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Autosomal Dominant Retinitis Pigmentosa (adRP; RP6): Cosegregation of RP6 and the Peripherin-RDS Locus in a Late-Onset Family of Irish Origin

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Summary

We recently reported the localization of a gene for late-onset autosomal dominant retinitis pigmentosa (adRP; RP6), on the short arm of chromosome 6, by linkage analysis in a large family of Irish origin. It is notable that the gene encoding peripherin-RDS, a photoreceptor-specific protein, recently has been physically mapped on 6p. In our own analysis, an intrageneic marker derived from this gene cosegregated with the adRP disease locus with zero recombination (lod score 5.46 at $q = .00$). Using the CEPH reference panel, we now report the mapping of the peripherin-RDS gene relative to other 6p markers in the CEPH data base. Incorporation of these data into a multipoint analysis produced a lod score for adRP of 8.21, maximizing at the peripherin-RDS locus. This study provides strong evidence suggesting a role for peripherin-RDS in the etiology of one form of adRP.

Introduction

Retinitis pigmentosa (RP) describes a heterogeneous group of inherited retinopathies which affect approximately 1 in 4,000 people (Bundley and Crews 1984; Bunker et al. 1984). The primary pathological change associated with RP is the degeneration of the photoreceptors and of the outer nuclear layers of the retina. Classical clinical features include optic-disk pallor, retinal arteriolar attenuation, pigmentary deposits, and reduced or extinguished electrophysiological responses (Heckenlively 1988; Flannery et al. 1989). Typically symptoms include reduced night and peripheral vision, eventually leading to loss of far-peripheral and central visual fields.

The presence of genetic heterogeneity is well established in RP; the disease may be inherited in autosomal dominant (adRP), autosomal recessive (arRP), or X-linked fashion. However, further heterogeneity exists within these categories. In the case of adRP, the disease genes segregating in a number of families have been localized to 3q, close to the gene encoding the retinal specific photoreceptor pigment rhodopsin (McWilliam et al. 1989; Farrar et al. 1990). In contrast to this, in other families the disease genes are excluded from the same region of 3q, hence indicating the presence of nonallelic heterogeneity in adRP (Farrar et al. 1990; Inglehearn et al. 1990). More recently we have localized an adRP locus on the short arm of chromosome 6 (Farrar et al. 1991*a*). In a parallel study Daiger and coworkers localized an adRP gene on 8p (Blanton et al. 1991). Furthermore, the presence of allelic genetic heterogeneity in adRP has been demonstrated, as a number of mutations within the rhodopsin gene have been identified in patients (Dryja et al. 1990, 1991; Inglehearn et al. 1991; Farrar et al. 1991*c*).

The photoreceptor cell–specific transmembrane protein peripherin-RDS (retinal degeneration slow) has been localized to the short arm of chromosome 6 by in situ hybridization (Travis et al. 1991). Our localization of a human retinopathy on 6p, together with the knowledge that the peripherin-RDS gene also maps on

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6p, prompted us to refine our previous adRP linkage and to more precisely map the human peripherin-RDS locus. To do this we have used both a recently identified mononucleotide repeat sequence in the peripherin-RDS gene (Kumar-Singh et al. 1991) and the panel of CEPH reference families (Dausset et al. 1990). We have defined highly informative dinucleotide repeat markers flanking the adRP gene on 6p. It is notable that the peripherin-RDS gene maps into the center of our linkage group, at zero recombination with respect to the adRP locus, hence strongly suggesting mutations in the peripherin-RDS gene as the underlying defect in this form of adRP.

Subjects and Methods

TCDG Pedigree

Both affected and unaffected members of the family have undergone funduscopic examination, Goldman perimetry, and electroretinograms (ERGs) according to a protocol based on that proposed by the international standardization committee (Marmor et al. 1989). The affected members of the family manifest a late-onset form of adRP, with individuals noting difficulty with night vision in their early twenties, with later onset of symptoms due to loss of peripheral visual fields (Kenna 1991). Electroretinographic assessment showed that rod-isolated responses were both significantly reduced in amplitude and delayed in time in young (age 5 years and older) affected members of the family. However, cone responses, although reduced, were still detectable in the older affected individuals. Two-color adaptometry revealed a concomitant rod and cone threshold elevation in a regionalized manner consistent with a Massof and Finkelstein (1981) type 2 adRP. Funduscopic examination demonstrated classical features associated with RP: disk pallor, arteriolar attenuation, and pigmentary deposits.

Polymorphic Markers and PCR Amplification

Individuals from TCDG were typed for the following markers: D6S89, D6S109, D6S105, HLA-DRA, and TCTE 1 from chromosome 6p (table 1A). Primers RDS1 (5′ CACTTGGTGCATAAGCACAGA 3′) and RDS2 (5′ AAGCAAACGGCCAACCTGTCA 3′), flanking our newly defined mononucleotide repeat polymorphism at the 3′ end of the peripherin-RDS gene, were generated and used to type individuals from TCDG and CEPH family members. Oligonucleotide primers were synthesized using an Applied Biosystems 391 DNA synthesizer. DNA was prepared from peripheral blood, and PCR reactions were carried out according to a method described elsewhere (Farrar et al. 1991*a*).

Data Analysis

Data were organized using the LINKSYS (version 3.1) data-management package (Attwood and Bryant 1988). Computations were performed with LIPED (Ott 1974) and LINKAGE version 3.5 (Lathrop et al. 1984) on a 512-K PC. Multipoint analyses were restricted to consideration of 32 haplotypes. The number of alleles for each marker locus was reduced to three or four, without loss of information with respect to adRP. Centimorgan distances between loci on 6p were fixed on the basis of the CEPH data base. Male and female recombinations were taken to be equal in all analyses. The calculated segregation ratio in TCDG (.51) did not differ significantly from that expected for a fully penetrant autosomal dominant gene (.5). In view of the late onset of the disorder, to be prudent we have assumed a 90% penetrance for the affected phenotype. The mononucleotide repeat polymorphism at the peripherin-RDS locus was analyzed in the CEPH reference families. Two-pair linkage analyses between peripherin-RDS and other 6p loci were carried out using the CEPH Data Base, version 4.0.

Results and Discussion

Members of a late-onset Irish adRP pedigree TCDG have been typed with the markers from chromosome 6p that are listed in table 1A. Many of these markers were highly informative mono- or dinucleotide repeat sequence polymorphisms, each showing between 9–13 alleles. Elsewhere we have described the localization of an adRP locus to 6p by using a number of these dinucleotide-repeat polymorphisms (Farrar et al. 1991*a*). In the present study we have updated and extended our original linkage analysis. For completeness we have included in table 1B all two-point lod scores and corresponding recombination fractions between adRP and the 6p marker loci. We have found that adRP is most closely linked both to the human major histocompatibility complex (HLA-DRA) and to the peripherin-RDS gene, both loci showing zero recombination between the disease gene, with lod scores of 4.36 and 5.46, respectively. The two-point lod score between adRP and the marker TCTE1, which maps proximal to HLA, maximized at $q = .05$,

Table 1

Summary of Genetic Markers and Linkage Data

A. Summary of Markers Used on Chromosome 6

Locus	Polymorphism Type[a]	Allele Size Range (bp)	PIC	Reference
D6S89	SSLP	199–227	.88	Litt and Luty 1990
D6S109	SSLP	169–193	.78	Ranum et al. 1991
D6S105	SSLP	116–138	.77	Weber et al. 1991
HLA-DRA	RFLP (*Dde*I)	482/158 274/208/168	.73	Elianou et al. 1990
RDS	SSLP	96–98	.54	Kumar-Singh et al. 1991
TCTE1	SSLP	171[b]	.73	Kwiatkowski et al. 1991

B. Two-Point Lod Scores between adRP Locus and 6p Locus

	Lod Score at Recombination Fraction of									
Locus	.000	.001	.05	.10	.15	.20	.25	.30	.35	.40
D6S89	.611	2.590	4.098	4.052	3.776	3.373	2.879	2.319	1.711	1.012
D6S109	−2.037	1.034	3.902	3.932	3.658	3.238	2.725	2.152	1.550	.960
D6S105	7.123	7.115	6.686	6.129	5.488	4.781	4.015	3.197	2.332	1.437
HLA-DRA	4.364	4.356	3.954	3.533	3.100	2.654	2.195	1.724	1.245	.774
RDS	5.376	5.366	4.877	4.356	3.815	3.256	2.680	2.092	1.498	.917
TCTE1	2.654	4.598	5.742	5.411	4.901	4.295	3.622	2.896	2.129	1.330

C. Two-Point Lod Scores between Peripherin-RDS Gene Locus and 6p Loci

Locus	Recombination (q)	Maximum Lod Score
HLA-A	.182	21.19
HLA-B	.180	26.25
HLA-DR	.180	21.50
GLO1	.156	10.39
CRI-L171	.089	17.46
KRAS	.186	13.11
CRI-R322	.303	2.59

[a] SSLP = simple sequence length polymorphism.
[b] Most prominent allele size for TCTE1.

with a lod score of 5.74. In our previous study, using the marker D6S109 which maps distal to HLA, we had obtained significant lod scores maximizing at a recombination fraction of .1. Hence we have now identified both proximal and distal genetic markers which show recombinants with respect to the disease gene.

Peripherin-RDS is a photoreceptor cell–specific transmembrane protein which was first identified in bovine rod outer-segment disks (Molday et al. 1987). Subsequently a mutation in the peripherin-RDS gene has been found to be the underlying biochemical defect in the retinal degeneration slow (*rds*) mouse (Travis et al. 1989; Connell et al. 1991). Mice homozygous for the mutation fail to elaborate outer segments, and photoreceptor degeneration occurs rapidly, typically within 1 year of birth (Sanyal and Jansen 1981). Heterozygous mice, although less seriously affected, show abnormal photoreceptor development followed by a slower progressive photoreceptor degeneration (Hawkins et al. 1985). It is notable that the pathological changes present in *rds* mice resemble those observed in RP patients (Travis et al. 1991).

The human peripherin-RDS gene has been mapped to 6p12 by in situ hybridization, but, to date, it has not been placed in a genetic linkage map (Travis et al. 1991). In view of the tight linkage between adRP and peripherin-RDS, which is a prime candidate for adRP, we have typed the peripherin-RDS gene in the CEPH reference panel. To do this we used a newly identified

poly-T repeat polymorphism which is present in the 3′ flanking region of the gene (Kumar-Singh et al. 1991). With the data contained in CEPH Data Base version 4.0, two-point lod scores between this gene and a number of the chromosome 6 loci in the data base were obtained (table 1C). It is notable that we found the peripherin-RDS locus to be proximal to the HLA complex, with lod scores of 21.19, 26.25, and 21.50 between peripherin-RDS and HLA-A, HLA-B, and HLA-DR, respectively, maximizing in each case at a recombination fraction of .18.

The markers used in the present study have previously been typed through all or a subset of the CEPH reference panel (Kwiatkowski et al. 1991; Weber et al. 1991; Zoghbi et al. 1991*a*; H. T. Orr, personal communication). The order and distances between marker loci on 6p were deduced from series of two-point analyses of each marker locus with respect to the other loci. The order obtained, tel–D6S89–D6S109–D6S105–HLA–DRA–RDS–TCTE1–cen, is in agreement both with the physical mapping data from radiation hybrids derived from 6p (Zoghbi et al. 1991*b*) and with the in situ hybridization data (Kwiatkowski et al. 1991; Travis et al. 1991). These loci on 6p form a continuous linkage group of approximately 50 cM (sex averaged).

The adRP locus has been moved through this 6p map to obtain an estimate of the most likely location for the disease gene (fig.1). The data from sequential multipoint analyses with two marker loci and the disease gene indicate that the most likely location for adRP is at the same position as that of the peripherin-RDS gene with a maximum lod score of 8.21. The lod scores at the distal and proximal markers D6S109 and TCTE1 were −1.85 and 4.6, respectively. On the basis of data from the second of the multipoint analyses (D6S109, D6S105, and adRP), the odds in favor of the disease locus being proximal to D6S109 versus

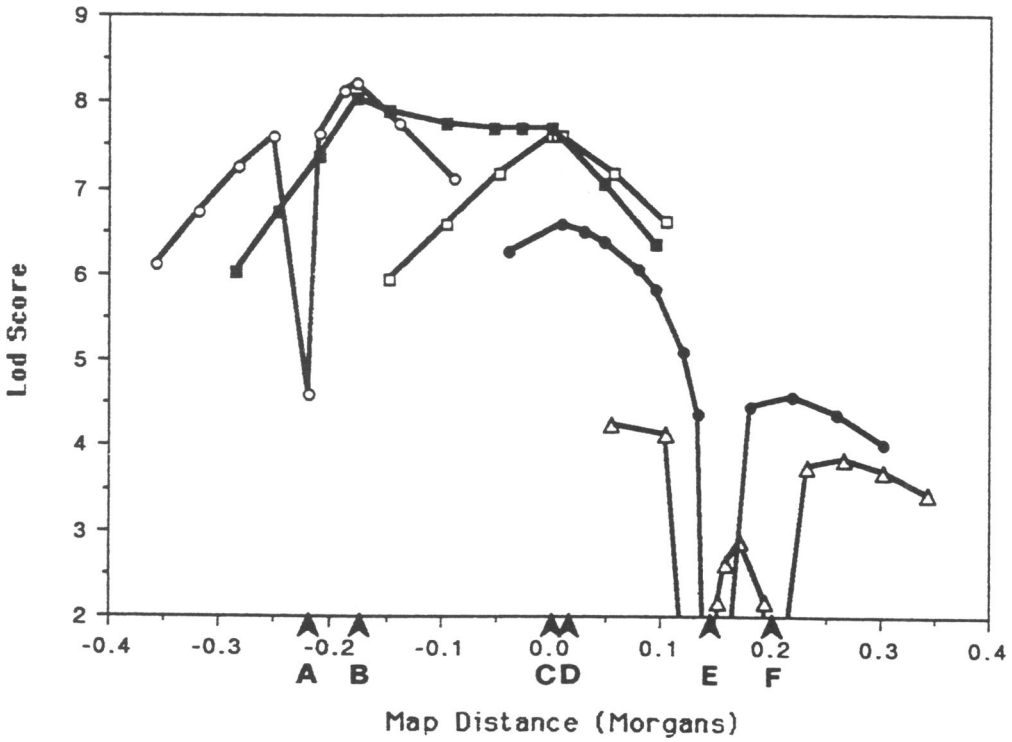

Figure 1 Series of overlapping sequential multipoint analyses with adRP locus and, in each case, two contiguous marker loci, performed using LINKMAP. The Kosambi mapping function was used to account for differences between genetic distances and recombination fractions. The 6p map is arbitrarily centered around the HLA locus; the HLA-DRA marker (D) was placed at .00. The other loci were positioned around it: TCTE1 (A) at −0.219, peripherin-RDS (B) at −0.177, D6S105 (C) at 0.01, D6S109 (E) at 0.145, and D6S89 (F) at 0.2. As each multipoint analysis is not directly comparable with the other analyses, it is technically more correct to include each analysis in the figure. Hence the five graphs from the sequential analyses have been superimposed in one figure. This is a best estimate of the actual linkage data, as we are unable to analyze all the data simultaneously, because of limited computer memory.

at this locus are approximately 1×10^8:1. The probability of the adRP gene being distal to D6S109 is approximately 1:100. The data from the fifth analysis (RDS, TCTE1, and adRP), indicates that the relative odds of the disease gene being localized at the RDS locus versus at the TCTE1 locus are approximately 4×10^3:1. However, the lod score proximal to TCTE1 maximizes at 7.6, indicating that adRP cannot be excluded from being proximal to this marker.

In all two-point and multipoint analyses we have, as a prudence measure, incorporated a 90% penetrance for the disease phenotype, because of the late onset of the disorder. However, this has resulted in a lower quantity of information being obtained from this pedigree—and in particular, from a number of key recombinant individuals. This is clearly exemplified by the positive lod score of 4.6 obtained for adRP at TCTE1, even though recombinants have been observed between adRP and TCTE1.

In conclusion, in our study, we have refined our localization of an adRP gene on the short arm of chromosome 6. We have identified both proximal and distal markers which show recombinants between the marker and the disease gene. Furthermore, we have placed the gene encoding the photoreceptor cell–specific protein peripherin-RDS in a linkage map of 6p and have found that the most likely location for adRP is at the same position as that of this retinal gene. This observation, together with data on the *rds* mouse, in which peripherin-RDS has been implicated as causing a photoreceptor degeneration, strongly suggests peripherin-RDS as a prime candidate for adRP. Screening of groups of unrelated patients for sequence alterations in this gene is the next logical step. In this regard, techniques such as single-strand conformation-polymorphism electrophoresis and heteroduplex analysis will prove to be valuable.

Note added in proof.—While as yet no mutation at the peripherin-RDS locus has been detected in this family under study, a mutation in a second adRP family of Irish origin has been identified (Farrar et al. 1991*b*).

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References

Attwood J, Bryant S (1988) A computer programme to make analysis with LIPED and LINKAGE easier to perform and less prone to input errors. Ann Hum Genet 52:259

Blanton SH, Heckenlively JR, Cottingham AW, Friedman J, Sadler LA, Wagner M, Friedman LH, et al (1991) Linkage mapping of autosomal dominant retinitis pigmentosa (RP1) to the pericentric region of human chromosome 8. Genomics 11:857–869

Bundley S, Crews SJ (1984) A study of retinitis pigmentosa in the city of Birmingham. II. Prevalence. J Med Genet 21:417–420

Bunker CH, Berson EL, Bromley WC, Hayes RP, Roderick TH (1984) Prevalence of retinitis pigmentosa in Maine. Am J Ophthalmol 97:357–365

Connell G, Bascom R, Molday L, Reid D, McInnes RR, Molday RS (1991) Photoreceptor peripherin is the normal product of the gene responsible for retinal degeneration in the *rds* mouse. Proc Natl Acad Sci 88:723–726

Dausset J, Cann H, Cohen D, Lathrop M, Lalouel J-M, White R (1990) Centre d'Etude du Polymorphisme Humaine (CEPH): comparative genetic mapping of the human genome. Genomics 6:575–577

Dryja TP, McGee TL, Hahn LB, Cowley GS, Olsson JE, Reichel E, Sandberg MA, et al (1991) Mutations within the rhodopsin gene in patients with autosomal dominant retinitis pigmentosa. N Engl J Med 323:1302–1307

Dryja TD, McGee TL, Reichel E, Hahn LB, Cowley GS, Yandell DN, Sandberg MA et al (1990) A point mutation of the rhodopsin gene in one form of retinitis pigmentosa. Nature 343:364–366

Elianou JF, Pinet V, Clot J (1990) *Dde* I polymorphism in the HLA-DRA regulatory region. Nucleic Acids Res 18: 7195

Farrar GJ, Jordan SA, Kenna P, Humphries MM, Kumar-Singh R, McWilliam P, Allamand V, et al (1991*a*) Autosomal dominant retinitis pigmentosa: Localisation of a disease gene (RP6) to the short arm of chromosome 6. Genomics 11:870–874

Farrar GJ, Kenna P, Jordan SA, Kumar-Singh R, Humphries MM, Sharp EM, Shiels DM, et al (1991*b*) A three-base-pair deletion in the peripherin-RDS gene in one form of retinitis pigmentosa. Nature 354:478–480

Farrar GJ, Kenna P, Redmond R, Shiels D, McWilliam P, Humphries MM, Sharp EM, et al (1991*c*) Autosomal dominant retinitis pigmentosa: a mutation in codon 178 of the rhodopsin gene in 2 adRP families of Celtic origin. Genomics 11:1170–1171

Farrar GJ, McWilliam P, Bradley DG, Kenna P, Lawler M, Sharp EM, Humphries M, et al (1990) Autosomal dominant retinitis pigmentosa: linkage to rhodopsin and evidence for genetic heterogeneity. Genomics 8:35–40

Flannery JG, Farber DB, Bird AC, Bok D (1989) Degenerative changes in a retina affected with autosomal dominant retinitis pigmentosa. Invest Ophthlmol Vis Sci 30:191–211

Hawkins RK, Jansen HG, Sanyal S (1985) Development and degeneration of retina in rds mutant mice: photoreceptor abnormalities in the heterozygotes. Exp Eye Res 41:701–720

Heckenlively JR (1988) Autosomal dominant retinitis pigmentosa. In: Ewan H (ed) Retinitis pigmentosa, Lippincott, Philadelphia, pp 125–149

Inglehearn CF, Bashir R, Leister DH, Jay M, Bird AC, Bhattacharya SS (1991) A 3-bp deletion in the rhodopsin gene in a family with autosomal dominant retinitis pigmentosa. Am J Hum Genet 48:26–30

Inglehearn CF, Jay M, Lester DH, Bashir R, Jay B, Bird AC, Wright AF, et al (1990) No evidence for linkage between late onset autosomal dominant retinitis pigmentosa and chromosome 3 locus D3S47 (C17): Evidence for genetic heterogeneity. Genomics 6:168–173

Kenna P (1991) Characterization of Irish autosomal dominant retinitis pigmentosa kindreds showing genetic heterogeneity. In: Humphries P, Bhattacharya SS, Bird A (eds) Degenerative retinopathies: advances in clinical and genetic research. CRC, Boca Raton, FL, pp 105–110

Kumar-Singh R, Jordan SA, Farrar GJ, Humphries P (1991) Poly (T/A) polymorphism at the human retinal degeneration slow (RDS) locus. Nucleic Acids Res 19:5800

Kwiatkowski TJ, Beaudet AL, Trask BL, Zoghbi HY (1991) Linkage mapping and fluoresence in situ hybridization of TCTE1 on human chromosome 6p: analysis of dinucleotide polymorphisms on native gels. Genomics 10:921–926

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3343–3346

Litt M, Luty JA (1990) Dinucleotide repeat polymorphism at the D6S89 locus. Nucleic Acids Res 18:4301

McWilliam P, Farrar GJ, Kenna P, Bradley DG, Humphries MM, Sharp EM, McConnell DJ, et al (1989) Autosomal dominant retinitis pigmentosa (ARDP): localization of an ADRP gene to the long arm of chromosome 3. Genomics 5:619–622

Marmor MF, Arden GB, Nilsson SEG, Zrenner E (1989) Standard for clinical electroretinography. Arch Ophthalmol 107:816–819

Massof RW, Finkelstein D (1981) Two forms of autosomal dominant primary retinitis pigmentosa. Doc Ophthalmol 51:28–346

Molday RS, Hicks D, Molday L (1987) Peripherin: a rim-specific membrane protein of rod outer segment discs. Invest Ophthalmol Vis Sci 28:50–61

Ott J (1974) Estimation of the recombination fraction in human pedigrees: efficient computation of the likelihood for human linkage studies. Am J Hum Genet 26:588–597

Ranum LPW, Chung M-Y, Duvick LA, Zoghbi HY, Orr HT (1991) Dinucleotide repeat polymorphism at the D6S109 locus. Nucleic Acids Res 19:1171

Sanyal S, Jansen H (1981) Absence of receptor outer segments in the retina of *rds* mutant mice. Neurosci Lett 21: 23–26

Travis GH, Brennan MB, Danielson PE, Kozak CA, Sutcliffe JG (1989) Identification of a photoreceptor specific mRNA encoded by the gene responsible for retinal degeneration slow (*rds*). Nature 338:338–70–73

Travis GH, Christerson L, Danielson PE, Klisak I, Sparkes RS, Hahn LB, Dryja TP, et al (1991) The human retinal degeneration slow (RDS) gene: chromosome assignment and structure of the mRNA. Genomics 10:733–739

Weber JL, Kwitek AE, May PE, Zoghbi HY (1991) Dinucleotide repeat polymorphism at the D6S105 locus. Nucleic Acids Res 19:968

Zoghbi HY, Jodice C, Sandkuijl LA, Kwiatkowski TJ Jr, McCall AE, Huntoon SA, Lulli P, et al. (1991*a*) The gene for autosomal dominant spinocerebellar ataxia (SCA1) maps telomeric to the HLA complex and is closely linked to the D6S89 locus in three large kindreds. Am J Hum Genet 49:23–30

Zoghbi HY, McCall AE, LeBorgne-Demarquoy F (1991*b*) Sixty-five radiation hybrids for the short arm of human chromosome 6: their value as a mapping panel and as a source for rapid isolation of new probes using repeat mediated PCR. Genomics 9:713–720