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

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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 Xp21.1–p11.4. The other locus (*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[1 2] 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.[3] Subsequent linkage studies have supported locations both proximal[4–9] and distal[10–13] 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 $6{\cdot}4\times10^9$:1 in favour of two loci versus a single XLRP locus.[14] 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.[14] There was some additional evidence for a third locus between *DXS28* and *DXS164*,[14] but evidence from patients with deletions in this region tends not to support this.[15]

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.[16 17] The available evidence from this source localises *RP3* to a small (<400 kb) region between *CGD* and the proximal BB deletion breakpoint.[15] 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,[18 19] in which the gene responsible is consistent with an *RP2* location and which refines the localisation of this locus.

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Materials and methods

ASCERTAINMENT AND DIAGNOSIS OF FAMILY MEMBERS

The M 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.[19] The diagnoses were

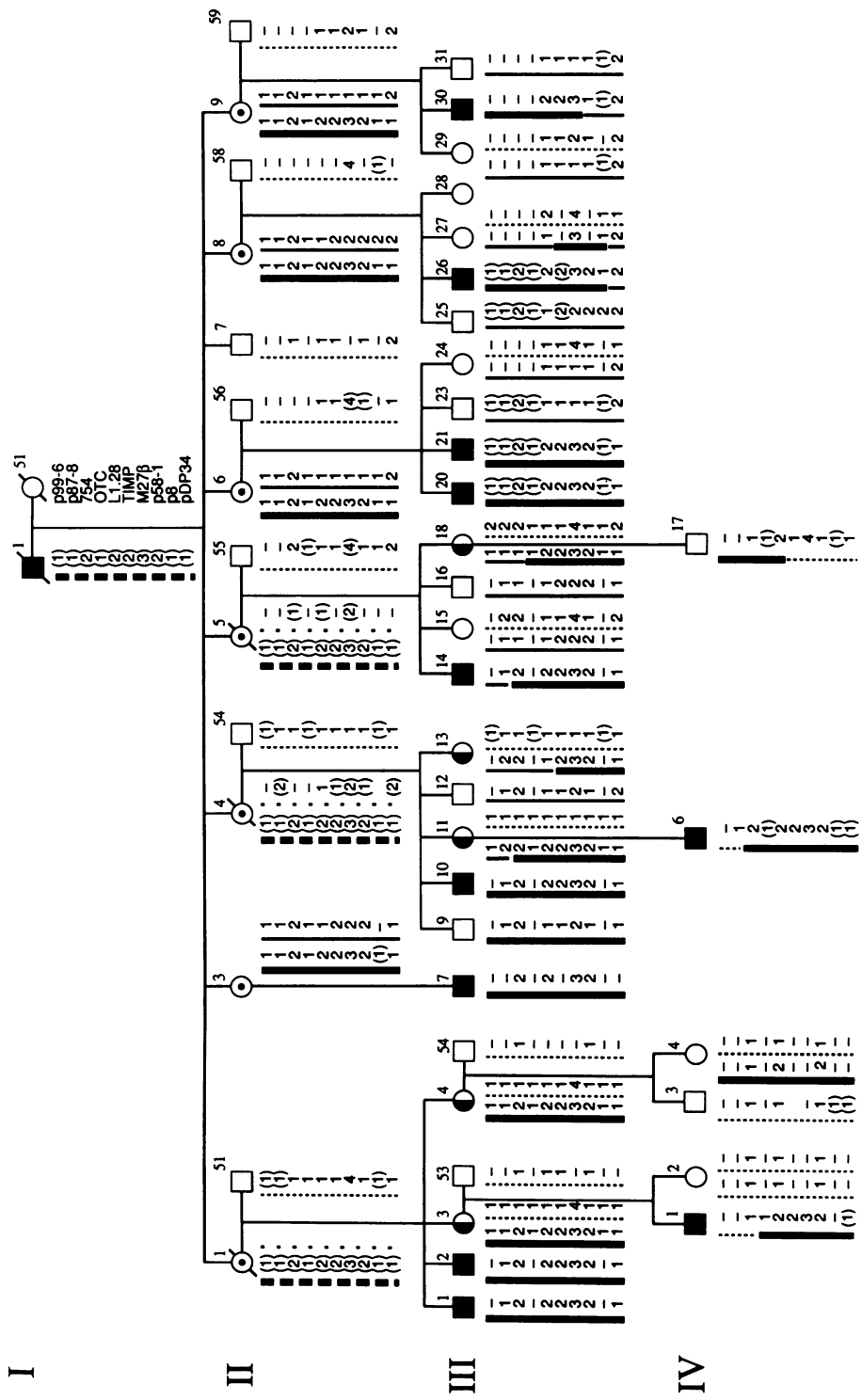

Figure 1 *Pedigree of the M kindred showing the results in each subject. The order of probes used and corresponding alleles (1, 2 represent A1, A2, etc) are shown below each subject tested. The clinical status is given, with normal males (□), affected males (■), females shown to be carriers on testing (◐), obligate carrier females (○).*

as described previously[18 19] 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.*[20] 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.[21] Probes were labelled with and without isolation of the insert in low melting temperature agarose, by random priming or nick translation, hybridised, and washed at 65°C as described previously.[6] The *TIMP* polymorphisms were analysed as described by Aldred and Wright[22] by means of the polymerase chain reaction, followed by digestion with *Bgl*I and *Bgl*II, agarose gel electrophoresis and staining with ethidium bromide. The combined heterozygosity at this locus is 68%.[22] The probes used and their associated polymorphisms are shown in table 1.

LINKAGE ANALYSIS

Linkage was analysed using the LINKAGE program package.[24] 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 combined 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 *DXS146*. 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 (θmax=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 (θmax) of 0·08 (Zmax=4·21), as does the proximal Xp locus *DXS14* (Xp11.21) (θmax=0·07, Zmax=2·85). The proximal Xq locus *DXS1* Xq11.2–q12) shows no evidence of recombination with *XLRP* in the M kindred, although this is not statistically significant (θmax=0·00, Zmax=1·8), while the more distal long arm locus *DXYS1*, situated close to the choroideremia gene in Xq21, shows only loose linkage to *XLRP* (θmax=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[14] and Mahtani and Willard.[23] The TIMP locus was amplified by means of the polymerase chain reaction as described by Aldred and Wright.[22]

Locus	Probe	Location (cM)	RFLP	Fragment size	Frequency
DXS164	pERT87–8	Xp21.2 (−32·1)	*Taq*I	1·1, 2·7/3·8	0·74/0·26
DXS84	754	Xp21.1 (−28·0)	*Pst*I	12·0/9·0	0·62/0·38
DXS7	L1.28	Xp11.4–p11.3 (−13·0)	*Taq*I	12·0/9·0	0·68/0·32
TIMP		Xp11.3–p11.23 (−3·0)	*Bgl*II	3·1/3·0	0·66/0·34
			*Bgl*I	2·6/1·9/0·7	0·55/0·45
DXS255	M27β	Xp11.22 (0)	*Pst*I	Variable	Variable
DXS14	p58–1	Xp11.21 (+6)	*Msp*I	4·0/2·5	0·65/0·35
DXS1	p8	Xq11.2–q12 (+20·0)	*Taq*I	15·0/9·0	0·84/0·16
DXYS1X	pDP34	Xq21.31 (−30·0)	*Taq*I	11·0/12·0	0·60/0·40

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 (θmax) and lod score (Zmax) are shown.

Locus	0·00	0·01	0·05	0·10	0·20	0·30	0·40	θmax	Zmax
DXS164	−∞	−8·02	−4·57	−3·12	−1·71	−0·92	−0·39	0·50	0·00
DXS84	−∞	−3·66	−1·66	−0·89	−0·26	−0·01	0·05	0·40	0·05
DXS7	−∞	3·10	4·11	4·19	3·67	2·75	1·53	0·08	4·21
TIMP	4·14	4·07	3·82	3·48	2·76	1·96	1·05	0·00	4·14
DXS255	6·92	6·82	6·41	5·87	4·69	3·36	1·82	0·00	6·92
DXS14	−∞	2·40	2·84	2·80	2·38	1·75	0·96	0·07	2·85
DXS1	1·06	1·04	0·97	0·88	0·68	0·48	0·25	0·00	1·06
DXYS1	−∞	−3·88	−1·28	−0·31	0·37	0·50	0·36	0·29	0·50

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*,[14] which is consistent with the physical mapping data.[15] 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.*[14] 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.

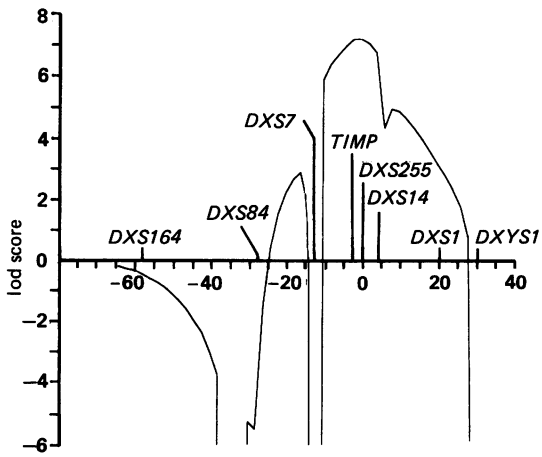

Figure 2 Results of multipoint analysis of X linked retinitis pigmentosa and eight polymorphic markers in the M kindred. The analysis was carried out as described in the text. The multipoint lod scores are plotted against genetic location on a linear genetic map (see table 1).

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.[25] 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,[26] 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.[27]

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 choroido-retinal dystrophy as distinct from retinitis pigmentosa.[18] However, it is now thought to be relatively typical of XLRP,[19] 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,[28] 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,[18] 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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