Physical and Genetic Mapping of Polymorphic Loci in Xq28 (DXS15, DXS52, and DXS134): Analysis of a Cosmid Clone and a Yeast Artificial Chromosome

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Summary
Sequences corresponding to the Xq28 loci DXS15, DXS52, DXS134, and DXS130 were shown to be present in a 140-kb yeast artificial chromosome (YAC XY58, isolated by Little et al.). This YAC clone appears to contain a faithful copy of this genomic region, as shown by comparison with human DNA and with a cosmid clone that contains probes StI1c (part of the DXS52 sequences) and cpX67 (DXS134). cpX67 and StI1c are contained in 11 kb and detect the same MspI RFLP polymorphism. A comparison of the YAC restriction map and pulsed-field gel electrophoresis data leads us to propose the following order of loci: DXS52(VNTR)–DXS33–DXF2253–DXSI10–DXS134–DXS125–DXS15–DXS52, this whole cluster being comprised within 575 kb. The physical proximity of the DXS15, DXS52, and DXS134 loci led us to reinvestigate recombination events that had been reported between these loci in families from the Centre d'Étude du Polymorphisme Humain. Our results do not support the assumption that this region shows increased recombination.

Introduction
The Xq28 region of the human X chromosome contains many disease loci. The biochemical defect of most of these diseases is poorly known, and the loci have been mapped genetically with respect to polymorphic markers in this region, notably DXS15 and the hyper-variable locus DXS52. (Mandel et al. 1989). For the localization of these disease loci, detailed and unambiguous genetic and physical maps are necessary. Physical mapping by pulsed-field gel electrophoresis (PFGE) has allowed the linking of key DNA sequences within two clusters. The first one was shown to contain the polymorphic loci DXS15, DXS33, DXS52, and DXSI34 (Patterson et al. 1987; Bell et al. 1989). However, one complicating factor is the dispersion, within this region, of the sequence family detected by the StI1c probe, which defines the DXS52 locus (Arveiler et al. 1989; Bell et al. 1989). The second cluster contains several well-known genes encoding the red and green color pigments, glucose 6-phosphate dehydrogenase, and coagulation factor VIII (Arveiler et al. 1989). The genetic map has been the object of some controversy concerning both the relative order of the two clusters with respect to the telomere and the recombination fraction between loci.

The cloning of large DNA fragments in yeast artificial chromosomes (YAC) appears a powerful tool to map such regions of the human genome (Burke et al. 1987), and it is important to establish at this stage the fidelity of the technique. A library of YAC clones for the Xq24–q28 region is being constructed from a somatic hybrid cell line (Little et al. 1989). A YAC clone containing sequences detected by the StI4-I and DXI3 probes (DXS52 and DXS15) has been isolated by Little et al. (1989). This clone was further shown to contain DXS130 and DXS134 (Wada et al. 1990). Here we describe the detailed physical mapping of both this clone and an independently selected cosmid clone. This allowed us
to locate precisely DXS15, DXS10, and DXS134 and one polymorphic member of the DXS52 family. We report also genetic mapping information related to these loci.

**Material and Methods**

Cosmid 33-3A1 was isolated from a nonamplified 49XXXXY genomic DNA cosmid library, constructed by Heilig et al. (1987), screened with the St14-I probe (a 3-kb EcoRI fragment at the DXS52 locus). The 49XXXXY DNA, used to make this cosmid library and used as control DNA in Southern blot experiments, originates from the lymphoblastoid cell line GM1202 (Human Genetic Mutant Cell Repository, Camden, NJ). The recombinant yeast clone XY58 has been isolated by Little et al. (1989), by screening with St14-I and DX13, and was provided to us by Dr. D. Schlessinger (St. Louis). Yeast cells were grown in minimal medium (uracil minus); yeast DNA was isolated according to the protocol of Holm et al. (1986). Isolation of genomic DNA, electrophoresis, blotting (on diazobenzyloxymethyl paper or on Hybond-N), and hybridization were performed according to a method described elsewhere (Oberlé et al. 1986a). Probes were labeled by random priming (Feinberg and Vogelstein 1983). The following probes (shown with their sources) were used to hybridize genomic blots: FIX-P1 (F9 locus) (Oberlé et al. 1986b), St14-I (3-kb EcoRI fragment), and St14c (10-kb EcoRI fragment), the latter two being from the DXS52 locus (Oberlé et al. 1986b); cX55.7 (DXS105), cpX6 (DXS130), and cpX67 (DXS134) (Hofker et al. 1987); and MN12 (DXS33) (Patterson et al. 1987), G1.3c (DXF2253) (Bardoni et al. 1988), DX13 (DXS15), and S2A (DXS51) (Drayna et al. 1984). DNA from 3-generation pedigrees was obtained from the Centre d’Etude du Polymorphism Human (CEPH). For restriction mapping of YAC XY58, high-molecular-weight DNA from YAC XY58 was digested to completion with BamHI, BssHII, MluI, NotI, NruI, PvuI, SacII, SalI, SfiI, or XhoI. Indirect end-label mapping of XY58 was carried out after partial digestion with increasing concentrations of BamHI, SacII, and XhoI. PFGE was performed using a CHEF apparatus. Agarose gels (1%) were run at 13°C in 0.5 × TBE buffer (20 × TBE = 1 M Tris, 830 mM boric acid, 10 mM EDTA) at 160 V for 15 h; pulse times were 2.5–7.5 s. Size-fractionated DNA was transferred to nylon membranes (ONCOR SUREBLOT) in denaturing solution (0.5 N NaOH, 1 M NaCl) after an initial 15-min depurination in 0.2 N HCl. Filters were prehybridized in 1 M NaCl, 1% SDS, 10% dextran sulfate at 65°C for 2 h; denatured probe (2 × 10^6 cpn/ml) and herring sperm DNA (75 µg/ml) were added, and hybridization was for 20 h. Washing was performed as for the genomic Southern blots.

**Results**

**Mapping of XY58 and Comparison with a Cosmid Clone That Contains the St14c and cpX67 Sequences**

The St14-I probe (a 3-kb EcoRI fragment at the DXS52 locus) was used to screen a cosmid library (Heilig et al. 1987), yielding cosmid 33-3A1 which contains a 43-kb insert. This cosmid was studied in comparison with YAC XY58, described by Little et al. (1989) as hybridizing to the St14-I and DX13 (DXS15) probes, and it was further shown to contain probes cpX6 and cpX67 (Wada et al. 1990).

Probes St14-I and cpX67 (DXS134) hybridized to restriction fragments that have identical sizes in the cosmid and YAC clones (results not shown, see fig. 1 for results on XY58). However, a 10-kb EcoRI fragment is detected by St14-I, which does not correspond to the cognate fragment (3 kb). This suggested that the cosmid and YAC clones contain the St14c fragment, the first probe cloned from the St14 sequence family (Oberlé et al. 1985). Indeed, St14c hybridized to the cosmid (not shown) and to XY58, detecting a fragment of the same size as the cognate EcoRI fragment in genomic DNA (fig. 1A). XY58 contains only a single EcoRI fragment detected by St14-I or St14c, while in genomic DNA these probes detect as many as six EcoRI fragments. Thus, only a minor part of the St14 sequence family is present within XY58, as already has been noted by Little et al. (1989). In particular the VNTR region responsible for the multiallelic RFLP at the DXS52 locus (Mandel et al. 1986; Heilig et al. 1987) is not present in XY58 (it is 2 kb from St14-I; authors’ unpublished data). Sequences corresponding to cpX6 (DXS130) and DX13 were present only in YAC XY58. The probes MN12 (DXS33) and G1.3c (DXF22S3, a member of a small dispersed X-specific sequence family), both known to be physically linked to the DXS52 locus (Patterson et al. 1987; Arveiler et al. 1989), were absent from both cosmid 33-3A1 and YAC XY58.

In order to determine the fidelity of the sequences inserted in YAC XY58, we compared the St14c, cpX67c, and DX13-hybridizing fragments in genomic DNA and in YAC XY58. All St14c-hybridizing XY58 fragments
Figure 1  Comparison between genomic and YAC XY58 restriction fragments detected by probes St14c and St14-l (DXS52), cpX67 (DXS134), and DX13 (DXS15). A, Southern blot with BamHI, HindIII, TaqI, and EcoRI digests of a control male (lanes 1), the 49XXXXY cell line GM1202 (lanes 2), and YAC XY58 (lanes Y). Lanes 1 and 2 contain 10 μg digested DNA, and lanes Y contain 0.2 μg digested DNA. This Southern blot was hybridized with the St14c probe. B–D, Autoradiographs of the same blot as in fig. 1A, hybridized with the probes St14-l, cpX67, and DX13, respectively. The cpX67 hybridizing genomic DNA fragments are faint or not visible in fig. 1C; on the original X-ray film all hybridizing YAC XY58 fragments show a clearly visible corresponding genomic fragment. Sizes of marker DNA bands are indicated in kilobases. V = fragment giving a positive hybridization with pBR322 vector sequences. * = fragment which hybridizes with both St14c and cpX67; R = fragment hybridizing to probe R (the amplified 500-bp end fragment located at the right arm side of YAC XY58).
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are also present in genomic DNA. The Stl4-I probe detects in XY58 a subset of the fragments that hybridize to Stl4c (fig. 1B).

The cpX67 probe also hybridized to the same fragments in genomic DNA and in XY58. cpX67 and Stl4c detected common fragments, i.e., a 6.6-kb BamHI and a 10-kb HindIII fragment (marked with an asterisk in fig. 1C). In the HindIII digest of XY58, cpX67 hybridizes to the 10-kb fragment and to a 11-kb fragment. This means that cpX67 and Stl4c are contained within an 11-kb region.

Stl4-I, Stl4c, and cpX67 detected fragments which have the same size in genomic DNA as in XY58, but this was not the case for the DX13 probe. The BamHI and TaqI fragments detected by DX13 have a different size in the YAC clone than in genomic DNA, while the 2.2-kb EcoRI fragment (which corresponds to the probe itself) and the HindIII fragment have the same size in the two DNAs (fig. 1D). Two of the abnormal fragments (the 4.5-kb BamHI fragment and the 1.6-kb TaqI fragment, labeled V in fig. 1D) also hybridize to pBR322. These fragments thus contain both DX13 and YAC vector sequences (pYAC-4 contains pBR322-derived sequences [Burke et al. 1987]). As the library from which XY58 was isolated was constructed by cloning a partial EcoRI digest in the EcoRI site of pYAC-4 (Little et al. 1989), it appeared likely that the DX13 2.2-kb EcoRI fragment lies at one of the two extremities of the XY58 insert. We amplified the two extremities of the insert by using a recently developed technique based on the polymerase chain reaction (R. Heilig, unpublished data). We obtained a 1,200-bp fragment for the left arm (L) and a 500-bp fragment for the right arm (R). (The left arm of the vector contains the ampicillin-resistance sequences.) L, used as probe, did not hybridize to XY58 restriction fragments which are recognized by Stl4c, Stl4-I, cpX67, or DX13. In all four digests the R probe hybridized to restriction fragments recognized by DX13 (in fig. 1D these fragments are indicated by the letter R). In the BamHI and TaqI digests, only the fragments that contain both pYAC-4 vector sequences and DX13 sequences hybridized to the R probe. Furthermore, R detects the same BglII RFLP as DX13 (not shown). We conclude that the DX13 fragment lies at the extremity of the YAC XY58 insert (on the R side).

To further search for possible rearrangements within XY58, we compared the 43-kb insert of cosmID 33-3A1 with the corresponding region of XY58. DNA of XY58 and of cosmID 33-3A1 was digested with four restriction enzymes, and fragments hybridizing to the whole cosmID 33-3A1 were compared. All but one of the cosmID fragments which do not hybridize to vector DNA have the same size as in XY58 DNA (fig. 2). The exception was a 3.6-kb TaqI restriction fragment that also hybridizes to the Stl4c probe. This fragment and a 1.9-kb TaqI fragment observed in XY58 might correspond to alleles of a TaqI RFLP detected by the Stl4c probe. We conclude that the insert sequences in cosmID 33-3A1 are entirely contained in XY58 and that the YAC clone is not rearranged for these 43 kb.

**Restriction Mapping of the YAC Clone**

We performed detailed restriction mapping of XY58

![Figure 2](image-url)  
**Figure 2** Comparison of fragment size in XY58 and cosmID 33-3A1. DNA from cosmID 33-3A1 and YAC XY58 DNA was digested with BamHI, HindIII, EcoRI, and TaqI and hybridized to the entire cosmID 33-3A1. Length of the size-marker fragments are indicated in kilobases. Y = YAC XY58 DNA (1 µg/lane); C = cosmID 33-3A1 DNA (10 ng/lane); V = fragment hybridizing to cosmID vector sequences; * = fragments that differ in size between the two clones. These fragments hybridize to Stl4c and might represent variants of the genomic DNA (see Mandel et al. 1986).
constructed gouspart of the polylinker of cosmid 724.

Figure that partial cisely includesthe Patterson high tween is found, aszymes, veileret digested studies. Since DX13 and found to vector map performed three density (and sites, are analyzed bythesites (DXS134, Sf1i and Stl4c (DXS52), and DX13 (DXS15), as well as to vector probes corresponding to L or R of YAC. For three additional enzymes (BamHI, SacII, and Xbol) partial digestion and mapping by indirect end-labeling was performed (using the vector probes).

This placed the DXS134–DXS52 (Stl4c) cluster between DXS130 and DXS15, at about 40 kb from the latter (and from the right end of the YAC clone). A very high density of sites for "rare-cutter" enzymes was found, confirming and extending the observations of Patterson et al. (1987), especially in the region that includes the Stl4c probe, which was therefore more precisely mapped in the cosmid 33-3A1. The 5-kb segment that contains the Stl4-I cross-hybridizing sequences contains three SfiI sites, two MluI sites, one NotI, and one SacII site. The latter two sites are often found in CpG (HTF) islands (Lindsay and Bird 1987). Another potential CpG island (with one BssHII site and one SacII site) is found 12 kb from the NotI site, in the direction of DXS15.

Stl4c Detects the Same RFLP as cpX67

Our analysis showed that Stl4c and cpX67 are comprised within 11 kb. Stl4c was previously reported to detect three MspI RFLPs (Oberlé et al. 1985). The independently isolated cpX67 probe also detects an MspI RFLP with allelic fragments estimated at 3.7 and 3.4 kb (Hofker et al. 1987). These fragments fall in a similar size range as the allelic MspI fragments 1 and 2 detected by Stl4c (previously estimated at 4.4 and 3.6 kb). We verified that, in MspI digests of genomic DNAs, allelic fragments 1 and 2 detected by Stl4c are the same as the ones detected by cpX67 (fig. 4). It should be noted that the second MspI polymorphic system detected by Stl4c (allelic fragments 3 and 4) was found to be in very strong linkage disequilibrium with the VNTR RFLP and is within 5 kb of Stl4-I (Oberlé et al. 1985).

Reinvestigation of Two CEPH Pedigrees Reported to Show Recombination between DXS15 and DXS52 or DXS134

Because Patterson et al. (1987) reported that the physical distance between Stl4 sequences and DX13 could be as little as 60 kb, it was suggested that this region contains a hot spot of recombination (Bell et al. 1989). In the first genetic map of the X chromosome (Drayna and White 1985) the map distance between DX13 and Stl4 was reported to be 5.5 cM, while only 0.06 cM would be expected on the basis of the average relationship of 1 cM/1,000 kb. However, the only three recombinations in 54 informative meiosis occurred in the same

![Figure 3](image-url)  
**Figure 3** Restriction maps of YAC XY58 and cosmid 33-3A1. The upper part shows the rare-cutter restriction map of YAC XY58 constructed by using PFGE; the black boxes indicate sequences which contain the DXS130, DXS134, DXS52 (Stl4c), and DXS15 loci. Arrowheads indicate BamHI restriction sites. L and R = left-arm side and right-arm side, respectively, of YAC XY58. The rare-cutter restriction map of cosmid 33-3A1 is shown beneath; the box indicates the DXS134-DXS52 (Stl4c) cluster, with cpX67 directly adjacent to the Stl4c fragment. The black and the striped parts of the box correspond to the Stl4c fragment, with the striped part representing the Stl4-I homologous part of Stl4c. The small, open part of the box at the left indicates the cpX67 fragment. S and C = Sall side and Clal side, respectively, of the polylinker of cosmid 33-3A1; B = BstHII; C = Clal; M = MluI; N = NotI; Nr = NruI; P = PvuII; S = Sall; Sa = SacII; Sf = SfiI; X = XhoI.
and DXS15 and one between DXS52 and both DXS15 and DXS134. We have retyped this pedigree (fig. 5) and found one recombinant between DXS52 and both DXS15 and DXS134 but none between DXS134 and DXS15. Furthermore, individual 9 of this pedigree, reported to present a recombination event between the loci F9 and DXS105, was typed by us as being a recombinant between DXS105 and DXS52. (As an internal check, DXS52 was typed both on the TaqI blot also used for F9, DXS51, and DXS105 and on the MspI blot used for DXS134.)

Thus, of the five recombination events that in CEPH families were reported to occur between DXS52, DXS15, and DXS134, only one has been confirmed. This weakens the support for a hot spot of recombination between these physically closely linked markers.

Discussion

Our analysis of a YAC containing well-known markers from the Xq28 region has allowed us to clarify their respective positions. This illustrates well the usefulness of this new technology for genome analysis. Comparison of selected regions of the YAC clone with corresponding regions in either genomic DNA or a cosmid suggests that the YAC clone contains a faithful representation of human sequences.

Recently, Bell et al. (1989) reported two recombination events in a CEPH pedigree: one between DXS134 and DXS15 and another between DXS15 and DXS52 in another X-chromosome genetic map based on the same data (Donis-Keller et al. 1987). In the genotype data base of the CEPH (version 1) we identified this peculiar family as family 1377. Since, if true, this typing could have been due to either a chromosomal inversion or some other kind of rearrangement, we retyped the family. Our DXS15 typing agreed with that reported in the CEPH data base, but the typing for DXS52 (St14c probe) differed for three DNAs, and, as a result, no recombination was found between the two loci (this could be further checked by using a probe nearer to the VNTR at DXS52, which allowed the typing on the same BglII digests as used for the DX13 typing).

Figure 4  An MspI RFLP detected by St14c that is identical to the MspI RFLP detected by cpX67. A Southern blot with MspI-digested DNA of five female individuals (1–5) was hybridized to St14c and cpX67. The St14c probe detects three MspI RFLP systems: a first system with alleles 1 and 2 (4.4 and 3.6 kb), a second system with alleles 3 and 4 (2.0 and 1.6 kb), and a third system which concerns the presence/absence of allele 5 (1.0 kb) (also see Oberlé et al. 1985).

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Figure 4  An MspI RFLP detected by St14c that is identical to the MspI RFLP detected by cpX67. A Southern blot with MspI-digested DNA of five female individuals (1–5) was hybridized to St14c and cpX67. The St14c probe detects three MspI RFLP systems: a first system with alleles 1 and 2 (4.4 and 3.6 kb), a second system with alleles 3 and 4 (2.0 and 1.6 kb), and a third system which concerns the presence/absence of allele 5 (1.0 kb) (also see Oberlé et al. 1985).

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A single member of the St14 sequence family was found in XY58, that corresponds to the 10-kb segment (St14c) originally described by Oberlé et al. (1985). Since St14c is at 40 or 100 kb of either ends of the clone, this confirms that the St14 family is dispersed, as proposed on the basis of pulsed-field mapping studies (Arveiler et al. 1989; Bell et al. 1989). In particular, the VNTR region responsible for the multiallelic polymorphism at the DXS52 locus (Mandel et al. 1986) is not present in XY58. We have shown that DXS134 and St14c are adjacent fragments, within 11 kb. DXS134 previously had been reported to be within the same BssHII 300-kb fragment as an unidentified member of the St14 family (Bell et al. 1989). In fact, one of the three MspI RFLPs described for St14c (Oberlé et al. 1985) is the same as the RFLP independently described for cpX67 (Hofker et al. 1987). St14c is also 30 kb from the probe DX13 (DXS15). This confirms and extends the findings of Patterson et al. (1987) showing linkage within 60 kb of DXS15 and a member of the St14 family. It is astonishing that four independently derived probes are contained within the 140-kb region. Given the pool of mapped X-specific probes (~200–250) from which the presented studies were drawn, an average of 1 probe/1,000 kb would be expected. The four probes have been isolated from two libraries by using two different strategies (Davies et al. 1981; Oberlé et al. 1985; Hofker et al. 1987). An overrepresentation of probes for a region of chromosome 21 has also been observed (Gardiner et al. 1988). This suggests some bias in probe isolation, possibly favoring regions of the genome which are less methylated. For both libraries, mcr A + B + bacterial strains were used, which contain restriction systems that degrade DNA methylated at CpG dinucleotides. It recently has been shown that the use of mcr A + B + host strains and packaging extracts considerably reduces the efficiency of cloning methylated DNA (Kretz et al. 1989).

The mapping of a NotI site and a BssHII site between St14c and DXS15 clarifies the map of the region. Bell et al. (1989) have shown that DXS15 and DXS134 are on separate NotI and BssHII fragments, each of them hybridizing to the St14-l probe. The cpX67-hybridizing BssHII fragment of Bell et al. (1989) appears identical to a fragment hybridizing both to the St14-l probe and to G1.3c, a member of a dispersed X-specific sequence family. Furthermore, probe MN12 (DXS33) was found on a third 150–180-kb BssHII fragment, together with another St14 sequence (Patterson et al. 1988; Arveiler et al. 1989; Bell et al. 1989). This fragment contains the VNTR sequence (Bell et al. 1989) and can be further cleaved in some cell lines by a BssHII site that separates DXS33 from the St14 sequence (Arveiler et al. 1989; Bell et al. 1989). Their relative order is given by a 400-kb SfiI fragment common to DXS33 and DXS134 (Bell et al. 1989). One of the SfiI sites is within St14c, and the finding that the 400-kb SfiI fragment does not hybridize to St14-l is not contradictory, since the part of the St14c fragment at the left of the SfiI site does not hybridize to St14-l (fig. 3). The three BssHII fragments must be contiguous, since a 575-kb BssHII fragment (a result of incomplete digestion) contains DXS15, DXS33, DXS52, and DX22S3 (Arveiler et al. 1989).

The final map of the region is shown in figure 6. The St14 family appears dispersed over a 575–600-kb region in Xq28. The signification of this organization is not known, but it might be of interest to point out that two other sequence families appear dispersed in regions of the X chromosome: the G1.3 family (one member

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**Figure 6** Summary of the DXS52-DXS15 region. Restriction sites for BssHII (B), NotI (N), and SfiI (S) are indicated. The BssHII site in parentheses is cleaved only in some cell lines, while the BssHII site marked with a square was not cleaved detectably in the same studies (Arveiler et al. 1989; Bell et al. 1989). The localization of the asterisked SfiI site is only based on the size (400 kb) of the DXS33 and DXS134 hybridizing fragment and is therefore not precise. The arrowed bars indicate the range for localization of various markers. The region cloned in YAC XY58 is represented by a heavy line. B = BssHII; S = SfiI; N = NotI.
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is in the Xq28 region analyzed here, but the others are in Xp22.2-p22.3 (Bardoni et al. 1988; Ballabio et al. 1989) and the ornithine amino transferase pseudogene family in Xp21.1-11.2 (Lafreniere et al. 1989). The St14 sequence is conserved in evolution (Mandel et al. 1986), and this was the basis of the mapping of corresponding sequences on the mouse X chromosome. The presence of probable CpG islands within the St14c fragment (NotI and SacII sites) is an additional element suggesting that the St14 family is an expressed-gene family.

When the proximity of St14 sequences and DXS15 was demonstrated, it was suggested that the region might be unusually prone to recombination (Patterson et al. 1987; Brown et al. 1988; Bell et al. 1989). We know now that the VNTR polymorphism that defines genetically the DXS52 locus is 400–500 kb from DXS15.

The recombination between DXS15 and DXS52 has been reported, in various studies, to be 1–5 cM (Drayna and White 1983; Brown et al. 1988; Lehesjoki et al. 1989). In our own studies we found a maximum lod score $z = 41.44$ at 0.6 cM (I. Oberlé, unpublished data). Here we have shown that four of the five recombination events previously reported between DXS15 and DXS52 in CEPH families cannot be confirmed. Fewer data are available for the linkage of DXS134 to either DXS15 or DXS32, and one should perhaps reinvestigate the large family that showed rather high recombination between DXS52 and DXS134 ($\theta_{max} = .15$) or between DXS15 and DXS134 ($\theta = .08$) (Veenema et al. 1987). At present we think there is little support for the existence of a particularly high recombination rate in this region.

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References

Arveiler B, Vincent A, Mandel JL (1989) Toward a physical map of the Xq28 region in man: linking color vision, G6PD and coagulation factor VIII genes to an X-Y homology region. Genomics 4:460–471

Ballabio A, Carrozzo R, Andria G, Persico G, Bick DP, Ropers HH, Ferguson-Smith MA, et al (1989) Deletion map of the Xp22.3-pter region obtained by the study of patients with contiguous gene syndromes. Human Gene Mapping 10. Cytogenet Cell Genet 51:93–7

Bardoni B, Guioli S, Raimondi E, Heilig R, Mandel JL, Ottolenghi S, Camerino G (1988) Isolation and characterization of a family of sequences dispersed on the human X chromosome. Genomics 3:32–38

Bell MV, Patterson MN, Dorkins HR, Davies KE (1989) Physical mapping of DXS134 close to the DXS52 locus. Hum Genet 82:27–30

Brown WT, Gross A, Chan C, Jenkins EC, Mandel JL, Oberlé I, Arveiler B, et al (1988) Multilocus analysis of the fragile X syndrome. Hum Genet 78:201–205

Burke DT, Carle GF, Olson MV (1987) Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. Science 236:806–812

Davies KE, Young BD, Ellis RG, Hill ME, Williamson R (1981) Cloning of a representative genomic library of the human X chromosome after sorting by flow cytometry. Nature 293:374–376

Donis-Keller H, Green P, Helms C, Cartinhour S, Weißenbach B, Stephens K, Keith TP, et al (1987) A genetic linkage map of the human genome. Cell 51:319–337

Drayna D, Davies K, Hartley DA, Mandel JL, Camerino G, Williamson R, White R (1984) Genetic mapping of the human X chromosome by using restriction fragment length polymorphisms. Proc Natl Acad Sci USA 81:2836–2839

Drayna D, White R (1985) The genetic linkage map of the human X chromosome. Science 230:753–758

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137:266–267

Gardiner K, Watkins P, Münke M, Drabkin H, Jones H, Patterson D (1988) Partial physical map of human chromosome 21. Somatic Cell Mol Genet 14:623–638

Heilig R, Lemaire C, Mandel JL (1987) A 230 kb cosm id walk in the Duchenne muscular dystrophy gene: detection of a conserved sequence and of a possible deletion prone region. Nucleic Acids Res 15:9129–9142

Hofker MH, Bergen AAB, Skraastad MI, Carpenter NJ, Veenema H, Connor JM, Bakker E, et al (1987) Efficient isolation of X chromosome–specific single-copy probes from a cosm id library of a human X/hamster hybrid-cell line: mapping of new probes close to the locus for X-linked mental retardation. Am J Hum Genet 40:312–328

Holm C, Meeks-Wagner DW, Fangman WL, Botstein D (1986) A rapid efficient method for isolating DNA from yeast. Gene 42:169–173

Kretz PL, Reid CH, Greener A, Short JM (1989) Effect of lambda packaging extract mcr restriction activity on DNA cloning. Nucleic Acids Res 17:5409

Lafreniere RG, Mahtani MM, Brown CJ, Sharp CB, Davies
KE, Willard HF (1989) Assignment of DNA markers of the pericentromeric region of the human X chromosome. Human Gene Mapping 10. Cytogenet Cell Genet 51:1028
Lehesjoki AE, Sistonen P, Rasi V, de la Chapelle A (1989) Linkage and gene order studies in hemophilia A. Human Gene Mapping 10. Cytogenet Cell Genet 51:1031
Lindsay S, Bird AP (1987) Use of restriction enzymes to detect potential gene sequences in mammalian DNA. Nature 327:336–338
Little RD, Porta G, Carle GF, Schlessinger D, d’Urso M (1989) Yeast artificial chromosomes with 200–800 kilobase inserts of human DNA containing HLA, Vk, S5, and Xq24-28 sequences. Proc Natl Acad Sci USA 86:1598–1602
Mandel JL, Arveiler B, Camerino G, Hanauer A, Heilig R, Koenig M, Oberlé I (1986) Genetic mapping of the human X chromosome: linkage analysis of the q26-q28 region that includes the fragile X locus and isolation of expressed sequences. Cold Spring Harbor Symp Quant Biol 51:195–196
Mandel JL, Willard HF, Nussbaum RL, Romeo G, Puck JM, Davies K (1989) Report of the Committee on the Genetic Constitution of the X Chromosome. Human Gene Mapping 10. Cytogenet Cell Genet 51:384–437
Oberlé I, Drayna D, Camerino G, White R, Mandel JL (1985) The telomeric region of the human X chromosome long arm: presence of a highly polymorphic DNA marker and analysis of recombination frequency. Proc Natl Acad Sci USA 82:2824–2828
Oberlé I, Camerino G, Kloepfer C, Moisan JP, Grzeschik KH, Hellkühl B, Hors-Cayla MC (1986a) Characterization of a set of X-linked sequences and of a panel of somatic cell hybrids useful for the region mapping of the human X chromosome. Hum Genet 72:43–49
Oberlé I, Heilig R, Moisan JP, Kloepfer C, Mattei MG, Mattei JF, Boué J, et al (1986b) Genetic analysis of the fragile-X mental retardation syndrome with two flanking polymorphic DNA markers. Proc Natl Acad Sci USA 83:1016–1020
Patterson M, Bell M, Schwartz C, Davies K (1988) Pulsed-field gel mapping studies in the vicinity of the fragile site at Xq27.3. Am J Med Genet 30:581–591
Patterson M, Kenwrick S, Thibodeau S, Faulk K, Mattei MG, Mattei JF, Davies KE (1987) Mapping of DNA markers close to the fragile site on the human X chromosome at Xq27.3. Nucleic Acids Res 15:2639–2651
Veenema H, Carpenter NJ, Bakker E, Hofker MH, Millington Ward A, Pearson PL (1987) The fragile X syndrome in a large family. III. Investigations on linkage of flanking DNA markers with the fragile site Xq27. J Med Genet 24:413–421
Wada M, Little RD, Abidi F, Porta G, Labella T, Cooper T, Della Valle G, et al (1990) Human Xq24-Xq28: approaches to mapping with yeast artificial chromosomes. Am J Hum Genet 46:95–106