Linkage studies and deletion screening in choroideremia

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Abstract

Fourteen families with choroideremia (TCD) have been examined for linkage to nine genetic markers located on the proximal long arm of the X chromosome. Linkage to three markers (*DXYS1*, *DXS72*, *DXS3*) located in Xq21 was found with a four point lod score of 8.25. No evidence of submicroscopic deletions was observed using *DXS233* and *DXS232*, both thought to lie within about 1 Mb of the TCD gene.

Choroideremia (McKusick No 30310; tapetochoroidal dystrophy, TCD) is a progressive, X linked degeneration of the choroid and retina. Onset is usually in the first or second decade in males, with pigmentary changes and choroidal atrophy, progressing to loss of central vision usually in the sixth decade. Females are generally asymptomatic although they usually show characteristic fundus changes, ranging from a minimal pigmentary disturbance to focal areas of loss of retinal pigment epithelium. Linkage was first reported by Nussbaum *et al*¹ between TCD and the *DXYS1* locus, located on the proximal long arm of the X chromosome at Xq13-q21. This finding was confirmed by Jay *et al*,² Schwartz *et al*,³ and Sankila *et al*.⁴ Other loci found to show significant evidence of linkage to TCD include *DXYS12* and *DXS3*,^{4,5} both

located in proximal Xq. Lesko *et al*⁵ carried out a multipoint linkage analysis of 12 TCD families using nine DNA probes and found significant evidence of linkage to five loci (*PGK*, *DXYS1*, *DXS72*, *DXYS12*, and *DXS3*). They proposed a gene order putting TCD proximal to *DXS3* with odds of 96:1 (*DXS1-DXYS1-TCD-DXS3-DXS17* or *DXS1-TCD-DXYS1-DXS3-DXS17*) in contrast to others⁶ who had suggested a distal location in a study of two TCD families.

Further support for a location close to *DXYS1* has come from the study of TCD patients carrying microscopic and macroscopic deletions of this and neighbouring loci.⁷⁻⁹ The first report was by Rosenberg *et al*⁷ who reinvestigated a male carrying an interstitial deletion in Xq13-q21.3. This patient was severely mentally retarded with agenesis of the corpus callosum, cleft lip and palate, and on ophthalmological examination was found to have TCD. The *DXYS1* locus was found to be deleted while *DXS17* was present. Hodgson *et al*⁸ also reported a cytogenetically visible deletion in subband Xq21.1 in a male with TCD, mental retardation, and minor dysmorphic features. Two probes were also deleted in this subject, at the *DXYS1* and *DXS3* loci, respectively. Nussbaum *et al*⁹ found deletions in two further families in which TCD was associated with mental retardation and deafness, only one of which (XL-62) showed a cytogenetically detectable deletion in Xq21, also associated with loss of *DXYS1* and *DXS72*. The second family (XL-45) showed no visible deletion or loss of signal at the above loci. However, two clones (*DXS232*, *DXS233*) were isolated using the phenol enhanced reassociation technique (pERT) which were found to be deleted in this family.⁹ Finally, Cremers *et al*¹⁰ reported finding deletions in two out of eight unrelated classical TCD patients with probe p1bD5 (*DXS165*), also located in Xq12-q21.3. We report here the results of further linkage studies in 14 TCD families using nine DNA probes and the results of deletion screening at the *DXS232* and *DXS233* loci.

Methods

Fourteen TCD families (C1-5, 9-12, 14-16, 20, 22), were ascertained at the Genetic Clinic, Moorfields Eye Hospital, London, and included in the study. The diagnosis of TCD in males and carrier females

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was made as described previously.² Females over the age of 14 years showing no clinical evidence of carrier status were taken to be normal.² DNA was extracted from whole blood by the method of Kunkel *et al.*¹¹ The DNA was digested with restriction endonuclease (3 to 5 units μg^{-1} DNA), separated by electrophoresis in 0.8% agarose, and transferred to nylon filters by the method of Southern.¹² The probes and their associated restriction fragment length polymorphisms are shown in table 1. Probes were labelled by random priming and hybridisations carried out as described previously.^{5, 14}

Linkage analyses were carried out using the linkage programs described by Clayton¹⁵ (two point analyses) and the LINKMAP subroutine of LINKAGE¹⁶ (multipoint analysis). Approximate 90% confidence limits were calculated by reducing the lod score at the maximum value of the recombination fraction by one unit.¹⁷

Results

LINKAGE ANALYSES

The results of two point linkage analyses using nine DNA probes are shown in table 2. Three loci (*DXS1*, *DXYS1*, *DXS72*) show no definite recombination with *TCD* in these families. The strongest evidence for close linkage is between *TCD* and *DXYS1* with a lod score of 5.16 at zero recombination (90% limits,

$\theta=0-0.10$). The *DXS1* locus located more proximally (table 1) also shows no recombination (lod score 1.98), nor does the *DXS72* locus (lod score 1.97). With more distal probes (*DXS17*, *DXS11*, *DXS178*, *DXS177*) the recombination fractions (θ) are larger and the lod scores low, as is the case with the proximal X short arm marker, *DXS14*. The *DXS3* locus has been reported to show significant evidence of linkage to *TCD* at θ values of 0.04 (lod score 12.32) and 0.05 (lod score 5.81) respectively.^{4, 5} We found a maximum likelihood value of θ at 0.11 at a lod score of 1.62. Since recombination has been observed with *DXS3*, a multipoint analysis was run with *TCD*, *DXYS1*, *DXS72*, and *DXS3*, using LINKMAP.¹⁶ The result is shown in table 3. The most likely order, with *TCD* proximal to *DXYS1* and *DXS3* is only 4.4 fold more

Table 3 Results of a multipoint linkage analysis of *TCD* and three loci (*DXYS1*, *DXS72*, *DXS3*), to try and establish the most likely order of these loci. The results were obtained with the program LINKMAP. Location scores, lod scores (z), and odds were obtained as described by Lathrop *et al.*¹⁶

Order	Location score (z)	Odds
A <i>TCD-DXS72-DXYS1-DXS3</i>	37.97 (8.25)	A v D 4.4:1
B <i>DXS72-TCD-DXYS1-DXS3</i>	37.97 (8.25)	B v D 4.4:1
C <i>DXS72-DXYS1-TCD-DXS3</i>	36.41 (7.92)	C v B 2.2:1
D <i>DXS72-DXYS1-DXS3-TCD</i>	35.00 (7.61)	D v C 2.0:1

Table 1 Summary of the probes used in this investigation with their locations and associated polymorphisms. Nomenclature and assignments as in Mandel *et al.*¹³

Locus	Probe	Region	RFLP enzyme	Alleles (kb)	Allele frequencies
<i>DXS14</i>	p58-1	Xp11.21	<i>MspI</i>	4.0/2.5	0.65/0.35
<i>DXS1</i>	p8	Xq11.2-q12	<i>TaqI</i>	15.0/9.0	0.84/0.16
<i>DXS72</i>	pX65H7	Xq21.1	<i>HindIII</i>	7.2/0.7	0.45/0.55
<i>DXS232</i>	pJL68	Xq21.1-q21.2	—	—	—
<i>DXS233</i>	pJL8	Xq21.1-q21.2	—	—	—
<i>DXS276</i>	pJL77	Xq21.1	—	—	—
<i>DXYS1</i>	pDP34	Xq21.31	<i>TaqI</i>	11.0/12.0	0.60/0.40
<i>DXS3</i>	p19-2	Xq21.3	<i>MspI</i>	11.0/4.3	0.79/0.21
			<i>TaqI</i>	2.95, 2.15/5.1	0.62/0.38
<i>DXS17</i>	S21,S9	Xq22	<i>TaqI</i>	2.2/2.0	0.65/0.35
<i>DXS178</i>	p212/9	Xq21.33-q22	<i>TaqI</i>	3.2/1.8	0.70/0.30
<i>DXS11</i>	p22-33	Xq24-q25	<i>TaqI</i>	11.0/20.0	0.83/0.17
<i>DXS177</i>	pλ.2.7	Xq26	<i>EcoRI</i>	7.0/6.0	0.80/0.20

Table 2 Table of lod scores between *TCD* and the markers shown, with the maximum likelihood values of the recombination fraction (θ_{max}) and the corresponding lod scores (lod_{max}).

Locus	Recombination fraction								Lodmax	θ_{max}
	0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.40		
<i>DXS14</i>	—∞	—1.78	—0.80	—0.34	—0.08	0.05	0.11	0.10	0.12	0.34
<i>DXS1</i>	1.98	1.82	1.65	1.48	1.30	1.11	0.91	0.48	1.98	0.00
<i>DXS72</i>	1.97	1.79	1.60	1.40	1.19	0.97	0.75	0.28	1.97	0.00
<i>DXYS1</i>	5.16	4.68	4.19	3.68	3.14	2.60	2.05	0.97	5.16	0.00
<i>DXS3</i>	—∞	1.38	1.61	1.58	1.44	1.25	1.03	0.56	1.62	0.11
<i>DXS17</i>	—∞	—0.56	0.33	0.70	0.85	0.86	0.77	0.38	0.87	0.23
<i>DXS11</i>	—∞	1.42	1.49	1.42	1.28	1.10	0.88	0.40	1.49	0.09
<i>DXS178</i>	—∞	0.05	0.91	1.16	1.19	1.11	0.94	0.46	1.20	0.19
<i>DXS177</i>	—∞	—0.23	0.02	0.13	0.19	0.21	0.20	0.14	0.21	0.27

likely than that with the disease locus located distal to *DXS3*. The four point lod scores for the most likely orders (A, B in table 3) with *TCD* proximal to *DXYS1* are 8.25, providing highly significant evidence of linkage. These results assume that the three marker loci are each separated by a recombination fraction of 0.02 in the order *DXS72-DXYS1-DXS3*.^{5 18}

DELETION SCREENING

None of the nine probes examined was found to be deleted in any of these families. Affected family members were screened with three additional probes isolated by Nussbaum *et al*: pJL8 (*DXS233*), pJL68 (*DXS232*), and pJL77 (*DXS276*). No evidence of deletion or rearrangement was found with these three loci by Southern analysis of patient's DNA digested and separated by conventional electrophoresis. A rare restriction fragment length polymorphism was seen for *DXS233* with the enzyme *PvuII* in one family (alleles 5 kb and 9 kb), but no other variants were identified.

Discussion

These linkage results are consistent with those reported previously, with *TCD* tightly linked to *DXYS1* (Xq21.31) and neighbouring probes *DXS72* and *DXS3* (four point lod score 8.25). The *DXS1* locus located more proximally in Xq11-q13 shows no definite recombination either (lod score 1.98), consistent with the possibility that this proximal, centromeric region shows relatively low rates of recombination. More distally located probes (*DXS17*, *DXS178*, *DXS11*, *DXS177*) show higher recombination rates with the *TCD* locus, consistent with a localisation for *TCD* in the Xq21.2-q21.3 region.^{1 5 18}

A number of microscopic and submicroscopic deletions have been identified in patients in whom *TCD* forms part of more complex syndromes, including mental retardation, deafness, and cleft lip and palate.⁷⁻⁹ More recently, microdeletions were found in two out of eight unrelated *TCD* males with no other clinical abnormalities,¹⁰ using probe p1bD5 (*DXS165*). We therefore wished to exclude the possibility of similar microdeletions in this sample of patients from classical *TCD* families using three loci, two of which (*DXS232*, *DXS233*) lie within a deletion (XL-45) containing the *TCD* locus which is estimated to be <1250 kb in size.⁹ The results show that there is no evidence of microdeletions detectable with the above probes. We were also unable to detect any submicroscopic deletions in these families with p1bD5 (*DXS165*), although one small (120 to 150 kb) deletion was found in family C3 using two clones located distal to p1bD5 (F P M Cremers *et al*, submitted). Merry *et al*¹⁹ also failed to find deletions in any of 42 unrelated *TCD* probands using probe p1bD5, but detected a translocation breakpoint in a

woman with choroideremia and a de novo X;13 translocation.

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