

## Linkage Studies in a Large Fragile X Family

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### Summary

We have analyzed the segregation of five loci in the region Xq27/28 in a large family affected by the fragile X syndrome. The marker DXS115 (767) is shown to be polymorphic with the enzyme *Pst*I, as well as with *Bst*XI. This marker will be useful in the analysis of both fragile X and haemophilia A families. The data presented here are consistent with the following order of loci: Xcen-F9-DXS105(cX55.7,55E)-DXS98(4D-8)-FRAXA-DXS52(St14)-DXS115(767)-qter.

### Introduction

After Down syndrome the fragile X syndrome (Martin-Bell syndrome) is one of the most frequent genetic causes of mental retardation. Recent prevalence figures suggest that the frequency is 0.3-1/1,000 among males and 0.2-0.6/1,000 among females (Turner et al. 1986; Webb et al. 1986; Kahkonen et al. 1987). The associated cytogenetic finding of a fragile site at Xq27, which is expressed in 2%-50% of cells in affected individuals, is not a reliable tool for carrier detection and fails to detect both unaffected hemizygous male carriers and most unaffected heterozygotes. The fragile X syndrome differs from other X-linked disorders because of its unusual segregation within families. About 20% of hemizygous gene carriers do not express the defect and are unaffected; on the other hand, about 30% of female heterozygotes are to differing degrees mentally retarded, which cannot be fully explained as a consequence of selective X chromosome inactivation (Froster-Iskenius et al. 1985; 1986; Sherman et al. 1985; Wilhelm et al., in press). Hemizygous male carriers and the majority of the unaffected female heterozygotes will be undetectable by cytogenetic techniques and will thus fail to be diagnosed as being at risk before they reproduce.

Received May 11, 1988; revision received June 27, 1988.

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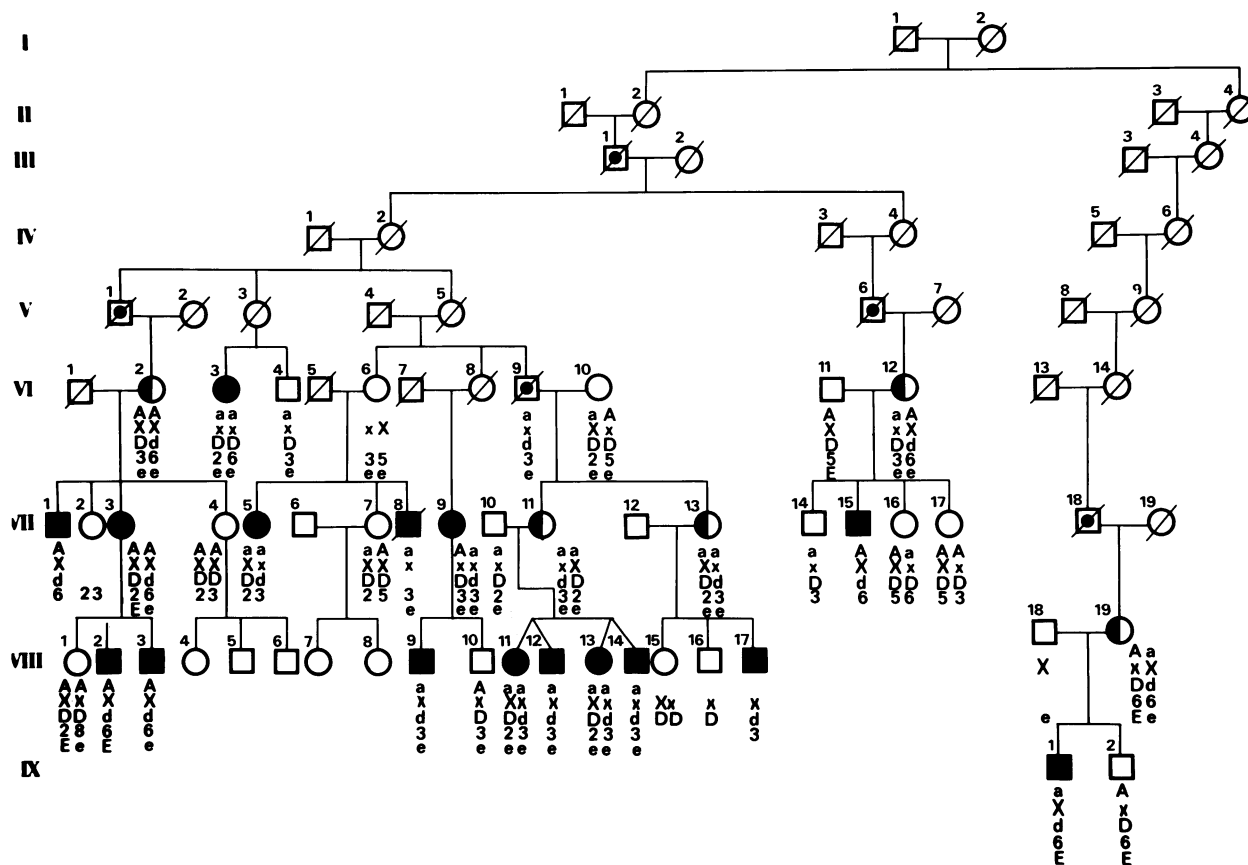
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Several DNA markers flanking the fragile X locus (FRAXA) have been identified (Oberle et al. 1986; Veenema et al. 1987; Arveiler et al. 1988; Brown et al. 1988, and in press; Heilig et al., in press; Thibodeau et al., in press; see Davies et al. 1988 for review). DXS98 on the proximal side and DXS52 on the distal side of the fragile site have been shown to be the loci most closely linked to the mutation (Brown et al. 1987; Arveiler et al. 1988; Mulley et al., in press). However, the high recombination rate in this region of the X chromosome still limits the usefulness of DNA markers for prenatal diagnosis and prospective carrier detection. It has been suggested that F8C might be closer to the fragile X locus than is DXS52 (Davies et al. 1988). The data presented here do not support this view, since they place DXS115, a locus that we have previously shown to be physically linked to F8C, distal to DXS52 (Patterson et al., in press).

### Material and Methods

#### *Patients and Family Studies*

The pedigree shown in figure 1 was ascertained through a follow-up of a previous screening investigation in a home for the mentally disabled (Froster-Iskenius et al. 1983). Cytogenetic and clinical data on the family have been reported previously elsewhere, together with a detailed pedigree (Froster-Iskenius et al. 1985; family I).



**Figure 1** Large kindred segregating for the fragile X syndrome. Symbols used are as follows: □, ○ = normal male, female; ■, ● = mentally retarded fragile X-positive male, female; □, ○ = phenotypically normal obligate carrier male, female. A diagonal line is used to indicate that an individual is deceased. Genotypes are indicated using letters as follows: Aa, F9, Xx, DXS105, Dd, DXS98; Ee, DXS115. Numbers are used to indicate the genotype at the DXS52 locus (Oberle et al. 1985).

**DNA Probes**

The DNA probes used are summarized in table 1. We are grateful to Professor Brownlee (FIX), Professor Pearson (cx55.7 and 767), Dr. Mandel (St14, 55E), and Dr. Nussbaum (4D8) for making probes available.

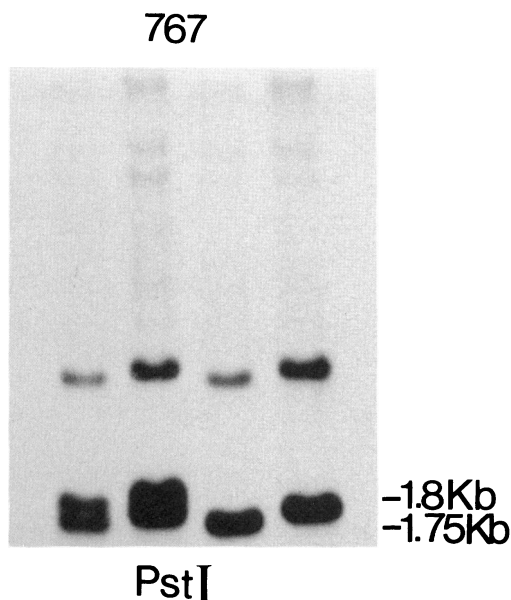
**DNA Analysis**

Blood samples were collected in heparin (1 ml Li-quemin (Roche)/20 ml blood). DNA was extracted from peripheral lymphocytes or lymphoblastoid cell cultures according to standard methods (Kunkel et al. 1977).

**Table 1**

**Characteristics of RFLPs Used in This Analysis**

Locus (Probe)	Enzyme	RFLP Band Sizes (kb)	Heterozygosity	Reference
F9 (FIX) . . . . .	<i>TaqI</i>	1.8/1.3	.4	Camerino et al. 1983
DXS105 (cx55.7) . . .	<i>TaqI</i>	4.5/3.2	.25	Hofker et al. 1987
DXS105 (55E) . . . . .	<i>PstI</i>	11/9	.4	Present study and Heilig et al., in press
DXS98 (4D-8) . . . . .	<i>MspI</i>	25/7.8	.27	Boggs and Nussbaum 1984
DXS115 (767) . . . . .	<i>PstI</i>	1.8/1.75	.38	Present study
DXS52 (St14) . . . . .	<i>TaqI</i>	Multiallelic	.80	Oberle et al. 1985



**Figure 2** RFLP revealed by 767. DNA samples were digested with *Pst*I and hybridized as described in Material and Methods.

Genomic DNA was digested to completion with the appropriate restriction endonucleases according to the manufacturer's instructions. Resulting DNA fragments were separated electrophoretically on 0.7% agarose gels and transferred to nitrocellulose or Hybond® N membranes (American International) by Southern transfer (Southern 1975).

Probes were either nick-translated (Rigby et al. 1977) or labeled by random priming (Feinberg and Vogelstein 1983). Filters were hybridized according to standard methods and were washed at high stringency ( $0.1 \times$

SSC, 0.1% SDS, 65 C) (Maniatis et al. 1982). Autoradiography was performed with intensifying screens at  $-70$  C for 1–3 days.

#### Genetic Analysis

Lod scores were calculated using the computer program LINKAGE (Lathrop and Lalouel 1984). A penetrance of 80% in males and 56% in females was used, as in our previous analyses (Thibodeau et al., in press).

#### Results

Following our observation of physical linkage between F8C and the locus DXS115 (767), we decided to investigate DXS115 for a useful polymorphism. The *Pst*I RFLP revealed by this marker is shown in figure 2. The gels were run for longer times than the standard ones in order to reveal the polymorphic bands clearly at 1.75 and 1.8 kb with allele frequencies of .77 and .23, respectively (44 X chromosomes tested). This is a useful RFLP, since we have found that the insertion-deletion polymorphism detected by 55E (DXS105) (Heilig et al., in press) can also be observed with *Pst*I (table 1).

DNA from 39 individuals of the pedigree shown in figure 1, including one unaffected hemizygous carrier and 10 affected hemizygotes, was obtained. The segregation of F9, DXS105, (cx55.7, 55E), DXS98, DXS52, and DXS115 was studied. The allele frequencies for the RFLPs used are summarized in table 1. The peak lod scores ( $Z_{\max}$ ) and the corresponding recombination fractions ( $\theta_{\max}$ ) are given in table 2. DXS98 shows close linkage with the fragile X mutation, giving a  $Z_{\max}$  of 4.95 at a  $\theta_{\max}$  of .03.

Matings informative for several loci in the family

**Table 2**

**Lod Scores for the Linkage Between Loci Defined by Four DNA Markers and the Fragile X Mutation (FRAXA).**

LocI	$\theta_{\max}$	$Z_{\max}$	95% Confidence Limits
F9-DXS105 . . . . .	.08	1.35	.01-.35
F9-DXS98 . . . . .	.12	.70	. . .
F9-FRAXA . . . . .	.12	.93	. . .
F9-DXS52 . . . . .	.26	.25	. . .
DXS105-DXS98 . . . . .	.09	1.69	.01-.35
DXS105-FRAXA . . . . .	.11	1.43	.00-.40
DXS105-DXS52 . . . . .	.08	2.01	.01-.32
DXS98-FRAXA . . . . .	.03	4.95	.00-.16
DXS98-DXS52 . . . . .	.09	2.38	.01-.32
FRAXA-DXS52 . . . . .	.04	4.09	.00-.19

NOTE.—Approximate 95% confidence limits are given (where possible) as the value for  $\theta$  at a lod score 1.0 lower than the peak value (Conneally et al. 1985).

shown in figure 1 provide data regarding the order of loci. The putative recombinant individual VII-17 suggests that DXS98 and DXS105 lie between F9 and FRAXA, as has been suggested by genetic (Brown et al. 1987; Veneema et al. 1987) and physical (Patterson et al., in press) studies. Individual VIII-16 suggests that DXS98 is closer to the fragile site than is DXS105, consistent with previous genetic and physical mapping data (see Davies et al. 1988). Four-point analysis of the data from the proximal markers also suggested that the locus order F9-DXS105-DXS98-FRAXA is approximately eight times more likely than any other. In this multipoint analysis the  $\theta_{\max}$ 's for the three intervals were estimated as .076, .091, and .026, respectively.

On the distal side of the fragile site, DXS115 was not sufficiently informative to give a significant lod score in either two-point or multipoint analyses. However, evidence for tight linkage between DXS115 and DXS52 ( $\theta_{\max} = 0$ ;  $Z_{\max} = 4.56$ ) has been presented elsewhere (Arveiler et al. 1988). In addition, information on the relative order of the loci was derived from a phase-known recombination event in figure 1. Individual VII-3 is heterozygous for both DXS52 and DXS115 and must have inherited the affected chromosome with haplotype A,X,d,6,e. Her affected sons have the haplotypes A,X,d,6,E and A,X,d,6,e, indicating co-segregation of the mutation with DXS52 and a recombination event in individual VIII-2 between DXS52 and DXS115. If we assume that double recombination events are unlikely in this distance, and since DXS52 maps distal to the fragile site, these data suggest that DXS115 lies further distal from DXS52. When all the data are taken together, the results are consistent with the order Xcen-F9-DXS105-DXS98-FRAXA-DXS52-DXS115-Xqter.

### Discussion

We have analyzed a large kindred affected by the fragile X syndrome by using five polymorphic markers both distal and proximal to the fragile site. Our analysis incorporates a new RFLP, with *Pst*I, detected by the probe 767 at the DXS115 locus. A *Bst*XI RFLP has already been reported for this probe (Arveiler et al. 1988), but this represents a useful addition, as the probe can be used on the same blots as those required for 55E (DXS105), which also reveals an RFLP with *Pst*I. DXS115 was only informative in a few individuals in our large pedigree, although two recombination events were observed. For one of these, information was available for DXS52. Recombination occurred between DXS52 and DXS115 (fig. 1, VIII-2), with the fragile

X mutation segregating with DXS52. This suggests the order FRAXA-DXS52-DXS115-Xqter. DXS115 may therefore not be very useful for the diagnosis of the fragile X syndrome but should be helpful in the study of other disease loci that map distal to DXS52.

Physical mapping data have suggested that DXS115 may lie within 250 kb of the F8 gene (Patterson et al., in press). The 767 sequence lies within an intron of the F8 gene that is reiterated once elsewhere in the Xq28 region (Wion et al. 1986; Patterson et al. 1988). The polymorphism used here maps in the region outside the F8 intron. We are currently attempting to define its exact position relative to other probes in the Xq28 region.

Our analysis using loci lying proximal to the fragile site suggests that DXS98 is the closest marker to the fragile site ( $Z_{\max} = 4.95$ ;  $\theta_{\max} = .03$ ), consistent with recent reports (Brown et al. 1987; Mulley et al., in press; also see Davies et al. 1988). Unfortunately, this marker is not very informative, which limits its usefulness in the counseling of families. In summary, our data suggest the order Xcen-F9-DXS105(cx55.7, 55E)-DXS98(4D8)-FRAXA-DXS52(St14)-DXS115(767).

### Acknowledgments

We are grateful to Helen Blaber for typing the manuscript, to R. Warneke and Tracey Flint for expert technical assistance, and to Professor E. Schwinger for helpful discussions. We thank the Medical Research Council of Great Britain and the Boehringer Ingelheim Foundation for financial support.

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