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A Contiguous, 3-Mb Physical Map of Xq28 Extending from the Colorblindness Locus to DXS15

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Summary

Using pulsed-field gel electrophoresis (PFGE), we have generated a 3-megabase (Mb) physical map of Xq28, a region of the human genome known to contain many disease loci. We have determined the location of the genes for protan/deutan colorblindness (R/GCP), factor VIII (F8), glucose-6-phosphate dehydrogenase (G6PD), and a series of RFLPs and have derived the following order for this region: R/GCP–GdX–G6PD–F8–DXS115–DXS33–DXS134–DXS15. Using newly isolated probes, we have also established the direction of transcription of F8 within the map and have localized CpG islands flanking this gene.

Introduction

Many X-linked diseases map to the subchromosomal region Xq28, distal to the fragile site at Xq27.3 (reviewed in Davies et al. 1988). Cloned DNA is available for only three of these loci, hemophilia A (factor VIII [F8]; Gitschier et al. 1984; Wood et al. 1984), glucose-6-phosphate dehydrogenase deficiency (G6PD; Persico et al. 1986), and protan/deutan colorblindness (R/GCP for red and green cone pigments; Nathans et al. 1986*b*). Other diseases of unknown biochemical basis, including adrenoleukodystrophy (Aubourg et al. 1987), Emery-Dreifuss muscular dystrophy (Boswinkel et al. 1985), nephrogenic diabetes insipidus (Knoers et al. 1988), dyskeratosis congenita (Connor et al. 1986), Hunter syndrome (Upadhyaya et al. 1986), and a form of spastic paraplegia (Kenwrick et al. 1986), are closely linked to RFLPs in Xq28 or to the protein polymorphism exhibited by G6PD.

Attempts have been made by genetic analysis to determine relative positions for disease loci and for RFLPs in Xq28. However, segregation analysis has yielded little information regarding probe order, because of the paucity of multiply informative individuals. Documented examples include recombinants that place F8 distal to St14 (DXS52) and DX13 (DXS15) (Mulligan et al. 1987; Patterson et al. 1989), and others that put F8 proximal to these probes (Gross et al. 1988). Multipoint linkage analysis has also provided contradictory results about the relative order of these loci (Upadhyaya et al. 1986; Hofker et al. 1987). Precise localization of disease genes in Xq28 will not be achieved until a consistent order for genetic markers in this region is established.

Pulsed-field gel electrophoresis (PFGE) has provided the means for generating large-scale restriction maps following fragmentation with restriction enzymes that cleave DNA infrequently. Physical mapping of Xq28 by PFGE has been initiated by Patterson et al. (1987*a*, 1987*b*, 1988). In these studies two clusters of Xq28 loci were identified. The genes for F8, G6PD, and anonymous expressed sequence GdX were physically linked, within 650 kb, and polymorphic loci MN12 (DXS33) and DX13 (DXS15) were found to bracket St14 within a distance of 550 kb. We have extended these studies to include RFLP markers 767 (DXS115) and cpX67 (DXS134) and the R/GCP locus, which varies in size in both normal and color-blind individuals (Nathans et al. 1986*a*, 1986*b*; Vollrath et al. 1988). The resultant map links the DX13/St14/MN12 cluster and R/GCP to the F8/G6PD/ GdX cluster, to provide a contiguous, large-scale map of about 3 megabases (Mb).

A number of important conclusions can be derived

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from the generation of this map. First, a comparison of physical and genetic distance is possible. Second, flanking markers for disease loci such as hemophilia A can be determined. Finally, the ascertainment of probe order will allow a more directed search for other disease loci.

Material and Methods

Cell Lines

Lymphoblastoid cell lines were maintained using standard methods. Cells with the karyotype 49,XXXXY (GM1202A) were acquired from NIGMS Human Genetic Mutant Cell Repository, Camden, NJ. Lymphoblastoid cell line L558, derived from a hemophilia patient with a complete F8 gene deletion, was obtained from Drs. Mario Pirastu and Emilia Vitale (Casarino et al. 1986). B cell lines from male (A and B) and female individuals with a normal karyotype were also used.

DNA Probes

The DNA probes used for the present study are listed in table 1. All DNA probes were incorporated with ^{32}P by using the Amersham multiprime labeling kit (Feinberg and Vogelstein 1983) to a specific activity of at least 10^9 cpm/μg.

Pulsed-Field Gel Electrophoresis

Lymphocytes, isolated from whole blood by using ficoll/hypaque, or cultured cells were suspended at a final concentration of 1.25×10^7/ml in 0.5% low-melting-point agarose (Ultrapure; BRL). These blocks were processed for restriction digestion according to a method described elsewhere (Kenwrick et al. 1987). Digested samples were run on a CHEF DRII electrophoresis system (Biorad) using running conditions as recommended by the manufacturer. Unless otherwise stated, gels were 1% agarose and electrophoresis was at 200 V and 14°C. Additional details for individual gels are given in the figure legends. For molecular-weight markers, lambda concatamers and chromosomes from either *Saccharomyces cerevisiae* (YNN.295) or *Candida albicans* were used (Clontech).

Southern Blot Analysis

Gels were acid treated (0.1 M HCl, 2 × 20 min) before conventional denaturation and neutralization for Southern blotting onto Hybond® N filters (Amersham). The filters were UV-irradiated (1 min, shortwave UV) and baked for 1 h at 80°C. Hybridization conditions were as described elsewhere (Kenwrick et al. 1987). Filters were washed at high stringency (0.2 × SSC, 0.1% SDS, 65°C). All radioactive signal was removed by boiling in distilled water between sequential hybridizations, and removal was verified by exposure to film.

Results

An Xq28 physical map resulting from the data in the present paper is summarized in figure 1, where loci are shown above their respective *NotI* fragments. Subse-

Table 1

Xq28 DNA Probes

Probe	Locus	Size/Description	Reference
hs7	R/GCP	1.4 kb/cDNA of red pigment gene	Nathans et al. 1986*b*
767	DXS115	1.3 kb/RFLP marker with homologous copy in intron-22 of F8 gene	Hofker et al. 1985 Patterson et al. 1989
Gd512	GdX	.9 kb/cDNA probe from a transcribed gene located 40 kb downstream of G6PD gene	Martini et al. 1986
G6PD	G6PD	3.0 kb/cDNA of G6PD gene	Persico et al. 1986
625.8	F8	3.5 kb/CpG island probe located 30 kb 3′ of F8 gene	J. Gitschier, unpublished data
F8	F8	9 kb/cDNA of F8 gene	Wood et al. 1984
MN12	DXS33	2.8 kb/RFLP marker	Patterson et al. 1987*a*
DX13	DXS15	2.0 kb/RFLP marker	Harper et al. 1984
St14	DXS52	3.0 kb/RFLP marker	Oberle et al. 1985
cpX67	DXS134	.6 kb/RFLP marker	Hofker et al. 1987
700.1	F8	.6 kb/CpG island sequence located 4 kb 5′ of F8 gene	Present paper, fig. 1
701.1	F8	1.3 kb/Single-copy sequence located 17 kb 5′ of F8 gene	Present paper, fig. 1
710.1	DXS115	1.3 kb/Single-copy sequence located 6 kb from polymorphic copy of 767	Present paper, fig. 1

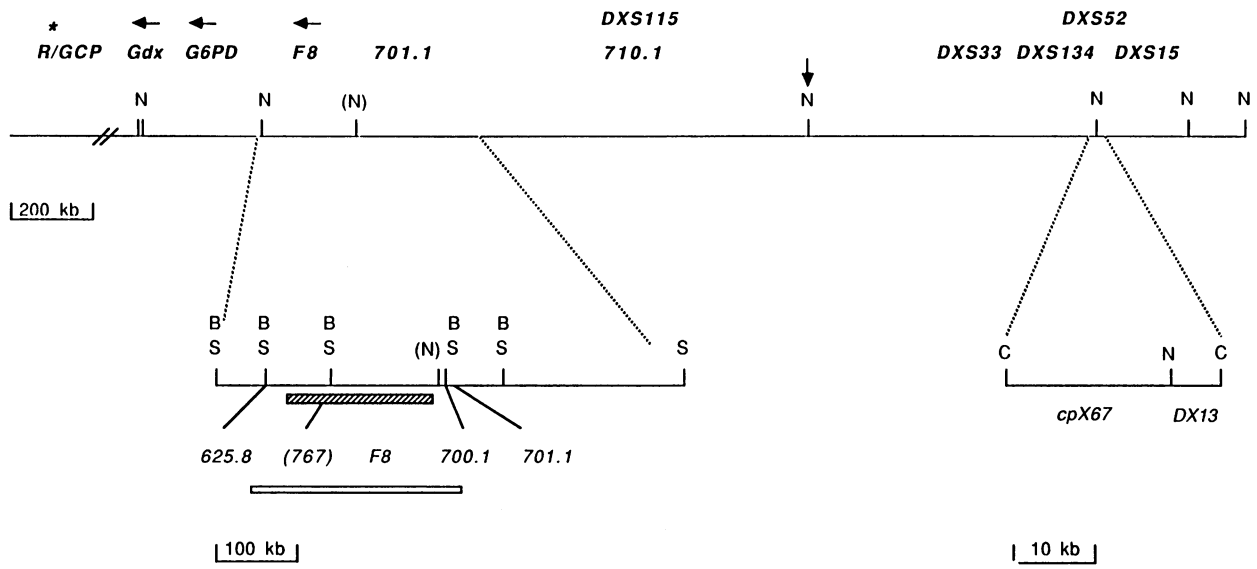

Figure 1 Physical map representing more than 3 Mb of Xq28. Loci are positioned above their respective *Not*I (N) fragments. An asterisk (*) indicates that the size of the fragment for R/GCP is variable and may be separated from GdX by more than one site (see text). The horizontal arrows indicate the direction of transcription for GdX, G6PD, and F8 genes, and references in the text to 5′ or upstream are relative to this orientation. The *Not*I site that is digested in the female DNA (see text and fig. 2) is indicated in parentheses. The vertical arrow points to a *Not*I site is absent or undigested in DNA from patient L558. Two sections of the map are enlarged to show the positions of additional restriction-enzyme sites: *Bss*HII (B) and *Sac*II (S) for the F8 region and *Cla*I (C) for the cpX67/DX13 region. For the F8 map, the open box represents the extent of a chromosomal walk and the hatched box shows the position of the F8 gene. Five CpG islands, indicated by the presence of both *Bss*HII and *Sac*II sites, are shown. The F8 sequence homologous to 767 is located a few kilobases downstream of the internal CpG island. The close proximity of the cpX67 (DXS134) and DX13 (DXS15) loci within a small *Cla*I fragment is illustrated. Size scales for the *Not*I map and the two enlarged sections are indicated.

quent sections will describe the generation of this map by using pulsed-field gel electrophoresis with the series of probes listed in table 1. In addition to previously described Xq28 markers, we have employed the following sequences generated during a chromosomal walk around the F8 gene: 625.8, 700.1 and 701.1. The relative positions of these probes are indicated in figure 1 and are described in table 1. The RFLP marker 767 has a homologous but nonidentical copy within the F8 gene (Patterson et al. 1989). To facilitate unambiguous mapping of the polymorphic copy of the probe, we have isolated a single-copy sequence (710.1) that is 6 kb from 767 and is not homologous to part of the F8 gene. This sequence was isolated from a library of size-selected fragments, chosen to contain only the non-F8 copy of 767 (S. Kenwrick, unpublished data).

Linkage of the F8 Gene to 767

Previous attempts to link the polymorphic probe 767 to other sequences in Xq28 have been complicated by the presence of a homologous, nonpolymorphic copy of the probe within intron 22 of the F8 gene (Patterson et al. 1989). Using DNA probe 710.1, described above, we show that both copies of 767 and the F8 gene reside within a *Not*I fragment of about 1.3 Mb. To confirm this linkage we have utilized DNA from a hemophilia patient (L558) that is deleted for the whole of the F8 gene (and hence for the intronic copy of 767) but that retains 625.8 (a sequence 30 kb downstream of F8) and the polymorphic version of 767. DNA probes were sequentially hybridized to the Southern blot of *Not*I-digested DNA shown in figure 2.

For normal individuals (fig. 2, lanes 1, 3, and 4) a *Not*I fragment of about 1.3 Mb is detected by 710.1 and all sequences from the F8 region. This band is partially dissected in the female DNA in lane 3 to yield 1.1-Mb and 230-kb fragments. The 1.1-Mb band is detected by 710.1 and 701.1, and the 230-kb band is detected by F8 and 625.8. Predictably, 767 identifies all three bands in this sample, because of its duplication within this region (not shown). The fragmentation of the female DNA is due to cleavage at a *Not*I site 1.7 kb 5′ to the first exon of the F8 gene (see fig. 1, *Not*I site in parentheses). This site was identified during a walk from the F8 gene, and a probe upstream of this site, 701.1, identifies the 1.1-Mb and not the 230-kb

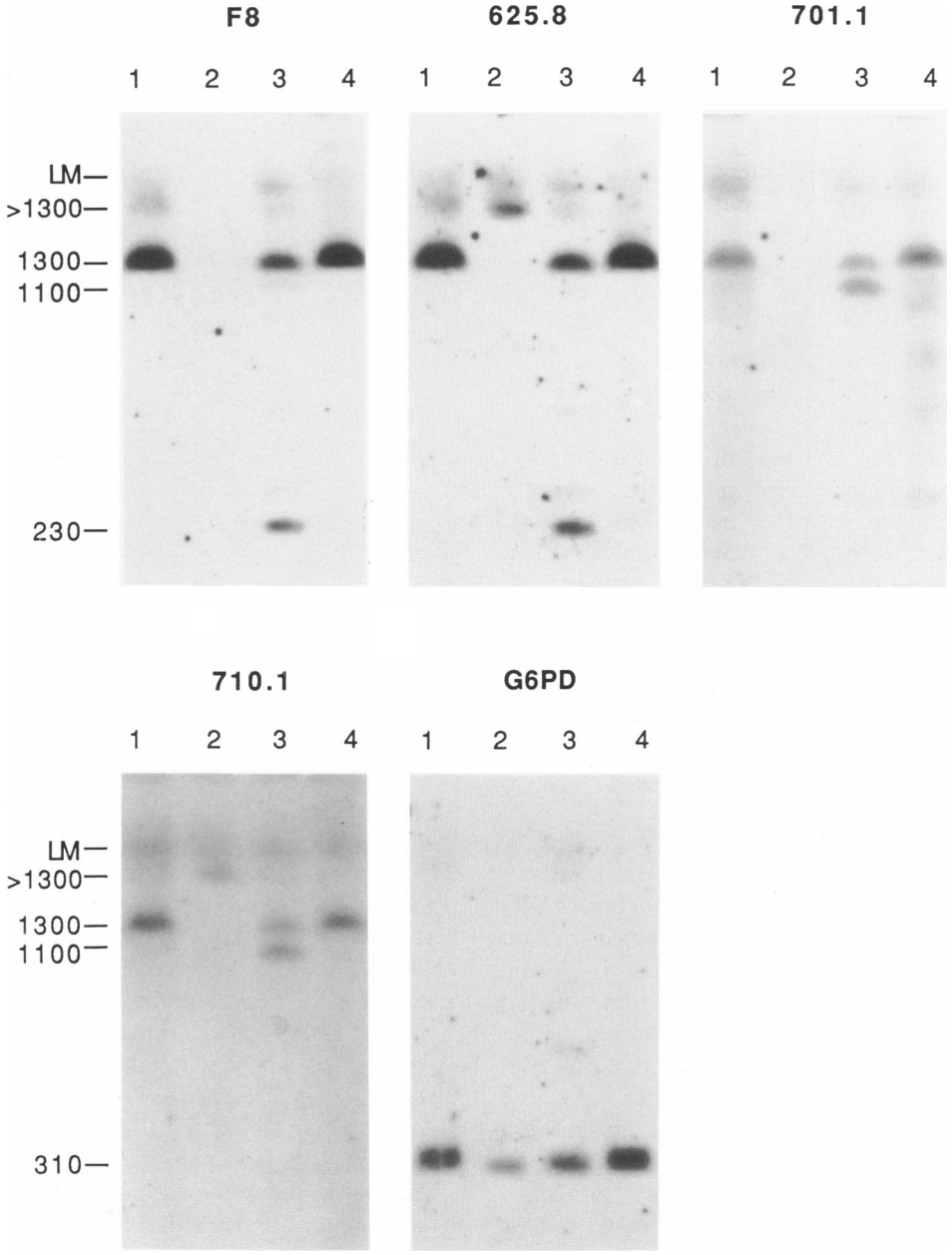

Figure 2 Linkage of 767 to F8. Five DNA probes, identified above each autoradiograph, were hybridized to *Not*I-digested DNA from lymphoblastoid cell lines. Lane 1, male A; lane 2, L558; lane 3, female; lane 4, male B. Samples were resolved using 70-s pulse time for 16 h followed by 120 s for 14 h. Fragment sizes in this and all subsequent figures were estimated by comparison with lambda concatamers and chromosomes from YNN.295 yeast, and all are given in kilobases. The size of the large altered fragment in lane 2 cannot be accurately determined but is smaller than a yeast chromosome of 1,600 kb. LM is the region of limited mobility which contains unresolved DNA. As for all Southern blots shown in the present paper, the origin is represented by the top of the photograph.

band. We were able to confirm this interpretation with conventional agarose gels by showing that an 18-kb *Bam*HI fragment identified by 700.1 is partially cleaved by *Not*I to generate a 1.5-kb fragment in DNA from the individual in lane 3 (data not shown). Furthermore, 710.1 and 625.8 identify the same altered band of approximately 1.5 Mb in the hemophilia patient DNA, a result that would be expected for probes linked on a single *Not*I fragment. An increase rather than a decrease in the size of the band indicates that, in DNA

from this patient, a flanking *NotI* site is either absent or not cleaved (potentially because of polymorphism or increased methylation). Further evidence to support this conclusion will be presented in the next section. Linkage of the 767 region to F8 is also supported by the finding that 710.1 and F8 identify a 1.3-Mb fragment in DNA from two peripheral blood lymphocyte samples (data not shown). The results presented above are summarized in the composite map in figure 1.

Linkage of the MN12/DX13 Cluster to F8

MN12 and DX13 were also hybridized to the *NotI* Southern blot described above, and the results are shown in figure 3. DX13 reveals 220-kb and 370-kb bands in all DNA samples tested, although differences in the degree of digestion around this sequence are apparent. The smallest fragment identified by MN12 is 700 kb. This band and partial bands of 950 kb and 1,100 kb, also observed using DX13, are not present for the patient DNA (L558) in lane 2. In this case MN12 identifies the same large fragment (~1,500 kb) observed previously for 710.1 and 625.8 (sequences that bracket the deletion). This result implies that the *NotI* site, discussed in the previous section and that is absent or refractory to digestion in L558 DNA, lies between 710.1 and MN12 (indicated by a vertical arrow in fig. 1) and that these two sequences are linked within a distance of approximately 1.8 Mb, the sum of the smallest fragments seen when these probes are used. The linkage of MN12 to 767, however, must be considered tentative until such a linkage can be confirmed in normal DNA.

Orientation of the F8 Gene

From the results presented in figure 2 we can also determine the orientation of the F8 gene within the *NotI* map. Probes upstream of F8 hybridize to the 1.1-Mb band detected by 710.1 in the female sample, whereas G6PD reveals a band of 300 kb. GdX, G6PD, and F8 have been shown to be linked, in that order, within a distance of 650 kb by using the enzyme *NruI* (Patterson et al. 1987*b*). Gd512 is routinely found to identify both an 8-kb *NotI* band on conventional Southern blots and a partial band of about 300 kb when PFGE is used. In combination, these result show that the direction of transcription for F8 is the same as that for G6PD.

Linkage of cpX67 to DX13, MN12, and St14

The following evidence shows that RFLP marker cpX67 lies between probes DX13 and MN12. In *NotI* digests, cpX67 identifies a 700-kb fragment identical in size to that observed when MN12 is used, whereas

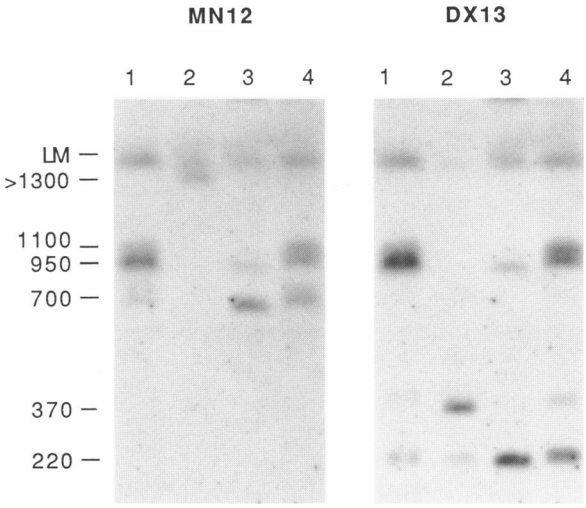

Figure 3 Linkage of MN12 and DX13. MN12 and DX13 were hybridized to the same Southern blot described in fig. 2. Note that the patient DNA in lane 2 possesses an altered fragment in place of the normal band for MN12.

DX13 reveals a band of 220 kb (fig. 4*a*). However, when enzyme *ClaI* is used, cpX67 identifies the same three-band pattern as does DX13 (fig. 4*b*). The smallest of these bands is smaller than 50 kb. We have analyzed this linkage further by using double digestion with *NotI* and *ClaI*. DX13 and cpX67 reveal bands of 6 kb and 20 kb, respectively, following digestion with both of these enzymes, indicating that they lie within 26 kb of each other. Furthermore, we observe an identical pattern with DX13 and cpX67 in *MluI* digests, including a partial band of 550 kb in common with MN12. The close linkage of DX13 and cpX67 is a surprising result, given a level of 3% recombination between these loci (Lehesjoki et al., submitted).

The highly polymorphic probe St14 hybridizes to *NotI* fragments in common with both MN12 and DX13, confirming that several copies of this sequence are distributed across this region (data not shown). No additional St14-specific fragments are observed. The exact location of the polymorphism detected by St14 within the cluster MN12, cpx67, and DX13 cannot be determined at this stage.

Linkage of the Colorblindness Cluster to G6PD

We have exploited the variation in size exhibited by the R/GCP gene cluster to establish linkage of this locus to GdX, a gene 40 kb downstream of G6PD. Initially, hs7, Gd512 (cDNA probes from R/GCP and GdX loci, respectively), and G6PD sequences were hybridized to

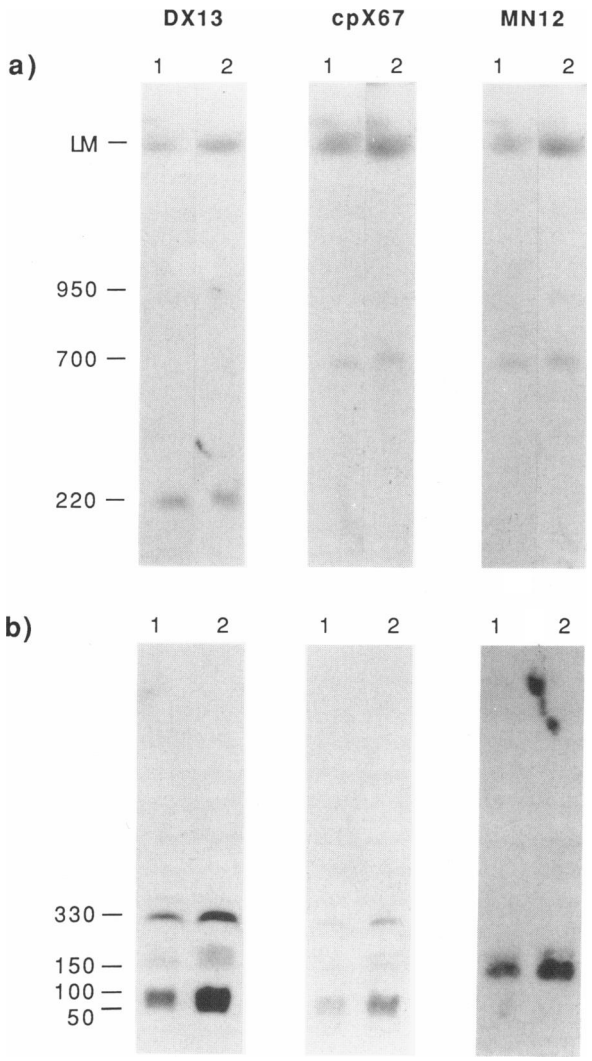

Figure 4 Linkage of cpX67 to DX13 and MN12. *a,* Southern blot showing hybridization of DX13, cpX67, and MN12 to *Not*I-digested cell line DNA, resolved using a 120-s pulse time and 150 V for 50 h. Lane 1, male A; lane 2, female; *b,* Hybridization of the same three probes to *Cla*I digests of DNA from cell lines separated for 24 h by using 70-s pulse time with 200 V. Lane 1, female; lane 2, GM1202A. LM is the region of limited mobility.

*Cla*I digests of DNA from normal female and GM1202A (49XXXXY) cell lines (fig. 5*a*, lanes 1 and 2, respectively). The partial bands shared by hs7 and Gd512 differ in size between the two samples. The common bands are 320 kb and 400 kb, respectively. In lane 2, faint partial bands of 750 kb and 900 kb are also shared. Smaller bands revealed by Gd512 are the same size for both samples and are not revealed by hs7. Several of these (100 kb, 200 kb, and 250 kb) are identified by G6PD, which lies only 40 kb upstream of GdX, indicating that the GdX locus lies between R/GCP and G6PD.

This suggestion of linkage between Gd512 and hs7 was investigated further by using *Cla*I-digested DNA from the peripheral blood lymphocytes of a series of color-blind individuals. We would predict that partial bands revealed by hs7 should exhibit a wide variation, because of rearrangements affecting the size of the R/GCP cluster, and that partial bands revealed by Gd512 should show a concomitant variation if the two loci are closely linked. Figure 5*b* shows that such a covariation of partial bands is observed (indicated by *), confirming close linkage of the GdX and R/GCP loci. In this experiment an internal control for artifactual variation is that the smallest bands identified by Gd512 are the same size (120 kb and <50 kb) for all DNA samples. The differences in digestion pattern between DNA in figures 5*a* and 5*b* may be due either to differences in methylation at specific *Cla*I sites for cell lines versus lymphocytes isolated from blood or to the presence of inactive X chromosomes in female and GM1202A cells. It is difficult to derive a *Cla*I map based on these results, because of the number of partially digested sites in this region. Additional experiments indicate that *Cla*I bands as small as 4.5 kb are observed when Gd512 is used. This high density of sites may be a reflection of the CpG islands in the vicinity of the GdX locus (Toniolo et al. 1988). Although variation in the size of the R/GCP cluster was reflected by the sizes of the *Not*I fragments for these samples, we were unable to link hs7 and Gd512 by using this enzyme, resulting in a break in the *Not*I map shown in figure 1. We can conclude, however, from the *Cla*I data described above that the R/GCP and GdX loci are within 320 kb of each other.

CpG Islands Flanking the F8 Gene

Five CpG islands are in the region of the F8 gene. One of these is located within intron 22 of the F8 gene and has been shown to be associated with a transcript (Levinson et al. 1988). Two additional islands, represented by sequences 700.1 and 625.8 (fig. 1), have been isolated during genomic walks from both ends of the locus. Following digestion with *Sac*II and *Bss*HII, two more CpG islands, 70 kb 5′ and 90 kb 3′ to F8, are revealed by hybridization with these probes. The relative positions of islands associated with the F8 gene are shown in figure 1. The possibility that the four flanking islands are expressed is being examined.

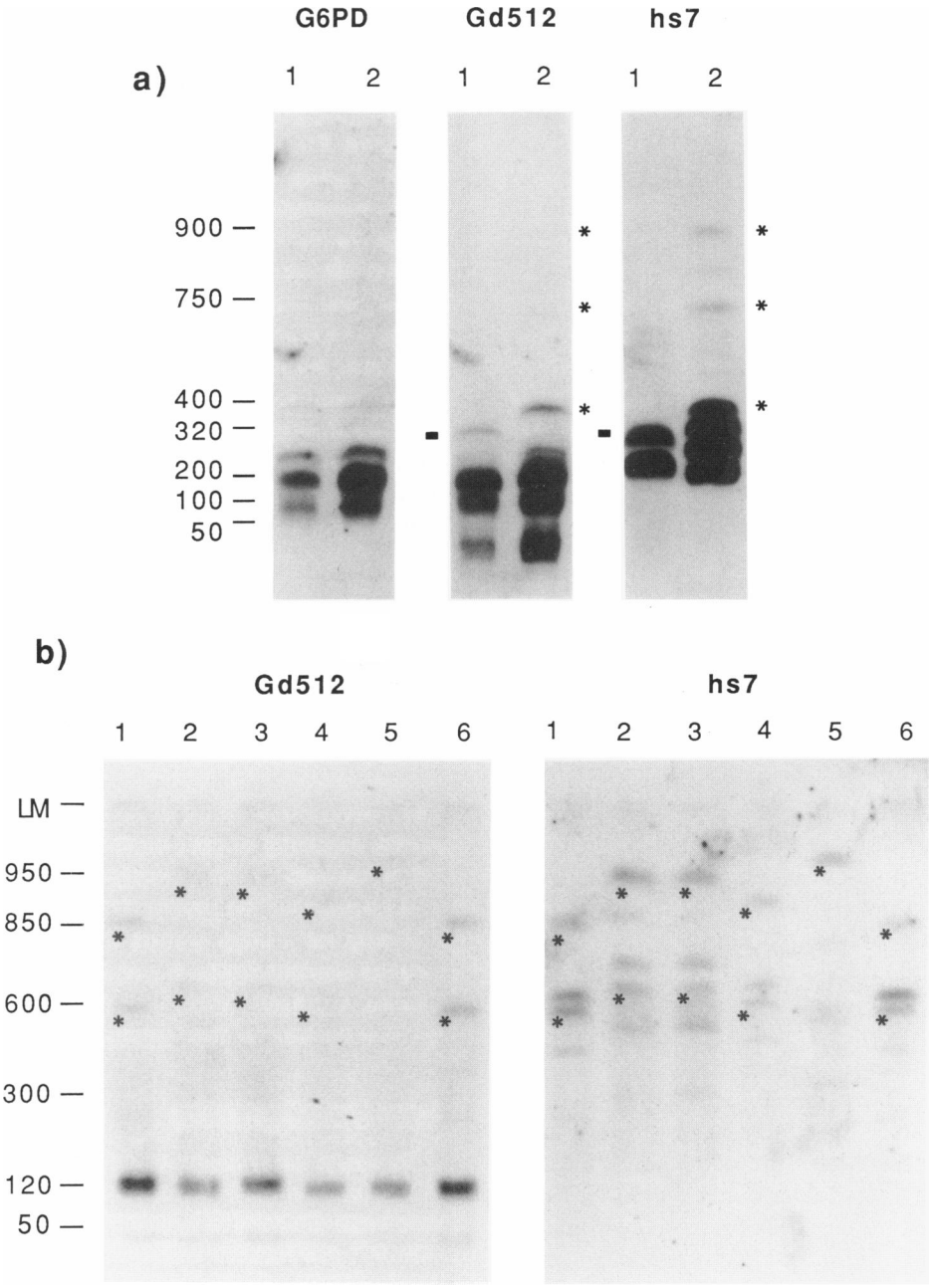

Figure 5 Linkage of colorblindness locus to GdX. *a,* Hybridization of G6PD, Gd512, and hs7 to the Southern blot of *Cla*I-digested DNA described in fig. 4*b*. Lane 1, female; lane 2, GM1202. Horizontal bars and asterisks indicate the positions of bands of equivalent size for Gd512 and hs7 in DNA samples from female (lane 1) and GM1202 (lane 2) cell lines, respectively. *b,* Southern blot of *Cla*I-digested DNA from white blood cells from a series of red/green color-blind individuals. Samples were resolved for 24 h by using 70-s pulse time and hybridized sequentially to Gd512 and hs7. Asterisks (*) highlight the fragments that cohybridize to the two probes.

Discussion

We have generated a 3-Mb physical map around the F8 gene incorporating polymorphic loci frequently used for analysis of diseases in Xq28. According to a recent estimate of 5–6 Mb for the size of Xq28 (Warren et al. 1989), the map presented here represents a significant fraction of this region. A number of disease loci, e.g., adrenoleukodystrophy and Emery-Dreifuss muscular

dystrophy, are closely linked to F8 and St14 and therefore may lie within this 3 Mb of DNA. The physical order for polymorphic markers will allow the positioning of recombinational events during segregation analysis and hence assist in the localization of these disease genes within Xq28. A comparison of genetic and physical distance for this region is also now possible; for example, a genetic distance for the interval St14 to F8 has been estimated as 3% recombination (z = 14.62) (Mulligan et al. 1987), whereas the physical data presented here suggest that they are 1–2 Mb apart. A more surprising result is that DX13 and cpX67 are less than 30 kb apart, as several crossovers have been observed between these two probes (Hofker et al. 1987; Lehesjoki et al., submitted). This interval may, therefore, contain a hot spot for recombination. From the probe order generated, we can also determine that polymorphic probes from the 767 and colorblindness loci flank the F8 gene, a result that will complement the use of intragenic probes for hemophilia A.

During the preparation of the present paper, Arveiler et al. (1989) reported evidence for physical linkage of GdX and R/GCP within a distance of 750 kb by using the enzyme *Cla*I and DNA from GM1202. The data we present from use of the same enzyme and a series of DNA samples from red/green color-blind individuals show that the distance between these sequences is maximally 600 kb. In our hands, use of GM1202 and a cell line from a normal female refined this distance to as little as 320 kb.

We have also used the results of physical mapping experiments to determine the positions of a number of CpG islands near the F8 gene. These represent potentially expressed—and therefore candidate—genes for disease loci in Xq28. Although one of these islands is located only 4 kb 5′ to the first exon of F8, it is not associated with the expression of F8.

It is tempting to speculate that a region for which contradictory results have been presented with respect to probe order may be subject to gross rearrangement leading to a difference in physical order from family to family. Relevant to this is the fact that many of these studies, as well as the multipoint linkage analyses, have been conducted in families with a genetic disorder in Xq28, often the fragile X syndrome. During our analysis using a variety of DNA sources, we have found no evidence to support a variation of probe order. However, a much more extensive analysis including DNA from the controversial families would be needed to address this question fully. Demonstration of physical linkage for the sequences in the presented map has for the most part been confirmed by using a series of DNA samples. The exception is the linkage of 767 to the MN12 cluster, which was observed when DNA from a hemophilia patient with a deletion of the F8 gene was used. The possibility remains that this patient possesses a gross rearrangement of this region, resulting in an abnormal probe order. One argument against this is that the *Not*I fragments for G6PD and DX13 are unaltered. We are currently examining this linkage further.

It is clear from the generated map that some regions contain a greater number of polymorphic probes than do others; for example, the St14 region is rich in markers, whereas the interval between F8 and St14 is not. In order to improve the possibility of localizing disease genes, more probes should be isolated from the stretches of DNA that are underrepresented by RFLPs. Now that a physical map has been established, libraries greatly enriched for these regions can be generated using PFGE fractionation of DNA from human/rodent hybrids containing part or all of a single X chromosome (Anand et al. 1988). This approach could also be used to generate markers from the ends of this physical map, in order to link the map to probes lying proximal to the fragile site, and to the Xq telomere. In addition, obtaining cDNAs for expressed sequences associated with CpG islands may result in the isolation of single-copy probes dispersed across a much larger region of genomic DNA because of the presence of introns.

In conclusion, the generation of this contiguous physical map for a large section of Xq28 will allow both a better interpretation of genetic data and a more systematic approach to the isolation of candidate genes for disease loci in this region.

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References

Anand R, Honeycombe J, Whittaker PA, Elder JK, Southern EM (1988) Clones from an 840-kb fragment containing the 5′ region of the DMD locus enriched by pulsed field gel electrophoresis. Genomics 3:177–186

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

Aubourg PR, Sack GH, Meyers DA, Lease JJ, Moser HW (1987) Linkage of adrenoleukodystrophy to a polymorphic DNA probe. Ann Neurol 21:349–352

Boswinkel E, Walker A, Hodgson S, Benham F, Bobrow M, Davies K, Dubowitz V, et al (1985) Linkage analysis using eight DNA polymorphisms along the length X chromosome locates the gene for Emery-Dreifuss muscular dystrophy to distal Xq. Cytogenet Cell Genet 40:586

Casarino L, Pecorara M, Mori PG, Morfini M, Mancuso G, Scrivano L, Molinari AC, et al (1986) Molecular basis for hemophilia A in Italians. Res Clin Lab 16:227

Connor JM, Gatherer D, Gray FC, Pirrit LA, Affara NA (1986) Assignment of the gene for dyskeratosis congenita to Xq28. Hum Genet 72:348–351

Davies KE, Mandel JL, Weissenback J, Fellous M (1988) Report of the Committee on the Genetic Constitution of the X and Y Chromosomes. Cytogenet Cell Genet 46:277–315

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Gitschier J, Wood WI, Goralka TM, Wion KL, Chen EY, Eaton DE, Vehar GA, et al (1984) Characterization of the human factor VIII gene. Nature 312:326–330

Gross AC, Brown WT, Ferrando CJ, Dobkin CS, Jenkins EC (1988) Linkage study of RFLP markers distal to fragile X. Am J Hum Genet 43:A145

Harper K, Winter RM, Pembrey ME, Hartley D, Davies KE, Tuddenham EGD (1984) A clinically useful DNA probe closely linked to haemophilia A. Lancet 2:6–8

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 cosmid 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

Hofker MH, Wapenaar MC, Goor N, Bakker B, van Ommen GJB, Pearson PL (1985) Isolation of probes detecting restriction fragment length polymorphisms from X chromosome specific libraries: potential use for diagnosis of Duchenne muscular dystrophy. Hum Genet 70:148–156

Kenwrick S, Ionasescu VV, Ionasescu G, Searby C, King A, Dubowitz M, Davies KE (1986) Linkage studies of X-linked recessive spastic paraplegia using DNA probes. Hum Genet 73:264–266

Kenwrick S, Patterson MN, Speer A, Fischbeck K, Davies K (1987) Molecular analysis of the Duchenne muscular dystrophy region using pulsed-field gel electrophoresis. Cell 48:351–357

Knoers N, van der Heyden H, van Oost BA, Ropers HH, Monnens L, Willems J (1988) Nephrogenic diabetes insipidus: close linkage with markers from the distal long arm of the human X chromosome. Hum Genet 80:31–38

Lehesjoki AE, Sistonen P, Rasi V, de la Chapelle A. Hemophilia A: genetic prediction and linkage studies in all available families in Finland. (submitted)

Levinson B, Lakich D, Silvera P, Kenwrick S, Gitschier J (1988) A gene contained within a factor VIII intron is identified by a CpG island. Am J Hum Genet 43:A192

Martini G, Toniolo D, Vulliamy T, Luzzatto L, Dono R, Viglietto G, Paonessa G, et al (1986) Structural analysis of the X-linked gene encoding human glucose-6-phosphate dehydrogenase. EMBO J 5:1849–1855

Mulligan LM, Grover HJ, Blanchette VS, Giles AR, Lillicrap DL, Phillips A, Holden JJA, et al (1987) Recombination between the factor VIII gene and the DXS52 locus gives the most probable genetic order as centromere-Fra(X)-DXS15-DXS52-F8C-telomere. Am J Med Genet 26:751–760

Nathans J, Piantanida TP, Eddy RL, Shows TB, Hogness DS (1986*a*) Molecular genetics of inherited variation in human color vision. Science 232:203–210

Nathans J, Thomas D, Hogness DS (1986*b*) Molecular genetics of human color vision: the genes encoding blue, green, and red pigments. Science 232:193–202

Oberle 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

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, Gitschier J, Bloomfield J, Bell M, Dorkins H, Froster-Iskenius U, Sommer S, et al (1989) An intronic region within the human factor VIII gene is duplicated within Xq28 and is homologous to the polymorphic locus DXS115 (767). Am J Hum Genet 44:679–685

Patterson M, Kenwrick S, Thibodeau S, Faulk K, Mattei MG, Mattei JF, Davies KE (1987*a*) Mapping of DNA markers close to the fragile site on the human X chromosome at Xq27.3. Nucleic Acids Res 15:2639–2651

Patterson M, Schwartz C, Bell M, Sauer S, Hofker M, Trask B, van den Engh G, et al (1987*b*) Physical mapping studies on the human X chromosome in the region Xq27-Xqter. Genomics 1:297–306

Persico MG, Viglietto G, Martini G, Toniolo D, Paonessa G, Moscatelli C, Dono R, et al (1986) Isolation of human glucose-6-phosphate dehydrogenase (G6PD) cDNA clones: primary structure of the protein and unusual 5′ noncoding region. Nucleic Acids Res 14:2511–2522

Toniolo D, Martini G, Migeon BR, Dono R (1988) Expression of the G6PD locus on the human X chromosome is associated with demethylation of three CpG islands within 100 kb of DNA. EMBO J 7:401–406

Upadhyaya M, Sarfarazi M, Bamforth JS, Thomas NS, Oberle I, Young I (1986) Localization of the gene for Hunter syndrome on the long arm of X chromosome. Hum Genet 74:391–398

Vollrath D, Nathans J, Davis RW (1988) Tandem array of human visual pigment genes at Xq28. Science 240:1669–1672

Warren ST, Knight S, Zhang F, Peters JF, Stayton C, Sutcliffe J, Consalez G (1989) Molecular analysis of the human band Xq28. In: Genome mapping and sequencing. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p 126

Wood WI, Capon DJ, Simonsen CC, Eaton DL, Gitschier J, Keyt B, Seeburg PH, et al (1984) Expression of active human factor VIII from recombinant DNA clones. Nature 312:330–337