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RP2-associated retinal disorder in a Japanese cohort: Report of novel variants and a literature review, identifying a genotype–phenotype association

Kaoru Fujinami[1,2,3,4] | Xiao Liu[1,2,5] | Shinji Ueno[6] | Atsushi Mizota[7] | Kei Shinoda[7,8] | Kazuki Kuniyoshi[9] | Yu Fujinami-Yokokawa[1,3,10,11] | Lizhu Yang[1,2] | Gavin Arno[1,3,4,12] | Nikolas Pontikos[1,3,4] | Shuhei Kameya[13] | Taro Kominami[6] | Hiroko Terasaki[6] | Hiroyuki Sakuramoto[9] | Natsuko Nakamura[1,7,14] | Toshihide Kurihara[2] | Kazuo Tsubota[2] | Yozo Miyake[1,15,16] | Kazutoshi Yoshiake[17] | Takeshi Iwata[17] | Kazushige Tsunoda[1] | Japan Eye Genetics Consortium Study Group

[1]Laboratory of Visual Physiology, Division of Vision Research, National Institute of Sensory Organs, National Hospital Organization Tokyo Medical Center, Tokyo, Japan

[2]Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan

[3]UCL Institute of Ophthalmology, London, UK

[4]Moorfields Eye Hospital, London, UK

[5]Southwest Hospital/Southwest Eye Hospital, Third Military Medical University (Army Medical University), Chongqing, China

[6]Department of Ophthalmology, Nagoya University Graduate School of Medicine, Nagoya, Japan

[7]Department of Ophthalmology, Teikyo University, Tokyo, Japan

[8]Department of Ophthalmology, Saitama Medical University, Moroyama Campus, Saitama, Japan

[9]Department of Ophthalmology, Kindai University Faculty of Medicine, Osaka-Sayama, Japan

[10]Department of Health Policy and Management, Keio University School of Medicine, Tokyo, Japan

[11]Division of Public Health, Yokokawa Clinic, Suita, Japan

[12]North East Thames Regional Genetics Service, UCL Great Ormond Street Institute of Child Health, NHS Foundation Trust, London, UK

[13]Department of Ophthalmology, Nippon Medical School Chiba Hokusoh Hospital, Inzai, Japan

[14]Department of Ophthalmology, The University of Tokyo, Tokyo, Japan

[15]Aichi Medical University, Nagakute, Japan

[16]Next vision, Kobe Eye Center, Kobe, Japan

[17]Division of Molecular and Cellular Biology, National Institute of Sensory Organs, National Hospital Organization Tokyo Medical Center, Tokyo, Japan

Correspondence
Kaoru Fujinami, Laboratory of Visual Physiology, Division for Vision Research, National Institute of Sensory Organs, National Hospital Organization Tokyo Medical Center, No. 2-5-1, Higashigaoka, Meguro-ku, Tokyo

Abstract

The retinitis pigmentosa 2 (*RP2*) gene is one of the causative genes for X-linked inherited retinal disorder. We characterized the clinical/genetic features of four patients with *RP2*-associated retinal disorder (*RP2*-RD) from four Japanese families in

Kaoru Fujinami and Xiao Liu are joint first authors of this study.

152-8902 Japan.
Email: k.fujinami@ucl.ac.uk

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a nationwide cohort. A systematic review of *RP2*-RD in the Japanese population was also performed. All four patients were clinically diagnosed with retinitis pigmentosa (RP). The mean age at examination was 36.5 (10–47) years, and the mean visual acuity in the right/left eye was 1.40 (0.52–2.0)/1.10 (0.52–1.7) in the logarithm of the minimum angle of resolution unit, respectively. Three patients showed extensive retinal atrophy with macular involvement, and one had central retinal atrophy. Four *RP2* variants were identified, including two novel missense (p.Ser6Phe, p.Leu189Pro) and two previously reported truncating variants (p.Arg120Ter, p.Glu269CysfsTer3). The phenotypes of two patients with truncating variants were more severe than the phenotypes of two patients with missense variants. A systematic review revealed additional 11 variants, including three missense and eight deleterious (null) variants, and a statistically significant association between phenotype severity and genotype severity was revealed. The clinical and genetic spectrum of *RP2*-RD was illustrated in the Japanese population, identifying the characteristic features of a severe form of RP with early macular involvement.

KEYWORDS
inherited retinal disorder, retinitis pigmentosa, *RP2* gene, X-linked recessive

1 | INTRODUCTION

Inherited retinal disorder (IRD) is one of the major causes of blindness in developed countries in both children and the working population (Liew, Michaelides, & Bunce, 2014; Sohocki et al., 2001; Solebo, Teoh, & Rahi, 2017). Retinitis pigmentosa (RP) represents a heterogeneous group of RDs characterized by progressive bilateral degeneration of rod and cone photoreceptors, which affects approximately 1:3000 individuals (Boughman, Conneally, & Nance, 1980; Chizzolini et al., 2011; Lyraki, Megaw, & Hurd, 2016; Prokisch, Hartig, Hellinger, Meitinger, & Rosenberg, 2007). Various inheritance patterns have been identified in RP and allied disorders, including autosomal dominant, autosomal recessive (AR), X-linked recessive (XL), mitochondrial inheritance, and others (Wright, Chakarova, Abd El-Aziz, & Bhattacharya, 2010).

XLRP is observed in approximately 10 to 20% of RP cases (Breuer et al., 2002; Fishman, 1978; Haim, 1992; Prokisch et al., 2007; Wright et al., 2010) and is associated with the most severe form of the disease (Fishman, 1978). Three causative genes for XLRP are the RP GTPase regulator (*RPGR*; OMIM: 312610), the retinitis pigmentosa 2 (*RP2*; OMIM: 312600), and orofaciodigital syndrome 1 (*OFD1*; OMIM: 300170) genes. *RGPR* and *RP2* account for 70–90% and 7–18% of XLRP cases, respectively (Hardcastle et al., 1999; Neidhardt et al., 2008; Pelletier et al., 2007; Sahel, Marazova, & Audo, 2014; Vervoort et al., 2000).

RP2 was first identified by linkage analysis and encodes the RP2 protein, which consists of 350 residues (Schwahn et al., 1998). The RP2 protein is localized to the plasma membrane of rod/cone photoreceptors, the retinal pigment epithelium (RPE), and other retinal cells in human (Grayson et al., 2002), as well as in the Golgi complex, the primary cilia, and the basal body of the connecting cilium in mice (Evans et al., 2010; T. Hurd et al., 2010; T. W. Hurd, Fan, & Margolis, 2011; Lyraki et al., 2016). RP2 goes through dual acylation at the extreme N-terminus, and this modification is crucial for plasma membrane localization and connecting cilium targeting (Chapple et al., 2000; Chapple, Hardcastle, Grayson, Willison, & Cheetham, 2002; Evans et al., 2010; T. Hurd et al., 2010; Lyraki et al., 2016). Cone-dominated retinal degeneration was reported in mouse models (Li et al., 2013; Li, Rao, & Khanna, 2019; H. Zhang et al., 2015).

Since the discovery of *RP2* as a causative gene for RP, 133 disease-associated variants have been identified, including 43 missense variants, 14 nonsense variants, 15 splice site alterations, 50 small insertions/deletions, nine gross deletions, one gross insertion, and others (HGMD; https://portal.biobase-international.com; Supporting Information), and patients with *RP2*-associated retinal disorder (*RP2*-RD) often present a severe and "atypical" form for RP, with early macular involvement causing central visual loss (Andreasson et al., 2003; Carss et al., 2017; Dandekar et al., 2004; Hosono et al., 2018; Jayasundera et al., 2010; Ji et al., 2010; Jin, Liu, Hayakawa, Murakami, & Nao-i, 2006; Maeda et al., 2018; Mashima et al., 2000; Mashima, Saga, Akeo, & Oguchi, 2001; Mears et al., 1999; Miano et al., 2001; Prokisch et al., 2007; Sharon et al., 2000; Sharon et al., 2003; Vorster et al., 2004; Wada, Nakazawa, Abe, & Tamai, 2000; Wang et al., 2014; Yang et al., 2014). A number of studies have been published about *RP2*-RD, especially in the European population; however, only a limited number of case reports/series have described the clinical and genetic features of *RP2*-RD in the East Asian population (Dan, Huang, Xing, & Shen, 2020; Hosono et al., 2018; Ji et al., 2010; Jiang et al., 2017; Jin et al., 2006; Kim et al., 2019; Koyanagi et al., 2019; Kurata et al., 2019; Lim, Park, Lee, & Taek Lim, 2016; Maeda et al., 2018; Mashima et al., 2001; Mashima et al., 2000; Pan et al., 2014; Wada et al., 2000; Xu et al., 2019; J. Zhang et al., 2019).

Therefore, the purpose of this study was to characterize the clinical and genetic features of patients with *RP2*-RD in a large nationwide Japanese cohort. A systematic review of *RP2*-RD in the Japanese population was also performed to clarify the genetic background and establish a genotype–phenotype association.

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 of the Japan Eye Genetics Consortium (JEGC; http://www.jegc.org/). The principal institute is National Institute of Sensory Organs (NISO), National Hospital Organization Tokyo Medical Center (Reference: R18-029) (World Medical Association, 2013).

2.1 | Participants

Patients with a clinical diagnosis of IRD and available whole-exome sequencing (WES) genetic data were studied between 2008 and 2018. A total of 1,294 subjects from 730 families for whom genotype–phenotype association studies were completed, were surveyed, including 47 families with XLRP and 141 families with sporadic RP (Fujinami et al., 2016; Fujinami et al., 2019; Fujinami-Yokokawa et al., 2019; Fujinami-Yokokawa et al., 2020; Kameya et al., 2019; Katagiri et al., 2020; Kondo et al., 2019; Maeda-Katahira et al., 2019; Mawatari et al., 2019; Mizobuchi et al., 2019; Nakamura et al., 2019; Nakanishi et al., 2016; Pontikos et al., 2020; Xiao Liu et al., 2020; Yang et al., 2020).

2.2 | Clinical examinations

Clinical information is available in the NISO online database, including ethnicity, medical and family history, chief complaints of visual symptoms, onset of disease (of when the visual loss was first noted by the patient or when an abnormal retinal finding was first detected), measurement of refractive errors, best-corrected decimal visual acuity (BCVA) converted to the logarithm of the minimum angle of resolution (LogMAR) unit, fundus photographs, fundus autofluorescence (FAF) images, spectral-domain optical coherence tomographic (SD-OCT) images, kinetic visual fields, and electrophysiological responses recorded in accordance with the international standards of the International Society for Clinical Electrophysiology of Vision (ISCEV) (McCulloch et al., 2015a, 2015b).

2.3 | Variant detection

Genomic DNA was extracted from all affected subjects and unaffected family members (where available) for co-segregation analysis. WES with target sequence analysis of 301 retinal disease-associated genes mainly listed in a public database (RetNet https://sph.uth.edu/retnet/home.htm) was performed (Fujinami et al., 2016; Xiao Liu et al., 2020). The called variants were filtered based on the allele frequencies in the general Japanese population (less than 1%) of the Human Genetic Variation Database (HGVD; http://www.hgvd.genome.med.kyoto-u.ac.jp/). Hypomorphic variants with high allele frequencies (>1%) were analyzed for three particular genes (*EYS*, *ABCA4*, *USH2A*) (Yang et al., 2020). Depth and coverage for the target areas were assessed using the integrative Genomics Viewer (http://www.broadinstitute.org/igv/). Sanger bi-direct sequencing was performed to confirm the detected *RP2* variants and to conduct co-segregation analysis. Primer sequences are provided in Table S1.

Together with the clinical features (phenotype categorization) and the patterns of inheritance, disease-causing variants were determined from the detected/filtered variants in the retinal disease-associated genes (Fujinami-Yokokawa et al., 2020; Xiao Liu et al., 2020).

2.4 | In silico molecular genetic analysis

The allele frequencies of all called variants for the Japanese, East Asian, South Asian, European, and African populations were established based on the HGVD (Japanese), Integrative Japanese Genome Variation (iJGVD 3.5k, 4.7k; https://jmorp.megabank.tohoku.ac.jp/ijgvd/; Japanese), 1,000 Genomes (http://www.internationalgenome.org/; total), and the Genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org/; East Asian, South Asian, European [non-Finish], and African).

All detected variants in the *RP2* gene were analyzed with general and 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/), SIFT (https://www.sift.co.uk/), PROVEAN (http://

provean.jcvi.org/index.php), Polyphen 2 (http://genetics.bwh.harvard.edu/pph2/), and Human Splicing Finder (http://www.umd.be/HSF3/). The evolutionary conservation scores were evaluated with the UCSC database (https://genome.ucsc.edu/index.html).

The location of the detected *RP2* variants was analyzed with a schematic genetic and protein structure of RP2 (ENST00000218340.3), and multiple alignments of eight species of RP2 were performed with the Clustal Omega program (https://www.ebi.ac.uk/Tools/msa/clustalo/). Molecular modeling of missense variants was performed with YASARA software (http://www.yasara.org/) based on a Swiss model (O75695; XRP2_HUMAN; https://swissmodel.expasy.org/).

The 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 | A systematic review of *RP2*-RD

A systemic review of peer-reviewed articles that describe Japanese cases with *RP2*-RD was performed. A public search engine (PubMed; https://www.ncbi.nlm.nih.gov/pubmed/) was used to identify articles, and clinical and genetic information was collected. For the previously reported *RP2* variants, in silico molecular genetic analyses were performed in the same way as in the current study.

2.6 | Analysis of genotype–phenotype association

Patients in the current study and previously reported cases were classified into two genotype groups based on the presence of null *RP2* variants such as nonsense variants, frameshift variants, and splice site alterations: genotype group A with null variants and genotype group B with missense variants. For the purpose of this analysis, probands in the current cohort and previous publications were classified into two phenotype groups based on disease onset and BCVA: (a) a mild phenotype group showing both late-onset (≧10 years) and moderate or better VA (between 0.22 and 1.0 LogMAR unit in the better eye) and (b) a severe phenotype group with both early-onset (<10 years) and severe VA (1.0 LogMAR unit or worse in the better eye). Patients who did not meet any of the two criteria were classified into an intermediate phenotype group. Probands with available data in families were selected for the further analyses and patients with unavailable data of either onset or VA were excluded.

An association between the genotype group classification and the phenotype severity group classification was investigated with Cochran-Armitage Test. A p value <.05 was considered statistically significant.

3 | RESULTS

3.1 | Demographics

Four affected males from four families who had a clinical diagnosis of IRD and were harboring *RP2* variants were identified. The detailed demographics are described in Table 1. All four patients were clinically diagnosed with RP by attending doctors. The pedigrees of the four families are presented in Figure 1. All four families were originally from Japan and any mixture with other ethnicity was not reported. XL family history was clearly reported or possible in three families (Families #2, #3, and #4), and no affected subjects except for the proband were reported in one family (Family #1). One patient had a medical history of severe uveitis in the left eye (Patient 2), and retinal imaging, visual field testing and electrophysiological assessment were unavailable due to the dense corneal opacity. Cataracts were reported in two patients (Patients 2 and 4), and one patient underwent cataract surgery in the right eye (Patient 2). The mean age at the latest examination of four patients was 32.5 years (range, 10–47).

3.2 | Onset, chief complaint, refraction, and visual acuity

The mean age of onset was 11.3 (range, 3–28) years in the three patients with available records. Two of these three patients had early-onset of 3 years (Patients 1 and 2). Chief complaints at the initial visit of four patients with available records were night blindness in two patients (Patients 2 and 3), photophobia in one (Patient 1), and reduced visual acuity in one (Patient 4).

The mean spherical equivalent of the refractive errors of three phakic patients with available records was −2.17 diopter (−6.0 to 0.0) in the right eye and −2.33 diopter in the left eye (−6.0 to −0.50). Two patients had high myopia (Patients 2 and 4). One patient had an intraocular lens in the right eye (Patient 2). The median values of BCVA in the right and left eyes of the four patients with available records were 1.27 (0.52–2.00), and 1.12 (0.52–1.70) LogMAR units, respectively. There were three patients with severe VA (1.0 or worse LogMAR units in the better eye) (Patients 1, 2, and 4), and one with moderate VA (between 0.22 and 1.0 LogMAR unit in the better eye) (Patient 3).

3.3 | Retinal images and morphological findings

Fundus photographs were obtained in all four patients, and FAF images were available in one patient (Patient 1). The representative images are presented in Figure 2, and the detailed findings are described in Table 2. Extensive atrophic changes were observed in two patients (Patients 1 and 2). There was one patient with peripheral atrophy (Patient 3) and one with atrophic changes at the posterior pole (Patient 4). Preserved foveal appearance was shown in two patients (Patients 1 and 3), and preserved peripheral appearance was found in one patient (Patient 4). Bone spicule pigmentation at the periphery was identified in one patient (Patient 2), and macular pigmentation was detected in two patients (Patients 2 and 4). Retinal vessel attenuation was observed in three patients (Patients 1–3), and optic disc pallor was shown in two patients (Patients 2 and 3).

SD-OCT was obtained in four patients (Patients 1–4). Representative images are presented in Figure 3. Loss of photoreceptor layers

TABLE 1 Demographics and detected variants of four Japanese patients with *RP2*-associated retinal disorder (*RP2*-RD)

Family no	Patient no	Patient ID	Inheritance	Sex	Age (at latest examination)	Onset	Chief complaint/ other symptoms	Refractive errors		BCVA in the LogMAR unit		RP2 variants	Phenotype severity group	Genotype group
								RE (diopter)	LE (diopter)	RE	LE			
1 (TMC-01)	1-II:1 (patient 1)	1-II:3	Sporadic	M	10	3	Photophobia/poor VA/ night blindness	0.0	−0.5	1.3	1.15	C.358C>T, p.Arg120Ter	Severe	A
2 (NU-01)	2-II:3 (patient 2)	2-II:3	XL	M	35	3	Night blindness/poor VA/ peripheral visual field defect	−6.0	NA	2	NLP	c.801_804del, p.Glu269CysfsTer3	Severe	A
3 (TU-01)	3-III:1 (patient 3)	3-III:1	XL	M	38	28	Night blindness	−0.5	−0.5	0.52	0.52	c.17C>T, p.Ser6Phe	Mild	B
4 (KDU-01)	4-II:1 (patient 4)	4-II:1	XL	M	47	NA	Reduced visual acuity	−6.0	−6.0	1.7	1.7	c.566T>C, p.Leu189Pro	NA	B

Note: 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 when an abnormal retinal finding was first detected. Severe post-uveitic changes with dense corneal opacity (invisible fundus) were found in the left eye of patient 2. Cataracts were reported in two patients (patients 2 and 4), and one patient underwent cataract surgery in the right eye (patient 2). RP2 transcript ID: NM_006915.2. Whole-exome sequencing with target analysis of 301 retinal disease-associated genes mainly listed on a public database (RetNet https://sph.uth.edu/retnet/home.htm) was performed. Genotype A: Null variants, severe group; genotype B: Missense variants, mild group.

Abbreviations: AR, autosomal recessive; BCVA, best corrected deimal visual acuity converted to the logarithm of the minimum angle of resolution (LogMAR) unit; F, female; LE, left eye; M, male; NA, not available; NLP, no light perception; no, number; RE, right eye; XL, x-linked recessive.

was observed at the entire retina in three patients (Patients 1, 2, and 4) and at the peripheral retina in one patient (Patient 3). Relatively preserved foveal structure, including slight changes of fluid in the inner layers were identified in one patient (Patient 3).

3.4 | Visual fields and electrophysiological findings

Visual field testing was performed in four patients with Goldmann kinetic perimetry (Table 2). Peripheral visual field loss with central scotoma was observed in two patients (Patients 1 and 3). There was one patient with an entire visual field defect (Patient 2) and one with a large central scotoma and preserved peripheral field (Patient 4). Electrophysiological assessment was performed in four patients (Patients 1–4) (Table 2). Extinguished responses in both dark-adapted and light-adapted conditions were recorded in three patients (Patients 1–3). Relatively preserved responses in both dark-adapted and light-adapted conditions were observed in one patient (Patient 4).

3.5 | *RP2* variants

Four affected probands (males) were tested with WES with target analysis of 301 retinal disease-associated genes: 1-II:1 (Patient 1), 2-II:3 (Patient 2), 3-III:1 (Patient 3), and 4-II:1 (Patient 4). In addition, two unaffected family members from Family 1 and two unaffected family members in Family 3 were examined for segregation: 1-I:1 (father of Patien1), 1-I:2 (mother of Patient 1), 3-II:7 (father of Patient 3), and 3-II:8 (mother of Patient 3) (Figure 1, Table S2). Two mothers from two families (Families 1 and 3) were proved to be carriers: 1-I:2 (mother of Patient 1) and 3-II:8 (mother of Patient 3).

Variants data of four patients are summarized in Table 1 and Figure 1. Four hemizygous *RP2* variants were identified: c.17C>T (p.Ser6Phe); c.358C>T (p.Arg120Ter); c.566T>C (p.Leu189Pro); and c.801_804del (p.Glu269CysfsTer3) (NM_006915.2). Two variants have been previously reported: p.Arg120Ter in eight articles(Carss et al., 2017; Hardcastle et al., 1999; Jin et al., 2006; Kurata et al., 2019; Mashima et al., 2001; Mears et al., 1999; Vorster et al., 2004; Wang et al., 2014) and p.Glu269CysfsTer3 in one article (Pelletier et al., 2007). The other two variants have never been reported: c.17C>T (p.Ser6Phe) and c.566T>C (p.Leu189Pro).

A schematic of the RP2 protein structure showing the positions of the four detected variants in the current study is presented in Figure 4. There was one missense variant located in exon 1 (p.Ser6-Phe), one nonsense variant (p.Arg120Ter), and one missense variant (p.Leu189Pro) in exon 2, and one frameshift variant in exon 3 (p.Glu269CysfsTer3).

3.6 | *In silico* molecular genetic analysis

The detailed results of *in silico* molecular genetic analyses for the four detected *RP2* variants in the current study are provided in Table 3.

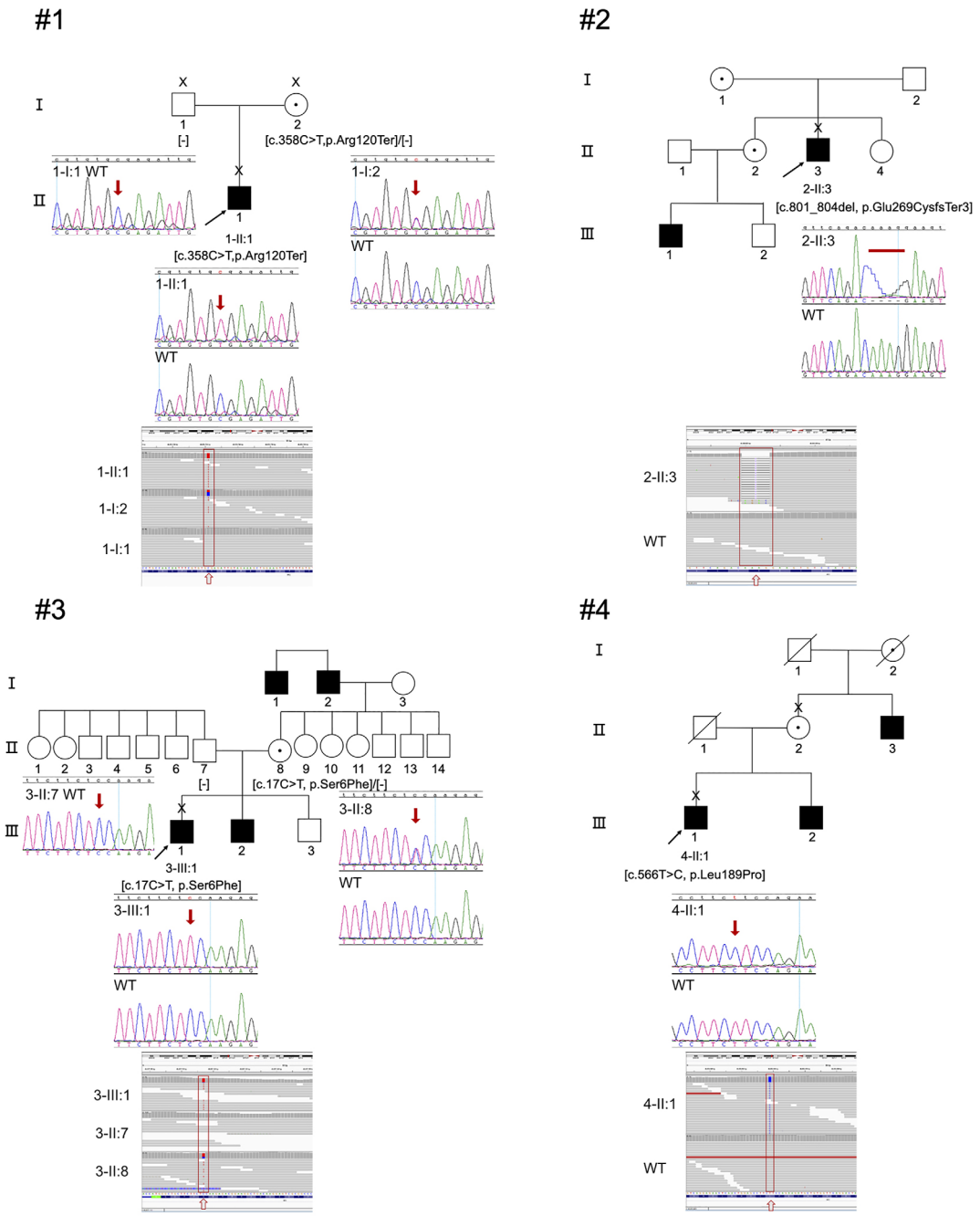


FIGURE 1 Pedigrees of four Japanese families with retinitis pigmentosa harboring hemizygous *RP2* variants. The affected males are represented by solid squares (men), and unaffected family members are represented by white icons. The slash symbol indicates deceased individuals. The generation is numbered on the left. The probands and the clinically examined individuals are marked by an arrow and a cross, respectively. Depth and coverage for the target areas by next-generation sequencing were assessed using the integrative Genomics Viewer (http://www.broadinstitute.org/igv/). Sanger bi-direct sequencing was also performed to confirm each variant

These four *RP2* variants were well-covered with WES, but no subjects in the general population had these variants, which confirmed the rarity of these detected variants (Table 3, Table S3).

Three general (MutationTaster, FATHMM, CADD) and three functional (SIFT, PROVEAN, Polyphen2) prediction programs were applied for two missense variants (p.Ser6Phe, p.Leu189Pro), and all programs predicted disease-causing/damaging effects. The evolutionary conservation scores obtained with the UCSC database indicated high conservation of the two missense variants (Figure 5). Molecular modeling of these missense variants is shown in Figure S1. Pathogenicity classifications, according to the ACMG guidelines, were pathogenic for the two truncating variants (p.Glu269CysfsTer3, p.Arg120Ter), likely pathogenic for the one missense variant (p.Ser6Phe,) and uncertain significance for the one missense variant (p.Leu189Pro).

Overall, given the inheritance and the phenotype, two disease-causing variants (p.Glu269CysfsTer3, p.Arg120Ter) and two putative

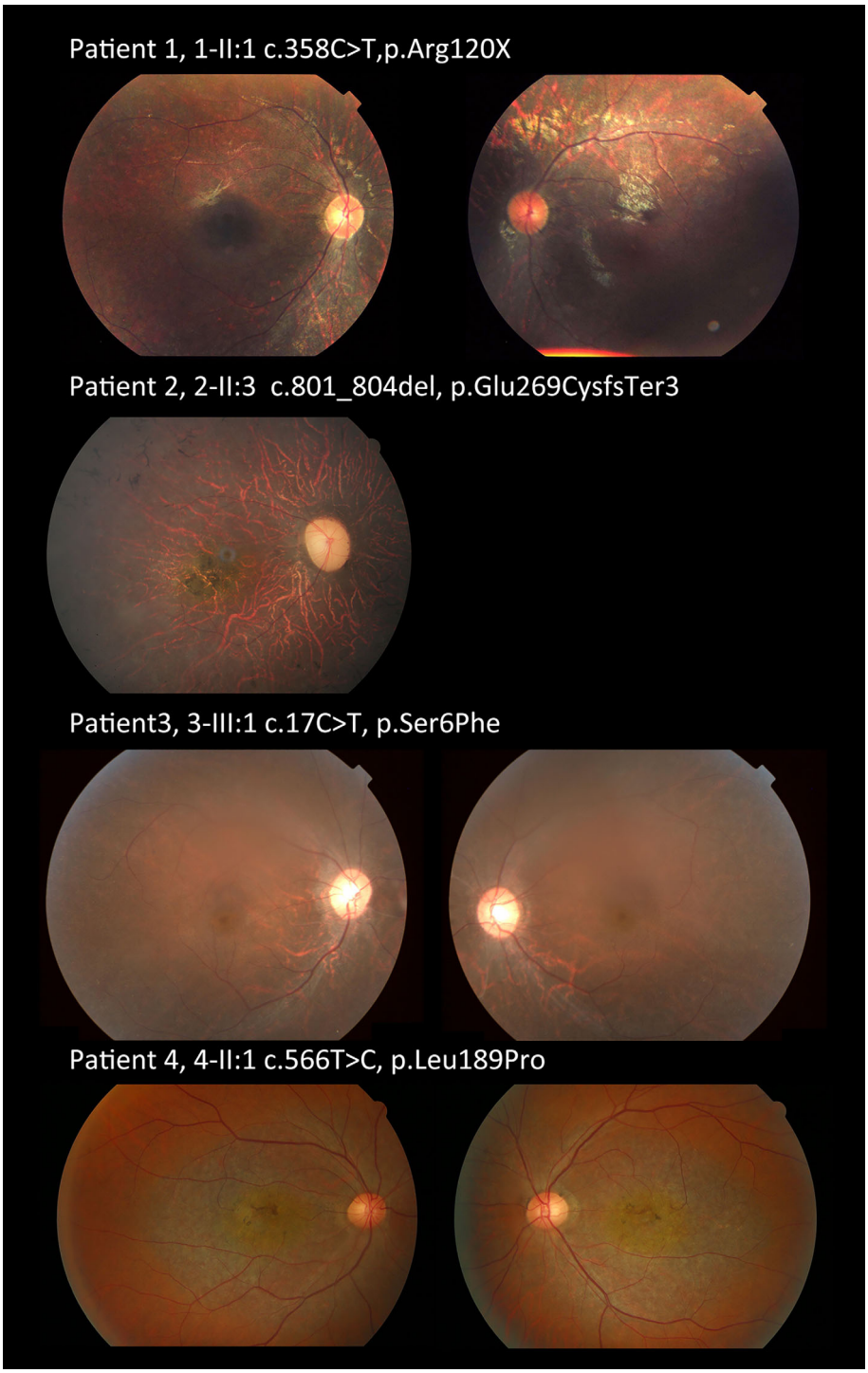


FIGURE 2 Fundus photographs and fundus autofluorescence images of *RP2*-associated retinal disorder (*RP2*-RD). Patient 1: Extensive retinal atrophic changes with relatively preserved foveal appearance and vessel attenuation. Patient 2: Extensive retinal atrophic changes with bone spicule pigmentation at the periphery and patchy pigmentation at the macula, vessel attenuation, and disc pallor. Patient 3: Atrophic changes at the peripheral retina with relatively preserved foveal appearance, vessel attenuation, and disc pallor. Patient 4: Atrophic changes at the posterior pole with pigmentation at the macula

disease-causing variants (p.Ser6Phe, p.Leu189Pro) were determined in four families with XLRP.

3.7 | Nineteen cases from 14 Japanese families with *RP2*-RD in previous reports

There are eight previous reports of *RP2*-RD in the Japanese population (Hosono et al., 2018; Jin et al., 2006; Koyanagi et al., 2019; Kurata et al., 2019; Maeda et al., 2018; Mashima et al., 2000; Mashima et al., 2001; Wada et al., 2000). The summarized data are presented in Table 4. Nineteen affected males from 14 families were reported in total. There were 17 patients with RP and two patients with Leber congenital amaurosis (LCA).

The mean age at the latest examination among the 16 patients with available data was 31.2 (16–61) years, and the mean age at onset of the eight patients with available data was 5.75 (3–11) years. Other descriptions about the age of onset were as follows: in the first

TABLE 2 Retinal, morphological, visual field, and electrophysiological findings of four Japanese patients with *RP2*-RD

Patient no	Fundus/FAF findings	SD-OCT findings	Visual field	Electrophysiological assessment
1	Extensive retinal atrophic changes with relatively preserved foveal appearance and vessel attenuation.	Loss of photoreceptor layers at the entire retina with relatively preserved other sensory retinal layers and RPE layer.	Peripheral visual field loss with central scotoma.	Extinguished responses in both dark-adapted and light-adapted conditions.
2	Extensive retinal atrophic changes with bone spicule pigmentation at the periphery and patchy pigmentation at the macula, vessel attenuation, and disc pallor.	Loss of photoreceptor layers at the entire retina with thinned RPE.	Entire visual field loss.	Extinguished responses in both dark-adapted and light-adapted conditions.
3	Atrophic changes at the peripheral retina with relatively preserved foveal appearance, vessel attenuation, and disc pallor.	Loss of photoreceptor layers at the peripheral retina with relatively preserved foveal structure including slight changes of fluid in the inner layers.	Peripheral visual field loss with central scotoma.	Extinguished responses in both dark-adapted and light-adapted conditions.
4	Atrophic changes at the posterior pole with pigmentation at the macula.	Loss of photoreceptor layers at the entire retina with thinned RPE.	Large central scotoma with preserved peripheral field.	Relatively preserved responses in both dark-adapted and light-adapted conditions.

Note: Retinal imaging, visual field testing, and electrophysiological assessment were unavailable due to the dense corneal opacity after severe uveitis in the left eye of Patient 2.
Abbreviations: FAF, fundus autofluorescence; LE, left eye; RE, right eye; RPE, retinal pigment epithelium; SD-OCT, spectral-domain optical coherence tomography.

decade (two patients), early teens (one patient), within 1 year (one patient), and childhood (one patient). Night blindness was noticed as the chief complaint in 10 out of the 12 patients (10/12, 83%) with available data. The mean spherical equivalent of refractive errors of 10 patients with available data was −6.6 diopter (−12.0–0.75) in the right eye and −6.1 diopter in the left eye (−10.0–0.50). The mean BCVA in the right and left eyes of 12 patients with available data was 1.14 (0.70–1.52) and 1.25 (0.52–1.70) LogMAR units, respectively. There were five eyes with hand motion, three eyes with light perception, and one eye with non-light perception. Electrophysiological responses were undetectable in 12 patients with available data.

The detailed results of in silico molecular genetic analyses for the 12 *RP2* variants in the previous Japanese reports are provided in Table 3. There were four frameshift variants, three nonsense variants, two splice site alterations, and three missense variants: c.87G>A (p.Trp29Ter); c.102+1G>A; c.217delT (p.Tyr73IlefsTer18); c.353G>A (p.Arg118His); c.358C>T (p.Arg120Ter); c.413A>G (p.Glu138Gly); c.677delG (p.Gly226ValfsTer12); c.685C>T (p.Gln229Ter); c.758T>G (p.Leu253Arg); c.769–2A>G; c.882delA (p.Gly295ValfsTer14); and c.831_832dupTC (p.Gln278LeufsTer16). Eight variants are unique in the Japanese population. With regard to four variants, there are reports from other populations: c.102+1G>A; p.Arg118His; p.Arg120Ter; and p.Glu138Gly. One common variant (p.Arg120Ter) was identified in three Japanese families in the previous reports (Jin et al., 2006; Kurata et al., 2019; Mashima et al., 2001).

3.8 | Genotype–phenotype association

For the analysis, a total of 10 probands with available onset age and BCVA were studied: three from the current study and seven from previously reported cases. There were eight patients in genotype group A (null variants) and two in genotype group B (non-null variants) (Table S4). Seven patients had a severe phenotype with earlier onset of the disease and severe VA loss, and three had a mild phenotype with later onset and moderate VA loss. A statistically significant association between genotype group classification and phenotype severity classification was revealed ($p < .05$).

4 | DISCUSSION

The clinical and genetic spectrum of *RP2*-RD was documented in a nationwide cohort of the Japanese population, detecting four variants, two of which have never been reported. A severe RP phenotype with early macular involvement causing central visual loss was identified and a genotype–phenotype association based on the presence of null variants was illustrated.

In the present study, *RP2*-RD accounted for 6.4% of XLRP families (3/47 families with XLRP) and 0.7% of sporadic RP cases (1/141 families with sporadic RP) in the JEGC cohort with IRD. Koyanagi et al. reported genetic results of a large cohort of 1,209 patients with RP and revealed that three of 18 patients (3/18, 16.7%) with a family

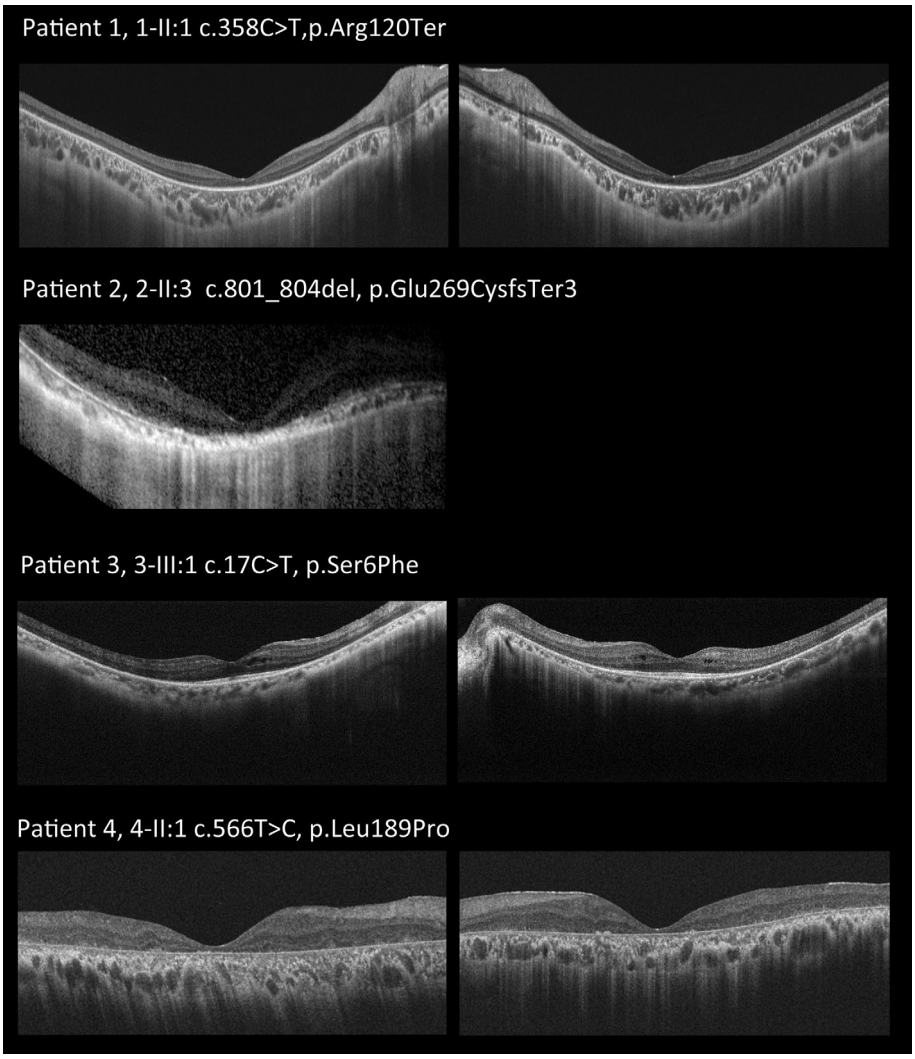
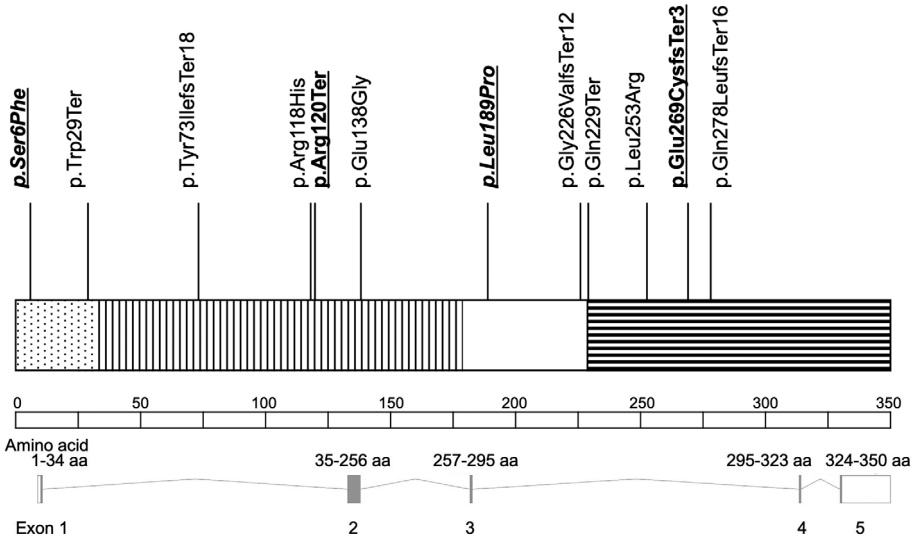


FIGURE 3 Optical coherence tomographic images of *RP2*-RD. Patient 1: Loss of photoreceptor layers at the entire retina with relatively preserved other sensory retinal layers and retinal pigment epithelial (RPE) layer. Patient 2: Loss of photoreceptor layers at the entire retina with thinned RPE. Patient 3: Loss of photoreceptor layers at the peripheral retina with relatively preserved foveal structure, including slight changes of fluid in the inner layers. Patient 4: Loss of photoreceptor layers at the entire retina with thinned RPE



Dot: myristoylation part (1-34 amino acid) (Karin Kuhnel et al. 2006)
Vertical stripe: C-CAP/cofactor C-like domain (24-179 amino acid) (Uniprot)
Horizontal stripe : ferredoxin-like domain (229–350 amino acid) (Karin Kuhnel et al. 2006)

FIGURE 4 A schematic genetic and protein structure of *RP2* and the location of the detected variants. The *RP2* gene (ENST00000218340.3) contains five exons that encode a 350 amino acid protein containing a myristoylation part, a cofactor C (Arl3 binding) domain, and a ferredoxin-like domain (Jayasundera et al., 2010). Four variants detected in the current study are underlined (c.17C>T (p.Ser6Phe); c.358C>T (p.Arg120Ter); c.566T>C (p.Leu189Pro); and c.801_804del (p.Glu269CysfsTer3)), and previously reported variants in the Japanese populations are shown without an underline. Two detected variants (p.Ser6Phe, p.Leu189Pro), which have never been reported, are shown in italic

TABLE 3 *In silico* molecular genetic analysis results for four detected variants in the current study and 10 previously reported variants in Japanese patients with *RP2*-RD

Nucleotide change	Amino acid change/ effect	Position	Coding impact	Location	dbSNP ID	HGVD	iJGVD		1000 genome	Allele frequency (genome)					
							3.5K	4.7K		East Asian	South Asian	African	European (Non-Finnish)	Total	Male
c.17C>T	p.Ser6Phe	46696552	Missense	Exon 1 of 5 position 75 of 160 (coding)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.87G>A	p.Trp29Ter	46696622	Nonsense	Exon 1 of 5 position 145 of 160 (coding, NMD)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.102+1G>A	Splice site alteration	46696638	Splice site alteration	Intron 1 of 4 position 1 of 16273 (splicing-ACMG, splicing, intronic)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.217delT	p.Tyr73IlefsTer18	46713025	Frameshift	Exon 2 of 5 position 115 of 666 (coding, NMD)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.353G>A	p.Arg118His	46713161	Missense	Exon 2 of 5 position 251 of 666 (coding)	rs28933687	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.358C>T, p. Arg120Ter	p.Arg120Ter	46713166	Nonsense	Exon 2 of 5 position 256 of 666 (coding, NMD)	rs104894927	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.413A>G	p.Glu138Gly	46713221	Missense	Exon 2 of 5 position 311 of 666 (coding)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.566T>C	p.Leu189Pro	46713374	Missense	Exon 2 of 5 position 464 of 666 (coding)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.677delG	p.Gly226ValfsTer12	46713485	Frameshift	Exon 2 of 5 position 575 of 666 (coding, NMD)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.685C>T	p.Gln229Ter	46713493	Nonsense	Exon 2 of 5 position 583 of 666 (coding, NMD)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.758T>G	p.Leu253Arg	46713566	Missense	Exon 2 of 5 position 656 of 666 (coding)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.769−2A>G	Splice site alteration	46719421	Splice site alteration	Intron 2 of 4 position 5845 of 5846 (splicing-ACMG, splicing, intronic)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.801_804delAAAG	p.Glu269CysfsTer3	46719455	Frameshift	Exon 3 of 5 position 33–36 of 115 (coding, NMD)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

TABLE 3 (Continued)

Nucleotide change	Amino acid change/ effect	Position	Coding impact	Location	dbSNP ID	HGVD	iJGVD 3.5K	iJGVD 4.7K	1000 genome	Allele frequency (genome) East Asian	South Asian	African	European (Non-Finnish)	Total	Male
c.831_832dupTC	p.Gln278LeufsTer16	46719484	Frameshift	Exon 3 of 5 before position 65 of 115 (coding, NMD)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.882delA	p.Gly295ValfsTer14	46719536	Frameshift	Exon 3 of 5 position 114 of 115 (splicing-ACMG, splicing, coding, NMD)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Nucleotide change	Amino acid change/ effect	General prediction: MutationTaster Prediction	MutationTaster Accuracy	MutationTaster Converted rankscore	FATHMM Prediction	FATHMM Score	FATHMM Converted rankscore	CADD Score	Functional prediction: SIFT Prediction	Human Splice Finder 3.0
c.17C>T	p.Ser6Phe	Disease causing	0.9993	0.4646	Damaging	−2.81	0.9113	24	Damaging	Probably no impact on splicing
c.87G>A	p.Trp29Ter	Disease causing automatic	1	0.81	Damaging	0.936	0.5866	37	NA	Potential alteration of splicing
c.102+1G>A	Splice site alteration	Disease causing	1	0.81	Damaging	0.9426	0.6059	33	NA	Most probably affecting splicing
c.217delT	p.Tyr73IlefsTer18	NA	NA	NA	NA	NA	NA	**26.5**	NA	Probably no impact on splicing
c.353G>A	p.Arg118His	Disease causing	1	0.81	Damaging	−2.73	0.9068	29.1	Damaging	Potential alteration of splicing
c.358C>T, p. Arg120Ter	p.Arg120Ter	Disease causing automatic	1	0.81	Damaging	0.7834	0.3863	34	NA	Potential alteration of splicing
c.413A>G	p.Glu138Gly	Disease causing	1	0.81	Damaging	−2.81	0.9113	27.8	Damaging	Potential alteration of splicing
c.566T>C	p.Leu189Pro	Disease causing	1	0.81	Damaging	−3.01	0.9221	27.1	Damaging	Potential alteration of splicing
c.677delG	p.Gly226ValfsTer12	NA	NA	NA	NA	NA	NA	27.5	NA	Potential alteration of splicing.
c.685C>T	p.Gln229Ter	Disease causing automatic	1	0.81	Damaging	0.947	0.6204	36	NA	Potential alteration of splicing
c.758T>G	p.Leu253Arg	Disease causing	0.9998	0.4908	Tolerated	−1.12	0.7759	24.7	Damaging	This mutation has probably no impact on splicing.
c.769−2A>G	Splice site alteration	NA	NA	NA	NA	NA	NA	34	NA	Most probably affecting splicing
c.801_804delAAAG	p.Glu269CysfsTer3	NA	NA	NA	NA	NA	NA	11.53	NA	Potential alteration of splicing
c.831_832dupTC	p.Gln278LeufsTer16	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.882delA	p.Gly295ValfsTer14	NA	NA	NA	NA	NA	NA	NA	NA	Potential alteration of splicing

(Continues)

Nucleotide change	Amino acid change/effect	Conservation				Conservation				ACMG Classification							
		PhyloP46way		PhastCons46way		PhyloP100way		PhastCons100way			Identified classification rules						
		Mammalian	Mammalian rankscore	Mammalian	Mammalian rankscore	vertebrate	vertebrate rankscore	vertebrate	vertebrate rankscore	Verdict	Factor1	Factor2	Factor3	Factor4	Factor5	References in the Japanese population	References in other population
c.17C>T	p.Ser6Phe	2.072	NA	1	NA	2.226	0.4261	1	0.7164	Likely pathogenic	PM2	PP1	PP2	PP3		This study	NA
c.87G>A	p.Trp29Ter	2.134	NA	1	NA	4.5009	0.6011	1	0.7164	Pathogenic	PVS1	PM2	PP3			Koyanagi et al., 2019	NA
c.102+1G>A	Splice site alteration	2.134	NA	0.994	NA	4.5009	0.6011	1	0.7164	Pathogenic	PVS1	PP1	PM2	PP3		Kurata et al., 2019	Sharon et al., 2000
c.217delT	p.Tyr73IlefsTer18	4.494	NA	1	NA	NA	NA	NA	NA	Pathogenic	PVS1	PP1	PM2	PP3		Kurata et al., 2019	NA
c.353G>A	p.Arg118His	5.5	NA	1	NA	9.4499	0.9677	1	0.7164	Likely Pathogenic	PM2	PM5	PP2	PP3	PP5	Koyanagi et al., 2019	Schwahn et al., 1998 and others
c.358C>T	p.Arg120Ter	NA	NA	NA	NA	0.8289	0.271	0.8659	0.3072	Pathogenic	PVS1	PP1	PM2	PP3	PP5	This study, Mashima et al., 2001; Jin et al., 2006; Kurata et al., 2019	Mears et al., 1999 and others
c.413A>G	p.Glu138Gly	2.134	NA	0.994	NA	8.805	0.9154	1	0.7164	Likely pathogenic	PM2	PP1	PP2	PP3	PP5	Kurata et al., 2019	Miano et al., 2001
c.566T>C	p.Leu189Pro	4.5	NA	1	NA	7.5549	0.8117	1	0.7164	Uncertain Significance	PM2	PP3				This study	NA
c.677delG	p.Gly226ValfsTer12	5.506	NA	1	NA	NA	NA	NA	NA	Pathogenic	PVS1	PM2	PP3			Koyanagi et al., 2019	NA
c.685C>T	p.Gln229Ter	3.8	NA	1	NA	4.504	0.6014	1	0.7164	Pathogenic	PVS1	PP1	PM2	PP3		Kurata et al., 2019	NA
c.758T>G	p.Leu253Arg	4.542	NA	1	NA	7.5549	0.8117	1	0.7164	Likely pathogenic	PS3	PM2	PP1	PP3	PP5	Wada et al., 2000	NA
c.769−2A>G	Splice site alteration	4.319	NA	1	NA	8.211	0.8971	1	0.7164	Pathogenic	PVS1	PM2	PP1	PP3		Hosono et al., 2018	NA
c.801_804delAAAG	p.Glu269CysfsTer3	4.319	NA	1	NA	NA	NA	NA	NA	Pathogenic	PVS1	PM2	PP3			This study	Pelletier et al., 2007
c.831_832dupTC	p.Gln278LeufsTer16	NA	NA	NA	NA	NA	NA	NA	NA	Pathogenic	PVS1	PM2	PP1	PP3		Mashima et al., 2000	NA
c.882delA	p.Gly295ValfsTer14	NA	NA	NA	NA	NA	NA	NA	NA	Pathogenic	PVS1	PM2	PP3			Maeda et al., 2018	NA

Note: Chr—chromosome; Het—heterozygous; ND—not detected. Reference: NM_006915.2, ENST00000218340.3, GRCh37.p13. The allele frequency of all called variants for Japanese, East Asian, South Asian, European, and African was established based on the HGVD (Japanese), Integrative Japanese Genome Variation (iJGVD 3.5k, 4.7k; https://jmorp.megabank.tohoku.ac.jp/ijgvd/; Japanese), 1000 genome (http://www.internationalgenome.org/; total), and the genome aggregation database (gnomAD; http://gnomad.broadinstitute.org/; East Asian, South Asian, European (non-Finish), and African).All detected variants in

history of XL harbored pathogenic *RP2* variants (Koyanagi et al., 2019). The prevalence of *RP2*-RD in Japan can be slightly lower than that in Europe (21.6% in Denmark; 15.9% in France) (Pelletier et al., 2007; Prokisch et al., 2007). In total, four out of 287 families with RP with any inheritance (4/287, 1.4%) were diagnosed with *RP2*-RD in the JEGC cohort, and this proportion was lower than that in the United States (18/611, 2.9%) (Jayasundera et al., 2010).

In the current study, three patients presented extensive/peripheral retinal atrophy with macular involvement, and one had constricted retinal atrophic changes at the posterior pole. Thus, the characteristic clinical findings of an "atypical" form of early macular involvement were identified, as reported previously (Dandekar et al., 2004; Jayasundera et al., 2010). High myopia (≤−6.0 diopters) was identified in a half (50%) of our four Japanese patients and its prevalence is similar to that of *RP2*-RD in a different cohort (12/25, 48.0%) (Jayasundera et al., 2010). This prevalence of high myopia in *RP2*-RD was much higher than that of the general Japanese population (5.8–11.8%) reported in previous reports (Ueda et al., 2019; Yotsukura et al., 2019). Central visual loss was also found in all four patients, which was likely caused by macular dysfunction in *RP2*-RD. Although the onset of disease was variable, it is notable that the presence of macular involvement is crucial for the impairment of visual acuity in *RP2*-RD.

Two novel and two previously reported variants were identified in four Japanese families in the current study. Two truncating variants (p.Arg120Ter, p.Glu269CysfsTer3) are located in exons 2 and 3, and functional loss of the RP2 protein was predicted. One missense variant (p.Leu189Pro) was located in the ARL3 binding domain, and the other missense variant (p.Ser6Phe) was located in the myristoylation region of the RP2 protein (Figure 4) (Jayasundera et al., 2010; Pelletier et al., 2007; Schwahn et al., 1998). Although functional analysis has not been performed, the clinical findings and the suggested inheritance highly support the disease causation with the XL recessive inheritance.

Mashima et al. reported detailed clinical findings of a patient with p.Arg120Ter: a 24-year-old Japanese male presented a severe form of RP with early macular involvement (Mashima et al., 2001). Similar clinical findings were observed in our patient with p.Arg120Ter (Patient 1). Likewise, Kurata et al. reported the severe phenotype of a patient with this variant. Although there are four reports from other populations, a founder effect should be considered for this allele in the Japanese population, given the high prevalence of this allele (4/18 families; 22.2%) in patients with *RP2*-RD.

The current study and literature search of *RP2*-RD in the Japanese population revealed a high proportion of null variants (11/15; 73.3%), which is in keeping with the findings among the European and North American populations (9/13; 69.2% in France; 11/17; 64.8% in the United States). This finding supports that the complete loss of function is the main mechanism of *RP2*-RD shared between the Japanese and European populations.

Ten unique *RP2* variants in the Japanese population were analyzed: two variants detected in the current study and eight previously reported variants. This high proportion (10/15, 66.7%) of unique

the RP2 gene were analyzed with general and 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/), SIFT (https://www.sift.co.uk/), PROVEAN (http://provean.jcvi.org/index.php), Polyphen 2 (http://genetics.bwh.harvard.edu/pph2/), and Human splicing finder (http://www.umd.be/HSF3/). Evolutional conservation score was evaluated with the UCSC database (https://genome.ucsc.edu/index.html). 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); PM5 (Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before); PP1 (Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease.); PP3(Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.); PP4 (Patient's phenotype or family history is highly specific for a disease with a single 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).

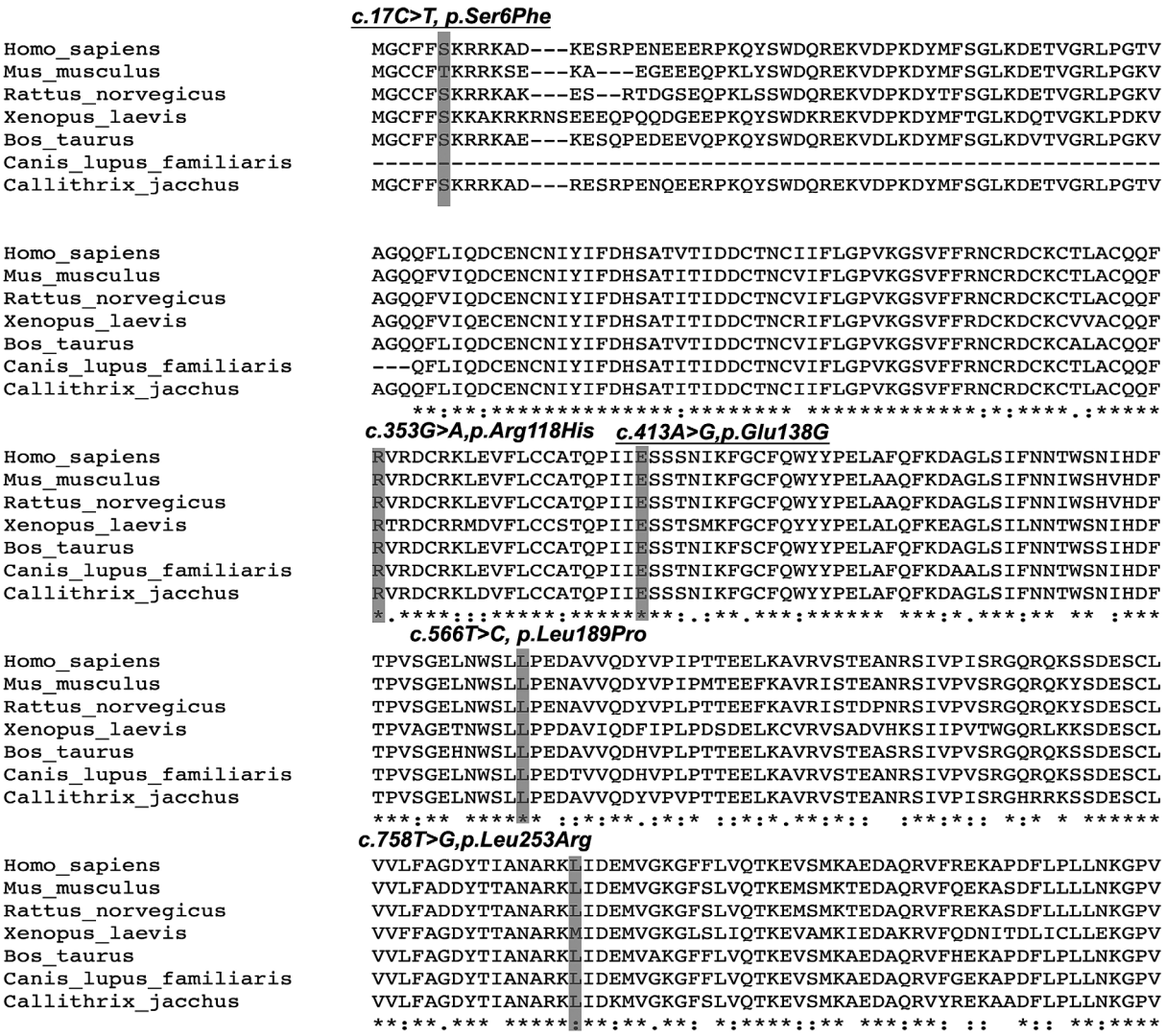


FIGURE 5 Multiple alignments of eight species of *RP2*. The alignment was performed with the Clustal Omega program (https://www.ebi.ac.uk/Tools/msa/clustalo/), and the amino acid sequence alignment is numbered in accordance with the *Homo sapiens RP2* sequence (ENST00000218340.3). An asterisk indicates complete conservation across the eight species. The positions of variant residues are highlighted with a gray background; c.17C>T (p.Ser6Phe) and c.566T>C (p.Leu189Pro) detected in the current study and c.353G>A (p.Arg118His); c.566T.C (p.Leu189Pro); and c.758T>G (p.Leu253Arg) from previous reports

variants suggests the distinct genetic background of the Japanese population with regard to the *RP2* gene.

A genotype–phenotype association based on the presence of null variants was revealed in the current study. A more severe phenotype with early-onset disease was associated with a severe genotype with null variants, while a milder phenotype with relatively preserved visual acuity was associated with a mild genotype with missense variants. This genotype–phenotype association is in keeping with that reported in the previous literature, and such information should be useful in predicting disease prognosis (Jayasundera et al., 2010; Pelletier et al., 2007).

There are limitations in the current study. First, the clinical assessments of mothers (carriers) of the probands were unavailable and co-segregation analysis was not performed in two families. Additional analysis of mothers of the proband both in regard to clinical and genetic aspects could further validate the clinical and molecular genetic diagnosis of *RP2*-RD. Second, the molecular disease-causing mechanisms of the novel two variants are not yet known; therefore, further functional analysis is needed to elucidate the nature of novel variants. Third, the data obtained by the literature search were not standardized, and it could be difficult to compare the data with each other. Last, the sample size for genotype–phenotype association analysis in the current study was still small, so further studies in larger cohorts could help to elucidate the disease mechanism.

In conclusion, phenotypic and genotypic characteristics of *RP2*-RD were illustrated in the Japanese population. A distinct genetic background in the Japanese population was identified; however, a significant genotype–phenotype association was confirmed, as in other populations. This information should be helpful to monitor and counsel patients, as well as to in selecting patients for future therapeutic trials.

TABLE 4 Clinical information of 19 patients from 14 Japanese families with *RP2*-RD

RP2 variants	Patient ID in the original article	Phenotype	Inheritance	Sex	Age (at latest examination)	Onset	Chief complaint	Refractive errors		BCVA in the LogMAR unit		Electrophysiological assessment		Phenotype severity group	Genotype group	References
								RE (dioptor)	LE (dioptor)	RE	LE	Dark-adapted condition	Light-adapted condition			
c.87G>A,p.Trp29Ter	YWC-116	RP	XL	M	24	NA	NA	NA	NA	NA	NA	NA	NA	NA	A	Koyanagi et al., 2019
c. 102+1G>A, splie site alteration	F8-P8	RP	XL	M	16	11	Night blindness	−8.5	−4.5	0.82	0.52	Non-recordable	NA	Mild	A	Kurata et al., 2019
c.217delT, p.Tyr73llefsTer18	F9-P9	RP	XL	M	30	9	Night blindness	−7	−6.5	1.52	1.7	Non-recordable	Non-recordable	Severe	A	Kurata et al., 2019
c.353G>A,p.Arg118His	N-212	RP	XL	M	61	NA	NA	NA	NA	NA	NA	NA	NA	NA	B	Koyanagi et al., 2019
c.358C>T, p.Arg120Ter	I-III-1	RP	XL	M	24	5	Night blindness	−3	−3	1.15	1.15	NA	NA	Severe	A	Mashima et al., 2001
c.358C>T, p.Arg120Ter	I-II-2	RP	XL	M	48	<10 years	Night blindness	NA	NA	LP	LP	NA	NA	Severe	A	Mashima et al., 2001
C.358C>T, p.Arg120Ter	I-II-3	RP	XL	M	44	<10 years	Night blindness/ poor vision	NA	NA	LP	HM	NA	NA	Severe	A	Mashima et al., 2001
c.358C>T, p.Arg120Ter	E-3	RP	XL	M	NA	Childhood	Night blindness	NA	NA	NA	NA	Non-recordable	Non-recordable	NA	A	Jin et al., 2006
0.358C>T, p.Arg120Ter	F10-P10	RP	XL	M	17	6	Visual loss	0.75	0.5	1.1	1.3	Non-recordable	Non-recordable	Severe	A	Kurata et al., 2019
c.413A>G,p.Glu138Gly	F11-P11	RP	XL	M	41	NA	Night blindness/ poor visual acuity	−12	−10	HM	HM	Non-recordable	NA	NA	B	Kurata et al., 2019
c.413A>G,p.Glu138Gly	F11-P12	RP	XL	M	38	NA	NA	−10	−8.5	1.15	1.15	Non-recordable	NA	NA	B	Kurata et al., 2019
c.677delG, p.Gly226ValfsTer12	OPH-619	RP	XL	M	36	NA	NA	NA	NA	NA	NA	NA	NA	NA	A	Koyanagi et al., 2019
c.685C>T,p.Gln229Ter	F12-P13	RP	XL	M	30	3	Visual loss	−5.25	−5.75	1.52	1.7	Non-recordable	Non-recordable	Severe	A	Kurata et al., 2019
c.758T>G,p.Leu253Arg	III-4	RP	XL	M	29	6	Night blindness/ poor visual acuity	NA	NA	NA	NA	NA	NA	NA	B	Wada et al., 2000
c.758T>G,p.Leu253Arg	III-1	RP	XL	M	NA	Early teens	NA	−6.5	−9	0.7	1.22	Non-recordable	Non-recordable	Mild	B	Koyanagi et al., 2019
c.769−2A>G	NA	LCA	NA	M	NA	<1 year	NA	NA	NA	Severe visual impairment	Severe visual impairment	Severely reduced or non-detectable ERG	Severely reduced or non-detectable ERG	Severe	A	Hosono et al., 2018
c.882delA, p.Gly295ValfsTer14	40	RP	XL	M	25	NA	NA	NA	NA	NA	NA	NA	NA	NA	A	Maeda et al., 2018
c.831_832dupTC, p. Gln278LeufsTer16	IV-1	RP	XL	M	19	3	Night blindness/ photophobia	−6.75	−7.0	HM	HM	NA	NA	Severe	A	Mashima et al., 2000
c.831_832dupTC, p. Gln278LeufsTer16	IV-2	LCA	XL	M	17	3	Night blindness/ photophobia	−8	−8	1.15	1.3	NA	NA	Severe	A	Mashima et al., 2000

Note: A systemic review of peer-reviewed articles which describe Japanese cases with RP2-RD was performed.
Abbreviations: ERG, electroretinogram; ID, identification; LCA, Leber congenital amaurosis; RP, retinitis pigmentosa.

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ORCID

Kaoru Fujinami https://orcid.org/0000-0003-4248-0033
Gavin Arno https://orcid.org/0000-0002-6165-7888
Nikolas Pontikos https://orcid.org/0000-0003-1782-4711

REFERENCES

Andreasson, S., Breuer, D. K., Eksandh, L., Ponjavic, V., Frennesson, C., Hiriyanna, S., … Swaroop, A. (2003). Clinical studies of X-linked

retinitis pigmentosa in three Swedish families with newly identified mutations in the RP2 and RPGR-ORF15 genes. *Ophthalmic Genetics*, *24*(4), 215–223. https://doi.org/10.1076/opge.24.4.215.17228

Boughman, J. A., Conneally, P. M., & Nance, W. E. (1980). Population genetic studies of retinitis pigmentosa. *American Journal of Human Genetics*, *32*(2), 223–235.

Breuer, D. K., Yashar, B. M., Filippova, E., Hiriyanna, S., Lyons, R. H., Mears, A. J., … Swaroop, A. (2002). A comprehensive mutation analysis of RP2 and RPGR in a north American cohort of families with X-linked retinitis pigmentosa. *American Journal of Human Genetics*, *70*(6), 1545–1554. https://doi.org/10.1086/340848

Carss, K. J., Arno, G., Erwood, M., Stephens, J., Sanchis-Juan, A., Hull, S., … Raymond, F. L. (2017). Comprehensive rare variant analysis via whole-genome sequencing to determine the molecular pathology of inherited retinal disease. *American Journal of Human Genetics*, *100*(1), 75–90. https://doi.org/10.1016/j.ajhg.2016.12.003

Chapple, J. P., Hardcastle, A. J., Grayson, C., Spackman, L. A., Willison, K. R., & Cheetham, M. E. (2000). Mutations in the N-terminus of the X-linked retinitis pigmentosa protein RP2 interfere with the normal targeting of the protein to the plasma membrane. *Human Molecular Genetics*, *9*(13), 1919–1926.

Chapple, J. P., Hardcastle, A. J., Grayson, C., Willison, K. R., & Cheetham, M. E. (2002). Delineation of the plasma membrane targeting domain of the X-linked retinitis pigmentosa protein RP2. *Investigative Ophthalmology & Visual Science*, *43*(6), 2015–2020.

Chizzolini, M., Galan, A., Milan, E., Sebastiani, A., Costagliola, C., & Parmeggiani, F. (2011). Good epidemiologic practice in retinitis pigmentosa: From phenotyping to biobanking. *Current Genomics*, *12*(4), 260–266. https://doi.org/10.2174/138920211795860071

Dan, H., Huang, X., Xing, Y., & Shen, Y. (2020). Application of targeted panel sequencing and whole exome sequencing for 76 Chinese families with retinitis pigmentosa. *Molecular Genetics & Genomic Medicine*, *8*(3), e1131. https://doi.org/10.1002/mgg3.1131

Dandekar, S. S., Ebenezer, N. D., Grayson, C., Chapple, J. P., Egan, C. A., Holder, G. E., … Hardcastle, A. J. (2004). An atypical phenotype of macular and peripapillary retinal atrophy caused by a mutation in the RP2 gene. *The British Journal of Ophthalmology*, *88*(4), 528–532. https://doi.org/10.1136/bjo.2003.027979

Evans, R. J., Schwarz, N., Nagel-Wolfrum, K., Wolfrum, U., Hardcastle, A. J., & Cheetham, M. E. (2010). The retinitis pigmentosa protein RP2 links pericentriolar vesicle transport between the Golgi and the primary cilium. *Human Molecular Genetics*, *19*(7), 1358–1367. https://doi.org/10.1093/hmg/ddq012

Fishman, G. A. (1978). Retinitis pigmentosa. Genetic percentages. *Archives of Ophthalmology*, *96*(5), 822–826.

Fujinami-Yokokawa, Y., Pontikos, N., Yang, L., Tsunoda, K., Yoshitake, K., Iwata, T., … Japan Eye Genetics Consortium. (2019). Prediction of causative genes in Inherited Retinal disorders from spectral-domain optical coherence tomography utilizing deep learning techniques. *Journal of Ophthalmology*, *2019*, 1691064. https://doi.org/10.1155/2019/1691064

Fujinami-Yokokawa, Y., Kuniyoshi, K., Hayashi, T., Ueno, S., Mizota, A., Shinoda, K., … Japan Eye Genetics Consortium. (2020). Clinical and genetic characteristics of 18 patients from 13 Japanese families with CRX-associated Retinal disorder: Identification of genotype-phenotype associations. *Scientific Reports*, in press, *10*, 9531.

Fujinami, K., Kameya, S., Kikuchi, S., Ueno, S., Kondo, M., Hayashi, T., … Tsunoda, K. (2016). Novel RP1L1 variants and genotype-photoreceptor microstructural phenotype associations in cohort of Japanese patients with occult macular dystrophy. *Investigative Ophthalmology & Visual Science*, *57*(11), 4837–4846. https://doi.org/10.1167/iovs.16-19670

Fujinami, K., Yang, L., Joo, K., Tsunoda, K., Kameya, S., Hanazono, G., … East Asia Inherited Retinal Disease Society Study Group. (2019). Clinical and genetic characteristics of east Asian patients with occult macular dystrophy (Miyake disease): East Asia occult macular dystrophy studies report number 1. *Ophthalmology*, *126*(10), 1432–1444. https://doi.org/10.1016/j.ophtha.2019.04.032

Grayson, C., Bartolini, F., Chapple, J. P., Willison, K. R., Bhamidipati, A., Lewis, S. A., … Cheetham, M. E. (2002). Localization in the human retina of the X-linked retinitis pigmentosa protein RP2, its homologue cofactor C and the RP2 interacting protein Arl3. *Human Molecular Genetics*, *11*(24), 3065–3074.

Haim, M. (1992). Prevalence of retinitis pigmentosa and allied disorders in Denmark. III. Hereditary pattern. *Acta Ophthalmologica*, *70*(5), 615–624.

Hardcastle, A. J., Thiselton, D. L., Van Maldergem, L., Saha, B. K., Jay, M., Plant, C., … Bhattacharya, S. (1999). Mutations in the RP2 gene cause disease in 10% of families with familial X-linked retinitis pigmentosa assessed in this study. *American Journal of Human Genetics*, *64*(4), 1210–1215.

Hosono, K., Nishina, S., Yokoi, T., Katagiri, S., Saitsu, H., Kurata, K., … Hotta, Y. (2018). Molecular diagnosis of 34 Japanese families with Leber congenital Amaurosis using targeted next generation sequencing. *Scientific Reports*, *8*(1), 8279. https://doi.org/10.1038/s41598-018-26524-z

Hurd, T., Zhou, W., Jenkins, P., Liu, C. J., Swaroop, A., Khanna, H., … Margolis, B. (2010). The retinitis pigmentosa protein RP2 interacts with polycystin 2 and regulates cilia-mediated vertebrate development. *Human Molecular Genetics*, *19*(22), 4330–4344. https://doi.org/10.1093/hmg/ddq355

Hurd, T. W., Fan, S., & Margolis, B. L. (2011). Localization of retinitis pigmentosa 2 to cilia is regulated by importin beta2. *Journal of Cell Science*, *124*(Pt 5), 718–726. https://doi.org/10.1242/jcs.070839

Jayasundera, T., Branham, K. E., Othman, M., Rhoades, W. R., Karoukis, A. J., Khanna, H., … Heckenlively, J. R. (2010). RP2 phenotype and pathogenetic correlations in X-linked retinitis pigmentosa. *Archives of Ophthalmology*, *128*(7), 915–923. https://doi.org/10.1001/archophthalmol.2010.122

Ji, Y., Wang, J., Xiao, X., Li, S., Guo, X., & Zhang, Q. (2010). Mutations in RPGR and RP2 of Chinese patients with X-linked retinitis pigmentosa. *Current Eye Research*, *35*(1), 73–79. https://doi.org/10.3109/02713680903395299

Jiang, J., Wu, X., Shen, D., Dong, L., Jiao, X., Hejtmancik, J. F., & Li, N. (2017). Analysis of RP2 and RPGR mutations in five X-linked Chinese families with retinitis Pigmentosa. *Scientific Reports*, *7*, 44465. https://doi.org/10.1038/srep44465

Jin, Z. B., Liu, X. Q., Hayakawa, M., Murakami, A., & Nao-i, N. (2006). Mutational analysis of RPGR and RP2 genes in Japanese patients with retinitis pigmentosa: Identification of four mutations. *Molecular Vision*, *12*, 1167–1174.

Kameya, S., Fujinami, K., Ueno, S., Hayashi, T., Kuniyoshi, K., Ideta, R., … Japan Eye Genetics, C. (2019). Phenotypical characteristics of POC1B-associated retinopathy in Japanese cohort: Cone dystrophy with normal funduscopic appearance. *Investigative Ophthalmology & Visual Science*, *60*(10), 3432–3446. https://doi.org/10.1167/iovs.19-26650

Katagiri, S., Hayashi, T., Nakamura, M., Mizobuchi, K., Gekka, T., Komori, S., … Nakano, T. (2020). RDH5-related fundus Albipunctatus in a large Japanese cohort. *Investigative Ophthalmology & Visual Science*, *61*(3), 53. https://doi.org/10.1167/iovs.61.3.53

Kim, M. S., Joo, K., Seong, M. W., Kim, M. J., Park, K. H., Park, S. S., & Woo, S. J. (2019). Genetic mutation profiles in Korean patients with Inherited Retinal diseases. *Journal of Korean Medical Science*, *34*(21), e161. https://doi.org/10.3346/jkms.2019.34.e161

Kondo, H., Oku, K., Katagiri, S., Hayashi, T., Nakano, T., Iwata, A., … Iwata, T. (2019). Novel mutations in the RS1 gene in Japanese patients with X-linked congenital retinoschisis. *Human Genome Variation*, *6*, 3. https://doi.org/10.1038/s41439-018-0034-6

Koyanagi, Y., Akiyama, M., Nishiguchi, K. M., Momozawa, Y., Kamatani, Y., Takata, S., … Sonoda, K. H. (2019). Genetic characteristics of retinitis

pigmentosa in 1204 Japanese patients. *Journal of Medical Genetics*, *56*(10), 662–670. https://doi.org/10.1136/jmedgenet-2018-105691

Kurata, K., Hosono, K., Hayashi, T., Mizobuchi, K., Katagiri, S., Miyamichi, D., … Hotta, Y. (2019). X-linked retinitis pigmentosa in Japan: Clinical and genetic findings in male patients and female carriers. *International Journal of Molecular Sciences*, *20*(6), 1518–1518. https://doi.org/10.3390/ijms20061518

Li, L., Khan, N., Hurd, T., Ghosh, A. K., Cheng, C., Molday, R., … Khanna, H. (2013). Ablation of the X-linked retinitis pigmentosa 2 (Rp2) gene in mice results in opsin mislocalization and photoreceptor degeneration. *Investigative Ophthalmology & Visual Science*, *54*(7), 4503–4511. https://doi.org/10.1167/iovs.13-12140

Li, L., Rao, K. N., & Khanna, H. (2019). Structural but not functional alterations in cones in the absence of the Retinal disease protein retinitis Pigmentosa 2 (RP2) in a cone-only retina. *Frontiers in Genetics*, *10*, 323. https://doi.org/10.3389/fgene.2019.00323

Liew, G., Michaelides, M., & Bunce, C. (2014). A comparison of the causes of blindness certifications in England and Wales in working age adults (16-64 years), 1999-2000 with 2009-2010. *BMJ Open*, *4*(2), e004015. https://doi.org/10.1136/bmjopen-2013-004015

Lim, H., Park, Y. M., Lee, J. K., & Taek Lim, H. (2016). Single-exome sequencing identified a novel RP2 mutation in a child with X-linked retinitis pigmentosa. *Canadian Journal of Ophthalmology*, *51*(5), 326–330. https://doi.org/10.1016/j.jcjo.2016.03.017

Lyraki, R., Megaw, R., & Hurd, T. (2016). Disease mechanisms of X-linked retinitis pigmentosa due to RP2 and RPGR mutations. *Biochemical Society Transactions*, *44*(5), 1235–1244. https://doi.org/10.1042/BST20160148

Maeda-Katahira, A., Nakamura, N., Hayashi, T., Katagiri, S., Shimizu, S., Ohde, H., … Tsunoda, K. (2019). Autosomal dominant optic atrophy with OPA1 gene mutations accompanied by auditory neuropathy and other systemic complications in a Japanese cohort. *Molecular Vision*, *25*, 559–573.

Maeda, A., Yoshida, A., Kawai, K., Arai, Y., Akiba, R., Inaba, A., … Takahashi, M. (2018). Development of a molecular diagnostic test for retinitis Pigmentosa in the Japanese population. *Japanese Journal of Ophthalmology*, *62*(4), 451–457. https://doi.org/10.1007/s10384-018-0601-x

Mashima, Y., Saga, M., Akeo, K., & Oguchi, Y. (2001). Phenotype associated with an R120X nonsense mutation in the RP2 gene in a Japanese family with X-linked retinitis pigmentosa. *Ophthalmic Genetics*, *22*(1), 43–47.

Mashima, Y., Saga, M., Hiida, Y., Imamura, Y., Kudoh, J., & Shimizu, N. (2000). Novel mutation in RP2 gene in two brothers with X-linked retinitis pigmentosa and mtDNA mutation of leber hereditary optic neuropathy who showed marked differences in clinical severity. *American Journal of Ophthalmology*, *130*(3), 357–359. https://doi.org/10.1016/s0002-9394(00)00553-5

Mawatari, G., Fujinami, K., Liu, X., Yang, L., Yokokawa, Y. F., Komori, S., … JEGC Study Group. (2019). Clinical and genetic characteristics of 14 patients from 13 Japanese families with RPGR-associated retinal disorder: Report of eight novel variants. *Human Genome Variation*, *6*, 34. https://doi.org/10.1038/s41439-019-0065-7

McCulloch, D. L., Marmor, M. F., Brigell, M. G., Hamilton, R., Holder, G. E., Tzekov, R., & Bach, M. (2015a). Erratum to: ISCEV standard for full-field clinical electroretinography (2015 update). *Documenta Ophthalmologica*, *131*(1), 81–83. https://doi.org/10.1007/s10633-015-9504-z

McCulloch, D. L., Marmor, M. F., Brigell, M. G., Hamilton, R., Holder, G. E., Tzekov, R., & Bach, M. (2015b). ISCEV standard for full-field clinical electroretinography (2015 update). *Documenta Ophthalmologica*, *130*(1), 1–12. https://doi.org/10.1007/s10633-014-9473-7

Mears, A. J., Gieser, L., Yan, D., Chen, C., Fahrner, S., Hiriyanna, S., … Swaroop, A. (1999). Protein-truncation mutations in the RP2 gene in a north American cohort of families with X-linked retinitis pigmentosa. *American Journal of Human Genetics*, *64*(3), 897–900. https://doi.org/10.1086/302298

Miano, M. G., Testa, F., Filippini, F., Trujillo, M., Conte, I., Lanzara, C., … Ciccodicola, A. (2001). Identification of novel RP2 mutations in a subset of X-linked retinitis pigmentosa families and prediction of new domains. *Human Mutation*, *18*(2), 109–119. https://doi.org/10.1002/humu.1160

Mizobuchi, K., Hayashi, T., Katagiri, S., Yoshitake, K., Fujinami, K., Yang, L., … Nakano, T. (2019). Characterization of GUCA1A-associated dominant cone/cone-rod dystrophy: Low prevalence among Japanese patients with inherited retinal dystrophies. *Scientific Reports*, *9*(1), 16851. https://doi.org/10.1038/s41598-019-52660-1

Nakamura, N., Tsunoda, K., Mizuno, Y., Usui, T., Hatase, T., Ueno, S., … Miyake, Y. (2019). Clinical stages of occult macular Dystrophy based on optical coherence tomographic findings. *Investigative Ophthalmology & Visual Science*, *60*(14), 4691–4700. https://doi.org/10.1167/iovs.19-27486

Nakanishi, A., Ueno, S., Hayashi, T., Katagiri, S., Kominami, T., Ito, Y., … Terasaki, H. (2016). Clinical and genetic findings of autosomal recessive Bestrophinopathy in Japanese cohort. *American Journal of Ophthalmology*, *168*, 86–94. https://doi.org/10.1016/j.ajo.2016.04.023

Neidhardt, J., Glaus, E., Lorenz, B., Netzer, C., Li, Y., Schambeck, M., … Berger, W. (2008). Identification of novel mutations in X-linked retinitis pigmentosa families and implications for diagnostic testing. *Molecular Vision*, *14*, 1081–1093.

Pan, X., Chen, X., Liu, X., Gao, X., Kang, X., Xu, Q., … Zhao, C. (2014). Mutation analysis of pre-mRNA splicing genes in Chinese families with retinitis pigmentosa. *Molecular Vision*, *20*, 770–779.

Pelletier, V., Jambou, M., Delphin, N., Zinovieva, E., Stum, M., Gigarel, N., … Rozet, J. M. (2007). Comprehensive survey of mutations in RP2 and RPGR in patients affected with distinct retinal dystrophies: Genotype-phenotype correlations and impact on genetic counseling. *Human Mutation*, *28*(1), 81–91. https://doi.org/10.1002/humu.20417

Pontikos, N., Murphy, C., Moghul, I., Arno, G., Fujinami, K., Fujinami, Y., … UK Inherited Retinal Dystrophy Consortium, Phenopolis Consortium. (2020). Phenogenon: Gene to phenotype associations for rare genetic diseases. *PLoS One*, *15*(4), e0230587. https://doi.org/10.1371/journal.pone.0230587

Prokisch, H., Hartig, M., Hellinger, R., Meitinger, T., & Rosenberg, T. (2007). A population-based epidemiological and genetic study of X-linked retinitis pigmentosa. *Investigative Ophthalmology & Visual Science*, *48*(9), 4012–4018. https://doi.org/10.1167/iovs.07-0071

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., … Committee, A. L. Q. A. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, *17*(5), 405–424. https://doi.org/10.1038/gim.2015.30

Sahel, J. A., Marazova, K., & Audo, I. (2014). Clinical characteristics and current therapies for inherited retinal degenerations. *Cold Spring Harbor Perspectives in Medicine*, *5*(2), a017111. https://doi.org/10.1101/cshperspect.a017111

Schwahn, U., Lenzner, S., Dong, J., Feil, S., Hinzmann, B., van Duijnhoven, G., … Berger, W. (1998). Positional cloning of the gene for X-linked retinitis pigmentosa 2. *Nature Genetics*, *19*(4), 327–332. https://doi.org/10.1038/1214

Sharon, D., Bruns, G. A., McGee, T. L., Sandberg, M. A., Berson, E. L., & Dryja, T. P. (2000). X-linked retinitis pigmentosa: Mutation spectrum of the RPGR and RP2 genes and correlation with visual function. *Investigative Ophthalmology & Visual Science*, *41*(9), 2712–2721.

Sharon, D., Sandberg, M. A., Rabe, V. W., Stillberger, M., Dryja, T. P., & Berson, E. L. (2003). RP2 and RPGR mutations and clinical correlations in patients with X-linked retinitis pigmentosa. *American Journal of Human Genetics*, *73*(5), 1131–1146. https://doi.org/10.1086/379379

Sohocki, M. M., Daiger, S. P., Bowne, S. J., Rodriquez, J. A., Northrup, H., Heckenlively, J. R., … Sullivan, L. S. (2001). Prevalence of mutations

causing retinitis pigmentosa and other inherited retinopathies. *Human Mutation*, *17*(1), 42–51. https://doi.org/10.1002/1098-1004(2001)17:1<42::AID-HUMU5>3.0.CO;2-K

Solebo, A. L., Teoh, L., & Rahi, J. (2017). Epidemiology of blindness in children. *Archives of Disease in Childhood*, *102*(9), 853–857. https://doi.org/10.1136/archdischild-2016-310532

Ueda, E., Yasuda, M., Fujiwara, K., Hashimoto, S., Ohno-Matsui, K., Hata, J., … Sonoda, K. H. (2019). Trends in the prevalence of myopia and myopic Maculopathy in a Japanese population: The Hisayama study. *Investigative Ophthalmology & Visual Science*, *60*(8), 2781–2786. https://doi.org/10.1167/iovs.19-26580

Vervoort, R., Lennon, A., Bird, A. C., Tulloch, B., Axton, R., Miano, M. G., … Wright, A. F. (2000). Mutational hot spot within a new RPGR exon in X-linked retinitis pigmentosa. *Nature Genetics*, *25*(4), 462–466. https://doi.org/10.1038/78182

Vorster, A. A., Rebello, M. T., Coutts, N., Ehrenreich, L., Gama, A. D., Roberts, L. J., … Greenberg, L. J. (2004). Arg120stop nonsense mutation in the RP2 gene: Mutational hotspot and germ line mosaicism? *Clinical Genetics*, *65*(1), 7–10. https://doi.org/10.1111/j..2004.00163.x

Wada, Y., Nakazawa, M., Abe, T., & Tamai, M. (2000). A new Leu253Arg mutation in the RP2 gene in a Japanese family with X-linked retinitis pigmentosa. *Investigative Ophthalmology & Visual Science*, *41*(1), 290–293.

Wang, J., Zhang, V. W., Feng, Y., Tian, X., Li, F. Y., Truong, C., … Wong, L. J. (2014). Dependable and efficient clinical utility of target capture-based deep sequencing in molecular diagnosis of retinitis pigmentosa. *Investigative Ophthalmology & Visual Science*, *55*(10), 6213–6223. https://doi.org/10.1167/iovs.14-14936

World Medical Association. (2013). World medical association declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA*, *310*(20), 2191–2194. https://doi.org/10.1001/jama.2013.281053

Wright, A. F., Chakarova, C. F., Abd El-Aziz, M. M., & Bhattacharya, S. S. (2010). Photoreceptor degeneration: Genetic and mechanistic dissection of a complex trait. *Nature Reviews. Genetics*, *11*(4), 273–284. https://doi.org/10.1038/nrg2717

Xiao Liu, K. F., Kuniyoshi, K., Kondo, M., Ueno, S., Hayashi, T., Mochizuki, K., … Consortium, J. E. G. (2020). Clinical and genetic characteristics of 15 affected patients from 12 Japanese families with GUCY2D-associated retinal disorder. *Translational Vision Science & Technology*, *9*(6), 2.

Xu, K., Xie, Y., Sun, T., Zhang, X., Chen, C., & Li, Y. (2019). Genetic and clinical findings in a Chinese cohort with Leber congenital amaurosis and early onset severe retinal dystrophy. *The British Journal of Ophthalmology*, *104*, 932–937. https://doi.org/10.1136/bjophthalmol-2019-314281

Yang, L., Fujinami, K., Ueno, S., Kuniyoshi, K., Hayashi, T., Kondo, M., … JEGC Study Group. (2020). Genetic Spectrum of EYS-associated Retinal disease in a large Japanese cohort: Identification of disease-associated variants with relatively high allele frequency. *Scientific Reports*, *10*(1), 5497. https://doi.org/10.1038/s41598-020-62119-3

Yang, L., Yin, X., Feng, L., You, D., Wu, L., Chen, N., … Ma, Z. (2014). Novel mutations of RPGR in Chinese retinitis pigmentosa patients and the genotype-phenotype correlation. *PLoS One*, *9*(1), e85752. https://doi.org/10.1371/journal.pone.0085752

Yotsukura, E., Torii, H., Inokuchi, M., Tokumura, M., Uchino, M., Nakamura, K., … Tsubota, K. (2019). Current prevalence of myopia and Association of Myopia with Environmental Factors among Schoolchildren in Japan. *JAMA Ophthalmology*, *137*, 1233. https://doi.org/10.1001/jamaophthalmol.2019.3103

Zhang, H., Hanke-Gogokhia, C., Jiang, L., Li, X., Wang, P., Gerstner, C. D., … Baehr, W. (2015). Mistrafficking of prenylated proteins causes retinitis pigmentosa 2. *The FASEB Journal*, *29*(3), 932–942. https://doi.org/10.1096/fj.14-257915

Zhang, J., Gao, F., Du, C., Wang, J., Pi, X., Guo, W., … Cui, X. (2019). A novel RP2 missense mutation Q158P identified in an X-linked retinitis pigmentosa family impaired RP2 protein stability. *Gene*, *707*, 86–92. https://doi.org/10.1016/j.gene.2019.05.006

SUPPORTING INFORMATION

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

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