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Autosomal Dominant Retinitis Pigmentosa: Exclusion of the Gene from the Short Arm of Chromosome 1 including the Region surrounding the Rhesus Locus

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Summary

Members of a large Irish pedigree exhibiting early-onset autosomal dominant retinitis pigmentosa (ADRP) were typed for the rhesus blood group and nine DNA markers on chromosome 1. Close linkage between the ADRP locus and any of the marker loci was excluded using two-point analysis. With use of the sex-averaged maps of Dracopoli et al. and Donis-Keller et al. and a strategy of rolling multipoint analyses, support was gained for the exclusion of ADRP from a 224-cM region of the chromosome, including almost the entire short arm. The disease locus was significantly excluded from within at least 50 cM of the rhesus locus and, as a loose linkage between these two genes has been suggested by other studies, this result may support the possibility of genetic heterogeneity within the autosomal dominant subgroup of retinitis pigmentosa.

Introduction

Retinitis pigmentosa (RP) is the term given to a genetically heterogeneous set of disorders characterized by progressive loss of peripheral and night vision, photoreceptor dysfunction, retinal changes including "bone-spicule" pigmentary deposits, and a diminished or absent electroretinogram (ERG). The incidence of the disorder, a major cause of blindness in adults, is approximately 1/4,000. The mode of inheritance in Ireland is autosomal dominant in 15% of cases, autosomal recessive in 30%, X-linked in 17%, and sporadic or isolated in 38% (G. J. Farrar and J. M. B. Maloney, unpublished data).

The difficulties in distinguishing between these three subtypes by using the limited clinical and pedigree data usually available to the genetic counselor (Rothberg et al. 1982; Beaty and Boughman 1986) are one indication of the usefulness of finding closely linked genetic markers. Indeed, a linked genetic marker has already been established for X-linked RP (Bhattacharya et al. 1984; Nussbaum et al. 1985; Friedrich et al. 1985; Farrar et al. 1988). Further, these linkage studies yielded some suggestion of genetic heterogeneity within this subgroup (Davies et al. 1987).

Controversial subdivisions of autosomal dominant RP (ADRP) have also been advanced, originally on the basis of age of onset of night blindness, giving a type I, with onset before 10 years of age, and type II, with onset typically in adulthood (Massof and Finkelstein 1981; Farber et al. 1985). A more refined division, based on static perimetry of retinal visual fields and ERG data and that maintained some correspondence to the previous categories, has been suggested by Lyness et al. (1985) and correlated significantly with intrafamily groupings.

The problems posed by this suspected heterogeneity have been avoided in our work by the typing of individuals from a single large pedigree (fig. 1). Linkage studies with ADRP have been conducted with a number of pedigrees and to date have excluded the disease locus from more than 950 cM, approximately 30% of the genome (Rywlin 1951; Hussels-Maumenee et al. 1975;

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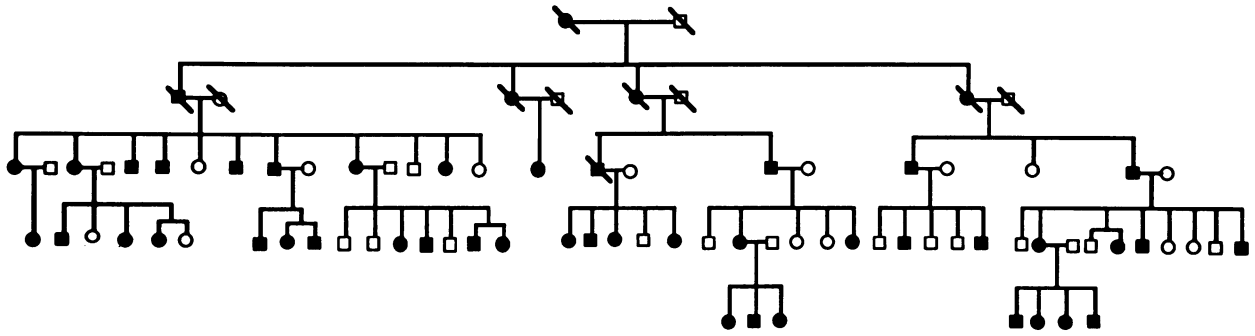

Figure 1 The 84-member Irish pedigree TCDM1 exhibiting ADRP. Samples have been procured from all live individuals shown.

Spence et al. 1977*a*, 1977*b*; Field et al. 1982; Daiger et al. 1987).

The most promising suggestion of linkage to emerge has been that to the rhesus locus on chromosome 1, giving a maximum lod score of 1.89 at a recombination fraction (θ) of $\theta_m = 0.20$, $\theta_f = 0.40$, using data from studies on American pedigrees (Field et al. 1982; Heckenlively et al. 1982; Daiger et al. 1987). There is also supportive but unconfirmed evidence from a Chinese study (Yijian et al. 1987).

Here we present the results of multipoint analyses which support exclusion of the disease gene, segregating in a single pedigree exhibiting type I ADRP, from a 224-cM region including the rhesus blood group and most of the short arm of chromosome 1.

Material and Methods

Members of a 84-person Irish kindred exhibiting ADRP, TCDM1 (fig. 1), were studied. Affected status was assigned by using totally extinguished ERG and/or symptoms characteristic of RP, including nyctolopia and peripheral visual field loss. In addition, these individuals all exhibited fundascopic disturbances typical of the disease: disk pallor, attenuation of retinal vessels, and classical bone-spicule pigmentary deposits in the retinal periphery. Affected members, without exception, reported difficulty with night vision before the age of 10 years, and extinguished ERG patterns coupled with fundascopic disturbance have been obtained in four children aged 6–10 years. This early onset of disease was suggestive of type I ADRP (Massof and Finkelstein 1981) and enabled a confident assignment of normal phenotype to the at-risk individuals shown as unaffected (Berson 1981; Field et al. 1982). The youngest of these was 16 years of age.

Available samples were typed for nine polymorphic DNA loci mapping to chromosome 1, namely, D1S11, D1S14, D1S15, D1S16, D1S19 (Dracopoli et al. 1988), D1S57 (Nakamura et al. 1987), NGFB (Breakefield et al. 1984), REN (Hobart et al. 1984), and PND (Nemer et al. 1986), and a large subset of the pedigree was tested for the rhesus blood factors. DNA was prepared directly from blood samples, and standard Southern blotting methods were used. Details of the RFLP systems used are given in table 1. Our data analyses were carried out using the data management package LINKSYS (Attwood and Bryant 1988), in conjunction with the programs LIPED (Ott 1974), and LINKMAP from the LINKAGE (version 3.5) package (Lathrop et al. 1984), and were performed on a 512-K PC.

As well as pairwise calculations, a series of joint analyses of ADRP and two or three contagious markers were conducted. Because of computer memory limitation, our multipoint analyses were restricted to considering a maximum of 32 haplotypes, and, as a result, only the most informative of the three rhesus alleles was employed, although other loci data were fully considered. Distances between loci were fixed according to the sex-averaged genetic map by Dracopoli (Dracopoli et al. 1988), except for the NGFB-REN interval, which was constructed using positions relative to the Duffy (Fy) locus given in the maps of Dracopoli et al. (1988) and Donis-Keller et al. (1987). The relative order of all loci in the map had been established with odds in favor of at least 100:1 (Dracopoli et al. 1988). Each joint analysis was performed twice; in the first instance θ_m values and θ_f values were considered to be equal, and in the second analysis a constant map ratio of 2.566 was used, to allow for θ_f values being higher than θ_m values (Farrer et al. 1987; Dracopoli et al. 1988).

Table 1

DNA Polymorphisms on Chromosome 1

Chromosome	Locus	Probe	Enzyme	Alleles	Frequency
1p22.1	NGFB	5'Sub	*Taq*I	A1	.87
				A2	.13
1p36	PND	JA110	*Xho*I	A1	.54
				A2	.46
1q32	REN	3' Rennin cDNA	*Hin*dIII	A1	.38
				A2	.62
1p22	D1S11	p1-18	*Bcl*I	A1	.57
				A2	.43
1p22	D1S14	p6-02	*Taq*I	A1	.53
				A2	.47
1p31-pter	D1S15	p1-11B	*Bgl*II	A1	.56
				A2	.30
				A3	.14
1p31-pter	D1S16	p2-32	*Bgl*II	A1	.75
				A2	.25
1p31-qter	D1S19	p4-03	*Sac*I	A1	.41
				A2	.59
1p31-pter	D1S57	pYNZ2	*Taq*I	A1	.33
				A2	.42
				A3	.25

NOTE.—Loci information was taken from Dracopoli et al. (1988) and from the DNA committee RFLP table presented at HGM9, a preliminary to Pearson et al. (1987).

Results

On the basis of pairwise analysis, each of the 10 linked loci from the short arm region of chromosome 1 were excluded from close linkage to ADRP lod scores, (table 2, fig. 2).

From the results of multipoint analysis assuming that $\theta_m = \theta_f$ and except for a narrow region between D1S14 and D1S16, the disease locus was significantly excluded, to the accepted log likelihood of −2, from any position in the 140.2-cM region between the most distal marker, PND, and the proximal locus, NGFB. In the segment between D1S14 and D1S16, location scores climb above a log likelihood of −2 for a distance of 5 cM but remain below −1.8, giving relative odds against linkage of at least 60:1. The ADRP locus was also excluded

Table 2

Results of Pairwise Linkage Analysis Between ADRP and Chromosome 1 Markers

	θ ($\theta_m = \theta_f$)						
MARKER	.00	.001	.05	.10	.20	.30	.40
NGFB.....	−∞	−10.69	−2.67	−1.43	−.47	−.15	−.06
PND.....	−∞	−12.45	−4.02	−2.56	−1.18	−.53	−.19
REN.....	−∞	−7.81	−2.34	−1.35	−.50	−.16	−.02
Rh	−∞	−6.78	−1.76	−.94	−.31	−.11	−.05
D1S11	−∞	−16.74	−4.26	−2.25	−.66	−.12	−.01
D1S14	−∞	−3.74	−.49	−.03	.27	.30	.18
D1S15	−∞	−13.68	−3.46	−1.81	−.53	−.11	−.01
D1S16	−∞	−13.64	−3.37	−1.52	−.06	.33	.22
D1S19	−∞	−7.24	−2.16	−1.24	−.40	−.08	.01
D1S57	−∞	−28.32	−8.24	−4.94	−2.06	−.80	−.23

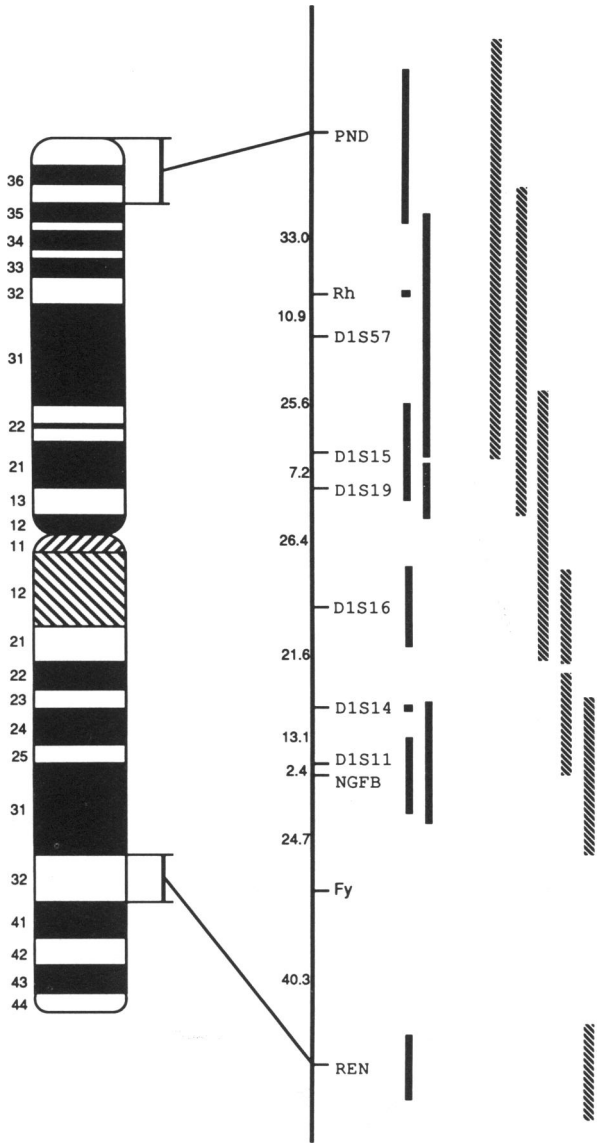

Figure 2 Genetic map (from Donis-Keller et al. 1987; Dracopoli et al. 1988) and physical assignments (from Morton and Burns 1988) of loci on chromosome 1. Genetic distances are given in centimorgans. Thicker lines to the right of the map are to scale and indicate regions from which the ADRP gene is excluded by a lod score of less than −2, using two-point analyses (filled lines) and multipoint analyses (hatched lines).

from 20.0 cM beyond PND, from 16.0 cM beyond NGFB, and from a 19.1-cM region around the REN locus (fig. 2).

The introduction of a high sex-specific difference in recombination frequency, with use of a θ_f:θ_m ratio of 2.566, gave some reduction in the support for exclusion of the ADRP locus at each map position (fig. 3). However, log likelihoods remained below −1.5 in the region bounded by D1S14 and D1S16 and below −2.0 in the rest of the interval bounded by NGFB and PND.

Discussion

Using multipoint analysis, we report exclusion of the ADRP locus from regions of the sex-averaged map of chromosome 1 that total at least 190 cM. Furthermore, it remains highly improbable that the disease locus is located in a narrow interval between D1S14 and D1S16, where its location score climbs marginally above the accepted limit for significant exclusion (Morton 1955). In addition, the Duffy (Fy) blood locus has elsewhere been reported in pairwise linkage studies with ADRP (Field et al. 1982; Daiger et al. 1987) and has yielded powerful exclusion data with lod scores <-2.00 at $\theta_m = \theta_f < .24$. This is an effective negation of the other interval containing weak information in our study: the central region between the NGFB and REN loci.

Thus there is support for the exclusion of the ADRP locus from a continuous region of 224 cM including probably the entire short arm of chromosome 1. Evidence for exclusion around the most distal marker, PND, is strong, although it is not certain how far the genetic map extends beyond it. Dracopoli et al. (1988) have suggested that at least a further 23 cM exists on the male genetic map.

That the ADRP locus is significantly excluded from at least within 50 cM of the rhesus locus in this family may indicate genetic heterogeneity in the autosomal dominant form of the disease. However, other studies' reported evidence for loose linkage is quite inconclusive. In the case of both the American (Field et al. 1982; Heckenlively et al. 1982; Daiger et al. 1987) and the Chinese (Yijian et al. 1987) sources, data were collected from several pedigrees, and, in the former at least, results from both putative clinical types (Massof and Finkelstein 1981) were combined. Without a separation of the proposed clinical subtypes of the disease, no support for a corresponding genetic subdivision may be given. Further work, including a multipoint analysis involving the largest American pedigree, RP01 (Field et al. 1982), and flanking markers may be more conclusive.

That a single pedigree exhibiting early-onset or type 1 ADRP (Massof and Finkelstein 1981) can provide adequate information for a conclusive analysis of an extensive, continuous chromosomal region is a considerable advantage in our investigations. The pedigree's 5-generation structure is given in fig. 1. Most living

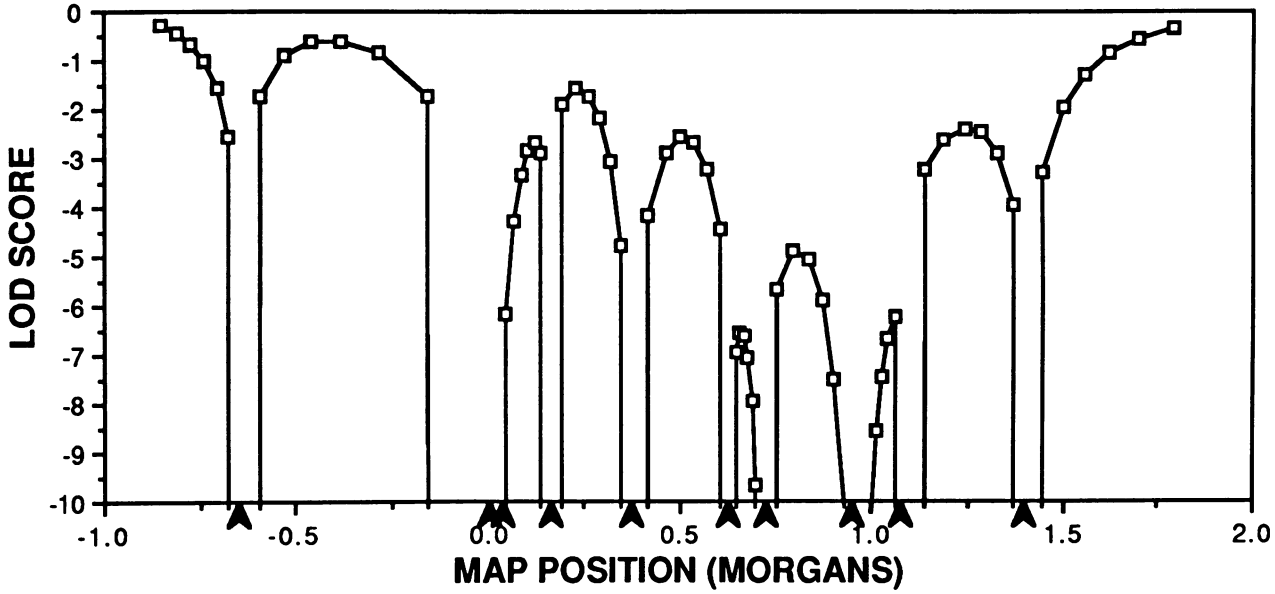

Figure 3 Support for exclusion of ADRP from a region including the short arm of chromosome 1. The locus NGFB was arbitrarily set at .00, and the other loci were positioned from it according to the genetic distances given by Donis-Keller et al. (1987) and Dracopoli et al. (1988). REN was set at −0.65 Morgans, D1S11 at 0.024 Morgans, D1S14 at 0.155 Morgans, D1S16 at 0.371 Morgans, D1S19 at 0.635 Morgans, D1S15 at 0.707 Morgans, D1S57 at 0.963 Morgans, rhesus (Rh) at 1,072 Morgans, and PND at 1.402 Morgans. Locations of these loci are denoted by arrowheads on the map position axis. The curve shows the lod scores resulting from multipoint analyses under the assumption of a constant $\theta_f:\theta_m$ ratio of 2.566 (from the maps of Dracopoli et al. 1988). Analyses assuming that $\theta_m = \theta_f$ gave lod scores that were negative to a greater degree at the same map positions but are not shown, to avoid clutter.

members shown were typed at some point in our study, but the core of our results were obtained from only five of its nuclear families. Therefore it is to be expected that, because of the present fuller availability of DNA samples and the provision of highly informative mapped probes (Donis-Keller et al. 1987; Nakamura et al. 1987), our further studies will require a lower map density of RFLP markers.

The efficacy of multipoint mapping in a study of this kind has been well documented (Wainwright et al. 1986; Farrer et al. 1987). Although a large analysis including all marker data would give a more complete result (Ott 1985), it was found that combining analyses of ADRP versus contigious groups of two or three loci gave adequate support for the exclusion of the disease gene from the region under consideration. The performing of these calculations by using the limited power of a personal computer was facilitated by the simplicity of our pedigree input and the fact that an age-related qualification of unaffected status is not necessary with the early-onset form of ADRP (Field et al. 1982).

Exclusions of disease loci from whole chromosomes by the use of linkage maps and multipoint mapping have been reported elsewhere (Wainwright et al. 1986; Farrer et al. 1987), and the present investigation contains one of the larger regions to have been treated in such a fashion.

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