Genetic localisation of the RP2 type of X linked retinitis pigmentosa in a large kindred

A F Wright, S S Bhattacharya, M A Aldred, M Jay, A D Carothers, N S T Thomas, A C Bird, B Jay, H J Evans

Abstract
Genetic linkage and deletion studies have led to the proposal that there are at least two loci on the X chromosome which are responsible for X linked retinitis pigmentosa (XLRP). One locus (RP3) has been closely defined by genetic linkage and deletion analyses and localised to the region between the ornithine transcarbamylase (OTC) and chronic granulomatous disease (CYBB) loci in Xp11.1–p11.4. The other region (RP2) has been assigned by linkage analysis alone to region Xp11.4–p11.2, but its localisation is less well defined. The results of a multipoint linkage analysis of a single large XLRP kindred using eight informative loci provide further evidence on the localisation of RP2 to this region. The maximum likelihood location of this locus shows a multipoint lod score of 7.17 close to DXS255 (in Xp11.22) and TIMP (in Xp11.3–p11.23), neither of which show recombination with RP2, in an area extending from 2 cM proximal to DXS7 to 1 cM distal to DXS14 (approximate 95% confidence limits).

X linked retinitis pigmentosa (XLRP) is a severe form of outer retinal dystrophy characterised by onset of night blindness in the first or second decade followed by progressive narrowing of the visual fields and usually loss of central vision before the fourth decade. At least 14 to 15% of families and 15 to 28% of retinitis pigmentosa patients in the UK have the X linked disorder so that the population prevalence is in the region of 1 in 10 000 to 30 000.

XLRP was first mapped to the proximal short arm of the X chromosome by genetic linkage to DXS7, localised to Xp11.4–p11.3. Subsequent linkage studies have supported locations both proximal and distal to the DXS7 locus. The situation was clarified by a heterogeneity analysis of 62 XLRP kindreds from nine centres, in which it was shown that the overall likelihood was 6.4×10^5 in favour of two loci versus a single XLRP locus. The majority of families (60 to 75%) were associated with an RP3 locus at 1 to 4 cM distal to OTC, but 25 to 40% of families were consistent with an RP2 locus localised to a broader region between 3 cM proximal to DXS7 and 1 cM distal to the centromeric clone DXZ1, representing about 16 cM on the genetic map. There was some additional evidence for a third locus between DXS28 and DXS164, but evidence from patients with deletions in this region tends not to support this.

The site of the RP3 locus has been further defined by the analysis of two patients with X chromosomal deletions showing complex phenotypes, including retinitis pigmentosa. The available evidence from this source localises RP3 to a small (<400 kb) region between CGD and the proximal BB deletion breakpoint. No deletions have been found in association with the RP2 locus.

This study presents the results of a linkage analysis of a large XLRP kindred described previously, in which the gene responsible is consistent with an RP2 location and which refines the localisation of this locus.

Materials and methods

ASCERTAINMENT AND DIAGNOSIS OF FAMILY MEMBERS

The kindred (RP22, F15) was ascertained through the Genetic Clinic, Moorfields Eye Hospital, London. Diagnoses were made by ophthalmological examination including fundus examination, visual field tests, electroretinogram, and, in the case of at risk females, by photopic flicker sensitivity. The diagnoses were...
Figure 1. Pedigree of the A1 kindred showing the results in each subject. The order of probes used and corresponding alleles (1, 2 represent A1, A2, etc.) are shown below each subject tested. The clinical status is given, with normal males (♂), female shown to be carriers (♀), obligate carrier female (○).
as described previously with the exception of one at risk female (III-29, fig 1) who on re-evaluation was found to show only equivocal evidence of the carrier state so was reclassified as genetic status unknown.

DNA METHODS
DNA was extracted from whole blood by the method of Kunkel et al. It was digested with restriction endonuclease using 3 to 5 units µg⁻¹ DNA, separated by gel electrophoresis in 0.8% agarose and 1 × TBE buffer, and transferred to nylon (Genescreen Plus, Nytran) filters by the method of Southern. Probes were labelled with and without isolation of the insert in low melting agarose, by random priming or nick translation, hybridised, and washed at 65°C as described previously. The TIMP polymorphisms were analysed as described by Aldred and Wright by means of the polymerase chain reaction, followed by digestion with BglII and BglIII, agarose gel electrophoresis and staining with ethidium bromide. The combined heterozygosity at this locus is 68%. The probes used and their associated polymorphisms are shown in table 1.

LINKAGE ANALYSIS
Linkage was analysed using the LINKAGE program package. Serial four point analyses were run using the LINKMAP sub-routine and the resultant recombination fractions were converted to a linear genetic map of 50 points using the Kosambi mapping function. The multipoint lod scores were compared at each of the 50 genetic map points over all analyses. The genetic map included fixed locations for the eight informative loci (DXS164, DXS84, DXS7, TIMP, DXS255, DXS14, DXS1, and DXYS1) as shown in table 1. Uninformative loci included DXS41, OTC, OATL1, and DXSI46. The likelihoods were used to compute the posterior probability of linkage to RP3 (set at 23 ± 2 cM) and RP2 (set at 0 ± 10 cM) respectively, after integration over the multipoint likelihood curve in these regions.

Results
TWO POINT ANALYSES
The results of two point analyses are shown in table 2. Two loci, located in Xp11.22 and Xp11.3–p11.23 respectively, show no evidence of recombination with XLRP in this kindred, namely DXS255 (Zmax=6.92) and TIMP (Zmax=4.14). By contrast, Xp21 loci such as DXS164 and DXS84, both of which are closely linked to RP3, show no evidence of linkage to XLRP in this kindred (Zmax=0.50, 0.40; Zmax=0.00, 0.05). Probe DXS7 (Xp11.4–p11.3) shows significant evidence of linkage to XLRP at a recombination fraction (Zmax=0.29) as does the proximal Xq locus DXS14 (Xp11.21) (Zmax=0.50, 0.40, 0.29). The proximal Xq locus DXS14 (Xq11.2–q12) shows no evidence of recombination with XLRP in the M kindred, although this is not statistically significant (Zmax=0.00, Zmax=1.8), while the more distal long arm locus DXS11, situated close to the choroideremia gene in Xq21, shows only loose linkage to XLRP (Zmax=0.29, Zmax=0.50).

Table 1 Table of probes and their associated polymorphisms used in this study. The chromosomal assignment of each locus is shown together with their assumed locations on a linear genetic map based on the data of Ott et al and Mahanta and Willard. The TIMP locus was amplified by means of the polymerase chain reaction as described by Aldred and Wright.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Probe</th>
<th>Location (cM)</th>
<th>RFLP</th>
<th>Fragment size</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXS164</td>
<td>pERT87-8</td>
<td>Xp21.2 (32-1)</td>
<td>Taq1</td>
<td>1-1, 2-7/3-8</td>
<td>0-74/0-26</td>
</tr>
<tr>
<td>DXS84</td>
<td>754</td>
<td>Xp21.1 (28-0)</td>
<td>Ps1</td>
<td>12-0/9-0</td>
<td>0-62/0-38</td>
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<tr>
<td>DXS7</td>
<td>L1.28</td>
<td>Xp11.4–p11.3 (13-0)</td>
<td>Taq2</td>
<td>12-0/9-0</td>
<td>0-68/0-32</td>
</tr>
<tr>
<td>TIMP</td>
<td></td>
<td>Xp11.3–p11.23 (3-0)</td>
<td>BglII</td>
<td>3-1/3-0</td>
<td>0-66/0-34</td>
</tr>
<tr>
<td>DXS255</td>
<td>M27β</td>
<td>Xp11.2 (22-0)</td>
<td>Ps2</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>DXS14</td>
<td>p58-1</td>
<td>Xp11.21 (+6)</td>
<td>Msp1</td>
<td>4-0/2-5</td>
<td>0-65/0-35</td>
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<tr>
<td>DXS1</td>
<td>p8</td>
<td>Xp11.2–q12 (+20-0)</td>
<td>Taq2</td>
<td>15-0/9-0</td>
<td>0-84/0-16</td>
</tr>
<tr>
<td>DXYS1X</td>
<td>pDP34</td>
<td>Xq21.31 (30-0)</td>
<td>Msp1</td>
<td>11-0/12-0</td>
<td>0-60/0-40</td>
</tr>
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</table>

Table 2 Results of two point linkage analysis between X linked retinitis pigmentosa and eight polymorphic markers in the M kindred. The maximum likelihood values of the recombination fraction (Zmax) and lod score (Zmax) are shown.

<table>
<thead>
<tr>
<th>Locus</th>
<th>0-00</th>
<th>0-01</th>
<th>0-05</th>
<th>0-10</th>
<th>0-20</th>
<th>0-30</th>
<th>0-40</th>
<th>Zmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXS164</td>
<td>-∞</td>
<td>-∞</td>
<td>-∞</td>
<td>-∞</td>
<td>-∞</td>
<td>-∞</td>
<td>-∞</td>
<td>0-00</td>
</tr>
<tr>
<td>DXS84</td>
<td>-∞</td>
<td>-∞</td>
<td>-∞</td>
<td>-∞</td>
<td>-∞</td>
<td>-∞</td>
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<td>0-00</td>
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<tr>
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<td>-∞</td>
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<td>-∞</td>
<td>-∞</td>
<td>-∞</td>
<td>-∞</td>
<td>0-00</td>
</tr>
<tr>
<td>TIMP</td>
<td>4-14</td>
<td>4-07</td>
<td>3-82</td>
<td>3-48</td>
<td>2-76</td>
<td>1-96</td>
<td>1-05</td>
<td>0-00</td>
</tr>
<tr>
<td>DXS255</td>
<td>6-92</td>
<td>6-82</td>
<td>6-41</td>
<td>5-87</td>
<td>4-69</td>
<td>3-36</td>
<td>1-82</td>
<td>0-00</td>
</tr>
<tr>
<td>DXS14</td>
<td>-∞</td>
<td>2-40</td>
<td>2-94</td>
<td>2-80</td>
<td>2-38</td>
<td>1-75</td>
<td>0-96</td>
<td>0-00</td>
</tr>
<tr>
<td>DXS1</td>
<td>1-06</td>
<td>1-04</td>
<td>0-97</td>
<td>0-88</td>
<td>0-68</td>
<td>0-48</td>
<td>0-25</td>
<td>0-00</td>
</tr>
<tr>
<td>DXYS1</td>
<td>-∞</td>
<td>-∞</td>
<td>-∞</td>
<td>-∞</td>
<td>-∞</td>
<td>-∞</td>
<td>-∞</td>
<td>0-50</td>
</tr>
</tbody>
</table>
MULTIPOINT ANALYSES
The results of the multipoint analysis are shown in fig 2. The maximum likelihood location of the XLRP locus segregating in this family is between DXS255 and TIMP (Zmax=7:17) although there is a broad peak with the approximate 95% confidence limits extending between a point 2 cM proximal to DXS7 and another 1 cM distal to DXS14. These results are confirmed by reference to the pedigree shown in fig 1. The probe results are shown and individual haplotypes inferred so that the location of crossovers can be identified. Two recombinants are found between DXS7 and RP2, in subjects III-13 and IV-17, neither of which are recombinant with TIMP, or other proximal probes, while the distal probes DXS164 (III-13) and DXS84 (IV-17) are also recombinant. These results are both consistent with an XLRP locus proximal to DXS7. Similarly, there is a single definite recombinant with DXS14 which is non-recombinant with DXS255 (III-30), supporting a location distal to DXS14.

PROBABILITY OF RP2 VERSUS RP3 IN THE M KINDRED
The posterior probability of an RP2 versus an RP3 locus was computed on the basis of the multipoint likelihoods. This requires assumptions firstly about the location of the RP3 locus in Xp21.1–p11.4. This locus has been defined genetically as lying 1 cM distal to OTC, which is consistent with the physical mapping data. The weighted likelihood integral was therefore calculated using as weighting function a Gaussian prior distribution centred at −23 cM (fig 2) with a standard deviation of 1 cM. Similarly for RP2, the locus was defined as lying within 10 cM on either side of the DXS255 locus (corresponding to −10 cM to +10 cM, fig 2), which is consistent with the localisation of Ott et al. In this case, the Gaussian weighting function was therefore centred at 0 cM with a standard deviation of 5 cM. The prior probability of each locus was assumed to be equal and the posterior probabilities derived for RP2 and RP3. The results showed that the posterior probability of RP2 is 1:00 in this kindred, while that for RP3 is zero.

Discussion
One of the major problems in refining the genetic localisation of RP2 is the presence of genetic heterogeneity and the difficulty of assigning small or moderate sized families to one or other locus. The M kindred, however, is large enough to provide an unambiguous assignment to either RP3 or RP2. The results of linkage analysis using eight informative polymorphic loci show that the posterior probability of RP2 in this kindred is 1:00, while that for RP3 is zero. No recombination was observed between RP2 and DXS255 (Zmax=6:92) or TIMP (Zmax=4:14), assigned to Xp11.22 and Xp11.3–p11.23 respectively. A third locus in this region, DXS426, located in Xp11.4–p11.22, has also been found to show no evidence of recombination with RP2 in this family (Zmax=5:13), as reported elsewhere. Low recombination frequencies were observed with two other loci in the Xp11 region, DXS7 (Xp11.4–p11.3) and DXS14 (Xp11.21), which showed values of 0:08 (Zmax=4:21) and 0:07 (Zmax=2:85) respectively. By contrast, significant recombination was found between RP2 and the Xp21 loci DXS164 and DXS84, as well as with the proximal Xq marker DXYS1 (table 2). The results with DXS164 showed that the disease locus can be excluded for distances up to a recombination fraction of 0:17 (Z=−2:04). The estimated recombination fraction between RP3 and DXS164 is 0:09 (see table 1 and above), which therefore excludes this locus. These results tentatively suggest that the most likely chromosomal location for RP2 is in R band Xp11.23, since it has been suggested that most genes are located in R rather than G bands, although it is impossible to exclude the presence of the gene in a small R sub-band within a G band. The matter is not of purely academic significance in the light of the ability to microdissect and isolate large numbers of clones from chromosomal bands.

The multipoint analysis showed a broad likelihood peak between DXS7 and DXS14 (fig 2), consistent with the observed pattern of recombination (fig 1), which argues for a location proximal to DXS7 and distal to DXS14. Analysis of further RP2 families in which there is recombination between RP2 and TIMP or DXS255 will be required to establish whether RP2 is proximal or distal to these loci.
Are there clinical features in this family that might help to distinguish RP2 from RP3 type families? This is an important question since many moderate and small families with XLRP cannot be assigned unambiguously, which complicates or excludes the use of probes for diagnostic purposes and limits the sample of families available for detecting rare recombinants and hence refining the genetic localisation. The clinical picture in affected males is relatively uniform in the M family with onset of night blindness in childhood, sometimes as early as 3 years of age, followed by a progressive generalised contraction of visual fields and loss of central vision in the older men. Males show a moderate degree of myopia (−6 D to −10 D). The fundus appearances and clinical features were initially described as a choroidoretinal dystrophy as distinct from retinitis pigmentosa. However, it is now thought to be relatively typical of XLRP, since choroidal atrophy is seen commonly with longstanding outer retinal atrophy. Carrier females in this family show no evidence of a tapetal reflex, in contrast to at least some RP3 families, but show varying degrees of peripheral retinal pigment epithelial atrophy with or without pigment migration. None of these features suggests an absolute distinction between RP3 and RP2 loci, since all the reported features have been described in association with RP3 loci, although myopia in hemizygotes may be more common with RP2 and tapetal reflex in carriers may be found exclusively with RP3. It is interesting to note that all affected males are myopic while all of their unaffected sibs are emmetropic, perhaps suggesting a pleiotropic effect of RP2, but if so it may be non-specific and related more to severity than to type. The wide variation in expression of XLRP both within and between many families suggests that gross clinical differences are unlikely to provide a useful means of distinguishing these loci.

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