Clinical Characteristics and Genetic Variants in Taiwanese Patients With *PROM1*-Related Inherited Retinal Disorders

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Purpose. This study investigated the clinical characteristics of patients with *PROM1*-related inherited retinal diseases (IRDs).

Methods. Patients diagnosed with IRDs who had mutations in *PROM1* were identified at Linkou Chang Gung Memorial Hospital and Kaohsiung Medical University Hospital in Taiwan. Information on clinical characteristics and best-corrected visual acuity was recorded. Color fundus (CF) images, fundus autofluorescence photography (FAF), spectral-domain optical coherence tomography (SD-OCT), and electroretinograms (ERGs) were analyzed to examine patient phenotypes. *PROM1* variants were detected using whole exome sequencing and verified by Sanger sequencing.

Results. Fourteen patients from nine families with *PROM1*-related IRDs were analyzed. Most patients exhibited chorioretinal atrophy in the macular area, with or without extramacular involvement on CF. Similarly, hypo-autofluorescence confined to the macular area, with or without extramacular involvement, was present for most patients on FAF. Furthermore, SD-OCT revealed outer retinal tubulations and focal or diffuse retinal thinning. ERGs showed variable findings, including maculopathy with normal ERG, subnormal cone response, and extinguished rod and cone responses. We detected five variants of the *PROM1* gene, including c.139del, c.794del, c.1238T>A, c.2110C>T, and c.1117C>T.

Conclusions. In this study, we evaluated 14 Taiwanese patients with five *PROM1* variants. Additionally, incomplete penetrance of heterozygous *PROM1* variants was observed. Furthermore, patients with autosomal dominant *PROM1* variants had lesions in the macular area and the peripheral region of the retina. SD-OCT serves as a useful tool for early detection of *PROM1*-related IRDs, as it captures certain signs of such diseases.

Keywords: retinitis pigmentosa, macular dystrophy, outer retinal tubulation, *PROM1*

The *PROM1* gene (OMIM 604365) is located on chromosome 4p15.32 and encodes the protein prominin-1.[1] Initially identified as a marker for hematopoietic stem cells, prominin-1 was later found to be expressed in retinoblastoma cell lines and adult retina.[2–4] Maw et al.[1] analyzed a consanguineous Indian pedigree with autosomal recessive (AR) retinal degeneration and identified a homozygous 1-bp deletion in the *PROM1* gene in affected individuals. Since then, variants in the *PROM1* gene have been linked to various inherited retinal diseases (IRDs), including Stargardt disease 4 (OMIM 603786; autosomal dominant [AD]),[5] retinal macular dystrophy 2 (OMIM 608051; AD),[6] retinitis pigmentosa 41 (OMIM 612095; AR),[7] and cone–rod dystrophy 12 (OMIM 612657; AD and AR).[8] Previous studies have reported variable and overlapping phenotypes associated with *PROM1* variants, which can be difficult to differentiate

from other IRDs based on clinical manifestations and ophthalmic examinations.[7,9–14] Therefore, genetic analysis is essential for the diagnosis of these conditions. With the development of high-throughput sequencing technology, whole exome sequencing (WES) provides sufficient power to detect causative variants and confirm clinical diagnoses.[15]

The *PROM1* gene plays a pivotal role in retinal development, producing a protein situated in the outer segments of photoreceptors.[16] This transmembrane glycoprotein is believed to influence disc morphogenesis, photopigment sorting, and photoreceptor autophagy.[1] Despite this understanding, the clinical features of patients afflicted with *PROM1*-related IRDs currently lack a clear explanation based on the identified variants. For example, the connection between inheritance patterns and phenotype expression remains largely elusive. Although recessive and dominant *PROM1* variants are thought to be linked to lesions in the peripheral and central retina respectively, cases with dominant *PROM1* variants have also exhibited peripheral pigmentary deposition. Consequently, there is a need for further investigation to discern the primary impact of *PROM1* variants on rod or cone cells. This study aimed to investigate the clinical characteristics and identify genetic variants associated with *PROM1*-related IRDs in Taiwan. Specifically, we evaluated patients using color fundus (CF) imaging, fundus autofluorescence photography (FAF), spectral-domain optical coherence tomography (SD-OCT), and full-field electroretinography (ERG).

Methods

The protocol of this study adheres to the tenets of the Declaration of Helsinki. It has been approved by the Institutional Review Board of Chang Gung Medical Foundation (IRB No. 201601569B0C602) and Kaohsiung Medical University Hospital (IRB No. KMUHIRB-SV(I)-20230033) in Taiwan. All patients and their accessible family members included in this study provided written informed consent.

Patient Inclusion

In this study, we conducted a retrospective search of databases and enrolled patients with IRDs at Linkou Chang Gung Memorial Hospital and Kaohsiung Medical University Hospital in Taiwan, spanning the period from 2010 to 2022. Notably, Linkou Chang Gung Memorial Hospital and Kaohsiung Medical University Hospital serve as prominent medical centers in northern and southern Taiwan, respectively. Patients with *PROM1* variants were included in our studies. We did not exclude patients with other variants, but only one patient (case 5) exhibited the *CACNA1F* variant (c.3115G>T). To evaluate the inheritance patterns and clinical manifestations of the *PROM1*-related IRDs, we also included accessible family members of the affected patients in our study.

Clinical Assessment

All patients included in this study, along with their accessible family members, underwent comprehensive assessments, which included an analysis of their general and ocular medical records. Additionally, we recorded the patients' best-corrected visual acuity and collected data from patients' CF images, FAFs, SD-OCTs, and full-field ERGs. T-YL, EY-CK, P-KL, and N-KW were responsible for determining the clinical image findings, and any discrepancies were resolved through discussions.

Color Fundus

The CF images were captured using a digital fundus camera (Nonmyd-α-DIII; Kowa Optimed, Chou-ku, Japan). Ultra-widefield fundus images were taken with an Optos 200Tx (Optos PLC, Dunfermline, UK).

Fundus Autofluorescence

To perform FAF, we used a confocal scanning laser ophthalmoscope (Heidelberg Retina Angiograph; Heidelberg Engineering, Heidelberg, Germany), which enabled us to obtain high-resolution photographs. Automated eye tracking was utilized to improve the accuracy and quality of the images.

Spectral-Domain Optical Coherence Tomography

SD-OCT images were obtained using either the RTVue (Optovue, Inc., Fremont, CA, USA) or SPECTRALIS HRA+OCT (Heidelberg Engineering).

Full-Field ERG

Full-field electroretinography was performed in accordance with the International Society for Clinical Electrophysiology of Vision (ISCEV) standards,[17,18] using Burian Allen contact lens electrodes and either the UTAS-E3000 system (LKC Technologies, Gaithersburg, MD, USA) or the Espion Visual Electrophysiology System (Diagnosys LLC, Lowell, MA, USA), as previously described.[19,20]

Genetic Analysis

The DNA of all included patients and their accessible family members was extracted from peripheral blood using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Whole-exome sequencing was performed as previously described,[21] and variants of the *PROM1* gene identified were subsequently verified through Sanger sequencing. The pathogenicity assessment result of each detected variant is presented according to the American College of Medical Genetics and Genomics (ACMG) guidelines.[22]

Results

Demographic Data

A total of 14 patients from nine families diagnosed with IRDs and who had at least one *PROM1* variant were included in our study. Detailed demographic information is summarized in Table 1. The age range of patients at evaluation was from 7 to 63 years; the five males and nine females were all from Taiwan. The pedigrees of the nine families are presented in Figure 1. Cases 7 and 8 were cousins; cases 9 and 10 are the father and aunt, respectively, of case 11; and case 12 is the mother of cases 13 and 14. Although the family history of most patients was unremarkable, case 2 had consanguineous parents who were first cousins.

Clinical Findings

The results of ophthalmic examination are presented in Table 2. Blurred vision was the most common symptom, reported by eight out of 14 patients (57.1%); one patient reported each of the following symptoms: night blindness, photophobia, distorted vision, and color impairment. None

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Table 1. Demographic Data and Genetic Profiles of Patients With Inherited Retinal Diseases

Case	Age of Onset, Y	Age of OPD Visit, Y[*]	Sex	Diagnosis	Genotype	Gene	Nucleotide Change[†]	Amino Acid Change	Variant Effect	Pathogenicity[‡]
1	16	22	M	Retinitis pigmentosa	Homozygous	*PROM1*	c.139del	p.His47IlefsTer12	Frameshift	Pathogenic
2[§]	25	36	F	Retinitis pigmentosa	Homozygous	*PROM1*	c.139del	p.His47IlefsTer12	Frameshift	Pathogenic
3	44	51	F	MD	Heterozygous	*PROM1*	c.139del	p.His47IlefsTer12	Frameshift	Pathogenic
4	30s	54	F	MD	Heterozygous	*PROM1*	c.139del	p.His47IlefsTer12	Frameshift	Pathogenic
5[||]	6	10	M	MD with CSNB	Heterozygous	*PROM1*	c.139del	p.His47IlefsTer12	Frameshift	Pathogenic
6	41	62	F	Retinitis pigmentosa	Heterozygous	*PROM1*	c.794del	p.Glu265GlyfsTer9	Frameshift	Pathogenic
					Heterozygous	*PROM1*	c.1238T>A	p.Val413Asp	Missense	Likely pathogenic
7[¶]	Childhood	43	F	Cone–rod dystrophy	Heterozygous	*PROM1*	c.1117C>T	p.Arg373Cys	Missense	Pathogenic
8[¶]	28	32	F	MD	Heterozygous	*PROM1*	c.1117C>T	p.Arg373Cys	Missense	Pathogenic
9[#]	30	59	F	MD	Heterozygous	*PROM1*	c.1117C>T	p.Arg373Cys	Missense	Pathogenic
10[#]	45	63	M	Cone–rod dystrophy	Heterozygous	*PROM1*	c.1117C>T	p.Arg373Cys	Missense	Pathogenic
11[#]	2	35	F	Retinitis pigmentosa	Heterozygous	*PROM1*	c.1117C>T	p.Arg373Cys	Missense	Pathogenic
12[**]	16	50	F	Cone–rod dystrophy	Heterozygous	*PROM1*	c.2110C>T	p.Arg704Cys	Missense	Pathogenic
13[**]	N/A	12	M	MD	Heterozygous	*PROM1*	c.2110C>T	p.Arg704Cys	Missense	Pathogenic
14[**]	N/A	7	M	MD	Heterozygous	*PROM1*	c.2110C>T	p.Arg704Cys	Missense	Pathogenic

AD, autosomal dominant; AR, autosomal recessive; CSNB; congenital stationary night blindness; F, female; FH, family history; OPD, outpatient department; M, male; MD, macular dystrophy.

[*] The age of OPD visit indicates the age at the most recent OPD visit.

[†] The variant was presented based on NM_006017.3.

[‡] The pathogenicity of the variant was presented according to the ACMG guidelines.

[§] This patient had consanguineous parents who were first cousins.

[||] This patient had another variant, the c.3115G>T variant of *CACNA1F* gene, which was presented based on NM_001256789.3.

[¶] These patients are cousins.

[#] These patients are from family 8.

[**] These patients are from family 9.

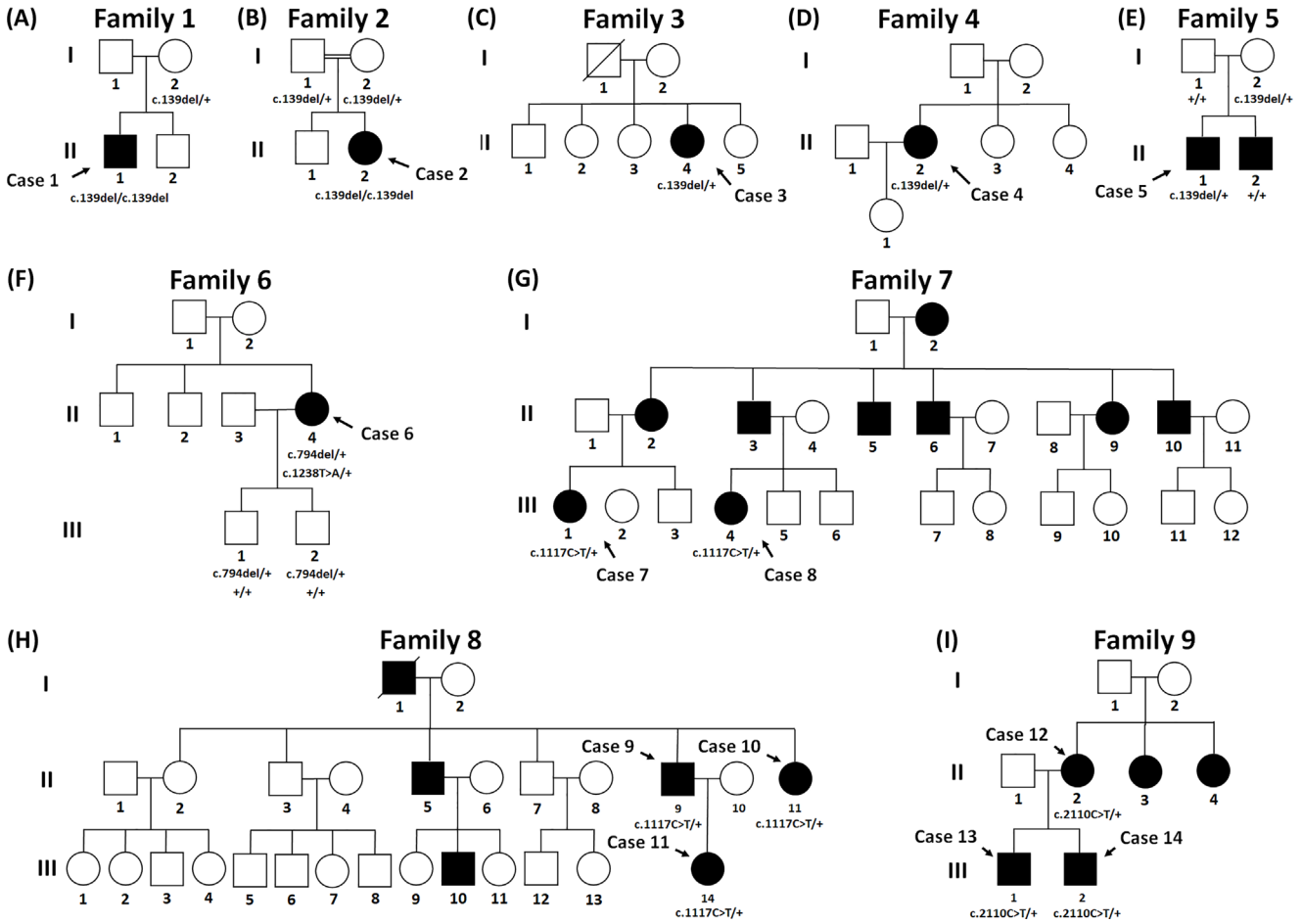

Figure 1. Family trees of the patients. The *black box* indicates the person with affected vision. Genetic analysis was conducted on all included patients and their accessible family members, and the genetic profiles are included under the *boxes*. Notably, case 2 had consanguineous parents who were first cousins.

of the patients had any systemic involvement, and most denied any history of ocular surgery (12/14, 85.7%). The only exceptions were case 4 and case 12. In case 4, a vitrectomy was conducted to address retinal detachment in the right eye, and another medical center had administered an intravitreal injection to manage a previous macular lesion in the left eye. This macular lesion was deemed to be connected with age-related macular degeneration. In case 12, surgical interventions were undertaken in both eyes. Cataract surgery was performed, and scleral buckling and vitrectomy procedures were undertaken to address retinal detachment. Best-corrected visual acuity at the time of evaluation ranged from 20/20 to hand motion at 5 cm.

Color Fundus Images

CF images were obtained in all cases, as shown in Figure 2. Regarding the c.139del variant, four of the five patients (80.0%; cases 1, 2, 3, and 4) presented with pigmentary changes, which were located parafoveally and temporally in cases 1 and 2, respectively (Figs. 2A, 2B). In cases 3 and 4, pigmentary changes were located in the macular area (Figs. 2C, 2D). Case 5 exhibited prominent choroidal vessels, potentially linked to retinal thinning caused by congenital stationary night blindness and myopia (≤–5.00 D) (Fig. 2E).[19,23] Case 6, which had the c.794del variant, exhibited pigmentary changes in the diffuse retina (Fig. 2F). Three of the five patients with the c.1117C>T variant (60.0%; cases 7, 8, and 11) presented with pigmentary changes located in the macular area (cases 7 and 8) (Figs. 2G, 2H) and the diffuse retina (case 11) (Fig. 2K). Prominent choroidal vessels were noted in cases 9 and 10 which may be related to thinning of the retina (Figs. 2I, 2J). The three patients with the c.2110C>T variant (100.0%; cases 12, 13, and 14) presented with pigmentary changes located in the macula (Figs. 2L–2N).

Fundus Autofluorescence

FAF was obtained from all cases (Fig. 3). Four of the five patients with the c.139del variant (80.0%; cases 1, 2, 3, and 4) presented with hypo-AF spots, located in the diffuse retina (Figs. 3A, 3B, 3C, and 3D). Case 1 showed bull's eye maculopathy (Fig. 3A). However, case 5 did not show any hypo-AF or hyper-AF lesions in the retina, possibly due to the patient's young age (Fig. 3E). Only granular changes were observed in case 5. Case 6 which had the c.794del variant, exhibited hypo-AF lesions in the diffuse retina (Fig. 3F). Four of the five patients with the c.1117C>T variant (80.0%; cases 7, 8, 9, and 10) presented with hypo-AF lesions with surrounding hyper-AF rings in the macular area (Figs. 3G–3J). Case 8 showed bull's eye maculopathy (Fig. 3H), and

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Table 2. Clinical Characteristics of Patients With Inherited Retinal Diseases

Case	Symptom	Ocular History	BCVA (OD; OS)	Variant		Image Finding
1	Night blindness	Myopia (−7.00 D)	20/200; 20/100	c.139del	CF	Pigmentary change in parafoveal, arcade, and temporal area
					FAF	Bull's eye maculopathy and hypo-AF spots around arcades
					SD-OCT	ORT, diffuse retinal thinning, and loss of ellipsoid zone in fovea
					ERG	Extinguished response of rod and cone
2	Blurred vision	None	20/666; 20/286	c.139del	CF	Pigmentary change in parafoveal and temporal areas
					FAF	Hypo-AF lesion in macular area and hypo-AF spots around arcades
					SD-OCT	Diffuse retinal thinning
					ERG	Extinguished response of rods and cones
3	Photophobia	Myopia (−6.25 D)	20/200; 20/2000	c.139del	CF	Pigmentary change in macular area
					FAF	Hypo-AF lesion in macular area and hypo-AF spots around arcade
					SD-OCT	ORT, diffuse retinal thinning, and foveal atrophy
					ERG	N/A
4	Distorted vision	Myopia (−8.00 D) TPPV, OD IVI, OS	20/40; 20/100	c.139del	CF	Pigmentary change in macular area
					FAF	Hypo-AF lesion in macular area and hypo-AF spots around arcade
					SD-OCT	Loss of ellipsoid zone in fovea and retinal thinning
					ERG	N/A
5	Blurred vision	Myopia (−5.25 D) Exotropia Nystagmus	20/100; 20/100	c.139del	CF	Prominent choroidal vessels
					FAF	No hypo-AF or hyper-AF lesion and granular due to nystagmus
					SD-OCT	Loss of COST line and choroidal thinning at nasal macula
					ERG	Electronegative waveform
6	Blurred vision	None	CF/10 cm; HM/100 cm	c.794del c.1238T>A	CF	Diffuse pigmentary change in retina
					FAF	Diffuse hypo-AF lesions within and outside macula
					SD-OCT	ORT, diffuse retinal thinning, and foveal atrophy
					ERG	Extinguished scotopic and photopic responses
7	Blurred vision	None	CF/10 cm; 20/2000	c.1117C>T	CF	Pigmentary change in macular area
					FAF	Hypo-AF lesion with surrounding hyper-AF ring in macular area and hypo-AF spots outside the arcades
					SD-OCT	ORT and diffuse retinal thinning
					ERG	Cone–rod dystrophy
8	Color impairment	None	20/25; 20/22	c.1117C>T	CF	Pigmentary change in macular area
					FAF	Bull's eye maculopathy and hyper AF spots at parafovea
					SD-OCT	Outer nuclear thinning and relatively foveal preservation
					ERG	Maculopathy with subnormal scotopic and photopic responses

Table 2. Continued

Case	Symptom	Ocular History	BCVA (OD; OS)	Variant		Image Finding
9	Blurred vision	Myopia (−5.00 D)	20/1600; 20/1600	c.1117C>T	CF	Chorioretinal atrophy with prominent choroidal vessels at macula
					FAF	Hypo-AF lesion with surrounding hyper-AF ring in macular area
					SD-OCT	ORT, foveal atrophy
					ERG	Normal
10	Blurred vision	Myopia (−1.00 D)	20/800; 20/1600	c.1117C>T	CF	Chorioretinal atrophy with prominent choroidal vessels at macula
					FAF	Hypo-AF lesion with surrounding hyper-AF ring in macular area
					SD-OCT	ORT, foveal atrophy
					ERG	Cone–rod dystrophy
11	Blurred vision and color impairment	None	20/800; HM/5 cm	c.1117C>T	CF	Diffuse pigmentary change in retina and macula
					FAF	Hypo-AF lesions at macula and diffuse hypo- and hyper-AF lesions in retina
					SD-OCT	ORT, diffuse retinal thinning, and foveal atrophy
					ERG	Extinguished response of rod and cone
12	Blurred vision	Myopia (−5.00 D) Scleral buckle, OU; cataract surgery, OU	20/60; CF/20 cm	c.2110C>T	CF	Pigmentary change in macular area, OD; pigmentary change with multiple RPE atrophy and scarring in the posterior pole
					FAF	Hypo-AF lesion with surrounding hyper-AF ring in macular area and hypo-AF spots outside the arcades, OD; diffuse hypo- and hyper-AF lesions within and outside macula, OS
					SD-OCT	Diffuse retinal thinning, and foveal atrophy
					ERG	Cone–rod dystrophy
13	None	None	20/20; 20/20	c.2110C>T	CF	Pigment change at fovea
					FAF	Mild hyper-AF lesion at foveola with mild parafoveal surrounding hyper-AF ring
					SD-OCT	Blurring and interruption of ellipsoid and interdigitation zone at fovea
					ERG	Normal
14	None	None	20/20; 20/20	c.2110C>T	CF	Pigment change at fovea
					FAF	Mild hyper-AF lesion at foveola with mild parafoveal surrounding hyper-AF ring
					SD-OCT	Optical gap, blurring of ellipsoid and interdigitation zone at fovea
					ERG	Normal

AF, autofluorescence; BCVA, best-corrected visual acuity; CF, color fundus; COST, cone outer segment termination; CF, counting finger; ERG, electroretinogram; FAF, fundus autofluorescence; HM, hand motion; IVI, intravitreal injection; N/A, not applicable; OD, oculus dexter; OS, oculus sinister; SD-OCT, spectral-domain optical coherence tomography; ORT, outer retinal tubulation; TPPV, trans pars plana vitrectomy.

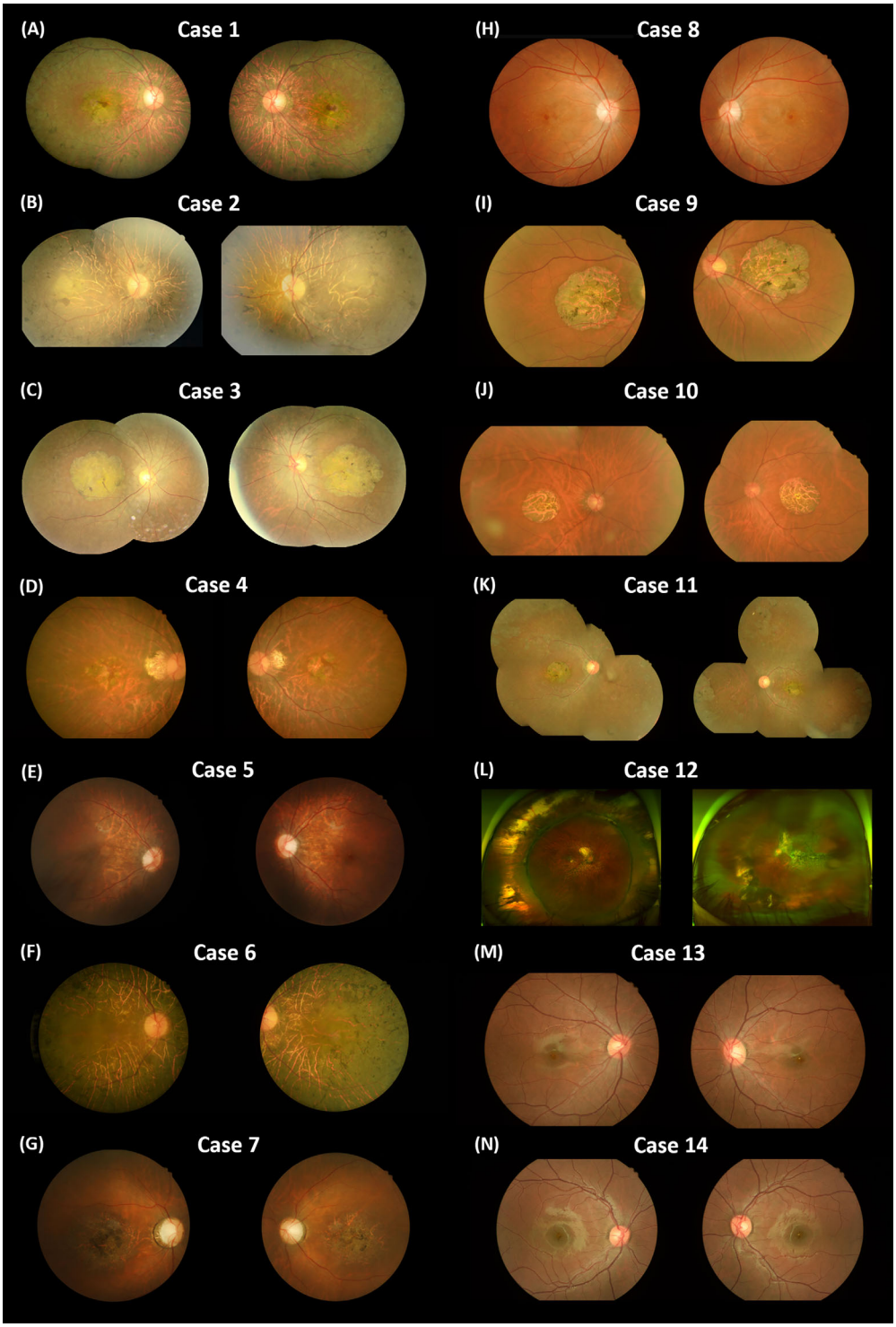

Figure 2. Color fundus of the patients. Most cases had pigmentary changes (all except for cases 5, 9, and 10), and two cases with *PROM1* heterozygous variants had lesions in the macular area and peripheral retina (cases 6 and 10). Three cases presented prominent choroidal vessels (cases 5, 9, and 10).

case 11 demonstrated hypo-AF lesions at macula and diffuse hypo- and hyper-AF lesions in the retina (Fig. 3K). Two of the three patients with the c.2110C>T variant (66.7%; cases 13 and 14) presented with mild hyper-AF lesions in the foveola with a mild hyper-AF ring parafoveally (Figs. 3M, 3N). Case 12 exhibited diffuse hypo- and hyper-AF lesions within and outside macula (Fig. 3L).

Spectral-Domain Optical Coherence Tomography

SD-OCT images were obtained from all cases (Fig. 4). Two of the five patients with the c.139del variant (40.0%; cases 1 and 3) presented with outer retinal tubulation (ORT) (Figs. 4A, 4C). Four of the five patients (80.0%; cases 1, 2, 3, and 4) reported diffuse retinal thinning (Figs. 4A–4D).

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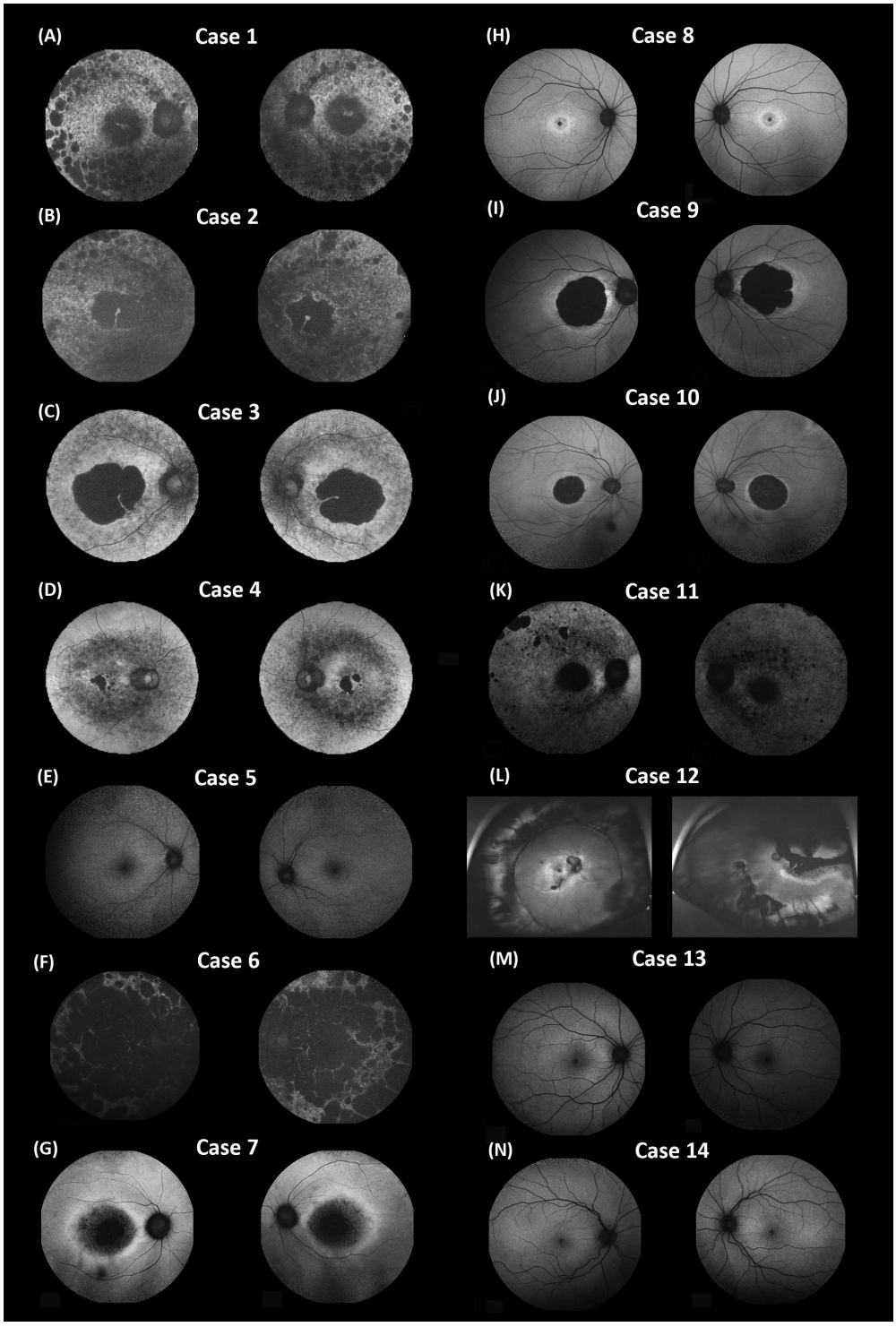

Figure 3. Fundus autofluorescence of the patients. Most cases had pigmentary changes (all except for cases 5, 8, 13, and 14). Two cases with *PROM1* heterozygous variants demonstrated lesions in the macular area and peripheral retina (cases 3, 4, 6, 7, 11, and 12). Eight cases showed hyper-AF lesions, and hyper-AF lesions in the diffuse retina were observed in two cases (cases 11 and 12). Additionally, two patients had bull's eye maculopathy (cases 1 and 8).

Two of the five patients (40.0%; cases 1 and 4) exhibited loss of the ellipsoid zone (EZ) in the fovea (Figs. 4A, 4D). Case 3 showed foveal atrophy (Fig. 4C). Case 5 demonstrated loss of the cone outer segment termination (COST) line and choroidal thinning at the nasal macula (Fig. 4E). Case 6 had the c.794del variant and demonstrated ORT, diffuse retinal thinning, and foveal atrophy (Fig. 4F). Two of the five patients with the c.1117C>T variant (40.0%; cases 7 and 11) reported diffuse retinal thinning (Figs. 4G, 4K). Three of the five patients (60.0%; cases 9, 10, and 11) reported foveal

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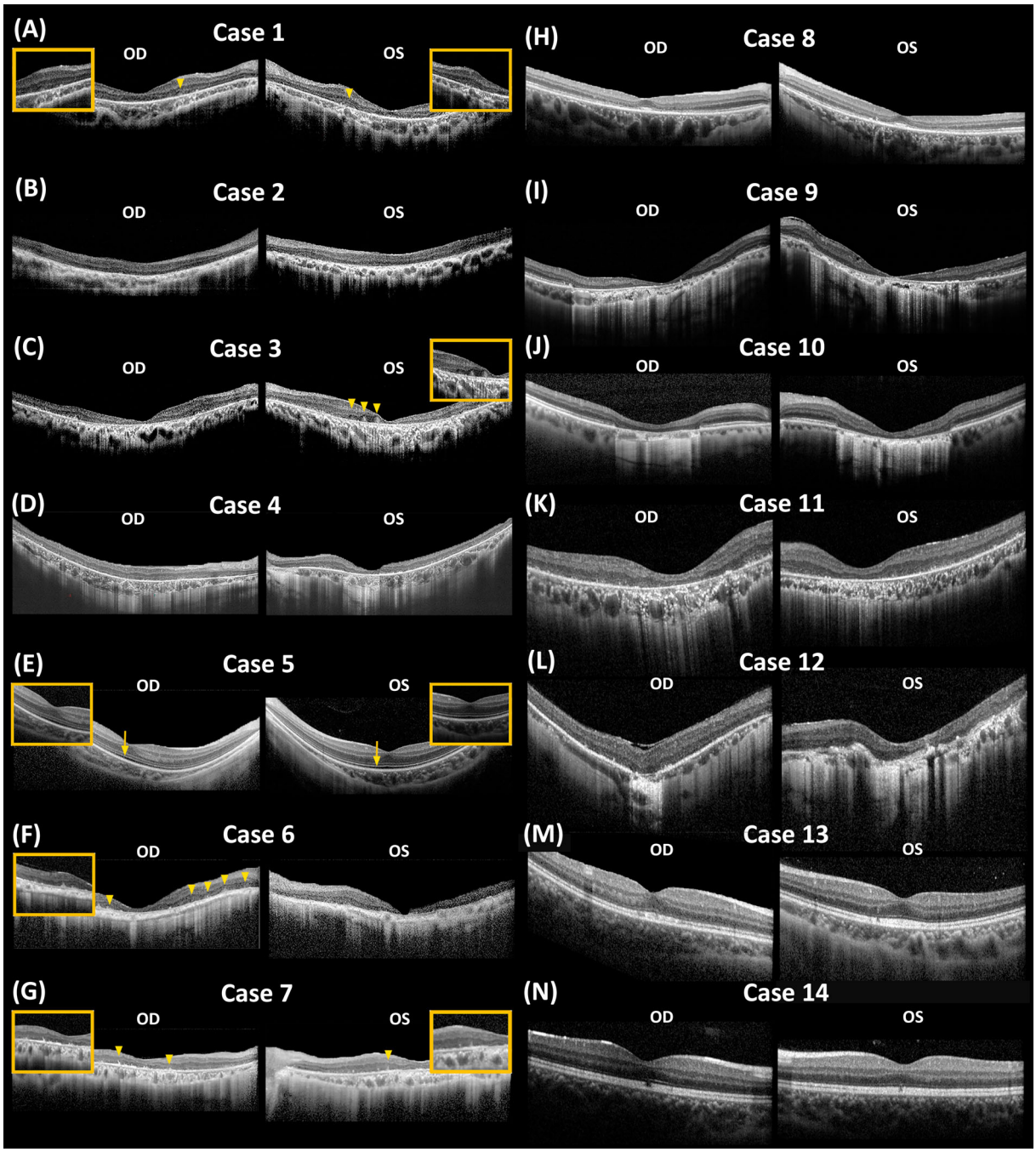

Figure 4. OCT of the patients. A *yellow arrow* indicates loss of the COST line. Four cases showed ORT (cases 1, 3, 6, and 7). Four cases reported impairment of the ellipsoid zone in the fovea (cases 1, 4, 13, and 14). Case 5 presented loss of the COST line, and case 14 had an optic gap in the foveal area.

atrophy (Figs. 4I–4K). Four of the five patients (80.0%; cases 7, 9, 10, and 11) showed ORT (Figs. 4G, 4I–4K). Case 8 demonstrated outer nuclear thinning and relatively foveal preservation (Fig. 4H). Two of the three patients with the c.2110C>T variant (66.7%; cases 13 and 14) presented with blurring of the ellipsoid and interdigitation zone at fovea (Figs. 4M, 4N). Case 12 reported diffuse retinal thinning and foveal atrophy (Fig. 4L). Case 14 showed an optical gap (Fig. 4N).

Electroretinogram

Electroretinography was performed on 12 patients, and the results are presented in Figure 5. Two of the patients with the c.139del variant (66.7%; cases 1 and 2) presented with an extinguished response of the rods and cones (Figs. 5A, 5B). Case 5 had an electronegative waveform (Fig. 5C). Case 6 had the c.794del variant and showed extinguished scotopic and photopic responses (Fig. 5D). Two of the five patients with the c.1117C>T variant (40.0%; case 7

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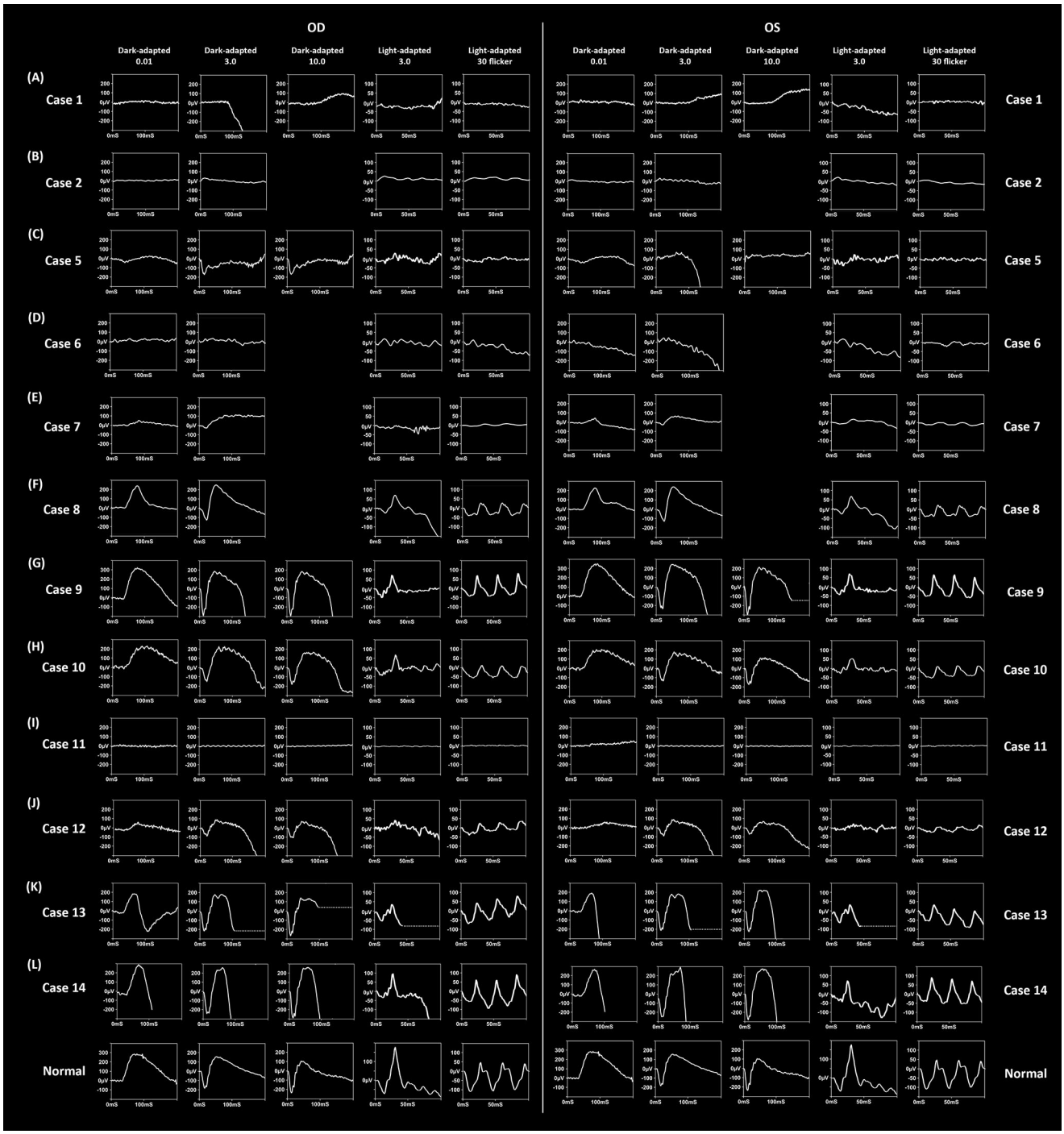

Figure 5. Electroretinograms of the patients. Three cases showed extinguished response of rod and cone (cases 1, 2, and 11). Three cases demonstrated cone–rod dystrophy (cases 7, 10, and 12). An electronegative waveform was present in case 5. Case 6 had extinguished scotopic and photopic responses, and case 8 had a maculopathy with subnormal scotopic and photopic responses.

and 10) exhibited cone–rod dystrophy (Figs. 5E, 5H). Case 8 exhibited subnormal scotopic and photopic responses (Fig. 5F). Case 9 had a normal ERG (Fig. 5G). Case 11 had extinguished responses of the rods and cones (Fig. 5I). Regarding the c.2110C>T variant, two of the three patients (66.7%; cases 13 and 14) presented with a normal ERG (Figs. 5K, 5L). Case 12 exhibited a cone–rod dystrophy (Fig. 5J).

Genetic Results

Genetic analysis by WES was performed in all cases. Detailed information regarding the genetic profiles is summarized in Table 1, and the pathogenicity results are presented in Supplementary Table S1. A total of five variants in the *PROM1* gene were detected in the 14 patients. The c.139del[24] (cases 1, 2, 3, 4, and 5), c.1238T>A[25] (case 6), c.2110C>T[26] (cases 7, 8, 9, 10, and 11), and c.1117C>T[10] (cases 12,

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13, and 14) variants were known, but the c.794del variant was novel according to the ClinVar database. The c.794del variant was classified using the ClinGen Sequence Variant Interpretation Working Group criteria of PVS1 and PM2. With respect to PVS1, this frameshift variant was anticipated to induce loss of function through nonsense-mediated mRNA decay, a well-established disease-causing mechanism associated with *PROM1*-related IRDs. The variant was absent in the Genome Aggregation Database (gnomAD), suggesting its rarity among the general population. The most common variants were c.139del and c.1117C>T (5/14, 35.7%), and other variants included c.794del, c.1238T>A, and c.2110C>T. Notably, the c.139del variant was homozygous in two patients and heterozygous in three patients. Other variants in different genes identified through WES encompassed the c.3115G>T (p.Glu1039Ter) variant within the *CACNA1F* gene (case 5). This variant was classified as likely pathogenic in accordance with the rules of PVS1 (null variant) and PM2 (absent from controls) of the ACMG guidelines. Their accessible family members were examined to determine the penetrance of these variants (see Supplementary Table S2). None of the reported family members associated with our cases exhibited any clinical symptoms, and, among them, four individuals had a history of myopia. The CF images, FAF, and SD-OCT of accessible family members are presented in Supplementary Figures S1, S2, and S3, respectively. These family members were found to be unaffected in the aforementioned ophthalmic examinations. Based on the clinical findings, genetic profile, and inheritance mode, the disease-causing variant was determined.

Discussion

This study documented 14 patients with variants in the *PROM1* gene who were recruited from two medical centers, one in northern Taiwan and the other in southern Taiwan. A novel variant, c.139del, was identified within the *PROM1* gene. This variant may to lead to IRDs through both AR and AD inheritance modes. Notably, it was observed that *PROM1* AD variants did not consistently result in manifestation of the associated phenotype, indicating incomplete penetrance. Interestingly, unlike previous reports on *PROM1*-related IRD cases where AD variants were primarily associated with central lesions in the retina and AR variants were typically linked with peripheral lesions, our cohort displayed widespread retinal involvement irrespective of their inheritance modes. Additionally, a novel pathogenic variant, c.794del, was detected in case 6. Furthermore, the severity of *PROM1*-related IRDs did not appear to correlate with the age of patients within our cohort. Among the younger cases in our series, we uncovered intriguing features within SD-OCT images that could potentially serve as early indicators of the disease. For example, case 5 exhibited a reduced width of the EZ line, whereas case 11 displayed an optic gap in the EZ line. In summary, this study offers a comprehensive evaluation of the clinical characteristics and genetic variants among patients affected by *PROM1*-related IRDs.

In our study, five out of the 14 patients were found to carry the c.139del variant in the *PROM1* gene, which has been mainly reported in Asians.[7,24,27–29] Significantly, this variant was associated with both AR and AD retinal diseases in our cases. To the best of our knowledge, this is the first study to showcase the c.139del variant within the *PROM1* gene manifesting in both AR and AD inheritance modes. Notably, the mother of case 1 and the parents of case 2 were identified as carriers of the same heterozygous c.139del variant in the *PROM1* gene as seen in case 3. However, these asymptomatic parents with the heterozygous c.139del variant could be explained by incomplete penetrance, leading to the lack of a discernible phenotype.[30] This may be attributed to unknown modifiers influencing the gene expression network, thereby contributing to incomplete penetrance. Lee et al.[31] proposed that the presence of an *ABCA4* variant might exacerbate the severity of *PROM1*-related IRDs. Nevertheless, we were unable to identify any *ABCA4* variants in case 1, the mother of case 1, case 2, or the parents of case 2. According to our review of the literature, no study has explored the penetrance of *PROM1* variants so far. Only a few publications investigating the c.1117C>T variant of the *PROM1* gene have provided detailed information on their accessible family members. For example, Michaelides et al.[10] annotated the genotypes of proband and certain family members, and complete penetrance of c.1117C>T variant of *PROM1* gene was mentioned. However, not all unaffected family members received full ophthalmic examinations, such as image studies and genetic analyses. It is possible that some asymptomatic family members have the mutation, which could indicate incomplete penetrance of the c.1117C>T variant. The collection of detailed information through retinal exams and genetic tests of all family members with or without symptoms is needed to further investigate penetrance of the *PROM1* variant.

The location of the affected retina may be associated with the inheritance mode of the gene variant in cases with the *PROM1* variant. Previous studies by Cehajic-Kapetanovic et al.[32] and Michaelides et al.[10] have reported that *PROM1* recessive variants were related to degeneration over the pan-retina. Michaelides et al.[6] and Del Pozo-Valero et al.[33] proposed that *PROM1* dominant variants were associated with lesions located in the central retina. Similarly, in our series, cases 1 and 2 had the homozygous c.139del variant and presented with bull's eye maculopathy initially and peripheral lesions subsequently, whereas cases 3 and 4 had the heterozygous c.139del variant and showed impairment over the macular area. However, the patients in our cohort with the heterozygous c.1117C>T variant demonstrated central and peripheral lesions. Previous publications have documented cases of *PROM1*-related IRDs in Asia, with a significant association identified primarily for the c.1117C>T variant.[34,35] Wang et al.[34] conducted a study in China, reporting on 10 individuals who exhibited a heterozygous presentation of the c.1117C>T variant. Their study revealed the presence of macular lesions in these cases, along with peripheral pigment disposition captured through widefield fundus photography. Notably, older individuals affected by the condition displayed more prominent pigmentary deposits compared to younger affected individuals within the same family. As a result, it was concluded that IRDs associated with the heterozygous c.1117C>T variant represent distinct stages of a singular disease process. This disease impacts central cones in the initial phase and progresses to affect peripheral rods in the later stage.[34] However, simultaneous or closely successive dysfunction of two photoreceptors often makes it difficult to distinguish their priority of impairment. The *PROM1* variant was associated with the production of transmembrane glycoprotein on the outer segments of photoreceptors and disk morphogenesis, photopigment sorting, and photoreceptor autophagy.[1,16]

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Notably, cones were more prone to dysfunction in *PROM1*-related retinal diseases of both AR and AD modes compared to rods. The lamellar membrane in the outer segments of cones expresses a wider distribution of *PROM1* than in rods. The more opened structure of cones allows greater exposure to extracellular spaces and deterioration via premature degeneration. More experiments are needed to understand the association among inheritance mode, photoreceptor structure, and location of the affected retina.

In case 6, we identified two likely pathogenic variants in the *PROM1* gene (c.794del and c.1238T>A) by whole exome sequencing according to ACMG guidelines. To further determine the segregation and phase of these compound heterozygous variants, we sequenced the *PROM1* genes of the two asymptomatic sons of case 6 and found that both of them carried the c.794del variant. However, when these two sons reached their 30s, their fundus photography, FAF, SD-OCT, and ERGs were all found to be normal. Unless this variant causes relatively late-onset macular or retinal degeneration, the normal phenotype in these two sons with the c.794del variant could be explained by an unknown modifier. Furthermore, no pathogenicity of the c.794del variant served as an alternative cause. Despite its being a truncating deletion, the available phase testing did not provide sufficient support for its pathogenicity. Further investigation is required, including a longer follow-up on these two sons, evaluating more cases with the c.794del variant, and additional study to investigate the pathogenesis of these *PROM1* variants in IRDs.

Unlike truncating variants that lead to loss of function through nonsense-mediated mRNA decay, missense variants could induce disease through different mechanisms. First, mutant proteins might interfere with wild-type proteins through a dominant-negative mechanism.[36] Regarding the c.1238T>A variant, the ocular symptoms and positive findings in the ophthalmic examination of case 6 could potentially be attributed to a dominant-negative effect. However, this variant was not present in the two sons of case 6, making it difficult to ascertain its dominant-negative effect. Furthermore, all of the cases with the heterozygous c.2110C>T variant (cases 12, 13, and 14) presented positive findings on their ophthalmic examinations, such as pigmentary changes in CF and hypo- or hyper-AF lesions in FAF. Although c.2110C>T could have a dominant-negative effect on wild-type *PROM1* protein in this family, further studies using knock-in mouse models are necessary to elucidate its effect. Also, *PROM1* missense variants might impact other protein products. Yang et al.[16] proposed that the c.1117C>T variant led to the mislocalization of both *PROM1* and *PCDH21*. They reported an overlapping mislocalized immunolabeling pattern in photoreceptors between patients with the *PROM1* and *PCDH21* variants. As a result, the loss of *PCDH21* function might be connected to the dominant effect of the *PROM1* c.1117C>T variant on retinal degeneration. Additional studies are required to explore the interactions involving *PROM1* and other proteins.

The severity of *PROM1*-related IRD might not be related to age. In our study, two families (families 7 and 8) carried the c.1117C>T variant. In family 7, the older cousin (case 7) showed a larger macular lesion and cone–rod dystrophy on ERG, but case 8 had a smaller macular lesion on imaging and maculopathy on ERG. However, in family 8, case 11, the youngest case in the family, had a diffuse retinal involvement, which was more severe than that of her father (case 9) and aunt (case 10). Thus, the youngest patient in the same family demonstrated a more severe phenotype than older affected family members. Similar with the outcomes of previous study, Fujinami et al.[35] detailed their observations of 10 patients from eight Japanese families afflicted with *PROM1*-associated retinal disorders. This group displayed distinctive characteristics, featuring macular atrophy coupled with cone (–rod) dysfunction. Notably, the c.1117C>T variant emerged as being predominant (66.7%) within the Japanese population, reflecting its prevalence in Europe, as well. Of particular interest, a case with an 11-year disease duration exhibited better visual acuity compared to cases with durations of 8 and 47 years. This observation suggests that there might not be a direct correlation between disease duration and the severity of *PROM1*-related IRDs. This difference could potentially be attributed to foveal sparing seen in bull's-eye maculopathy.[37] Further research is imperative to uncover the intricate relationship between age and the severity of *PROM1*-related IRDs.

When examining whether a genotype–phenotype correlation exists based on loss-of-function and missense variants, our observations unveiled distinct phenotypic outcomes, particularly in relation to the loss-of-function variant c.139del. Specifically, individuals with a homozygous c.139del variant (cases 1 and 2) exhibited more severe and widespread phenotypes, characterized by retinitis pigmentosa. In contrast, patients possessing a heterozygous c.139del variant (cases 3 and 4) presented with macular dystrophies. However, a significant aspect that remained uncertain was whether those with a heterozygous variant would progress to develop extensive retinitis pigmentosa as they age. Furthermore, we encountered an intriguing scenario in case 6, who carried the heterozygous loss-of-function variant c.794del. Notably, the severity of her phenotypes could be attributed to the presence of an additional missense c.1238T>A variant. This secondary variant resulted in the absence of the normal wild-type PROM1 protein, providing a plausible explanation for the heightened clinical manifestations observed in this case. Aside from the c.1238T>A variant, which remained challenging to assess due to its coexistence with the c.794del variant in case 6, it is noteworthy that patients harboring the other two missense variants (c.1117C>T and c.2110C>T) displayed varying degrees of phenotypic severity. Age alone may not be the sole determinant contributing to the observed variations in disease stages or phenotypes. This insight underscores the complex interplay of genetic and environmental factors that contribute to the clinical manifestations of these missense variants.

SD-OCT emerged as an effective tool for patients with *PROM1*-related IRDs, particularly when other testing methods failed to yield revealing results. Through SD-OCT scans, we identified some indicative signs, such as ORT, loss of the COST line, and an optical gap in the EZ line, among patients who carried *PROM1* variants. ORT was commonly observed in our cases (4/14, 28.6%) and is a prominent feature in various retinal diseases.[38] After retinal impairment, the rearrangement of photoreceptors presents as round hypo-AF spaces along with hyper-AF borders in the outer nuclear layer under SD-OCT.[39] In SD-OCT scans, we observed ORT in four of the 14 patients (28.6%) and early signs in two young patients (14.3%). Both case 5 and his younger brother initially presented with decreased vision during their first visit, yet CF and FAF did not exhibit any lesions. Notably, both cases carry the c.3115G>T variant in the *CACNA1F* gene, which is classified as likely pathogenic based on the rules of PVS1 (null variant) and PM2 (absent from controls) of the ACMG guidelines.[22] This variant can lead to congeni-

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tal stationary night blindness and electronegative ERGs. Our prior study suggested that *CACNA1F* gene variants causing X-linked IRDs could impact the response of cones and rods as detected on ERG but not the presentation on OCT.[19] In this context, these two boys shared a "hemizygous" variant in the *CACNA1F* gene, which also happened to be a nonsense variant considered likely pathogenic. However, we observed a thinner EZ line and loss of the COST line solely in case 5, not in his younger brother, who does not carry the *PROM1* variant. Therefore, the SD-OCT changes in case 5 could potentially be linked to the c.139del variant in the *PROM1* gene. On the other hand, the SD-OCT of case 14 showed an "optical gap" in the EZ line that has not been previously described in cases with *PROM1* variants. Compared to the relatively normal appearance on CF and FAF in young patients with *PROM1* variants, SD-OCT is more likely to detect subtle *PROM1*-related lesions in the early stages of disease. Regular monitoring with SD-OCT is crucial before arranging further evaluation for these young patients.

Our study evaluated the clinical manifestations of 14 patients with *PROM1*-related IRDs. However, there are some limitations of the study. First, this study was retrospective and cross-sectional. A prospective cohort study would be necessary to analyze the disease progression and variation of phenotype in patients with *PROM1*-related IRDs. Second, our study included only a limited number of patients due to the low prevalence of *PROM1*-related IRDs. However, we identified certain patients with the same *PROM1* variants, such as c.139del and c.1117C>T, for comparison. Third, not all affected family members underwent ophthalmic examination and genetic analysis. Nevertheless, we collected information from all included patients and their accessible family members to investigate the penetrance of the *PROM1* variant. Finally, the precise pathogenesis of *PROM1*-related IRDs remains unknown. Further research is needed to explore the association between the *PROM1* variant and IRDs.

Conclusions

This study details *PROM1*-related IRDs in patients from two centers in Taiwan and has identified five variants of the *PROM1* gene. Notably, patients carrying the c.139del variant exhibited *PROM1*-related IRDs through both AR and AD inheritance modes. Phenotypically normal individuals who carried the heterozygous *PROM1* variant may have incomplete penetrance modes of inheritance. For AD *PROM1*-related IRDs, the manifestations included macular lesions and peripheral retinal impairment. Furthermore, the missense variant c.1238T>A was identified as potentially leading to gene loss of function. The study observed that the severity of *PROM1*-related IRDs did not seem to correlate with the age of the patients. Distinct characteristics were detected in patients through OCT, including ORT, thinner EZ, loss of the COST line, and optic gaps, underlining the significance of SD-OCT for early detection of these conditions. The study emphasizes the need for further research to uncover the intricate relationships between *PROM1* genotypes and phenotypes.

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