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Further Refinement of the Location for Autosomal Dominant Retinitis Pigmentosa on Chromosome 7p (RP9)

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Summary

A form of autosomal dominant retinitis pigmentosa (adRP) mapping to chromosome 7p was recently reported by this laboratory, in a single large family from southeastern England. Further sampling of the family and the use a number of genetic markers from 7p have facilitated the construction of a series of multipoint linkage maps of the region with the most likely disease gene location. From this and haplotype data, the locus can now be placed between the markers D7S484 and D7S526, in an interval estimated to be 1.6–4 cM. Genetic distances between the markers previously reported to be linked to this region and those described in the recent whole-genome poly-CA map were estimated from data in this and other families. These data should assist in the construction of a physical map of the region and will help to identify candidate genes for the 7p adRP locus.

Introduction

Retinitis pigmentosa (RP) is the name given to a group of inherited retinal dystrophies characterized by photoreceptor atrophy and pigment deposition in the retinal periphery. Patients experience narrowing of visual fields (tunnel vision) and night blindness, often progressing to complete blindness in later life. RP can be inherited in X-linked, autosomal dominant, and autosomal recessive modes. In addition, there is both clinical and genetic heterogeneity within each category. Within the autosomal dominant category, the disease can be caused by mutations in rhodopsin (Dryja et al. 1990, 1991; Sung et al. 1991; Inglehearn et al. 1992) and rds/peripherin (Farrar et al. 1991; Kajiwara et al. 1991; Wells et al. 1992). Other autosomal dominant RP (adRP) families have revealed linkage to markers on chromosomes 8cen (Blanton et al. 1991), 7p (Inglehearn et al. 1993), and 7q (Jordan et al. 1993).

The report of adRP on chromosome 7p was based on linkage to the disease in a large family, known as "adRP7," from southeastern England, to two microsatellite markers D7S435 and D7S460 (Weber et al. 1990; Hudson et al. 1992). D7S435 has been physically located to 7p13-15.1, and we were able to demonstrate close linkage between this and D7S460. This locus has now been given the name "RP9" (MIM 180104). Recently, two second-generation human linkage maps have been made available to the research community. One uses previously existing markers and places these relative to new ones identified by researchers of the NIH/CEPH Collaborative Mapping Group (1992). D7S435 is already located on this map. The other is based entirely on a new set of highly informative poly-CA markers cloned by Weissenbach et al. (1992). In order to place the disease locus more accurately, we have used markers which are derived from the second of these maps and which span a 38-cM region of chromosome 7p (converted by the Kosambi mapping function). In addition, we have now sampled nine further affected individuals and two normal spouses from family adRP7 (fig. 1).

Patients and Methods

The phenotype in the adRP family known as adRP7, which shows linkage to chromosome 7p, has been described elsewhere (Moore et al. 1993). It has wide varia-

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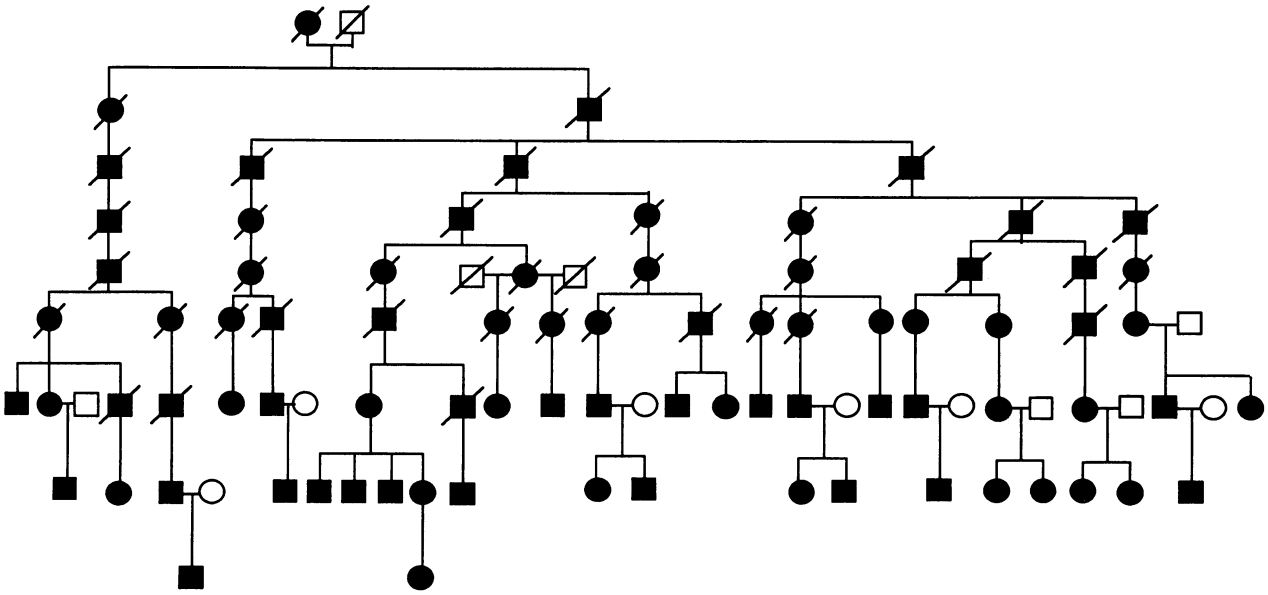

Figure 1 Pedigree showing all of the members of family adRP7 who were used in this analysis. Only gene carriers and their parents are included, so this is not a complete pedigree of the family. Males are represented by squares, females by circles; affected family members are shown as blackened symbols.

tion in severity of disease in gene carriers. To avoid problems generated by failure to detect the disease phenotype, only affected individuals were used for DNA analysis. For more accurate estimation of genetic distances between 7p markers, linkage data in four other families were also analyzed. These included two generations of a family with Usher syndrome, two generations of a family with adRP, a five-generation adRP pedigree, and a four-generation X-linked RP pedigree, totaling 97 potentially informative meioses. Allele frequencies were estimated from spouses of members of adRP7 and from a panel of 49 unrelated individuals of British origin.

Patient DNAs were prepared from peripheral blood lymphocytes by standard protocols. Marker systems were typed in the family by PCR amplification of the microsatellite region by using one unlabeled primer and one kinase labeled with γ-^{32}P ATP. The protocol used to amplify consisted of 30 cycles of 94°C for 1 min for denaturing, 55°C for 1 min for annealing, and 72°C for 1 min for extension, for all systems except D7S460, which amplified optimally at the slightly lower annealing temperature of 52°C. Products were size fractionated on 6% acrylamide denaturing gels run at 40°C–50°C in 5.3 M urea. Where possible, marker analyses were combined in twos or threes, and, to date, no combination was found not to work.

Data were prepared for linkage analysis using the Linksys Data Management package (Attwood and Bryant 1988). Analysis was performed using the LINKAGE package, version 5 (Lathrop et al. 1984).

Results

To locate markers D7S460 and D7S435, previously linked to 7p adRP, on the second-generation poly-CA genome map of Weissenbach et al. (1992), we selected five suitable families, including adRP7, and typed all sampled members for D7S435, D7S460, and 11 poly-CAs spanning the 7p region (data not shown). From this analysis, marker D7S435 was found to be genetically indistinguishable from D7S526, with a two-point lod score of 13.8 and no recombination. Similarly, marker D7S460 mapped along with markers D7S484 and D7S497, with two-point lod scores of 21.5 and 9.9, respectively, again with no recombination. A map of this chromosomal region is presented in figure 2.

The lod scores between the disease locus and all of these markers were then calculated in family adRP7. These data are shown in table 1. Two markers, D7S460 and D7S485, show very tight linkage to the disease locus, giving two-point lod scores of 7.3 and 7.8, respectively, with no recombination. Two other markers, D7S484 and D7S526, show linkage at 1.4 cM and 3.3

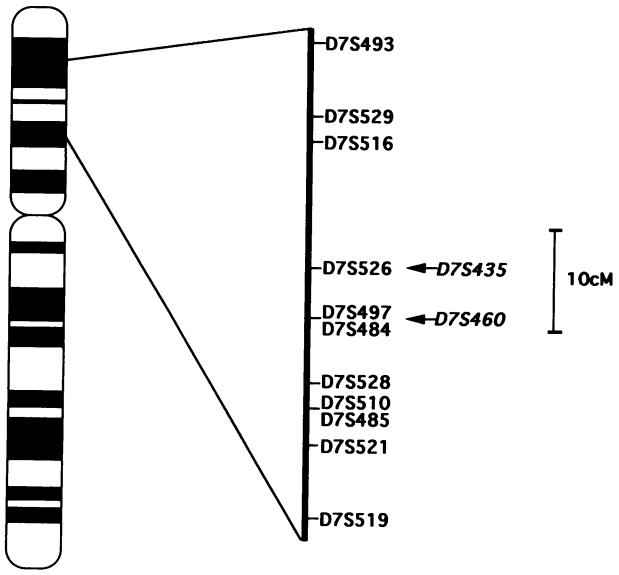

Figure 2 Ideogram of human chromosome 7, with an expanded genetic map of the region to which adRP localizes. Distances (i.e., θ) are those published by Weissenbach et al. (1992). The two markers shown in italics are those originally linked to this locus.

cM (by the Kosambi mapping function), with lod scores of 14.5 and 8.7, respectively.

A subset of these markers was then used to generate a multipoint map of the region, in order to identify the most likely location of the disease gene. The complexity of the family, the number of loci, and the number of alleles detected by each marker system was such that it was not possible to pool all the data available. To simplify the data analysis, rare alleles coming from unaffected spouses were renamed as though they were the same as the commoner unlinked alleles already segregating in the family. This reduced allele frequencies down to three or four for each marker system. Multipoint analysis was then performed as a series of six three-point analyses, with two markers and the disease, in order to calculate the likelihood of the disease gene being situated between these two markers. Seven of the most informative markers which spanned the region were used in this way on the U.K. Human Genome Mapping Project computing facility (described by Rysavy et al. 1992). To simplify the presentation of these data we have plotted on a single graph the between-marker intervals of each of the six graphs, as shown in figure 3. This is not a single multipoint analysis, and therefore lod scores between intervals cannot be directly compared. Nevertheless, these analyses give tentative evidence for a likely disease locus in the interval D7S484–D7S526, with a peak lod score of 17.8. This hypothesis is supported by haplotype analysis (data not shown), with two crossovers being identified by markers distal to the disease locus, including D7S526 and one different crossover identified by D7S484 and other markers proximal to this interval. The recombination fraction (θ) for this interval is estimated by Weissenbach and coworkers to be .04. Recalculating this interval in a collection of our own families, we obtained a peak lod score of 23.7 at θ = .016. However, the 1-lod (90%) confidence limit for this θ was .002–.06.

Table 1

Lod Scores between the Disease Locus and 7p Markers in adRP7

Marker	Lod Score at θ =							$\hat{\theta}$	Z_{max}	1-Lod Confidence Limit
	.00	.01	.05	.10	.20	.30	.40			
D7S493	−∞	−4.32	.50	1.92	2.31	1.68	.79	.17	2.36	.08–.33
D7S529	−∞	−2.74	1.24	2.21	2.13	1.36	.57	.14	2.35	.06–.30
D7S516	−∞	1.02	2.02	2.06	1.53	.87	.35	.08	2.10	.01–.26
D7S435	−∞	3.23	4.47	4.31	3.13	1.76	.64	.06	4.49	.01–.17
D7S526	−∞	8.24	8.58	7.85	5.75	3.50	1.46	.03	8.67	.005–.11
D7S460	7.29	7.08	6.28	5.30	3.46	1.88	.71	.00	7.29	.00–.05
D7S484	−∞	14.52	13.93	12.57	9.38	5.93	2.58	.01	14.54	.00–.06
D7S497	2.85	2.74	2.35	1.88	1.09	.52	.18	.00	2.85	.00–.10
D7S528	−∞	3.94	4.01	3.54	2.39	1.32	.50	.03	4.10	.002–.13
D7S510	−∞	4.14	4.69	4.24	2.89	1.60	.63	.04	4.70	.006–.14
D7S485	7.82	7.61	6.78	5.77	3.85	2.16	.84	.00	7.82	.00–.04
D7S521	−∞	6.74	6.59	5.83	4.09	2.43	1.02	.02	6.83	.001–.10
D7S519	−∞	−.67	2.67	3.38	2.96	1.88	.79	.12	3.41	.05–.25

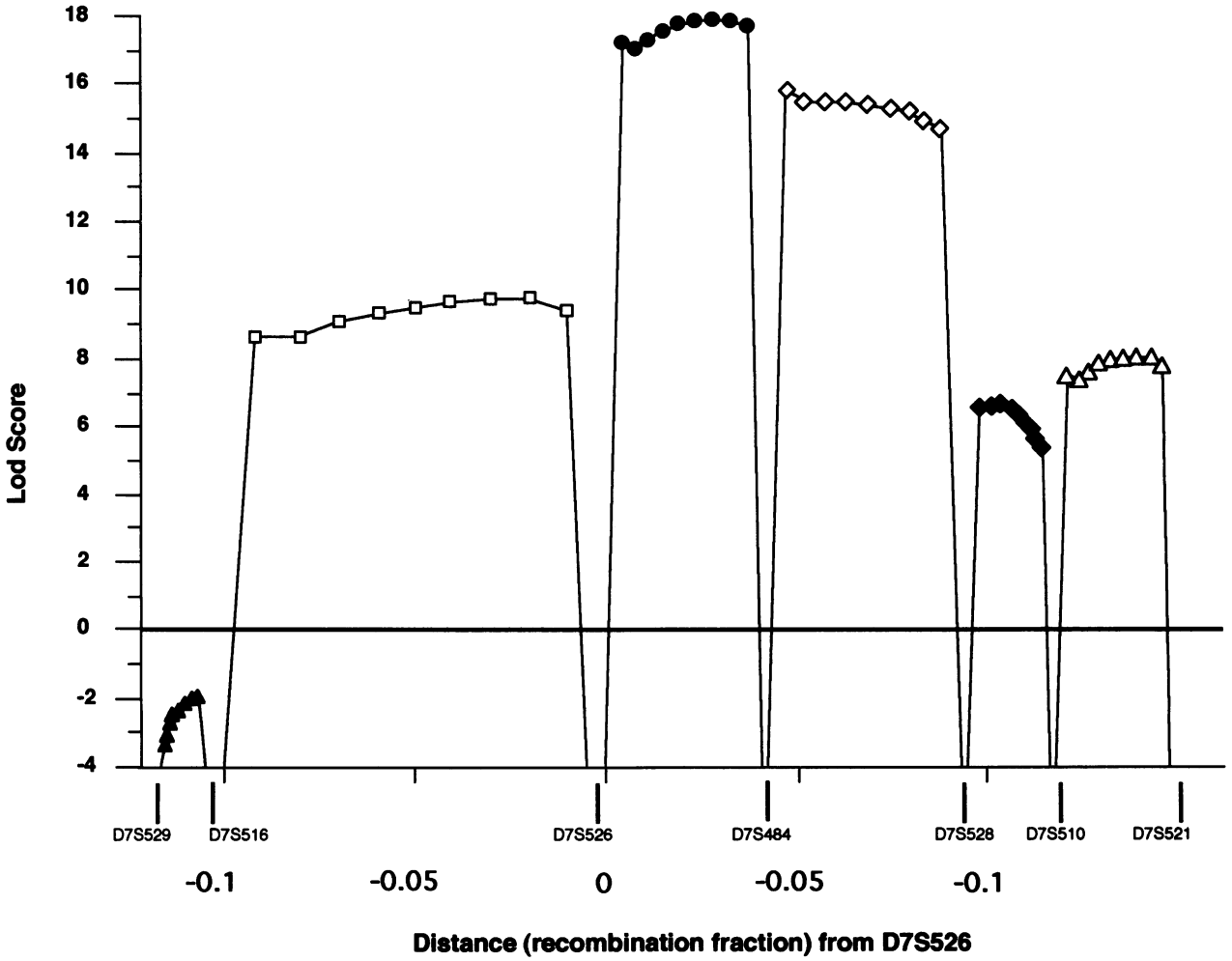

Figure 3 Pooled results of a series of six three-point analyses, of adRP, against two markers in family adRP7. Each set of symbols represents the central interval of a different three-point analysis, while data outside the two markers have not been plotted, to allow representation on a single graph.

Discussion

The data presented demonstrate that adRP in family adRP7 maps to the interval between markers D7S484 and D7S526. The θ value of this interval has been estimated by others to be .04, while this study estimates it to be .016 with a 1-lod confidence interval of θ = .002–.06. In addition, the disease gene in this family has now been located relative to markers on both of the high-resolution human genome maps currently available (NIH/CEPH Collaborative Mapping Group 1992; Weissenbach et al. 1992). Since both markers map immediately distal to the anchor marker TCRG, which is physically located to 7p15, the physical localization of the disease gene has also been refined. This information will assist in determining whether chromosome 7–specific retinal cDNAs such as those published by Zheng et al. (1992) are candidates for the disease locus. Also, this study has identified a highly informative set of markers which will quickly determine whether other families are linked to this locus. The markers D7S460, D7S526, and D7S484 can be amplified and resolved together, with allele sizes of 196–180 bp, 135–125 bp, and 113–99 bp, respectively, when they are used in a PCR protocol with an annealing temperature of 54°C (data not shown).

It is interesting to note that D7S484 gives a lod score nearly twice that given by any other marker, even though it detects one crossover with the disease locus.

This is a result of the unusual structure of family adRP7, which has been traced back many generations through extended relationships (loops) which inevitably cannot be sampled. When, for a given marker, a disease is linked to the same rare allele in all branches of the family, the MLINK program considers it likely that there has been no crossover in these unsampled loops and therefore scores them as nonrecombinant. This problem illustrates the importance of knowing the appropriate allele frequencies when such a family is used, as discussed elsewhere (Knowles et al. 1992; Ott 1992). For this reason, we estimated the frequencies from a random sample of ~120 chromosomes derived from the population of origin, rather than using frequencies available in the Johns Hopkins Genome Database.

The interval within which the disease must lie is now sufficiently small to consider physical mapping and positional cloning. To this end we now have YAC clones containing the markers described and are seeking to establish contiguous maps of the regions around markers D7S484 and D7S526. These maps will be starting points for a YAC-based chromosome walk in order to clone the region containing the adRP gene. This approach should lead to the identification of a gene implicated in adRP, which in turn will give further insights into other inherited eye diseases and into normal photoreceptor function.

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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, Freidman J, Sadler LA, Wagner M, Friedman L, et al (1991) Linkage mapping of autosomal dominant retinitis pigmentosa (RPI) to the pericentric region of human chromosome 8. Genomics 11:857–869

Dryja TP, Hahn LB, Cowley GS, McGee TL, Berson EL (1991) Mutation spectrum of the rhodopsin gene among patients with autosomal dominant retinitis pigmentosa. Proc Natl Acad Sci USA 88:9370–9374

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

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

Hudson TJ, Engelstein M, Lee MK, Ho EC, Rubenfield MJ, Adams CP, Houseman DE, et al (1992) Isolation and chromosomal assignment of 100 highly informative human simple sequence repeat polymorphisms. Genomics 13:622–629

Inglehearn CF, Carter SA, Keen TJ, Lindsey J, Stephenson AM, Bashir R, Al-Maghtheh M, et al (1993) A new locus for autosomal dominant retinitis pigmentosa on chromosome 7p. Nature Genet 4:51–53

Inglehearn CF, Keen TJ, Bashir R, Jay MR, Fitzke FW, Bird AC, Crombie A, et al (1992) A complete screen for mutations of the rhodopsin gene in a panel of patients with autosomal dominant retinitis pigmentosa. Hum Mol Genet 1:41–45

Jordan SA, Farrar GJ, Kenna P, Humphries MM, Sheils DM, Kumar-Singh R, Sharp EM, et al (1993) Localisation of an autosomal dominant retinitis pigmentosa gene to chromosome 7q. Nature Genet 4:54–57

Kajiwara K, Hahn LB, Mukai S, Travis GH, Berson EL, Dryja TP (1991) Mutations in the human retinal degeneration slow gene in autosomal dominant retinitis pigmentosa. Nature 354:480–483

Knowles JA, Vieland VJ, Gilliam TC (1992) Perils of gene mapping with microsatellite markers. Am J Hum Genet 51:905–909

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multipoint linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Moore AT, Fitzke FW, Jay MR, Arden GB, Inglehearn CF, Keen TJ, Bhattacharya SS, et al (1993) Autosomal dominant retinitis pigmentosa with apparent incomplete penetrance: a clinical, electrophysiological, psychophysical and molecular genetic study. Br J Ophthalmol 77:473–479

NIH/CEPH Collaborative Mapping Group (1992) A comprehensive genetic linkage map of the human genome. Science 255:67–86

Ott J (1992) Strategies for characterizing highly polymorphic markers in human gene mapping. Am J Hum Genet 51:283–290

Rysavy FR, Bishop MJ, Gibbs GP, Williams GW (1992) The UK Human Genome Mapping Project online computing service. Comput Appl Biosci 8:149–154

Sung CH, Davenport CM, Hennessey JC, Maumenee IM, Jacobson SG, Heckenlively JR, Nowakowski R, et al (1991)

Rhodopsin mutations in autosomal dominant retinitis pigmentosa. Proc Natl Acad Sci USA 88:6481–6485

Weber JL, Kwitek AE, May PE (1990) Dinucleotide repeat polymorphisms at the D7S435 and D7S440 loci. Nucleic Acids Res 18:4039

Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millasseau G, Vaysseix G, et al (1992) A second generation linkage map of the human genome. Nature 359:794–801

Wells J, Wroblewski J, Keen TJ, Inglehearn CF, Jubb C, Eckstein A, Jay MR, et al (1992) Mutations in the human retinal degeneration slow (RDS) gene can cause either retinitis pigmentosa or macular dystrophy. Nature Genet 3:213–218

Zheng K, Gieser L, Zu E, Swaroop A, Yang-Feng TL (1992) Chromosomal localisation of cDNA clones from subtracted eye libraries. Am J Hum Genet Suppl 51:986