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Unaffected Carrier Males in Families with Fragile X Syndrome

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SUMMARY

Males who transmit the fragile X chromosome but are themselves clinically normal have occasionally been observed. We have studied three families segregating the fragile X. In one family, there are three unaffected carrier males, and in each of the other two families, there is one unaffected carrier male. Three of these carrier males were studied cytogenetically, and none exhibited the fra(X)(q27) marker. The occurrence of carrier males and of other unusual genetic features in fragile X families suggest that this condition is not inherited as a standard recessive trait linked to the X chromosome.

INTRODUCTION

The fragile X syndrome is a relatively common form of mental retardation characterized by familial occurrence and predisposition for males. Affected individuals exhibit an inducible cytogenetic marker: the fra(X)(q27). Physical anomalies in persons with fragile X syndrome are mild and variable but may include megalotestes, long face with prominent ears, and generalized hyperextensibility of the joints [1].

Fragile X syndrome is considered to be an X-linked recessive disorder [2]. However, males who transmit the condition but are themselves clinically normal were observed in the first reported family with fragile X [3, 4] and have since been noted in other families [5–13]. We report here three additional families in which a total of five unaffected obligate carrier males were ob-

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served. Extensive cytogenetic evaluation of three of these carrier males in our laboratory revealed all to lack expression of the fragile site. Our data, when taken with other genetic features of the fragile X syndrome, suggest that this condition is not transmitted by a standard recessive mechanism linked to the X chromosome.

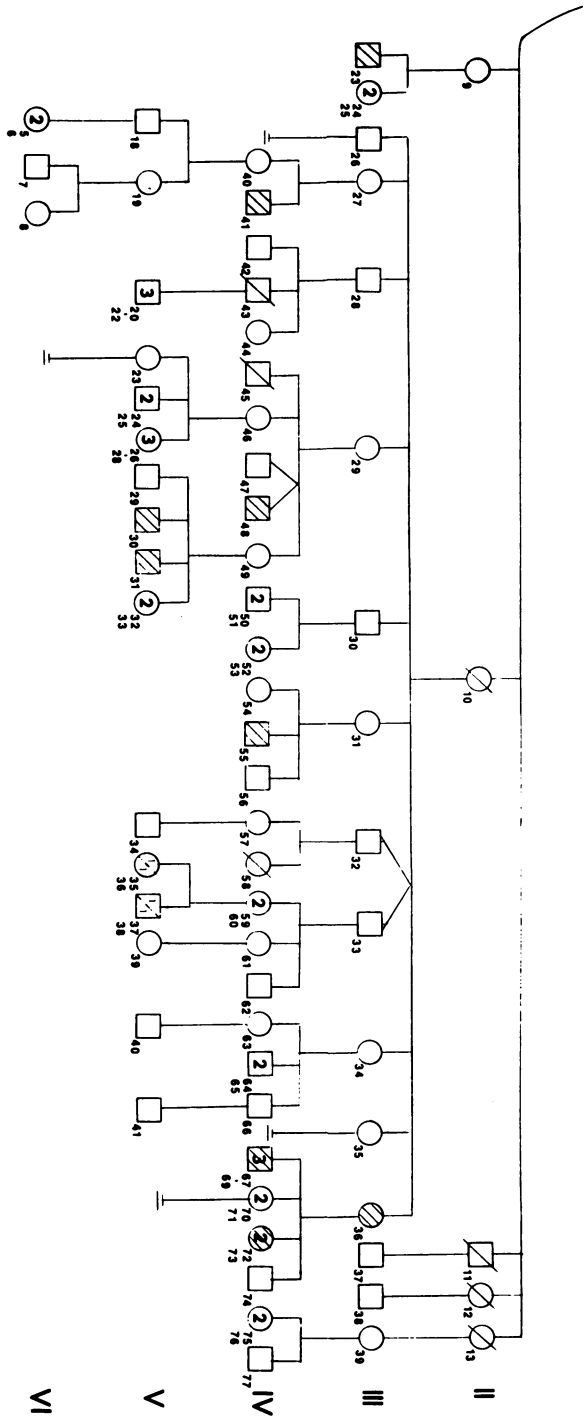
MATERIALS AND METHODS

Fragile X screening studies on each subject were done in at least two different media systems—low folic acid (medium 199) and exposure to FUdR or methotrexate using our standard procedure [14]. In fragile X families, a person is considered positive if 1% of the cells express the fragile X.

Family Reports

Family D1 (fig. 1) was ascertained at The University of Texas Health Science Center at Dallas by fragile X screening of the proband (V-5, table 1) at age 13 months. Fragile X studies on his maternal uncle (IV-13) were also positive (table 1). The maternal great-grandmother (II-4) had a negative family history, while an extensive history of mental retardation was recorded in the family of the maternal great-grandmother (II-5). His family is descended from a German Catholic couple (I-3 and I-4) who emigrated from Bavaria to south Texas in the early 1880s. Three unaffected carrier males (II-5, II-7, and IV-22) were found in this family. The affected male descendants of II-7 have seizures while the other branches do not. Individual IV-23 died in a state mental retardation facility; his brother (IV-22) is a college graduate and runs a successful business. His IQ tested at 148 during college. The chromosome analyses on individuals IV-26 and IV-28 were done by the Alberta Heredity Disease Program, Edmonton, Alberta, Canada. Individual III-26 may or may not be affected; family reports conflict. No formal psychometric testing of individual II-5 was available, but he has always been considered normal by the family. He worked in a service station prior to his retirement. Extensive testing of individuals II-5 and IV-22 for the fragile X repeatedly gave negative results (table 1). The only carrier female who was tested and found to be positive for the fragile X was III-29 (6/100 cells). Extensive studies of several other obligate carrier females (III-13, IV-11, V-10) always gave negative results. One affected female (IV-3) was tested and was negative (0/150). Lymphoblastoid lines are available from the Institute for Medical Research (IMR, Camden, N.J.) on 30 family members. (A list of IMR numbers is available from P. N. H.-P.)

Family D20 (fig. 2) was ascertained during evaluation of individual IV-1 for mental and development delay (table 1). The family history revealed that individual II-6 had some learning disability. Cytogenetic testing was done on individuals II-6 and II-7. This testing confirmed II-6 to have the fragile X; II-7, an unaffected carrier male, was negative both times tested (table 1). Individuals I-4 and I-5 were of Austrian Jewish descent and were first cousins as were individuals I-1 and I-2. I-4 and I-2 emigrated to the United States in 1914. II-5 had juvenile diabetes and died in her early 30s; II-4 reportedly died at age 12 with testicular cancer. II-6 presently lives with II-7 and apparently does have some mental impairment although he served in the U.S. Army during World War II and has been married twice. II-7 completed high school and is presently employed in an administrative governmental position in another state. All available information indicates that he has normal intelligence; he was not available for physical examination or formal IQ testing. Cytogenetic studies on individuals III-3 and III-4 were negative for the fragile X. During the course of this study, III-4 became pregnant and elected to have prenatal diagnosis. The fetus (IV-2) was found to be male, and the pregnancy was terminated. Chromosome studies on amniotic fluid cells predicted a fragile X negative male both in our laboratory (FUdR: 0/47) and a reference laboratory (FUdR: 0/27; methotrexate: 0/56). (It was noted at the reference laboratory that the cells were past the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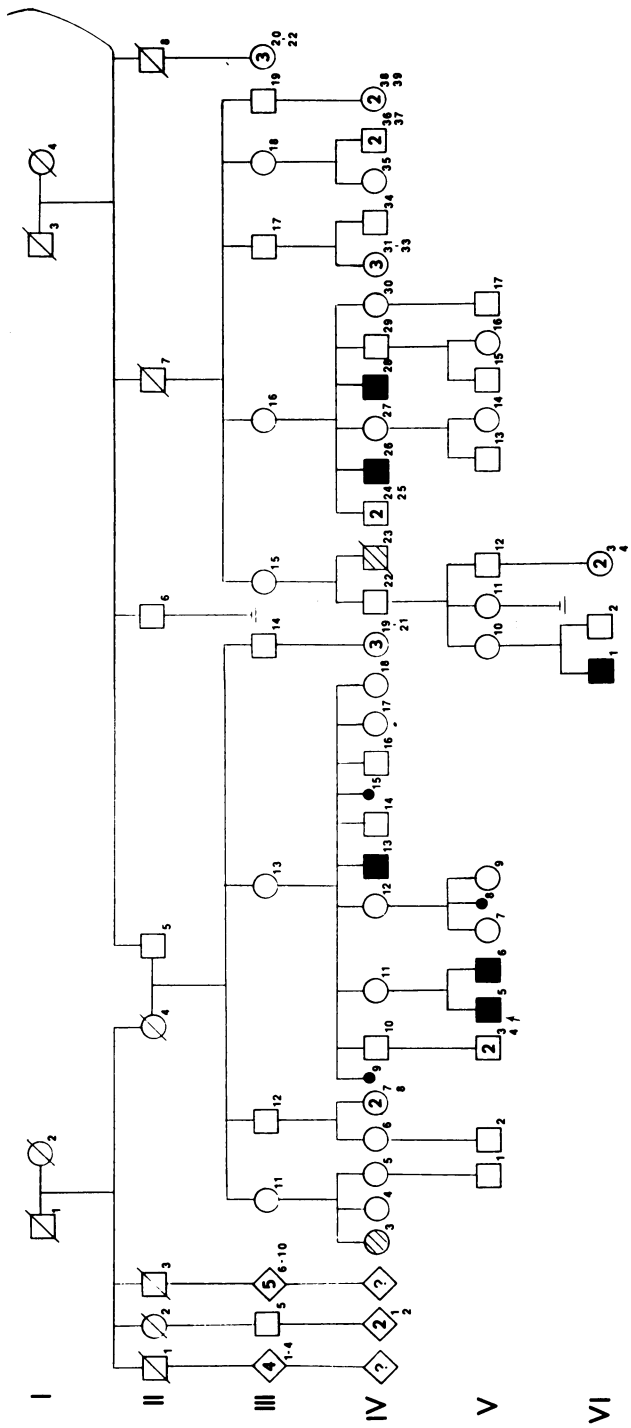

FIG. 1.—Pedigree of family D1. This is an expanded and updated version of pedigree HP1 in [15]. ■ ● = mentally retarded, fragile X identified cytogenetically; ▨ ⊘ = mentally retarded by family history.

TABLE 1

FRAGILE X EXPRESSION IN LYMPHOCYTES OF AFFECTED AND CARRIER MALES

FAMILY	INDIVIDUAL	FRAGILE X (+ CELLS/TOTAL CELLS)	
		Medium 199	MEM/FUdR
D1	IV-13	19/100	25/100
	V-5	5/50	11/50
	V-6	13/47	14/50
	VI-1	13/50	4/50
	II-5	0/150	0/125
	IV-22	0/100	0/175
D20	II-6	3/50	12/69*
	IV-1	14/50†	...
	IV-2	1/25	9/50
	II-7	0/50	0/150*
D22	IV-4	7/100	18/50
	V-7	25/100	20/100

* MEM/MTX instead of FUdR.
† Study done by Dr. Nancy Carpenter, Tulsa, Oklahoma.

optimal growth phase at the time of fragile X study.) Follow-up studies on cord blood were positive for the fragile X (table 1). Cell lines are available from IMR from four family members (II-7, III-3, III-4, IV-2).

Family D22 (fig. 3) was ascertained by the Mayo Clinic. Follow-up studies in our laboratory revealed the brother (V-7) as well as a cousin (IV-4) on the maternal grandfather's side of the family to be positive for the fragile X (table 1). The unaffected carrier grandfather (III-21) was killed in an automobile accident. The obligate carrier mother and aunt (IV-14, IV-20) did not express the fragile X.

DISCUSSION

Several features not characteristic of a classical X-linked recessive trait have been observed in families with the fragile X:

(1) The three families presented above (figs. 1–3) include at least five clinically normal males who are obligate carriers of the fragile X. Family D1 (fig. 1) exhibits transmission from an unaffected carrier female, through two unaffected males, to an affected male in the sixth generation. At least 10 other families reported previously demonstrate transmission of the fragile X through unaffected carrier males [3–13].

(2) The number of mentally retarded males in families segregating for fragile X is about 20% less than expected with X-linked recessive inheritance [15, 16].

(3) Mental retardation occurs in about 35% of females who are carriers of the fragile X trait [15, 16]. This frequency of serious clinical abnormalities among female carriers is much greater than that seen in typical X-linked recessive conditions such as hemophilia or Duchenne muscular dystrophy.

(4) Penetrance varies in sibships with different histories of expression of fragile X syndrome. (a) Unaffected carrier males have fewer daughters with

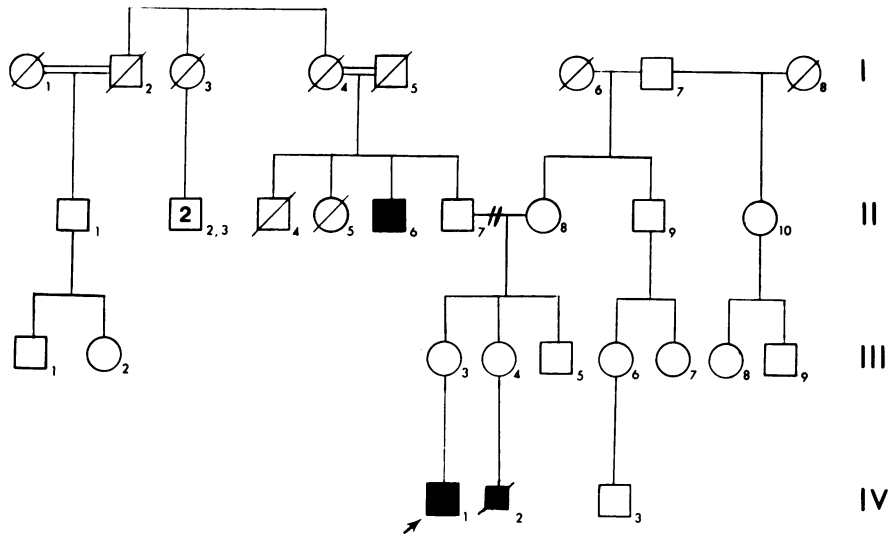

FIG. 2.—Pedigree of family D20. *Symbols* have the same meaning as in figure 1. This is an expanded and updated version of pedigree HP20 in [15].

mental retardation than unaffected carrier females [15, 17] even though twice as many affected daughters would be expected among the carrier fathers' offspring as compared to the daughters of carrier mothers. (b) Mental retardation is also less common among the brothers and sisters of unaffected carrier males than among children in other sibships born to carrier mothers [15, 17]. (c) On the other hand, mental retardation is more common among the offspring of mentally retarded women who carry the fragile X syndrome than among the offspring of unaffected women who carry this trait [15]. (d) Most difficult of all to explain by X-linked inheritance or any other conventional genetic mechanism is the observation that carrier mothers of unaffected carrier males are much less likely to have mentally retarded offspring than the unaffected carrier daughters of these same carrier males [15].

(5) Almost all mothers of boys with mental retardation due to fragile X have been found to carry the trait themselves [15, 16]. This implies that de novo mutants are rare among males with fragile X syndrome and that "mutation" of the "gene" for this disease occurs predominantly or only during male meiosis. The "mutation" frequency for this "gene" in sperm has been estimated to be 7×10^{-4} [16], a rate that is at least 10 times greater than that estimated for most other X-linked genes in humans [18].

(6) The fragile X syndrome differs from all other monogenetic diseases studied in that the abnormal phenotype is associated with the presence of an inducible cytogenetic marker, the fra(X)(q27). Moreover, expression of this marker appears to be correlated with expression of mental retardation in fragile X syndrome, at least qualitatively. The fra(X)(q27) could not be demonstrated in any of the unaffected carrier males in the families reported above despite extensive cytogenetic investigation. Similar findings in unaffected male carriers

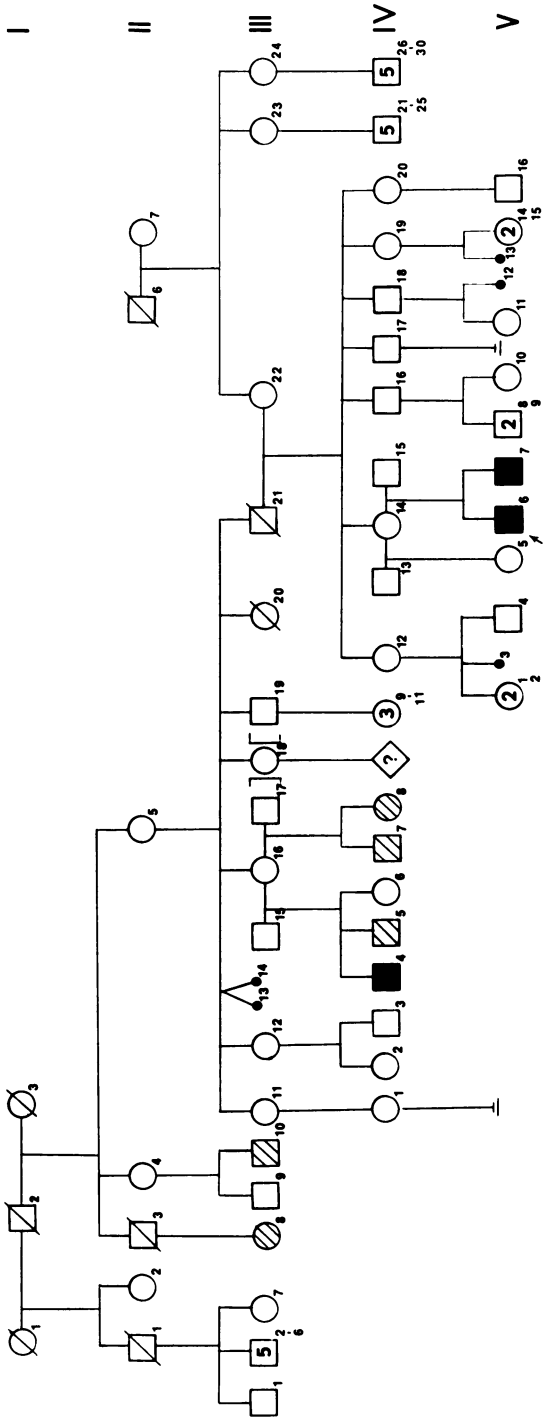

FIG. 3.—Pedigree of family D22. *Symbols* have the same meaning as in figure 1

to fragile X trait have been reported by Rhoads et al. [7], by Camerino et al. [19], and by Froster-Iskenius et al. [12]. In female carriers, intelligence appears to be inversely correlated with expression of the fra(X)(q27) [20, 21] and may be dependent on the extent to which the relevant X chromosome is inactivated [21, 22].

Clearly, the fragile X syndrome has unique genetic characteristics. Application of the techniques of modern molecular genetics should produce the critical evidence needed to explain the inheritance of this condition. Initial studies of this type have already been reported [19, 23].

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