RDH5-Related Fundus Albipunctatus in a Large Japanese Cohort

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PURPOSE. To investigate clinical characteristics of *RDH5*-related fundus albipunctatus (FAP) in a Japanese cohort.

METHODS. Twenty-five patients from 22 pedigrees with *RDH5*-related FAP were studied. Ophthalmic medical records were reviewed. For genetic analysis, either Sanger sequencing of the *RDH5* gene or whole-exome sequencing was performed.

RESULTS. Genetic analysis identified eight different *RDH5* variants, including seven known *RDH5* variants (p.G35S, p.G107R, p.R167H, p.A240GfsX19, p.R278X, p.R280H, and p.L310delinsEV) and a novel variant: c.259C>T (p.Q87X). The most frequently observed variant was p.L310delinsEV (65.2%, 30/46 alleles). Of 50 eyes examined, 44 eyes (88.0%) showed logMAR best-corrected visual acuity (BCVA) of 0.10 or better. In optical coherence tomography, macular involvement was observed in 12 patients (24 eyes). Ten patients (83.3%) who had good BCVA (0.10 or better) exhibited diffuse disruption of the outer retina with foveal sparing, and two patients (16.7%) exhibited diffuse disruption throughout the macula and decreased BCVA. Among the 24 eyes, ring-or crescent-shaped hyperautofluorescence or irregular autofluorescence around the fovea was observed in 15 eyes (83.3%) of 18 eyes examined by fundus autofluorescence imaging. Full-field electroretinography showed extinguished or severely decreased rod responses in all 23 examined patients, whereas decreased cone responses were seen in 17 patients (73.9%).

CONCLUSIONS. Multimodal imaging and electroretinography of *RDH5*-related FAP revealed high frequencies of macular involvement in older patients and decreased cone responses. Our findings suggest that progressive macular/cone dysfunction, as well as delayed rod function, may be key phenotypic features of *RDH5*-related FAP.

Keywords: fundus albipunctatus, *RDH5*, Japanese, next-generation sequencing, novel variant

The retinol dehydrogenase 5-encoding gene (*RDH5*) (OMIM *601617) encodes a 318-amino acid microsomal enzyme (11-*cis*-retinol dehydrogenase) that is specifically expressed in retinal pigment epithelium (RPE) and plays an important role in the retinoid (visual) cycle by converting 11-*cis*-retinol to 11-*cis*-retinal.[1,2] Dysfunction of 11-*cis*-retinol dehydrogenase leads to a disturbance of the visual cycle, notably including the abnormal accumulation of 11-*cis*-retinol and the lack of 11-*cis*-retinal.[3] In 1999, biallelic *RDH5* variants were reported as the cause of fundus albipunctatus (FAP) (OMIM #136880).[4,5]

FAP is a rare form of stationary night blindness characterized by night blindness from childhood, stationary or slow progression of rod abnormalities, frequent and

progressive cone abnormalities in older age, and numerous and dense white dots from midperipheral to peripheral retina. In FAP patients, full-field electroretinography (ERG) shows extremely reduced or absent rod responses in standard dark adaptation but reveals the recovery of rod function to nearly normal in extended dark adaptation.[5–7] As the same category of flecked retina syndrome, retinitis punctata albescens is reported, but retinitis punctata albescens is classified into a distinct clinical entity according to different clinical characteristics of progressive disease course, poor or absent rod-plus-cone ERG responses in standard dark adaptation, and no or partial recovery of ERG responses in extended dark adaptation.[8,9] To date, clinical examinations for *RDH5*-related FAP have focused on particular assays, such as electroretinograms[10,11] and fundus autofluorescence (FAF) imaging,[12] as well as other imaging modalities.[13–15] These clinical studies have clarified the high frequency of progressive macular involvement, including macular atrophy and macular/cone dystrophy in *RDH5*-related FAP.[10–15] However, as far as we know, there has been no large cohort study that evaluates using multimodal imaging and ERG in *RDH5*-related FAP.

Because the dysfunction of the RDH5 protein (11-*cis*-retinol dehydrogenase) leads to a lack of 11-*cis*-retinal, a pharmacological replacement therapy for the missing activity or product has been proposed as a clinical intervention. Oral 9-*cis*-retinoid therapy (derived from the alga *Dunaliella bardawil* and consisting of a 50:50 mix of all-*trans*-β-carotene and 9-*cis*-β-carotene) has been used in pharmacological attempts to recover the retinoid cycle by bypassing the missing function of RDH5.[16] Additionally, other inherited retinal disorders associated with the lack of retinoid isomerohydrolase RPE65 and lecithin retinol acyltransferase proteins, key enzymes for the retinoid cycle, as well as that of the RDH5 protein, have been clinically targeted via oral therapy using 9-*cis*-retinoid (QLT091001/9-*cis*-β-carotene).[17,18] Thus, oral 9-*cis*-retinoid therapy is a promising treatment for *RDH5*-related FAP and other retinoid cycle-related retinal dystrophies. For clinical trials, it is indispensable to investigate the clinical and genetic features of these retinal dystrophies in the Japanese population.

RDH5-related FAP is more common in the Japanese population than in Western populations. Here, we describe an investigation of 25 patients with *RDH5*-related FAP from 22 Japanese pedigrees who came from multiple centers. The purpose of this study was to investigate clinical and genetic characteristics of *RDH5*-related FAP patients for an upcoming clinical trial.

MATERIALS AND METHODS

The protocol used for this study was approved by the Institutional Review Board of The Jikei University School of Medicine (approval no. 24-232 6997), Kindai University Faculty of Medicine (approval no. 22-132), Mie University Graduate School of Medicine (approval no. 2429), Nagoya University Graduate School of Medicine (approval no. 2010-1067), National Hospital Organization Tokyo Medical Center (approval no. R18-029), and University of Occupational and Environmental Health (approval no. H29-03). The protocol adhered to the tenets of the Declaration of Helsinki, and informed consent was obtained from each participant.

Participants

Twenty-five patients, who were diagnosed with FAP and had pathogenic *RDH5* variants in homozygous or compound heterozygous states were studied. All 25 patients had never been reported previously except for one patient (patient II-1 in family 22: F22, II-1).[19]

Clinical Study

We retrospectively reviewed the medical records of patients. The medical records included decimal best-corrected visual acuity (BCVA), funduscopy, optical coherence tomography (OCT) (Cirrus HD-OCT, Carl Zeiss Meditec AG, Dublin, CA, USA; Spectralis, Heidelberg Engineering, Heidelberg, Germany; DRI OCT-1, Topcon, Tokyo, Japan), visual field testing using Goldmann perimetry (Haag Streit, Bern, Switzerland) or Humphrey field analyzer (Carl Zeiss Meditec AG), fundus autofluorescence imaging (Spectralis HRA or Spectralis HRA2, Heidelberg Engineering; Optos Panoramic 200MA, Optos PLC, Dunfermline, UK), and ERG, as well as the family history.[20–28] BCVA was determined at a distance of 5 m using Landolt C charts and was converted to logMAR units. ERG was performed according to the guidelines of the International Society for Clinical Electrophysiology of Vision including dark-adapted (DA) 0.01 ERG (stimulation, 0.01 cd·s·m^{-2}), DA 3.0 ERG (stimulation, 3.0 cd·s·m^{-2}), light-adapted (LA) 3.0 ERG (stimulation, 3.0 cd·s·m^{-2}; background, 30 cd·m^{-2}), and LA 30-Hz flicker (stimulation, 3.0 cd·s·m^{-2}; background, 30 cd·m^{-2}).[29] ERG was performed using Burian–Allen contact lens electrodes (Hansen Ophthalmic Laboratories, Iowa City, IA, USA) and Ganzfeld dome and neuropack,[24,30–32] a contact lens electrode with built-in white light-emitting diode system (LE-4000, Tomey Co., Nagoya, Japan),[19,23,33] or RETeval device with a handheld ERG recording system with skin electrodes (LKC Technologies, Gaithersburg, MD, USA).[34,35] The patients' ERG amplitudes were compared to controls recorded by each different ERG system.

Statistical Analysis

Statistical analyses were performed using PASW Statistics 18 software (SPSS Inc., Chicago, IL). A Mann–Whitney *U* test was used to compare the examination age of patients with or without macular involvement, because the examination age with macular involvement was not distributed normally, as judged by the Shapiro–Wilk test ($P < 0.05$). $P < 0.05$ was considered significant in both tests.

Identification of *RDH5* Variants

Genomic DNA was extracted from leukocytes in venous blood from the 25 affected patients and from four unaffected family members (families 10, 12, and 15) using a Gentra Puregene Blood Kit (Qiagen, Hilden, Germany) or by the phenol–chloroform method with subsequent ethanol precipitation of nucleic acids. The coding region of the *RDH5* gene was analyzed by direct sequencing using previously reported primer pairs[20] or by whole-exome sequencing (WES).[36,37] The filtering criteria of the WES data were as follows: (1) the patient had *RDH5* variants in the homozygous or compound heterozygous state, and (2) the *RDH5* variant was detected at a prevalence of <1% in the 1000 Genomes database
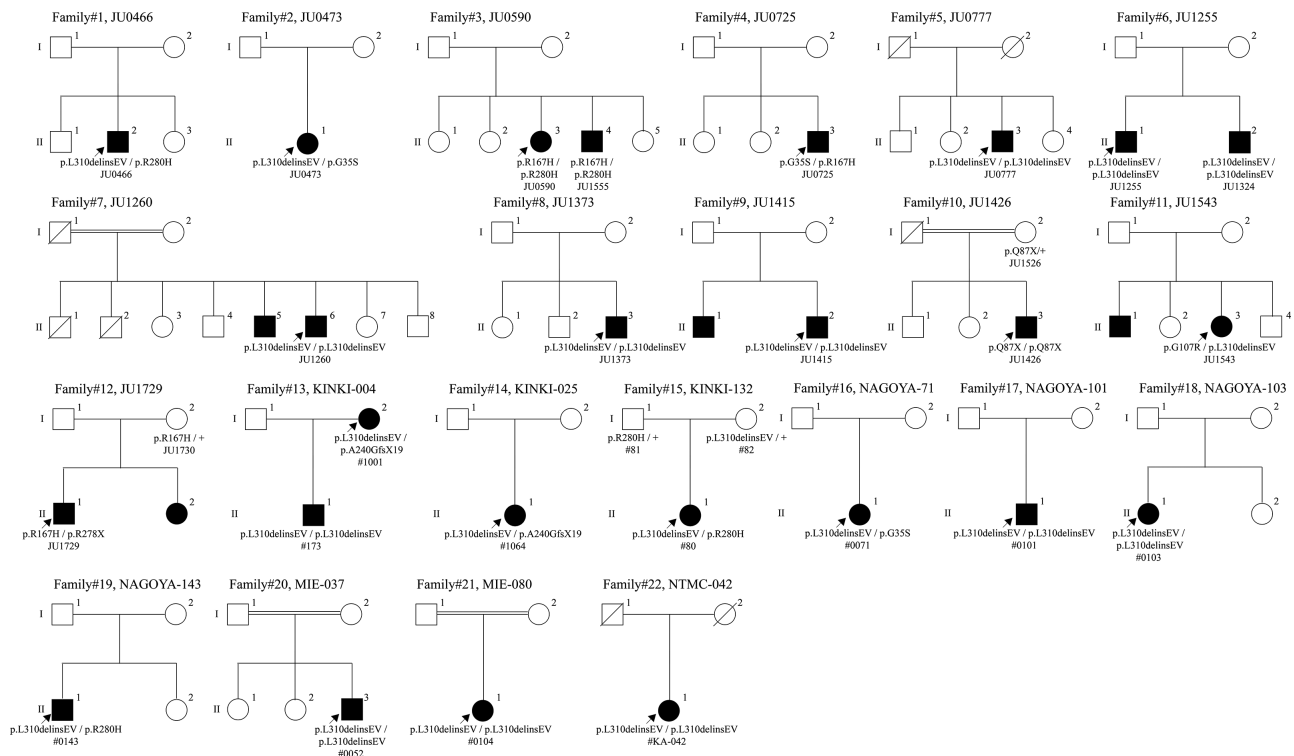
FIGURE 1. Pedigree charts of the families with fundus albipunctatus. Black arrows show the probands of the families. *Square*, male; *circle*, female; *solid*, affected; *open*, unaffected; *slash*, deceased. Four families (7, 10, 20, and 21) had a history of consanguineous marriage.

(http://www.1000genomes.org/), the Exome Aggregation Consortium database (http://exac.broadinstitute.org/), the Human Genetic Variation Database (http://www.genome.med.kyoto-u.ac.jp/SnpDB/), or the Tohoku Medical Megabank Organization database (https://ijgvd.megabank.tohoku.ac.jp/). Confirmation and segregation of the *RDH5* variants were assessed in each pedigree by direct sequencing. We determined the pathogenicity of the known *RDH5* variants based on the previous studies.[13,38,39] We evaluated the prevalence, segregation result, and impact on the RDH5 protein to determine the pathogenicity for novel *RDH5* variants. The inferred *RDH5* variants were compared with the sequences of the National Center for Biotechnology Information Reference Sequence (GenBank NM_001199771.1).

RESULTS

In total, 25 patients (representing 22 pedigrees), all of whom had been diagnosed with FAP with *RDH5* variants in the homozygous or compound heterozygous states, were studied in the present study, including four families (families 7, 10, 20, and 21) with a history of consanguineous marriage (Fig. 1). Clinical and genetic findings for the 25 patients are summarized in the Supplementary Table. Funduscopy, FAF, and OCT images are shown in Figures 2 and 3 and Supplemental Figures S1 and S2. All patients had a history of congenital night blindness. The age at which the comprehensive ophthalmic examinations were performed ranged from 9 to 79 years old (mean age, 43.4 years old; SD = 21.6 years; median age, 49 years). The duration of follow-up ranged from 0 to 19 years (mean, 6.6 years;

SD = 5.1 years; median, 7 years). Ten patients were female, and 15 were male. The value of refractive error is shown in the Supplementary Table. Two patients (F6, II-1; F7, II-6) had severe myopia, one of whom (F7, II-6) underwent cataract surgery with intraocular lens implantation.

Clinical Findings

BCVA was performed in all 50 eyes of the 25 patients. LogMAR BCVA values of 0 or better were detected in 35 eyes (70.0%); values 0.10 or better were detected in 44 eyes (88.0%). The six eyes for which BCVA was over 0.10 were as follows: F5, II-3 yielded a score of 0.15 in the left eye (LE); F7, II-6 had a score of 0.70 in the right eye and 0.82 in the left eye (BE); F11, II-3 exhibited a score of 0.22 in the right eye (RE); and F13, II-1 yielded scores of 0.52 (RE) and 1.00 (LE). Fourteen eyes of eight patients (28.0%) showed worsened visual acuity during follow-up examination. Among those eyes, six eyes of four patients (RE of F1, II-2; LE of F5, II-3; BE of F7, II-6; and BE of F13, II-1) exhibited markedly worsened logMAR BCVA (minimum; –0.08–0.40; maximum; 0.15–1.00).

Funduscopy was performed in all 50 eyes of the 25 patients, showing white dots from vascular arcade to peripheral retina in all 50 eyes, but the distributions and densities of the white dots were variable. Most patients showed dense white dots in the arcade vessels as well as in the midperipheral to peripheral retina (e.g., BE of F18, II-1; BE of F21, II-1). In several older patients, white dots were inconspicuous and localized only to part of the midperipheral to peripheral retina (e.g., BE of F10, II-3; BE of F11, II-3). On the other hand, fine white dots were
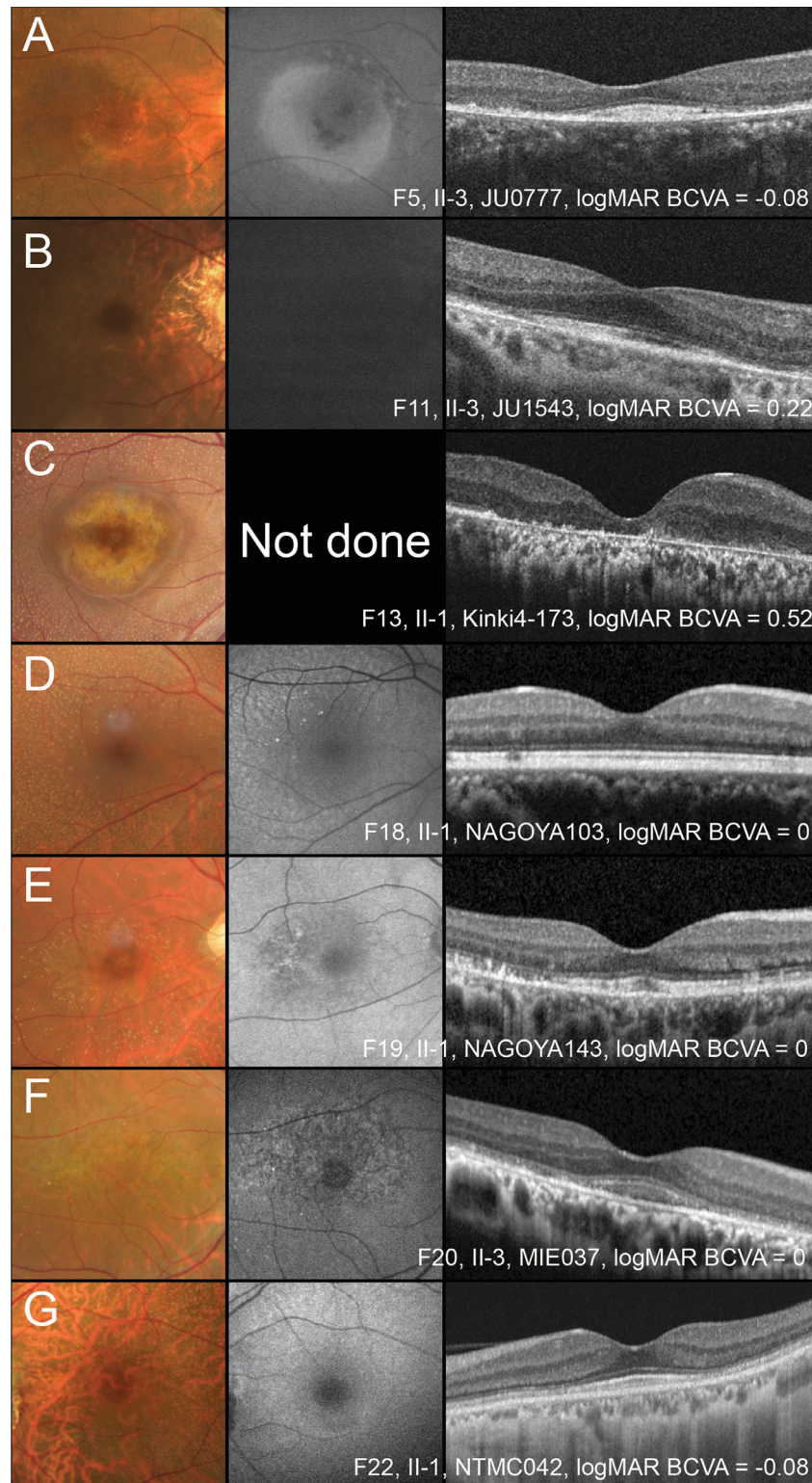
FIGURE 2. Representative funduscopy, FAF, and OCT images focused on macula (*left*, *middle*, and *right columns*, respectively). (**A**) Right eye (RE) of family 5, patient II-3 (F5, II-3) shows macular degeneration in fundus photographs, crescent-shaped hyperautofluorescence around the fovea and irregular autofluorescence around the fovea in the FAF images, and diffuse disruption of the external limiting membrane (ELM), ellipsoid zone (EZ), and interdigitation zone (IZ) and thinning of the outer nuclear layer (ONL) with sparing of the fovea in OCT images. (**B**) RE of F11, II-3 shows macular degeneration in fundus photographs, absent autofluorescence in FAF images, and diffuse disruption of the ELM, EZ, and IZ and thinning of the ONL with sparing of the fovea in OCT images. (**C**) RE of F13, II-1 shows ring-shaped macular discoloration in fundus photographs and diffuse disruption of ELM, EZ, and IZ and thinning of the ONL throughout the macula in OCT images. (**D**) RE of F18, II-1 shows no abnormalities at the macula in fundus photographs or in FAF and OCT images. (**E**) RE of F19, II-1 shows white dots around the fovea in fundus photographs, irregular autofluorescence around the fovea in FAF images, and diffuse disruption of

the ELM, EZ, and IZ and thinning of the ONL with sparing of the fovea in the OCT images. (**F**) RE of F20, II-3 shows macular degeneration in fundus photographs, irregular autofluorescence around the fovea in FAF images, and diffuse disruption of the ELM, EZ, and IZ and thinning of the ONL with sparing of the fovea in OCT images. (**G**) Left eye (LE) of F22, II-1 shows normal fundus appearance in fundus photographs, mild irregular autofluorescence around the fovea in FAF images, and diffuse EZ disruption with sparing of the fovea in OCT images.

localized in part of the midperipheral to peripheral retina in one young patient (BE of F4, II-3). Variable macular involvement was observed in 23 eyes from 12 patients (46.0%), including macular degeneration in 17 eyes (Figs. 2A, 2B, 2F, 3A, 3B), parafoveal white dots (sparse) in four eyes (BE of F13, I-2; BE of F19, II-1) (Fig. 2E), and ring-shaped macular discoloration in two eyes (BE of F13, II-1) (Fig. 2C).

FAF imaging was performed in 42 of 50 eyes. The FAF imaging revealed ring- or crescent-shaped hyperautofluorescence around the fovea in three eyes (BE of F7, II-6; RE of F22, II-1), irregular autofluorescence around the fovea in nine eyes (BE of F9, II-2; BE of F10, II-3; BE of F19, II-1; BE of F20, II-3; LE of F21, II-1) (Figs. 2E–2G, 3C, 3D), both findings in two eyes (BE of F5, II-3) (Fig. 2A), mild irregular autofluorescence in one eye (LE of F22, II-1) (Fig. 2G), no apparent macular abnormality in 21 eyes, and an inability to evaluate (due to reduced/absent autofluorescence in the posterior retina) in six eyes (BE of F11, II-3; BE of F6, II-1; BE of F15, II-1) (Fig. 2B). In extreme instances, FAF imaging showed absent autofluorescence with almost invisible retinal vessels (e.g., BE of F11, II-3; BE of F15, II-1). In patients with slightly reduced autofluorescence, FAF imaging showed dots of hyperautofluorescence in the areas of white dots in funduscopy (e.g., BE of F16, II-1). The reduced autofluorescence was seen in patients with various fundus appearances and ages; no association was apparent among these parameters.

Retinal OCT imaging was performed in 48 of 50 eyes. OCT revealed varying degrees of outer retinal changes represented by diffuse disruption of the external limiting membrane (ELM), ellipsoid zone (EZ), or interdigitation zone (IZ) and thinning of the outer nuclear layer (ONL) in 24 eyes in 12 patients, slight epiretinal membrane in two eyes (BE of F6, II-1), and no apparent abnormalities except for retinal changes related to the white dot lesions in 22 eyes in 11 patients. The patient with epiretinal membranes had normal macular appearance in funduscopy, and this finding is unlikely to be related to FAP. In 24 eyes with the outer retinal changes, 20 eyes showed sparing of the fovea, whereas four eyes (BE of F7, II-6; BE of F13, II-1) showed disruption of ELM and EZ/IZ throughout the macula. The more disruptive ELM and EZ/IZ were likely to be, the thinner the ONL. OCT images of white dot lesions occasionally were obtained in some patients; these images showed small hyperreflective linear lesions located at the level between ELM and RPE (e.g., BE of F17, II-1). Taken together, of the 24 eyes with macular involvement by OCT, 23 eyes (95.8%) also showed macular changes by funduscopy; among 18 of the 24 eyes that were also examined by FAF imaging, 15 eyes (83.3%) showed FAF abnormalities.

In the evaluation of central visual field, visual field testing using either Goldmann perimetry (17 patients) or Humphrey field analyzer (one patient) was performed in 36 eyes of 18 patients, including 16 eyes of eight patients with macular involvement by any of the examinations. Fourteen of these 16 eyes showed abnormalities in the central visual fields, except for one patient with sparse parafoveal white dots

(BE of F13, I-2). In 20 eyes of 10 patients without macular involvement, there were no abnormalities in the central visual fields. In the evaluation of peripheral visual field, visual field tests using Goldmann perimetry in 17 patients (34 eyes) showed preserved peripheral visual fields in 24 eyes, constriction of peripheral visual field by I-4e isopters in eight eyes, and absolute scotomas in the midperipheral visual field in two eyes (BE of F12, I-2).

The mean age of patients with macular involvement was 58.6 ± 11.1 years (range, 40–79 years; 12 patients), and they were significantly older than patients without macular involvement (29.3 ± 19.4 years; range, 9–58 years; 13 patients) ($P < 0.05$). In fact, macular involvement was seen in 12 of 17 patients (70.6%) who were ≥40 years of age and was not seen in any patient who was less than 40 years of age. Among the 12 patients with macular involvement, 10 patients (83.3%) had good BCVA, and two patients (16.7%) exhibited severely decreased BCVA (logMAR 0.70 in RE and 0.82 in LE in F7, II-6; logMAR 0.52 in RE and 1.00 in LE in F13, II-1).

Five patients (F6, II-1; F6, II-2; F8, II-3; F14, II-1; F18, II-1) without macular involvement, who were over 40 years of age, exhibited good BCVA but no remarkable differences of fundscopy and FAF imaging findings compared with patients over 40 years of age with macular involvement as seen by OCT. In terms of visual acuity, a total of six eyes of four patients who were ≥40 years of age exhibited decreased BCVA (worse than logMAR 0.10).

Other Funduscopic Findings

Visible choroidal vessels due to variable degrees of myopic atrophy were observed in ten eyes of five patients (BE of F6, II-1; BE of F7, II-6; BE of F11, II-3, BE of F19, II-1; BE of F22 II-1), two (F6, II-1; F7, II-6) of whom had severe myopia; the remaining three patients had mild myopia (Supplementary Table). Other complications were diabetic retinopathy (BE of F1, II-2) and myelinated nerve fibers (RE of F5, II-3).

Electroretinographic Findings

ERG results were symmetrical between right and left eyes in all 23 examined patients, 22 of whom underwent ERG testing after prolonged dark adaptation for 2 to 3 hours, in addition to standard dark adaptation for 20 to 30 minutes. Rod responses with standard dark adaptation (DA 0.01 ERG) showed extinguished b-waves in 21 patients and severely decreased b-waves in two patients. With a prolonged dark adaptation of 2 or 3 hours, b-waves recovered to normal ranges in 10 patients, partial recovery of b-waves was seen in 11 patients, and no recovery was seen in one patient (BE of F18, II-1).

Combined rod–cone responses with standard dark adaptation (DA 3.0 ERG) showed decreased a- and b-waves with electronegative wave form in 10 patients, decreased a- and b-waves in 12 patients, and a normal range in one patient. With prolonged dark adaptation of 2 or 3 hours, recovery of both a- and b-waves to normal ranges was seen in
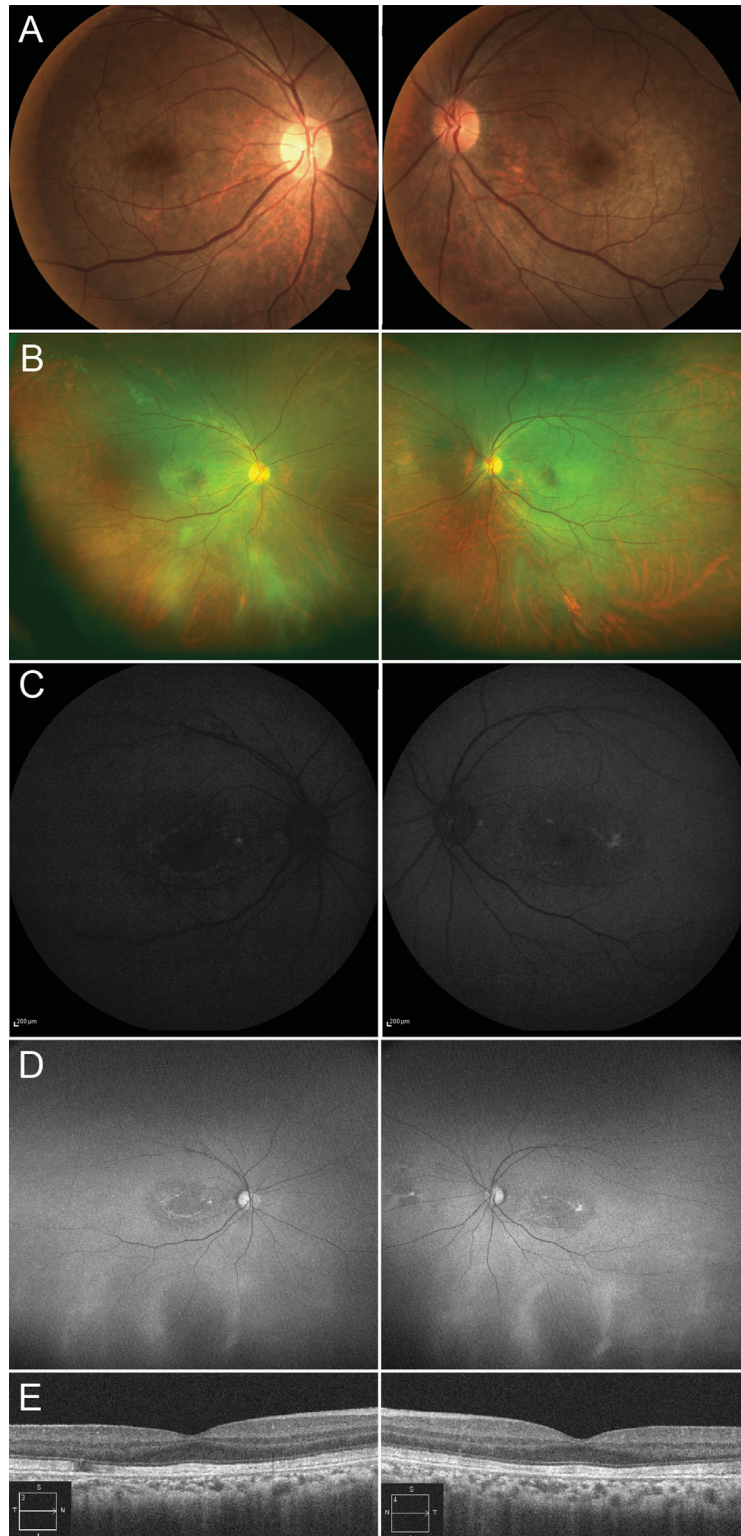
FIGURE 3. Funduscopy, FAF, and OCT images of family 10, patient II-3 (F10, II-3) with a novel homozygous *RDH5* variant (p.Q87X). (**A**) Fundus photographs in the posterior pole and (**B**) wide-field fundus photographs show inconspicuous, partial white dots at the peripheral retina and macular degeneration in both eyes. (**C**) FAF images in the posterior pole and (**D**) wide-field FAF images show reduced autofluorescence in the entire retina and irregular autofluorescence around the fovea in both eyes. (**E**) OCT images show a diffuse disruption of ellipsoid zone and interdigitation zone with sparing of the fovea in both eyes. Left and right columns correspond to left and right eyes, respectively.
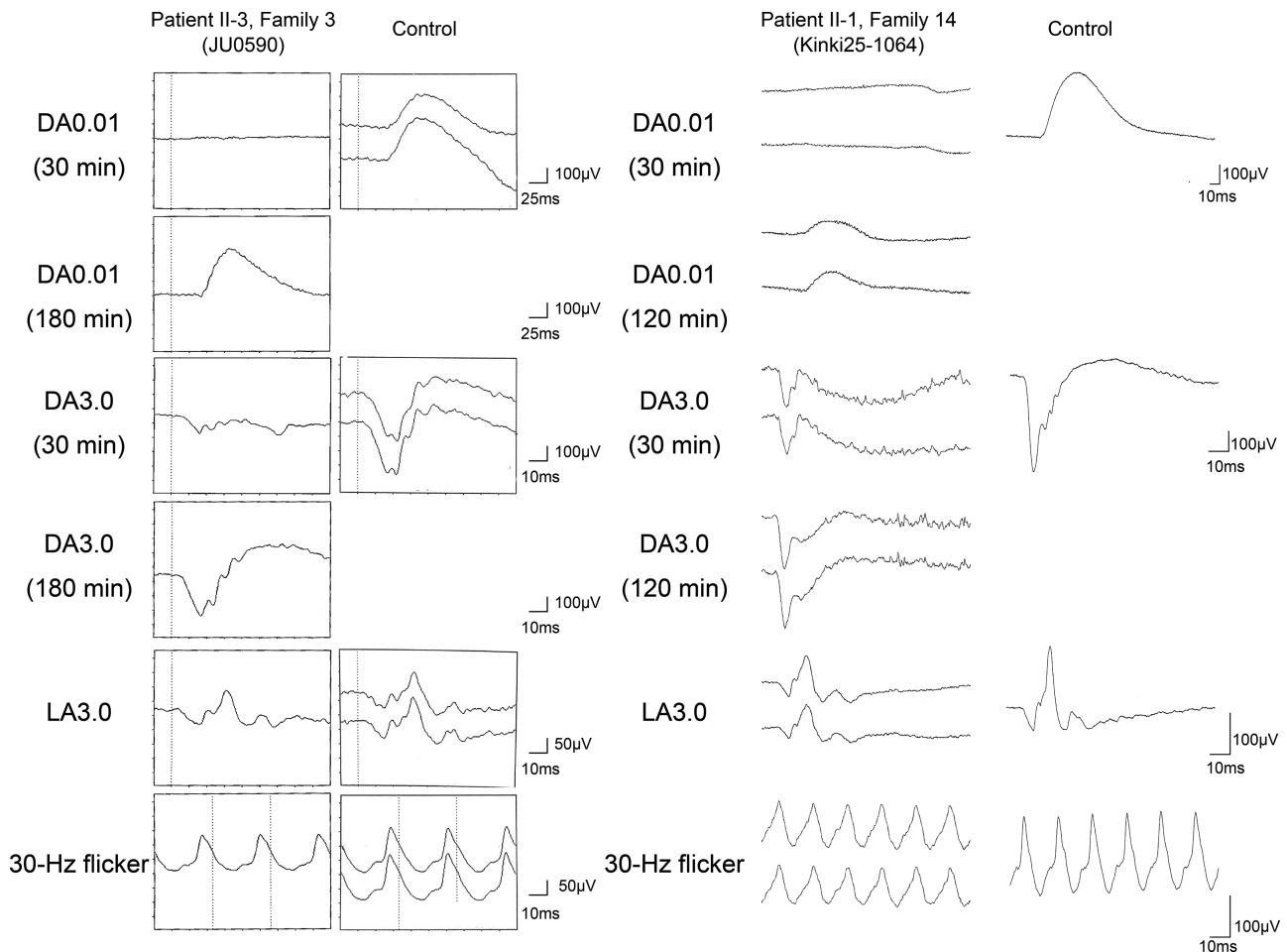
Figure 4. Representative full-field ERG. The ERG waveforms of two patients (F3, II-3; F14, II-1) are shown. Rod responses (DA 0.01) with standard dark adaptation show extinguished b-waves in both patients, whereas rod responses with prolonged dark adaptation (180 minutes) show full recovery of b-waves in F3, II-3 and partial recovery in F14, II-1. Combined rod–cone responses with standard dark adaptation (DA 3.0 ERG) show decreased a- and b-waves with an electronegative form in F3, II-3 and decreased a- and b-waves in F14, II-1. Rod responses (DA 3.0) with prolonged dark adaptation show full recovery of a- and b-waves in F3, II-3 but only partial recovery of a- and b-waves in F14, II-1. LA 3.0 and 30-Hz flicker responses are within the normal range in F3, II3 but slightly decreased in F14, II-1.

10 patients, partial recovery of a- and/or b-waves was seen in 11 patients, and one patient had no change because of normal a- and b-waves with standard dark adaptation.

Cone (LA 3.0 ERG) responses showed slightly decreased or decreased a- and b-waves in 17 of 23 patients (73.9%), including six patients without macular involvement, and normal a- and b-waves in six patients. The 30-Hz flicker responses showed decreased b-waves in 14 patients and normal b-waves in nine patients. Representative ERG waveforms from two patients (F3, II-3; F14, II-1) are shown in Figure 4.

Molecular Genetic Findings

RDH5 variants were identified by WES in seven pedigrees (families 14, 15, 17, 18, 19, 21, and 22), by the combination of WES and direct sequencing in one pedigree (family 13), and by direct sequencing in the remaining pedigrees. In family 13, WES analysis identified p.L310delinsEV in the heterozygous state in patient I-2, and screening by direct sequencing additionally identified p.A240GfsX19 in the heterozygous state in patient I-2 and p.L310delinsEV in the homozygous state in patient II-1. Inheritance exhibited an autosomal recessive inheritance pattern, except in family 13, where the disease showed a pseudo-dominant inheritance pattern.

Eight different *RDH5* variants were identified, including seven known *RDH5* variants—c.103G>A (p.G35S), c.319G>C (p.G107R), c.500G>A (p.R167H), c.718dupG (p.A240GfsX19), c.832C>T (p.R278X), c.839G>A (p.R280H), and c.928delCinsGAAG (p.L310delinsEV)—and a novel variant: c.259C>T (p.Q87X). The p.Q87X variant, which is absent from the database and (to our knowledge) from the previous literature, co-segregated. This variant is predicted to result in truncation of the RDH5 protein, and therefore is presumed to be loss-of-function for RDH5 protein. We concluded that p.Q87X is the pathogenic variant in family 10. p.L310delinsEV was the most frequently observed variant (65.2%, 30/46 alleles); the frequencies of the other variants were each less than 10% (Table). Although two patients (F13, I-2; F13, II-1) belonged to the same family (F13), they had different genotypes (p.L310delinsEV/p.A240GfsX19 in F13, I-2 and p.L310delinsEV/p.L310delinsEV in F13, II-1).

TABLE. *RDH5* Variants Identified in the 22 Pedigrees with Fundus Albipunctatus

Exon	Nucleotide	Amino Acid	Homo	Hetero	Variants/Allele (%)[*]	dbSNP ID	Ref.
2	c.103G>A	p.G35S	0	3	3/46 (6.5)	rs759359491	Nakamura et al.[10]
2	c.259C>T	p.Q87X	1	0	2/46 (4.3)	Not reported	This study
3	c.319G>C	p.G107R	0	1	1/46 (2.2)	Not reported	Nakamura et al.[10]
3	c.500G>A	p.R167H	0	3	3/46 (4.3)	rs199877211	Sekiya et al.[46]
4	c.718dupG	p.A240GfsX19	0	2	2/46 (4.3)	rs753378940	Nakamura et al.[10]
5	c.832C>T	p.R278X	0	1	1/46 (2.2)	rs751232605	Liu et al.[39]
5	c.839G>A	p.R280H	0	4	4/46 (8.7)	rs62638193	Gonzalez-Fernandez et al.[4]
5	c.928delCinsGAAG	p.L310delinsEV	11	8	30/46 (65.2)	rs768850783, rs761500612	Nakamura et al.[10]

[*] We treated Kinki 004-1001 (F13, I-2) and Kinki 004-173 (F13, II-1) as a single pedigree, despite the difference in genotype; however, the two genotypes were treated as different genotypes when we counted the number of *RDH5* variants.

We included these two patients in a single pedigree, despite their different genotypes; however, the two genotypes were dealt with as different genotypes when we counted the number of *RDH5* variants (Table).

Clinical Summary of Patients with the Frequent p.L310delinsEV Variant Homozygously

There were 12 patients with the p.L310delinsEV variant homozygously, including one young patient 17 years old and 11 patients ranging in age from 40 to 78 years. Six patients (F6, II-1, 58 years-old; F6, II-2, 56 years-old; F8, II-3, 44 years-old; F9, II-2, 58 years-old; F17, II-1, 17 years-old, F18, II-1, 51 years-old) did not exhibit macular involvement, whereas seven other patients (F5, II-3, 60 years old; F7, II-6, 78 years old; F9, II-2, 58 years old; F13, II-1, 40 years old; F20, II-3, 48 years old; F21, II-1, 58 years old; F22, II-1, 59 years old) had a variable degree of macular involvement. Cone ERG performed in 10 patients (Supplementary Table) showed normal responses in two patients, slightly decreased responses in three patients, and decreased responses in five patients. These results demonstrated that there was a phenotypic variability even in patients with the same variant.

DISCUSSION

In the present study, we examined clinical and genetic findings for 25 patients from 22 Japanese pedigrees with *RDH5*-related FAP. To our knowledge, this cohort represents the largest clinical analysis of *RDH5*-related FAP to date.

Macular Findings

Macular involvement a characteristics of *RDH5*-related FAP, especially for older patients.[10,11] Nakamura et al. HYPERLINK \l "bib10" [10] reported that six of 14 patients with *RDH5*-related FAP exhibited cone dystrophy, which is a complication of FAP, and they were assessed as having macular atrophy in addition to decreased cone responses in ERG. In subsequent studies, the same group reported that decreased cone responses in ERG were also observed in older patients, even in individuals without apparent abnormalities in fundus appearance, visual acuity, or visual field.[11,40] The finding of cone dysfunction subsequently has been re-evaluated and confirmed as being related to EZ and IZ abnormalities and thinning of the ONL in OCT images[13] and decreased cone density using adaptive optics tech-

nology.[41] Because the RDH5 protein (11-*cis*-retinol dehydrogenase) converts 11-*cis*-retinol to 11-*cis*-retinal in RPE cells,[1,2,5] the evaluation of RPE cells using FAF imaging is also important. FAF in *RDH5*-related FAP revealed a trend toward reduced autofluorescence in the entire retina, sometimes making it difficult to evaluate the macula.[12] However, Sergouniotis et al.[13] provided a series of FAF images for six of nine patients with *RDH5*-related FAP; these images show autofluorescence patterns similar to those seen in the present study. We evaluated macular involvement in 25 patients, including the use of FAF imaging in 21 patients (Fig. 2, Supplementary Fig. S1). As shown in the Results section, our patients exhibited broad phenotypic variability in each clinical examination but had features that were shared among patients. Notably, 12 of 25 patients showed macular involvement in fundus appearance and/or FAF accompanied by OCT abnormalities, whereas BCVA was relatively preserved. Although the funduscopy, FAF, and OCT imaging had enough sensitivity to detect macular involvement, OCT was the most sensitive examination. The macular involvement, including decreased cone function, was more readily apparent in older patients; thus, progressive macular involvement occurred in *RDH5*-related FAP with high frequency in the older patients.

There is a cone-specific visual cycle between Müller cell and the cone outer segment in chicken and mammalian retina.[42–44] In the cone-specific cycle, the 11-*cis*-retinol is also converted to 11-*cis*-retinal via 11-*cis*-retinol dehydrogenase activity apart from RDH5 protein in the cone outer segment,[43] whereas the canonical conversion by RDH5 protein occurs in RPE cells for the supply of 11-*cis*-retinal to both rod and cone systems.[3] The supply of 11-*cis*-retinol from the cone-specific cycle might not be enough to maintain normal cone function, especially in older patients, due to the decreased number of cone photoreceptors. This might explain the fact that the cone dysfunction usually occurs in older age compared with congenital rod-mediated dysfunction. There remains a question why *RDH5*-related FAP patients generally exhibit good visual acuity in spite of a high frequency of macular involvement or decreased cone ERG, as shown in the current and previous studies.[10,13,15] A few reports of a small number of *RDH5*-related FAP patients have described OCT abnormalities in the macula.[13,15,41,45] We investigated OCT findings of 12 patients with macular involvement and demonstrated that the 10 patients (83.3%) who had good BCVA exhibited diffuse disruption of ELM, EZ, and/or IZ with sparing of the fovea, whereas two patients (16.7%) exhibited diffuse disruption throughout the macula and decreased BCVA. Our OCT findings can explain that

good BCVA resulted from foveal sparing even though diffuse disruption of the outer retina and decreased cone ERG were observed.

Other Retinal Findings

Night blindness, white dots in the fundus, reduced autofluorescence in FAF, and progressive macular involvement are characteristic of *RDH5*-related FAP.[13] Previous studies have reported that the white dots became inconspicuous and macular involvement increases in older patients, whereas the pattern of white dots varies without any apparent regularity.[10,11,13,46] Although we obtained similar findings, our data did not detect an apparent relationship between white dots in the fundus and reduced autofluorescence in FAF. Accumulation of 11-*cis*-retinol and 11-*cis*-retinyl esters in RPE cells has been proposed as the cause of the formation of white dots,[47,48] although this hypothesis does not fully explain why this accumulation becomes focal to different degrees. In contrast, reduced autofluorescence, which has been described as a grainy pattern,[15] fuzzy and grainy images,[14] or the lack of autofluorescence,[12] is inferred to be the result of decreased A2E (*N*-retinylidene-*N*-retinylethanolamine) and lipofuscin formation due to impaired production of all-*trans*-retinal or restricted photopigment turnover by an incomplete visual cycle.[14] Thus, given that examinations by multimodal imaging permit evaluation of the retinal structure and function from different perspectives, this approach may provide new insights in the context of recently attempted therapies.

The ERG analysis revealed extremely reduced or absent rod responses in standard dark adaptation of 20 to 30 minutes and the recovery of rod responses to nearly normal in extended dark adaptation of 2 to 3 hours. These findings are characteristic for the diagnosis of FAP.[5–7] In the present study, almost all cases (21 of 22 patients, 95.5%) showed complete or partial recovery of rod responses in extended dark adaptation, whereas one case of a 61-year-old (BE of F19, II-1) showed extinguished b-waves in standard dark adaptation and no recovery in extended dark adaptation (Supplementary Table S1). Thus, it appears that the recovery of rod responses in extended dark adaptation is a clinical characteristic of FAP, although rare cases show no recovery of rod responses.

Molecular Genetic Findings

To date, 48 *RDH5* variants have been reported as the causes of FAP in HGMD Professional 2018.3. In the present study, we reported seven known variants and one novel variant. The p.L310delinsEV variant, which is the most frequently detected variant in the Japanese population,[10,49] was found at a high frequency (65.2%) in the present study (Table). The five known variants (p.G35S, p.G107R, p.R167H, p.A240GfsX19, and p.R280H) detected in the present study also have been reported previously in the Japanese population.[10,46] One novel variant (p.Q87X) was identified in the homozygous state in one Japanese family (family 10, JU1426) with a consanguineous marriage. Notably, all patients were classified into the same clinical entity as FAP, whereas phenotypic variability was seen even in patients with the same genotype of the homozygous p.L310delinsEV variant. Previous studies have also suggested no correlation between variant types and phenotypes.[40,50] In addition, there is a report that one of monozygotic sisters with *RDH5*-related FAP and cone dystrophy showed macular degeneration,

whereas the other sister showed normal macular appearance.[51] These findings suggest that non-genetic environmental factors or *trans*-acting variants in modifier genes might be associated with variability or severity of the disease phenotype.

Future Clinical Trials

An experimental study using *Rdh5*$^{-/-}$*Rdh11*$^{-/-}$ mice has reported that oral administration of the 9-*cis*-retinal improved cone function but not rod function.[48] In *RDH*-related FAP patients, the oral administration of the alga *Dunaliella bardawil* has been demonstrated to improve the peripheral visual field and rod function electroretinographically in a short-term period of 3 months.[16] The difference in improvement of rod versus cone function between the human and mouse model cannot be explained at present; nonetheless, oral delivery of 9-*cis*-retinal not only improves delayed dark adaptation but also might prevent further progressive deterioration of cone function in *RDH5*-related FAP. Intervention at earlier times (before macular involvement appears) is expected to lead to better clinical outcomes. An appropriate goal for clinical trials will be to retard the progression of cone dysfunction.

Conclusions

We reported the clinical and genetic characteristics of 25 patients from 22 Japanese pedigrees with *RDH5*-related FAP, including seven known *RDH5* variants and one novel *RDH5* variant. Multimodal imaging and ERG revealed high frequencies of macular involvement in older patients, as well as decreased cone responses, suggesting progressive loss of macular/cone function rather than a stationary condition. In addition, the OCT findings of patients with macular involvement revealed diffuse disruption of the outer retina with sparing of the fovea in most of patients, which can explain good BCVA in spite of macular involvement and/or decreased cone responses. Our data are expected to facilitate an upcoming clinical trial using 9-*cis*-retinoids for the treatment for *RDH5*-related FAP patients.

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