Linkage studies and deletion screening in choroideremia

A F Wright, R L Nussbaum, S S Bhattacharya, M Jay, J G Lesko, H J Evans, B Jay

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,\(^5\) both located in proximal Xq. Lesko et al\(^6\) 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 others\(^7\) 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 reassocation 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. Females over the age of 14 years showing no clinical evidence of carrier status were taken to be normal. DNA was extracted from whole blood by the method of Kunkel et al. 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. 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.

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

**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, θ = 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. We found a maximum likelihood value of θ at 0.011 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. The result is shown in table 3. The most likely order, with TCD proximal to DXYS1 and DXS3 is only 4-4 fold more likely than DXS3 proximal to DXYS1.

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

<table>
<thead>
<tr>
<th>Locus</th>
<th>Probe</th>
<th>Region</th>
<th>RFLP</th>
<th>Alleles (kb)</th>
<th>Allele frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXS14</td>
<td>p58-1</td>
<td>Xq11.21</td>
<td>MspI</td>
<td>4.0-2.5</td>
<td>0.65±0.35</td>
</tr>
<tr>
<td>DXS1</td>
<td>p8</td>
<td>Xq11.2-q12</td>
<td>TaqI</td>
<td>15.0-9.0</td>
<td>0.84±0.16</td>
</tr>
<tr>
<td>DXS72</td>
<td>pX65H7</td>
<td>Xq21.1</td>
<td>HindIII</td>
<td>7.2±0.7</td>
<td>0.45±0.55</td>
</tr>
<tr>
<td>DXS242</td>
<td>pJL68</td>
<td>Xq21.1-q21.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DXS233</td>
<td>pJL8</td>
<td>Xq21.1-q21.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DXS276</td>
<td>pJL77</td>
<td>Xq21.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DXYS1</td>
<td>pDP34</td>
<td>Xq21.3</td>
<td>TaqI</td>
<td>11.0-12.0</td>
<td>0.60±0.40</td>
</tr>
<tr>
<td>DXS3</td>
<td>p19-2</td>
<td>Xq21.3</td>
<td>MspI</td>
<td>11.0-12.0</td>
<td>0.79±0.21</td>
</tr>
<tr>
<td>DXS17</td>
<td>pS11.5</td>
<td>Xq22</td>
<td>TaqI</td>
<td>2.95±0.5</td>
<td>0.62±0.38</td>
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<tr>
<td>DXS178</td>
<td>p2129</td>
<td>Xq21.3-q22</td>
<td>TaqI</td>
<td>3.2±1.8</td>
<td>0.70±0.30</td>
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<tr>
<td>DXS1</td>
<td>p22-33</td>
<td>Xq24-q25</td>
<td>TaqI</td>
<td>11.0±2.0</td>
<td>0.83±0.17</td>
</tr>
<tr>
<td>DXS177</td>
<td>p6.7</td>
<td>Xq26</td>
<td>EcoRI</td>
<td>7.0±0.0</td>
<td>0.80±0.20</td>
</tr>
</tbody>
</table>

**Table 2** Table of lod scores between TCD and the markers shown, with the maximum likelihood values of the recombination fraction (θmax) and the corresponding lod scores (lodmax).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Recombination fraction</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0-00</td>
</tr>
<tr>
<td>DXS14</td>
<td>-∞</td>
</tr>
<tr>
<td>DXS7</td>
<td>1.98</td>
</tr>
<tr>
<td>DXS72</td>
<td>1.97</td>
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<tr>
<td>DXYS1</td>
<td>5.16</td>
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<tr>
<td>DXS3</td>
<td>-∞</td>
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<tr>
<td>DXS17</td>
<td>-∞</td>
</tr>
<tr>
<td>DXS11</td>
<td>-∞</td>
</tr>
<tr>
<td>DXS178</td>
<td>-∞</td>
</tr>
<tr>
<td>DXS177</td>
<td>-∞</td>
</tr>
</tbody>
</table>

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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.

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 definitive 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.

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. More recently, microdeletions were found in two out of eight unrelated TCD males with no other clinical abnormalities, 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. 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 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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