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New Polymorphic DNA Marker Close to the Fragile Site FRAXA

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DNA from a human–hamster hybrid cell line, 908-K1B17, containing a small terminal portion of the long arm of the human X chromosome as well as the pericentric region of 19q was used as starting material for the isolation of an X-chromosome-specific DNA segment, RN1 (DXS369), which identifies a *Xmn*I RFLP. Linkage analysis in fragile X families resulted in a maximum lod score of 15.3 at a recombination fraction of 0.05 between RN1 and fra(X). Analysis of recombinations around the fra(X) locus assigned RN1 proximal to fra(X) and distal to DXS105. Analysis of the marker content of hybrid cell line 908K1B17 suggests the localization of RN1 between DXS98 and fra(X). Heterozygosity of DXS369 is approximately 50%, which extends the diagnostic potential of RFLP analysis in fragile X families significantly.

INTRODUCTION

The fragile X [fra(X)] syndrome associated with a fragile site at Xq27.3 is the most common cause of inherited mental retardation (Turner *et al.*, 1986). The incidence is approximately 1 in 1500 newborn males (Webb *et al.*, 1986). The fragile site induced *in vitro* by thymidine deprivation is expressed in 1 to 80% of the cells analyzed (Lubs, 1969; Sutherland, 1977). Carrier diagnosis by cytogenetic analysis is restricted because about 50% of females and 20% of males are nonpenetrant for fra(X) expression as well as for mental retardation (Sherman *et al.*, 1985). The application of closely linked DNA markers can be a supplementary tool for the diagnosis of individuals at risk.

A genetic map of the region Xq27 has been constructed (Carpenter *et al.*, 1987; Brown *et al.*, 1988; Arveiler *et al.*, 1989). The probes cX55.7 (DXS105) and 4D-8 (DXS98) are estimated to be located at 10–15 cM proximal to the fra(X) locus (Vincent *et al.*, 1989). Distal to fra(X), loci F8 and ST14 (DXS52) are located at 10 and 13 cM, respectively. The frequencies of recombination are relatively high. Therefore more DNA markers are required for both developing antenatal and carrier diagnosis and studying the molecular basis of the disorder.

We report the isolation of a DNA marker that originates from the fra(X) region and detects an RFLP. The marker is tightly linked to the fra(X) locus and is located on the proximal side of the disease locus.

TABLE 1

Recombination Fraction (θ) Values and Lod Scores (Z) for Two-Point Crosses

	θ						
Linkage group	0.01	0.05	0.10	0.15	0.20	$\hat{Z}$	$\hat{\theta}$
Fra(X)–RN1	13.88	15.29	14.63	13.29	11.55	15.29	0.05
DXS105–RN1	−5.50	1.23	3.27	3.91	2.87	3.91	0.15
DXS105-fra(X)	−4.90	1.80	3.13	3.73	2.85	3.81	0.17

MATERIALS AND METHODS

Cell Hybrids

The construction of somatic cell hybrid 908K1B17, containing a small terminal segment of Xq and the pericentric region of 19q as a contiguous segment of unique human material, has been described in detail elsewhere (Schonk *et al.*, 1989; Wieringa *et al.*, 1988).

Construction of Human Xq–19q-Specific Phage Sublibrary

DNA from cell hybrid 908K1B17 (Schonk *et al.*, 1989) was isolated and partially cleaved with restriction

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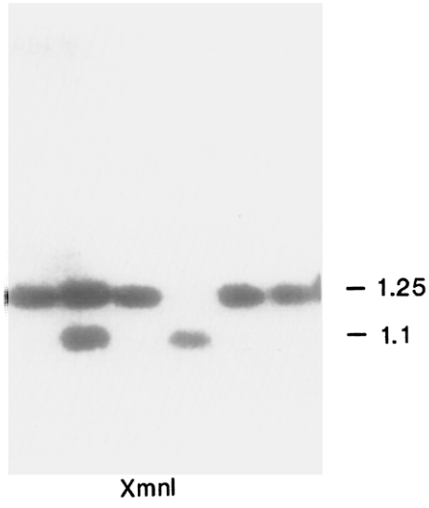

FIG. 1. Polymorphism detected by probe RN1. The sizes (in kb) of the allelic restriction fragments are indicated on the right.

enzyme *Sau*3A to a mean fragment size of 15–20 kb. Fragments of 10–20 kb were electrophoretically resolved on low-gel-temperature agarose, purified, and ligated into *Eco*RI and *Bam*HI doubly cleaved phage EMBL12 vector DNA (Natt and Scherer, 1986) using standard methods (Maniatis *et al.*, 1982). Next, phage DNA ligation mixtures were packaged using phage packaging extract (Promega Biotec) according to the protocol provided by the manufacturers.

Phage recombinants (500,000) were plated on *Escherichia coli* LE392 host cells and screened for human-specific *Alu*-positive inserts using ^{32}P-labeled pBLUR8 probe DNA and standard plaque-screening–hybridization protocols (for details see Hulsebos *et al.*, 1986). Approximately 0.1–0.2% of the phages contained *Alu*-positive inserts. Distinct individual *Alu*-positive phages were selected, plaque-purified, and subsequently used for DNA preparation (Maniatis *et al.*, 1982; Hulsebos *et al.*, 1986). For subcloning, DNAs were double digested with *Eco*RI and *Pst*I, and fragments were ligated into *Pst*I–*Eco*RI-cleaved pSP64 or pUC18 plasmid vector DNAs and cloned using standard procedures. Subclones containing single-copy human DNA were tested for the presence of human X-chromosomal DNA inserts using DNA from the hybrid cell lines containing either a unique human X chromosome or a unique chromosome 19.

DNA Analysis and Probes

Probes used in this study were F9 (Camerino *et al.*, 1985), cX55.7 (DXS105) (Veenema *et al.*, 1987; Hofker *et al.*, 1987), 4D-8 (DXS98) (Boggs and Nussbaum, 1984), F8C (Gitschier *et al.*, 1985), and ST14 (DXS52) (Oberle *et al.*, 1985).

DNA from peripheral blood was isolated as described (Miller *et al.*, 1988). Restriction enzyme digestion, gel electrophoresis, Southern blotting, and hybridization were performed according to standard procedures (Maniatis *et al.*, 1982) or to the manufacturer's instructions. DNA was labeled by primed synthesis according to Feinberg and Vogelstein (1983).

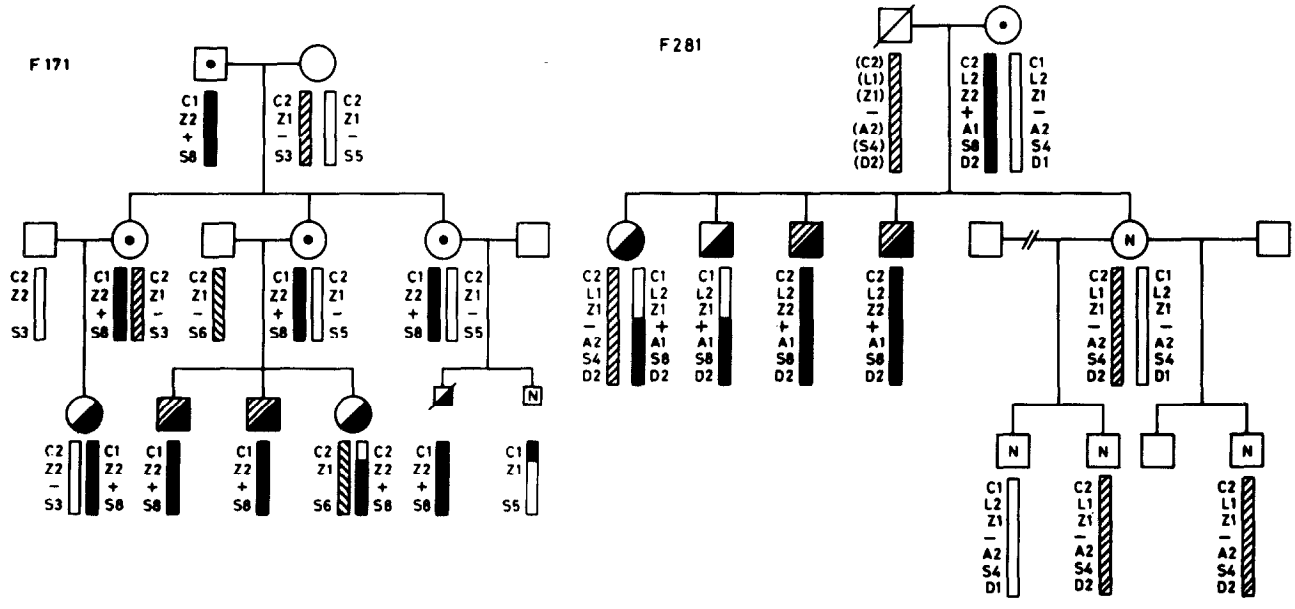

FIG. 2. Segregation analysis of DNA markers in two fragile X families. A plus sign (+) or a minus sign (−) indicates the presence or absence, respectively, of the fragile X mutation. Unaffected males and females are symbolized as □ and ○. The following symbols are used: ⊡ and ⊙, obligate carriers; ◪ and ◕, positive fragile X expression; ◪ mentally retarded; N, no fragile X expression. The polymorphisms shown for each individual are C, cX55.7; L, cX33.2; Z, RN1; A, F8; S, ST14; D, DX13.

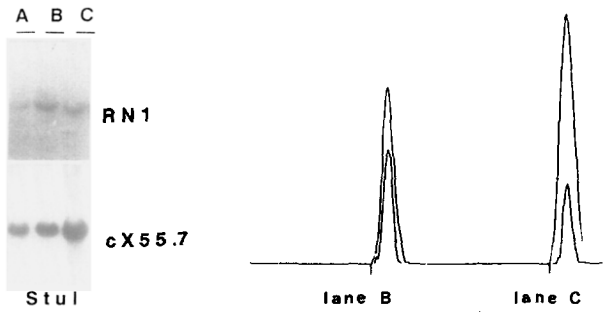

FIG. 3. Southern blot analysis of *StuI*-digested DNA. **Left:** The Southern blot was hybridized with RN1 and cX55.7. Lane **A**, DNA of 46XY; lane **B**, DNA of 46XX; lane **C**, DNA of cell line L with an interstitial deletion in Xq27. **Right:** The signals of the densitometer scan of lanes **B** and **C**, respectively. The upper signal and the lower signal are from cX55.7 and RN1, respectively.

Lod scores were calculated using the LINKAGE 5.03 computer program (Lathrop and Lalouel, 1984). A mutation rate of 1/2000 was used. The program allows variable penetrance for mental impairment or fra(X) expression. A penetrance of 56% in females and 80% in males was used (Sherman *et al.*, 1985).

RESULTS

The plasmid subclone pRN1, which was shown to have a human X-chromosome-specific *PstI–EcoRI* DNA insert (600 bp), was used to screen for RFLPs in a test panel of 20 unrelated X chromosomes by using 13 different restriction enzymes. A polymorphic site was found only with the enzyme *XmnI*, resulting in allelic fragments of 1.25 kb (frequency = 0.59) and 1.1 kb (frequency = 0.41) (Fig. 1). Linkage between RN1 and the fra(X) locus was examined in fra(X) pedigrees. A peak lod score of 15.3 was calculated at a recombination fraction of 0.05 between fra(X) and RN1 with a 90% confidence limit between 0.01 and 0.11 (Table 1). The recombination fractions and lod scores are given for cX55.7 and either fra(X) or RN1. They confirm a close linkage between RN1 and fra(X).

A more precise localization of RN1 was obtained by three-point analysis in some of the families in which a recombination was detected between the fra(X) locus and markers ST14 and cX55.7. Five recombinations were observed between fra(X) and ST14. In all cases RN1 cosegregated with fra(X). In family 171 (Fig. 2) two recombinations were noted between cX55.7 and the fra(X) locus. RN1 cosegregated with the fra(X) locus. These recombinants are indicative of localization of RN1 between cX55.7 and ST14. In family 281 (Fig. 2) two recombinations were found between fra(X) and cX55.7. RN1 cosegregated with cX55.7, placing RN1 proximal to the fra(X) locus.

Independent information was derived from a lymphoblastoid cell line from a female L with an X-chromosomal deletion including 4D-8 but not cX55.7 and the probes distal to fra(X) (Schmidt *et al.*, submitted for publication). DNA from this cell line was hybridized with RN1. The hybridization signal obtained was compared densitometrically with the signal of control DNA. The signal was 50% of that of control DNA (46XX) (Fig. 3).

On the basis of these data, RN1 was localized between fra(X) and cX55.7.

DISCUSSION

This study describes a polymorphic DNA probe close to the fragile site at Xq27. By genetic mapping in fragile X pedigrees, we have localized RN1 between fra(X) and cX55.7. A second argument for this localization is the reduction of hybridization to the cell line bearing a deletion between cX55.7 and F8. The DNA probe RN1 was derived from a human–hamster hybrid cell line with a small terminal long-arm segment of the X chromosome containing F8 and ST14 but not 4D-8 and cX55.7 (Schonk *et al.*, 1989). These data together suggest the localization of RN1 between 4D-8 and fra(X), making it the closest proximal probe isolated so far.

For diagnostic applications in carrier detection and prenatal diagnosis of fra(X), flanking probes are required. The frequencies of recombination of the probes located proximal to the fra(X) locus are relatively high. The informative marker RN1 is located proximal to fra(X) and extends the diagnostic potential of RFLP analysis in fragile X families. Brown *et al.* (1988) noted a significant variation in recombination between F9 and fra(X) in different fra(X) pedigrees. RN1 will also be valuable for testing the existence of genetic linkage heterogeneity. Further investigations are needed to confirm that the genetic distance between RN1 and fra(X) is about 5 cM.

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