: transmission also through normal males

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SUMMARY In the pedigree reported here two apparently normal males may have transmitted the fragile X chromosome. Eighteen family members were examined cytogenetically. The fragile X was detected in a high percentage of cells from nine mentally retarded members of this family (six males and three females) and in one female obligate carrier. Four other obligate carriers showed no or only a few cells with the fragile X.

Mental retardation has been considered to be a constant feature in males with the fragile X¹ whereas variable phenotypic expression has been recognised in heterozygous females.² Recently, it has been shown that the degree of mental retardation may also vary considerably in male patients.³ This point is illustrated further by the finding of the fragile X in two normal brothers⁴ and by reports of pedigrees with transmission of the fragile X through apparently normal males.^{5–9}

In this paper a large pedigree is reported in which several patients with the fragile X syndrome are related to one another through their maternal male ancestors. In addition, the cytogenetic results obtained in obligate female carriers in this pedigree may clarify the correlation between the age and clinical features of the donor, with the probability of detecting the fragile X in vitro.

Material and methods

In 1976 the parents of a child with Down's syndrome were referred for genetic counselling. This initiated the study of a family with several mentally retarded persons of both sexes.

All but one of the retarded persons were examined clinically and photographed after parental or personal consent or both was obtained. Whenever possible or relevant, testicular volume was assessed by the use of a Prader type orchidometer. Volumes of 25 ml or more were considered enlarged. Stature,

head circumference, and length of the right ear were measured. However, because data on ear size in age-matched normal people are unavailable, and the degree of prominence of the ears can hardly be assessed anthropometrically, subjective description of size and degree of prominence has been used.

Data on circumstances of birth, psychomotor development, and schooling progress were obtained by interviewing parents or other informed relatives. Results of formal psychometric testing were not available because all but an elderly institutionalised patient have always lived at home.

Chromosomes were obtained from phytohaemagglutinin stimulated whole blood cultures. Cells were cultured for 4 or 5 days in Hepes buffered medium 199, supplemented with 2% fetal calf serum and 1% penicillin-streptomycin. Trypsin-Giemsa and carbol-fuchsin stained metaphases were studied. Between 50 and 250 metaphases were examined in the males and up to 550 in the females.

Results

The family pedigree is shown in the figure. In tables 1 and 2 results of cytogenetic or clinical investigation or both in males and females are listed. The fragile X was found in 16 to 37% of cells in the six male patients, in 18 to 42% in the three female patients, and in 0.5 to 26% in some of their normal or nearly normal female relatives. Macro-orchidism was found in two adult males who were examined clinically but not in one elderly patient (III.11). In the two children (V.6 and V.7) testicular size was normal for age.

Since members of the first and second generation

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FIGURE Pedigree of family.

in this kindred died several decades ago, data on them consist of family photographs and of information obtained from surviving relatives.

I.1 and I.2, the ancestral couple, were normal, the husband being a bricklayer. Like his father, II.1 started out as a bricklayer but later became an independent building contractor. He did his own book-keeping. Throughout his life, and even after retirement, he was known as an avid reader of books. His wife was mentally and physically normal. This couple have two retarded grandchildren (IV.1 and IV.3) who live at home. They were found to have the fragile X chromosome. Their mother (III.6), examined clinically shortly before her death, was not mentally handicapped and their father (III.5) was normal.

II.2, the only sister in the second generation who has survived into adulthood, was normal and so was her husband. Among five children she had one

TABLE 2 Clinical and cytogenetic findings in the females.

Subject	Age (yr)	No of fra(X)/No of metaphases	%fra(X)	Remarks
Mentally retarded carriers				
IV.3	31	21/50	42	Moderate mental retardation
IV.7	35	17/50	34	Moderate mental retardation
IV.13	32	18/100	18	Moderate mental retardation
Obligate carriers				
III.25	78	0/60	0	
III.28	73	0/150	0	
III.30	65	2/300	0.7	
IV.9	39	13/50	26	Borderline IQ
V.4	19	2/185	1.1	
Potential carriers				
IV.2	35	0/100	0	A priori carrier risk 0.5
IV.12	33	3/550	0.5	A priori carrier risk 0.5
IV.6	41	0/100	0	A priori risk dependent on unknown population frequency of fra(X)

TABLE 1 Clinical and cytogenetic findings in the males.

	III.11	IV.1	IV.5	IV.10	V.6	V.7
Age (yr)	79	47	48	36	61	21
Height (cm)	169	174	170	189	75†	*
Head circumference (cm)	57	57	*	59	50†	50-75‡
Large and/or prominent ears	+	+	+	+	+	+
Macro-orchidism	-	+	*	+	-	-
Mental retardation	Severe	Moderate	Mild	Moderate	†	†
No of fra(X)/No of metaphases	12/50	8/50	12/50	25/100	82/250	37/100
% fra(X)	24	16	24	25	33	37

*Not measured.

†Delayed psychomotor development.

‡Centile instead of absolute measurement in growing children.

mentally retarded son (III.11) who lives in an institution for the retarded and is, at the age of 79, the oldest fragile X patient in this pedigree.

II.3, a bricklayer, and II.4, a carpenter, are said to have been normal with normal offspring. II.5 was a bricklayer too. Together with his brother (II.4) he established a family business which he himself represented in obtaining new contracts. His wife was normal and unrelated to the wife of his oldest brother. The children of II.5 are of normal intelligence. Among his grandchildren and great-grandchildren there are two adult males, two adult females, and two young boys with mental or developmental retardation.

The oldest of the males (IV.5) could not have an adequate clinical examination for social and psychological reasons. He is employed in a family business. He is married to a mentally retarded wife, who does not show the fragile X chromosome, and has a clinically normal daughter (IV.4) and son. The other adult male (IV.10) and the two females (IV.7 and IV.13) are employed in a workshop for the mentally handicapped. Psychomotor development of the children (V.6 and V.7) is delayed (see table 1).

All living female obligate carriers in this pedigree were examined cytogenetically. Four of them are of normal intelligence. In two of them (III.25 and III.28) no fragile X could be demonstrated. In the other two heterozygotes (III.30 and V.4) the frequency of the fragile X amounted to only 0.7% and 1.1%, respectively. The remaining female obligate heterozygote (IV.9) is of borderline normal intelligence and shows the fragile X in 26% of metaphases.

Two clinically normal presumptive female carriers were included in the study. One of them (IV.2) showed no fragile X while in the other (IV.12), the mother of the proband with Down's syndrome, three cells with the fragile X were found out of a total of 550 examined (see table 2).

Discussion

In the pedigree reported here two normal males (II.1 and II.5) may have transmitted the fragile X through their daughters to affected grandchildren and great-grandchildren. Definite proof of this hypothesis cannot be obtained but there are strong arguments in its favour. The wives of II.1 and II.5 were unrelated to each other. Their family histories are unremarkable with regard to mental retardation. Also a sister (II.2) in the second generation had a son, still surviving, with the fragile X syndrome. It is plausible to assume that subjects II.1, II.2, and II.5 had received the fragile X from their mother, the family history of whom, unfortunately, is not known.

Although probably hemizygous for the fragile X trait, males II.1 and II.5 have always been considered to be normal. Even after making allowance for the fact that in the less affluent and less educated society of several decades ago mild mental handicap may have remained undetected, it is clear that the mental ability in the ancestral generation largely surpassed that of the present day patients. This finding documents further the existence of a large range of intellectual capacity in males with the fragile X and confirms previous reports.^{3,4}

The important practical implication of these results is that normal male relatives of fragile X patients ought to be included in the cytogenetic study of families with possible X linked mental retardation. The X linked pattern of inheritance of the fragile X is illustrated by the pedigree in the figure. No male-to-male transmission was observed. None of the sons of II.1, II.5, and IV.5 was or is retarded, but all the daughters of these subjects should be carriers. Five of these seven females have reproduced. The finding of retarded offspring with the fragile X confirms the heterozygous genotype in all but one. The latter is said to have normal offspring but more detailed information is not available. As shown in table 2, in some instances it has been difficult to demonstrate the fragile X chromosome *in vitro*. The finding of the fragile X in only 1.1% of the metaphases from a 19-year-old, clinically normal, obligate female carrier (V.4) does not favour the hypothesis that the *in vitro* phenomenon may not be demonstrable in the lymphocytes of elderly females because of an age related factor.¹⁰ On the contrary, the cytogenetic data obtained from the females in this pedigree and those in other pedigrees published elsewhere,¹¹ confirm the existence of a positive correlation between mental deficiency and 'in vitro' expression of the fragile X.

This correlation has been observed by others.¹² If further documented, the latter conclusion has important implications for genetic counselling in mentally normal females who may be carriers. Failure to demonstrate the fragile X does not exclude the heterozygous state and even if the frequencies of positive cells are below the background level established in each cytogenetics laboratory, their presence should be interpreted as confirmatory of the carrier state, especially in females belonging to fragile X families.

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