Journal of Medical Genetics 1988, **25**, 222–226

Linkage analysis of X linked retinitis pigmentosa in the Irish population

G J FARRAR*, M T GERAGHTY*, J M B MOLONEY†, D J McCONNELL*, AND P HUMPHRIES*

*From *the Genetics Department, Lincoln Place Gate, Trinity College Dublin; and †the Research Foundation, The Royal Victoria Eye and Ear Hospital, Adelaide Road, Dublin 2, Ireland.*

SUMMARY There is significant evidence for genetic and phenotypic heterogeneity in X linked retinitis pigmentosa (XLRP). We have studied the linkage of XLRP in four Irish families to a number of polymorphic DNA markers. We report linkage between the *DXS7* (L1·28) locus and the *XLRP* locus (Z=3·445, θ=0·00). Combined with the previously published data on British and Danish families, the genetic distance between the *DXS7* and *XLRP* loci is now estimated at 5 cM with a maximum lod score of 13·026 and a 1-lod confidence interval of 0·75 to 9·5 cM. Linkage was also observed between 754 and *XLRP* (Z=3·41, θ=0·00) and between pERT87 and *XLRP* (Z=1·37, θ=0·1). The heterogeneity of XLRP is discussed in relation to these observations.

Retinitis pigmentosa (RP) is a group of heterogeneous disorders involving progressive night blindness, field restriction, and eventual blindness. The frequency of the X linked subtype (XLRP) is estimated at 1 to 2/15 000 representing 14 to 22% of the hereditary forms.[1–3]

Linkage between *XLRP* and the polymorphic DNA marker L1·28 (*DXS7*) on the short arm of the X chromosome (Xp11·0–11·3) was established by Bhattacharya *et al.*[4] The genetic distance between the *DXS7* marker and the *XLRP* locus was estimated at 3 cM with a 95% confidence interval of 0 to 15 cM. Subsequent studies have confirmed this linkage,[5 6] though there is significant evidence of genetic heterogeneity from both genetic and clinical observations.[7 8] In view of this suspected heterogeneity it is important to map *XLRP* in other populations, both to test the possibility of heterogeneity and as a prerequisite to genetic counselling.

We have analysed the inheritance of XLRP in the Irish population by compiling linkage data on Irish families using a number of polymorphic DNA probes around the *XLRP* locus. Here we report on linkage between *XLRP* and 754, L1·28, and pERT87. Our results suggest that the XLRP gene in the Irish population maps in the same position as that found in the British and Danish populations.[4 6]

Materials and methods

SOURCE OF FAMILIES

Four families with typical X linked retinitis pigmentosa were used in this study. These were ascertained from the records of The Research Foundation of The Royal Victoria Eye and Ear Hospital, Dublin. All ophthalmological and electrodiagnostic testing was also done in The Research Foundation of The Royal Victoria Eye and Ear Hospital.

Received for publication 26 February 1987.
Revised version accepted for publication 30 April 1987.

ANALYSIS OF DNA

DNA samples (10 μg) were digested to completion with the relevant restriction endonuclease, separated by electrophoresis in 1% agarose gels, and blot transferred onto nitrocellulose filters according to the method of Southern.[9] Filters were hybridised with 32^P labelled insert from one of the recombinant DNA probes L1·28,[4] 754,[8] pERT87·8,[10] pERT87·1,[10] and OTC[11] and washed to a final stringency of 0·1 × SSC at 65°C.

LINKAGE ANALYSIS

Linkage analysis was performed using the LIPED computer program.[12] Resultant lod scores for the L1·28 probe were summed with those previously published (table 1).

Results

DNA was purified from four Irish families and analysed using a number of polymorphic markers linked to *XLRP*. Each family was tested with the following DNA markers: 754, L1·28, pERT87, and OTC. At least two families were informative with each of these markers except OTC. Standard two point linkage analysis and lod scores were used

TABLE 1 *Lod scores for XLRP and the L1·28 probe.*

θ	*Reference*				*This paper*	*Total A*	*Total B*	*Total C*
	4	*5*	*6*	*7*				
0·00	—	—	1·692	—	3·445	—	—	—
0·025	7·86	1·62	1·875	1·37	3·291	13·026	14·646	16·016
0·05	7·79	2·10	1·887	1·98	3·144	12·821	14·921	16·901
0·075	7·55	2·24	1·885	2·20	2·989	12·424	14·664	16·864
0·1	7·27	2·29	1·862	2·38	2·829	11·961	14·251	16·631
0·125	6·90	2·27	1·806	2·54	2·666	11·372	13·642	16·187
0·15	6·55	2·21	1·744	2·37	2·499	10·793	13·003	10·793
0·2	5·80	2·00	1·599	2·18	2·153	9·552	11·552	13·732
0·3	4·62	1·38	1·180	1·5	1·413	7·210	8·593	9·493
0·4	2·03	0·64	0·642	0·62	0·620	3·292	3·932	4·552
0·5	0·00	0·00	0·000	0·00	0·000	0·000	0·000	0·000
Confidence interval (cM)	0–15	—	—	—	0–15·5	0·75–9·5	1–11·5	2–13·5

to detect linkage of these markers to *XLRP* and to each other.

DNA from 18 normal unrelated females was digested with the relevant restriction enzymes and analysed by blot hybridisation with L1·28. The frequency of the A1 (12 kb) and A2 (9 kb) alleles was found to be 0·75 and 0·25, respectively. These observations are similar to previous data,[4] and in this population imply that 37·5% of families will be informative with this probe. With L1·28 we obtained a maximum lqd score of 3·445 at 0 cM with a 1-lod confidence interval of 0 to 15·5 cM (table 1). This map position is similar to that found in the studies of Bhattacharya *et al*[4] and Friedrich *et al*.[6]

For the 754 marker the frequency of the 9 kb and 11 kb alleles in the normal population was found to be (0·35) and (0·65), respectively. The maximum lod score was 3·41 at 0 cM with a 1-lod confidence interval of 0 to 16 cM (table 2). Probe 754 has been localised to the region Xp11·3-Xp21 by hybridisation to a somatic cell panel[13] and further localised to Xp21·2 as a result of its absence in a male DMD patient with a deletion in this region[8] (table 3, figure). Since 754 has been placed close to the *DMD* locus at Xp21 one would expect tight linkage of *DMD* to 754. Bakker *et al*[14] estimated the distance between 754 and *DMD* at 3 cM but other studies have indicated distances of 12 cM and 21 cM.[15–17] In our study we found significant tight linkage of 754 to *XLRP* suggesting 754 may not be so tightly linked to *DMD*.

We analysed linkage between *XLRP* and pERT87. Two sets of pERT87 alleles were used to increase the amount of information. These were the pERT87·8 alleles (2·2 kb and 4·4 kb at frequencies of 0·4 and 0·6) and the pERT87·1 alleles (7·5 kb and 8·7 kb at frequencies of 0·28 and 0·72). We found maximum lod scores of 1·37 and 1·2, both at 10 cM, with confidence intervals of 0 to 41 cM and 0 to 44 cM for pERT87·8 and pERT87·1, respectively (table 2). Although not definitive, these data indicate that the locus defined by pERT87 is likely to be further from the *XLRP* locus than either L1·28 or 754. This is consistent with earlier data which show that pERT87 maps close to the *DMD* locus[10 18] (figure).

TABLE 2 *Lod scores for XLRP, L1·28, 754, and pERT87.*

θ	*RP 754*	*RP pERT87·8*	*RP pERT87·1*	*L1·28 754*	*pERT87·8/1 L1·28*	*754 pERT87·8*	*754 pERT87·1*
0·000	3·413	—	—	—	—	—	—
0·025	3·276	1·105	0·950	1·659	0·801	0·923	0·769
0·05	3·135	1·294	1·141	1·832	1·015	1·184	0·965
0·075	2·992	1·357	1·205	1·876	1·102	1·186	1·034
0·1	2·844	1·367	1·218	1·866	1·134	1·200	1·051
0·125	2·693	1·346	1·201	1·824	1·136	1·184	1·039
0·15	2·538	1·307	1·165	1·761	1·117	1·148	1·007
0·2	2·214	1·187	1·058	1·589	1·036	1·036	0·907
0·3	1·507	0·850	0·758	1·116	0·755	0·710	0·618
0·4	0·702	0·428	0·382	0·505	0·361	0·305	0·259
0·5	0·000	0·000	0·000	0·000	0·000	0·000	0·000
Confidence interval (cM)	0–16	0–41	0–44	0–46	0–46	0–43	0–47·5

TABLE 3 *Possible locations and orders for XLRP and L1·28.*

Location for XLRP	*References*	*Linkage*	*Lod*	*Comment*
[*XLRP*-0cM-L1·28-0cM-*XLRP*]	This paper	θ=0·00 0–15·5 cM	3·445	—
[*XLRP*-3cM-L1·28-3cM-*XLRP*]	4	θ=0·03 0–15 cM	7·89	—
Xcen-*XLRP*-6cM-L1·28	6	θ=0·06	1·892	3 point linkage analysis
[*XLRP*-10cM-L1·28-10cM-*XLRP*]	5	θ=0·10		—
Xcen-L1·28-12·5cM-*XLRP*	7	θ=0·125	2·29 2·54	3 point linkage analysis
Xcen-L1.28-20cM-*XLRP(DMD)*	8			Deletion Xp 21·1–21·3 Patient: DMD, CGM, XLRP

As yet only one family has been partially informative with the OTC probe giving an insignificant maximum lod score of 0·6 at 0 cM.

The lod scores between the individual DNA markers were calculated (table 2). Linkage between L1·28 and 754 was 1·88 at 7·5 cM. For L1·28 and pERT87·8/1 the maximum lod score was 1·14 at 12·5 cM. Linkage between 754 and pERT87 was 1·2 and 1·05 at 10 cM for pERT87·8 and pERT87·1, respectively. These data are consistent with the previously reported order for these markers of Xcen-L1·28-754-pERT87[10 15 19 20] (figure).

Recombination has apparently occurred between pERT87 and *XLRP* in one affected male. This

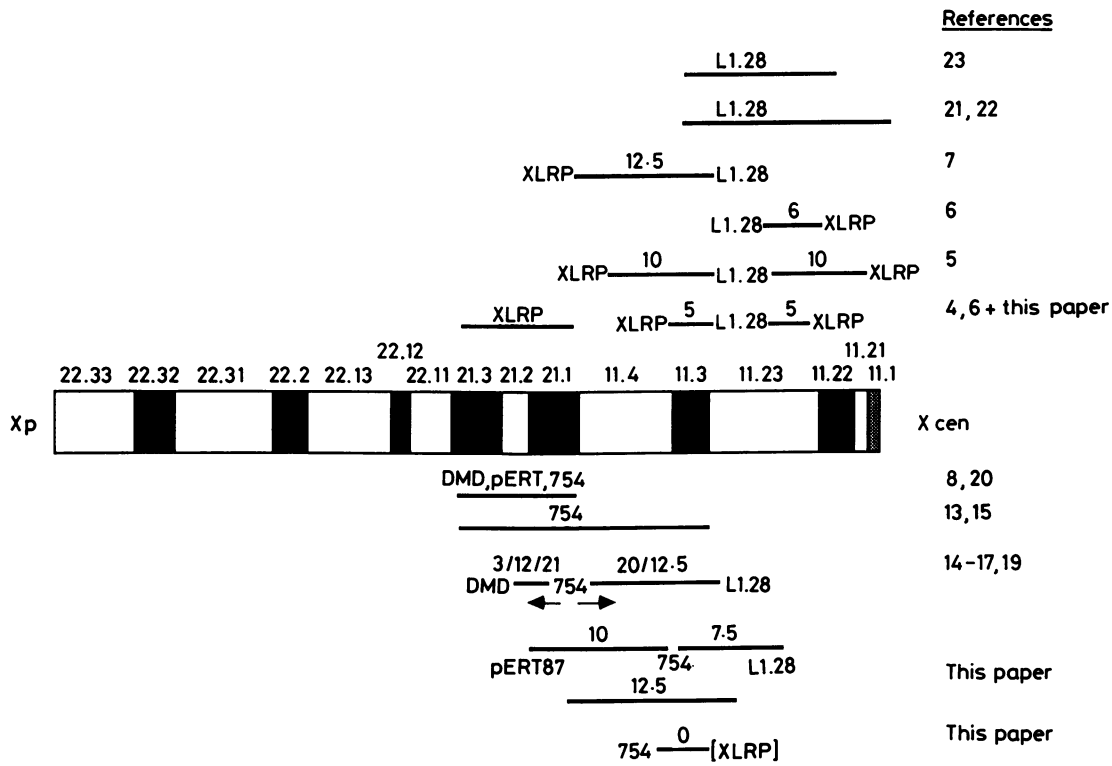

FIGURE *Summary of mapping data on XLRP. (Units=cM.)*

subject was informative for the pERT87·8/1, L1·28, and 754 markers. The data showed that recombination has occurred between the locus defined by pERT87 and the *XLRP* locus but not between either L1·28 and *XLRP* or 754 and *XLRP*. Taking into account data from other studies,[8 10 20] which show that both 754 and pERT87 are distal to *XLRP* on Xp, then the most probable order for these markers is Xcen-(*XLRP*-L1·28)-754-pERT87.

Discussion

As yet the order of *XLRP* and L1·28 is uncertain. Using three point linkage analysis two conflicting orders for *XLRP* and L1·28 have been found[7 8] (table 3). One report placed *XLRP* distal and the other proximal to L1·28 on Xp.[7 8] In our study both 754 and L1·28 are linked to *XLRP* with a maximum lod score of 3·4 at 0 cM. Since from our data the genetic distance between L1·28 and 754 is estimated at 7·5 cM, it is likely that 754 and L1·28 are on opposite sides of *XLRP*. However, an order for *XLRP* either proximal or distal to L1·28 on Xp would fall within the confidence intervals for the genetic distances estimated between these loci.

There has been some discussion based on phenotypic heterogeneity and genetic mapping about whether XLRP can be caused by mutations at more than one locus. Three clinically variant forms of XLRP have been described. (McKusick 31260, 30320, 30330).[21] The extension of linkage studies on XLRP to a new population is important in deciding whether there is genetic heterogeneity in XLRP. Moreover, the reliability of using linked markers for counselling on the inheritance of genetic disorders depends critically on the question of locus heterogeneity.

The first location for XLRP was identified by Bhattacharaya *et al.*[4] They showed linkage of *XLRP* to the locus *DXS7* where polymorphism is detected by the DNA probe L1·28. L1·28 had been assigned to Xp11·0-Xp11·3 using in situ hybridisation and somatic cell hybrids.[22 23] More recently, it has been located between Xp11·4 and Xp11·23 using a somatic cell hybrid constructed from a subject with an interstitial deletion of the short arm of the X chromosome[24] (figure). Bhattacharaya *et al*[4] analysed four families showing typical XLRP (McKusick 31260)[21] giving a maximum lod score of 3·45 at 0 cM. A fifth family, which was initially described as having a variant form of XLRP, an X linked choroidoretinal dystrophy (McKusick 30330),[21] but after further examination was thought to have typical XLRP, gave a maximum lod score of 4·65 at 4 cM. Combining the data gave an overall maximum lod score of 7·89 at 3 cM with a 95% confidence interval of 0 to 15 cM (tables 1 and 3).

This assignment of *XLRP* as closely linked to L1·28 was supported by a study on a Danish family by Freidrich *et al.*[6] They obtained a maximum lod score of 1·892 at 6 cM (tables 1 and 3). In our study of XLRP in four Irish families, L1·28 and *XLRP* were linked with an overall maximum lod score of 3·445 at 0 cM and a 1-lod confidence interval of 0 to 15·5 cM. Since the British, Irish, and Danish data seem to be in agreement, we may combine them, giving a maximum lod score of 13·026 at 2·5 cM with a 1-lod confidence interval of 0·75 to 9·5 cM (table 1A).

A location for the *XLRP* locus further from L1·28 was suggested by two sets of linkage data (tables 1 and 3). Mukai *et al*[5] found linkage between *XLRP* and L1·28 with a maximum lod score of 2·29 at 10 cM using four informative XLRP families, three British and one Korean. Nussbaum *et al*[7] studied one large Latin-American family having a clinical variant of XLRP which shows a golden-metallic or 'tapetal' reflex in the heterozygote (McKusick 30320).[21] In this family they found linkage of *XLRP* to L1·28 with a maximum lod score of 2·54 at 12·5 cM. Although a significant lod score of 3 was not obtained in either of these studies, when combined they give a maximum lod score of 4·81 at 12·5 cM with a 1-lod confidence interval of 4·5 to 22·5 cM.

The data of Mukai *et al*[5] and Nussbaum *et al*[7] are only suggestive of genetic heterogeneity in XLRP. More substantial evidence of genetic heterogeneity came from three point linkage analysis (table 3). Nussbaum *et al*[7] used both OTC and L1·28 to analyse the inheritance of the 'tapetal' reflex variant of XLRP and found that the most probable order was Xcen-L1·28-*XLRP*. Freidrich *et al*[6] also from three point linkage analysis using the C banding heteromorphism and L1·28 found the order Xcen-*XLRP*-L1·28. Further possible evidence for genetic heterogeneity in XLRP was reported by Franke *et al*[8] who found a deletion of Xp21·1–21·3 in a male patient presenting with DMD, CGM, RP, and McLeod syndrome. As this patient was a new mutation it was impossible to distinguish which form of RP he suffered from. If he did suffer from XLRP this deletion patient would provide further evidence of genetic heterogeneity in XLRP.

Taken together the genetic and clinical data do suggest that there is more than one locus for XLRP. However, given the intrinsic inaccuracies of mapping human genetic markers by lod scores, and the possibility that chromosomal rearrangements may be quite frequent on the X chromosome (see for example the results of Kunkel *et al*[20] on DMD and BMD), then the data for XLRP must not be taken

to be definitive. If the published data for linkage of *XLRP* and L1·28[4–7] are combined with our data we obtain a total lod score of 16·9 at 5 cM with a 1-lod confidence interval of 2 to 13·5 cM (table 1C). There are three grounds for excluding the data of Nussbaum *et al*[7] on one large family. The clinical presentation was different from typical XLRP, the maximum lod score was nearly significant at 2·54 at 12·5 cM, and three point linkage analysis placed *XLRP* distal to L1·28 on Xp. If the data of Nussbaum *et al*[7] are left out, the remaining data give a combined lod score of 14·92 at 5 cM with a 1-lod confidence interval of 1 to 11·5 cM (table 1B).

Conclusion

In this study of four Irish families we have mapped *XLRP* with respect to L1·28, 754, and pERT87 (figure). We found significant close linkage of *XLRP* to both L1·28 and 754 and looser linkage to pERT87, thus placing pERT87 further away from *XLRP* than either L1·28 or 754. We have shown that the XLRP gene in four Irish families has a similar location to that found in the British and Danish populations studied by Bhattacharya *et al*[4] and Freidrich *et al*[6] respectively, and may be the same as that found by Mukai *et al*.[5] The data add to those needed to reach a conclusion as to whether XLRP is genetically heterogeneous. This is essential before polymorphic DNA probes can be used in genetic counselling.

We wish to thank Dr Shomi Bhattacharya for useful discussions and the provision of the L1·28 and 754 probes. We also wish to thank Dr Kay Davies for the OTC probe and Dr Louis Kunkel for the pERT87·8 and pERT87·1 probes. This work was supported by RP Ireland-Fighting Blindness, RP America, and The British Retinitis Pigmentosa Society. We also acknowledge the support of the Trinity College Dublin Trust.

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Correspondence and requests for reprints to Dr P Humphries, The Genetics Department, Lincoln Place Gate, Trinity College Dublin, Dublin 2, Ireland.