Putative association of a mutant ROM1 allele with retinitis pigmentosa

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Abstract

Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous form of retinal degeneration. Several genes and loci have been shown to be involved in the disease, although each of them could only account for a few cases. Mutations in the gene encoding ROM1, a rod-specific protein, have been putatively associated with several forms of RP. Here we describe a double mutant allele of this gene, P60T and T108M, present in two affected sibs and also in two healthy members of a Spanish RP family. The same double mutant allele was previously considered to be responsible for autosomal dominant RP in one family. We now report data that questions the potential pathogenicity of these two ROM1 mutations.

Introduction

Retinitis Pigmentosa (RP) is a group of inherited retinal disorders characterized by a progressive constriction of peripheral visual fields, night blindness and, eventually, severe visual impairment (Heckenlively et al. 1988). Both linkage analysis and the candidate gene approach have identified several RP genes and loci (Dryja and Li 1995), but the genetic defect in most cases remains unknown.

One such gene is that encoding the rod outer segment membrane protein (ROM1) (Kajiwara et al. 1994; Sakuma et al. 1995; Bascom et al. 1995). Its product was first identified as a disk-rim protein, present in the rod outer segment and absent in cones (Bascom et al. 1992). However, recent experiments with anti-ROM1 antibodies suggest that it could be present in both photoreceptor cells (Moritz and Molday 1996).

To identify the molecular defect underlying the disease in an RP family, eight candidate genes were analyzed. The two affected sibs bore a double mutant allele of ROM1, P60T and T108M. This allele has been described as the cause of RP in another family (Bascom et al. 1995).

Materials and methods

Pedigree and clinical evaluation

The pedigree studied (referred to as V-8) comprises two branches with a total of three affected members (Fig.1). Clinical and electrophysiological studies (Table 1) included measurement of visual acuity, fundus examination, visual field testing, electroretinogram (ERG), electrooculogram (EOG) and dark adaptation. Ganzfeld ERGs were assessed, for both affected and non affected members of the family, according to the International Society for the Clinical Electrophysiology of Vision Standards (ISCEV). Rod-isolated responses were obtained under scotopic dark-adapted (20 min) conditions, mixed cone-rod responses with single flashes of white light in scotopic conditions and cone-isolated responses with 30 Hz white flickering light in fotopic conditions. EOG testing was performed following the ISCEV protocol using a Ganzfeld stimulator and a Compact Four Ca-2000 computer (Nicolet).

Informed consent was obtained from all subjects after the nature and possible consequences of the study had been explained.

Analysis of DNA polymorphisms

Genomic DNA was extracted from peripheral blood according to Miller et al. (1988).

Microsatellite markers at β-subunit of rod phosphodiesterase (PDEB), rhodopsin (RHO) and recoverin (D17S786) genes were from the MapPairs set (Research Genetics, Huntsville, AL). The polymorphisms at the peripherin/RDS gene were as described elsewhere (Kumar-Singh et al. 1991; Bayés et al. 1996). Two internal variants, 1447A/G and 1683delC, were used for cosegregation analyses of the γ subunit of rod phosphodiesterase (PDEG) (Hahn et al. 1994). Finally, cosegregation with S antigen (SAG) was studied using an intragenic tri-allelic polymorphism (Sheffield et al. 1992), detected by ASO hybridization.

PCR conditions were as follows: genomic DNA (300 ng) was amplified in a 25 μl reaction mixture containing 6.6 pmols of each primer, 1 unit of Dynazyme DNA polymerase (Fynzymes Oy, Espoo, Finland) in the recommended buffer and 200 μM dNTPs. The PCR amplification profile was: 94ºC 40 s, 50-60ºC 30 s (x35); 72ºC 5 min (x1).

Mutation analysis

A PCR-SSCP strategy was used for mutation screening. Oligonucleotide primers flanking the coding regions of PDEG, peripherin/RDS and ROM1 genes, were designed according to the published genomic sequences (Piriev et al. 1994; Travis et al. 1991; Bascom et al. 1993a). For Neural Retinal Leucine Zipper (NRL) gene, primers were kindly provided by Dr. A. Swaroop. Oligonucleotides to amplify the coding regions of PDEB and RHO were as described elsewhere (Riess et al. 1992; Keen et al. 1991).

PCR conditions were as described above except for a final reaction volume of 50 μl.

The protocol for SSCP analysis has been published elsewhere (Bayés et al. 1995). All samples were run under three sets of conditions, varying acrylamide and glycerol concentrations and running temperatures.

DNA sequencing

PCR products giving an aberrant SSCP pattern were purified by the QIAquick-spin PCR Purification Kit (Qiagen Inc, Chatsworth, USA) and cloned into pUC18 using the Sure Clone^{TM} Ligation Kit (Pharmacia Biotech, Uppsala, Sweden). Three to five mutant clones were sequenced in each case by the dideoxy chain-termination method using the T7 sequencing^{TM} Kit (Pharmacia). When the variant identified altered the recognition site for a restriction enzyme, PCR products were further digested with the corresponding enzyme to confirm the nucleotide change.

Results and Discussion

The pattern of inheritance of RP in family V-8 (Fig.1) is unclear. Individuals I.1 and I.2 are descendants from a consanguineous marriage and members I.2 and I.3 come from the same small village. These data suggest a recessive pattern of inheritance. However, the presence of affected individuals in two generations (three females), would favour the hypothesis of RP segregating as a dominant trait. If this is the case, incomplete penetrance would have to be assumed. Heterogeneity in phenotypic expression due to incomplete penetrance has already been documented in ADRP families (Berson and Simonoff 1979; Kim et al. 1994).

Clinical and electrophysiological data of the available affected and healthy members are shown in Table 1. The probands, individuals II.4 and II.5, present a typical form of RP. According to clinical records, individual I.1 had also been diagnosed as affected of RP. Individual I.2, the mother of the probands, showed decreased values of visual acuity and ERGs, which could be attributable to both cataracts and age, while her fundus examination appeared totally normal. The clinical parameters of the remaining members examined were all comprised within the normal ranges, although there was a high degree of variability for the ERG values. Individuals III.1, III.3 and III.4 were healthy at the time of the study but, considering the age of onset of RP in their mothers, they were still too young to be diagnosed with certainty. Finally, individuals II.1, II.2 and II.3 denied having any visual impairment.

The involvement in the disease of the PDEB, PDEG, peripherin/RDS, recoverin, RHO, NRL, SAG and ROM1 genes was assessed by combining cosegregation and mutation analyses. Four aberrant SSCP patterns were detected, corresponding to PCR fragments of exon 22 of PDEB, exon 3 of NRL and exons 1 and 2 of ROM1. Cloning and sequencing of the PDEB fragment revealed a known polymorphism, 2598G/A (Collins et al. 1992). Moreover, analysis of exon 3 of NRL identified a novel silent nucleotide substitution, 4723C/T.

The third aberrant SSCP pattern revealed a ROM1 allele with two nucleotide changes, each leading to an amino acid substitution, P60T and T108M. This double mutant allele is identical to that described by Bascom et al. (1995) in only the affected members of a small ADRP family. However, in V-8 the ROM1 allele is present in all the affected individuals as well as in some healthy members (Fig.1). To exclude minimal functional disturbances in some of these subjectively healthy carriers, individuals I.2, II.3 and II.6 were subjected to EOG and dark adaptation tests. Individual I.2 (aged 72) showed an EOG Arden ratio of 150 (normal values are above 180; see Table 1), and the rod threshold of the dark adaptation curve was slightly increased. However, these results could be attributable, again, to both cataracts and age. Individuals II.3 and II.6 showed EOG ratios of 200 and their dark adaptation curves were normal.

Finally, analysis of the fourth aberrant SSCP pattern, corresponding to exon 2 of ROM1, identified the 1071G/C polymorphism (Bascom et al. 1993b) and a segregation study showed that the two affected sisters had inherited different paternal alleles (Fig. 1). Taking these data together, the involvement of the ROM1 gene in a recessive form of the disease is ruled out in this family.

Kajiwara et al. (1994) reported a digenic form of RP in three families due to mutations in ROM1 and RDS genes. To test this model, we analyzed the peripherin/RDS internal polymorphisms 558C/T and 1294C/T in our family, which showed that the affected sibs did not share the paternal peripherin/RDS allele. Thus, the hypothesis of a digenic ROM1/RDS pattern of inheritance is ruled out.

The role of the ROM1 double mutant allele in RP is still unclear. Nevertheless, in family V8 there is a correlation between its presence and abnormal or low ERG values. Unaffected individuals bearing this allele showed the lowest ERG recordings (see Table 1). Additionally, the mutant allele has been only described in another RP family but not found in the 200 control chromosomes analyzed by Bascom et al. (1995), nor in a sample of 76 chromosomes studied here.

The mutant ROM1 allele could be directly involved in the disease as a dominant mutation with incomplete penetrance or highly variable expressivity. Some ADRP families showing highly heterogeneous phenotypic expression have been reported recently (Kim et al. 1995; Evans et al. 1995; Xu et al. 1995). Alternatively, a digenic pattern involving ROM1 and another unidentified gene could be considered. Indeed, according to Bascom et al. (1992) a ROM1-peripherin/RDS complex of four subunits would associate with other proteins in rods. Alterations in any of these unknown proteins together with mutations in ROM1 could help explain this case of RP. However, in the absence of conclusive data, the pathogenicity of this mutant ROM1 allele is questionable.

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References

Bascom RA, Manara S, Collins L, Molday RS, Kalnins VI, Mcinnes RR (1992) Cloning of the cDNA for a novel photoreceptor membrane protein (rom-1) identifies a disk rim protein family implicated in human retinopathies. Neuron 8: 1171--1184

Bascom RA, Schappert K, McInnes RR (1993a) Cloning of the human and murine *ROM1* genes: genomic organization and sequence conservation. Hum Mol Genet 2: 385--391

Bascom RA, Liu L, Humphries P, Fishman GA, Murray JC, McInnes RR (1993b) Polymorphisms and rare sequence variants at the *ROM1* locus. Hum Mol Genet 2: 1975--1977

Bascom RA, Liu L, Heckenlively JR, Stone EM, McInnes R (1995) Mutation analysis of the ROM1 gene in retinitis pigmentosa. Hum Mol Genet 4: 1895--1902 (erratum: 4:2424)

Bayés M, Valverde D, Balcells S, Grinberg D, Vilageliu L, Benítez J, Ayuso C, Beneyto M, Baiget M, Gonzàlez-Duarte R (1995) Evidence against involvement of recoverin in autosomal recessive retinitis pigmentosa in 42 Spanish families. Hum Genet 96: 89--94

Bayés M, Martínez-Mir A, Valverde D, del Río E, Vilageliu LL, Grinberg D, Balcells S, Ayuso C, Baiget M, Gonzàlez-Duarte R (1996) Autosomal recessive retinitis pigmentosa in Spain: evaluation of 4 genes and 2 loci involved in the disease. Clin Genet (in press)

Berson EL, Simonoff EA (1979) Dominant retinitis pigmentosa with reduced penetrance. Arch Ophthalmol 97: 1286--1291

Collins C, Hutchinson G, Kowbel D, Riess O, Weber B, Hayden MR (1992) The human beta-subunit of rod photoreceptor cGMP phosphodiesterase - complete retinal cDNA sequence and evidence for expression in brain. Genomics 13: 698--704

Dryja TP, Li T (1995) Molecular genetics of retinitis pigmentosa. Hum Mol Genet 4: 1739--1743

Evans K, Almaghtheh M, Fitzke FW, Moore AT, Jay M, Inglehearn CF, Arden GB, Bird AC (1995) Bimodal expressivity in dominant retinitis pigmentosa genetically linked to chromosome 19q. Br J Ophthalmol 79: 841--846

Hahn LB, Berson EL, Dryja TP (1994) Evaluation of the gene encoding the gamma subunit of rod phosphodiesterase in retinitis pigmentosa. Invest Ophthalmol Visual Sci 35: 1077--1082

Heckenlively JR, Yoser SL, Friedman LH, Oversier JJ (1988) Clinical findings and common symptoms in retinitis pigmentosa. Am J Ophtalmol 105: 504--511

Kajiwara K, Berson EL, Dryja T (1994) Digenic retinitis pigmentosa due to mutations at the unlinked peripherin/RDS and ROM1 loci. Science 264: 1604--1608

Keen TJ, Inglehearn CF, Lester DH, Bashir R, Jay M, Bird AC, Jay B, Bhattacharya SS (1991) Autosomal dominant retinitis-pigmentosa - four new mutations in rhodopsin, one of them in the retinal attachment site. Genomics 11: 199--205

Kim SK, Haines JL, Berson EL, Dryja TP (1994) Nonallelic heterogeneity in autosomal dominant retinitis pigmentosa with incomplete penetrance. Genomics 22: 659--660

Kim RY, Fitzke FW, Moore AT, Jay M, Inglehearn C, Arden GB, Bhattacharya SS, Bird AC (1995) Autosomal dominant retinitis pigmentosa mapping to chromosome 7p exhibits variable expression. Br J Ophthalmol 79: 23--27

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

Miller SA, Dyke DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucl Acids Res 16: 1215

Moritz OL, Molday RS (1996) Molecular cloning, membrane topology, and localization of bovine rom-1 in rod and cone photoreceptor cells. Invest Ophthalmol Visual Sci 37: 352--362

Piriev NI, Khramtsov NV, Lipkin VM (1994) Cloning and characterization of the gene encoding the cGMP-phosphodiesterase gamma-subunit of human rod photoreceptor cells. Gene 151: 297--301

Riess O, Noerremoelle A, Weber B, Musarella MA, Hayden MR (1992) The search for mutations in the gene for the beta-subunit of the cGMP phosphodiesterase (PDEB) in patients with autosomal recessive retinitis-pigmentosa. Am J Hum Genet 51: 755--762

Sakuma H, Inana G, Murakami A, Yajima T, Weleber RG, Murphey WH, Gass JDM, Hotta Y, Hayakawa M, Fujiki K, Gao YQ, Danciger M, Farber DB, Cideciyan AV, Jacobson SG (1995) A heterozygous putative null mutation in ROM-1 without a mutation in peripherin/RDS in a family with retinitis pigmentosa. Genomics 27: 384--386

Sheffield VC, Beck JS, Nichols B, Cousineau A, Lidral AC, Stone EM (1992) Detection of multiallele polymorphisms within gene sequences by GC-clamped denaturing gradient gel electrophoresis. Am J Hum Genet 50: 567--575

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

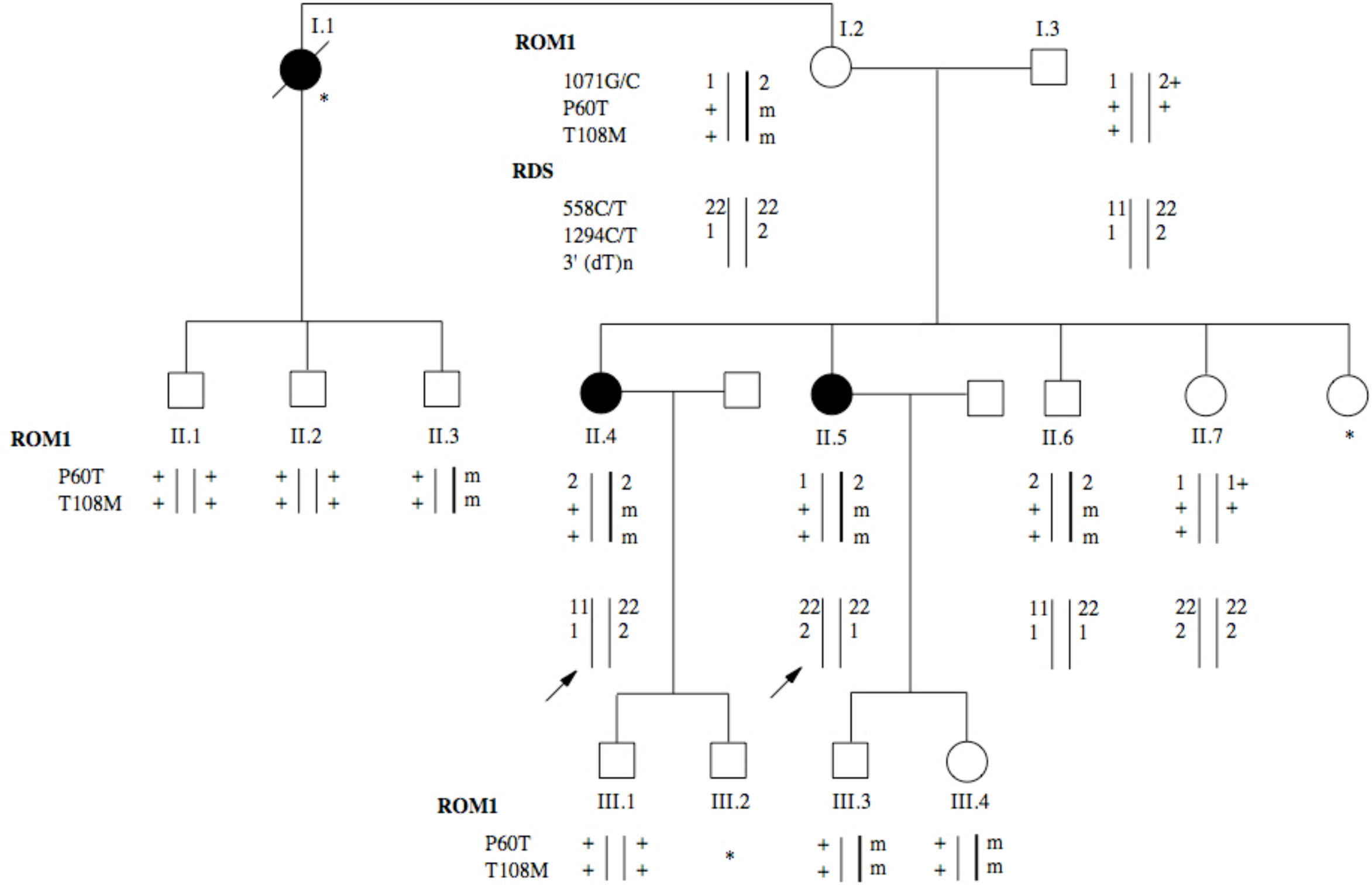
Xu SY, Nakazawa M, Tamai M, Gal A (1995) Autosomal dominant retinitis pigmentosa locus on chromosome 19q in a Japanese family. J Med Genet 32: 915--916

Fig.1. Pedigree of family V-8 showing ROM1 and peripherin/RDS haplotypes. Arrows indicate the peripherin/RDS alleles inherited from the father in the two affected sisters. Members whose DNA was not available are marked with an asterisk.

Table 1. Results of the ophthalmologic test in family V-8.

Patient	Age	Age at onset	Visual acuity[1]	Fundus[2]	Visual field[3]	ERGR[1,4]	ERGC[1,5]	ERGCR[1,6]	EOG[7]
I.2	72	-	OD-0.4 OS-0.6	NORMAL	NORMAL	OD-133 OS-106	OD-49 OS-47	OD-240 OS-204	<150
II.3	-	-	OD- OS-	-	-	OD- OS-	OD- OS-	OD- OS-	200
II.4	47	30	OD-0.7 OS-0.1	RP-D	TV	OD-30 OS-27	OD-0 OS-0	OD-43 OS-43	-
II.5	45	30	OD-FC OS-FC	RP-D	TV	OD-0 OS-0	OD-8 OS-7	OD-9 OS-8	-
II.6	39	-	OD-1 OS-1	NORMAL	NORMAL	OD-156 OS-160	OD-48 OS-53	OD-256 OS-270	200
II.7	34	-	OD-1 OS-1	NORMAL	NORMAL	OD-247 OS-238	OD-78 OS-77	OD-396 OS-388	-
III.1	25	-	OD-1 OS-1	NORMAL	NORMAL	OD-282 OS-277	OD-103 OS-95	OD-419 OS-398	-
III.3	18	-	OD-1 OS-1	NORMAL	NORMAL	OD-179 OS-208	OD-72 OS-71	OD-354 OS-376	-
III.4	16	-	OD-1 OS-1	NORMAL	NORMAL	OD-174 OS-183	OD-97 OS-90	OD-317 OS-318	-

1. OD: right eye; OS: left eye; FC: finger count; 2. RP-D: diffuse RP; 3. TV: tunnel vision; 4. Rod function, lower normal limit 10(micro-volt; 5. Cone function, lower normal limit 50 micro-volt; 6. Cone and rod function, lower normal limit 150 micro-volt; 7. EOC Arden ratio, lower normal limit 180.


I.1
I.2
I.3
*
ROM1
1071G/C 1 2
P60T + m
T108M + m
1 2+
+ +
+
RDS
558C/T 22 22
1294C/T 1 2
3' (dT)n
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1 2
ROM1
II.1
II.2
II.3
II.4
II.5
II.6
II.7
*
P60T + + + + + m
T108M + + + + + m
2 2
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2 2
+ m
+ m
1 1+
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11 22
1 2
22 22
2 1
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2 2
ROM1
III.1
III.2
III.3
III.4
P60T + + * + m + m
T108M + + + m + m