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Confirmation of linkage and refinement of the RP28 locus for autosomal recessive retinitis pigmentosa on chromosome 2p14-p15 in an Indian family

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Purpose: To report the linkage analysis of retinitis pigmentosa (RP) in an Indian family.
Methods: Individuals were examined for symptoms of retinitis pigmentosa and their blood samples were withdrawn for genetic analysis. The disorder was tested for linkage to known 14 adRP and 22 arRP loci using microsatellite markers.
Results: Seventeen individuals including seven affecteds participated in the study. All affected individuals had typical RP. The age of onset of the disease ranged from 8-18 years. The disorder in this family segregated either as an autosomal recessive trait with pseudodominance or an autosomal dominant trait. Linkage to an autosomal recessive locus RP28 on chromosome 2p14-p15 was positive with a maximum two-point lod score of 3.96 at theta=0 for D2S380. All affected individuals were homozygous for alleles at D2S2320, D2S2397, D2S380, and D2S136. Recombination events placed the minimum critical region (MCR) for the RP28 gene in a 1.06 cM region between D2S2225 and D2S296.
Conclusions: The present data confirmed linkage of arRP to the RP28 locus in a second Indian family. The RP28 locus was previously mapped to a 16 cM region between D2S1337 and D2S286 in a single Indian family. Haplotype analysis in this family has further narrowed the MCR for the RP28 locus to a 1.06 cM region between D2S2225 and D2S296. Of 15 genes reported in the MCR, 14 genes (*KIAA0903, OTX1, MDH1, UGP2, VPS54, PELI1, HSPC159, FLJ20080, TRIP-Br2, SLC1A4, KIAA0582, RAB1A, ACTR2,* and *SPRED2*) are either expressed in the eye or retina. Further study needs to be done to test which of these genes is mutated in patients with RP linked to the RP28 locus.

Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of retinal degenerations characterized by abnormalities of the photoreceptors or retinal pigment epithelium (RPE) leading to progressive loss of vision. Patients typically present with night blindness, followed by constriction of the peripheral visual fields. In the advance stages of the disease, the retina develops intraretinal and preretinal clumps of black melanin pigments appearing like bone spicules, attenuated retinal vessels, loss of RPE and paleness of the optic nerves [1]. The age of onset of the initial clinical symptom varies from childhood to middle age [2]. The incidence of RP is estimated to be 1 in 4,000 people in western populations [3]. The actual incidence of RP is not known in the Indian population. RP exhibits a variety of modes of inheritance. It can be inherited as an autosomal dominant, autosomal recessive, X-linked recessive, X-linked dominant or, in rare cases, as a digenic trait. Autosomal dominant RP (adRP) and autosomal recessive RP (arRP) each accounts for approximately 20% of RP cases, while X-linked RP accounts for 10% of RP cases. Approximately 50% of all RP patients are simplex cases, which include sporadic RP and arRP [4-6]. To date, a total of 22 loci for arRP and 14 loci for adRP have been reported (RetNet). In a majority of the cases, the linkage has been reported in either a single family or only a few families. To date, genes for a total of 17 arRP and 12 adRP loci have been isolated (RetNet). Gu et al. [2] have reported linkage of a consanguineous Indian family with arRP to the RP28 locus on chromosome 2p11.2-p15. We report here confirmation of this linkage in a second consanguineous Indian family. Gu et al. [2] have previously mapped this locus to a 16 cM region between D2S1337 and D2S286. Haplotype analysis in the present family has further narrowed this region to a 1.06 cM region between markers D2S2225 and D2S296.

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METHODS

Subjects: We have ascertained 17 members of a four-generation consanguineous family with RP from the state of Karnataka, India (Figure 1). There are two consanguinous matings in the pedigree. Individuals III-4 and III-5 are first cousins. Individuals II-1 and II-2 are known to be related; however, the exact relationship is unknown. A total of seven individuals were diagnosed with RP. The age of onset of nightblindness ranged from 8 to 18 years. Funduscopy revealed the typical features of RP including attenuated blood vessels, bone spicule pigmentation, waxy pallor of the disc and optic atrophy. Informed consent was obtained for research following the guidelines of the Indian Council of Medical Research, New Delhi.

Genotyping and linkage analyses: For genotyping, total genomic DNA sample was extracted from peripheral blood samples using a Wizard™ Genomic DNA Purification kit (Promega, Madison, WI). To determine if this family was linked to one of the known adRP or arRP loci, 2 to 4 microsatellite markers were selected from the candidate region of each of these loci and used to genotype the family. Microsatellite markers were synthesized commercially according to data from the Genome Database. Marker order and genetic distances were obtained from the Marshfield Medical Research Foundation web site. Amplification of microsatellite markers was performed as reported in Kumar et al. [7]. Radiolabelled PCR products were separated on a 6% denaturing polyacrylamide gels and subjected to either Phosphor Image analysis (Fuji Inc., Kanagawa, Japan) or exposed to X-ray films.

Two-point lod scores were calculated using the MLINK program from the LINKAGE package version 5.1 [8], under the assumption of autosomal recessive mode of inheritance and a disease gene frequency of 0.0001. Population specific allele frequencies are not available for the Indian population; therefore equal marker allele frequencies were assumed for linkage analysis for most of the markers. Allele frequencies were calculated for markers showing homozygosity using 30 control individuals. However, varying the allele frequencies did not substantially change the linkage results.

Table 1. Clinical descriptions of patients

Patient	Age now (age at onset)	Eye	BCVA	Refraction		Visual field
III-4	52 (-)	OU	LP	N/A		N/A
IV-1	23 (18)	OD	6/24	+0.5 x -1.5 40°	(6/24)	Tunnel vision
		OS	6/36	+0.5 x -1.5 140°	(6/36)	Tunnel vision
IV-2	20 (15)	OD	6/18	-4.0 x -1.0 20°	(6/18)	Tunnel vision
		OS	6/18	-4.5 x -1.0 110°	(6/18)	Tunnel vision
IV-3	18 (12)	OD	6/12	-8.0 x -3.0 50°	(6/12)	Tunnel vision
		OS	6/18	-8.0 x -2.5 140°	(6/18)	Tunnel vision
IV-4	13 (10)	OD	6/24	-3.0 x -2.0 20°	(6/36)	Tunnel vision
		OS	6/18	-3.0 x -2.0 160°	(6/18)	Tunnel vision
IV-5	11 (8)	OD	6/9	-8.0	(6/9)	Tunnel vision
		OS	6/9	-8.0	(6/9)	Tunnel vision
IV-7	16 (11)	OD	6/36	-2.75	(6/36)	Tunnel vision
		OS	6/18	-1.0 x -1.0 180°	(6/18)	N12

This table provides the age of onset of RP, BCVA, refraction and visual field data for all seven affected individuals. The best corrected Snellen visual acuity is abbreviated “BCVA”; light perception vision is abbreviated “LP”.

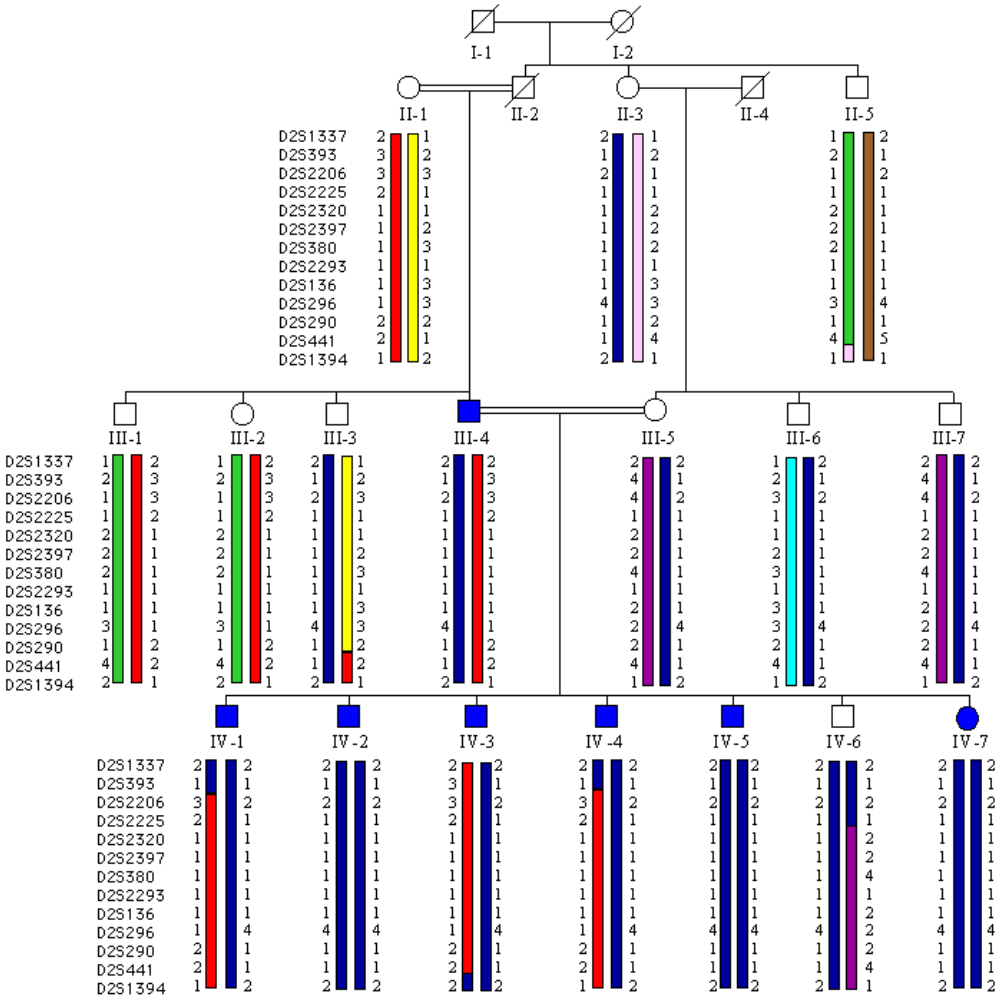

Figure 1. Pedigree diagram and haplotype analysis of the family IIS-2. Pedigree diagram and haplotype analysis of the family IIS-2. Blue symbols denote persons affected with RP.

RESULTS

All affected individuals from the family IIS-2 had typical RP including bone spicules and attenuated vessels. The age of onset of the disease (night blindness) ranged from 8-18 years (Table 1). Individual III-4 had completely lost his vision by the age of 40 years and had advanced stage RP involving the macula with bone spicule pigmentation all around the fundus. His perception of light was positive in both eyes. Individuals IV-1 and IV-7 had typical RP with early consecutive optic atrophy with atrophic macular degeneration. Individual IV-2 had typical RP with macular degeneration. Individual IV-3 had typical RP with perivascular bone spicule pigmentation, sheathed vessels with a tapetal reflex at the macula. Individual IV-4 had typical RP with a normal macula. Individual IV-5 had typical RP with early consecutive optic atrophy and normal macula. In addition to RP, individuals IV-2, IV-3, IV-4, IV-5, and IV-7 were also myopic.

Visual inspection of the pedigree suggested that RP is either segregating as an autosomal dominant trait or an autosomal recessive trait with pseudodominance in the family IIS-2 (Figure 1). Linkage analysis was carried out in this family using primers from candidate regions of each of the 14 adRP and 22 arRP loci. Linkage was excluded for all except arRP locus RP28 on chromosome 2p11.2-p15 (data not shown). Linkage to RP28 locus on chromosome 2p11.2-p15 was positive with a maximum two-point lod score of 3.96 at theta=0 for marker D2S380 (Table 2). All the affected individuals were homozygous for alleles for the markers D2S2320, D2S2397, D2S380, and D2S136 (Figure 1). None of the normal individuals were found to be homozygous for alleles at these marker loci. A disease haplotype 1-1-1-1 for these markers was segregated with the disease in this family. The telomeric boundary of the homozygous region was set by heterozygosity for the marker D2S2225 in affected individuals III-4, IV-1, IV-3, and IV-4. Similarly, the centromeric boundary was delineated by heterozygosity for the marker D2S296 in individu-

Table 2. Two-point lod scores at RP28 markers in the family IIS-2

	Lod scores at theta								
Markers	0.00	0.001	0.01	0.05	0.10	0.15	0.20	0.30	0.40
D2S1337	0.79	0.79	0.76	0.64	0.49	0.37	0.26	0.10	0.02
D2S393	-∞	1.51	0.42	1.49	1.69	1.64	1.48	1.04	0.52
D2S2206	-∞	5.05	-2.10	-0.20	0.44	0.69	0.78	0.70	0.42
D2S2225	-∞	7.31	-4.31	-2.25	-1.39	-0.92	-0.61	-0.24	-0.05
D2S2320	2.15	2.14	2.09	1.84	1.53	1.22	0.92	0.34	-0.10
D2S2397	2.95	2.94	2.87	2.56	2.19	1.84	1.50	0.88	0.34
D2S380	3.96	3.95	3.87	3.51	3.06	2.62	2.18	1.35	0.62
D2S2293	0.42	0.42	0.40	0.34	0.27	0.21	0.15	0.07	0.02
D2S136	2.24	2.24	2.20	2.03	1.81	1.60	1.38	0.96	0.51
D2S296	-∞	4.46	-1.52	0.29	0.83	0.99	1.01	0.82	0.48
D2S290	-∞	6.36	-3.40	-1.48	-0.80	-0.48	-0.29	-0.10	-0.02
D2S441	-∞	7.12	-3.18	-0.67	0.17	0.49	0.61	0.51	0.21
D2S1394	-∞	4.25	-2.22	-0.82	-0.32	-0.10	-0.01	0.03	0.01

The two-point lod scores were calculated for 13 microsatellite markers at the RP28 locus with theta values ranging from 0 to 0.40.

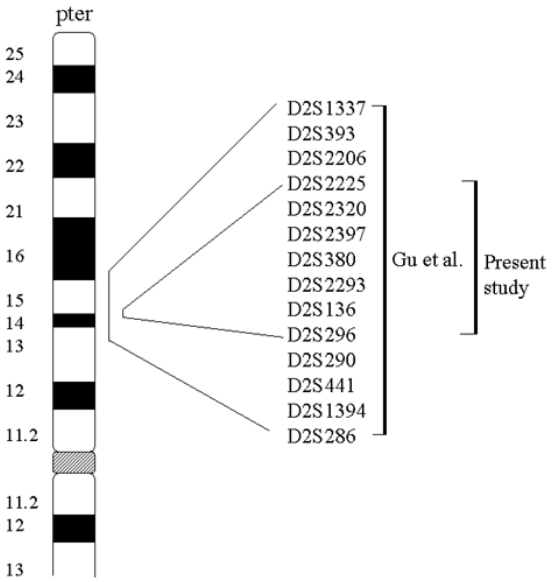

Figure 2. Positions of minimum critical regions for the RP28 locus on chromosome 2p. Schematic map of chromosome 2p showing positions of marker loci genotyped for linkage analysis by Gu et al. [2] and the present study. Minimum critical regions (MCRs) observed for the RP28 locus by Gu et al. [2] and during the present study are delineated. Marker positions are taken from the UCSC Genome Bioinformatics Site.

Table 3. Known arRP loci and genes

Serial number	Locus	Chromosome location	Gene
1	RP20	1p31.2	RPE65 (retinal-pigment epithelium-specific 65 kDa protein)
2	RP19	1p22.1	ABCR (ATP-binding cassette transporter-retinal)
3	RP12	1q31.3	CRB1 (crumbs homolog 1)
4		1q41	USH2A (encoding a protein with lamin FGF and fibronectin type III domain)
5	RP28	2p14-p15	
6		2q13	MERTK (c-mer protooncogene, receptor kinase)
7	RP26	2q31.2-q32.3	
8		2q37.1	SAG (arrestin s-antigen)
9	RP4	3q22.1	RHO (rhodopsin)
10		4p16.3	PDE6B (rod cGMP phosphodiesterase b-subunit)
11		4p12	CNGAI (cyclic nucleotide-gated channel alpha subunit)
12		4q32.1	LRAT (lecithin retinal acyltransferase)
13	RP29	4q32-q34	
14		5q33.1	PDE6A (rod cGMP-gated channel alpha subunit)
15	RP14	6p21.31	TULP1 (tubby-like protein 1)
16	RP25	6cen-q15	
17		8q12.3	TTPA (tocopherol transfer protein, alpha)
18		10q23.1	RGR (RPE-retinal G protein-coupled receptor)
19		15q23	NR2E3 (nuclear receptor subfamily 2 group E3)
20		15q26.1	RLBP1 (cellular retinaldehyde-binding protein)
21	RP22	16p12.1-p12.3	
22		16q13	CNGB1 (cyclic nucleotide-gated channel, beta-1)

Summary of 22 known arRP loci and 17 known genes responsible for autosomal recessive retinitis pigmentosa.

Table 4. Details of known genes between markers D2S2225 and D2S296

Serial number	Gene	Expressed in eye	UniGene cluster
1	KIAA0903 (uncharacterized gene)	Yes	16218
2	OTX1 (orthodenticle Drosophila homolog 1)	Yes	445340
3	LOC51057 (uncharacterized gene)	No	414952
4	MDH1 (malate dehydrogenase)	Yes	75375
5	UGP2 (uridine diphosphoglucose pyrophosphorylase 2)	Yes	417361
6	VPS54 (vacuolar protein sorting 54)	Yes	48499
7	PELI1 (Pellino Drosophila protein 1)	Yes	7886
8	HSPC159 (uncharacterized gene)	Yes	372208
9	FLJ20080 (uncharacterized gene)	Yes	7942
10	TRIP-Br2 (transcriptional regulator interacting with the PHS-bromodomain 2)	Yes	77293
11	SLC1A4 (soluble carrier family 1, glutamate/ neutral amino acid transporter, member 4)	Yes	323878
12	KIAA0582 (uncharacterized gene)	Yes	146007
13	RAB1A (Ras-related protein Rab-1A)	Yes	227327
14	ACTR2 (actin-related yeast homolog protein 2)	Yes	393201
15	SPRED2 (sprouty protein with EVH-1 domain 2)	Yes	173108

Known 15 candidate genes in the RP28 minimum critical region between markers D2S2225 and D2S296 on chromosome 2p14-p15.

als III-4, IV-1, IV-3, and IV-4. The homozygous region of 1.06 cM between D2S2225 and D2S296 at 2p14-p15 most probably represents the chromosomal segment homozygous by descent (Figure 2).

DISCUSSION

We have reported the linkage of a consanguineous family from India segregating with autosomal recessive RP to the RP28 locus on chromosome 2p14-p15. This confirms the mapping of the RP28 locus in a second Indian family. Gu et al. [2] have previously reported that the minimum critical region (MCR) for the RP28 locus spans 16 cM between D2S1337 and D2S286 in a single Indian family. Haplotype analysis in the family IIS-2 has further narrowed this region to a 1.06 cM region defined by markers D2S2225 and D2S296 (Figure 2). To the best of our knowledge, this family is from a different ethnic background and is not related to the family reported by Gu et al. [2].

Of the 22 known loci for arRP, genes for 17 loci have been isolated and characterized so far (Table 3; RetNet). However, genes for five loci (RP28, RP26, RP29, RP25, and RP22) have not been identified yet (Table 3). A total of 15 known genes have been reported between the markers D2S2225 and D2S296 (Table 4; UCSC Genome Bioinformatics Site) which span the RP28 locus. While none of these genes show any homology with known arRP genes, 14 of these 15 genes (*KIAA0903*, *OTX1*, *MDH1*, *UGP2*, *VPS54*, *PELI1*, *HSPC159*, *FLJ20080*, *TRIP-Br2*, *SLC1A4*, *KIAA0582*, *RAB1A*, *ACTR2*, and *SPRED2*) are either expressed in eye and/or retina (Table 4). It is possible that mutations in one of these genes result in the disease phenotype in the families linked to the RP28 locus.

In summary, we have confirmed linkage of arRP to the RP28 locus on chromosome 2p14-p15 in a second consanguineous Indian family. Haplotype analysis in the family has further narrowed the MCR of the RP28 locus to a 1.06 cM region between the markers D2S2225 and D2S296. These data will be useful in the isolation and characterization of the gene, mutations in which result in arRP in families linked to the RP28 locus. This will eventually lead to a proper patient management and genetic diagnosis in future.

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