Accepted Manuscript

Expanded phenotypic spectrum of retinopathies associated with autosomal recessive and dominant mutations in *PROM1*

Marta Del Pozo-Valero, Inmaculada Martin-Merida, Belen Jimenez-Rolando, Ana Arteche, Almudena Avila-Fernandez, Fiona Blanco-Kelly, Rosa Riveiro-Alvarez, Caroline Van Cauwenbergh, Elfride De Baere, Carlo Rivolta, Blanca Garcia-Sandoval, Marta Corton, Carmen Ayuso

PII: S0002-9394(19)30244-2

DOI: https://doi.org/10.1016/j.ajo.2019.05.014

Reference: AJOPHT 10959

To appear in: *American Journal of Ophthalmology*

Received Date: 19 October 2018

Revised Date: 8 May 2019

Accepted Date: 8 May 2019

Please cite this article as: Del Pozo-Valero M, Martin-Merida I, Jimenez-Rolando B, Arteche A, Avila-Fernandez A, Blanco-Kelly F, Riveiro-Alvarez R, Van Cauwenbergh C, De Baere E, Rivolta C, Garcia-Sandoval B, Corton M, Ayuso C, Expanded phenotypic spectrum of retinopathies associated with autosomal recessive and dominant mutations in *PROM1*, *American Journal of Ophthalmology* (2019), doi: https://doi.org/10.1016/j.ajo.2019.05.014.

ABSTRACT

Purpose: To describe the genetic and phenotypic characteristics of a cohort of patients with *PROM1* variants.

Design: Case-case study.

Method: We screened a cohort of 2216 families with inherited retinal dystrophies using classical molecular techniques and next-generation sequencing approaches. The clinical histories of 25 patients were reviewed to determine age of onset of symptoms, and the results of ophthalmoscopy, best corrected visual acuity, full-field electroretinography, and visual field studies. Fundus autofluorescence and spectral-domain optical coherence tomography were further assessed in 7 patients.

Results: *PROM1* variants were identified in 32 families. Disease-causing variants were found in 18 autosomal recessive and 4 autosomal dominant families. Monoallelic pathogenic variants or variants of unknown significance were identified in the remaining 10 families. Comprehensive phenotyping of 25 patients from 22 families carrying likely disease-causing variants revealed clinical heterogeneity associated with the *PROM1* gene. Most of these patients presented cone-rod dystrophy and some exhibited macular dystrophy or retinitis pigmentosa, while all presented macular damage. Phenotypic association of a dominant splicing variant with late-onset mild maculopathy was established. This variant is one of the 3 likely founder variants identified in our Spanish cohort.

Conclusions: We report the largest cohort of patients with *PROM1* variants, describing in detail the phenotype in 25 of them. Interestingly, within the variability of phenotypes due to this gene, macular involvement is a common feature in all patients.

Expanded phenotypic spectrum of retinopathies associated with autosomal recessive and dominant mutations in *PROM1*

Short title: Phenotypic spectrum of *PROM1*-associated retinopathy

Marta Del Pozo-Valero[1], Inmaculada Martin-Merida[1,2], Belen Jimenez-Rolando[3], Ana Arteche[1], Almudena Avila-Fernandez[1,2], Fiona Blanco-Kelly[1,2], Rosa Riveiro-Alvarez[1], Caroline Van Cauwenbergh[4,5], Elfride De Baere[4], Carlo Rivolta[6,7], Blanca Garcia-Sandoval[3], Marta Corton[1,2,†], Carmen Ayuso[1,2,†].

1. Department of Genetics, Instituto de Investigación Sanitaria–Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain.

2. Center for Biomedical Network Research on Rare Diseases (CIBERER), ISCIII, Madrid, Spain.

3. Department of Ophthalmology, Instituto de Investigación Sanitaria–Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain.

4. Center for Medical Genetics Ghent, Ghent University and Ghent University Hospital, Ghent, Belgium.

5. Department of Ophthalmology, Ghent University and Ghent University Hospital, Ghent, Belgium.

6. Department of Computational Biology, Unit of Medical Genetics, University of Lausanne, Lausanne, Switzerland.

7. Department of Genetics and Genome Biology, University of Leicester, Leicester, United Kingdom.

*Correspondence to: **Carmen Ayuso:** cayuso@fjd.es. Servicio de Genética, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz, Universidad Autónoma de Madrid, Av. Reyes Católicos, 2. Madrid, 28040 Spain. Tel: +34 609612728; Fax: +34 915504849

Supplemental Material available at AJO.com

INTRODUCTION

Inherited retinal dystrophy (IRD) refers to a group of progressive and degenerative diseases that affect the photoreceptor cells and lead to visual impairment[1]. Its prevalence is estimated at 1/3500 to 1/5000 worldwide. IRDs are subclassified as retinitis pigmentosa (RP), the most common type; cone-rod dystrophy (CRD); and cone dystrophy (CD). Patterns of inheritance include autosomal dominant (ad), autosomal recessive (ar), and X-linked (XL) transmission, though mitochondrial and digenic forms have also been described[2]. The first noticeable symptoms are commonly night blindness and progressive loss of the peripheral visual field among RP patients[3], due to the dysfunction or loss of rod photoreceptors and loss of visual acuity (VA) and photophobia in CD/CRDs patients[4] caused by cone degeneration. To date, 307 loci have been found to cause IRD, with 271 genes identified (https://sph.uth.edu/retnet), one of which is *PROM1* (RefSeq NM_006017; OMIM 604365).

Located on chromosome 4p15.32, the *PROM1* gene comprises 27 exons and encodes prominin-1, a five-transmembrane domain glycoprotein originally identified as a hematopoietic stem-cell antigen, CD133/AC133[5,6]. Prominin-1 is expressed in several stem and progenitor cells originating from various sources, including the neural and hematopoietic systems[7,8]. Photoreceptor, glial, and epithelial cells from developing and adult organs express prominin-1[9–11]. In the retina, PROM1 is located at the base of photoreceptor outer segments, where it is involved in disk membrane morphogenesis[12]. Seven alternative splice variants have been reported in human tissues[13], 2 of which are the most highly expressed isoforms in the retina[14]. Disease-causing *PROM1* variants have been associated with arRP with macular degeneration, arCRD, adCRD, and macular phenotypes, such as Stargardt-like and bull's-eye maculopathies[15,16]. In this work, we used state-of-the-art genotyping techniques to study the involvement of the *PROM1* gene as the potential cause of a variety of IRDs in a large cohort of 2216 families, mostly from Spain. We provide genetic data from a total of 32 families carrying novel and previously described *PROM1* variants. We also describe the phenotype associated with likely disease-causing *PROM1* variants by further detailed ophthalmic evaluation in 25 patients. Overall, these data are important for the development of future therapeutic trials, including gene-specific therapies for *PROM1*-related phenotypes.

SUBJECTS AND METHODS

Subjects and samples

All 2216 patients were clinically diagnosed with non-syndromic IRD and recruited at Fundación Jiménez Díaz University Hospital (FJD, Madrid, Spain). This study was designed in compliance with the tenets of the Helsinki Declaration, and patient enrolment was approved by the ethics committee of this institution. DNA samples were collected from the FJD biobank. Total RNA of 4 patients and 3 controls was obtained from saliva, whole blood, blood-isolated mononuclear cells, or fibroblasts using NucleoSpin RNA kits (NucleoSpin, MACHEREY-NAGEL, Duren, Germany) or TRI reagent (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's instructions. Prior to RNA extraction, saliva samples were collected using the Oragene RNA kit (DNA Genotek, Ottawa, Ontario, Canada).

Molecular screening

Molecular screening of the IRD patients making up this cohort has been performed over the past 27 years using different genetic tools. First, commercial genotyping microarrays (ASPER Ophthalmics, Tartu, Estonia) and/or sequencing of the most prevalent genes, depending on the patient's phenotype, were applied to 1762 index patients. Following this, 957 remained uncharacterized and were subsequently studied through different NGS strategies, including targeted gene panels[17], clinical exome, and/or whole-exome sequencing (WES), as previously described[18]. An additional 454 new cases were studied by NGS as the first approach.

The pathogenicity of unreported variants was established according to their allele frequency in the Exome Aggregation Consortium (ExAC) and gnomAD (http://gnomad.broadinstitute.org/); *in silico* prediction tools for new splice and missense variants, including CADD[19] and those included in the commercial Alamut software v2.9.0 (Interactive Biosoftware, Rouen, France); and cosegregation studies in the family when other relatives were available. The guidelines of the American College of Medical Genetics and Genomics (ACMG) were used for variant classification[20].

In addition, we performed high-resolution copy number variation (CNV) analysis of *PROM1* using the customized arrEYE Agilent CGH-8x60K microarray[21] (Agilent Technologies, Inc.,

Santa Clara, CA, USA) and Sanger sequencing to discard the intronic variants c.2077-521A>G[22] and the c.2281-20_2281-11del[23] in the *PROM1* gene in 6 patients carrying monoallelic variants in *PROM1* (RP-0365, RP-1740, MD-0289, RP-1243, RP-2831, and MD-0875) as well as the affected daughter of MD-0873, who presented another phenotype (Leber congenital amaurosis and congenital heart disease).

Haplotype analysis

Three microsatellite markers (D4S1602, D4S2960 and D4S1567) and 7 SNPs (rs73125627, rs3213710, rs2072313, rs6449209, rs3815344, rs2286455, and rs2078622) flanking 2 Mb around *PROM1* were studied in 14 families with the variants c.303+1G>A, c.1354dup;p.Tyr452Leufs*13, and c.1984-1G>T.

Expression analysis of the PROM1 gene

Total RNA obtained from the whole blood, blood mononuclear cells, and saliva of 4 individuals carrying the pathogenic splicing variants c.303+1G>A and c.303+2T>C, as well as 3 wild-type controls, was transcribed by using the SuperScript IV cDNA synthesis kit (Thermo Fisher Scientific) in a final volume of 20 µL. Fibroblast RNA from one control was also used specifically to evaluate *PROM1* expression in this tissue. RT-PCR assays were performed using specific primers for exons 2 and 5.

Clinical assessment

IRDs were classified as CD/CRD or RP phenotypes based on clinical examination together with presence and onset of either VA loss and photophobia or visual field constriction and night blindness as the initial symptoms, respectively. A macular dystrophy (MD) phenotype was defined in the patients with macular lesions and normal full-field electroretinogram (ERG) responses. A comprehensive ophthalmologic examination including detailed medical history, measurements of best-corrected visual acuity (BCVA), and ophthalmoscopy was available for 25 patients carrying *PROM1* variants. In addition, RP patients and patients carrying the c.303+1G>T variant were also tested using spectral domain optical coherence tomography (SD-OCT) and fundus autofluorescence (FAF). Fluorescein angiography (FA) was also performed in 1 patient from family MD-0934.

RESULTS

Molecular findings from PROM1 screening

PROM1 was studied using conventional methods and/or NGS (Supplementary Figure 1, available at AJO.com) We analyzed a cohort of 2216 patients, mostly comprising Spanish individuals and all of whom presented non-syndromic IRD, including RP, CD/CRD, and/or MD. Table 1 and 2 summarizes the variants identified in *PROM1* from a total of 32 families (Supplementary Figure 2, available at AJO.com).

In total, we identified 19 *PROM1* variants, 10 of which are novel. The pathogenicity of these variants was established in accordance with the ACMG classification[20] (Supplementary Table 1, available at AJO.com). The location of the likely pathogenic variants in the coding sequence of *PROM1* is shown in Supplementary Figure 3 (available at AJO.com).

Thirteen different variants were considered causative/likely causative; these appeared in 18 arRP or arCRD families, 3 adCRD/MD families, and 1 adRP family (Table 1). One was a novel missense homozygous variant (c.1435G>A;p.Gly479Arg) in family RP-1852.

The 6 remaining *PROM1* variants appeared in heterozygosis in 7 families (Table 2). In 4 families we identified 4 novel missense VUS, including 4 heterozygous missense variants, also present in their apparently healthy relatives (families MD-0875, MD-0001, MD-0059, and RP-2645). The previously reported variant c.604C>G;p.Arg202Gly was identified in families RP-0365 and RP-1740, and family RP-2680 carried the novel splicing variant c.2490-2A>G. Additionally, the known recessive variant c.1354dup was present in 3 monoallelic families (MD-0289, RP-1243, and RP-2831) (Table 2).

No CNVs or intronic variants could be identified in sporadic or recessive cases carrying monoallelic *PROM1* variants. We also assessed the presence of additional pathogenic variants in other IRD genes by means of targeted NGS, enabling us to identify 4 likely pathogenic variants in 3 cases (Supplementary Table 2, available at AJO.com). In proband RP-1740, who presented RP, we also identified a likely pathogenic recessive variant in heterozygosis in the *RP1* gene in addition to the p.Arg202Gly variant in *PROM1.* In family RP-2680, presenting arCRD, biallelic variants were found in the *ABCA4* gene, in addition to the splicing variant

c.2490-2A>G in *PROM1*. Segregation analysis showed recessive segregation of *ABCA4* alleles in the 2 affected siblings, together with a lack of segregation for the *PROM1* variant. Remarkably, the index case of family RP-2645 carried a novel *de novo* VUS in the *COL11A1* gene, along with the c.2408T>C;p.Val803Ala *PROM1* VUS. Upon clinical revaluation, we considered the variant in the *COL11A1* gene to be likely causative in this patient.

The most frequent pathogenic variant in our cohort was the c.1354dup variant, which leads to a frameshift p.Tyr452Leufs*13; this variant was discovered in 12 unrelated families, one of them from Ecuador (MD-0682). Nine of these variants fully explained the IRD phenotype, as it was found in homozygosis (7 families) or compound heterozygosis (2 families). In family RP-1110 we identified this variant segregating with a novel nonsense variant in the proband, and in homozygosis in an affected fourth-degree relative.

In 11 families we identified 6 splicing variants, 3 of which were novel. The variant c.303+1G>A, affecting the donor splice site of intron 3, was found in 5 patients from 3 families (MD-0235, MD-0934, and MD-1074), and behaved in a dominant manner. In family MD-1074 we could not establish the inheritance mode, as the heterozygous proband was conceived by *in vitro* fertilization with egg donation. No additional likely pathogenic variants were identified by WES in family MD-0235. The variants c.303+2T>C and c.630+1G>A, affecting the donor sites in introns 3 and 5, respectively, showed a recessive pattern of inheritance in 3 families (RP-0878, MD-0100, and MD-0649), and c.2490-2A>G, located at the acceptor site in intron 23, was identified in heterozygosis in the *ABCA4*-mutated patient mentioned above (RP-2680). Two previously reported splice variants were found in 4 unrelated families: the c.1984-1G>T in homozygosis in 3 families (MD-0123, MD-0762, and MD-0873) and the c.2130+2del variant in compound heterozygosis in family MD-0654.

In order to study the splicing effect of the c.303+1G>A and c.303+2T>C variants, RNA expression analyses were performed. The *PROM1* gene was expressed in whole blood, blood mononuclear cells, saliva, and fibroblasts. However, this study demonstrated that exon 3 was skipped in the isoforms expressed in these tissues (Supplementary Figure 4, available at AJO.com).

Considering all the variants identified in this study, the prevalence of *PROM1* variants in our IRD cohort was 1.4%, explaining the molecular defect in 22 families (~1%).

Identification of founder pathogenic variants in PROM1

In order to shed light on the possibility of founder effects for the 3 most prevalent pathogenic variants in our *PROM1* cohort, haplotype analysis was carried out in a total of 14 unrelated Spanish families. Haplotyping in 9 families (10 patients) carrying the common variant c.1354dup, in 2 families with the dominant allele c.303+1G>A, and in 3 families with the c.1984-1G>T variant revealed shared minimal genomic regions of 99 Kb, 17 Kb, and 457 Kb, respectively (Supplementary Figure 2).

Phenotype of patients carrying likely disease-causing variants in PROM1

The *PROM1*-associated phenotype and the clinical data available for 25 patients from 22 unrelated families were reviewed in detail and appear in Table 3 and Supplementary Table 3 (available at AJO.com). According to the clinical assessment, most patients harboring *PROM1* variants in our cohort had CRD, and only 3 and 2 patients were diagnosed with MD and RP, respectively. The CRD phenotype of the RP-1852 patient, who carried a novel missense variant in homozygosis in the *PROM1* gene, is also described in Supplementary Table 3. The first symptoms of the RP patients (RP-0878 and RP-2604) were night blindness (mean: 24±8.48 years) and loss of VF (mean: 28±2.83 years). At the age of examination, these RP patients presented very low BCVA, and extinguished rod and cone responses in the full-field ERG (Table 3). Fundus images, FAF, and SD-OCT revealed typical bone spicule-like pigmentation in the periphery as well as macular atrophy (Figure 1).

Considering the 20 CRD/MD patients for whom information was available, VA loss was uniformly the earliest presenting symptom (mean: 18.21±16.78 years) in all of them. The primary symptoms were similar between patients carrying the recessive variants c.1354dup and c.1984-1G>T (Table 3). Phenotypically, high variability was seen for nearly all ophthalmologic parameters analyzed (BCVA, ophthalmoscopy, VF, and full-field ERG), even within patients showing the same genotype and belonging to the same family. Four patients with the c.303+1G>A splicing variant showed a distinct phenotype characterized by a later onset (Table

3 and Figure 2). They presented VA loss in their fifth decade of life (mean: 46.75±11.70 years), and their BCVA was well preserved until that age. At the age of diagnosis, the only symptom presented by the youngest patient with the c.303+1G>A variant (MD-1074) was an alteration in color perception. FAF and SD-OCT images of all patients showed changes of varying severity in the macular region and the retinal layers (Figure 2). In the case of the child of MD-1074, the ophthalmologic findings were normal, with the exception of macular hyperfluorescence in the FAF. Patient MD-0934 III:1 has recently developed a choroidal neovascular membrane in the right eye (Supplementary Figure 5, available at AJO.com).

DISCUSSION

In this study we present the largest cohort of IRD patients carrying variants in *PROM1*, describing genetic data from 32 IRD families and phenotypic data from 25 patients carrying likely disease-causing variants. We found 19 rare *PROM1* variants in these families, 10 of which are novel. The prevalence of *PROM1* variants that seems to reliably explain the IRD phenotype is about 1%. The most prevalent pathogenic variant found in 12 unrelated families was c.1354dup. Haplotyping revealed a common 99-Kb genomic region linked to the variant in the probands analyzed, including one Latin American patient likely of Spanish ancestry. These findings indicate a possible founder effect for this pathogenic variant in our cohort of Spanish origin.

Around 12 pathogenic splicing *PROM1* variants have been reported to date. Here, we report a remarkably high proportion of splicing site alleles, explaining the phenotype in 10 families. Found in a total of 6 families, 2 splicing variants (c.303+1G>A and c.1984-1G>T) are frequent alleles in our cohort, likely due to founder effects as revealed by haplotype analysis. The longest shared region comprises the c.1984-1G>A splicing variant, which has been described in Spanish and French populations[24–26], thereby suggesting a founder effect in this geographical area. Additionally, the variants c.303+1G>A and c.303+2T>C showed different behavior in our families as concerns the underlying inheritance pattern, acting as dominant or recessive alleles, respectively. Although the heterozygous variant c.303+1G>A has been recently reported in a patient with Stargardt disease[27], no family data were available. Both variants affected the canonical donor site of exon 3, which is an alternative exon included in the 2 main retinal

PROM1 isoforms[14]. Skipping of exon 3 seems to occur naturally in extra-ocular tissues, which has prevented us from studying the potential splicing effect of these variants. It is therefore not possible to determine the molecular mechanism of the dominant splicing variant c.303+1G>A given the unavailability of retinal tissue from the patients. Moreover, due to the fact that exon 3 is presented in 97% of the isoforms in the retina[14] we suggest that exon 3 could play an important role in photoreceptors function and/or maintenance that should be further studied.

Variable phenotypes and different inheritance patterns are associated with pathogenic variants in the *PROM1* gene. It was previously considered that heterozygous missense variants are associated with autosomal dominant Stargardt-like and bull's eye maculopathies[28], while nonsense and frameshift variants are associated with arRP with macular degeneration[14,29,30]. However, our findings do not seem to support these observations. In our cohort, most of the identified *PROM1* variants were biallelic and seem to be associated with arCRD. These CRD patients showed both variable intra- and inter-familial age at onset, making it difficult to establish a genotype-phenotype correlation in the *PROM1* gene regarding specific variants. The RP-associated variants were less frequent, with only 2 cases. However, regardless of the initial diagnosis of primary cone or rod loss, all patients with *PROM1* disease-causing variants developed a macular involvement, which therefore should be considered a characteristic phenotypic finding of *PROM1*.

Remarkably, patients carrying the splicing variant c.303+1G>A showed particular phenotypic features not seen in other *PROM1*-associated cases in our cohort, including a later onset in the fifth decade with well-preserved VA until age 50. However, this variant was also found in a younger patient with color disturbances. As clinical data were not available in the other patients at such an early age, we could not establish whether the disease in this child would progress slowly, or if there could be a modifying factor making the patient's phenotype more severe.

We have identified a novel missense variant in homozygosis in family RP-1852. Though unable to perform segregation studies, we consider this variant a candidate to explain the CRD phenotype of the patient. Moreover, homozygous missense variants in the *PROM1* gene have been described before in a CRD patient[31].

Monoallelic *PROM1* variants, including 5 novel variants predicted to be pathogenic by *in-silico* programs and 2 variants reported previously, were also found in ad and ar families. We were able to rule out the *PROM1* gene as a causal factor in 2 families with likely disease causing variants in other IRD genes. The variant p.Arg202Gly reported as a dominant disease-causing variant[32] was identified in 2 ar families. The inconsistency of its inheritance pattern and its high frequency in population databases (ExAC; 0.015%) did not lend support to dominant-type pathogenicity[33], therefore it should be considered a VUS with unlikely causality in our patients. Three VUS in *PROM1* were segregating in healthy relatives of 3 families, excluding them as possibly disease-causing dominant alleles unless incomplete penetrance was present or symptoms were not yet developed. Further in-depth studies of the intronic regions should be needed to elucidate whether *PROM1* is the causal gene in the rest of ar cases carrying monoallelic *PROM1* variants.

In summary, we describe the clinical and genetic findings of the largest cohort of IRD patients with variants in the *PROM1* gene reported to date. NGS allows us to evaluate the contribution of the *PROM1* gene to IRD. This approach is crucial for accurate genetic counseling, disease prognosis, and for the development of future gene therapies. Disease-causing variants explain 1% of our cohort, supporting the idea that this gene is a rare cause of IRD. This study also highlights the great heterogeneity of *PROM1*-associated phenotypes regardless of genotype. All patients harboring disease-causing variants in this gene present characteristic macular involvement, which seems to support the pathogenicity of *PROM1* variants.

ACKNOWLEDGMENTS

a. Funding/Support

This work was supported by grants from the Instituto de Salud Carlos III (ISCIII) from the Spanish Ministry of Health, including CIBERER (06/07/0036), IIS-FJD Biobank PT13/0010/0012, and FIS (PI16/00425); and from the regional government of Madrid, RAREGenomics-CM (CAM, B2017/BMD-3721), all partially supported by FEDER (European Regional Development Fund). In addition, CNAG (2016 BBMRI-LPC Whole Exome Sequencing Call), the Spanish National Organization of the Blind (ONCE), the Spanish Fighting Blindness Foundation (FUNDALUCE), and the Ramon Areces Foundation also supported our work.

b. Financial disclosures

MDP-V is sponsored by the Conchita Rábago Foundation, IM-M by the CIBERER and MC is supported by the Miguel Servet Program (CP12/03256) from ISCIII.

† The authors Marta Corton and Carmen Ayuso have contributed equally.

c. Other acknowledgments

We would like to thank Oliver Shaw (Instituto de Investigación Sanitaria–Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM)) for proofreading the manuscript.

299 **REFERENCES**

300 1. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. Lancet 2006;368:1795–1809.

301 2. Parmeggiani F, S. Sorrentino F, Ponzin D, et al. Retinitis Pigmentosa: Genes and Disease
302 Mechanisms. Curr Genomics 2011;12:238–249. Available at:
303 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3131731/ [Accessed May 29, 2018].

304 3. Ayuso C, Millan JM. Retinitis pigmentosa and allied conditions today: A paradigm of translational
305 research. Genome Med 2010;2:34. Available at:
306 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2887078/ [Accessed May 29, 2018].

307 4. Thiadens AAHJ, Phan TML, Zekveld-Vroon RC, et al. Clinical course, genetic etiology, and visual
308 outcome in cone and cone-rod dystrophy. Ophthalmology 2012;119:819–826.

309 5. Miraglia S, Godfrey W, Yin A, Al E. A novel five-transmembrane hematopoietic stem cell antigen:
310 isolation, characterization, and molecular cloning. Blood 1997;90:5013–5021.

311 6. Yin A, Miraglia S. AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood
312 1997;90:5002–5012. Available at: http://bloodjournal.hematologylibrary.org/content/90/12/5002.short.

313 7. Fargeas CA, Fonseca A-V, Huttner WB, Corbeil D. Prominin-1 (CD133): from progenitor cells to
314 human diseases. Future Lipidol 2006;1:213–225. Available at:
315 https://www.tandfonline.com/doi/abs/10.2217/17460875.1.2.213 [Accessed May 29, 2018].

316 8. Bauer N, Fonseca AV, Florek M, et al. New insights into the cell biology of hematopoietic
317 progenitors by studying prominin-1 (CD133). In: *Cells Tissues Organs*.Vol 188.; 2008:127–138.

318 9. Weigmann A, Corbeil D, Hellwig A, Huttner WB. Prominin, a novel microvilli-specific polytopic
319 membrane protein of the apical surface of epithelial cells, is targeted to plasmalemmal protrusions of
320 non-epithelial cells. Proc Natl Acad Sci 1997;94:12425–12430. Available at:
321 http://www.pnas.org/cgi/doi/10.1073/pnas.94.23.12425.

322 10. Corbeil D, Röper K, Hellwig A, et al. The human AC133 hematopoietic stem cell antigen is also
323 expressed in epithelial cells and targeted to plasma membrane protrusions. J Biol Chem

2000;275:5512–20. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10681530.

11. Florek M, Haase M, Marzesco AM, et al. Prominin-1/CD133, a neural and hematopoietic stem cell marker, is expressed in adult human differentiated cells and certain types of kidney cancer. Cell Tissue Res 2005;319:15–26.

12. Yang Z, Chen Y, Lillo C, et al. Mutant prominin 1 found in patients with macular degeneration disrupts photoreceptor disk morphogenesis in mice. J Clin Invest 2008;118:2908–2916.

13. Fargeas CA, Huttner WB, Corbeil D. Nomenclature of prominin-1 (CD133) splice variants - An update. Tissue Antigens 2007;69:602–606.

14. Permanyer J, Navarro R, Friedman J, et al. Autosomal recessive retinitis pigmentosa with early macular affectation caused by premature truncation in PROM1. Investig Ophthalmol Vis Sci 2010;51:2656–2663. Available at: http://iovs.arvojournals.org/article.aspx?doi=10.1167/iovs.09-4857 [Accessed May 9, 2018].

15. Michaelides M, Johnson S, Poulson A, et al. An autosomal dominant bull's-eye macular dystrophy (MCDR2) that maps to the short arm of chromosome 4. Investig Ophthalmol Vis Sci 2003;44:1657–1662.

16. Kniazeva M, Chiang MF, Cutting GR, et al. Clinical and genetic studies of an autosomal dominant cone-rod dystrophy with features of Stargardt disease. Ophthalmic Genet 1999;20:71–81. Available at: https://doi.org/10.1076/opge.20.2.71.2287 [Accessed June 13, 2018].

17. Perez-Carro R, Corton M, Sánchez-Navarro I, et al. Panel-based NGS Reveals Novel Pathogenic Mutations in Autosomal Recessive Retinitis Pigmentosa. Sci Rep 2016;6. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4726392/ [Accessed June 13, 2018].

18. Martin-Merida I, Aguilera-Garcia D, Fernandez-San Jose P, et al. Toward the mutational landscape of autosomal dominant retinitis pigmentosa: A comprehensive analysis of 258 Spanish families. Investig Ophthalmol Vis Sci 2018;59:2345–2354.

19. Kircher M, Witten DM, Jain P, et al. A general framework for estimating the relative pathogenicity

of human genetic variants. Nat Genet 2014;46:310–315.

20. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–424. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4544753/ [Accessed June 13, 2018].

21. Van Cauwenbergh C, Van Schil K, Cannoodt R, et al. ArrEYE: A customized platform for high-resolution copy number analysis of coding and noncoding regions of known and candidate retinal dystrophy genes and retinal noncoding RNAs. Genet Med 2017;19:457–466. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5392597/ [Accessed June 20, 2018].

22. Mayer AK, Rohrschneider K, Strom TM, et al. Homozygosity mapping and whole-genome sequencing reveals a deep intronic PROM1 mutation causing cone-rod dystrophy by pseudoexon activation. Eur J Hum Genet 2016;24:459–462.

23. Eidinger O, Leibu R, Newman H, et al. An intronic deletion in the PROM1 gene leads to autosomal recessive cone-rod dystrophy. Mol Vis 2015;21:1295–306. Available at: http://www.ncbi.nlm.nih.gov/pubmed/4676936%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/26702251%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4676936%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/26702251%5Cnhttp://www.pubmedcentral.nih.gov/articleren.

24. De Castro-Miró M, Pomares E, Lorés-Motta L, et al. Combined Genetic and high-throughput strategies for molecular diagnosis of inherited retinal dystrophies. PLoS One 2014;9:e88410.

25. Boulanger-Scemama E, El Shamieh S, Démontant V, et al. Next-generation sequencing applied to a large French cone and cone-rod dystrophy cohort: Mutation spectrum and new genotype-phenotype correlation. Orphanet J Rare Dis 2015;10. Available at: http://www.ojrd.com/content/10/1/85 [Accessed June 15, 2018].

26. Bravo-Gil N, González-Del Pozo M, Martín-Sánchez M, et al. Unravelling the genetic basis of simplex Retinitis Pigmentosa cases. Sci Rep 2017;7:41937. Available at: http://www.nature.com/articles/srep41937 [Accessed June 15, 2018].

27. Bryant L, Lozynska O, Maguire AM, et al. Prescreening whole exome sequencing results from patients with retinal degeneration for variants in genes associated with retinal degeneration. Clin Ophthalmol 2018;12:49–63.

28. Michaelides M, Gaillard MC, Escher P, et al. The PROM1 mutation p.R373C causes an autosomal dominant bull's eye maculopathy associated with rod, rod-cone, and macular dystrophy. Investig Ophthalmol Vis Sci 2010;51:4771–4780. Available at: http://iovs.arvojournals.org/article.aspx?doi=10.1167/iovs.09-4561 [Accessed May 9, 2018].

29. Pras E, Abu A, Rotenstreich Y, et al. Cone-rod dystrophy and a frameshift mutation in the PROM1 gene. Mol Vis 2009;15:1709–1716.

30. Maw MA, Corbeil D, Koch J, et al. A frameshift mutation in prominin (mouse)-like 1 causes human retinal degeneration. Hum Mol Genet 2000;9:27–34. Available at: https://academic.oup.com/hmg/article-lookup/doi/10.1093/hmg/9.1.27 [Accessed May 9, 2018].

31. Tiwari A, Bahr A, Bähr L, et al. Next generation sequencing based identification of disease-associated mutations in Swiss patients with retinal dystrophies. Sci Rep 2016;6:28755.

32. Patel N, Aldahmesh MA, Alkuraya H, et al. Expanding the clinical, allelic, and locus heterogeneity of retinal dystrophies. Genet Med 2016;18:554–562. Available at: http://www.nature.com/articles/gim2015127 [Accessed May 9, 2018].

33. Sharon D, Kimchi A, Rivolta C. OR2W3 sequence variants are unlikely to cause inherited retinal diseases. Ophthalmic Genet 2016;37:366–368.

34. Zhao L, Wang F, Wang H, et al. Next-generation sequencing-based molecular diagnosis of 82 retinitis pigmentosa probands from Northern Ireland. Hum Genet 2015;134:217–230. Available at: http://link.springer.com/10.1007/s00439-014-1512-7 [Accessed July 4, 2018].

35. Carss K, Arno G, Erwood M, et al. Comprehensive Rare Variant Analysis via Whole-Genome Sequencing to Determine the Molecular Pathology of Inherited Retinal Disease. Am J Hum Genet 2017;100:75–90. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0002929716305274 [Accessed July 4, 2018].

401

402 **FIGURE CAPTIONS**

403 **Figure 1. Imaging findings in RP patients carrying *PROM1* variants.** A–C: Proband of RP-0878, carrying the
404 c.303+2T>C variant in homozygosis, 46 years of age. D–F: Proband of RP-2604, carrying the c.1117C>T;
405 p.Arg373Cys variant in heterozygosis, 56 years of age. Fundus autofluorescence (FAF) images show extensive
406 hypo-autofluorescent lesions located in the mid-periphery and the macular area. Fundus photographs reveal bone
407 spicule pigmentation in the periphery, retinal vessel attenuation, and severe retinal atrophy. SD-OCT showed
408 extensive loss of the ellipsoid band in both cases, as well as a severe foveal and choroidal atrophy in patient RP-
409 0878, and an epiretinal membrane with intraretinal cystic spaces in patient RP-2604. The absence of the ellipsoid
410 and retinal pigment epithelium layers in the SD-OCT images is consistent with the FAF findings.

411

412 **Figure 2. Imaging findings in patients with the c.303+1G>A variant in heterozygosis.** A: Fundus
413 photographs obtained at 52 years of age, fundus autofluorescence (FAF) and spectral domain optical coherence
414 tomography (SD-OCT) images obtained at 67 years of age from patient MD-0235. B: FAF, fundus photographs,
415 and SD-OCT images obtained of patient MD-0934 III:8 at 51 years of age. C: FAF, fundus photographs, and SD-
416 OCT images obtained of patient MD-0934 III:6 (sister of MD-0934 III:8) at age 53 years. D: FAF, fundus
417 photographs, and SD-OCT images obtained of patient MD-0934 III:1 (cousin of MD-0934 III:8) at 62 years of age.
418 E: FAF, fundus photographs, and SD-OCT images obtained of patient MD-1074 at age 6 years. The FAF
419 highlights lesions of variable severity limited to the macular area from a marked plaque of atrophy in patient A,
420 speckled hypo-autofluorescent in patients B and C, and hyper-autofluorescent in patient E. Patient D showed a
421 preserved macula in the right eye (RE) and a plaque of atrophy surrounding the foveal area in the left eye (LE).
422 Fundus photographs reveal macular atrophy in patients A-D sparing the fovea in the RE of patient A and the LE of
423 patient D. Retinal fundus photograph of patient E has a normal appearance. SD-OCT images demonstrat
424 perifoveal thinning of the outer retina in patient A and marked loss of photoreceptor inner segment/outer segment
425 band along with central choroid thickening in patients B and C. In patient C the fovea was preserved, evidencing a
426 “flying saucer” sign. Foveal sparing is observed in both eyes of patient A and in the LE of patient D. Patient E
427 shows normal appearance in the SD-OCT images.

Family	Phenotype	Allele 1				Allele 2				Segregation
		Exon/Intron	Nucleotide	Protein	Reference	Exon/Intron	Nucleotide	Protein	Reference	
Causative variants in ad families										
MD-0235	MD	Intron 3	c.303+1G>A	Splicing	(27)	-	-	-	-	Yes
MD-0934	CRD/MD	Intron 3	c.303+1G>A	Splicing	(27)	-	-	-	-	Yes
MD-1074	CRD	Intron 3	c.303+1G>A	Splicing	(27)	-	-	-	-	NA
RP-2604	RP	Exon 10	c.1117C>T	p.Arg373Cys	(30)	-	-	-	-	NA
Causative variants in ar families										
RP-0878	RP	Intron 3	c.303+2T>C	Splicing	This study	Intron 3	c.303+2T>C	Splicing	This study	Yes
MD-0100	CRD	Exon 5	c.622del	p.Thr208Leufs*23	(34)	Intron 5	c.630+1G>A	Splicing	This study	Yes
MD-0649	CRD	Intron 5	c.630+1G>A	Splicing	This study	Intron 5	c.630+1G>A	Splicing	This study	NA
RP-0586	CRD	Exon 8	c.869del	p.Ser290fs*2	(14)	Exon 11	c.1177_1178del	p.Ile393Argfs*21	(35)	NA
MD-0383	CRD	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	NA
MD-0803	CRD	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	NA
RP-1899	CRD	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	Yes
RP-0855	CRD	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	Yes
MD-0682	CRD	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	NA
RP-2752	CRD	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	NA
RP-2924	CRD	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	NA
RP-1110	CRD	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	Exon 15	c.1709_1710insAA	p.Tyr570*	This study	Yes
MD-0838	CRD	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	Intron 19	c.2130+2del	Splicing	(32)	Yes
MD-0654	CRD	Exon 12	c.1414del	p.Arg472Glufs*18	This study	Exon 12	c.1414del	p.Arg472Glufs*18	This study	NA
MD-0123	CRD	Intron 17	c.1984-1G>T	Splicing	(24)	Intron 17	c.1984-1G>T	Splicing	(24)	NA
MD-0762	CRD	Intron 17	c.1984-1G>T	Splicing	(24)	Intron 17	c.1984-1G>T	Splicing	(24)	Yes
MD-0873	CRD	Intron 17	c.1984-1G>T	Splicing	(24)	Intron 17	c.1984-1G>T	Splicing	(24)	NA
Likely causative variant										
RP-1852	CRD	Exon 12	c.1435G>A	p. Gly479Arg	This study	Exon 12	c.1435G>A	p. Gly479Arg	This study	NA

Table 1. Likely causative *PROM1* variants in our cohort of IRD patients. Abbreviations: ad, autosomal dominant; ar, autosomal recessive; MD, macular dystrophy; CRD, cone-rod dystrophy; RP, retinitis pigmentosa; NA, not available.

Family	Inheritance	Phenotype	*PROM1* variant in heterozygosis						Causative variants in other genes
			Exon/Intron	Nucleotide	Protein	Reference	Classification	Comments	
Families with a non causative variant in *PROM1*									
MD-0875	AR (sporadic)	CRD	Exon 4	c.314A>G	p.Tyr105Cys	This study	VUS	No cosegregation with the disease	-
MD-0001	AD	MD	Exon 4	c.437G>A	p. Arg146Gln	This study	VUS	No cosegregation with the disease	-
RP-0365	AR	RP	Exon 5	c.604C>G	p.Arg202Gly	(32)	VUS	Variant reclassified to VUS supported by ClinVar, population frequency and this study.	-
RP-1740	AR (sporadic)	RP	Exon 5	c.604C>G	p.Arg202Gly	(32)	VUS	Variant reclassified to VUS supported by ClinVar, population frequency and this study	*RP1*
MD-0059	AD	CRD	Exon 13	c.1472T>C	p.Phe491Ser	This study	VUS	No cosegregation with the disease	-
RP-2645	AR	RP	Exon 23	c.2408T>C	p.Val803Ala	This study	VUS	No cosegregation with the disease	*COL11A1*
RP-2680	AR	CRD	Intron 23	c.2490-2A>G	Splicing	This study	Likely Pathogenic	No cosegregation with the disease	*ABCA4*
Families incompletely characterized with a pathogenic recessive variant in *PROM1*									
MD-0289	AR (sporadic)	CRD	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	Pathogenic	Lack of a second allele	-
RP-1243	AR (sporadic)	RP	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	Pathogenic	Lack of a second allele	-
RP-2831	AR (sporadic)	RP	Exon 12	c.1354dup	p.Tyr452Leufs*13	(29)	Pathogenic	Lack of a second allele	-

Table 2. Other *PROM1* variants identified in our cohort of IRD patients. Abbreviations: AD: autosomal dominant; AR: autosomal recessive; MD, macular dystrophy; CRD, cone-rod dystrophy; RP, retinitis pigmentosa; VUS: variant of unknown significance.

Table 3. Clinical characteristics of RP and CRD/MD patients with causative variants in the *PROM1* gene. Patients with the c.1354dup, c.1984-1G>T, and c.303+1G>A were shown separately. *First symptom reported. Abbreviations: RP, retinitis pigmentosa; CRD, cone-rod dystrophy; MD, macular dystrophy; n., number of cases; SD, standard deviation; RPE, retinal pigment epithelium.

Characteristics	**RP**	**CRD/MD**	**(c.1354dup)**	**(c.1984-1G>T)**	**(c.303+1G>A)**
Age at diagnosis, n.	2	21	9	2	5
Mean ±SD	29.5 ± 4.95	23.76 ± 17.64	15.77 ± 8.21	22 ± 5.66	45.6 ± 24.42
Range	26-33	4-65	3-28	18-26	4-65
First symptoms					
Nyctalopia, n. (%)	2/2 (100)*	12/17 (71)	6/8 (75)	1/1 (100)	2/4 (50)
Mean ±SD	24 ± 8.48	26.83 ± 15.19	24.8± 17.96	25	46± 11.31
Visual field loss, n. (%)	2/2 (100)	15/20 (75)	6/9 (67)	3/3 (100)	1/5 (20)
Mean ±SD	28 ± 2.83	17 ± 10.33	15± 5.59	12± 6.56	50
Visual acuity loss, n. (%)	2/2 (100)	20/20 (100)*	9/9 (100)*	3/3 (100)*	4/4 (100)*
Mean ±SD	32	18.21 ± 16.78	12.13 ± 6.56	10.67 ± 8.08	46.75 ± 11.70
Funduscopic examination					
Typical RP, n. (%)	2/2 (100)	7/21 (33)	5/9 (55)	-	-
Macular RPE changes, n. (%)	2/2 (100)	20/20 (100)	8/8 (100)	3/3 (100)	5/5 (100)
Full-field electroretinography					
Scotopic and photopic extinguished, n. (%)	2/2 (100)	1/13 (8)	-	-	-
Rod-cone pattern, n. (%)	-	-	-	-	-
Cone-rod pattern, n. (%)	-	3/13 (23)	3/5 (60)	-	-
Cone pattern, n. (%)		-	-	-	-
Scotopic and photopic reduced, pattern not further specified n. (%)	-	6/13 (46)	2/5 (40)	2/2 (100)	2/5 (20)
Normal, n. (%)	-	3/13 (23)	-	-	3/5 (60)