Hum Genet (1988) 78:156–160

Human Genetics

Regional localization of polymorphic DNA loci on the proximal long arm of the X chromosome using deletions associated with choroideremia

M. Schwartz[1], H.-M. Yang[2], E. Niebuhr[2], T. Rosenberg[3], and D. C. Page[4]

[1]Section of Clinical Genetics 4062, Department of Pediatrics, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark
[2]Institute of Medical Genetics, University of Copenhagen, DK-2200 Copenhagen, Denmark
[3]National Eye Clinic for the Visually Impaired, DK-2900 Copenhagen, Denmark
[4]Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142, USA

Summary. In two unrelated families, males have been identified who suffer from choroideremia and at the same time have an interstitial deletion on the proximal long arm of the X chromosome. By high-resolution banding we have characterized the deletion chromosomes as del(X)(q21.1–q21.33) and del(X)(q21.2–q21.31) respectively. By Southern blot analysis we have mapped ten different polymorphic DNA loci relative to the position of the deletion and the choroideremia locus TCD. One probe, p31, was shown to cover one of the breakpoints of the smallest deletion. The following order of the loci was suggested by deletion mapping: cen-DXS106-DXS72-TCD-(DXYS1/DXYS23/DXYS5)-DXYS2-(DXYS12/DXS3)-(DXS17/DXS101)-Xqter.

Introduction

Choroideremia is an X-linked recessive trait leading to blindness during early adulthood in hemizygous males (Goedbloed 1942; Waardenburg 1942; Westerlund 1956). The disease is a well-defined clinical entity generally believed to be caused by a mutation at a single locus. There is no evidence of genetic heterogeneity (Kärna 1986). Female carriers are asymptomatic, but can be diagnosed with certainty due to a characteristic pigmentation pattern of the optic fundus (McCulloch and McCulloch 1948). Usually no other defects are associated with the disorder, although a syndrome that also includes retardation and anhidrotic ectodermal dysplasia has been reported (Van den Bosch 1959).

The choroideremia locus (TCD) has been shown by several groups to be closely linked to the DNA locus DXYS1, and patients with choroideremia and X-chromosomal deletions, including DXYS1, have been reported (Nussbaum et al. 1985; Schwartz et al. 1986; Sankila et al. 1987; Hodgson et al. 1987; Lesko et al. 1987). In this report we have refined the physical map of ten polymorphic DNA loci with respect to TCD by use of DNA from two independent families in which choroideremia is associated with cytogenetically detectable Xq deletions that include DXYS1: del(X)(q21.1–q21.33) (Schwartz et al. 1986) and del(X)(q21.2–q21.31) (Rosenberg et al. 1986).

Offprint requests to: M. Schwartz

Materials and methods

Family material

Family 1. A large interstitial deletion of the proximal part of the X chromosome was detected in a boy first described by Tabor et al. (1983) as being severely mentally retarded, with coarse facial features, a left-sided cleft lip and palate, hypertelorism, and macrocephaly. He was later found also to have choroideremia (Schwartz et al. 1986). His mother and sister were both found to be heterozygous for the X-chromosomal deletion (Tabor et al. 1983), as were subsequent carriers of choroideremia (Rosenberg et al. 1986).

Family 2. Two brothers were diagnosed as having choroideremia, sensorineural deafness, and mental retardation. A small interstitial deletion on the proximal long arm of the X chromosome was found in both brothers and in one of their mother's X chromosomes (Rosenberg et al. 1987). At ophthalmoscopic examination the mother was found to be a carrier of choroideremia. The mother's elder sister is mentally retarded but does not have the X chromosome deletion.

DNA markers

We have used probes for ten different polymorphic DNA loci all previously mapped to the proximal long arm of the X chromosome (Goodfellow et al. 1985). The DNA probes were kindly provided by investigators from different laboratories: cpX203 and cX52.5 from P. Pearson, pX65H7 from B. Schmeckpeper, St25–1 and St25–2 from J.-L. Mandel, p19–2 from G. Bruns, S21 from D. Drayna, p31 from J. Weissenbach. pDP7a is a subclone derived from p7b provided by J. Weissenbach. A list of the probes and their characteristics is given in Table 1.

Cytogenetics

Chromosome preparations were obtained from peripheral blood lymphocytes using a standard method for Q-banding, and by addition of BrdU during the final 5 h for R-banding with acridine orange (Dutrillaux et al. 1973) (Fig. 1). Cultures for high-resolution banding were synchronized with 10^{-7}M methotrexate for 24 h, then released by adding Leucovorin

Table 1. Polymorphic probes used

Probe[a]		Physical location		Enzyme	Polymorphic fragments (kb)	Y-specific fragments (kb)
Locus	Name	HGM 8	This report			
DXYS1	pDP34	Xp13–q21.1	Xp21.2–21.31	Taq I	12/11	15
DXYS2	pDP7a	Xq13–q22	Xq21.31–21.33	Pst I	11/7	11
DXYS5	p31	Xp13–q22	Xp21.2–q21.31	Taq I	4.8/2.8	4.8/2.8
DXYS12	St25–1	Xq13–q22	Xq21.31–21.33	Taq I	2.1/1.6	1.6
DXYS23	pDP132	Xq12–q22	Xp21.2–21.31	BamH I	22/21	10
DXS3	p19–2	Xp21.3–q22	Xp21.31–21.33	Msp I	16/4.6	
DXS17	S21	Xq21.3–q22	Xq21.33–q22	Taq I	2.2/2.0	
DXS72	pX65H7	Xq13–q22	Xq21.1–q21.2	Hind III	7.2/0.7	
DXS101	cX52.5	Xp21.3–q22	Xq21.33–q22	Msp I	7.7/7.5	
DXS106	cpX203	Xp11–q21.3	Xp11–q21.1	Bql II	5.8/1.0	

[a]Human Gene Mapping 8 (Goodfellow et al. 1985)

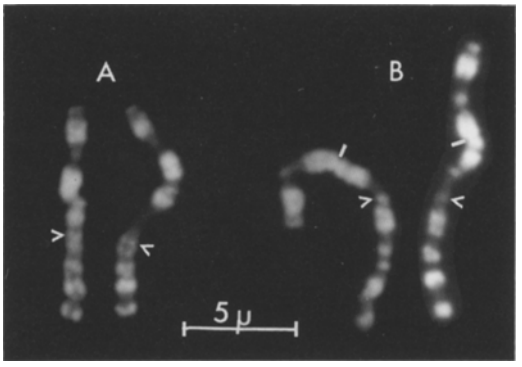

Fig. 1A, B. Normal X chromosomes (to the *right*) and X chromosomes with an interstititial deletion from the males in family 1 **(A)** and family 2 **(B)**. *Arrows* indicate the Xq22.1 band **(A)** and the Xq21.32 band **(B)**. The *bar* in **B** indicates the position of the centromere

$(3 \times 10^{-4}$ M), BrdU (10^{-4} M), and Hoechst 33258 (5×10^{-5} M) hours before harvest.

Southern blot analysis

DNA was isolated by routine methods from EDTA-stabilized blood. With the appropriate restriction enzymes, 5 μg DNA were digested according to the recommendations of the manufacturer (Boehringer, Mannheim). The resulting fragments were separated on agarose gel (Bio-Rad) followed by blotting onto Gene Screen Plus membranes (New England Nuclear). Radioactive probes were prepared by oligonucleotide-primed DNA synthesis using ^{32}P-dCTP (Feinberg and Vogelstein 1983). Before labeling, the insert was excised from the plasmid and purified on agarose gel. The filters were hybridized overnight at 68°C in 0.5 M Na-phosphate pH 7.2, 7% SDS, and 1 mM EDTA.

For some of the probes more stringent conditions were used for hybridization: 50% formamide, 5 × Denhardts, 5 × SSPE, 0.1% SDS, 10% dextran sulfate. Excess probe was washed off the filters in 0.04 M phosphate pH 7.2, 1% SDS and then in 0.02 M phosphate, 0.5% SDS. Signals were detected by autoradiography using Kodak X-O-MAT AR 5 films and intensifying screens.

Results

Cytogenetic studies

Family 1. Chromosome banding analysis confirmed the previous results, that the large, characteristic Q-positive/R-negative band Xq21 was almost completely deleted from the abnormal X chromosome (Tabor et al. (1983). A careful comparison between the high-resolution banding pattern of the del(X) and that of a large number of normal X chromosomes, analyzed at the same time by the same methods, showed that the Xq13.3 and Xq22.1 bands flanking Xq21 were present in the del(X). Therefore, the deletion can be described as del(X)(q21.1–q21.33). Since, however, the two R-positive bands Xq13 and Xq22 were observed not to be completely confluent, we conclude that a minute part of an R-negative band, either from Xq21.1 or from Xq21.33, is still present.

Family 2. Standard banding showed a small reduction in the size of band Xq21 in the affected brothers' and in one of their mother's X chromosomes. By high-resolution banding it could be demonstrated that the entire small proximal R-positive band, Xq21.2, and a minor part of the surrounding R-negative area are deleted. The deletion covers about one-fourth of the Xq21 band. The proximal breakpoint is located either within Xq21.1 or at the interface of Xq21.1 and Xq21.2. The deletion is thus described as del(X)(q21.2–q21.31) according to ISCN (1978). The deletion diagnosed in family 2 is completely included in that of family 1.

Deletion mapping

DNA from each relevant family member together with DNA from an unrelated individual was used to study the hybridization pattern of the various probes. Table 2 gives the results of the deletion mapping.

Because of the very close linkage that has been established between DXYS1 and TCD (Nussbaum et al. 1985; Schwartz et al. 1986; Sankila et al. 1987), the deletion of DXYS1 in the patients strongly suggests that TCD is located within both deletions. By comparing results with the previously reported localization of these X-chromosomal loci (Goodfellow et al. 1985), it was possible to divide them into those that are (A) not covered by either of the deletions, (B) covered by the larger deletion only, and (C) covered by both deletions.

Table 2. Analysis of the del(X) with the probes listed in Table 1

Probe name	Deletion[a]		Group[b]
	del (X) (q21.1–q21.33)	del (X) (q21.2–21.31)	
cpX203	+	+	
cX52.5	+	+	A
S21	+	+	
pX65h7	–	+	
St25–1	–	+	B
p19–2	–	+	
pDp7a	–	+	
pDp132	–	–	
pDP34	–	–	C
p31	–	*[c]	

[a]The presence (+) or absence (–) of specific X-chromosomal fragments detected by the probes
[b]See text for explanation
[c]p31 detects one of the deletion breakpoints in family 2

A: cpX203 (DXS106), cX52.5 (DXS101), S21 (DXS17). DXS106 has previously been assigned to Xp11–q21.3. It must then be proximal to both deletions and to TCD, hence physically assigned to Xp11–Xq21.1.

DXS101 and DXS17 were previously assigned to Xq21.3–Xq22. We can conclude that both are located distal to the deletions and TCD, hence physically assigned to Xq21.33–q22.

B: St25–1 (DXYS12), p19–2 (DXS3), pX65H7 (DXS72) and pDP7a (DXYS2). The loci defined by these probes are all present in DNA from the two brothers with the smaller deletion. DXS3 has been assigned to Xq21.3–q22, and DXYS12 to Xq13–q22. DXS3 must then be located distal to the smaller deletion. It is not possible from the present results to decide whether DXYS12 is distal or proximal to TCD. However, in a recombination event between TCD and DXS3 we have observed cosegregation of DXS3 and DXYS12. Therefore the most likely assignment of both loci is Xq21.31–q21.33.

DXS72 is present in the two brothers, and their mother is heterozygous for a restriction fragment polymorphism detected by pH65H7. It has been assigned to Xq13–q22 and it could be either proximal or distal to TCD. The same situation is found for locus DXYS2. However, from an informative meiosis is a linkage study we have seen cosegregation of DXS72/DXS106 haplotypes and DXYS2/TCD haplotypes respectively (in preparation). We conclude that DXS72 must be proximal to TCD, and DXYS2 distal to this locus.

C: pDP34 (DXYS1),pDP132 (DXYS23). The loci defined by these probes are absent from the genomic DNA of all three affected males. These loci are therefore expected to be closest to TCD. Recombinants have so far not been detected between TCD and DXYS23, while recombinations between DXYS1 and TCD have been reported (Gal et al. 1986a; Lesko et al. 1987). All other probes employed in this study have shown at least one recombination event. Both loci can thus be assigned within the deletion Xq21.2–q21.31, but no conclusion as to their order with respect to TCD can be reached. One probe showed a more remarkable result: p31 (DXYS5) was not detected in the larger deletion, but hybridization of p31 to Taq I blots of DNA from family 2 revealed a fragment with an altered size

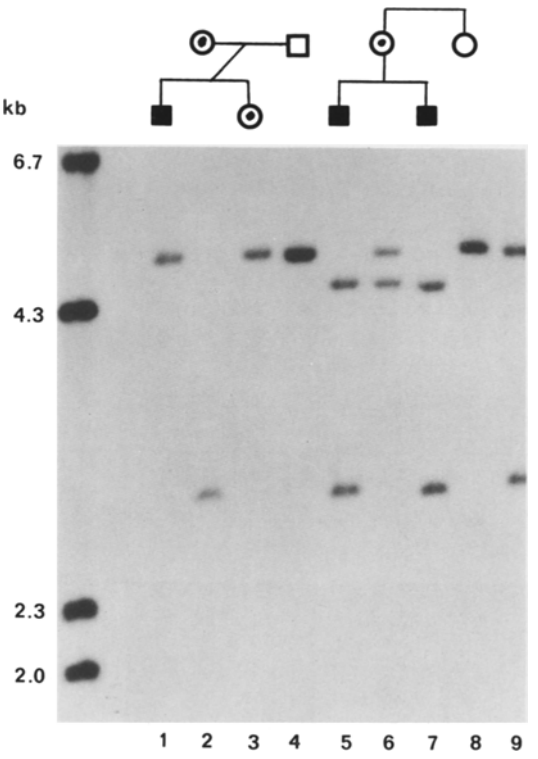

Fig. 2. Southern blot analysis of Taq I-digested DNA from family 1 *(lanes 1–4)*, family 2 *(lanes 5–8)*, and an unrelated control *(lane 9)* probed with the X-Y probe p31. This probe normally detects allelic fragments of 4.8 and 2.8 kb; either allele can be found on either an X chromosome or a Y chromosome. *Lane 1*, patient with the large deletion. Only the 4.8-kb fragment from his father *(lane 4)* is present, while there is no contribution from his mother. *Lanes 5, 6,* and *7* show the novel fragment of approximately 4.6 kb found in DNA from family 2 (smaller deletion)

compared with those of controls (Fig. 2). p31 normally detects allelic Taq I fragments of 2.8 kb and 4.8 kb. Either allele can be found on either an X chromosome or a Y chromosome. The heterozygous mother showed a 4.8-kb fragment from her normal X chromosome and a fragment of 4.6 kb from her deletion chromosome. The latter fragment was also found in her two sons, together with a 2.8-kb fragment from their Y chromosomes. This suggested that either a new polymorphic site is present in this family or the probe is covering one of the end points of the deletion. We therefore digested DNA from this family with several different enzymes and then carried out Southern analysis using the p31 probe. A novel fragment was found with all enzymes tested, confirming that this probe detects a deletion breakpoint.

Discussion

Deletion mapping has proved to be a powerful tool in genetic analysis. Well-characterized chromosomal deletions have been extremely valuable in the physical mapping of genes, including disease genes, as well as anonymous DNA sequences. The finding of a minor Xp21.2 deletion in a male with Duchenne muscular dystrophy (DMD) associated with chronic granulomatous disease (CGD), retinitis pigmentosa (RP), and McLeod syndrome has led to the precise localization of the loci for these diseases (Francke et al. 1985), cloning of

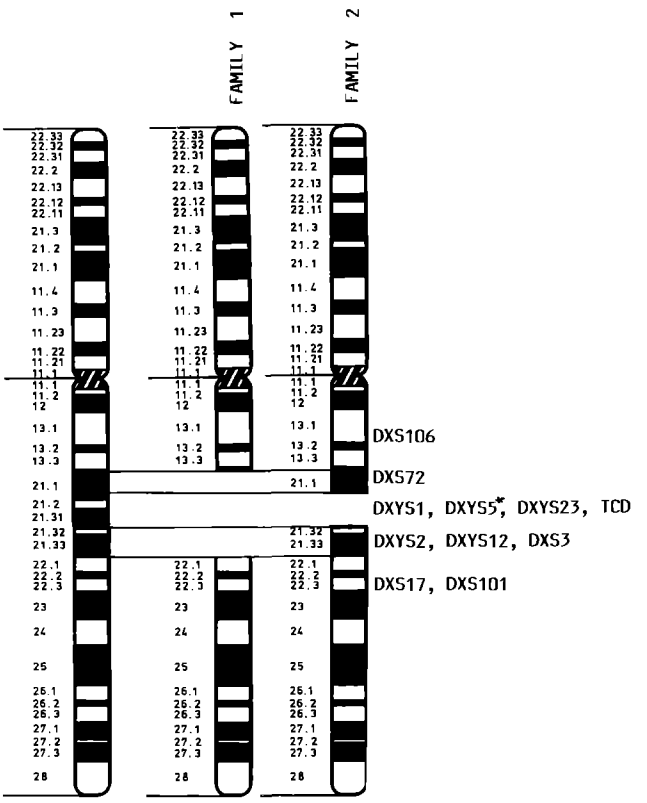

Fig. 3. Standard ideogram (ISCN 1981) of the human X chromosome *(left)* and two ideograms showing the two deletions. The physical localization of the DNA loci, based on the combined data from the deletion mapping described in the present paper and from previously published localizations (Goodfellow et al. 1985) are indicated to the *right*. *DXYS5 covers one of the deletion breakpoints in family 2

DNA probes from the deleted area (Kunkel et al. 1985), and recently the isolation of DNA sequences that may constitute the CGD and DMD genes (Royer-Pokora et al. 1986; Monaco et al. 1986).

We have analyzed DNA from individuals with deletions on the proximal long arm of the X chromosome. The males all suffer from choroideremia in addition to certain mental and physical handicaps. High-resolution banding of the chromosomes strongly suggests that the smaller deletion is included in the larger one. Results from DNA studies of these individuals made it possible to construct a deletion map of loci previously assigned to this part of the chromosome. DXYS1 and DXYS23, both X-Y homologous loci, were found to be deleted in both families, and the deletion we assume also includes TCD. A third X-Y homologous locus, DXYS5, apears to span one of the deletion breakpoints in family 2.

DXYS12 and DXS3 have previously been found to be closely linked to TCD (Gal et al. 1986a; Sankila et al. 1987). In the study by Gal et al. (1986a), the order DXS3-TCD-DXS11 was suggested. DXS11 (not included in our study) maps at Xq24 (Aldridge et al. 1984) distal to DXS17 and should therefore not be covered by any of the deletions described in the present investigation. This makes it unlikely that DXS3 and DXS11 are flanking TCD. A recent publication by Lesko et al. (1987) suggests that TCD is proximal to both DXYS1 and DXS3. Our data suggest the following order of loci: cen-DXS106-DXS72-TCD-(DXYS1/DXYS23/DXYS5)-DXYS2-(DXYS12/DXS3)-(DXS17/DXS101) Xqter (Fig. 3).

The genes for agammaglobulinemia (Kwan et al. 1986), Charcot-Marie-Tooth disease (Gal et al. 1986b), ectodermal dysplasia (McDermot et al. 1986; Kølvrå et al. 1986), and an X-linked form of cleft palate (Moore et al. 1987) have all been reported to be closely linked to DXYS1. None of our patients show signs of either agammaglobulinemia, Charcot-Marie-Tooth disease, or ectodermal dysplasia, strongly suggesting that the corresponding genes are located outside the deleted areas, and not as closely linked to DXYS1 as is TCD.

Our patient with the larger deletion does have a left cleft lip and palate, which is not present in the two brothers with the smaller deletion. The gene(s) for the X-linked form of cleft lip and palate may therefore map either proximally (Xq21.1–q21.2) or distally (Xq21.31–q21.33) to the smaller deletion. The two brothers suffer from sensorineural deafness, which was not found in the boy from family 1, nor was it present in the patient published by Hodgson et al. (1987). One explanation for this could be an inherited mutation independent of the deletion, although we find this unlikely. Although we have been able to map TCD physically, even the small deletion covers a rather large chromosomal area, and the physical distance among the X-Y homologous loci DXYS1, DXYS23, DXYS5, and TCD might well be large.

The deletion of Xq21.2 in family 2 is similar to the deletion at Xp21.2 in patient BB (Francke et al. 1985) in several respects. Both cases involve deletions of small R-positive bands within large R-negative bands. It is known that Q-positive/R-negative bands are generally late replicating, while Q-negative/R-positive bands are generally early replicating and more likely to harbor active structural genes (Comings 1978). By applying techniques which have been used so successfully in cloning the Xp21 region, e.g. pERT cloning (Kunkel et al. 1985), pulsed-field gel electrophoresis, and cloning of jumping fragments, it may be possible to isolate the TCD gene.

Acknowledgements. The excellent technical assistance of Karin Hjelm Knudsen, Bodil Mogensen, and Runa Hansen is greatly appreciated. We thank Dr. Søren Nørby for helpful discussion of the manuscript. This work has been supported by the Danish Medical Research Council (grant no. 12–5619), The Novo Foundation, The Carl Petersen Foundation (grant no. B1605), and the National Institutes of Health (HD 22532).

References

Aldridge JL, Kunkel G, Bruns G, Tantravahi U, Lalande M, Brewster T, Moreau E, Wilson M, Bromley W, Roderick T, Latt SA (1984) A strategy to reveal high-frequency RFLPs along the human X chromosome. Am J Hum Genet 36:546–564

Comings DE (1978) Mechanism of chromosome banding and implication for chromosome structure. Ann Rev Genet 12:25–46

Dutrillaux B, Laurent C, Couturier J, Lejeune J (1973) Coloration par l'acridine orange de chromosomes préalablement traités par le 5-bromodésoxyuridine (BUdR). CR Acad Sci [D] (Paris) 276: 3179–3181

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

Francke U, Ochs HD, de Martinville B, Giacalone J, Lindgren V, Distéche C, Pagon RA, Hofker MH, van Ommen GJB, Pearson PL, Wedgwood RJ (1985) Minor Xp21 chromosome deletion in a male associated with expression of Duchenne muscular dystrophy, chronic granulomatous disease, retinitis pigmentosa, and McLeod syndrome. Am J Hum Genet 37:250–267

Gal A, Brunsman F, Hogenkamp D, Rüther K, Aahlert D, Wienker TF, Hammerstein W, Pawlowitzki IH (1986a) Choroideremia-

locus maps between DXS3 and DXS11 on Xq. Hum Genet 73: 123–126
Gal A, Mücke J, Theile H, Wieacker PF, Ropers HH, Wienker TF (1986b) X-linked dominant Charcot-Marie-Tooth disease: suggestion of linkage with a cloned DNA sequence from the proximal Xq. Hum Genet 70:38–42
Goedbloed J (1942) Mode of inheritance of choroideremia. Ophthalmologica 104:308–315
Goodfellow PN, Davies KE, Ropers HH (1985) Report of the committee on the genetic constitution of the X and Y chromosomes. Cytogenet Cell Genet 40:296–355
Hodgson SV, Robertson ME, Fear CN, Goodship J, Malcolm S, Jay B, Bobrow M, Pembrey ME (1987) Prenatal diagnosis of X-linked choroideremia with mental retardation, associated with a cytogenetically detectable X chromosome deletion. Hum Genet 75:286–290
ISCN (1981) An international system for human cytogenetic nomenclature. High-resolution banding. Cytogenet Cell Genet 31:1–32
Kärnä J (1986) Choroideremia. A clinical and genetic study of 84 Finnish patients and 126 female carriers. Acta Ophthalmol [Suppl] (Copenh) 176:1–68
Kølvraa S, Kruse TA, Jensen PKA, Linde KH, Vestergård SR, Bolund L (1986) Close linkage between X-linked ectodermal dysplasia and a cloned DNA sequence detecting a two allele restriction fragment polymorphism in the region Xp11–q12. Hum Genet 74:284–287
Kunkel LM, Monaco AP, Middleswoth W, Ochs HD, Latt SA (1985) Specific cloning of DNA fragments absent from the DNA of a male patient with an X chromosome deletion. Proc Natl Acad Sci USA 82:4778–4782
Kwan SP, Kunkel L, Bruns G, Wedgwood RJ, Latt S, Rosen FS (1986) Mapping of the X-linked agammaglobulinemia locus by use of restriction fragment length polymorphism. J Clin Invest 77: 649–652
Lesko JG, Lewis RA, Nussbaum RL (1987) Multipoint linkage analysis of loci in the proximal long arm of the human X chromosome: application to mapping the choroideremia locus. Am J Hum Genet 40:303–311
MacDermot KD, Winther RM, Malcolm S (1986) Gene localisation of X-linked hypohidrotic ectodermal dysplasia (C-S-T syndrome). Hum Genet 74:172–174
McCulloch JC, McCulloch RJP (1948) A hereditary and clinical study of choroideremia. Trans Am Acad Ophthalmol Otolaryngol 52: 160–190
Monaco P, Neve RL, Colletti-Feener C, Bertelson CJ, Kurnit DM, Kunkel LM (1986) Isolation of candidate cDNAs for portions of the Duchenne muscular dystrophy gene. Nature 223:646–650
Moore GE, Ivens A, Chambers J, Farrall M, Williamson R, Page DC, Bjornsson A, Arnason A, Jensson O (1987) Linkage of an X-chromosome cleft palate gene. Nature 236:91–92
Nussbaum RL, Lewis RA, Lesco JG, Ferrell R (1985) Choroideremia is linked to the restriction fragment length polymorphism DXYS1 at Xq13–21. Am J Hum Genet 37:473–481
Rosenberg T, Schwartz M, Niebuhr E, Yang HM, Sardemann H, Andersen O, Lundsteen C (1986) Choroideremia in interstitial deletion of the X chromosome. Ophthalmic Paediatr Genet 7:205–210
Rosenberg T, Niebuhr E, Yang HM, Parving A, Schwartz M (1987) Choroideremia, congenital deafness and mental retardation in a family with an X chromosomal deletion. Ophthalmic Paediatr Genet (in press)
Royer-Pokora B, Kunkel LM, Monaco AP, Goff SC, Newburger PE, Baehner RL, Cole FS, Curnutte JT, Orkin SH (1986) Cloning the gene for an inherited human disorder – chronic granulomatous disease – on the basis of its chromosomal location. Nature 322: 32–38
Sankila EM, Chapelle A de la, Kärnä J, Forsius H, Frants R, Eriksson A (1987) Choroideremia: close linkage to DXYS1 and DXYS12 demonstrated by segregation analysis and historical-genealogical evidence. Clin Genet 31:315–322
Schwartz M, Rosenberg T, Niebuhr E, Lundsteen C, Sardemann H, Andersen O, Yang HM, Lamm LU (1986) Choroideremia: further evidence for assignment of the locus to Xq13–Xq21. Hum Genet 74:449–452
Tabor A, Andersen O, Lundsteen C, Niebuhr E, Sardemann H (1983) Interstitial deletion in the "critical region" of the long arm of the X chromosome in a mentally retarded boy and his normal mother. Hum Genet 64:196–199
Van den Bosch J (1959) A new syndrome in three generations of a Dutch family. Ophthalmologica 137:422–423
Waardenburg PJ (1942) Chorioideremie als Erbmerkmal. Acta Ophthalmol 20:235–274
Westerlund E (1956) Inheritance of choroideremia. Acta Ophthalmol 34:63–68

Received July 10, 1987 / Revised September 1, 1987