

Autosomal Recessive Retinitis Pigmentosa with Early Macular Affection Caused by Premature Truncation in *PROM1*

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PURPOSE. To identify the genetic basis of a large consanguineous Spanish pedigree affected with autosomal recessive retinitis pigmentosa (arRP) with premature macular atrophy and myopia.

METHODS. After a high-throughput cosegregation gene chip was used to exclude all known RP and Leber congenital amaurosis (LCA) candidates, genome-wide screening and linkage analysis were performed. Direct mutational screening identified the pathogenic mutation, and primers were designed to obtain the RT-PCR products for isoform characterization.

RESULTS. Mutational analysis detected a novel homozygous *PROM1* mutation, c.869delG in exon 8 cosegregating with the disease. This variant causes a frameshift that introduces a premature stop codon, producing truncation of approximately two-thirds of the protein. Analysis of *PROM1* expression in the lymphocytes of patients, carriers, and control subjects revealed an aberrant transcript that is degraded by the nonsense-mediated decay pathway, suggesting that the disease is caused by the absence of the *PROM1* protein. Three (s2, s11 and s12) of the seven alternatively spliced isoforms reported in humans, accounted for 98% of the transcripts in the retina. Given that these three contained exon 8, no *PROM1* isoform is expected in the affected retinas.

CONCLUSIONS. A remarkable clinical finding in the affected family is early macular atrophy with concentric spared areas. The authors propose that the hallmark of *PROM1* truncating mutations is early and severe progressive degeneration of both rods and cones and highlight this gene as a candidate of choice to prioritize in the molecular genetic study of patients with non-

canonical clinical peripheral and macular affection. (*Invest Ophthalmol Vis Sci.* 2010;51:2656–2663) DOI:10.1167/iivs.094857

Retinitis pigmentosa (RP [MIM268000]) is a genetically and clinically heterogeneous group of ocular diseases that cause rod and cone degeneration. It is characterized by night blindness, constriction of the visual field, and pigment spicule deposits in the mid periphery of the retina, which eventually lead to blindness. To date, it has been postulated that mutations in at least 60 genes may cause RP (see RetNet). RP is a major genetic cause of blindness in adults, with a worldwide prevalence of 1:3000 to 1:4000.^{1,2} Allelic heterogeneity stands out as a prominent feature of several RP genes, as exemplified by *ABCA4*,^{3–5} *CRB1*,^{2,6} *NRL*,⁷ *RDS*,⁸ *KLHL7*,⁹ and *CEP290*,¹⁰ where different mutations lead to distinct retinal disease phenotypes. In addition to RP, these genes are responsible for Stargardt disease, cone-rod dystrophy (CORD), macular degeneration, Leber congenital amaurosis (LCA), and pattern macular dystrophy, among other disorders. The wide range of clinical entities associated with these genetic variants support that the proteins encoded by many of these genes are essential for both cone and rod function, and yet each mutation produces a specific phenotypic effect.

Prominin 1 (*PROM1*, accession number: AF027208, Gene ID: 8842, also known as *PROML1*, *AC133*, and *CD133*; GenBank; <http://www.ncbi.nlm.nih.gov/Genbank/> NCBI) is located at 4p15.32 and at maximum length comprises 27 exons. The encoded protein, *PROM1*, is a five-transmembrane glycoprotein located at the plasma membrane protrusions, with two short N (extracellular)- and C (cytoplasmic)-terminal tails, and two large N-glycosylated extracellular loops (between TM2 and -3, and TM4 and -5). Seven *PROM1* protein isoforms produced by alternative splicing have been reported in human tissues,¹¹ although the alternatively spliced exons in the coding region only affect the short N- and the C-terminal domains. *PROM1* is expressed in both rod and cone photoreceptors. Moreover, *PROM1* expression has been detected in the cells of several other human tissues—among them CD34⁺ progenitor populations from adult blood and bone marrow cells—which has conferred on this protein the status of a valuable marker for human allogeneic transplantation.^{12,13} A paralogue of *PROM1*, *PROM2*, shares 60% of amino acid identity and displays the same characteristic of membrane topology.¹⁴ The pattern of *PROM2* expression largely overlaps that of *PROM1*, except that there is no expression in the retina.

PROM1 function in the retina is not known, although it is selectively associated with microvilli, making a relevant contribution to the generation of plasma membrane protrusions, their organization, and lipid composition, notably with respect

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to cholesterol.¹⁵ In rods, prominin appears to be concentrated in the plasma membrane evaginations at the nascent disc membranes at the base of the outer segments, which are essential structures in the biogenesis of photoreceptor discs and to which the contribution of PROM1 seems crucial.¹⁶ The gene and probably also its function are highly evolutionarily conserved. In the *Drosophila melanogaster* eye, *prom* (known as *eyes closed* or *eyc*) interacts with spacemaker (also known as *spam*, *eyes shut*, or *eyes*) and chaoptin to regulate the assembly of microvilli, ensure the structural integrity of the rhabdomeres, and guarantee the proper construction of an open rhabdom system.¹⁷ The human homologue of spacemaker, *EYS*, has been characterized as responsible for autosomal recessive retinitis pigmentosa.^{18,19} In mice, the absence of Prom 1 provokes progressive degeneration and functional deterioration of photoreceptors, due to impaired morphogenesis of the discs at the outer segment.^{16,20} In humans, mutations in *PROM1* have been associated with severe forms of retinal dystrophy. Missense mutations are associated with autosomal dominant Stargardt-like or bull's-eye macular dystrophy,¹⁶ whereas nonsense and frameshift mutations have been related to retinitis pigmentosa,^{21,22} and severe cone-rod dystrophy with macular degeneration and night blindness.²³

Herein, we describe a novel recessive mutation in the *PROM1* gene that is responsible for severe RP with macular degeneration and myopia in a consanguineous pedigree from Spain. The retinal degeneration in these patients seems to be associated with the loss of *PROM1* function as the nonsense-mediated decay machinery leads to an almost complete depletion of the mutated transcripts.

MATERIAL AND METHODS

DNA from Patients and Families

A consanguineous Spanish family affected with autosomal recessive RP (Fig. 1) was used in the present study. Informed consent from all the family members was obtained according to the tenets of the Declaration of Helsinki. The Bioethics Committee of the University of Barcelona (Barcelona, Spain) approved all the work concerning patient recruitment and sample collection. DNA was obtained from blood samples (Wizard Genomic DNA purification kit; Promega; Madison, WI). DNA from 203 matched Spanish control individuals was obtained from whole blood by the same method.

Clinical Examination

RP was diagnosed in all affected members after ophthalmic examination at the Instituto de Microcirugía Ocular (IMO, Barcelona, Spain) and the Hospital Universitario Central de Asturias (Oviedo, Spain). The clinical diagnosis included best corrected visual acuity and slit lamp biomicroscopy, followed by pupillary dilation and indirect ophthalmoscopy, fundus photography, fluorescein angiography, and full-field ERGs from both eyes (Fig. 2). The size and the extent of the visual-field defects within the central 30° were assessed with static perimetry (Fig. 3; Humphrey Field Analyzer; Carl Zeiss Meditec, Oberkochen, Germany). Electroretinograms (ERGs, EOGs) were recorded in accordance with the protocol of the International Society for Clinical Electrophysiology of Vision (ISCEV) at the IMO. A summary of the clinical features is provided in Table 1.

Genotyping and Cosegregation SNP Analysis with the RP-LCA Chip

DNA samples from eight related individuals, three affected and five unaffected, were genotyped with a high-throughput RP-LCA chip, which analyzes 240 SNPs of 40 genes responsible for autosomal dominant and recessive RP and LCA, as previously described.^{24,25} The SNPs were genotyped (SNPlex platform; Applied Biosystems, Inc. [ABI],

Foster City, CA), according to the instructions, protocol, and software provided by the manufacturer. The platform generated raw data genotypes that were then assigned to each individual. Haplotype and cosegregation analyses were performed by hand.

Whole-Genome Scan

The Nsp gene microarray (GeneChip Mapping 500K; Affymetrix; Santa Clara, CA) was used to genotype 262,000 SNPs for each individual according to the manufacturer's protocol. Genotype calls were determined by the Bayesian robust linear model with Mahalanobis distance algorithm (BRLMM).

Linkage Analysis

The BRLMM files were formatted with ALOHOMORA,²⁶ considering the allelic frequencies of the Caucasian population and using the Marshfield map as a reference. Pedstats²⁷ was used to discard Mendelian errors and all markers with a degree of heterozygosity in the family above 90% or below 10%. GRR software²⁸ was used to match the family relationships established in the pedigree and linkage was analyzed with Merlin.²⁹ Each chromosome was considered separately, and inheritance was analyzed under parametric conditions for a rare recessive allele (0.0001) assuming 100% penetrance.

PROM1 Mutation Screening

Twenty-six pairs of primers (Table 2) allowed the PCR amplification of the *PROM1* exons plus adjacent intronic sequences in the studied family members. All the fragments were sequenced (BigDye v. 3.1 kit; Prism 3730 DNA sequencer; ABI).

RT-PCR Analysis of *Prom1* and Characterization of Retinal Isoforms

A comprehensive data-mining search in the expression databases NCBI, UCSC, and Ensembl, was performed to identify the transcript and protein isoforms (see Appendix for database Web addresses).

Blood total RNA was obtained (RiboPure-Blood; Ambion, Austin, TX) from individuals IV1, IV3, IV4, and an unrelated control subject. To avoid RNA degradation, samples were mixed with RNA stabilizer (RNA-Later; Ambion) in a 1:3.5 ratio after blood collection. Total RNA (1.5 μg) was retrotranscribed (Transcriptor High Fidelity cDNA Synthesis Kit; Roche Applied Science, Indianapolis, IN) with random hexamers and oligo(dT)₁₈, according to the manufacturer's instructions.

The *Prom1* and *GAPDH* cDNAs were PCR amplified with specific primers (Table 2) in a final volume of 25 μL (GoTaq Flexi; Promega). Primers to detect all *PROM1* transcripts (Table 2, PROM1-exon5-F and PROM1-exon6-R) were used for amplification of blood cDNA, in a three-step PCR: denaturation for 3 minutes at 96°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 56°C, and 20 seconds at 72°C. *PROM1* retina isoforms differ on the presence or absence of exons 3, 25, 26b, and 27, and specific primers for the amplification of each isoform were therefore designed (Table 2). For amplification of retina cDNA (Biocat, Heidelberg, Germany), the following PCR conditions were used: denaturation at 96°C for 3 minutes, followed by 40 cycles of 30 seconds at 94°C, 30 seconds at 56°C, and 150 seconds at 72°C. PCR amplification of *GAPDH* (*GAPDH-F* and *GAPDH-R*) was performed as follows: denaturation for 2 minutes at 96°C, followed by 30 cycles of 20 seconds at 94°C and 2 minutes at 60°C.

The RT-PCR products were resolved by electrophoresis and a semiquantitative evaluation was obtained (Multi Gauge ver. 3.0 software; Fujifilm, Tokyo, Japan). Values were normalized against *GAPDH* levels and represented as a ratio of *PROM1/GAPDH*. The control wild-type ratio *PROM1/GAPDH* was arbitrarily set at 100%.

RESULTS

A Spanish pedigree of a consanguineous family with three severely visually impaired members was referred for clinical

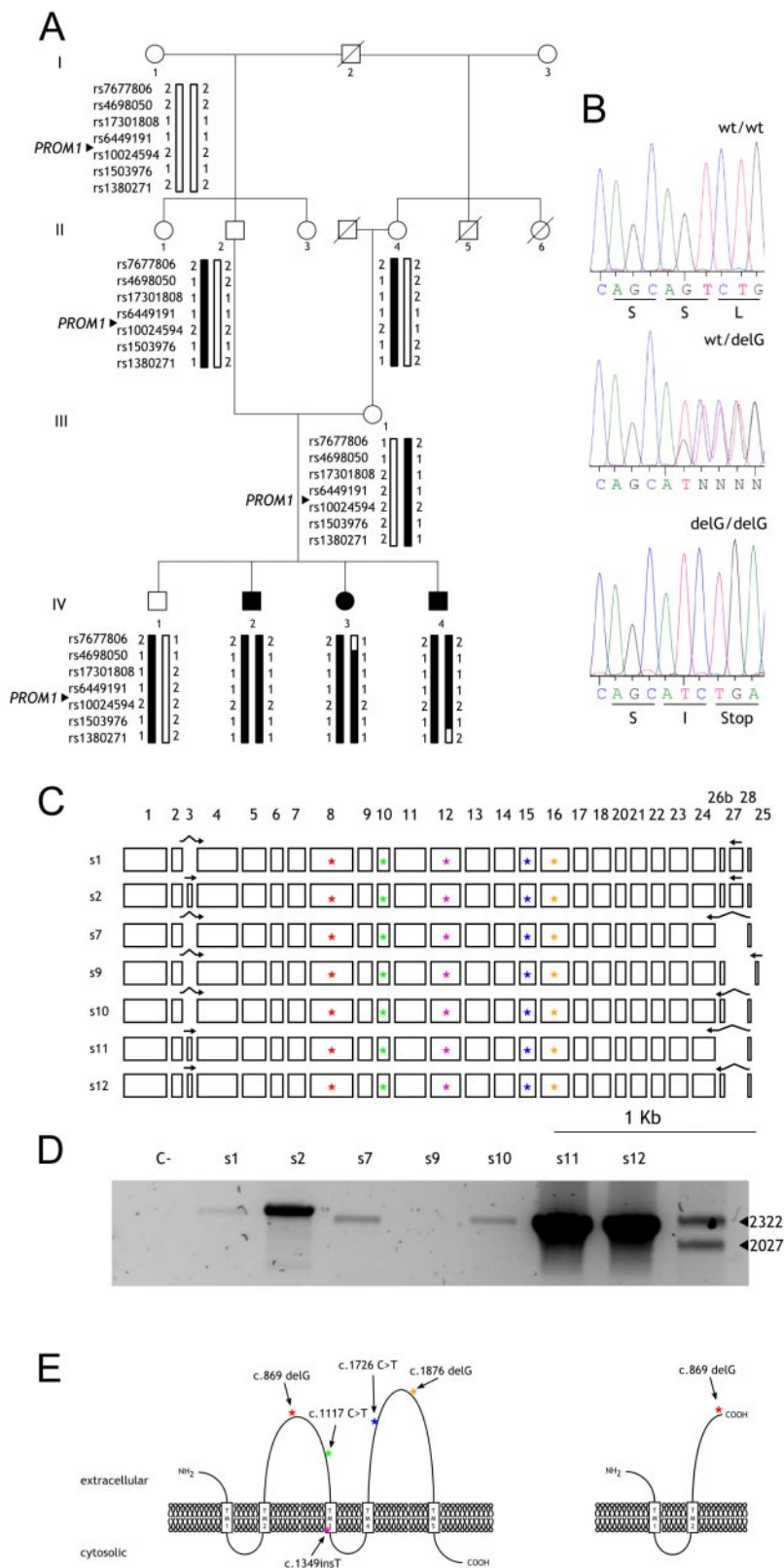


FIGURE 1. (A) Pedigree and SNP haplotypes on chromosome 4p16.1-p15.31 surrounding the *PROM1* locus. *Black bars* indicate the disease haplotype while *open bars* represent nondisease haplotypes. (B) Chromatograms identifying the mutation c.869delG, showing the wild-type exon 8 sequence (*top*), the heterozygous carrier (*middle*), and the homozygous patient (*bottom*). (C) *PROM1* exon structure showing the reported seven human isoforms (Fargeas et al.¹¹). They differ on the inclusion/exclusion of exons 3, 25, 26b, and 27, corresponding either to the N (3)- or C (25, 26b, and 27)-terminal tails. *Arrows*: specific primers for every isoform are indicated over the exons. The reported mutations are shown as colored stars. (D) RT-PCR analysis of *PROM1* isoforms in wild-type human retina; s11 and s12 are the most prominent isoforms; isoform s2 is expressed more faintly, whereas isoforms s1, s7, and s10 are barely detectable. (E) *PROM1* topology. *Left*: wild-type *PROM1* is predicted to consist of an extracellular N-terminal domain, five transmembrane domains (TM1-TM5) that define two small intracellular and two large extracellular loops and a C-terminal cytoplasmic tail. The location of the novel c.869delG mutation and the previously described c.1117 C>T, c.1349insT, c.1726 C>T, and c.1876delG mutations are also shown. *Right*: the assumed *PROM1* topology representation of the truncated protein encoded by the mutant allele.

assessment (Fig. 1). In particular, they reported night blindness in early childhood and bilateral progressive decline in visual acuity. An unaffected carrier sibling was also clinically assessed and had normal findings in an ophthalmic examination (Table 1; Figs. 2A, 2B). After the ophthalmic survey, the three patients were diagnosed with a retinal dystrophy form in which not just

rods, but also cones, were severely affected. Patient IV3 had noncongenital nystagmus. Slit-lamp biomicroscopic assessment of the anterior segment was normal in all affected siblings. However, in the three cases, fundusoscopic examination revealed waxy-pale discs, discrete attenuation of retinal arterioles, and in patients IV2 and IV3, pigmentary bone spicules

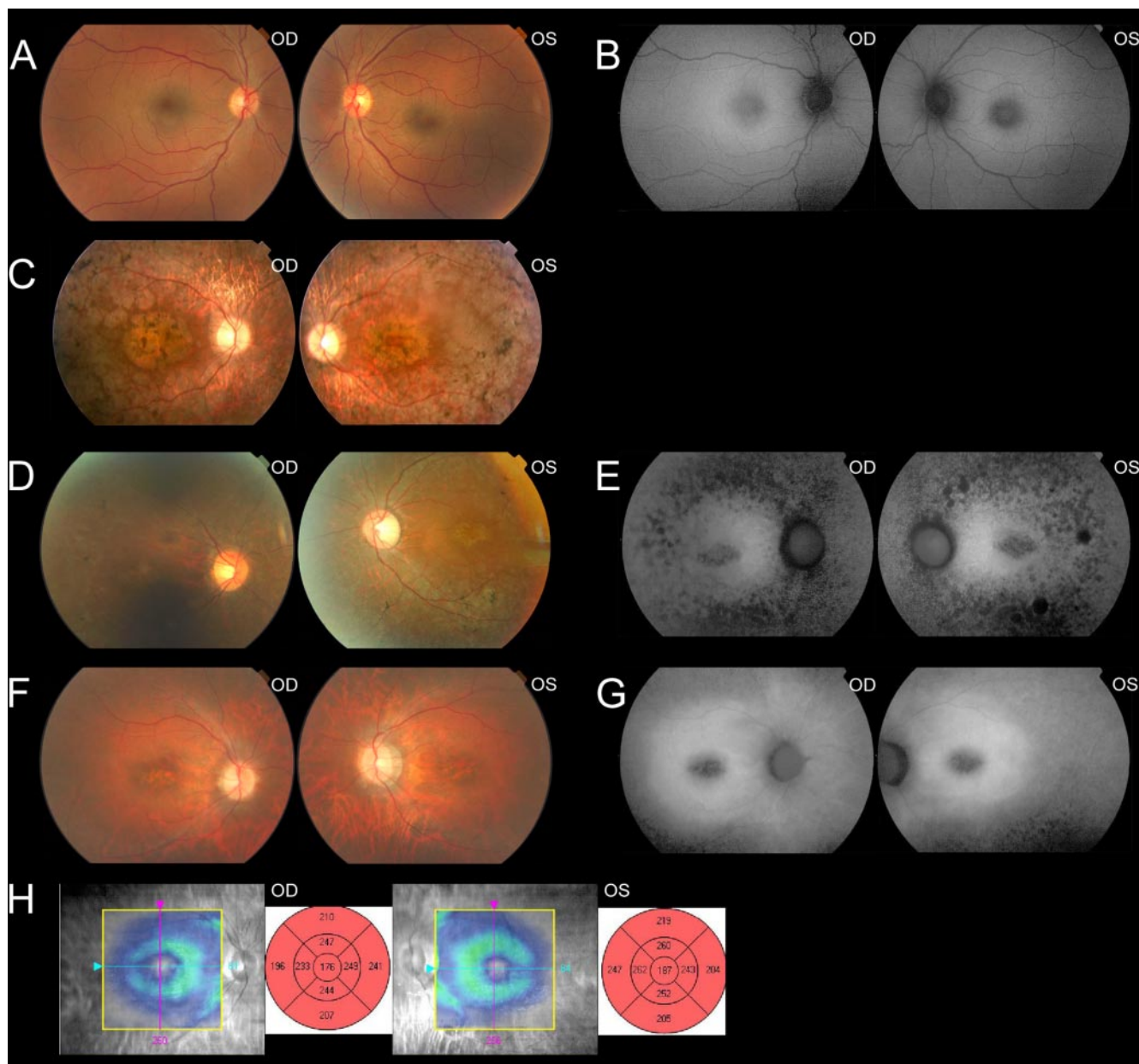


FIGURE 2. Fundus eye photographs, autofluorescence images, and optical coherence tomography (OCT) from affected and unaffected family members. Images correspond to nonaffected heterozygous carrier IV1 (A, B), patient IV2 (C), patient IV3 (D, E), and patient IV4 (F-H). The OCT macular scans from patient IV4 show bilaterally neurosensory atrophy in the macular area.

were apparent in the midperipheral retina, as well. Alteration in the retinal pigment epithelium in the macular area was remarkable in the three affected cases (Table 1; Figs. 2C, 2D, 2F). Moreover, autofluorescence images disclosed marked decreased autofluorescence in the periphery and macular areas due to severe changes in the pigment epithelium, with sparing of the RPE in discrete areas of the posterior pole in patients IV3 and IV4. (Figs. 2E, 2G). The three affected siblings presented with myopia; two of them, IV3 and IV4, showed myopic refractive error exceeding -5 D (Table 1). Patient IV3 had severe nystagmus; therefore, complete optical coherence tomography (OCT) scans of the maculae could not be obtained. Macular OCT scan of patient IV4 showed discrete bilaterally reduced retinal thickness (Fig. 2H). Electroretinograms were undetectable bilaterally for two of the affected siblings (IV3 and IV4). In addition, their visual field tests showed extensive constriction in both eyes (Fig. 3).

Overall, with both the macular and retinal periphery pathologically altered and both types of ERG abolished, the clinical association of symptoms with concentric periphery alterations and ophthalmoscopy findings and the results of visual function tests supported that the patients had diffuse retinal dystrophy, with traits assignable to severe RP with a distinct added feature of premature macular affection (Table 1).

Genome-wide Screening

A comprehensive cosegregation SNP chip containing 40 RP-LCA known genes²⁵ was used to genotype all members of this consanguineous family (Fig. 1A). This chip allows the genotyping of 240 SNPs (6 per gene) located close to each presumptive candidate. On the stringent criteria of both cosegregation and homozygosity, all these candidates were discarded as the cause

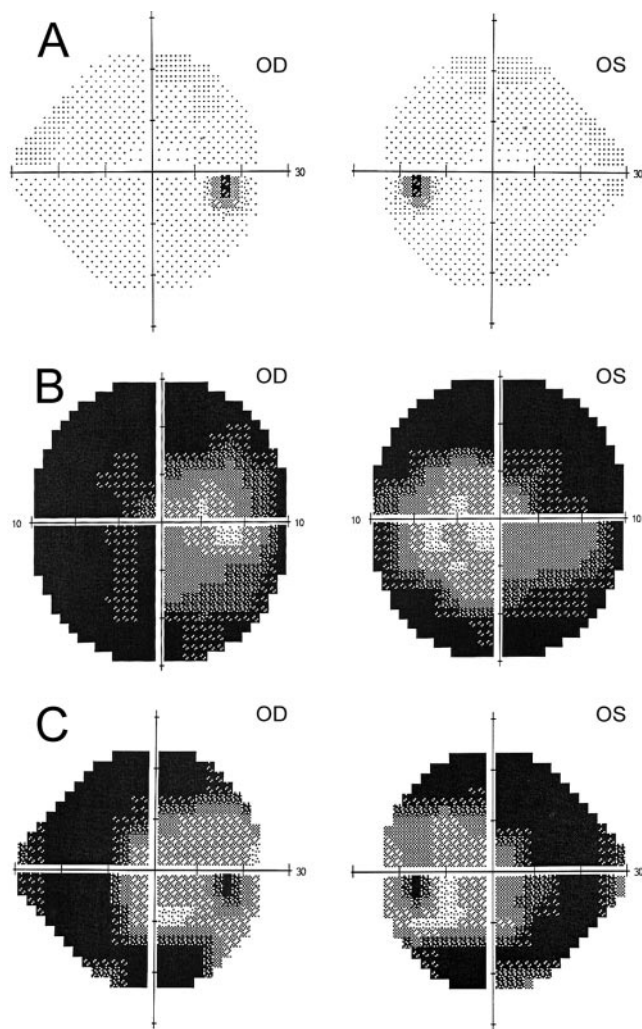


FIGURE 3. Humphrey's visual field test from affected and unaffected family members, carrier IV1 (A), patient IV3 (B), and patient IV4 (C). Note the correspondence of the preserved central area around the macula in this test with the autofluorescence images of the posterior pole in Figure 2.

of the disease. Then, a genome-wide search was considered. Eight related individuals, three affected and five unaffected (Fig. 1A), were analyzed by whole-genome genotyping. The linkage analysis revealed a 11.3-Mb homozygous region on chromosome 4, between SNPs rs7677806 (4p16.1) and rs1380271 (4p15.31), both excluded with a maximum LOD score of 2.532. One of the genes reported within the homozygous interval was *PROM1* (4p15.32), previously associated with severe retinal degenerations.^{16,21-23} After all the exons and flanking intronic regions of *PROM1* were sequenced in one affected individual, a homozygous deletion in exon 8 (c.869delG, Fig. 1B) was observed. This mutation cosegregated with the disease in the family, as it was present in homozygosity in all the affected siblings and in heterozygosity in four of the five unaffected members. Moreover, this variant was not detected in 406 chromosomes from unrelated Spanish control subjects. This nucleotide deletion resulted in a frameshift from codon 289 onward and caused a premature STOP codon after the addition of 1 amino acid (Fig. 1B). The predicted protein, if translated, would probably not be functional, as more than half of the protein is missing, including the two extracellular loops (Fig. 1E).

TABLE 1. Clinical Characteristics of the Three Affected (IV.2; IV.3, IV.4) and One Nonaffected (IV.1) Siblings of the Analyzed Consanguineous Pedigree

Individual	Age* (y)	RP Symptoms	Nystagmus	Progression	Visual Acuity (OD; OS)	Refraction (OD; OS)	Visual Field	Fundus	Myopia Axial Length (mm) (OD; OS)	ERG
IV1	37	No	NA	—	20/20; 20/20 LP: 20/60	Normal -0.50; -0.75 × 60° -1.00; -2.25 × 115°	Normal Extensive constriction on both eyes	Normal Bone-spicule pigmentation in the mid-peripheral retina; vessel attenuation; diffuse RPE atrophy;	—; — 23.62; 24.22	Normal Notperformed
IV2	35	Yes	No	Severe				severe macular alteration		
IV3	27	Yes	Yes	Severe	20/800; 20/400	-6.25; -0.50 × 130° -5.50; -0.75 × 65°	Extensive constriction on both eyes	Bone-spicule pigmentation in the mid-peripheral retina; subtle vessel attenuation; diffuse RPE alteration; severe macular atrophy	23.84; 23.39	Nonrecordable
IV4	18	Yes	No	Severe	20/100; 20/60	-10.00; -1.00 × 25° -8.75; -1.50 × 175°	Extensive constriction on both eyes	RPE depigmentation in the mid-peripheral retina without bone spicules; RPE alteration in the macula area	25.96; 25.75	Nonrecordable

ERG, electroretinogram; NA, not applicable; LP, light perception; RPE, retinal pigmented epithelium.

* Current age

TABLE 2. Sequence of Gene-Specific Primers Used for *PROM1* gDNA and RNA Amplification

Primer	Sequence	Primer	Sequence
PROM1-5'NCF	CGTCCAGGGCTCGGGTTTC	PROM1-15R	AAGAAAGACAACCTGGTCCGGCA
PROM1-5'NCR	AAAAGTTTGGGTTGGACGGGC	PROM1-16F	TGGAGGCTTAGAAGCCATGGGA
PROM1-1F	TCCCGAACCCATAAAGGGTCTG	PROM1-16R	TGTGAATGTACTCAATGCCACC
PROM1-1R	GCTTCTGTGCAAGCAATCGTAA	PROM1-17F	TGCAAAATGTTGCCACCTGTTT
PROM1-2F	AAGCTGTATGCGGTTTGCTGGT	PROM1-17R	GCAATGGCTGTGGACGGAAA
PROM1-2R	GGTTCAAATGGGATTGTAAGGTGG	PROM1-18F	GAAGGAGGGTGTCTTGGCAC
PROM1-3F	TGCTGCCGTTGGTTCTGGAG	PROM1-18R	GGCCTGCTCACAGCAATGGA
PROM1-3R	TCCAGTGCTTTGTTGATTGTGTTGA	PROM1-19F	AGTACACATTGTTAATTGTGTTGG
PROM1-4F	CTCAATTCTCTGCTTCTCTCTTTCAA	PROM1-19R	GGCACTGAGGTTTGGGATTGTG
PROM1-4R	GGAGCTGCTGTGCTGGGAGG	PROM1-20F	GCTCATCTCCTTCCTGCC
PROM1-5F	CAGTCCTTCTCGGGGCTCCT	PROM1-20R	TGGTCTGACACATCAATGTCCTT
PROM1-5R	AAACACCAATTCTGAAATTCGGC	PROM1-21F	TTCTCTGCTGTGGAGCCAGTT
PROM1-6F	TCTGGGCAGGAAGCAGCCTA	PROM1-21R	TGAGAAATCTGCACACCCGTGA
PROM1-6R	GGTCTGCTGCTGTGAAACA	PROM1-22F	GTTTGGAGTGGCCTAGATTCCG
PROM1-7F	TGCATGCGGAGACCCTGAAGA	PROM1-22R	TTCACCTGAACAGAAAGTCCAAA
PROM1-7R	TGCGTATGGCTGTGTTCCGA	PROM1-23F	CTTTCAACATGGGTCTTTCTCG
PROM1-8F	CCCTTGCACTGTGTCCTCTCTCA	PROM1-23R	TGGACTGAACATTTAAACTCATGGCA
PROM1-8R	CCTTTGCTCCTGCTGTGGTCA	PROM1-24F	GGTCCCTGCGGAACCTTCCAT
PROM1-9F	TGCTTGTCAAGGAGGTCTGAGC	PROM1-24R	ATGTGGAACCTGCAGGTACAG
PROM1-9R	TGGGAACCTGGAAGGATGAACACA	PROM1-3' NCF	TGCAACAAACATATTGCTGTGCCT
PROM1-10F	ACACAATCCCAGCAGCACCC	PROM1-3' NCR	TCCAAGTGAACATGGCCAATC
PROM1-10R	TAACGTCCGAATGACACAATTG	PROM1-exon5-F	GGCATCTTCTATGGTTTTGTGG
PROM1-11F	TGCATGGCTTGGCTATATTCTATGC	PROM1-exon6-R	TTACAGTCTGTGAACGCTTGT
PROM1-11R	TGTGCTGCCTGGTCTAAGCGA	GAPDH-F	TGAAGTCCGGTGTGAACGGATTGG
PROM1-12F	TCCGCTGGTTGAATTGGAAGG	GAPDH-R	AGGCCATGAGGTCCACCAC
PROM1-12R	TCTCTCCTCCTCGCGACCTG	PROM1-exon3-F	CCAGAACTGTAATCTTAGGTCT
PROM1-13F	ACCCTTGCTTCTCCTGGAGC	PROM1-NOexon3-F	GATTATGACAAGATTGCTACTATG
PROM1-13R	GCAATCCACATTGAGCGGCA	PROM1-exon27-R	TGTCATAACAGGATTGTGAATACC
PROM1-14F	AACAGAGCAAGACTCTGTCTCA	PROM1-exon25-R	CACTGAACAGAAAGTACCACAAC
PROM1-14R	TTCCAAGGTCTCAAAGGCTTTC	PROM1-NOexon27-R	GTTGTGATGGGTTTTTTCATGGG
PROM1-15F	CAGAAGTGGTGGGTGCTGGG	PROM1-Noexons26b_27-R	GTTGTGATGGGTATCGTACAC

Reduced *PROM1* RNA Expression Caused by the c.869delG Mutation

The mutation c.869delG introduces a stop codon on exon 8, 1661 bp upstream of the wild-type termination codon. Transcripts containing premature termination codons are reportedly degraded by nonsense-mediated decay (NMD).^{30,31} To assess whether the c.869delG mutation results in reduced levels of *PROM1* transcripts, we performed a comparative semiquantitative RT-PCR analysis of *PROM1* expression in white blood cells from two affected siblings (IV3 and IV4): one carrier (IV1) and a nonrelated control subject (Fig. 4A). The *PROM1* mRNA fragment used to test the NMD amplified 179 bp of exons 5 and 6, was proximal to the mutation, and was shared by all the human *PROM1* isoforms. The transcript levels

observed in the affected siblings were much lower than those in the wild-type control, whereas the carrier sibling yielded a midrange *PROM1* transcript level. The relative quantification of the normalized *PROM1* transcript showed that the *PROM1* level of the carrier was 33% that of the control, whereas the values decreased to 9% to 18% in patients (Fig. 4B), thereby supporting that the NMD machinery specifically degrades the transcript produced by the mutated allele.

PROM1 Isoforms in the Retina

PROM1 is a widely expressed gene with an as yet obscure function. After a comprehensive in silico search, at least nine different transcripts of *PROM1* in humans due to alternative splicing and the use of five alternative promoters were identi-

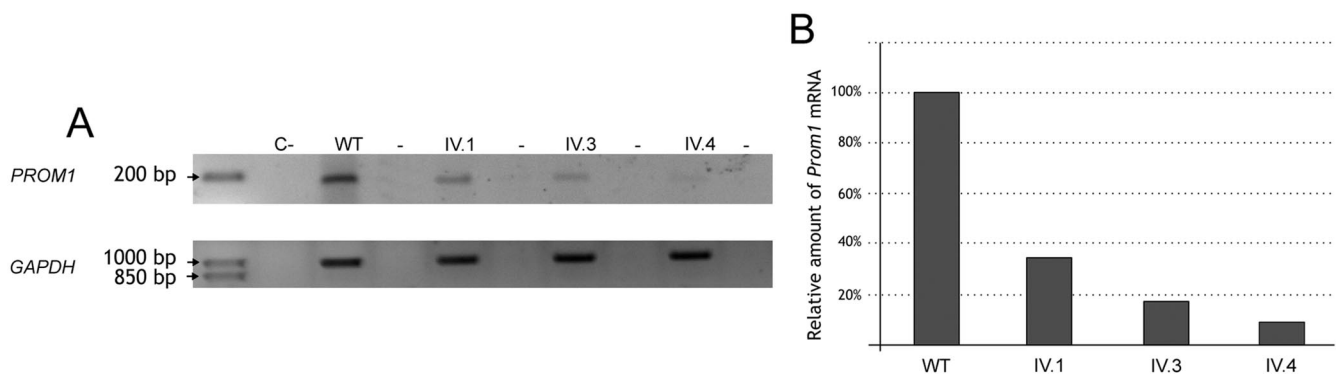


FIGURE 4. RT-PCR analysis of *PROM1* mRNAs from blood of patients IV3 and IV4, the heterozygous carrier and a control individual. (A) Patients IV3 and IV4 showed lower, although detectable levels of *PROM1* transcripts compared with carrier IV1 or the control (WT). *GAPDH* was used as control for normalization. (B) Semiquantitative analysis of *PROM1* levels, with *GAPDH* expression used for normalization and the *PROM1* levels of the wild-type control set at 100%.

fied. However, only seven protein isoforms, ranging from 826 to 866 amino acids, seem to be produced.¹¹ The discrepancy between the number of transcripts and protein isoforms arises from variations at the 5' and 3' untranslated region (UTR). Given that *PROM1* splicing events have not been studied in retinal tissues, we designed specific primers to amplify each reported protein isoform in the human retina (Fig. 2C). The most prominent *PROM1* isoforms in the retina are the s11 and s12 (around 47% and 43% respectively). In contrast, the s2 isoform, which spans all the coding exons, was represented at a much lower level (8% of the isoforms), whereas the s1, s7, and s10 isoforms were barely detectable. No traces of s9 isoform expression were detected under our conditions.

DISCUSSION

In this report, a consanguineous Spanish family with three affected siblings is described. The mode of inheritance and the main clinical features correspond to autosomal recessive RP but with a striking premature affection of cones. The fundus examination revealed attenuation of blood vessels, waxy pale discs, and bone spicules in the mid periphery. The autofluorescence images showed concentric affection of the retina, with the typical lesions at the periphery and the macula but with a considerable preservation of the RPE around the macula. Therefore, although both rods and cones were affected, the overall features—particularly, the aforementioned preservation area around the macula—led to the designation of the phenotype as RP rather than CORD. In addition, the three patients had myopia, two of them with enlarged axial lengths.

Genome-wide linkage analysis of the pedigree revealed a homozygous-by-descent chromosomal region on 4p15, where the *PROM1* gene, already implicated in retinal degeneration diseases, lies. Sequence analysis identified a novel single nucleotide deletion, c.869delG on exon 8, which fully segregates with the disease. This deletion generates a frameshift, which is predicted to result in a prematurely truncated product, missing more than two thirds of the protein and, in principle, assignable to a recessive trait. Although only a limited number of mutations have been described in *PROM1* (five, including this work) a genotype-phenotype pattern is beginning to emerge. Missense mutations have been associated with a dominant pattern of inheritance and a clinically mild degeneration of the macula, classified as Stargardt's-like and bull's-eye macular degeneration.¹⁶ In contrast, frameshift and null mutations have been associated with recessive retinal dystrophies—mainly RP^{21,22} and one recent report of CORD.²³ In these reports, the authors emphasize that, with gradually evolving degeneration, both rods and cones become affected. These previous results, together with our report, strongly suggest that the pathogenicity of *PROM1* mutations includes both types of photoreceptors, but the tempo and order of their affection is likely to be dependent on the type and location of the mutation. The severity and progression of the disease may also depend on other as yet unknown modifier genes.

Of note, this is the second report of *PROM1* mutations associated with high myopia. The fact that this gene is not highly expressed in the sclera²³ and that this feature is not constantly observed in patients but is present in two consanguineous families, points to an independent mutation in a closely linked locus, and/or some common modifier variants shared by the affected siblings.

The identified mutation in the present work, c.869delG, is the most upstream mutation described to date. The resulting frameshift would generate a very short protein, with only two transmembrane domains and devoid of the two large extracellular loops, which have been described to be glycosylated and

are crucial for the interaction with other protein partners.¹⁵ The quantification of *PROM1* transcript in the blood of our family showed that the carrier sibling presented around 50% of *PROM1* mRNA levels (but no affection of the retina), and the patients produced around 10% of the normal transcript levels. Therefore, at least for the c.869delG mutation, protein synthesis is compromised by the specific degradation of the mutant mRNA by the NMD pathway and thus, very low amounts of the aberrant protein, if any, reach the cell surface. It has been argued that the pathogenicity of the truncated *PROM1* mutant forms is due to a mislocalized protein or an aberrant role during protein trafficking in the endoplasmic reticulum (ER) and Golgi.¹⁶ However, our results support that the cause of the disease is the absence of the wild-type *PROM1* protein, rather than the gain of function or ER stress caused by the truncated mutant forms. Given that most mutations in *PROM1* reported to date generate prematurely truncated proteins, it is conceivable that also in these cases, the NMD machinery degrades the mutated allele transcript, thus providing a rationale for the severe retinal disorder associated with null and frameshift *PROM1* mutations. However, we cannot rule out that on mutations that produce a longer protein fragment, the intracellular toxicity of the spared protein could add to the retinal pathogenesis.

Our analysis of *PROM1* expression in the retina revealed that the three main isoforms (overall, accounting for >97% of *PROM1* transcripts) all contain exon 3, and the main differences lie in the C-terminal encoding exons, with exon 27 being the least represented. The difference between the two more highly expressed isoforms, is the inclusion/exclusion of exon 26b (included in the s12 isoform). That both isoforms are the most prominent in retina and expressed at similar levels suggests a distinct and relevant function for these two isoforms based on the peptide encoded by this distinctive exon. Notably, the identified mutation (c.869delG) will affect all isoforms in the retina, as it is embedded in an exon not affected by alternative splicing. The eventual phenotype of the truncated *PROM1* mutant forms would affect the correct folding and sealing of the photoreceptor membrane discs, resulting in an abnormal morphogenesis.

Prominin 1 has been the object of study from very different fields, which explains the multiplicity of names it has received. Originally, it was identified as an antigenic marker (AC133) in human hematopoietic stem cells and some tumoral cells, and was considered to be an antigen associated with undifferentiated replicating cells. The murine Prom (later prom1) was cloned instead as a protein selectively concentrated at the plasma protrusions of neuroepithelial progenitor cells and kidney. The identification of visual disorders associated with *PROM1* mutations has shifted its original role from a mere proliferation antigen to a prominent function in the microdomain structure of the plasma membrane, particularly relevant in photoreceptor disc morphogenesis and phototransduction. Although *PROM1* is widely expressed, only the retina is affected in patients and *prom1*-knockout mice. Given that *PROM2* shares 60% amino acid identities with *PROM1* and the two are concurrently expressed except in the retina, the former may account for the phenotype preservation in the remaining tissues.¹⁴ The phenotypic rescue due to partially overlapping of paralogue genes, as shown for *REP2* and *REP1* in choroideremia,³² is not an uncommon genetic event, but unfortunately no conclusive evidence has been gathered for *PROM2*.

The extremely high heterogeneity of retinal disorders has hampered molecular diagnosis and genotype-phenotype correlations. In this context, identifying distinct features associated with the clinical status of the patients is invaluable. In light of our results and those of others, we propose that early and

severe progressive degeneration of both rods and cones (with peripheral and macular affection) are the hallmark of *PROM1* truncating mutations. In patients in whom these symptoms concur, particularly if high myopia is present, *PROM1* would be the candidate of choice to prioritize in molecular genetic study.

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APPENDIX

Web Resources

The URLs for data presented in this work are as follows:

Ensembl: http://www.ensembl.org/Homo_sapiens/Info/Index.³⁵

Entrez: <http://www.ncbi.nlm.nih.gov/entrez/> National Center for Biotechnology Information (NCBI), Bethesda, MD.

GenBank; <http://www.ncbi.nlm.nih.gov/Genbank/> NCBI.

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> NCBI.

RetNet, <http://www.sph.uth.tmc.edu/RetNet/>; University of Texas Houston Health Science Center, Houston, TX.

UCSC Genome Browser, <http://genome.ucsc.edu/cgi-bin/hgGateway>, University of California Santa Cruz.

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