

Autosomal recessive retinitis pigmentosa locus *RP28* maps between D2S1337 and D2S286 on chromosome 2p11-p15 in an Indian family

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Abstract

Retinitis pigmentosa (RP) is a group of clinically and genetically heterogeneous disorders characterised by night blindness, constriction of visual field, and dystrophic changes of the retina. Previous genetic studies have shown extensive allelic and non-allelic genetic heterogeneity of RP. Here we describe an Indian family with multiple consanguineous marriages and a total of four patients with autosomal recessive (AR) RP. The homozygosity mapping strategy was successfully used and indicated close linkage between the disease locus and D2S380, D2S441, D2S291, and D2S1394 with maximum lod scores between 1.51-3.07 at $\theta=0.00$. The analysis of multiply informative meioses maps the locus (*RP28*) for ARRP in this family between D1S1337 and D2S286 on 2p11-p15. The involvement of visinin (*VSNLI*), a promising candidate gene assigned to chromosome 2p by previous studies, has been excluded by the absence of linkage.

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Retinitis pigmentosa (RP) is a heterogeneous group of inherited degenerative retinal disorders characterised by night blindness, constriction of visual field, and fundus changes including abnormal accumulation of pigment deposits in the retina. The age of onset of clinical symptoms varies from childhood/youth to middle age. RP is inherited most frequently as an autosomal recessive (AR) trait. Molecular genetic studies of the past several years have shown extensive allelic and non-allelic genetic heterogeneity within the ARRP group. To date, four different loci have been mapped on chromosomes 1q31-q32.1, 2q31-q33, 6p11-q15, and 16p12.3-p13.11. In addition, disease relevant mutations in a total of eight different genes expressed in the neuroretina or the retinal pigment epithelium have been identified in patients/families with ARRP (for a recent compilation see the RetNet home page in ut-sph.uth.tmc.edu). Yet, mutations of all these genes explain only a minority (<25%) of the cases as their frequency is usually very low (2-5% each) among the ARRP alleles.

Homozygosity mapping is a powerful tool for mapping the loci of rare autosomal recessive

traits in children from consanguineous marriages.¹ The method is based on the principle that a rare disease in the affected offspring of a consanguineous mating is the result of inheritance of two identical copies of the disease gene and nearby loci from the common ancestor, the feature termed "homozygosity by descent". Although a few other regions may also be homozygous (by descent) in any given child, these regions will vary from one child to another within the same sibship.

In the study presented here, the above strategy was used successfully both for a targeted and a genome wide search to map the ARRP locus in an Indian family. Family PMK146 contains multiple consanguineous marriages (fig 1). A total of four affected subjects were diagnosed in generations V and VI. Age of onset was in the first two decades and varied between five and 15 years. The three patients from generation V, aged 39 to 47 years, had severe visual handicap. The youngest patient, VI.2, had a visual acuity of 3/60 and counting fingers for the left and right eye, respectively, at the age of 15 years. The fundus picture of the probands showed the known features of RP, that is, narrowing of the arteries, bone corpuscle pigments, waxy pallor of the disc, and optic atrophy.

Genomic DNA of the family members analysed (fig 1) was extracted from whole blood spotted on filter papers. Scanning for homozygosity in the four affected subjects was performed using microsatellite repeat markers (with or without fluorescence labelling) of versions 6 and 6a of the MaiPairs™ Screening Set (Research Genetics, Huntsville, AL, USA). At first targeted search for homozygosity in affected family members was initiated using intragenic or nearby DNA polymorphisms for the eight known ARRP genes and those at loci closely linked to the four ARRP loci mapped so far. In all cases, heterozygosity was seen in one or more of the patients and linkage analysis gave highly negative lod scores for all values of the recombination fraction below 0.20 (data not shown). Subsequently, a random genome wide search for homozygosity was performed on the four affected subjects using polymorphic microsatellite markers. During this screening, all four affected subjects (V.1, 2, 3, and VI.2), but none of their healthy relatives, were found to be homozygous for the polymorphism at D2S1394 on the short arm of chromosome 2 (fig 1). In view of these results, further analysis was performed using addi-

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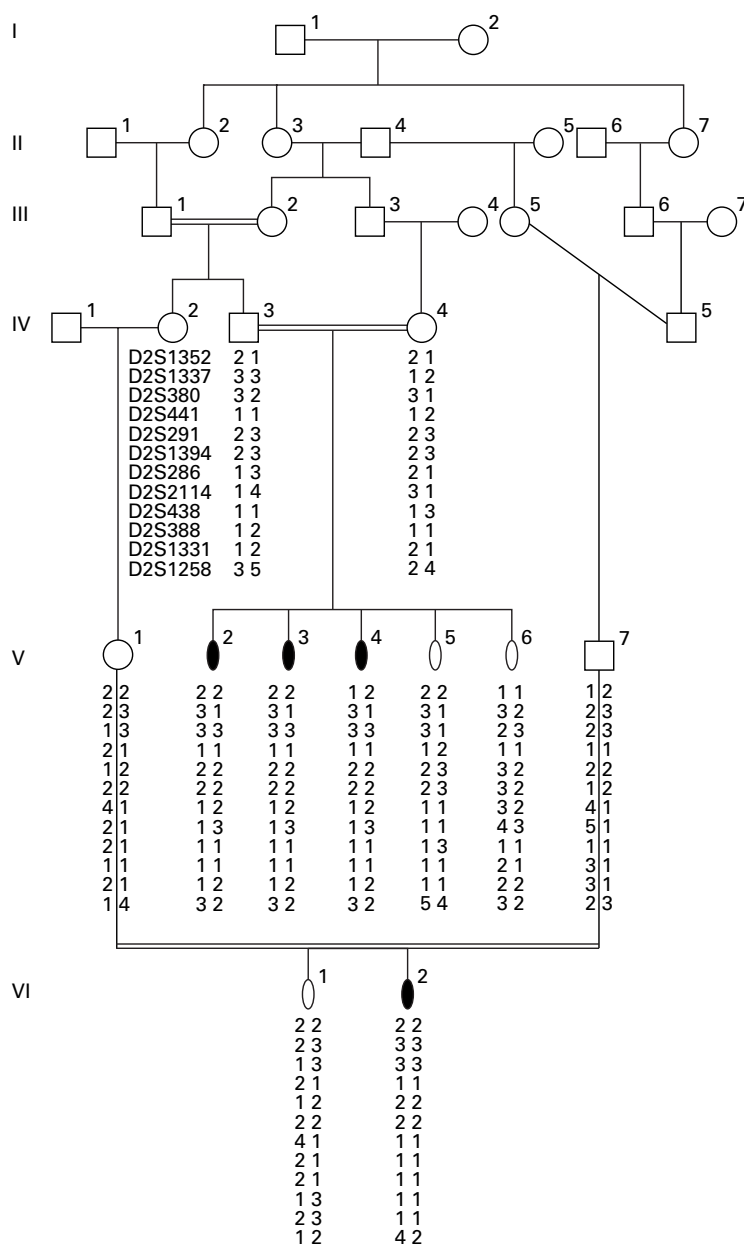


Figure 1 Simplified pedigree of PMK146. The identity of family members in generations V and VI has been encrypted for privacy. Genotypes are arranged according to the most likely haplotypes.

Table 1 Linkage relationship between the locus for autosomal recessive RP and 14 loci from chromosome 2p11-p15 in family PMK146

	Lod score (Z) at recombination fraction (θ)						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
D2S1352	$-\infty$	-1.55	-0.36	0.01	0.17	0.13	0.05
D2S1337	-0.71	-0.05	0.41	0.50	0.41	0.24	0.08
D2S380	3.07	2.99	2.65	2.24	1.46	0.78	0.27
D2S441	1.51	1.48	1.33	1.15	0.80	0.49	0.21
Haplotype*	3.38	3.29	2.94	2.50	1.65	0.91	0.33
D2S291	3.02	2.94	2.63	2.24	1.48	0.79	0.25
D2S1394	2.56	2.49	2.20	1.86	1.19	0.61	0.18
D2S286	0.47	1.09	1.39	1.28	0.84	0.39	0.09
D2S2114	0.57	1.18	1.48	1.36	0.89	0.43	0.11
D2S438	1.84	1.79	1.62	1.41	1.00	0.61	0.27
D2S388	1.92	1.86	1.65	1.38	0.87	0.44	0.13
D2S1331	-0.81	0.07	0.46	0.44	0.24	0.08	0.01
D2S1258	$-\infty$	-0.42	0.65	0.89	0.79	0.48	0.18
VSNL1	$-\infty$	-4.73	-2.19	-1.23	-0.47	-0.17	-0.03

*As no recombination was found in the region from (and including) D2S380 to D2S1394, haplotypes were constructed and used to calculate lod scores.

tional polymorphic markers from that chromosomal region. All four affected subjects showed a homozygous and identical pattern for a total of four anonymous markers (fig 1). The telomeric boundary of the homozygous region is set by heterozygosity for D2S1337 in affected subjects V.1, 2, and 3 that is most likely because a recombination occurred during transmission in one of the two previous generations. Similarly, heterozygosity was found in all patients in generation V for D2S286, marking the centromeric end of the chromosomal segment homozygous by descent and defining a region of about 16 cM harbouring the ARRP gene in this family. Using all genotype data, a haplotype (3-1-2-2) was constructed for the four markers between D2S1337 and D2S286 (fig 1), which was constantly cosegregating with the ARRP phenotype in family PMK146. As expected, positive lod scores with maxima at $\theta=0.00$ were obtained for all loci mapped within the region of homozygosity; the two point lod score of 3.07 for D2S380 reflects only the high degree of informativity of the corresponding polymorphism in family PMK146. The highest lod score value of 3.38 at $\theta=0.00$ was calculated if the haplotype was used (table 1). Taken together, the data from linkage analysis and homozygosity mapping strongly suggest that the ARRP locus *RP28* maps in 2p11-p15 (fig 2).

Visinin and visinin-like peptides represent a retina specific protein family. These peptides belong to a calcium binding protein family and are believed to be involved in the process of phototransduction in cone photoreceptors. A human homologue (*VSNL1*) of the rat visinin-like peptide gene has been mapped to 2p by linkage analysis and radiation hybrid mapping.² Using PCR primers to amplify a (CA)_n microsatellite repeat from the 3' untranslated region of the gene,² we genotyped all family members and found several recombinations (data not shown) thus excluding *VSNL1* as a candidate gene of the ARRP segregating in this family. In two point analyses, no linkage has been found between *VSNL1* and *RP28* (table 1) or the 12 loci from 2p studied (data not shown), suggesting that *VSNL1* maps outside the region analysed here.

The genetic length of the region harbouring this novel ARRP locus on the short arm of chromosome 2 is about 16 cM. The size of the critical region cannot be narrowed further by the analysis of this family as no recombination has been detected in the interval between D2S1337 and D2S286 using a total of four markers.

To date, it is not known what proportion of all ARRP cases is the result of mutations in the corresponding gene. Genotyping of 20 additional, similar sized Indian families for one or more of the polymorphisms given in table 1 has not shown any other kindred with similar linkage data (data not shown), suggesting that we are not dealing with a major locus for ARRP. However, a final answer to this question can only be given once the disease gene has been identified and a larger collection of unrelated patients has been screened for mutations. The

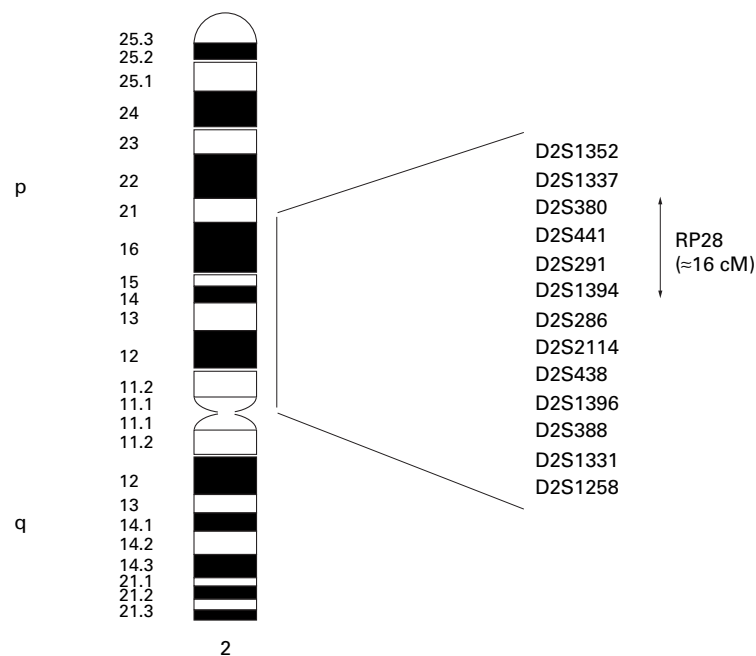


Figure 2 Schematic map of chromosome 2p showing the position of the loci genotyped for the linkage study. The relative position of the loci shown is taken from the Human Genetic Location Database (Idb: <http://cedar.genetics.soton.ac.uk/public.html>).

assumption that this gene is only one of the many genes implicated in the pathogenesis of ARRP is in line with the observation that mutations in the eight known ARRP genes identified and analysed so far also account for a small portion, up to 2-5% each, of all disease cases.^{3,4}

The chromosomal region harbouring the ARRP gene is about 14 Mb. Therefore a direct search for the corresponding gene by positional cloning is not feasible. The gene encoding the α subunit of the cone photoreceptor cGMP gated cation channel and mutated in achromatopsia, an autosomal recessive form of colour blindness, has been mapped on 2q11, most likely between D2S338 and D2S373.^{5,6} As there is no overlap between the intervals for achromatopsia and RP28, it is unlikely that the two disorders are allelic. Similarly, the gene *VSNL1*, encoding a visinin-like protein, a cone photoreceptor specific 24 kDa calcium binding peptide, has also been excluded as a likely candidate gene for the disease as no linkage was found between the two loci.

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