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Genetic Mapping of the Xq27-q28 Region: New RFLP Markers Useful for Diagnostic Applications in Fragile-X and Hemophilia-B Families

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Summary

We have characterized and genetically mapped new polymorphic DNA markers in the q27-q28 region of the X chromosome. New informative RFLPs have been found for DXS105, DXS115, and DXS152. In particular, heterozygosity at the DXS105 locus has been increased from 25% to 52%. We have shown that DXS105 and DXS152 are contained within a 40-kb region. A multipoint linkage analysis was performed in fragile-X families and in large normal families from the Centre d'Etudes du Polymorphisme Humain (CEPH). This has allowed us to establish the order centromere-DXS144-DXS51-DXS102-F9-DXS105-FRAXA-(F8, DXS15, DXS52, DXS115). DXS102 is close to the hemophilia-B locus ($z[\hat{\theta}] = 13.6$ at $\hat{\theta} = .02$) and might thus be used as an alternative probe for diagnosis in Hemophila-B families not informative for intragenic RFLPs. DXS105 is 8% recombination closer to the fragile-X locus than F9 ($z[\hat{\theta}] = 14.6$ at $\hat{\theta} = .08$ for the F9-DXS105 linkage) and should thus be a better marker for analysis of fragile-X families. However, the DXS105 locus appears to be still loosely linked to the fragile-X locus in some families. The multipoint estimation for recombination between DXS105 and FRAXA is .16 in our set of data. Our data indicate that the region responsible for the heterogeneity in recombination between F9 and the fragile-X locus is within the DXS105-FRAXA interval.

Introduction

The distal portion of the human X chromosome long arm contains many disease loci. For some of them, the biochemical defect is known, e.g., hemophilia A and B, glucose-6-phosphate dehydrogenase deficiency, adrenoleukodystrophy, and Hunter syndrome. For other rarer diseases—such as Emery-Dreyfuss muscular dystrophy, spastic paraplegia, one form of skeletal dysplasia, and dyskeratosis congenita—the gene has been traced and mapped only by means of linkage analysis to RFLP markers (Davies et al., in press).

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This region also contains the locus for the fragile-X mental retardation syndrome, the most frequent of all X-linked diseases (with an incidence of ~1/1,500 newborn males) and probably the most common cause of genetically inherited mental retardation (Turner and Jacobs 1983; Turner et al. 1986). The phenotypic trait associates mental retardation and presence of a fragile site in Xq27.3 induced in vitro by thymidine deprivation. Its segregation departs from classical recessive X-linked inheritance (Sherman et al. 1985). This disease poses diagnostic problems (since cytogenetic analysis detects only 50% of carrier females and may not be completely reliable for prenatal diagnosis) (Sherman et al. 1985; Jenkins et al. 1986).

The establishment of a fine genetic and physical map of the region is an important goal since it should allow a more precise localization of disease genes and thus provide more reliable diagnostic markers as well as tools for the cloning of these genes.

We have elsewhere reported that the coagulation-factor-IX gene (F9) and the hypervariable locus DXS52 (defined by probe St14) flank the disease locus (Oberlé et al. 1986*b*). Both markers appear to be located within clusters of closely linked loci (Szabo et al. 1984; Oberlé et al. 1985*b*, 1987; Oberlé and Mandel 1986). The distal cluster that contains DXS52, G6PD, and hemophilia A is at ~12% recombination from the fragile-X locus (FRAXA). Linkage between the factor-IX gene (F9), in the proximal cluster, and FRAXA shows a striking heterogeneity—as yet unexplained—since some families show tight linkage (Camerino et al. 1983) whereas others show only loose linkage (Brown et al. 1986, 1987*a*; Oberlé et al. 1986*b*). Furthermore, the density of loci in the two clusters contrasts with the paucity of markers between them, although they are separated by ~30% recombination. Very recently two markers (DXS98 and DXS105) have been described that appear to be closer to the fragile X on the proximal side than is F9, although the relatively low lod scores did not allow a precise estimate of their recombination frequency with either the fragile-X or the F9 loci (Brown et al. 1987*b*; Hofker et al. 1987). Moreover, their relatively low heterozygosity (~.25) limits their use in genetic analysis.

A large series of X-linked probes have been isolated by some of us (Hofker et al. 1985, 1987). We have systematically searched for RFLPs for eight probes assigned to the q27-q28 region and, by analysis of a large set of fragile-X families and normal families from the Centre d'Etudes du Polymorphisme Humain (CEPH), used the new RFLPs and some of the previously described ones to generate a multipoint linkage map. The markers and the genetic map described here should be useful for diagnostic applications and for more precise localization of disease loci in the q27-q28 region, as well as for a better understanding of the genetic peculiarities of the fragile-X syndrome.

Material and Methods

Family Studies

Presented here are the results of typing of 535 individuals in 23 fragile-X families, one large hemophilia-B family, and 16 normal families (pedigrees 2, 17, 28, 37, 45, 66, 884, 1331, 1332, 1344, 1346, 1347, 1375, 1408, 1413, and 1423) from the CEPH. They include and update the data concerning all fragile-X and normal families previously analyzed by Oberlé et al. (1986*b*, 1987) but do not include families typed in the Leiden laboratory. Genotypes and clinical data for disease pedigrees are available on request. Genotypes of CEPH families have been communicated to the CEPH data base.

Genomic DNA and Southern Blot Analysis

Total genomic DNA was extracted from human leukocytes or cultured cells, digested to completion with restriction endonucleases, fractionated by electrophoresis on 0.8% or 0.9% agarose gels, and blotted onto diazobenzyloxymethyl paper (Oberlé et al. 1986*a*) or Hybond-N membrane (Amersham).

For searching for RFLPs, batch hybridizations were performed. Ten to 15 blots were hybridized for 48 h in 50–60 ml of hybridization mix (3–4 ng of probe/ml; specific activity $1–2 \times 10^8$ cpm/μg DNA). Composition of the hybridization mix and washing conditions were as described elsewhere (Oberlé et al. 1986*a*).

For hybridization with repeat-containing cosmids, we prehybridized the probes with a large excess of nonradioactive sonicated human DNA (Litt and White 1985). The nick-translated cosmid (0.1–0.2 μg) was precipitated by means of 2 mM spermine. The pellet was dissolved in 30 μl of 0.5 M sodium phosphate buffer, and 250 μl of sonicated human DNA (2.5 mg/ml) was added. The mixture was boiled 10 min and then incubated 2 h at 65 C. Hybridization was performed in 50% formamide at 50 C.

Linkage Analysis

The LINKAGE program (Lathrop and Lalouel 1984; Lathrop et al. 1985) was used for two-point and multipoint analysis. Penetrance parameters in fragile-X families were as defined elsewhere (Oberlé et al. 1987).

Results

Search for New RFLPs

With a set of 10–31 restriction enzymes, a systematic search for new RFLPs was performed with eight single-copy probes localized to the Xq26-q28 region (Hofker et al. 1985, 1987), with at least nine independent X chromosomes tested (four females and one male). Restriction digests were blotted onto diazobenzyloxymethyl (DBM) paper, and hybridization was usually performed (as described above in Mate-

rial and Methods) in batches of as many as 15 blots, with purified inserts used as the probe.

This approach was fruitful, since it allowed us to detect new polymorphisms with several of the probes (table 1). Several RFLPs were found with probe cX38.1 (DXS102). The *Bst*XI and *Dde*I RFLPs (fig. 1*A*) have been studied in some detail and appear in very strong (perhaps complete) linkage disequilibrium with the *Taq*I RFLP (of 20 females tested, 15 were found to be homozygous for allele 1 of both the *Taq*I and the *Bst*XI RFLPs and five were heterozygous for both RFLPs). Furthermore, in a limited test of six individuals, polymorphisms were also found with *Pvu*II, *Msp*I, and *Hin*dIII, the same two individuals appearing as heterozygous in all three digests (fig. 1*B*). Although the latter three polymorphisms have not been tested for Mendelian segregation, we are confident that they are real, since the blot has been tested with many other probes so that artifacts due to contamination with plasmid DNA or to incomplete digestion can be eliminated. This suggests that most or all RFLPs at this locus might be due to an insertion-deletion mechanism. We also found a very rare (occurrence observed in two unrelated families) 3.9-kb allele in *Taq*I digests (fig. 1*A*).

Other useful RFLPs were obtained with two previously noninformative loci; a *Bst*XI RFLP with probe 767 (DXS115) and an *Apa*LI RFLP with probe cX33.2 (DXS152) showed heterozygosities of .25

Table 1

Polymorphic Probes

Probe Name (Locus) and Enzyme	Allele Size[a] (kb)	Frequency (*N*)
cX38.1 (DXS102):		
*Taq*I	11.8	.85 (160)
	1.65	.13
	3.9	.02
*Bst*XI	3.4	.86 (51)
	1.55	.14
*Dde*I	1.25	.76 (25)
	1.05	.24
780 (DXS119):		
*Bcl*I	10.5	.04 (27)
	6.8 + 3.7	.96
cos 55 (DXS105):		
*Eco*RI	7.5	.28 (54)
	5.8 + 4.2	.72
*Hin*dIII	11.1	.04 (48)
	9.0	.65
	7.1	.31
cX33.2 (DXS152, DXS105):		
*Apa*LI	12.0	.36 (50)
	8.3	.64
767 (DXS115):		
*Bst*XI	6.4	.86 (51)
	4.2	.14

NOTE. Numbers in parentheses are number of chromosomes tested. The following probes, tested with at least 10 restriction enzymes, did not detect RFLPs: cpX206 (DXS160), cX44.1 (DXS155), and 781 (DXS120).

[a] In addition, probes may detect constant fragments.

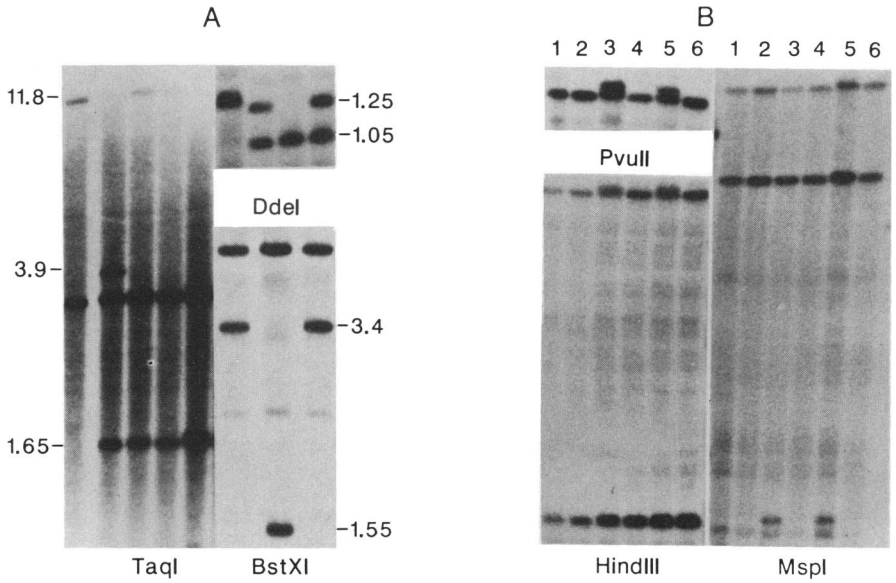

Figure 1 Polymorphisms at the DXS102 locus (probe cX38.1). *A*, Sizes of the allelic restriction fragments (in kb) are indicated. *B*, RFLPs detected in *Pvu*II, *Hin*dIII, and *Msp*I digests of the same six genomic DNAs.

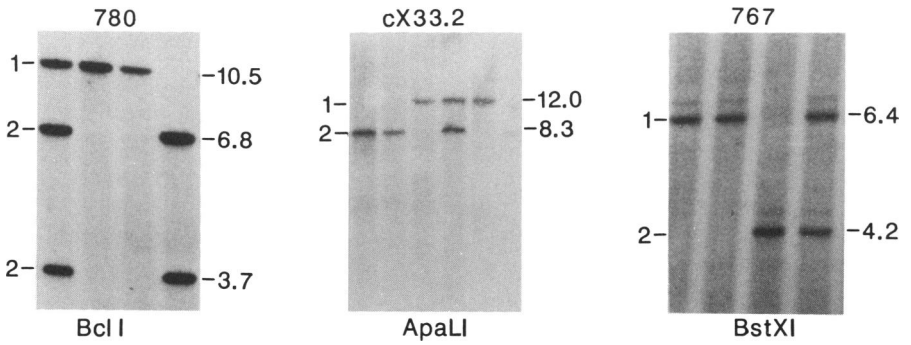

Figure 2 Polymorphisms detected by probes 780, cX33.2, and 767. Allelic restriction fragments are numbered on the left, and their sizes (in kb) are indicated on the right.

and .46, respectively (fig. 2, table 1). A very rare *Bcl*I RFLP was found for probe 780 (DXS119) that was only observed in a single family, in which it showed Mendelian segregation (fig. 2; also see below). We were unable to confirm the very rare cX55.7 *Eco*RI variant, cited elsewhere (Hofker et al. 1987) but not tested for Mendelian segregation. However, owing to its very low frequency, it could very well have escaped our notice.

Test for RFLPs with Whole Cosmids Reveals New Informative RFLP for DXS105

Since complete cosmids corresponding to some of the single-copy probes were available, we also performed a search for RFLPs by using the complete cosmids as probe; the search was done in the presence of a large excess of human DNA to compete out repetitive sequences and used highly stringent hybridization conditions (Litt and White 1985; also see Material and Methods). Scorable results were obtained for the three cosmids tested (cos 44, cos 48, and cos 55). These results revealed very useful RFLPs for cos 55 (DXS105) in both *Hin*dIII and *Eco*RI digests. The *Hin*dIII RFLP has two common alleles and a rare one, whereas the *Eco*RI RFLP has two alleles (fig. 3, table 1). The heterozygosity of the two RFLPs is very similar, and they appear to be in very strong (perhaps complete) linkage disequilibrium. Combined analysis of the two RFLPs does not give additional information (table 2). However, analysis of either of the two RFLPs can be combined with analysis of the *Taq*I RFLP, and this results in an important improvement in the informativeness of the DXS105 locus, since the combined heterozygosity is .52, compared with .25 when the *Taq*I RFLP is used alone (table 2).

Since the use of a whole cosmid as probe is not easily adapted to the routine of a diagnostic laboratory, we tried to subclone a single-copy probe that

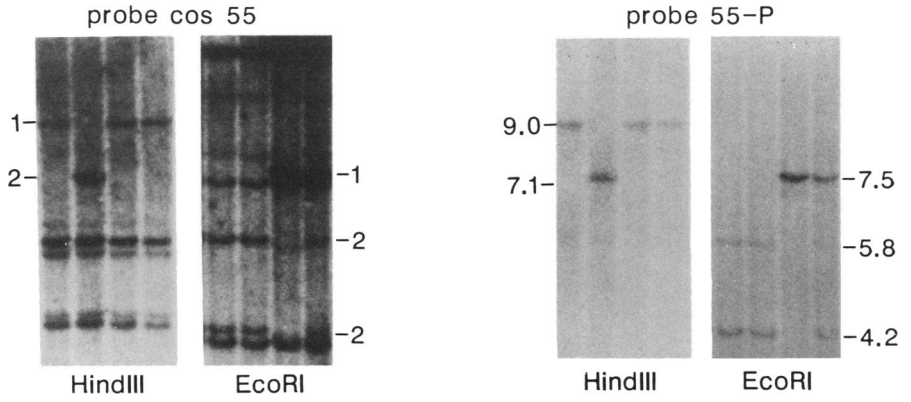

Figure 3 Polymorphisms at the DXS105 locus. The entire cosmid probe cos 55 or its subclone 55-P were hybridized to the same blot containing genomic DNAs digested with *Hin*dIII or *Eco*RI. Probes were prehybridized to sonicated human DNA as described in Material and Methods. Allelic fragments are numbered on the left panel, and their sizes are indicated on the right one.

Table 2

Haplotypes at the DXS105 Locus

Haplotypes		No. of X Chromosomes Found/Expected
cos 55 *Eco*RI	cos 55 *Hin*dIII	
1	1	0/3.5
1	2	2/1.5
2	2	0/4
2	1	16/9
cX33.2 *Apa*LI	cos 55 *Eco*RI	
1	1	6/2.5
1	2	0/6
2	2	18/11
2	1	0/4.5
cos 55 *Eco*RI	cX55.7 *Taq*I	
1	1	1/1
1	2	9/9
2	2	23/23
2	1	3/3
cX33.2 *Apa*LI	cX55.7 *Taq*I	
1	1	4/2.5
1	2	19/18
2	2	25/31
2	1	8/4.5

would detect the *Eco*RI and/or *Hin*dIII RFLPs. A *Bgl*II digest of cos 55 was subcloned into a plasmid vector, and recombinants corresponding to the various fragments were used as probes on genomic blots. This showed that a 7-kb *Bgl*II (cX55.B) fragment and a 2.4-kb *Pvu*II fragment (cX55.P) derived from it detect the two RFLPs (fig. 3); however, they still contain repetitive sequences and have to be used with an excess of competing human DNA.

DXS105 (cX55.7) and DXS152 (cX33.2) Are Derived from the Same or Overlapping Cosmids

While this work was being completed, we noticed that the probe cX55.P detects an *Apa*LI RFLP in linkage disequilibrium with the *Apa*LI RFLP detected by cX33.2. Furthermore, linkage analysis had shown that DXS105 and DXS152 appear very tightly linked ($z[\hat{\theta}] = 13.24$ at $\hat{\theta} = .0$; results not shown). Examination of the hybridization patterns revealed that restriction fragments corresponding to allele 1 of both *Apa*LI RFLPs have the same size whereas the size of the smaller alleles (allele 2) differed. This suggested that the two probes might flank the same polymorphic site. This was confirmed by the finding that cX33.2 hybridizes specifically to the genomic insert of cos 55 (results not shown). We conclude that the cX33.2 and cX55.7 probes derive from the same or overlapping cosmid clones. The *Apa*LI RFLP detected by cX33.2 appears to be in complete linkage disequilibrium with the *Eco*RI (or *Hin*dIII) RFLPs detected by the cos 55–derived probes and thus does not give additional information (table 2). All three RFLPs might in fact be due to a deletion-insertion polymorphism (B. Arveiler, A. Hanaver, I. Oberlé, A. Vincent, and J. L. Mandel, unpublished observations).

Linkage Analysis

To genetically map the loci DXS102 (cX38.1), DXS105 (cX55.7, cos 55, and cX33.2), DXS115 (767), and DXS119 (780), we analyzed the segregation of RFLPs in CEPH normal families, in families with fragile-X mental retardation, and in one large family with hemophilia B (see Material and Methods). All these families previously had been typed at several loci from the q26-q28 region, including loci DXS100 (45h), DXS144 (c11), DXS51 (52A), DXS52 (St14), and F9 (Oberlé et al. 1987). A few families had also been tested for DXS86 (St1), DXS15 (DX13), and F8C (coagulation-factor-VIII gene). We have typed most of the available fragile-X families for the *Taq*I RFLPs corresponding to the DXS102 and DXS105 loci, since blots already had been prepared during previous studies. These RFLPs are, however, not very informative (heterozygosity $\leq$ .25). The *Hin*dIII and *Apa*LI RFLPs corresponding to DXS105 were tested almost exclusively in families previously shown to exhibit recombination between F9 and the fragile-X locus. This results in a bias for the direct estimation of the linkage relationships between DXS105 and the fragile-X locus.

Two-point and multipoint linkage analysis was performed using the LINKAGE program (Lathrop and Lalouel 1984; Lathrop et al. 1985). Lod scores and recombination frequencies for pairs of markers are presented in table 3. High (>10) lod scores have been obtained for several pairs, which indicates that the data should allow accurate estimation of order and genetic-map distances.

DXS102 shows close linkage (with high lod scores) to F9, DXS51, and DXS144. In fact we found a single recombination event (in CEPH family 1413) between

Table 3

Lod Scores for Linkages between Loci in the Xq26-q28 Region

			Recombination Fraction (θ)						
	$z(\hat{\theta})$	$\hat{\theta}$	.001	.01	.05	.1	.15	.2	.3
DXS144-DXS51	14.26	.03	12.07	13.84	14.16	13.34	12.17	10.81	7.62
-DXS102 ...	10.46	.05	6.63	9.40	10.46	10.01	9.11	7.97	5.21
-F9	18.90	.03	15.58	18.24	18.79	17.66	16.05	14.16	9.74
DXS51-DXS102	4.95	.06	2.34	4.11	4.94	4.80	4.38	3.82	2.42
-F9	12.90	.03	10.71	12.48	12.80	12.00	10.88	9.57	6.52
DXS102-F9	13.59	.02	12.80	13.56	13.17	12.06	10.76	9.33	6.16
F9-DXS105	14.62	.08	0.99	9.61	14.14	14.57	13.76	12.37	8.55
-FRAXA	5.44	.17	−16.65	−6.75	1.80	4.55	5.37	5.36	4.09
DXS105-FRAXA ...	1.75	.24	−12.01	−7.53	−2.44	0.05	1.17	1.65	1.60
-DXS52	2.16	.30	−60.87	−31.14	−11.45	−4.15	−0.72	1.07	2.16
FRAXA-DXS52	15.83	.11	−2.99	6.29	13.98	15.77	15.51	14.30	10.27
DXS52-DXS115	4.56	.00	4.55	4.49	4.17	3.77	3.34	2.88	1.89
-F8C	10.65	.00	10.63	10.32	9.56	8.46	7.33	6.18	3.89

Note.—We included some very informative ($z[\hat{\theta}] > 10$) or important (F9-FRAXA and DXS105-DXS52) linkage between nonadjacent loci. Linkage results for DXS52-F8C include the results obtained in hemophilia-A families and previously described by Oberlé et al. 1985*a* but for which detailed lod-score information had not been published.

F9 and DXS102, and in this meiotic event DXS102 segregated as DXS100 whereas F9 segregated as DXS52. This suggested that, like DXS100, DXS102 is proximal to F9. One recombinant was found in a large fragile-X family, between DXS51 and DXS102. In this meiosis, DXS51 segregated with more proximal markers (DXS144 and DXS86) and recombined with respect to FRAXA whereas DXS102 segregated with FRAXA, suggesting that DXS102 is distal to DXS51. Multipoint mapping using the ILINK program confirms that DXS102 is located between DXS51 and F9, since all other orders have a much lower likelihood (table 4).

DXS105 appears to be located at 0.08 map units from F9, with a high (14.6) lod score. One fragile-X family previously studied with many probes showed that, of two meioses recombinant between F9 and the fragile-X locus, only one is recombinant between DXS105 and FRAXA, suggesting that DXS105 is between F9 and FRAXA (fig. 4*A*). The situation was

Table 4

Mapping of DXS102 and DXS105 by Four-Point Linkage Analysis

Order of Loci	Respective % Recombination between Adjacent Loci[a]	Relative Likelihood[a]
F9-DXS105-FRAXA-DXS52	7.0-17.1-11.6	220×10^{13}
DXS105-F9-FRAXA-DXS52	9.0-19.4-12.0	4.8×10^{13}
F9-FRAXA-DXS52-DXS105	16.8-11.3-26.5	496
F9-FRAXA-DXS105-DXS52	12.7-8.1-17.2	1
DXS102-F9-DXS105-DXS52	2.1-9.4-25.1	17
F9-DXS102-DXS105-DXS52	1.1-9.1-25.1	1
DXS144-DXS51-DXS102-FRAXA	1.9-2.7-18.3	1,956
DXS102-DXS144-DXS51-FRAXA	4.2-2.5-19.5	28
DXS144-DXS102-DXS51-FRAXA	3.3-3.1-19.8	1

[a] Calculated for various locations of DXS102 and DXS105 with respect to the map previously established, i.e., DXS144-DXS51-F9-FRAXA-DXS52 (Oberlé et al. 1987).

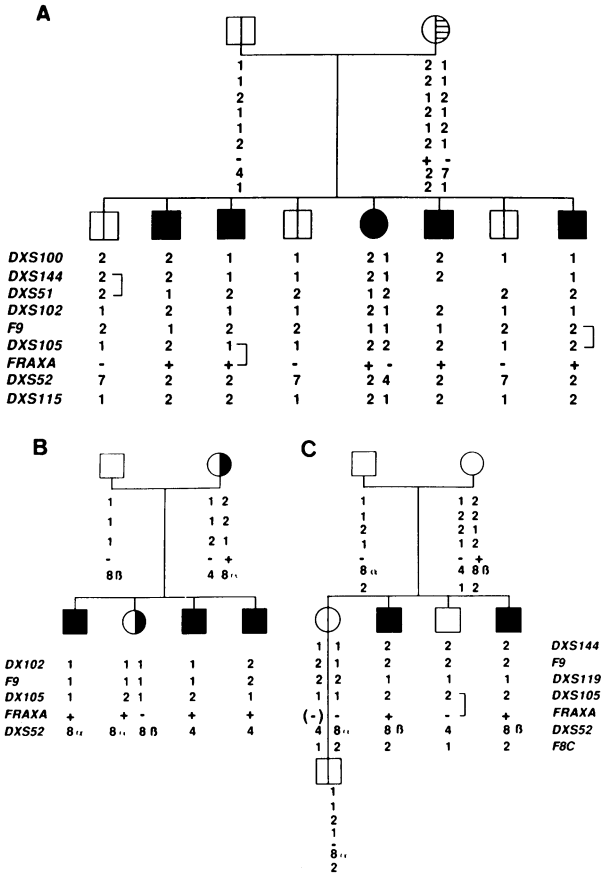

Figure 4 Segregation analysis of DNA markers from the Xq26-q28 region in three fragile-X families. Family A corresponds to family B of Oberlé et al. [1987]. A plus sign (+) or a minus sign (−) indicates the presence or absence, respectively, of the fragile-X mutation (− in parenthesis indicates that a normal carrier status has been inferred on the basis of cytogenetic analysis). Squares and circles represent males and females, respectively. □ and ○ = Phenotypically normal but not examined. In the following symbols the left half denotes the clinical status and the right half denotes the cytogenetic: ◫ and ◑ = normal with no fragile X; ▤ and ◍ = dull with 1%–2% fragile X; ■ and ● = mentally retarded with >2% fragile X. The vertical arrangement of alleles corresponds to the most likely phase in females. Phase is virtually known in family A, given the large size of the sibship. Recombination events are indicated by a bracket linking informative markers that flank the crossover (except in family B, in which a most likely phase cannot be defined for the fragile-X locus and its flanking markers).

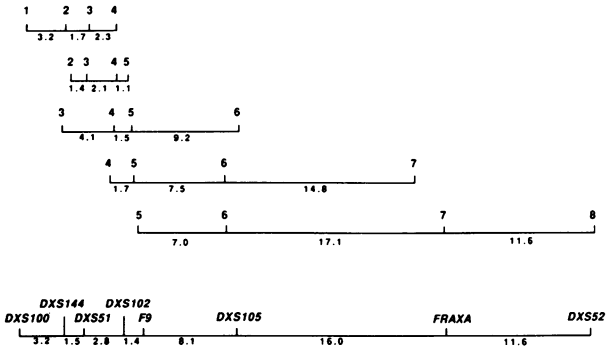

Figure 5 Genetic map of the Xq26-q28 region. Genetic map distances are derived on the basis of the four-point linkage tests indicated. Numbering of loci is as follows: 1, DXS100; 2, DXS144; 3, DXS51; 4, DXS102; 5, F9; 6, DXS105; 7, FRAXA; and 8, DXS52. Estimation of recombination frequency (indicated in % under each interval) was obtained using the ILINK program. The bottom map is a summary of all data.

less clear in family B, since there were two recombinations in four meioses between DXS105 and FRAXA, whereas the data with F9, being phase unknown, could be interpreted as being either 3 or 1 recombinant (the latter hypothesis would seem more likely in view of the previous knowledge on F9-FRAXA linkage [Oberlé et al. 1986*b*; Brown et al. 1987*a*]). However, if marker-to-marker and marker-to-disease data are taken into account in multipoint linkage analysis, it can be seen that DXS105 is distal to F9 and proximal to the fragile X and that all other orders are excluded (table 4, fig. 5). These results place DXS105 at 16% recombination from the fragile-X locus. The contradiction with the two-point results is only apparent, since there was a bias in the selection of the families used to analyze segregation for DXS105 (see above) whereas all families are included in the multipoint analysis.

For the *Bst*XI RFLP detected by probe 767 (DXS115), we obtained only limited data from two fragile-X families that showed complete cosegregation between DXS115 and DXS52 (St14-1) in 17 meioses, including meioses with recombination between DXS52 and the fragile-X locus (fig. 4*A*). These data suggest that DXS115 is part of the q28 cluster of loci that contains DXS52, HEMA, G6PD, and many other probes or disease loci (Szabo et al. 1984; Oberlé et al. 1985*b*; Oberlé and Mandel 1986; Patterson et al. 1987) but do not provide information on order within this cluster. Finally, a single family was informative for the very rare *Bcl*I RFLP detected by probe 780 (DXS119). One meiosis that showed recombination between fragile X and DXS119 also showed recombination with DXS105 and DXS144 (fig. 4C). This single event suggests a localization proximal to FRAXA but does not allow further ordering with the other markers.

Discussion

Our study has allowed us to better characterize markers in the Xq27-q28 region by (1) providing new informative RFLPs and (2) ordering the markers in a precise genetic map. We have shown by both two-point and multipoint mapping that DXS102 is very closely linked to the hemophilia B (F9) locus (z = 13.6 at θ = .02), and we were able to define the order DXS51-DXS102-F9. This order also agrees with physical mapping results obtained by means of analysis of a panel of rearranged X chromosomes (I. Oberlé, B. Arveiler, A. Vincent, and J. L. Mandel, unpublished data). Previous data of Connor et al. (1987) had placed DXS102 proximal to F9 but could not order it with respect to DXS51. Their linkage data would suggest a much greater distance between DXS102 and F9 (they obtained five recombinants in 23 meioses). It should also be noted that rather different results have been reported with respect to the map distance between F9 and DXS51. Although our data (θ = .03–.04) agree with those of Drayna and White (1985) (one recombinant in 55 meioses in normal families) and Brown et al. (1987*a*) (z = 8.86 at θ = .02 in fragile-X families), several groups reported much larger recombination values of .15–.22 (Davies et al. 1985; Connor et al. 1987; Veenema et al. 1987). It remains to be established whether this apparent discrepancy is due to small-sample variation, typing errors, or heterogeneity in the map distances found in different families. It should be noted in this respect that in the single large family analyzed by Veenema et al. (1987) (a family that includes family F2 of Davies et al. [1985]), there are eight recombinants in 32 informative meioses between DXS51 and F9.

On the basis of our genetic and physical mapping results, we suggest that, when factor IX probes are not informative (which is the case in approximately one-third of the families), DXS102 could be used as an alternative marker for diagnostic purposes in hemophilia-B families. This is made practical by the fact that both *Taq*I and *Dde*I detect RFLPs at the F9 and DXS102 loci (Camerino et al. 1983; Winship et al. 1984), allowing use of the same blots for the two loci. However, heterozygosity at the DXS102 locus is only 26% when the *Taq*I RFLP is used, and, owing to linkage disequilibrium, the new *Bst*XI and *Dde*I RFLPs described here do not appear to improve this figure. Mulligan et al. (1987) reported very recently that the DXS99 locus, corresponding to an informative *Sst*I RFLP, is very close to the hemophilia-B locus ($z[\hat{\theta}]$ = 9.79 at $\hat{\theta}$ = .0) and proposed its use as an alternate marker for diagnosis. However, in the absence of multipoint analysis and given the 95% confidence limits on their estimation of θ (.0–.06), it cannot be concluded whether this marker is closer to F9 than DXS102 or even than DXS51.

By means of segregation analysis in a single large family, DXS105 previously has been shown to map between F9 and the fragile-X locus (Veenema et al. 1987). In this family, DXS105 appeared to be close to the fragile-X locus (z = 5.01 at θ = .04) and at 8% recombination from F9. For eventual use of this marker in a diagnostic context it was important to establish the validity of this result in a larger set of families. Our data confirm the order F9-DXS105-FRAXA and the map distance between F9 and DXS105 ($z[\theta]$ = 14.62 at θ = .08). However, we find a much larger recombination fraction between DXS105 and the fragile-X locus (θ = .16 in multipoint linkage analysis). Some families show high recombination between DXS105 and FRAXA (see family B of fig. 4, in which there are two recombinants out of four meioses in a single sibship). Significant heterogeneity of recombination in different families previously had been shown by analysis of the F9-FRAXA linkage, and data were consistent, with ~20% of the families showing very tight linkage and 80% showing loose linkage (Brown et al. 1987*a*, and in press). Our results, combined with those of Veenema et al. (1987), suggest that the region responsible for this phenomenon is between DXS105 and the fragile-X locus, since the recombination that we have observed between FRAXA and DXS105 over all families is much larger than that recombination between F9 and FRAXA in tightly linked families.

We have performed heterogeneity tests by using the HOMOG program (Ott 1985, pp. 97–119, 200–203) (results not shown). Analysis of the three-point linkage data obtained for F9-DXS105-FRAXA in our set of families also indicated the existence of heterogeneity, although formal statistical significance was not reached (P = .1 if one assumes 2 df). On the other hand, data for the DXS52-FRAXA linkage indicated homogeneity, confirming earlier observations (Oberlé et al. 1986*b*; Brown et al. 1987*a*). The looser linkage (compared with the results of Veenema et al. [1987]) that we observed for DXS105-FRAXA is also consistent with the ~30% recombination found between DXS105 and DXS52 in normal and fragile-X families (see tables 3, 4). It should be noted that we

did not detect significant differences for the recombination fractions between the markers DXS51, F9, DXS105, and DXS52 when data from normal and fragile-X families were computed separately in a four-point analysis (results not shown).

Since DXS105 is closer than the F9 locus on the proximal side of FRAXA, it should become an important marker for genetic analysis in fragile-X families—for example, in (1) diagnosis of female carriers who do not express the fragile site and (2) detection of normal male transmitters. However, its use was limited by the low informativeness of the *Taq*I RFLP detected by probe cX55.7. Our finding of new RFLPs with either *Hin*dIII or *Eco*RI, which increase heterozygosity to .52, thus represents a significant improvement.

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