Retinitis pigmentosa families showing apparent X linked inheritance but unlinked to the RP2 or RP3 loci

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Abstract

Three families with retinitis pigmentosa (RP) are described in which the disorder shows apparent X linked inheritance but does not show linkage to the RP2 and RP3 regions of the short arm of the X chromosome. The families are also inconsistent with a localisation of the disease gene between DXS164 and DXS28. In one case, reassessment of the family in the light of these results suggested that the family may have an autosomal dominant form of RP. The remaining two families are consistent with X linkage and suggest the possibility of a new X linked RP (XLRP) locus.

These families highlight the difficulties in determining the mode of inheritance on the basis of pedigree structure and clinical data alone. Molecular genetics plays an important role in confirming the mode of inheritance and in detecting potential misclassifications, particularly in a group of disorders as heterogeneous as RP. They emphasise that caution is required in genetic counselling of RP families, particularly in the absence of any molecular genetic analysis.

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linkage analysis and deletion patients.⁴⁻¹² It is flanked distally by DXS84 and proximally by OTC and the proximal deletion breakpoint in the patient BB.¹¹ RP2 has been mapped by linkage analysis alone and is flanked by the markers DXS7 and DXS255.^{13,14}

In the course of analysing the linkage results in 40 XLRP families,¹⁵ we identified three families which fit neither an RP2 nor an RP3 location. All three families were originally considered to have XLRP. We discuss whether there might be other XLRP loci, or whether these families might have autosomal dominant RP (ADRP).

Materials and methods

DNA extraction and analysis was carried out as previously described.^{16,17} The markers used in this study are shown in table 1. Linkage analysis was carried out using the LINKAGE v5.03 program package. Families were assumed to show X linked segregation and multipoint lod scores were calculated using LINKMAP. Females were classified as obligate carriers if they had an affected father or son, and otherwise as of unknown genotype. Posterior probabilities of linkage to RP2 or RP3 were calculated as described by Wright *et al.*¹⁶ and van Dorp *et al.*¹⁸ Locations for the two loci were assumed to be RP3 0.4 cM distal to OTC and RP2 6.5 cM proximal to DXS7.¹⁵

FAMILY 23

This family was ascertained through the Genetic Eye Clinic in Birmingham and the pedigree is shown in fig 1. Three of seven brothers have severe retinitis pigmentosa with onset in early childhood. The mother, now asymptomatic in her eighties, was reported to show signs of being a carrier for X linked RP when examined

Retinitis pigmentosa is a group of retinal disorders characterised by progressive retinal degeneration with pigmentary retinopathy, night blindness, and progressive reduction of the visual fields. It may be inherited as an autosomal dominant, autosomal recessive, or X linked recessive trait. Of these, X linked RP is one of the most severe forms, with affected males commonly losing their central vision by the fourth or fifth decade of life. The family history is very important in determining the mode of inheritance, as the different forms of RP are not sufficiently distinct to allow a differential diagnosis to be made solely on clinical grounds.^{1,2}

The X linked type of RP is itself genetically heterogeneous. There are at least two X linked genes, RP2 at Xp11.4-p11.2 and RP3 at Xp21.1-p11.4.³ The existence of a third XLRP gene, localised between DXS164 and DXS28, has been postulated.^{4,5} However, patients deleted for this region of Xp have not been shown to have RP.⁶⁻⁸ The region containing the RP3 gene has been defined by

Table 1 Polymorphic markers used in DNA analysis

Marker	Location
DXS85	Xp22.3-p22.2
DXS41	Xp22.1
DXS164	Xp21.1
DXS206	Xp21.1
DXS84	Xp21.1
OTC	Xp21.1
DXS228	Xp11.4-p11.3
DXS7	Xp11.4-p11.3
SYN/ARAF1	Xp11.23
TIMP	Xp11.3-p11.23
OATL1	Xp11.3-p11.23
DXS255	Xp11.22
DXS146	Xp11.22
DXS1	Xq11.2-q12
DXYS1X	Xq21.31

Figure 1 Segregation of marker alleles in F23. The affected haplotype is shown as a thick solid line and recombinants as a change in shading. Before examination of markers between DXS84 and DXS255, this family was thought to be RP2 type, as RP apparently segregated with allele 2 at DXS255 with only one recombinant (II.7). However, additional markers and haplotype analysis showed that subjects II.6 and II.7 have different alleles at all marker loci except for DXS85. Thus it is unlikely that RP2 or RP3 could be the disease locus.

30 years ago. The father died at age 60 having had a normal fundus examination. There is no known earlier history of RP in this family. The daughters of affected male II.6 show patches of pigmentation in the periphery of their fundi, characteristic of the carrier state, and the single daughter of II.7 shows patchy peripheral pigmentation and a slightly reduced scotopic response on electroretinography (ERG). None of these females has a tapetal reflex. The family was therefore considered to have X linked RP.

FAMILY 54

This family, shown in fig 2, was ascertained through the Genetic Clinic at Moorfields Eye

Hospital, London. The pedigree structure is more immediately suggestive of X linked inheritance than F23, although again there are no affected males documented in the early generations. Subject III.3 was first diagnosed as having RP at the age of 24 and is now registered as partially sighted. Subject II.2 has suffered night blindness from an early age. II.1 has refused to be examined.

FAMILY 56

This family, also ascertained through the Genetic Clinic at Moorfields Eye Hospital, has been previously reported by Redmond *et al.*¹⁹ It contains a large sibship with five males severely affected with RP (fig 3). They all developed night blindness before the age of 10, have extinguished ERGs, poor visual acuities, posterior subcapsular lens opacities, and advanced fundus changes. These clinical details are shown in table 2. Their mother has peripheral bone spicule retinopathy and subnormal ERG with good vision at the age of 68, suggesting that she is a carrier of XLRP. Three of her daughters have scattered pigmentation of the retina or thinning of the retinal pigment epithelium or both, but normal vision, normal electrophysiology, and no night blindness, indicating that they are carriers of XLRP. A male in the third generation who is the son of a potential carrier female has recently been reported to show pigmentation of the fundus at 10 years of age. The boy is asymptomatic at present. It is hoped that psychophysical tests can be performed to establish whether or not he is affected by RP. There is no family history of RP in previous generations.

Results

HAPLOTYPE ANALYSIS

The pedigrees and segregation of marker alleles are shown in figs 1 to 3. The haplotypes shown are those which minimise the total number of recombinants.

FAMILY 23

Eleven markers were informative (fig 1), covering most of Xp and extending to proximal Xq. At all of these loci except DXS85, subjects II.6 and II.7, who are both affected, have different alleles. On the basis of these results, together with those from the rest of the sibship, it is unlikely that the disease locus resides within the region covered by these markers, unless more complicated haplotypes with double recombinants are invoked.

FAMILY 54

Nine loci were informative in one or both of the carriers II.1 and II.2 (fig 2). Subject III.1, an unaffected male, and his affected half brother III.3 share the same alleles at all loci examined. In contrast, III.1 only has the same allele as his unaffected half brother III.4 and unaffected cousin III.7 at DXS41. These results indicate that neither an RP2 nor an RP3 location for the disease gene is likely.

Figure 2 Segregation of marker alleles in F54. The pedigree structure suggests an X linked disorder, with affected cousins (III.3 and III.6) and transmission through females. Affected male III.3 and his unaffected half brother III.1 have alleles in common at all informative marker loci examined, indicating that RP in this family is probably not the result of mutation in the RP2 or RP3 loci.

FAMILY 56

This family was informative for six marker loci (fig 3). The results are similar to the previous two families. Subject II.4, who is unaffected, has alleles in common with his affected sibs II.2, II.3, and II.5 at all six loci. Affected male II.6 has the unaffected haplotype at DXS206, DXS84, and TIMP, recombining to the affected

haplotype at DXS255. Subject II.13 (also affected) shows the reverse of this situation, with the affected haplotype for DXS206 and DXS84, but the unaffected haplotype for TIMP and DXS255. Neither an RP3 nor an RP2 mutation seems likely in this family, given these haplotypes. If RP2 was proximal to DXS255, as previously suggested,³ then cross-overs would be required between DXS255 and RP2 in subjects II.4 and II.13.

POSTERIOR PROBABILITIES OF LINKAGE

Posterior probabilities of linkage to RP3, RP2, or neither were calculated (table 3). Each of the three families showed a greater than 80% probability of the disease being unlinked either to RP2 or to RP3, supporting the haplotype analyses.

Discussion

We have described three families thought to have the X linked form of RP on the basis of pedigree structure and because the males are severely affected, while the females show only mild changes typical of the carrier state. However, multipoint linkage analysis showed strongly negative lod scores across the entire region containing the RP2 and RP3 loci. Haplotype analysis also showed that the disease locus is unlikely to be RP2 or RP3. A locus between DXS84 and DXS28, as proposed by Musarella *et al*,⁴⁵ is also unlikely in these families (for example, II.7 in F23 and II.4 in F56). Posterior probabilities indicate that each family has a probability of greater than 0.8 of the disorder being unlinked to either locus.

If RP in these families is not caused by the RP2 or RP3 genes, then there are several possible explanations for these results. The disease gene could still be X linked, as the statistical analysis does not exclude the pos-

Figure 3 Segregation of marker alleles in F56. Unaffected male II.4 has the same marker alleles as his affected brothers (II.2, II.3, II.5) at all loci. Affected male II.6 has the unaffected haplotype distal to DXS255 while, conversely, II.13 (also affected) has the unaffected haplotype at TIMP and more proximal markers but the affected haplotype at DXS84 and more distal markers. These conflicting results make it unlikely that the disease gene is localised within the region spanned by these marker loci.

Table 2 Clinical details of four affected males in family 56

ID	Age	Age of onset	Visual acuity	Lens	Retina	ERG
II.2	42	8	PL	Cataract	Advanced RP	Extinguished
II.3	41	9	PL	Cataract	Advanced RP	Extinguished
II.5	38	8	HM	Cataract	Advanced RP	Not tested
II.12	20	10	6/60	Cataract	Advanced RP	Extinguished

PL=perception of light; HM=hand movements.

Table 3 Posterior probabilities for families 23, 54, and 56

Family	RP3	RP2	Unlinked
23	0.025	0.041	0.934
54	0.092	0.043	0.865
56	0.084	0.006	0.910

sibility of linkage to other regions of the X chromosome. If this is the case, the disease locus could be located either more distally on Xp or on Xq.

Germline mosaicism is a possible source of problems in mapping and prenatal diagnosis of other X linked diseases, such as Duchenne muscular dystrophy^{20,21} and Wiskott-Aldrich syndrome.²² This phenomenon results from a mutation which occurs early in gametogenesis and is present in a proportion of gametes, but not in the somatic cells. It usually manifests as an unaffected transmitting male, or a female who is presumed to be an obligate carrier but does not show any evidence of the mutation somatically, and it can be mistaken for a recurrent new mutation. However, mosaicism would not explain these RP families, where affected and unaffected brothers have the same marker alleles and where their mothers do have a mild disease phenotype. Even if the mothers in F23 and F56 were also somatic mosaics, complex mitotic recombination in early gametogenesis would be required to produce the observed haplotypes.

Another possible explanation, still assuming X linked inheritance, is that the disease gene is only partially penetrant. Thus unaffected males with the affected haplotype (subjects III.I in F54 and II.4 in F56) could carry a non-penetrant RP mutation. However, there are no published reports of incomplete penetrance of XLRP. This hypothesis would not explain the results in F23, where two affected males (II.6 and II.7) have different haplotypes. It is possible that family 23 has autosomal recessive RP (ARRP) and that the children of affected males are manifesting obligate heterozygotes. Clinically detectable decreases in rod sensitivity have been reported in ARRP carriers heterozygous for rhodopsin null alleles²³ and ERG in two carriers heterozygous for β phosphodiesterase mutations showed reduced maximum rod signal.²⁴ However, ARRP carriers do not commonly show fundus changes and this would argue against an autosomal recessive mode of inheritance in family 23.

An alternative hypothesis is that any of these families may have autosomal dominant RP and, by chance, affected males have a more severe phenotype than affected females. ADRP families do not necessarily show evidence of male to male transmission and with reduced penetrance and variable expressivity there is the opportunity for misclassification as XLRP.

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The results of the DNA analyses have prompted a re-evaluation of the clinical information in all three families. In view of the relatively mild symptoms of affected male III.3 in family 54, it now seems more likely that this family may have ADRP. However, in families 23 and 56 where the severe phenotype of the affected males contrasts with a very mild one in females, the clinical data still suggest X linked inheritance of RP in these two families. The results of the DNA analysis then raise the possibility that there is another locus for XLRP, although some caution is required as both families have affected males in only one generation. Further genetic studies are under way to investigate this question. Clinical studies will also continue with particular attention to the third generation of each family and if the young male in family 56 proves to have RP, this would further strengthen the evidence for an X linked mode of inheritance in this family.

These families highlight the difficulties in differentiating the genetic types of RP. Genetic linkage can be of great help in confirming the mode of inheritance, in distinguishing between the different genes responsible for ADRP and XLRP, and in identifying potential misclassifications that have important implications both for genetic counselling and for research. Of the three families presented here, such studies have identified probable misclassification of ADRP as XLRP in one case and could suggest the presence of a new XLRP locus in the remaining two. Both possible explanations are being examined further, while these results emphasise the caution which needs to be exercised in the genetic counselling of families with a condition as heterogeneous as RP.

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