Autosomal recessive (AR) PROM1 retinal dystrophy was first reported in a family with an "early-onset severe form of retinal degeneration." Later reports demonstrated that AR PROM1 can be associated with an early (childhood) onset of retinal dystrophy that manifests as either cone-rod dystrophy (CRD) or retinitis pigmentosa (RP) phenotypes with prominent macular involvement. Severe visual loss and a non-detectable full-field ERG, either by or within the third decade of life, are common in AR PROM1 cases, independent of whether the diagnosis is CRD or RP.

PROM1 (OMIM 612657) codes for a transmembrane glycoprotein (Prominin-1) that localizes to the base of the rod and cone outer segments and is involved in disc assembly and maintenance of outer segment structure. PROM1 is associated with both autosomal dominant (AD) and AR retinal dystrophies, with the AD forms tending to be later onset and milder. PROM1 is responsible for between 1.0% and 9.5% of AR CRD (arCRD) cases. Few identifiable findings in AR PROM1 patients have been reported. One report found that the combination of high myopia, nystagmus, and early-onset CRD is suggestive of AR PROM1. In this report we demonstrate four cases of PROM1-associated arCRD that showed identifiable macular and peripheral retinal, as well as full-field ERG findings.

Patients

Four patients from three families were diagnosed with CRD by one of the authors (GAF). Informed consent was obtained from each of the four patients, as well as from participating unaffected parents and siblings. Procedures adhered to the tenets of the Declaration of Helsinki, and the protocol was approved by the Western Institutional Review Board (IRB) and Columbia University IRB.

Genetic Analysis

Thirty-nine patients (from 34 families) with CRD were initially tested for mutations in the ABCA4 gene by direct sequencing. In 13 of these cases, two disease-associated ABCA4 variants
were found, whereas ABCA4 testing was negative in all four cases that are the focus of this study. Based on the availability of unaffected relatives to aid in analysis, eight CRD cases (either with no detected ABCA4 or one ABCA4 variant) were subjected to whole exome sequencing (WES) at Macrogen, Inc. (Seoul, South Korea), using the Agilent (Santa Clara, CA, USA) SureSelect V5 capture kit and Illumina (San Diego, CA, USA) HiSeq 4000. Resulting sequences were analyzed with BWA-MEM, Picard, GATK, and SnvEff tools. The average sequence depth of target regions was 100x resulting, on average, in identification of approximately 90,000 single nucleotide polymorphisms. These variants were filtered based on minor allele frequency (>0.0001) and with in silico prediction programs (PolyPhen2, REVEL, CADD). Possibly pathogenic variants were confirmed by Sanger sequencing and segregation with the disease in families. WES identified two compound heterozygous stop-gain variants of the PROM1 gene in patient 1 (family A; Table 1, Fig. 1) and segregation of these with the disease was confirmed in family A (including both parents and an unaffected sibling). One possibly pathogenic PROM1 variant was found in each of the remaining patients. The remaining four CRD patients who underwent WES either remain unsolved, or their phenotype was attributed to a gene other than PROM1. DNA of patients 2, 3, and 4 (families B and C; Fig. 1) were subjected to whole genome sequencing (WGS) at Macrogen, Inc., using Illumina TrueSeq PCR-free library kit and 150 base pair (bp) paired-end sequencing on NovaSeq 6000 (Illumina) with approximately 30x depth. Deleterious deep intronic mutations were found by WGS in each of the remaining families, which segregated with the disease (Table 1, Fig. 1). The available clinical histories, chart notes, imaging, and ERG findings of each patient were retrospectively reviewed for this study.

### Imaging

Color fundus photography was performed using a Canon CR-1 Mark II camera (Canon USA, Melville, NY, USA). Spectral-domain optical coherence tomography (SD-OCT), infrared reflectance scanning laser ophthalmoscopy (IR-SLO), near-infrared autofluorescence (NIR-AF), and short-wavelength autofluorescence (SW-AF) images were obtained using the Heidelberg Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany).

### Full-field ERG

Review of the prior ophthalmic records of patient 1 showed that he had undergone a full-field ERG at an outside clinic with an LKC Technologies UTAS-E 2000 unit (LKC Technologies, Gaithersburg, MD). Patient 4 had also undergone a full-field ERG at another outside clinic. In two cases (patient 2 and patient 4), a clinical full-field ERG was obtained on a Nicolet Viking IV system (Nicolet Biomedical Inc, Madison, WI, USA), under previously described conditions.13,14 The recording electrode was a unipolar Burian-Allen contact lens electrode. Dark-adapted ERG stimuli included a rod-isolated short-wavelength stimulus of 0.005 (photopic) cd-s-m⁻² and a rod and cone combined response to a white single-flash stimulus of 4.65 cd-s-m⁻². Light-adapted ERG conditions, tested on a 15.4 cd-s-m⁻² white background, included a white single-flash stimulus of 4.65 cd-s-m⁻² and a 32-Hz flicker stimulus. Control ranges for amplitudes and implicit times were obtained from more than 50 normally sighted subjects with an age range of 24 to 38 years.

### RESULTS

#### Genetic Analysis

Pedigrees for the three families are shown in Figure 1, and PROM1 mutations are listed in Table 1. For patient 1, WES found two PROM1 stop-gain variants in compound heterozygosity. Both mutations have been described before as causal in arCRD.15–17 In patients 2 and 3 (the brothers in family B), WES found one new, possibly pathogenic, PROM1 variant, which deletes seven amino acids (p.Asn395_Pro401del). In patient 4 (family C), WES detected a single, predicted pathogenic, PROM1 variant, which affects splicing. Because the ERG, autofluorescence, and retinal appearance (described below) of cases in both families suggested recessive disease caused by PROM1 mutations, the 1-mutation cases were subjected to WGS, which uncovered a large deletion removing two full exons in patient 2. This variant was subsequently verified also in his affected brother. WGS also detected a deep intronic variant in patient 4 (c.2077–521A>G), which has been described as a severe, splicing-affecting variant.18 All four affected cases were determined to be compound heterozygous for the predicted or demonstrated pathogenic PROM1 variants via segregation analyses in the three families (Fig. 1).

### Ocular, Medical, and Family Histories

Patient 1 had been previously diagnosed with CRD at 3 years of age by an outside clinic. He felt that his central vision had never been sharp, even with correction. In his early teens, he had noted photoversion. He felt that his night vision had always been at least mildly reduced, although he drove without difficulty at night until his late teens, when he noted worsening nyctalopia and slight loss of peripheral vision. He was first examined by one of the authors (GAF) at the age of 22. At that visit, he reported his symptoms of reduced color, central, and
night vision had all progressed significantly within the previous year. He also noted occasional brief photopsias since childhood. Nystagmus was not noted at that visit, and it was not reported in the ocular history. Medically, the patient had diagnoses of borderline hypertension, bipolar disorder, anxiety, and hyperhidrosis, as well as a past history of a heart murmur that had resolved by the age of 12. His maternal grandfather was believed to have been color deficient, but no other family history of retinal or visual problems was noted. The patient's father was of Syrian/Lebanese Christian ancestry, and his mother was of mixed Bohemian, Scotch-Irish, and Greek ancestry.

Patients 2 and 3 (brothers from family B) were of Polish ancestry. Patient 2 had failed a school vision screening in first grade, and his acuity reportedly had never been fully correctable with spectacles. His first examination was at age 15, at which time he reported no visual complaints. Nystagmus was not noted on examination or in the ocular history. He had been referred by an outside eye doctor who found macular abnormalities. He denied any medical conditions. Patient 3 had less severe symptoms than his brother. He was first seen at age 21, at which time he reported mild color vision complaints that had begun in his mid-late teens and mild photoaversion. He denied central, peripheral or night vision complaints. Mild micronystagmus was noted on examination. His medical history was unremarkable aside from asthma.

Patient 4 was a female patient of mixed Western European ancestry who presented at age 21. Her first visual symptoms were noted at age 4 when she failed a school acuity screening. At age 21, she noted photoaversion (specifically, poor vision in bright light), which had begun in her mid-late teens, as well as poor color vision. She denied nyctalopia and peripheral vision complaints.
complaints. Photoaversion complaints increased throughout the patient’s 20s. Nystagmus was not noted either on examination or by history. By age 25 she reported slow dark adaptation. Her family history was noncontributory. She was diagnosed with bipolar disorder in her mid-20s and treated with Lamictal and Prozac; however, by age 30 she was off medication. The three unaffected relatives in family B, as well as the father in family C, each received undilated direct ophthalmoscopy, which was described as normal. The father in family A had SD-OCT of the macula available, which was normal in the right eye and showed a few small drusenoid deposits in the left eye.

Clinical Psychophysical Testing

Best corrected visual acuity (BCVA) and refractive error at the initial and most recent clinic visits are shown in Table 2. Legal blindness (worse than 20/100 BCVA in the better eye) was reached in the third decade in patients 1, 2, and 4, whereas legal blindness had not been reached as of the most recent visit (age 29) in patient 3. The four patients each failed to identify more than the control plate on the Ishihara color vision test at their respective first visits. As a group, Goldmann visual fields generally showed expanding and deepening central scotomas, as well as at least moderate constriction of the peripheral boundaries, which increased throughout the third decade.

FIGURE 2. Color fundus photographs of the macula of the right eye in AR PROM1 patients 1 (A), 2 (B), 3 (C), and 4 (D), taken at ages 24, 27, 22, and 29 years, respectively. Each case showed mild vascular attenuation, peripapillary atrophy, and hypopigmentation to varying degrees in the fovea. A horizontally oval margin of either hyperpigmentation or relative sparing of macular pigment was most evident in patients 1 and 2, and a few drusenoid deposits were most evident in the macula of patient 1.
Macular Changes

Representative macular color fundus photographs of the right eye for each patient are shown in Figure 2. Each case showed normal optic nerves, varying degrees of parapapillary atrophy, and mild vessel attenuation. Each patient had atrophic lesions in the fovea, with an oval shape that was most evident in patient 1 (Fig. 2A). Patient 1 also showed a few small drusenoid lesions outside the central hypopigmented area. The central lesion in patient 2 (Fig. 2B, age 27) was larger and more deeply atrophic, whereas patients 3 and 4 showed smaller, less well-defined areas of foveal hypopigmentation. None of these images show definite fleck-like lesions, as might be seen in Stargardt disease.

Peripheral Retinal Changes

Figure 3 shows montage color fundus photographs, which demonstrate diffuse midperipheral granularity, as well as round, well-defined atrophic lesions in the retinal periphery of patients 1, 2, and 3. The lesions varied in size from approximately one-half to two disc diameters (approximately 0.75 to 3 mm diameter). Many of the atrophic lesions also had hypertrophic pigmentary changes, most evident at the borders of the lesions. The peripheral changes were progressive in these patients, with more bone spicule-type pigment developing in patient 1 as of his most recent visit at age 27. Patient 4 was not found to have midperipheral or peripheral lesions as of her most recent dilated fundus examination at age 33.

Imaging: Autofluorescence and SD-OCT

Figure 4 shows NIR-AF at selected visits for each patient. Small, circular lesions were hypoautofluorescent (hypo-AF), on a homogeneous background in the central 30° in patients 1, 3, and 4. There was variation in the distribution of these small hypo-AF lesions, occurring either in a cluster centered on the macula, as in patient 1, or predominantly between the disc and the fovea, as in patients 3 and 4. The horizontally oval foveal lesions seen on color photos were evident on NIR-AF as well.

Figure 5 shows multimodal imaging from patient 1. On SD-OCT (Fig. 5A), this patient demonstrated identifiable, but attenuated external limiting membrane (ELM) and inner segment ellipsoid (ISe) bands in the fovea at age 22. His inner retinal lamination appeared essentially normal, as it was in the other three patients, at least on their earlier scans. Outside the foveal area there appeared to be a residual thinned outer nuclear layer and ELM, but no ISe band. The foveal ISe was no longer present on a follow-up scan at age 27, consistent with a substantial loss of acuity in that time frame. Patients 3 and 4 showed SD-OCT findings similar to the age 22 scan of patient 1. However, unlike patient 1, patient 4 had severe BCVA loss. IR-SLO imaging in patient 1 (Fig. 5B), also at age 22, showed a cluster of small, round, hyporeflective lesions in the macula. These lesions were less apparent on IR-SLO images at later visits, and they were not consistently observed in the other three patients with that modality.

Patient 1 underwent SW-AF, NIR-AF, and en face SD-OCT at age 26 (Figs. 5C–F). The NIR-AF and SW-AF were largely similar to one another. Within the macula, the two modalities were essentially indistinguishable. Outside the macula, there was a heterogeneous background and several hypo-AF lesions that had not been present during an earlier visit at age 22 (Fig. 4A). The en face SD-OCT was most similar to the AF images when measured at the level of the anterior aspect of the RPE (Figs. 5E, 5F). At this depth, en face imaging demonstrated hyporeflective foci, many of which corresponded to those seen on the AF images. In Figure 5F, it also can be seen that the inner retinal lamination had become somewhat disrupted temporal to the fovea by age 26. Patient 2 had a very similar appearance at his more recent visits, at approximately age 32, with inner retinal lamination becoming less distinct, most
evidently temporal to the fovea. Patients 3 and 4 maintained a normal inner retinal layer appearance.

**Full-field ERG**

Patients 1 and 4, as noted, had been tested by separate outside clinics with clinical full-field ERGs at the ages of 12 and 4, respectively (ERGs not shown). Review of the ERG from patient 1 showed nondetectable light-adapted single-flash and 30-Hz flicker responses, as well as nondetectable rod-isolated blue single-flash responses. The scotopic combined “rods and cones” single-flash (approximately 2.5 cd·s·m⁻²) responses were subnormal, at approximately half of the reported lower limit of normal for the b-wave right and left eyes. The a-wave was minimally present in the right eye and notably reduced in the left eye. Repeat ERG by the same outside clinic at age 21 was deemed nondetectable for all stimuli. We obtained a copy of the full-field ERG findings on patient 4 that did not specifically note the stimulus parameters. Nonetheless, we were able to ascertain a minimal a-wave amplitude in proportion to the b-wave under the highest luminance scotopic single-flash stimulus condition that was used.

Patients 2 and 4 had full-field ERG performed on a Viking II system at ages 15 and 21, respectively (Fig. 6). Cone-mediated responses (Fig. 6, bottom) in both cases were essentially nondetectable, with the exception of a delayed and markedly reduced, 12-microvolt 32-Hz flicker response in patient 4. Under scotopic conditions (Fig. 6, top), the rod-isolated responses were markedly delayed and reduced in amplitude to below half the lower limit of normal. The scotopic combined (rod and cone) single-flash response demonstrated a markedly reduced a-wave (10%-15% of normal), as well as markedly delayed b-waves that were approximately one-third (patient 2) and one-half (patient 4) of the lower limit for amplitude. With the small a-waves and delayed b-waves, the combined response waveforms more closely resembled a control’s rod-isolated response than a combined response. The b/a amplitude ratio was 3.7 and 5.7, respectively, which compares to a control range of 1.25 to 2.2.

**DISCUSSION**

In this report, we described four cases of arCRD caused by mutations in PROM1 from three families who showed identifiable macular findings on multiple modes of retinal imaging. All four patients demonstrated oval foveal lesions, as well as small hypo-AF foci in the posterior pole on NIR-AF imaging. Three of the cases demonstrated peripheral circular lesions as well. One patient from each of the three families demonstrated an ERG phenotype in which the dark-adapted combined response had a very small a-wave and delayed b-wave.

All PROM1 variants reported in this study are predicted or were shown to be deleterious, including two variants resulting in premature stop codons; one small 21-bp deletion; one large genomic deletion, resulting in a loss of two full exons; one variant in splice consensus sequences, predicted to result in abolished splicing; and one deep intronic variant, which has been demonstrated to completely abolish correct splicing.¹⁸
Three of the six variants were previously reported,15–18 and the remaining three variants are new (Table 1). This study also underscores an importance of resorting to WGS in some cases in which the causal variants are not discovered by WES, but in which phenotype guides the genetic screening, and deep intronic variants (point mutations or large copy number variants) are expected to occur.

The horizontally oval macular lesions found in our patients can be seen in several previously published cases with color photography and SW-AF imaging, in patients whose ages varied from 17 to 27 years.2–4,6 Pras et al.2 described these oval macular lesions as a "peri-foveal ring of RPE hypertrophy bordered centrally and peripherally with RPE atrophy."

The NIR-AF findings of small, circular areas of hypo-AF were notable in that the lesions were not readily apparent on fundoscopy or color photography. The autofluorescent pigment imaged with NIR-AF is believed to be melanin,19 and the small hypo-AF lesions likely represent focal RPE atrophy. Reinforcing this assumption is our finding of hyporeflective foci corresponding to the hypo-AF lesions at the anterior aspect of the RPE on en face OCT imaging (Fig. 5E). Two AR PROM1 cases from previous studies have shown focal hypo-AF that were concentrated more in the arcades than in the macula and less prominently circular.3,6 In a third case, a 19-year-old, fluorescein angiography was performed, demonstrating a cluster of hyperfluorescent circular lesions in the macula that were of a very similar size and distribution to the hypo-AF lesions of patient 1 in the present study.2 NIR-AF in patient 1 showed some hypo-AF lesions that were not present on the corresponding SW-AF (Figs. 5C, 5D). This finding is consistent with reports on ABCA4-associated retinopathy and age-related macular degeneration, which have demonstrated that NIR-AF tends to show changes earlier and to a greater extent than SW-AF.19,20

Larger circular lesions were found in the mid-periphery of three of the PROM1-associated arCRD cases in this report, all of whom were in their third decade of life. These circular atrophic lesions are most reminiscent of pavingstone (or cobblestone) degeneration, which is a common, benign finding that is associated with myopia and is more often seen in older adults.21,22 As pavingstone degeneration is uncommon in young adults, the circular peripheral RPE changes in our AR PROM1 patients are likely related to the underlying retinal degeneration.

At issue is whether and to what extent the findings described in this study are specific to AR PROM1. One genetic type of retinal dystrophy caused by the CDHR1 gene shares several phenotypic similarities to AR PROM1. The proteins coded for by PROM1 and CDHR1 (also known as PCDH21) strongly interact, colocalizing to the base of the photoreceptor outer segments and playing interrelated roles in outer segment morphogenesis.9 As in PROM1, CDHR1 may be diagnosed as either cone-rod dystrophy or RP with prominent macular involvement.23 Round lesions in the mid-periphery were present in at least a quarter of reported CDHR1 cases.24,25 A phenotype very similar to the small hypo-AF dots seen in this study can be found in at least some cases of CDHR1 retinal degeneration.

FIGURE 5. Multimodal imaging of AR PROM1 patient 1. A vertical SD-OCT b-scan obtained at age 22 is shown in (A), with the corresponding IR-SLO image shown in (B). SW-AF (C) and NIR-AF (D) are overall similar, but locally more extensive changes are observable with the NIR-AF. The SW-AF shows very little focal hyperautofluorescence. The en face image in (E) is a mean intensity projection covering an area of 20° by 20°, at a distance of 24 μm anterior to Bruch’s membrane, with a thickness of 10 μm. This depth location, at the anterior aspect of the retinal pigment epithelium, is demarcated by a red band on a horizontal SD-OCT b-scan in (F). The en face image (E) showed hyporeflective foci, which largely corresponded to the patterns of hypouautofluorescence in (C) and (D).
Ocular features of AR PROM1 CRD

Dark-adapted ERG

Control

Patient 2

Patient 4

Blue flash

White flash

Light-adapted ERG

32Hz white

White flash

Amplitude (100 μV/division)

Time (ms)

0 40 80 120 160

Amplitude (100 μV/division)

Time (ms)

0 40 80 120 160

FIGURE 6. Full-field ERGs of AR PROM1 patient 2 (age 15) and patient 4 (age 21). The top panel shows the dark-adapted responses, with the rod-isolated blue stimulus above and the combined response stimulus below. The bottom panel shows light-adapted responses, with the 32Hz stimulus above, and the single-flash white stimulus below. Gray areas represent normative ranges for a- and b-wave amplitudes and implicit times. A control subject ERG is shown in black, whereas the two AR PROM1 patients are shown in red (patient 2) and blue (patient 4).

dystrophy as well.23,26 Review of published cases in which autofluorescence (primarily SW-AF) was performed in other genetic forms of CRD (neither PROM1 nor CDHR1) showed that, with this imaging modality, a substantial majority did not resemble the cases presented here.12,26-55 However, isolated cases of CRD associated with C8orf37, CERKL, GUCA1A, and RDH12 mutations did show moderate resemblances to the AF images presented here.34-37

The ERG phenotype discussed in this study can be found in three cases published by Eidinger et al.,7 the youngest of whom was 8 years old at the time of testing. One AR PROM1 patient who did not fit this ERG phenotype was a 25-year-old published by Pras et al.,4 in whom the combined response a-wave was relatively prominent, and therefore the b/a ratio appeared to be normal. AR PROM1 full-field ERGs have been reported to be non-detectable in most cases by the third decade of life,11,15,56 hence, setting an upper age limit to the utility of these ERG findings.

There are similarities between AR PROM1 and CDHR1 phenotypes on full-field ERG. Many CDHR1 ERGs are non-detectable by the third or fourth decade.38-40 Of the few well-described, detectable full-field ERGs that have been published, most shared the features described in this report, including a dark-adapted combined/maximal response that has an a-wave that is substantially reduced in proportion to the b-wave.26,41,42 It might be predicted that marked cone dysfunction in conjunction with a loss of rod sensitivity would produce this ERG phenotype in diffuse photoreceptor degenerations (CRD and RP)13,44; however, a review of the literature for ERG waveforms in at least 16 genetic forms of nonsyndromic CRD did not find this proportionally small a-wave to be present in a notable majority of published cases.11,12,27,34,35,37,42,45-54 Among the reviewed CRD cases, only a single CNGA3-associated CRD and six related individuals with RDH12-associated CRD were reported to have proportionally small a-waves with a dark-adapted combined response stimulus.15,55 Also, two cases of CNGB3-associated achromatopsia with “impaired rod-mediated function” demonstrated a “selective a-wave reduction.”56 Thus, the proportionally reduced a-wave finding alone is not specific to AR PROM1 (and CDHR1). In a less exhaustive search of non-CRD retinal dystrophies, isolated cases of EYS (AR RP and RP1 (AD RP)), and two cases of AR GUCY2D “congenital night blindness” also demonstrated proportionally small a-waves to combined/maximal response stimuli.57-59 These RP and congenital night blindness cases,15,55 however, do not demonstrate the grossly delayed waveforms seen in some types of CRD, as in those from this study, or in the above-cited studies on CRD associated with PROM1, CDHR1, or CNGA3.5,26,55 One more example in which the b/a ratio could be increased is KCNV2 retinopathy, in which the a-wave tends to be approximately at the lower limit of normal, whereas the scotopic b-wave tends to be in the upper range of normal or even “super-normal.”56-60 Such findings would not likely be confused with PROM1-associated CRD.

The markedly reduced a-wave in combination with the delayed b-wave is likely due to the loss of cone contribution to the mixed rod-cone response in combination with substantially reduced rod pathway sensitivity. However, when a similar relative preservation of the b-wave (or relative reduction of the a-wave) was found in a rat model of RHO-ADRP, it was suggested that a more active process could be responsible for this finding, such as the development of ectopic rod to bipolar cell synapses in the degenerating retina.56-61 Two studies of PROM1 knockout mice have included a dark-adapted ERG intensity series. In both studies, the a-wave was relatively reduced to the highest luminance scotopic flashes, similar to the RHO-ADRP rat.62,63

High myopia and nystagmus have been reported to occur in AR PROM1, but are not pathognomonic.7 The imaging and electrophysiological findings presented here are also not pathognomonic, and they are not evident at all ages. These findings represent observations that may aid in genotyping, either in targeted genetic testing of the PROM1/CDHR1 genes, or in interpretation of broader genetic screens.

Patient Consent

Written consent to publish this article was obtained from the study subjects. Written informed consent was obtained under both Western IRB and the IRB of Columbia University.
Ocular features of AR PROM1 CRD

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