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Clinical and genetic characteristics of 10 Japanese patients with *PROM1*-associated retinal disorder: A report of the phenotype spectrum and a literature review in the Japanese population

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Abstract

Variants in the *PROM1* gene are associated with cone (–rod) dystrophy, macular dystrophy, and other phenotypes. We describe the clinical and genetic characteristics of 10 patients from eight Japanese families with *PROM1*-associated retinal disorder (*PROM1*-RD) in a nationwide cohort. A literature review of *PROM1*-RD in the Japanese population was also performed. The median age at onset/examination of 10 patients was 31.0 (range, 10–45)/44.5 (22–73) years. All 10 patients showed atrophic macular changes. Seven patients (70.0%) had spared fovea to various degrees, approximately half of whom had maintained visual acuity. Generalized cone (–rod) dysfunction was demonstrated in all nine subjects with available electrophysiological data. Three *PROM1* variants were identified in this study: one recurrent disease-causing variant (p.Arg373Cys), one novel putative disease-causing variant (p.Cys112Arg), and one novel variant of uncertain significance (VUS; p.Gly53Asp). Characteristic features of macular atrophy with generalized cone-dominated retinal dysfunction were shared among all 10 subjects with *PROM1*-RD, and the presence of foveal sparing was crucial in maintaining visual acuity. Together with the three previously reported variants [p.R373C, c.1551+1G>A (pathogenic), p.Asn580His (likely benign)] in the literature of Japanese patients, one prevalent missense variant (p.Arg373Cys, 6/9 families, 66.7%) detected in multiple studies was determined in the Japanese population, which was also frequently detected in the European population.

KEYWORDS
autosomal dominant, cone dystrophy, cone rod dystrophy, macular dystrophy, PROM1

1 | INTRODUCTION

Inherited retinal disorder (IRD) is one of the major causes of blindness in developed countries, (Liew, Michaelides, & Bunce, 2014; Sohocki et al., 2001; Solebo, Teoh, & Rahi, 2017) including retinitis pigmentosa (RP), cone-rod dystrophy (CORD), cone dystrophy (COD), Stargardt disease (STGD), macular dystrophy (MD), Leber congenital amaurosis and others (Gill, Georgiou, Kalitzeos, Moore, & Michaelides, 2019; Hirji, Aboshiha, Georgiou, Bainbridge, & Michaelides, 2018; Kumaran, Moore, Weleber, & Michaelides, 2017; Michaelides, Hardcastle, Hunt, & Moore, 2006; Michaelides, Hunt, & Moore, 2003; Oishi et al., 2014, 2016; Rahman, Georgiou, Khan, & Michaelides, 2020; Tanna, Strauss, Fujinami, & Michaelides, 2017; Tee, Smith, Hardcastle, & Michaelides, 2016).

The clinical and genetic spectra overlap among IRDs. Pathogenic variants in the same gene may present different phenotypes. For example, relatively similar phenotypes of CORD, COD, STGD, and MD share causative genes such as *ABCA4*, *BEST1*, *PRPH2*, *RPGR*, *CRX*, *GUCY2D*, *RS1*, *POC1B*, *PROM1*, *CNGA3*, *CNGB3*, *GUCA1A*, *KCNV2*, and *RIMS1* (Ba-Abbad, Robson, MacPhee, Webster, & Michaelides, 2019; Bouzia et al., 2020; Fujinami-Yokokawa et al., 2020; Fujinami, Lois, Davidson, et al., 2013; Fujinami, Lois, Mukherjee, et al., 2013; Fujinami et al., 2015; Georgiou et al., 2020; Gill et al., 2019; Hirji et al., 2018; Hunt, Buch, & Michaelides, 2010; Kameya et al., 2019; Kominami et al., 2018; Kondo

et al., 2019; Liu et al., 2020; Mawatari et al., 2019; Michaelides et al., 2010; Mizobuchi et al., 2019; Nakanishi et al., 2016; Oishi et al., 2016; Rahman et al., 2020; Sisodiya et al., 2007; Strauss et al., 2016, 2018; Tanna et al., 2017; Tee et al., 2016, 2019). There are genes that are associated with different phenotypes of CORD/COD/MD and RP; *EYS*, *CRX*, *PRPH2*, *GUCY2D*, and *RP1L1* (Ba-Abbad et al., 2019; Bouzia et al., 2020; Davidson et al., 2013; Fujinami-Yokokawa et al., 2019, 2020; Fujinami et al., 2019; Hull et al., 2014; Katagiri et al., 2018; Koyanagi et al., 2019; Liu et al., 2020; Nakamura et al., 2019; Oishi et al., 2014, 2016; L. Yang et al., 2020). Moreover, different inheritances such as autosomal dominant (AD) and autosomal recessive (AR) are associated with differences in phenotypes (Bouzia et al., 2020; Fujinami-Yokokawa et al., 2020; Gill et al., 2019; Hull et al., 2014; Liu et al., 2020; Rahman et al., 2020).

PROM1 (OMIM: 604365), denoted as prominin 1, encodes a prominin pentaspan transmembrane glycoprotein selectively localized at the apical surface of murine neuroepithelial cells and is also known as CD133 and AC133 (Maw et al., 2000). The AC133 antigen was initially identified as a cell surface antigen and is expressed in hematopoietic stem cells, endothelial progenitor cells, and others (Maw et al., 2000; Miraglia et al., 1997; Yin et al., 1997). A later study by Maw et al. reported that prominin is concentrated in the base of the photoreceptor outer segments and that loss of prominin causes retinal degeneration with abnormal generation/conversion of the evaginations to disks (Maw et al., 2000). Recently, PROM1 has been associated with the regulation of photoreceptor autophagy in the retinal pigment epithelium (RPE) (Bhattacharya et al., 2017).

Variants in the *PROM1* gene have been associated with CORD/COD, MD, STGD, and RP (Arai et al., 2015; Arrigoni et al., 2011; Beryozkin et al., 2014; Birtel et al., 2018; Boulanger-Scemama et al., 2015; Carss et al., 2017; Cehajic-Kapetanovic et al., 2019; Collison et al., 2019; Eidinger et al., 2015; Eisenberger et al., 2013; Imani et al., 2018; Jinda et al., 2014; Khan & Bolz, 2015; Kim et al., 2017, 2019; Kniazeva et al., 1999; Liang et al., 2019; Littink et al., 2010; Liu et al., 2016; Maw et al., 2000; Mayer et al., 2016; Michaelides et al., 2005, 2010; Michaelides, Johnson, et al., 2003; Permanyer et al., 2010; Pras et al., 2009; Ragi et al., 2019; Salles et al., 2017; Song et al., 2011; Strauss et al., 2018; Wawrocka et al., 2018; Yang et al., 2008; Zhang et al., 2007; Zhao et al., 2015). Over 90 disease-associated *PROM1* variants have been identified in AD and AR manners (The Human Gene Mutation Database; http://www.hgmd.cf.ac.uk/ac/index.php) (Supporting Information 1). However, the clinical and molecular genetic characteristics remain uncertain due to the lack of large cohort studies, especially in the Asian population.

The purpose of this study was to characterize the clinical and molecular genetic features of *PROM1*-RD in a large Japanese cohort with IRD. A literature review of *PROM1*-RD was also performed to understand the genetic spectrum in the Japanese population.

2 | METHODS

The protocol of this study adhered to the tenets of the Declaration of Helsinki and was approved by the ethics committee of the participating institutions from Japan (National Institute of Sensory Organs, National Hospital Organization, Tokyo Medical Center (Reference: R18-029), and Kyoto University Graduate School of Medicine (Reference: G0746)).

2.1 | Participants

Patients in the Japan Eye Genetics Consortium (JEGC; http://www.jegc.org/) with a clinical diagnosis of IRD and available genetic data were studied between 2008 and 2018. A total of 1,294 subjects from 730 families were surveyed.

2.2 | Clinical examinations

Medical and family history was obtained in all affected subjects and unaffected family members (where available), including ethnicity, chief complaints of visual symptoms, onset of disease, and duration of disease (defined as the term between the onset and the latest examination).

Comprehensive ophthalmological examinations were performed in all affected subjects and unaffected family members (where available), including measurements of best-corrected decimal visual acuity (BCVA) converted to the logarithm of the minimum angle of resolution (LogMAR), ophthalmoscopy, fundus photography, fundus autofluorescence (FAF) imaging, spectral-domain optical coherence tomography (SD-OCT), kinetic and static visual field testing, and electrophysiological assessments according to the international standards of the International Society for Clinical Electrophysiology of Vision (ISCEV) (Hood et al., 2012; McCulloch et al., 2015a, 2015b; Robson et al., 2018).

2.3 | Genetic screening in the *PROM1* gene

Genomic DNA was extracted from all affected subjects and unaffected family members (where available for co-segregation analysis). Whole-exome sequencing with target analysis of retinal disease-associated genes (RetNet https://sph.uth.edu/retnet/) was performed according to the previously published methods (Fujinami et al., 2016; Oishi et al., 2016; Pontikos et al., 2020; Yang et al., 2020). The identified variants were filtered using allele frequency (less than 1%) in the Human Genetic Variation Database (HGVD; http://www.hgvd.genome.med.kyoto-u.ac.jp/), which provides allele frequencies of the general Japanese population. Depth and coverage for the target exons were interrogated using the integrative Genomics Viewer (http://www.broadinstitute.org/igv/). Sanger direct sequencing was performed to confirm the detected variants in the *PROM1* gene, and to conduct co-segregation analysis.

Together with the clinical features of affected subjects and the model of inheritance in the pedigree in consideration of the results of co-segregation analysis, disease-causing variants were determined from the called/detected variants in the retinal disease-associated genes.

2.4 | In silico molecular genetic analysis

The allele frequency of all detected variants in the HGVD, Integrative Japanese Genome Variation (iJGVD 3.5 k, 4.7 k; https://jmorp.megabank.tohoku.ac.jp/ijgvd/), 1000 Genomes (http://www.internationalgenome.org/), and the Genome Aggregation Database (gnomAD) was established (http://gnomad.broadinstitute.org/). All variants were analyzed with four general prediction programs and three functional prediction programs: MutationTaster (http://www.mutationtaster.org), FATHMM (http://fathmm.biocompute.org.uk/9), Combined Annotation Dependent Depletion (CADD; https://cadd.gs.washington.edu/), REVEAL (https://labworm.com/tool/revel), SIFT (https://www.sift.co.uk/), PROVEAN (http://provean.jcvi.org/index.php), and Polyphen 2 (http://genetics.bwh.harvard.edu/pph2/). Prediction of splice site alteration was performed with Human Splicing Finder (http://www.umd.be/HSF3/). The evolutionary conservation score for each variant was calculated from the UCSC database (https://genome.ucsc.edu/index.html). Variant classification was performed for all detected variants according to the guidelines of the American College of Medical Genetics and Genomics (ACMG) (Richards et al., 2015).

2.5 | Literature search

Peer-reviewed articles that report *PROM1* variants in the Japanese population were searched using the public search engine (PubMed; https://www.ncbi.nlm.nih.gov/pubmed/). Phenotypes of reported cases were surveyed, and in silico molecular genetic analysis for previously reported variants was performed in the same manner applied for the detected variants in the current study.

3 | RESULTS

3.1 | Participants

Ten affected subjects from eight families with a clinical diagnosis of IRD and who harbored heterozygous pathogenic *PROM1* variants were ascertained in this study. Some data for one patient have been published elsewhere (Oishi et al., 2016). Eight out of 30 families (8/30, 26.7%) with AD COD/CORD/MD/STGD in the JEGC IRD cohort with available whole-exome sequencing results were associated with AD *PROM1*-RD. There were no families in the JEGC cohort with autosomal recessive *PROM1*-RD in the 529 families with AR or sporadic IRD.

The detailed demographic data are described in Table 1. All subjects were originally from Japan. There are five females and five males. The pedigrees of eight families are presented in Figure 1. AD family history was clearly reported in all eight families. The median age at the latest examination of 10 affected subjects was 44.5 years (range, 22–73).

3.2 | Onset, chief complaint, and visual acuity

The median age of onset of 10 affected subjects was 31.0 years (range, 10–45). Three subjects had childhood onset of 10 years (3/10, 30%; Patients 5, 8, 10). Late onset of 45 years was reported in one subject (1/10, 10%; Patient 1). The other six subjects had intermediate onset between 15 and 45 years (6/10, 60%; Patients 2–4, 6, 7, 9). The median duration of disease of 10 affected subjects was 18.5 years (range, 8–47). Reduced visual acuity was reported as the chief complaint in eight subjects at the initial visit to the clinic (8/10, 80%; Patients 1–4, 6–8, 10).

The median values of BCVA in the right and left eyes of 10 affected subjects were 0.70 (range, 0.05–1.40, Snellen equivalent of 20/100) and 0.91 (0.1–1.22, Snellen equivalent of 20/160) in the LogMAR unit, respectively. Three subjects (3/10, 30.0%, Patients 2, 3, 7) had relatively favorable VA (LogMAR 0.22 or better in the better eye), four (4/10, 40.0%, Patients 6, 8–10) had moderate VA (between Log MAR 0.22 and 1.0 in the better eye), and three (3/10, 30.0%; Patients 1, 4, 5) had poor VA (LogMAR 1.0 or worse in the better eye). Asymmetric VA between the eyes (LogMAR 0.3 or more difference between the eyes) was observed in six subjects (6/10, 60%; Patients 1–3, 6, 8, 9).

3.3 | Retinal imaging and morphological findings

Detailed findings of fundus, FAF, and SD-OCT images are presented in Table 2. Representative fundus and FAF images are shown in Figure 2. Central retinal atrophy was demonstrated in all 10 affected subjects. Pigmentation was noted to various degrees in the atrophic area. Central retinal atrophy was more evident in FAF. Mottled and patchy areas of decreased AF were present at the central retina corresponding to retinal atrophy in all subjects. Central atrophy was surrounded by a ring of increased AF in seven subjects (7/10, 70%; Patients 1–3, 7–10). A more diffuse area of increased AF with less evident increased AF surrounding the atrophy was observed in three subjects (3/10; Patients 4–6).

Foveal sparing (defined as remaining foveal AF signal surrounded by the area of decreased AF) was observed on FAF in three eyes of two subjects (3/20 eyes, 15.0%; Patients 7, 9). The median BCVA of the three eyes with foveal sparing was 0.10 (range, 0.05–0.3) in the LogMAR unit, respectively.

Representative SD-OCT images are shown in Figure 3. Outer retinal disruption was observed at the fovea and parafovea in six subjects (6/10, 60.0%; Patients 1–4, 6, 8). Outer retinal disruption at the parafovea was found in four subjects (4/10, 40.0%; Patients 5, 7 9, 10), and one of these subjects had outer retinal disruption from the parafovea to the periphery (1/10, 10.0%; Patient 5). Increased signal of the choroid, indicating RPE atrophy, was observed in nine subjects (9/10, 90.0%; Patients 1–9), and no evidence of hypertransmission was found in one subject (1/10, 10%; Patient 10).

The ellipsoid zone was preserved at the fovea in seven eyes of six subjects (7/20, 35.0%; Patients 2, 3, 5, 7, 9, 10). The median BCVA of seven eyes with preserved ellipsoid zones was 0.15 (range, 0.05–0.7) in the LogMAR unit.

TABLE 1 Demographic features of 10 Japanese patients from eight families with *PROM1*-associated retinal disorder (*PROM1*-RD)

Family no	Patient no	Inheritance	Sex	Age (at latest examination)	Onset	Chief complaint	Best corrected visual acuity in the logMAR unit	
							RE	LE
1	Patient 1(1-II:3) (TMC01-01)	AD	M	73	45	Reduced VA	1.4	1
1	Patient 2(1-III:2) (TMC01-02)	AD	F	41	31	Reduced VA	0.7	0.15
2	Patient 3(2-II:2) (JU01-01)	AD	M	44	31	Reduced VA	0.7	0.1
2	Patient 4(2-I:1) (JU01-02)	AD	M	68	40	Reduced VA	1.05	1.3
3	Patient 5(3-II:2) (TMC02-01)	AD	F	40	10	Photophobia	1.3	1.22
4	Patient 6(4-III:1) (TMC03-01)	AD	F	38	30	Reduced VA	0.7	1
5	Patient 7(5-II:4) (TU01-01)	AD	M	45	34	Reduced VA	0.05	0.1
6	Patient 8(6-IV:1) (KYU01-01)	AD	F	22	10	Reduced VA	1	0.7
7	Patient 9(7-II:1) (TMC04-01)	AD	M	64	40	Central visual field loss	0.3	1
8	Patient 10(8-II:2) (TMC05-01)	AD	F	57	10	Reduced VA	0.7	0.82

Note: Autosomal dominant family history (at least having two affected subjects in two consecutive generations) was clearly reported in all eight families. Age was defined the age when the latest examination was performed. The age of onset was defined as either the age at which visual loss was first noted by the patient or, in the "asymptomatic" patients, when an abnormal retinal finding was first detected.
Abbreviations: AD, autosomal dominant; F, female; LE, left eye; LogMAR, logarithm of the minimum angle of resolution; M, male; No., number; RE, right eye.

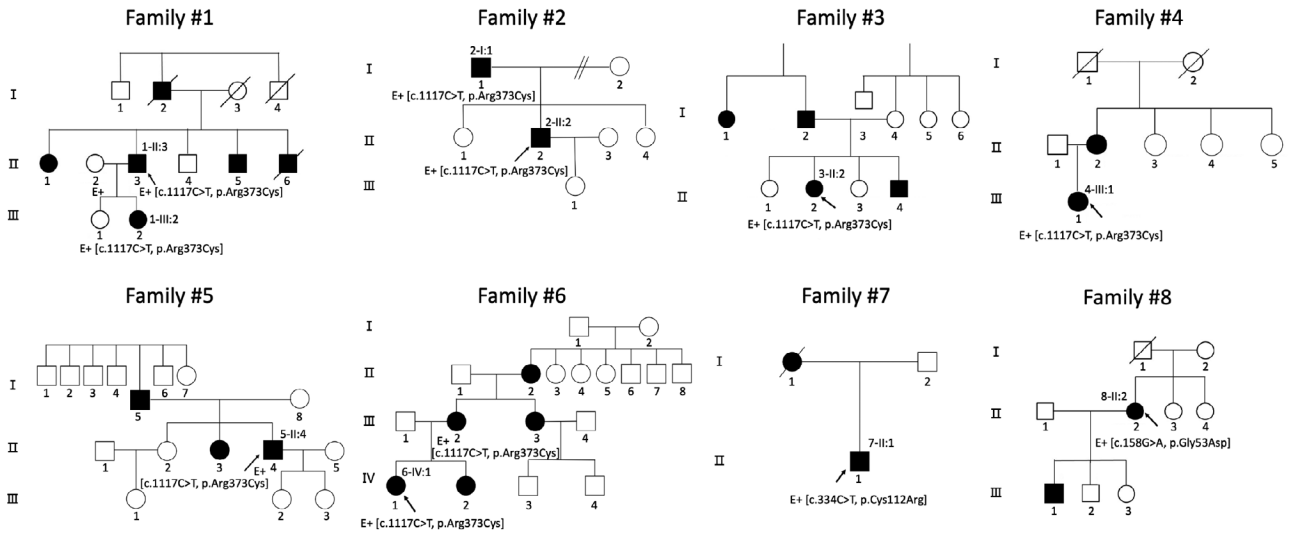

FIGURE 1 Pedigrees of eight Japanese families with inherited retinal disorder harboring *PROM1* variants. The solid squares (men) and circles (women) represent the affected patients and the white icons represent the unaffected family members. The slash symbol indicates deceased individuals. The generation number is shown on the left. The proband is marked by an arrow and the clinically examined individuals are indicated by a cross. Autosomal dominant family history was clearly reported in all eight families, and three heterozygous *PROM1* variants were identified, including one recurrent variant (c.1117C>T, p.Arg373Cys) and two novel variants (c.334T>C, p.Cys112Arg; c.158G>A, p.Gly53Asp)

3.4 | Visual field and electrophysiological findings

Detailed findings of visual fields and electrophysiological assessment are presented in Table 2. Visual field testing was performed in all affected subjects except for two subjects (Patients 4, 6), with Goldmann perimetry (GP; 8 subjects) and Humphrey visual field analyzer (HFA; 3 subjects). Central scotoma was observed in all eight subjects (8/8, 100.0%, Patients 1–3, 5, 7–10), with peripheral constriction found in two subjects (2/8, 25.0%; Patients 1, 5).

Electrophysiological testing was performed in all affected subjects except for one subject (Patient 6). Mildly decreased generalized cone responses (amplitude reduction less than 50% of normal reference) were demonstrated in five subjects (5/9, 55.6%; Patients 1, 2, 8–10). Moderately decreased generalized cone responses (amplitude reduction

TABLE 2 Retinal imaging, morphological findings, Visual fields, and electrophysiological assessments of 10 patients with *PROM1*-RD

	Fundus		FAF					SD-OCT					Visual field			Electrophysiological assessment	
Patient no	Central atrophy	Pigmentation	Mottled/patchy area of decreased AF	Ring of increased AF	Diffuse area of increased AF	FS (RE)	FS (LE)	Outer retinal disruption at the fovea	Outer retinal disruption at the parafovea	Increased signal of the choroid	EZ preservation at the fovea (RE)	EZ preservation at the fovea (LE)	Method	CS	Peripheral constriction	Responses in dark-adapted condition	Responses in light-adapted condition
1	Yes	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	No	No	GP	Yes	Yes	Severely decreased	Mildly decreased
2	Yes	Yes	Yes	Yes	No	No	Yes (slight)	Yes	Yes	Yes	No	Yes	GP	Yes	No	Preserved	Mildly decreased
3	Yes	Yes	Yes	Yes	No	Yes (slight)	Yes (slight)	Yes	Yes	Yes	No	Yes	GP	Yes	No	Preserved	Moderately decreased
4	Yes	No	Yes	No	Yes	No	No	Yes	Yes	Yes	No	No	NA	NA	NA	Severely decreased	Severely decreased
5	Yes	No	Yes	No	Yes	Yes (slight)	Yes (slight)	No	Yes (extended)	Yes	Yes	No	GP/HFA	Yes	Yes	Severely decreased	Severely decreased
6	Yes	No	Yes	No	Yes	No	Yes (slight)	Yes	Yes	Yes	No	No	NA	NA	No	NA	NA
7	Yes	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	GP/HFA	Yes	No	Moderately decreased	Moderately decreased
8	Yes	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	No	No	GP	Yes	No	Preserved	Mildly decreased
9	Yes	No	Yes	Yes	No	Yes	Yes (slight)	No	Yes	Yes	Yes	No	GP/HFA	Yes	No	Preserved	Mildly decreased
10	Yes	No	Yes	Yes	No	No	No	No	Yes	No	Yes	No	GP	Yes	No	Preserved	Severely decreased

Note: Foveal sparing was defined as remaining foveal AF signal surrounded by the area of decreased AF.

Abbreviations: BE, both eyes; CS, central scotoma; EZ, ellipsoid zone; FAF, fundus autofluorescence; FS, foveal sparing; GP, Goldmann Perimetry; HFA, Humphrey visual field analyzer; LE, left eye; M, male; NA, not available; RE, right eye; SD-OCT, spectral domain optical coherence tomography.

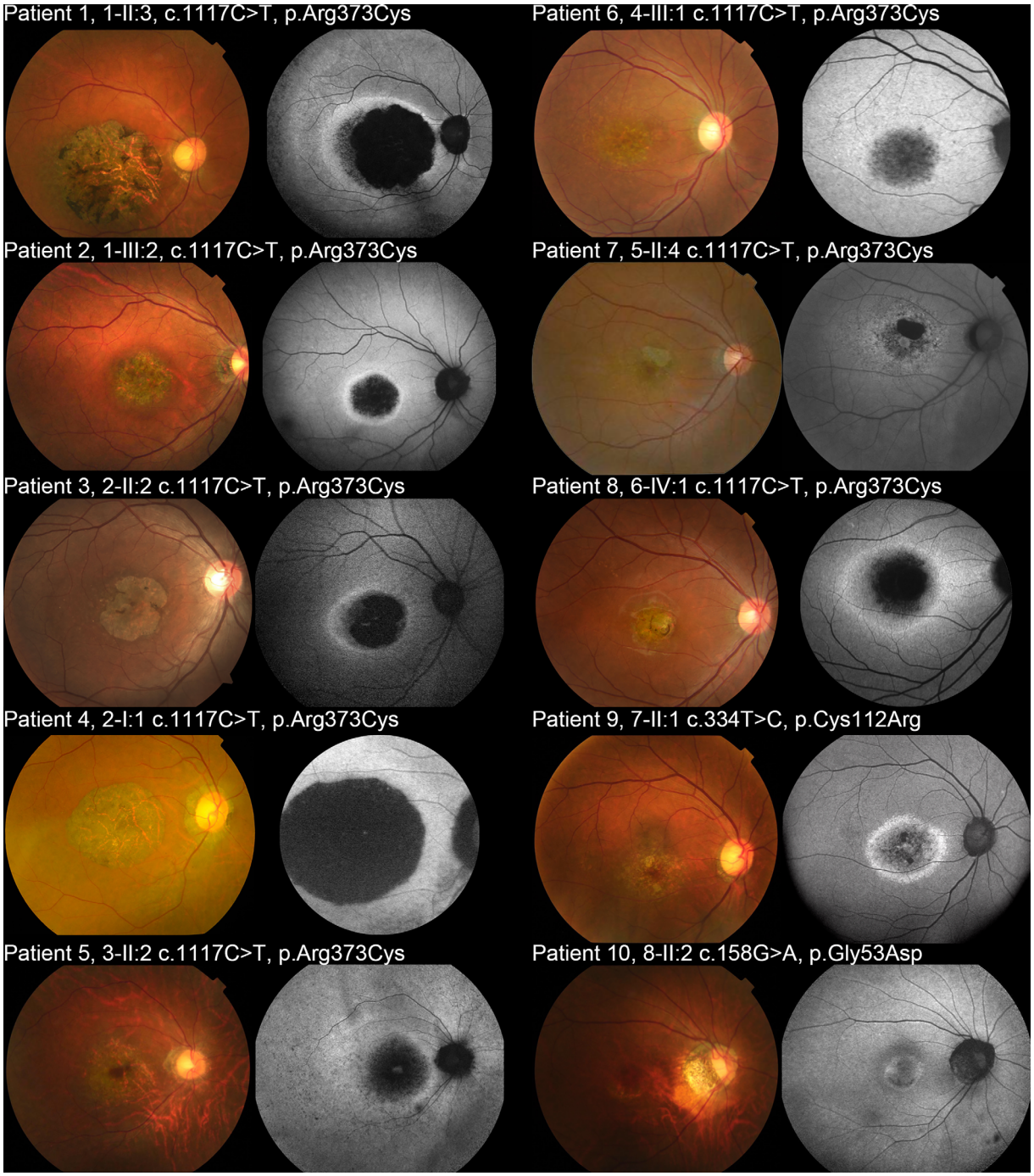

FIGURE 2 Fundus photographs and fundus autofluorescence images of 10 patients with *PROM1*-associated retinal disorder (*PROM1*-RD). Fundus photographs of the right eye show central retinal atrophy in 10 affected subjects. Fundus autofluorescence (FAF) images present mottled and patchy area of decreased AF at the central retina corresponding to retinal atrophy in all subjects, which is surrounded by a ring of increased AF in seven subjects (Patient 1–3, 7–10) and by diffuse high density of AF in one subject (Patient 5). Foveal sparing is observed in two subjects (Patients 7, 9)

between 25 and 50% of normal reference) were shown in two subjects (2/9, 22.2%; Patients 3, 7). Severely decreased generalized cone responses (amplitude reduction more than 75% of normal reference) with preserved rod responses were found in one subject (1/9, 11.1%; Patient 10). Severely decreased generalized cone and rod responses were identified in three subjects (3/9, 33.3%; Patients 1, 4, 5).

3.5 | *PROM1* variants

Variant data of 11 affected and two unaffected subjects of eight families are summarized in Table S1. Three heterozygous *PROM1* variants were identified by whole-exome sequencing with target analysis of retinal disease-associated genes (Table S2): c.1117C>T, (p.Arg373Cys);

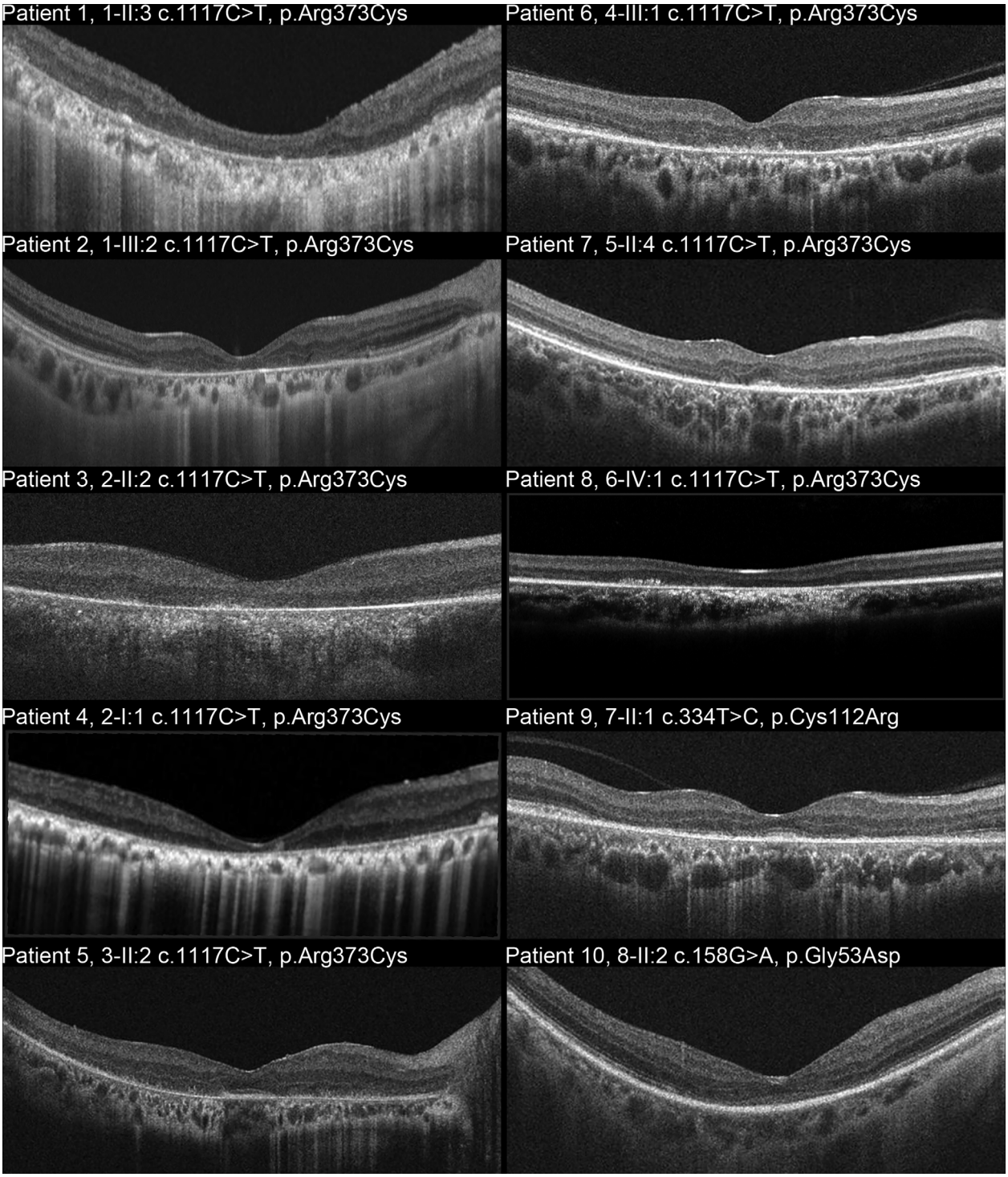

FIGURE 3 Spectral-domain optical coherence tomographic images of 10 patients with *PROM1*-RD. Spectral domain optical coherence tomography of the right eye shows outer retinal disruption at the fovea and parafovea in six subjects (Patients 1–4, 6, 8), at the parafovea in three subjects (Patients 7, 9, 10), and from the parafovea to the periphery in one subject (Patient 5). Increased signal of the choroid, indicating retinal pigment epithelial atrophy was observed in nine subjects (Patients 1–9), and no evidence of hypertransmission is found in one subject (Patient 10)

c.334T>C, (p.Cys112Arg); c.158G>A, (p.Gly53Asp) (NM_006017.2). One variant (p.Arg373Cys) was previously reported, and two variants have never been reported (p.Cys112Arg, p.Gly53Asp) (Michaelides et al., 2005). These three *PROM1* variants were confirmed with direct sequencing, and two variants were co-segregated within the families (p.Arg373Cys, p.Gly53Asp; Families #1, #2, #6, #8).

One variant was recurrent; p.Arg373Cys (6/8 families, 75.0%). Each of the other two variants was found in a single family

TABLE 3 In silico molecular genetics analyses of three detected and two previously reported *PROM1* variants in the Japanese population

Nucleotide change, amino acid change/effect	Study	Position	Coding impact	Location	dbSNP ID	HGVD	iJGVD		1,000 genome
							3.5 k	4.7 k	
c.1738A>C,p.Asn580His	Arai et al. *Journal of Ophthalmology* 2015	15995639	Missense	Exon 16 of 28 position 56 of 85 (coding)	rs199674847	0.0054	0.0075	0.0074	NA
c.1578+1G>A, splice site alteration (denoted asc.1551+1G>A in the original report; NM_001145847.2)	Koyanagi et al. *Journal of Medical Genetics* 2018	16002118	Splice site alteration	Intron 14 of 27 position 1 of 2007 (splicing, intronic)	rs1553901823	NA	NA	NA	NA
c.1117C>T,p.Arg373Cys	This study/Koyanagi et al. *Journal of Medical Genetics* 2018	16014922	Missense	Exon 11 of 28 position 40 of 64 (coding)	rs137853006	NA	NA	NA	NA
c.334T>C, p.Cys112Arg	This study	16035102	Missense	Exon 5 of 28 position 31 of 206 (coding)	NA	NA	NA	NA	NA
c.158G>A, p.Gly53Asp	This study	16077372	Missense	Exon 2 of 28 position 370 of 432 (coding)	rs755064227	0.0021	0.0017	0.0019	4.82E-05

Nucleotide change, amino acid change/effect	GnomAD																	
	Allele frequency (exome)							Allele frequency (genome)							Coverage in GnomAD Exomes samples			
	East Asian	South Asian	African	European (non-Finnish)	Total	Male	Female	East Asian	South Asian	African	European (non-Finnish)	Total	Male	Female	Mean coverage	Median coverage	% of samples over 20x coverage	
c.1738A>C,p.Asn580His	0.00167	NA	NA	NA	0.00012	0.0000962	0.000149	NA	NA	NA	NA	NA	NA	NA	65.9	65	99.47	
c.1578+1G>A, splice site alteration (denoted asc.1551+1G>A in the original report; NM_001145847.2)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	26.4	23	67.96	
c.1117C>T,p.Arg373Cys	NA	NA	NA	NA	0.00000401	NA	0.00000877	NA	NA	NA	NA	NA	NA	NA	63.8	59	99.23	
c.334T>C, p.Cys112Arg	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	33.4	31	75.29	
c.158G>A, p.Gly53Asp	0.000612	0.0000328	NA	NA	0.0000482	0.0000519	0.0000439	NA	NA	NA	NA	NA	NA	NA	58.6	59	94.01	

(Continues)

TABLE 3 (Continued)

	General prediction								Functional prediction									
	Mutation taster			FATHMM			CADD		REVEAL		SIFT			PROVEAN			Polyphen2	
Nucleotide change, amino acid change/ effect	Prediction	Accuracy	Converted rank score	Prediction	Score	Converted rank score	Score	Prediction	Score	Rank score	Prediction	Score	Converted rank score	Prediction	Score	Converted rank score	Prediction	Score
c.1738A>C, p.Asn580His	Polymorphism	0.7083, 0.6447	0.3058	Tolerated	0.66	0.5242	18.87	Benign	0.3059	0.6309	Tolerated	0.052, 0.055	0.3909	Damaging	−4.01, −3.91	0.7419	Possibly damaging	0.924
c.1578+1G>A, splice site alteration (denoted as c.1551 +1G>A in the original report; NM_001145847.2)	Disease causing	1	0.81	Damaging	0.9845	0.8287	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.1117C>T,p. Arg373Cys	Disease causing automatic	1.58E-10	0.08975	Tolerated	0.86	0.4678	6.971	Benign	0.2809	0.6024	Damaging	0.002	0.7215	Damaging	−2.67	0.5711	Possibly damaging	0.936
c.334T>C, p. Cys112Arg	Polymorphism	0.9989, 0.9981	0.2234	Tolerated	0.77	0.4964	21.4	Benign	0.187	0.4672	Damaging	0.002	0.7215	Damaging	−8.78, −8.98	0.9804	Probably damaging	0.996
c.158G>A, p. Gly53Asp	Polymorphism	1	0.08975	Tolerated	1.45, 1.06	0.3978	20.7	Benign	0.141	0.3826	Tolerated	0.083, 0.084, 0.253, 0.076, 0.08	0.3427	Damaging	−2.52, −3.55, −2.54	0.6876	Possibly damaging	0.538

TABLE 3 (Continued)

Nucleotide change, amino acid change/effect	Human splice finder 3.0	Conservation				Conservation				Verdict	ACMG classification					
		PhyloP46way		PhastCons46way		PhyloP100way		PhastCons100way			Identified classification rules					
		Mammalian	Mammalian rank score	Mammalian	Mammalian rank score	Vertebrate	Vertebrate rank score	Vertebrate	Vertebrate rank score		Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
c.1738A>C,p.Asn580His	No impact	1.068	NA	0.944	NA	4.2049	0.5827	1	0.7164	Likely benign	BS1	BP4				
c.1578+1G>A, splice site alteration (denoted as c.1551+1G>A in the original report; NM_001145847.2)	NA	NA	NA	NA	NA	7.662	0.8279	1	0.7164	Pathogenic	PVS1	PM2	PP5			
c.1117C>T,p.Arg373Cys	Potential alteration	0.336	NA	0.002	NA	0.303	0.1896	0.001	0.1379	Pathogenic	PS3	PM2	PP4	PP5	BP4	
c.334T>C, p.Cys112Arg	No impact	1.356	NA	0.002	NA	1.445	0.3469	0.001	0.1379	Likely pathogenic	PM2	PP4	PP5	BP4		
c.158G>A, p.Gly53Asp	Potential alteration	0.118	NA	0	NA	−0.2119	0.09323	0	0.06391	Uncertain significance	PM2	PP4	BP4			

Note: Reference: NM_006017.2, ENST00000510224.1, GRCh37.p13. Sequence variant nomenclature was obtained according to the guidelines of the Human Genome Variation Society (HGVS) by using Mutalyzer (https://mutalyzer.nl/). The allele frequency of all detected variants in the HGVD, Integrative Japanese Genome Variation (iJGVD 3.5 k, 4.7 k; https://jmorp.megabank.tohoku.ac.jp/ijgvd/), 1000 genome (http://www.internationalgenome.org/), and the genome Aggregation Database (gnomAD) was established (http://gnomad.broadinstitute.org/). All variants were analyzed with four general predictions programs and three functional prediction programs; MutationTaster (http://www.mutationtaster.org), FATHMM (http://fathmm.biocompute.org.uk/9), Combined Annotation Dependent Depletion (CADD; https://cadd.gs.washington.edu/), REVEAL (https://labworm.com/tool/revel), SIFT (https://www.sift.co.uk/), PROVEAN (http://provean.jcvi.org/index.php), Polyphen 2 (http://genetics.bwh.harvard.edu/pph2/). Prediction on splice site alteration was performed with Human splicing finder (http://www.umd.be/HSF3/). Evolutional conservation score for each variant was calculated from the UCSC database (https://genome.ucsc.edu/index.html). Variant classification was performed for all detected variants, according to the guidelines of the American College of Medical Genetics and Genomics (ACMG). Classification of predictions by the American College of Medical Genetics and Genomics (ACMG) was also applied for all detected variants; PVS1 (Null variant [nonsense, frameshift, canonical 1 or 2 splice sites, initiation codon, single or multiexon deletion] in a gene where loss of function is a known mechanism of disease); PS3 (Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product); PM2 (pathogenicity moderate 2; absent from controls in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium); PP4 (Patients phenotype or family history is highly specific for a disease with asingle genetic etiology); PP5 (pathogenicity supporting 5; reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation); BS1 (Allele frequency is greater than expected for disorder); BP4 (benign supporting 4; Multiple lines of computational evidence suggest no impact on gene or gene product).

(p.Cys112Arg for Family #7, p.Gly53Asp for Family #8). Called variants detected by whole-exome sequencing with targeted analysis in Family #7 were c.334T>C, (p.Cys112Arg) in the *PROM1* gene; c.3404C>T, (p.Pro1135Leu) in the *C2orf71* gene; c.1405G>T, (p.Val469Phe) in the *MERTK* gene; c.404T>C, (p.Ile135Thr) in the *SLC7A14* gene; c.3489T>A, (p.Asn1163Lys) in the *EYS* gene; c.1282G>A, (p.Asp428Asn) in the *CDH23* gene; and c.47G>A, (p.Gly16Asp) in the *FZD* gene. Two variants with AD inheritance (*PROM1*, *FZD4*) and low allele frequency (<0.5% of HGVD) were included. Called variants in Family #8 were c.158G>A, (p.Gly53Asp) in the *PROM1* gene; c.1327C>T, (p.Arg443Trp) in the *CYP4V2* gene; and c.8053G>A, (p.Val2685Ile) in the *VCAN* gene. Two variants with AD inheritance (*PROM1*, *VCAN*) and low allele frequency (<0.5% of HGVD) were included. Together with the clinical features of affected subjects and the model of inheritance in the pedigree, disease-causing variants in the *PROM1* gene were determined.

3.6 | In silico molecular genetic analysis for detected variants in the current study

The detailed results of in silico molecular genetic analyses for the three detected *PROM1* variants are presented in Table 3. The schematic genetic and protein structure of PROM1 and the location of the variants are shown in Figure 4, and multiple alignments of eight species of PROM1 is demonstrated in Figure 5.

Allele frequency for the three *PROM1* variants (p.Arg373Cys, p.Cys112Arg, p.Gly53Asp) in the general population was 0.00041, 0.0, 0.0045%, respectively. The allele frequency of East Asian/South Asian/African/European (non-Finish) for these three *PROM1* variants (p.Arg373Cys, p.Cys112Arg, p.Gly53Asp) was 0.0/0.0/0.0/0.0, 0.0/0.0/0.0/0.0%, and 0.058/0.0033/0.0/0.0%, respectively. There was one variant (p.Gly53Asp) which had a significantly higher allele frequency in the East Asian population than in other populations ($p < .001$, Fisher's exact test).

General prediction, functional prediction, and conservation were assessed for the three *PROM1* variants, and pathogenicity classification according to the ACMG guidelines (Richards et al., 2015) was pathogenic for p.Arg373Cys, likely pathogenic for p.Cys112Arg, and uncertain significance for p.Gly53Asp, respectively. The specific evidence levels used in the ACMG classification for each *PROM1* variant is presented in Table 3.

Overall, one disease-causing variant (p.Arg373Cys), one putative disease-causing variant (p.Cys112Arg) and one variant of uncertain significance (VUS) (p.Gly53Asp) in the *PROM1* gene were ascertained in eight families with AD COD/CORD/MD.

3.7 | Previously reported *PROM1* variants in the Japanese population

There are two reports describing *PROM1*-RD in the Japanese population other than the cases reported here. Koyanagi et al. reported two patients in a cohort of 1,204 Japanese patients with RP (Koyanagi et al., 2019). One "solved" 69-year-old female with RP harbored a heterozygous *PROM1* variant (c.1117C>T, (p.Arg373Cys)), as well as an

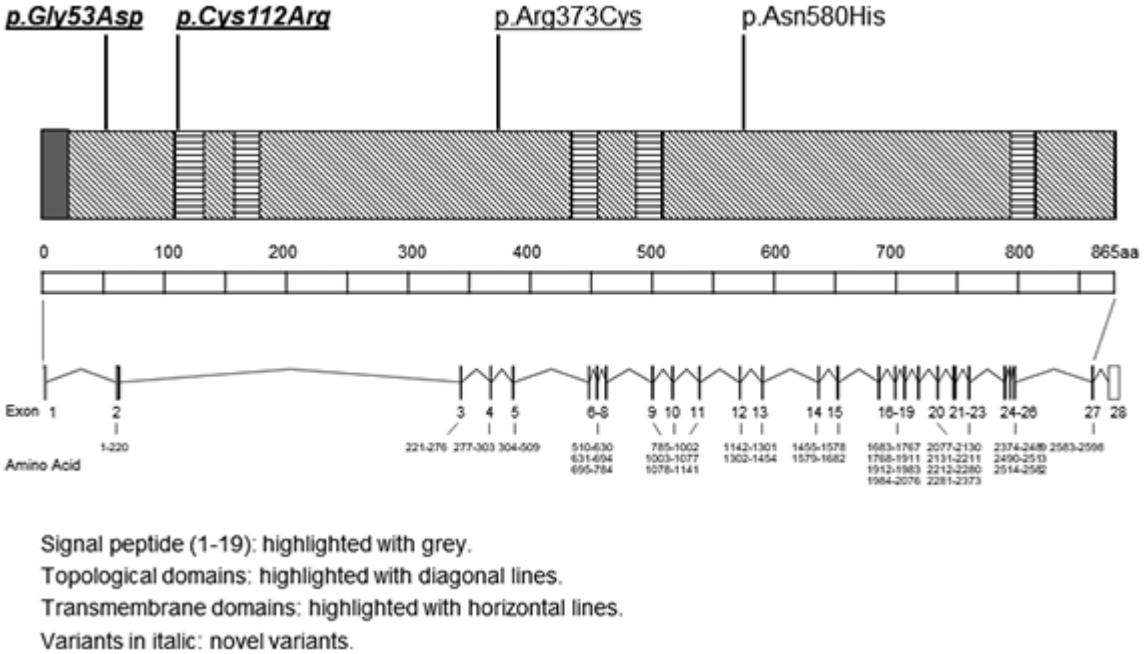

FIGURE 4 A schematic genetic and protein structure of PROM1 and the location of the missense variants detected in the Japanese population. The PROM1 gene (ENST00000510224.1) contains 28 exons and encodes an 866 amino acid protein containing topological domains (highlighted with diagonal lines) and transmembrane domains (highlighted with horizontal lines). Variants detected in the current study are underlined, and a previously reported variant in the Japanese population is shown without. Two detected variants (c.334T>C, (p.Cys112Arg); c.158G>A, (p.Gly53Asp)) which have never been reported, are shown in italics

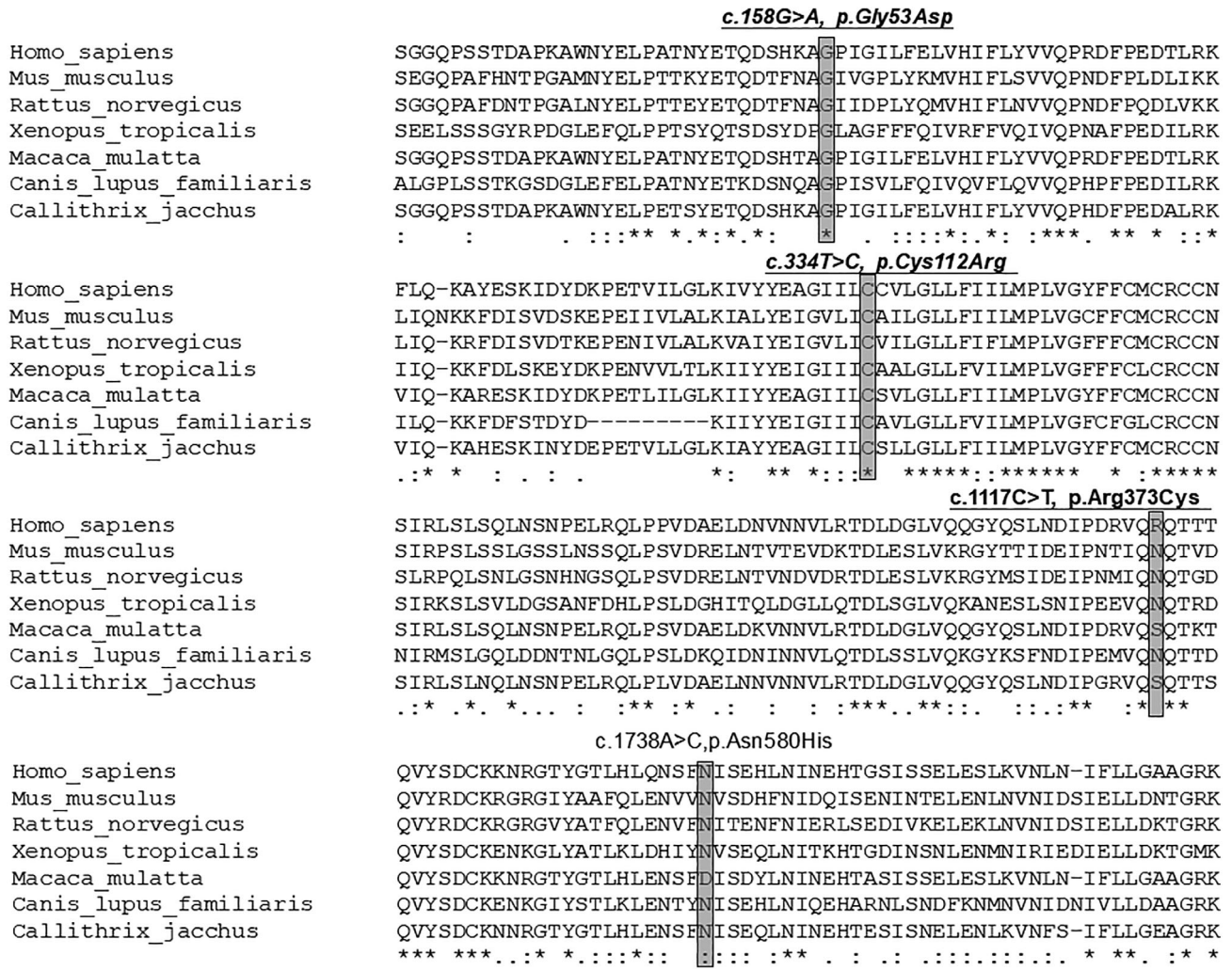

FIGURE 5 Multiple alignments of eight species of PROM1. The alignment was performed with the Clustal Omega program (https://www.ebi.ac.uk/Tools/msa/clustalo/), and the amino acid-sequence alignment was numbered in accordance with the *Homo sapiens* PROM1 sequence (ENST00000510224.1). An asterisk indicates complete conservation across the eight species. The positions of variant residues (c.1738A>C, (p.Asn580His); c.1117C>T, (p.Arg373Cys); c.334T>C, (p.Cys112Arg); c.158G>A, p.Gly53Asp), are highlighted with gray background

ABCA4 variant (c.4462T>C, [p.Cys1488Arg]—likely pathogenic) and an *SAG* variant (c.926delA, p.Asn309ThrfsTer12—pathogenic). One "unsolved" 61-year-old female with RP had a heterozygous canonical splice variant [(c.1578+1G>A; denoted as c.1551+1G>A in the original report (NM_001145847.2)]. No further detailed phenotypic information is available in these two cases.

Arai et al. reported four cases of AD *PROM1*-RD (Arai et al., 2015). Three unrelated cases shared a heterozygous *PROM1* variant (c.1738A>C,[p.Asn580His]). The other *PROM1* variant was not described. One 37-year-old patient with this variant (p.Asn580His) showed a phenotype of cone dystrophy. One patient with the AD RP phenotype harboring the *PROM1* variant (p.Asn580His) also harbored a heterozygous *EYS* variant (c.768A>G, p.Ile256Met –VUS) and a heterozygous *CRB1* variant (c.2306G>A, p.Arg769His—benign). Another patient with AD RP phenotype harboring the *PROM1* variant (p.Asn580His) had a heterozygous *EYS* variant (c.4957dupA, p.Ser1653LysfsTer2—pathogenic) and a heterozygous *CRB1* variant (c.2306G>A, p.Arg769His—benign). Association of *EYS*, *PROM1*, and *CRB1* in retinal dystrophies were proposed in the literature.

3.8 | In silico molecular genetic analysis for previously reported variants

The detailed results of in silico molecular genetic analyses for the three *PROM1* variants (c.1117C>T, p.Arg373Cys, c.1551+1G>A, c.1738A>C,p.Asn580His) described in previous reports are presented in Table 3. The first variant (p.Arg373Cys) was also identified in the current study and the latter two variants (c.1551+1G>A, c.1738A>C, (p.Asn580His)) were described only in the two Japanese reports. A schematic genetic and protein structure is shown in Figure 4, and multiple alignment of eight species is demonstrated in Figure 5.

Allele frequencies for the two previously reported *PROM1* variants (p.Asn580His, c.1551+1G>A) in the general population were

0.012 and 0.0%, respectively. The allele frequencies of East Asian/South Asian/African/European (non-Finish) for these two *PROM1* variants (p.Asn580His, c.1551+1G>A) were 0.167/0.0/0.0/0.0% and 0.0/0.0/0.0/0.0%, respectively. One variant (p.Asn580His) had a significantly higher allele frequency in the East Asian population than in other populations ($p < .001$, Fisher's exact test).

General prediction, functional prediction, and conservation were assessed for the two *PROM1* variants, and pathogenicity classification according to the ACMG guidelines was likely benign for p.Asn580His and pathogenic for c.1551+1G>A.

4 | DISCUSSION

Detailed clinical and genetic characteristics of a Japanese cohort of 10 affected subjects from eight families with AD *PROM1*-RD are illustrated. Characteristic features of macular atrophy and generalized cone-dominated retinal dysfunction were identified in all available cases. Foveal sparing was frequently found in AD *PROM1*-RD: 15.0% on FAF and 35.0% on SD-OCT. One common missense variant (p.Arg373Cys, 6/9 families, 66.7%) found both in the current and previous studies was determined out of five *PROM1* variants, in keeping with the common phenotype of MD/COD/CORD in the Japanese population.

The detailed data of a large cohort of Japanese patients with AD *PROM1*-RD are first described in the present study, while there are two large cohort studies of the detailed phenotype of European families with AD or AD/AR *PROM1*-RD (Cehajic-Kapetanovic et al., 2019; Michaelides et al., 2010). *PROM1*-RD accounts for 26.7% of AD COD/CORD/MD/STGD in the JEGC IRD cohort, in contrast with a lower prevalence of *PROM1*-RD in the other population (11.3% in United Kingdom) (Gill et al., 2019). There were no families with AR *PROM1*-RD in the current study, although a higher prevalence (approximately twice) of AR *PROM1*-RD than AD *PROM1*-RD was reported in another U.K. report (Cehajic-Kapetanovic et al., 2019). These facts indicate the relatively high prevalence of *PROM1*-RD in AD IRD predominantly affecting the macula/cone system in the Japanese population.

The age of onset of AD *PROM1*-RD in our cohort was variable, ranging from childhood to the forties. The early onset and long duration of the disease were not necessarily associated with poor vision; the duration of disease was similar in Patients 6 and 7 (8 vs. 11 years), but much better vision was found in Patient 6. In addition, the duration of disease was 47 years in Patient 10, but this patient still had a similar level of vision compared to a patient with 8 years of disease (Patient 6). These findings suggest that age of onset and duration of disease are not clearly related to the severity of visual acuity in *PROM1*-RD, unlike other macular dystrophies (Fujinami, Sergouniotis, Davidson, Wright, et al., 2013; Fujinami, Sergouniotis, Davidson, Mackay, et al., 2013; Fujinami et al., 2014, 2015, 2019; Nakamura et al., 2019). These are probably because of the frequent presence of foveal sparing (bull's eye maculopathy) in *PROM1*-RD.[5]

In our cohort, all 10 affected subjects showed concentrated macular atrophy in fundus photographs, and the atrophic or mottled areas were clearly demonstrated on FAF images. The very similar macular findings on fundus and FAF images found in our cohort were identified in previous studies of a specific variant (p.Arg373Cys) (Cehajic-Kapetanovic et al., 2019; Kim et al., 2017; Michaelides et al., 2010). It is of note, however, the peripheral atrophic changes were also found in a previous study (Michaelides et al., 2010).

All nine subjects with available data in our cohort demonstrated generalized cone or cone rod dysfunction. Five of nine subjects (55.6%) had preserved rod function. Four subjects (44.4%) with good VA showed slight or moderate reduction in cone responses; thus, the maintained generalized cone function could be an indicator for good vision. As shown in the previous study, the cone-dominated reduction of generalized retinal function is a key feature of AD *PROM1*-RD (Michaelides et al., 2010).

One disease-causing heterozygous variant, one putative disease-causing heterozygous variant, and one VUS were identified in our cohort (p.Arg373Cys, p.Cys112Arg, and p.Gly53Asp). Three variants (c.1117C>T, (p.Arg373Cys); c.1551+1G>A; c.1738A>C, (p.Asn580His)) were previously reported in the Japanese population. The recurrent variant (p.Arg373Cys) was reported in several publications of IRD in European/South Asian/African populations and our six AD Japanese families demonstrated very similar clinical findings, although the phenotypic information of a previously reported family was limited. Due to the limited genetic data source for haplotype analysis in this study to determine a single or multiple founder events, additional investigations are necessary to discern these. This variant introduces an additional cysteine residue in the extracellular loop of PROM1 (O43490; UniProt; https://www.uniprot.org/; Figure 4), which can cause disulfide bridge network disruption and abnormal homophilic protein interactions (Yang et al., 2008). The Arg373Cys mutant protein is mislocalized and causes mislocalization of the wild-type PROM1 protein and wild-type PCDH21 protein, which eventually leads to retinal degeneration (i.e., dominant-negative effect) (Yang et al., 2008).

One putative disease-causing variant (p.Cys112Arg) and a VUS (p.Gly53Asp) are located in a transmembrane domain and an extracellular domain of the PROM1 protein (Figure 4). Perfect evolutionary conservation was shown in the locations for both variants (p.Cys112Arg and p.Gly53Asp) (Figure 5). Given the extremely low allele frequency and the in silico prediction of the former variant (p.Cys112Arg; none in the general population detected by gnomAD whole exome sequence), this variant may represent a cause of AD *PROM1*-RD, although co-segregation analysis of family members was unavailable in this family (Family #7). The latter variant (p.Gly53Asp) with a relatively high allele frequency (0.0000482 detected by gnomAD whole exome sequence) in the general population suggests little supporting evidence for causation. The clinical effect of these variants is still uncertain, and function analysis is therefore needed to further understand the disease mechanism caused by these two variants.

Four of five *PROM1* variants (p.Gly53Asp; p.Cys112Arg; c.1551+1G>A; p.Asn580His) identified in the Japanese population have never been found in other populations. This finding suggests the distinct genetic background in the Japanese population with regard to the *PROM1* gene. Significantly high allele frequencies of two variants (p.Gly53Asp; p.Asn580His) in the general East Asian population could support this finding; although the provided information for the two unique variants (c.1551+1G>A—unsolved; p.Asn580His—a modifier) reported in previous Japanese reports was limited; (Arai et al., 2015; Koyanagi et al., 2019) thus, further genomic and functional analyses would help to interpret how these two variants have clinical effects as well as determine an inheritance manner.

There are limitations in this study. The selection bias related to the disease severity cannot be excluded, since it is unusual for subjects genetically at risk who show foveal sparing and no impaired visual acuity to visit clinics/hospitals. Moreover, this study is a cross-sectional retrospective study; thus, longitudinal natural history studies in a larger cohort could provide more accurate information for the phenotypic variation and the disease progression of *PROM1*-RD. The molecular mechanisms of most autosomal dominant *PROM1* variants are not yet known, and further functional investigation is required to conclude the disease-causation. The allele frequency provided by the public databases was applied for assessment of pathogenicity for each variant, however, it would be expected for individuals with the late-onset disease to be potentially included in the general population databases. The number of studies on *PROM1*-RD in the Japanese population obtained by a literature search was still limited, and further data accumulation should improve the achievements obtained by reviewing articles.

In conclusion, this large cohort study in the Japanese population determined the clinical and genetic characteristics of AD *PROM1*-RD. Concentrated macular atrophy on retinal imaging is a striking feature, with generalized cone-dominated retinal dysfunction. Disease onset and natural course was not clearly associated with severe visual impairment, and preservation of foveal structure and function is crucial for maintaining visual acuity. One prevalent missense variant (c.1117C>T, p.Arg373Cys) was determined in the Japanese population, which was also frequently detected in the European population. This information helps to monitor and counsel patients, as well as in designing future therapeutic trials.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

Kaoru Fujinami and **Akio Oishi**: Have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. **Kaoru Fujinami**, **Akio Oishi**, **Akitaka Tujikawa**, **Kazushige Tsunoda**: Research design. **Kaoru Fujinami**, **Akio Oishi**, **Lizhu Yang**, **Gavin Arno**, **Nikolas Pontikos**, **Kazutoshi Yoshitake**, **Yu Fujinami-Yokokawa**, **Xiao Liu**, **Takaaki Hayashi**, **Satoshi Katagiri**, **Kei Mizobuchi**, **Atsushi Mizota**, **Kei Shinoda**, **Natsuko Nakamura**, **Toshihide Kurihara**, **Kazuo Tsubota**, **Yozo Miyake**, **Takeshi Iwata**, **Akitaka Tsujikawa**, **Kazushige Tsunoda**; **Japan Eye Genetics Consortium study group:** Data acquisition and/or research execution. **Kaoru Fujinami**, **Akio Oishi**, **Lizhu Yang**, **Gavin Arno**, **Nikolas Pontikos**, **Kazutoshi Yoshitake**, **Yu Fujinami-Yokokawa**, **Xiao Liu**, **Takaaki Hayashi**, **Satoshi Katagiri**, **Kei Mizobuchi**, **Atsushi Mizota**, **Kei Shinoda**, **Natsuko Nakamura**, **Toshihide Kurihara**, **Kazuo Tsubota**, **Yozo Miyake**, **Takeshi Iwata**, **Akitaka Tsujikawa**, **Kazushige Tsunoda**; **Japan Eye Genetics Consortium study group:** Data analysis and/or interpretation. **Kaoru Fujinami**, **Akio Oishi**, **Lizhu Yang**, **Yu Yokokawa-Fujinami**, **Xiao Liu**, **Kei Shinoda**, **Akitaka Tujikawa**, **Kazushige Tsunoda:** Manuscript preparation.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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