GENOMICS 6, 609–615 (1990)

DXS165 Detects a Translocation Breakpoint in a Woman with Choroideremia and a *de Novo* X;13 Translocation

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Received May 26, 1989; revised November 16, 1989

The search for the gene for choroideremia (MIM 30310), a rare retinal dystrophy, has been of great interest due to the existence of several choroideremia patients with well-defined structural chromosome aberrations, thus providing the basis for a reverse genetics approach to the isolation of this disease gene. This report details our molecular studies of a woman with choroideremia and a *de novo* X;13 translocation. Pulsed-field gel electrophoresis using a contour-clamped homogeneous electric field apparatus has allowed detection of the translocation breakpoint with the anonymous DNA marker p1bD5 (DXS165) and the mapping of this probe to within 120 kb of the breakpoint. In addition, we have used this probe to isolate a clone (pCH4) from a 100-kb jumping library which has crossed a rare-cutting restriction site (*Xho*I) between DXS165 and the choroideremia gene and detects the translocation breakpoint using this enzyme. Although DXS165 lies within 120 kb of the breakpoint and Cremers *et al.* (1987, *Clin. Genet.* 32: 421–423; 1989, *PNAS* 86: 7510–7514) have detected deletions of DXS165 in 3 of 30 choroideremia probands, we have detected no deletions of this marker or of pCH4 in 42 unrelated probands with this retinal disease.

INTRODUCTION

Progress in the characterization of large regions of the genome by reverse genetics techniques is of great interest because this approach to the cloning of human disease genes has been successful for relatively few loci. These include the genes for Duchenne muscular dystrophy (Koenig *et al.*, 1987), chronic granulomatous disease (Royer-Pokora *et al.*, 1986), retinoblastoma (Friend *et al.*, 1986; Lee *et al.*, 1987), and, more recently, cystic fibrosis (Rommens *et al.*, 1989; Riordan *et al.*, 1989; Kerem *et al.*, 1989). The molecular study of patients with structural chromosomal alterations and the identification of physical landmarks can be extremely important in precisely localizing and ultimately isolating human disease genes.

Choroideremia (MIM 30310) is a progressive X-linked retinal dystrophy that produces visual field constriction and night blindness beginning in adolescence and typically results in complete blindness in the fourth or fifth decade (McCulloch and McCulloch, 1948; Sorsby *et al.*, 1952). The gene for choroideremia has been mapped to Xq21.1–q21.2 by tight linkage to DXYS1 and DXS72 (Schwartz *et al.*, 1986; Nussbaum *et al.*, 1985; Gal *et al.*, 1986; Lesko *et al.*, 1987; MacDonald *et al.*, 1987; Sankila *et al.*, 1987). This map assignment has been confirmed by the identification of several individuals with choroideremia as part of a complex syndrome, including mental retardation, deafness, epilepsy, and cleft lip and palate, with deletions involving Xq21 (Rosenberg *et al.*, 1986; Schwartz *et al.*, 1986; Hodgson *et al.*, 1987; Nussbaum *et al.*, 1987; Cremers *et al.*, 1988, 1989a; Schwartz *et al.*, 1988). Three anonymous DNA markers, DXS95, DXS165, and DXS233, were shown to be deleted in the smallest region of overlap between all of these characterized deletions (Nussbaum *et al.*, 1987; Cremers *et al.*, 1987, 1989a). Through a combination of chromosome jumping and long-range restriction mapping with pulsed-field gel electrophoresis, more precise mapping of these markers and the genes for choroideremia and X-linked mixed deafness with perilymphatic gusher has been achieved (Cremers *et al.*, 1989a; Merry *et al.*, 1989). The recent identification of a woman with choroideremia and an X;13 translocation (Siu *et al.*, 1988) has

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provided an additional, precise landmark for the choroideremia gene. This woman, whose normal X chromosome is inactive (Siu *et al.*, 1988), has choroideremia presumably because the choroideremia gene has been disrupted or deleted by the translocation event.

The anonymous DNA locus DXS165 was shown by Cremers *et al.* (1987, 1989b) to be deleted in 3 of 30 probands with isolated choroideremia, and a clone generated by chromosome jumping from DXS165 has recently been shown to detect one additional deletion in such probands (Cremers *et al.*, 1989b). The smallest region of overlap between these deletions (15–20 kb) spans the t(X;13) breakpoint and lends support to the notion that a part or all of the choroideremia gene lies in the vicinity of the t(X;13) breakpoint. In this report, we describe the detection of the X;13 translocation breakpoint by p1bD5 (DXS165), using pulsed-field gel electrophoresis. Using chromosome jumping, we have isolated a DNA clone (pCH4) that has crossed an *Xho*I site and lies within 45 kb of the translocation breakpoint. We also show that neither DXS165 nor pCH4 is deleted in any of 42 unrelated simple choroideremia probands.

MATERIALS AND METHODS

Probes and Cell Lines

The establishment of lymphoblast and fibroblast cell lines from the t(X;13) proband [46,X,t(X;13)-(q21.2;p12)] has been previously described (Siu *et al.*, 1988). Hybrid CIII-1 is a mouse/human somatic cell hybrid constructed with fibroblasts from the translocation patient (Siu *et al.*, 1988) and selected in HAT medium for retention of the derivative chromosome 13, which contains the active HPRT gene. Cell line 790 is a mouse/human hybrid containing an intact human X chromosome. GM1416 is a lymphoblastoid cell line with 48,XXXX karyotype obtained from the Mutant Cell Repository, Institute for Medical Research.

The DNA probes used in this study were p1bD5 (DXS165) and pXG7c (DXS95). p1bD5 was subcloned from pUC8 into pGEM3-blue.

Southern Blot Analysis

High-molecular-weight DNA was isolated from cell lines according to Aldridge *et al.* (1984). DNA fragments were separated by electrophoresis in 0.8% agarose gels and transferred to nylon filters (Zetabind) with 10×SSC. Prehybridization and hybridization conditions have been described (Boggs and Nussbaum, 1984). DNA probes were uniformly labeled to a specific activity of 1×10^9 cpm/μg by the random priming method (Feinberg and Vogelstein, 1983).

Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis was carried out using a contour-clamped homogeneous electric field (CHEF) apparatus (Chu *et al.*, 1986). High-molecular-weight DNAs from GM1416 and t(X;13) lymphoblasts and from t(X;13) fibroblasts were prepared in agarose plugs (Bio-Rad) as previously described (Merry *et al.*, 1989). Conditions for enzyme digestion and Southern transfer have also been described (Merry *et al.*, 1989). Double enzyme digestions were performed sequentially. DNA fragments were electrophoresed on a CHEF apparatus using an 80-s switching interval at 200 V (for analysis of fragments up to 1000 kb in size) or a 35-s switching interval at 150 V (for analysis of fragments up to 400 kb in size) for 24–40 h. Temperature was maintained at 10°C. Hybridization and washing conditions have also been described (Boggs and Nussbaum, 1984). DNA probes were uniformly labeled to a specific activity of 1×10^9 cpm/μg by the random priming method (Feinberg and Vogelstein, 1983). Autoradiographic exposures were from 1 to 3 days.

Phage Library Screening

Chromosome jumping was accomplished using a 100-kb jumping library (Collins *et al.*, 1987) constructed in a modified Charon 3A phage vector. Phage were plated on the bacterial host strain MC1061. Approximately 3×10^6 clones were screened with p1bD5 by the method of Benton and Davis (1977). A radioactively labeled riboprobe of p1bD5 was prepared using T7 RNA polymerase (Promega Biotec) to transcribe the insert. Prehybridization and hybridization conditions have been described (Merry *et al.*, 1989). Miniprep phage DNA was prepared according to standard protocols (Maniatis *et al.*, 1982). Phage clone inserts were subcloned into pGEM3-blue and used to transform the bacterial strain DH5α or HB101.

RESULTS

Ordering DXS165 and DXS95 with Hybrid CIII-1

DNA from hybrid clone CIII-1 containing the derivative chromosome 13 and lacking the derivative X and normal X was studied by Southern hybridization with the probes p1bD5 (DXS165) and pXG7c (DXS95). Probe pJL8 (DXS233) has been shown previously to be absent from this hybrid, placing it proximal to the t(X;13) breakpoint (Siu *et al.*, 1988). As shown in Fig. 1, probe p1bD5 (DXS165) detects no signal in hybrid CIII-1, while showing a strong signal in DNA from normal male and female and from another hybrid (790) containing the entire human X chromosome. In contrast, probe pXG7c (DXS95) hybridizes to restriction

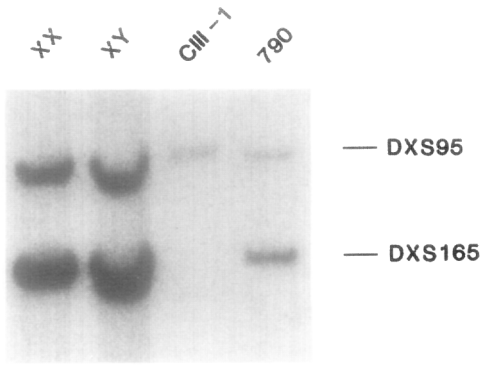

FIG. 1. Autoradiograph of a Southern blot containing *Eco*RI-digested DNAs from normal male and female lymphoblast cell lines and CIII-1 and 790 hybrid cell lines, hybridized sequentially with p1bD5 (DXS165) and pXG7c (DXS95).

fragments in hybrid CIII-1, placing this probe distal to the t(X;13) breakpoint and to p1bD5.

Detection of the t(X;13) Breakpoint

Probe p1bD5 (DXS165) was hybridized to Southern filters of CHEF gels containing DNAs from fibroblasts and lymphoblasts derived from normal individuals and from the translocation patient digested with several rare-cutting enzymes. p1bD5 detects altered fragments in DNA from the translocation patient when digested with *Sal*I, *Sfi* I, *Mlu*I, and *Eag*I, but not with *Xho*I (Fig. 2). These results place probe p1bD5 within 120 kb of the t(X;13) breakpoint. While the normal and altered fragments detected with *Sal*I, *Sfi* I, and *Mlu*I in t(X;13) DNA are of equal intensity (Figs. 2A and B), the altered 120-kb *Eag*I fragment always appears more intense than the normal 370-kb fragment (Figs. 2C and D). (A longer exposure of panel C reveals the 370-kb *Eag*I fragment.) The reason for this difference in intensities is unclear.

The identification of altered restriction fragments in t(X;13) DNA with p1bD5, combined with data from single and double digests of normal DNAs using the enzymes *Eag*I, *Sal*I, and *Sfi* I (Fig. 3), allowed the construction of a long-range restriction map around DXS165 (Fig. 4). The presence of the translocation breakpoint allows the centromere–telomere orientation of this map.

As can be seen in Fig. 3, the presence of a smaller fragment of approximately 380 kb in the *Eag*I/*Sal*I double digest indicates that the *Sal*I and *Eag*I fragments detected by p1bD5 overlap. The altered *Eag*I and *Sal*I fragments of 120 and 280 kb, respectively, in t(X;13) DNA (Fig. 2) allow the ordering of the proximal *Eag*I and *Sal*I sites and therefore also the distal sites. [On the basis of the location of the *Sal*I sites in the ribosomal RNA gene cluster on chromosome 13 (J. Sylvester, personal communication) and the demon-

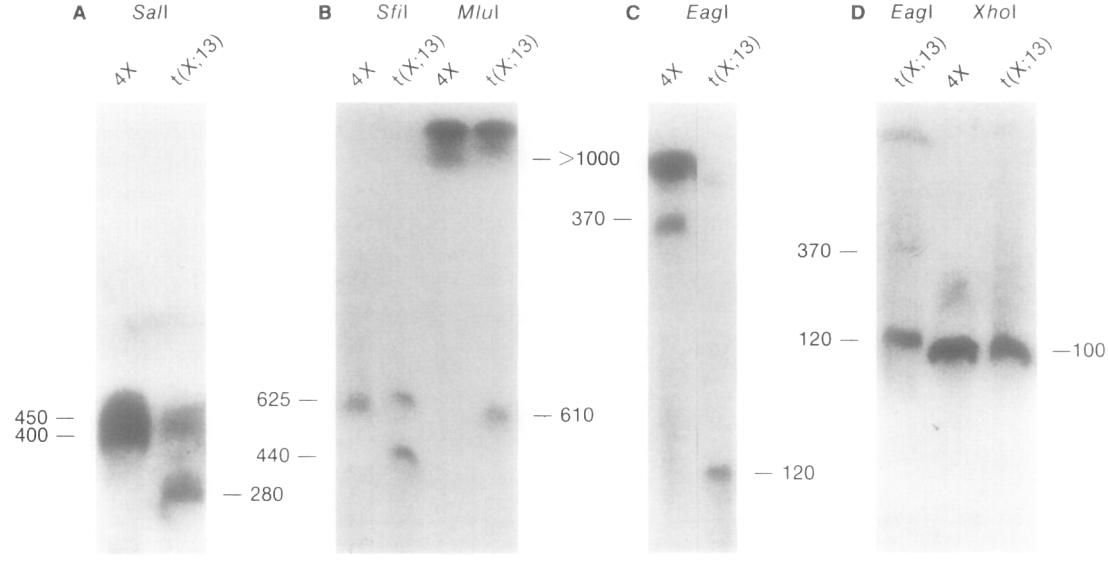

FIG. 2. Autoradiographs of CHEF filters containing DNAs from 4X and t(X;13) lymphoblast cell lines, hybridized with p1bD5 (DXS165). Numbers indicate the length of each fragment in kilobases. **(A)** Southern analysis of *Sal*I-digested DNAs. An altered 280-kb fragment is present in t(X;13) in addition to a normal fragment that migrates at approximately 450 kb. **(B)** Southern analysis of *Sfi* I- and *Mlu*I-digested DNAs. Altered *Sfi* I and *Mlu*I fragments of 440 and 610 kb, respectively, can be seen in addition to normal fragments. **(C)** Southern analysis of *Eag*I-digested DNAs. Note the altered 120-kb *Eag*I fragment in t(X;13) DNA. **(D)** Southern analysis of *Eag*I- and *Xho*I-digested DNAs. Note the presence of the normal 370-kb *Eag*I fragment in addition to the altered 120-kb fragment in t(X;13) DNA, as well as the unaltered *Xho*I fragment of 100 kb.

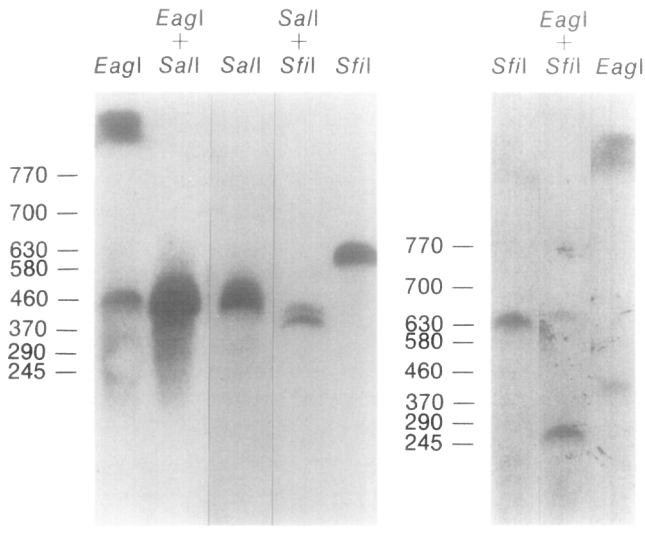

FIG. 3. Autoradiographs of CHEF filters containing DNA from the 4X lymphoblast cell line, hybridized with p1bD5 (DXS165). Numbers indicate the length of each fragment in kilobases.

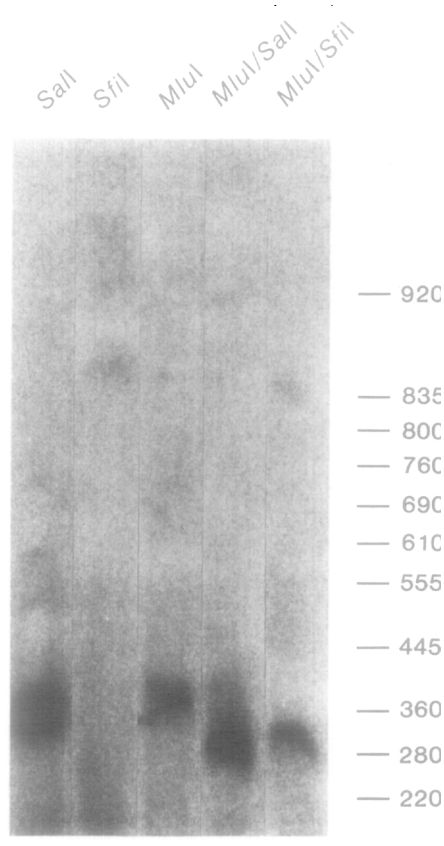

FIG. 5. Autoradiograph of a CHEF filter containing DNA from the 4X lymphoblast cell line, hybridized with pXG7c (DXS95). Note the weak 850-kb band in the lane containing *Sfi* I-digested DNA. Compare the *Sfi* I and *Mlu*I lanes from this autoradiograph with those in Fig. 2B, which represent sequential hybridizations of p1bD5 and pXG7c to the same CHEF blot, following stripping.

stration of undermethylation using probes from this region, the *Sal*I sites on this side of the t(X;13) chromosome are likely to be digested.]

The identification of an approximately 610-kb *Mlu*I fragment with p1bD5 that is present in t(X;13) DNA but not in normal DNA places an *Mlu*I site within 610 kb proximal to the t(X;13) breakpoint. This site was not placed on the map since the location of the chromosome 13 *Mlu*I site flanking this fragment and the breakpoint is not known. The normal *Mlu*I fragment is greater than 1000 kb and is not well resolved under these conditions.

An *Eag*I/*Xho*I double digest is identical to *Xho*I alone (data not shown); these data combined with the fact that the 100-kb *Xho*I fragment is unaltered in t(X;13) DNA (Fig. 2) place this fragment entirely within the altered 120-kb *Eag*I fragment.

Probe pXG7c (DXS95) was also used to screen CHEF blots of normal and t(X;13) DNAs. pXG7c detects *Sal*I and *Mlu*I fragments of approximately 360 kb (Fig. 5) that are unaltered in the translocation patient (data not shown). (While sizing of large restriction fragments always involves estimation, these fragments have been more accurately sized on other CHEF gels at 300 kb.) In *Sfi* I-digested t(X;13) DNA, however, pXG7c detects a 450-kb fragment in addition to the

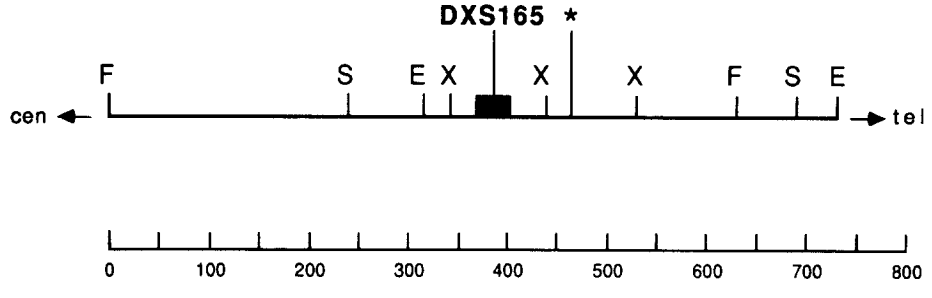

FIG. 4. Long-range restriction map around DXS165, constructed using probe p1bD5. *, Translocation breakpoint; E, *Eag*I; F, *Sfi* I; S, *Sal*I; X, *Xho*I.

normal 850-kb *Sfi* I fragment. A band of approximately 450-kb was also seen when DNAs from additional normal female lymphoblasts were analyzed, however, suggesting that this fragment results from a polymorphism or a methylation variation. Hybridization of pXG7c with *Not*I-, *Nru*I-, and *Pvu*I-digested DNAs from t(X;13) was uninformative.

pXG7c was hybridized to CHEF blots of single and double digests of control DNA using the enzymes *Sal*I, *Sfi* I, and *Mlu*I (Fig. 5); these data were compiled to construct the restriction map shown in Fig. 6. The precise positioning of *Sal*I sites is possible from double digest data of Cremers *et al.* (1989a). The centromere–telomere orientation of this map cannot be determined without additional physical landmarks. Consecutive hybridizations of CHEF filters with p1bD5 and pXG7c showed no common cross-hybridizing restriction fragments (Figs. 2B and 5), preventing the linking of these two restriction maps. A long-range restriction map for the anonymous marker DXS233 has already been described (Merry *et al.*, 1989). The restriction map for DXS233 could not be linked with the DXS165 restriction map, following consecutive hybridizations of CHEF filters with p1bD5 and pJL8 (DXS233) (data not shown).

Chromosome Jumping from DXS165

To move toward both the translocation breakpoint and the choroideremia gene, we screened a 100-kb jumping library (Collins *et al.*, 1987) with probe p1bD5. Three independent phage clones were isolated and plaque-purified. After subcloning into a plasmid vector, the portion of each jump clone representing the landing point was determined and used in Southern hybridizations of CHEF filters that were previously hybridized with p1bD5. One clone, pCH4, detects the same altered *Eag*I fragment as p1bD5 in t(X;13) DNA (Fig. 7, compare *Eag*I panel to that in Fig. 2C) but identifies a different *Xho*I fragment of approximately 95 kb in normal DNA. In addition, pCH4 detects an altered *Xho*I fragment of approximately 45 kb in DNA from the translocation patient (Fig. 7). This fragment was not seen when additional females, representing 12 independent X chromosomes, were analyzed in this manner.

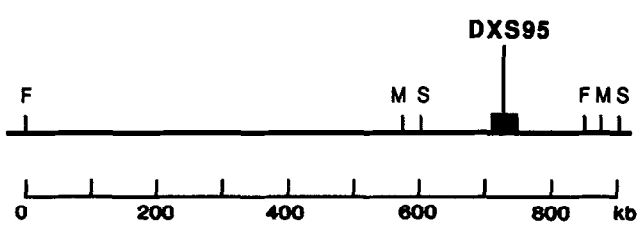

FIG. 6. Long-range restriction map around DXS95, using probe pXG7c. F, *Sfi* I; M, *Mlu*I; S, *Sal*I.

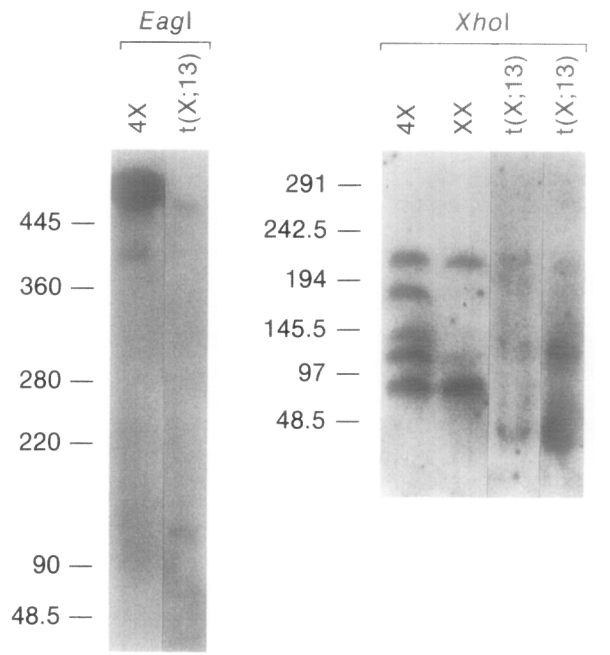

FIG. 7. Autoradiographs of CHEF filters containing DNAs from 4X and t(X;13) cell lines, hybridized with the jump clone pCH4. Note that the same *Eag*I fragments are detected by pCH4 and p1bD5 (see Fig. 2C). This is demonstrated by using the same Southern blot for sequential hybridizations, following stripping. In this figure, however, note the new *Xho*I pattern seen with pCH4 in normal DNAs and the altered *Xho*I fragment of approximately 45 kb in t(X;13) DNA.

No Proband Alterations of DXS165 or pCH4

Probes p1bD5 (DXS165) and pCH4 were used to screen routine Southern blots containing DNAs from 42 unrelated patients with choroideremia. In contrast to the finding of Cremers *et al.* (1987, 1989b) that 3 of 30 choroideremia probands were deleted for DXS165, we have not found this locus or pCH4 to be deleted or altered in any of our probands with isolated choroideremia. However, as expected, DXS165 and pCH4 are deleted from our patients with choroideremia as part of a contiguous gene deletion syndrome due to large deletions in Xq21 (XL-45, XL-62, MBU) (data not shown; Cremers *et al.*, 1989a).

DISCUSSION

The identification of individuals with choroideremia and chromosomal rearrangements has provided a number of physical landmarks with which to localize and identify the choroideremia gene. The identification of a woman with choroideremia and an X;13 translo-

cation is of particular importance because this translocation provides a single precise landmark for the gene, based on the assumption that the translocation disrupts the choroideremia gene and causes the disease in this patient. This translocation also provides a physical landmark in a region of Xq21 comprising several megabases of DNA in which very few landmarks have previously been identified. This facilitates the mapping and ordering of loci separated by this landmark.

Our pulsed-field gel and somatic cell hybrid results indicate that DXS165 and DXS95 flank the t(X;13) breakpoint, with DXS165 proximal to and within 120 kb of the breakpoint. Therefore, it is likely that DXS165 is proximal to the choroideremia gene, although it is possible that DXS165 lies within a very large intron of the gene. However, given the very low frequency of this disease in the population (1/300,000 to 1/400,000) (Lewis *et al.*, 1985), reflecting a small number of new mutations, we do not expect this gene to be extremely large. On the assumption that coding sequences for the choroideremia gene may be located close to the translocation breakpoint, we have moved in the direction of the choroideremia gene by chromosome jumping from DXS165. We are currently screening phage and cosmid libraries with pCH4 to move toward the translocation breakpoint and to isolate candidate sequences for the choroideremia gene. In addition, we are continuing to perform chromosome jumping experiments from pCH4 to isolate DNA clones from the other side of the breakpoint.

ACKNOWLEDGMENTS

We thank Donna Sosnoski for technical assistance and Dr. Paul Szabo for probe pXG7c (DXS95). D.E.M. is a predoctoral fellow of the March of Dimes/Birth Defects Foundation. V.S. acknowledges support from the Ontario Ministry of Community and Social Services. F.S.C. is an Associate Investigator of the Howard Hughes Medical Institute and acknowledges support from NIH Grant GM34960. R.A.L. acknowledges support from the Retinitis Pigmentosa Foundation Fighting Blindness/George Gund Foundation and Research to Prevent Blindness. R.L.N. acknowledges grant support from NIH RO1-06566 and the Retinitis Pigmentosa Foundation/George Gund Foundation. The laboratory work was performed in the Howard Hughes Medical Institute Laboratory in Philadelphia, where R.L.N. is an Associate Investigator and J.G.L. is a research technician.

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