

Genetic and Clinical Features of ABCA4-Associated Retinopathy in a Japanese Nationwide Cohort



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- **PURPOSE:** To clarify the genetic and clinical features of Japanese patients with ABCA4-associated retinopathy.
- **DESIGN:** Retrospective, multicenter cohort study.
- **METHODS:** Patients with retinal degeneration and biallelic ABCA4 variants were recruited from 13 different hospitals. Whole exome sequencing analysis was used for genetic testing. Comprehensive ophthalmic examinations were performed on matched patients. The primary outcome measure was identifying multimodal retinal imaging findings associated with disease progression.
- **RESULTS:** This study included 63 patients: 19 with missense/missense, 23 with missense/truncation, and 21 with truncation/truncation genotypes. In total, 62 variants were identified, including 29 novel variants. Six patients had a mild phenotype characterized by foveal-sparing or preserved foveal structure, including 4 with missense/missense and 2 with missense/truncation genotypes. The p.Arg212His variant was the most frequent in patients with mild phenotypes (4/12 alleles). Clinical

findings showed a disease duration-dependent worsening of the phenotypic stage. Patients with the truncation/truncation genotype exhibited rapid retinal degeneration within a few years and definite fundus autofluorescence imaging patterns, including hyper autofluorescence at the macula and few or no flecks.

- **CONCLUSIONS:** Our results indicate that missense/missense or missense/truncation genotypes, including the p.Arg212His variant, are associated with a relatively mild phenotype. In contrast, the truncation/truncation genotype causes rapid and severe retinal degeneration in Japanese patients with ABCA4-associated retinopathy. These data are vital in predicting patient prognosis, guiding genetic counseling, and stratifying patients for future clinical trials. (Am J Ophthalmol 2024;264: 36–43. © 2024 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>))

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IN 1997, STARGARDT DISEASE (STGD1, MIM: 248200) WAS first reported to be caused by biallelic variants of the ATP-binding cassette transporter, alpha 4 subunit (ABCA4) gene.¹ These variants are associated with several retinal conditions, including bull's-eye maculopathy,² macular atrophy,³ fundus flavimaculatus,⁴ cone-rod dystrophy, and retinitis pigmentosa.^{5–7} ABCA4-associated retinopathy is a commonly inherited retinal disorder, affecting approximately 1 in 8000 to 10,000 people.^{8,9} A recent national survey in the United Kingdom reported a yearly incidence of 0.127 per 100,000 population,¹⁰ which is 5 times higher than that observed in a study from the Nationwide Epidemiological Survey of Macular Dystrophy in Japan.¹¹ Previous large cohort studies have identified genotype-phenotype correlations in patients with ABCA4-associated retinopathy, showing that disease severity correlates with variant severity and worsens over time.^{12,13}

Notably, several therapies, including oral treatments¹⁴ and gene therapies using adeno-associated viruses,¹⁵ other large vectors,^{16,17} and antisense oligonucleotides,¹⁸

have recently been explored. However, *ABCA4*-associated retinopathy is rare among the Japanese, accounting for 1.7% of inherited retinal disorder cases (20 of 1210 pedigrees).¹⁹ With imminent clinical trials, it is crucial to thoroughly investigate this retinopathy's genetic and clinical characteristics in Japanese patients to precisely evaluate treatment efficacy and timing of interventions. This study represents the first nationwide Japanese cohort focused on *ABCA4*-associated retinopathy, aiming to elucidate the genetic and clinical profiles of patients with this condition, particularly concerning disease progression and genotype.

METHODS

• **ETHICS STATEMENT:** The Institutional Review Boards of The Jikei University School of Medicine (approval number 24-231 6997), Nihon University School of Medicine (approval number 20211105), Kindai University Faculty of Medicine (approval number 22-132 and R05-071), Kyushu University (approval number 536-08), The University of Tokyo (approval number 2018191G), Hamamatsu University School of Medicine (approval number 14-040), Teikyo University (approval number 10-007-5), Kobe City Eye Hospital (approval number E19002), Nagoya University Graduate School of Medicine (approval Number: 2020-0598), University of Miyazaki (approval number G-0118), Mie University Graduate School of Medicine (approval number MIE-2429), and the National Hospital Organization Tokyo Medical Center (approval number R18-029) approved this study. The study protocol followed the Declaration of Helsinki, and all participants provided written informed consent.

• **INCLUSION CRITERIA:** We studied 3324 patients with inherited retinal disorders who underwent genetic analysis. The inclusion criteria for patients were retinal degeneration conditions, such as macular dystrophy, cone-rod dystrophy, and retinitis pigmentosa phenotypes. Eligible patients exhibited either homozygous or compound heterozygous variants in the *ABCA4* gene as confirmed by genetic analysis across the consortium of 13 participating hospitals. The phenotypes were determined using findings from multimodal retinal imaging, visual field testing, and full-field electroretinography.

• **MOLECULAR GENETIC ANALYSIS:** Genetic testing was performed using whole exome sequencing analysis based on previously described methods.²⁰⁻²³ The pathogenicity of the *ABCA4* variants was evaluated using the Human Gene Mutation Database Professional (HGMD, <http://www.hgmd.cf.ac.uk/>), ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>), Genome Aggregation Database (gnomAD, <https://gnomad.broadinstitute.org/>), and the Japanese Multi Omics Reference Panel (jMorP; <https://jmorp.megabank.tohoku.ac.jp/>).

Patients with biallelic *ABCA4* variants were classified into 3 groups according to their genotypes (missense/missense, missense/truncation, and truncation/truncation) based on a modified method from a previous study.²⁴ Consequently, canonical and non-canonical splice site, frameshift, and stop-gain variants were defined as truncation variants.

• **CLINICAL EXAMINATIONS:** Comprehensive ophthalmic examinations, including medical review (age at onset and chief complaint), decimal best-corrected visual acuity (BCVA), fundus photograph, and short-wavelength fundus autofluorescence imaging (FAF) were performed using Spectralis HRA (Heidelberg Engineering, Heidelberg, Germany), and/or Optos 200Tx/California, ultrawidefield retinal imaging system (Optos, Dunfermline, UK), optical coherence tomography (OCT; Carl Zeiss Meditec AG, Dublin, CA, USA), and Goldmann perimetry (Haag Streit, Bern, Switzerland).

Phenotypic stage analysis

We analyzed the correlations between the phenotypic stage, disease duration, and BCVA across 3 genotypes. Based on the findings of well-established studies,^{4,13,25} the ophthalmologists K.M. and T.H. conducted independent evaluations of the phenotypic stages, utilizing fundus imaging and specific criteria outlined below:

Stage I: Macular lesions limited to the macula, exhibiting irregular pigmentary alterations, a bull's-eye maculopathy, or a distinctive "beaten-bronze" appearance.

Stage II: Macular lesions with characteristic yellowish flecks that emanate from the macula and extend beyond the vascular arcades and optic disc.

Stage III: Diffuse atrophy of the macular choriocapillaris with associated resorption of flecks within the macular area.

Stage IV: Extensive atrophy of the choriocapillaris accompanied by pigment deposition in the central posterior pole or further.

Representative images of each stage are shown in Figure 1.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corp, Armonk, NY, USA), R and R-studio (version 3.6.3; <http://www.R-project.org/>). Bonferroni's multiple comparison test was used to determine the significant differences in the age at onset and patients with each genotype. Decimal BCVA was converted to a logarithm of the minimum angle of resolution (logMAR) units for statistical analysis. Furthermore, the BCVA of counting fingers, hand motions, and light perceptions were converted to 2.0, 2.4, and 2.7 logMAR units, respectively.²⁶ Kaplan-Meier survival curves with the log-rank test were used to compare survival experiences (logMAR BCVA) among the

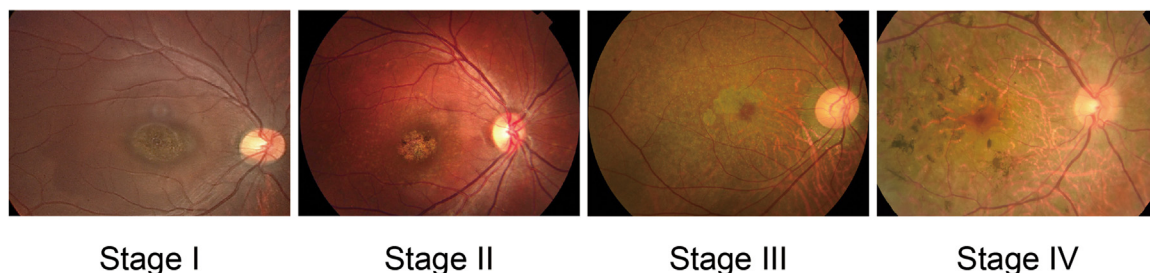


FIGURE 1. Representative imaging of each stage in patients with ABCA4-associated retinopathy.

Stage I: Macular lesions limited to the macula, exhibiting irregular pigmentary alterations, a bull's-eye maculopathy, or a distinctive "beaten-bronze" appearance. Stage II: Macular lesions with characteristic yellowish flecks that emanate from the macula and extend beyond the vascular arcades and optic disc. Stage III: Diffuse atrophy of the macular choriocapillaris with associated resorption of flecks within the macular area. Stage IV: Extensive atrophy of the choriocapillaris accompanied by pigment deposition in the central posterior pole or further.

3 genotypes. A significance threshold of $P < .05$ was established, and for cases where this was achieved among the 3 genotypes, a Bonferroni correction was applied to pinpoint which genotype pairs exhibited significant differences, using an adjusted significance level of $P < .017$.

RESULTS

• **MOLECULAR GENETIC FINDINGS:** In total, 63 patients who met the inclusion criteria were recruited, and 62 variants were identified, including 29 novel ones (Supplemental Tables 1 and 2). Of these variants, 42 were missense (67.7%), 9 were stop-gain (14.5%), 6 were frameshift (9.7%), and 5 were canonical/noncanonical splice-site variants (8.1%). The patients were categorized into 3 genotype groups: 19 (30.2%), 23 (36.5%), and 21 (33.3%) with missense/missense, missense/truncation, and truncation/truncation genotypes, respectively.

• **CLINICAL FINDINGS:** Supplemental Table 1 presents the clinical findings in the 63 patients with biallelic ABCA4 variants, including the age at onset, symptoms, disease duration (from onset to examination), BCVA (first and last examinations), and the phenotypic stage (first and last examinations).

Age at onset

Patients with truncation/truncation genotypes had a younger age at onset (7.29 ± 2.37 years; range, 3-11 years) than did those with missense/missense genotypes (26.44 ± 19.46 years; range, 6-68 years; $P = .000$) and missense/truncation (17.81 ± 11.75 years; range, 6-40 years; $P = .030$) genotypes. However, the patients with missense/missense and missense/truncation genotypes ($P = .234$) showed no differences in the age at onset (Figure 2).

Survival curves of visual acuity

Kaplan–Meier survival curves were initially plotted using a cutoff of 1.0 logMAR unit (equivalent to 0.1 a decimal visual acuity) to estimate the changes in BCVA during the disease course in all 63 patients. The survival curves indicated that BCVA required a median duration of 42 years to reach a 1.0 logMAR unit. Subsequently, comparing the BCVA progression among the 3 genotypes (missense/missense, missense/truncation, and truncation/truncation) showed that the median age for BCVA to reach a 1.0 logMAR unit was the late 60s, approximately 50 years, and approximately 30 years in patients with the missense/missense, missense/truncation, and truncation/truncation genotypes, respectively (Figure 3). The genotype comparison results were as follows: a significant difference was observed between the missense/missense and truncation/truncation genotypes ($P = .003$), as well as between the missense/truncation and truncation/truncation genotypes ($P = .003$). However, no significant difference was noted when comparing the missense/missense genotype with the missense/truncation genotype ($P = .900$) (Figure 3).

Phenotypic stage

Supplemental Figure 1 illustrates the correlations among the phenotypic stage, disease duration, and logMAR BCVA for the 3 genotypes. Among 15 patients with the missense/missense genotype, all but 5 patients (JU0266, JU2182, JU2189, JU2211, and Teikyo1024) exhibited an early stage (stage I or II) within the first 20 years of disease onset. Patients exhibited varying correlations between phenotypic stage and disease duration, and between phenotypic stage and logMAR BCVA. In 18 patients with the missense/truncation genotype, there was a tendency for the phenotypic stage to progressively worsen over time; however, some (JU0717, JU2154, and Kinki141-107) remained at an early stage (stage I or II) despite having had the disease for >20 years. This pattern was also observed in the correlation between the phenotypic stage and logMAR BCVA.

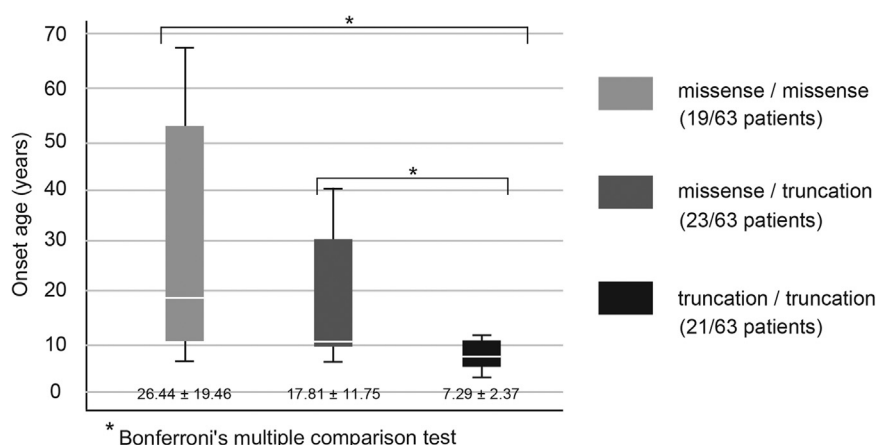


FIGURE 2. Age at onset across genotypes. Patients with the truncation/truncation genotype have a significantly younger age at onset (7.29 ± 2.37 years; range, 3-11 years) compared with those with missense/missense (23.60 ± 16.45 years; range, 6-50 years) and missense/truncation (16.90 ± 11.27 years; range, 6-40 years) genotypes, with P -values of <0.001 and 0.020 , respectively. However, the missense/missense and missense/truncation genotypes show no significant differences in age at onset ($P = .234$).

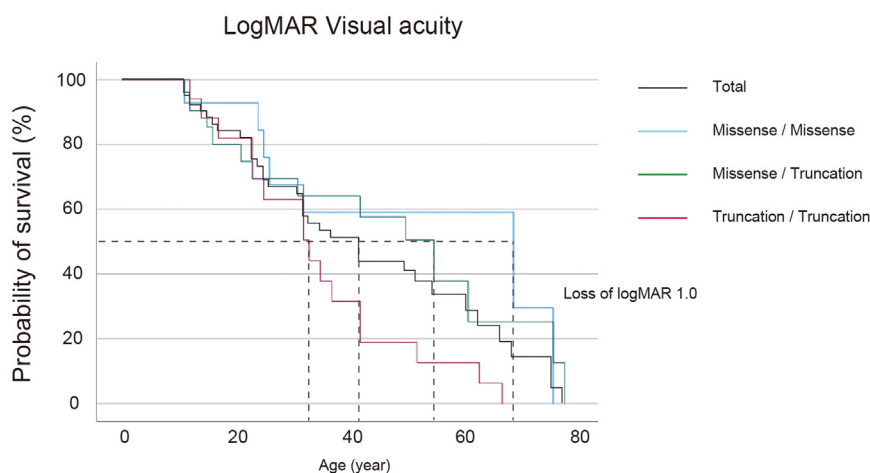


FIGURE 3. Survival curves and clinical course of visual acuity. Kaplan–Meier survival curves are plotted using a cut-off of 1.0 logMAR unit, equivalent to 0.1 a decimal visual acuity. In all 63 patients, the survival curves indicate that the median duration required for best-corrected visual acuity (BCVA) to decline to a 1.0 logMAR unit in half of the patients is 42 years. When comparing progression among the 3 genotypes, we find that the median age in patients when BCVA reaches a 1.0 logMAR unit is the late 60s, approximately 50 years, and approximately 30 years for the missense/missense, missense/truncation, and truncation/truncation genotypes, respectively. Log-rank test with Bonferroni correction is applied for the comparisons. The genotype comparison results show significant differences between missense/missense and truncation/truncation ($P = .003$) and between missense/truncation and truncation/truncation ($P = .003$) but not between missense/missense and missense/truncation ($P = .900$).

In 15 patients with the truncation/truncation genotype, a clear time-dependent progression of the phenotypic stage was observed. Furthermore, logMAR BCVA also deteriorated with the worsening of the phenotypic stage.

Multimodal retinal imaging

Supplemental Figure 2 shows the detailed multimodal retinal images of representative patients across the 3 genotypes.

Missense/missense genotype

In patients with missense/missense genotypes, fundus photography findings generally showed a gradually worsened

phenotypic stage over time. However, this trend was not observed in patients with foveal sparing or a preserved foveal structure (JU2151, JU2182, and JU2211). Flecks were observed in all early stage patients (KA304, KA241, KA115, and JU2151) but were absent in those at the end-stage (Teikyo1024, JU0266, JU2182, JU2189, and JU2211). FAF imaging revealed hypo-AF in the macular region, indicating macular degeneration or atrophy with a combination of hypo-AF and hyper-AF. This was consistent with flecks in the surrounding area in early-stage patients. In contrast, end-stage patients showed hypo-AF extending beyond the arcade vessels. The OCT

findings demonstrated foveal-sparing or preserved foveal structure in some patients, specifically those identified as JU2151 (p.Arg212His and p.His1865Tyr), JU2182 (p.Asp586Glu and p.Gly2041Asp), JU2189 (p.Arg212His and p.Arg212His), and JU2211 (p.Arg212His and p.Asn269Ser). Conversely, a different group of patients showed a disruption of the outer retinal layers at the fovea, including KA304 (p.Arg511Cys and p.Val675Ile), KA241 (p.Gly1623Ser and p.Arg1862Cys), and KA115 (p.Thr1019Met and p.Cys1488Tyr). Over the 8-year follow-up, the 68-year-old patient (JU2189) exhibited progressive damage to the outer retinal layers at the fovea.

Missense/truncation genotype

Fundus photography findings indicated that the phenotypic stage consistently worsened with increasing disease duration in patients with the missense/truncated genotype. Notably, all patients but one (KA074) had flecks throughout the disease course. The FAF findings revealed hypo-AF in the macular area, consistent with macular degeneration or atrophy, with or without additional hypo-AF and hyper-AF around the area, corresponding to flecks in all patients. OCT findings revealed foveal sparing in 2 patients (JU0657 and JU2208) and a disruption of the outer retinal layers at the fovea in other patients (N528, JU0099, JU0666, and KA074). However, 1 patient (JU0657) exhibited this disruption in the outer retinal layers at the fovea over time.

Truncation/truncation genotype

Fundus photography initially showed discoloration or a normal appearance in patients with the truncation/truncation genotype; however, all patients had rapidly increased retinal degeneration from the arcade vessels to the peripheral retina as the disease progressed. Only a few or no flecks were observed throughout the disease course. During the first examination, FAF findings revealed a normal appearance or hypo-AF in the macular region, with hyper-AF around the area (beyond the arcade vessels). Over time, all patients but one (JU1315) exhibited an expansion of hypo-AF beyond the arcades of vessels. In patient JU1315, hypo-AF was localized to the macular region, a finding that aligns with the expected macular degeneration or atrophy, accompanied by areas of both hypo-AF and hyper-AF nearby. The OCT findings revealed that during the disease course, there was a disruption in the outer retinal layers at the fovea in all patients studied.

DISCUSSION

This study characterized the genetic and clinical features in 63 Japanese patients with biallelic ABCA4 variants.

In total, 62 distinct ABCA4 variants were identified, including 41 missense (67.2%), 9 stop-gain (14.8%), 6

frameshifts (9.8%), and 5 splice site variants (8.2%). These findings are consistent with those of previous studies.^{12,13} A previously proposed genotype-phenotype correlation, with phenotypic severity dependent on variant severity, was observed in our cohort, particularly regarding age at onset (Figure 2) and visual acuity (Figure 3). Regarding the mild phenotype characterized by foveal sparing or preserved foveal structure, a large Spanish cohort of 506 patients with STGD1 reported only 8 cases of foveal sparing¹² predominantly with genotypes comprising 6 missense/missense and 2 missense/truncation genotypes. Our study observed a mild phenotype in 6 out of 63 patients, with 4 (JU2151, JU2182, JU2189, and JU2211) and 2 (JU0657 and JU2208) with the missense/missense and missense/truncation genotypes, respectively. Previous research suggests that milder phenotypes are strongly associated with the hypomorphic variants (p.Gly1961Glu and p.Arg2030Gln),²⁷⁻³⁰ and these vary across ethnic groups.²⁵ Notably, the p.Arg212His variant was the most frequently observed (4 of 12 alleles) in our patients with a mild phenotype (JU0657, JU2151, JU2182, JU2189, JU2208, and JU2211), suggesting it as a possible hypomorphic variant in Japanese patients with ABCA4-associated retinopathy. The p.Arg212His variant has also been detected in previously reported cases of STGD1³¹⁻³³; however, detailed clinical features have not been described. In contrast, a previous study has shown that the alternative variant (p.Arg212Cys) in a homozygous state is associated with a mild phenotype with retention of some ABCA4 protein function.³⁰ This supports our findings indicating that the p.Arg212His variant is also associated with a mild phenotype.

Previous large cohort studies have elucidated the clinical features and disease progression in patients with biallelic ABCA4 variants.^{12,13} Wang et al. described the disease progression in 42 patients with long-term follow-up, suggesting that the phenotypic stage progressed in a time-dependent manner.¹³ Consistent with these findings, our study observed a similar time-dependent progression of the phenotypic stage (retinal degeneration) (Supplemental Figure 1). However, no correlation was found between the phenotypic stage and disease duration in patients with a mild phenotype. This suggests that the actual disease duration may be longer than perceived because of subtle symptoms. Our research also investigated clinical variations based on genotype. A previous study found distinct clinical features in patients with rapid-onset chorioretinopathy (ROC), linked to more deleterious variants, such as truncating variant, differing from other ABCA4-associated retinopathy variants.³⁴ This study observed that deterioration of the macula began with an intense/hyper-AF and homogeneous signal on FAF, followed by a coalescing pattern of ROC within the subsequent decade.³⁴ Similarly, disease progression is characterized by an expansion of areas with decreased AF of the macula.^{35,36} Particularly in cases where the disease onset occurs in childhood, the rate of progression tends to be more rapid than that in adult-onset.³⁷ In

our cohort, hyper-AF was also observed in patients with the truncation/truncation genotype, subsequently, areas of decreased AF appeared and expanded (Supplemental Figure 2). Consequently, the previous and current studies indicate that hyper-AF at the macula is a characteristic finding in early-stage patients with the truncation/truncation genotype.

Furthermore, the early-stage disease differences in the presence of flecks based on the genotype were also investigated. The term “fundus flavimaculatus” is often used to describe the phenotype of retinal flecks without atrophy. Flecks are thought to correlate with disease severity. They originate from degenerating photoreceptor cells, impaired by the functional failure of the retinal pigment epithelium (RPE),^{38,39} and correlate with disease severity.⁴⁰ Our findings revealed that flecks were prevalent in patients with missense/missense and missense/truncation genotypes but are less common in those with truncation/truncation genotypes (Supplemental Figure 2). Longitudinal studies of flecks observed on OCT may help understand the differences in flecks observed using FAF imaging.^{38,39} Flecks initially appear as hyperreflective bands from the RPE to the outer nuclear layer (ONL), followed by the thinning of the ONL on OCT. Subsequently, as the fleck deposition dissipates, OCT reveals the disruption of the outer retinal layers, including the ellipsoid zone (EZ). These results indicate that the rapid disease progression leads to little or no fleck deposition, resulting in the sudden appearance of atrophic retinal areas. The presence of few or no flecks in the early stages of the disease may also characterize patients with the truncation/truncation genotype. The differences in fleck presentation in the early stages of the disease could predict the degree of progression and genotype.

Additionally, recent studies have investigated the progression of ABCA4-associated retinopathy with respect to retinal imaging, microperimetry, and electroretinogram (ERG) findings. Both cross-sectional and longitudinal assessments have reported changes in retinal sensitivity measured by microperimetry and alterations in the EZ observed through OCT in childhood-onset STGD1.^{41,42} Assessment using microperimetry has shown that the rate of progression in children is significantly greater than that in adults.⁴¹ Moreover, OCT studies have revealed that quantifying the area of EZ loss offers greater sensitivity than measurements of EZ loss width for evaluating progression.⁴² One study has outlined the potential of using ERG evaluation and FAF imaging results to predict disease progression,⁴³ with patients categorized into 3 ERG groups based on retinal function²⁹ and 3 FAF groups according to the extent of hypo-AF and retinal background appearance.⁴⁴ This study has found a strong correlation between ERG and FAF results. It is sug-

gested that patients assessed in early childhood who possess at least 1 truncation variant and exhibit poor initial BCVA or both, are likely to show a more extensive retinal involvement or progress to a more severe phenotype over time than what baseline FAF imaging might indicate.⁴³ This enables the possibility of informing patients by comparing FAF with the current gold standard of ERG, thereby aiding in prognostication. They may help determine the appropriate timing for therapeutic intervention and assess the effectiveness of treatment in ongoing gene therapy.

This study has some limitations, including the small cohort size and the absence of whole-genome sequencing data, which may have overlooked additional deep intronic variants.²⁵ Furthermore, the limitations encompass the lack of microperimetry assessment, grouping of ERG and ultrawidefield FAF evaluation, and assessment of EZ loss. Further research using a larger patient cohort, comprehensive genomic data, and imaging and functional evaluation of the retina is necessary to substantiate our findings.

In conclusion, our study suggests that the missense/missense or missense/truncation genotypes, including the p.Arg212His variant, are associated with a milder phenotype of ABCA4-associated retinopathy. However, the truncation/truncation genotype is associated with a more rapid and severe retinal degeneration in Japanese patients with ABCA4-associated retinopathy. These findings are crucial for predicting patient prognosis, providing genetic counseling, and stratifying patients for future clinical trials.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Kei Mizobuchi: Writing – original draft, Formal analysis, Conceptualization. **Takaaki Hayashi:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Koji Tanaka:** Data curation. **Kazuki Kuniyoshi:** Data curation. **Yusuke Murakami:** Data curation. **Natsuko Nakamura:** Data curation. **Kaoruko Torii:** Data curation. **Atsushi Mizota:** Data curation. **Daiki Sakai:** Data curation. **Akiko Maeda:** Data curation. **Taro Kominami:** Data curation. **Shinji Ueno:** Data curation. **Shunji Kusaka:** Data curation. **Koji M Nishiguchi:** Writing – review & editing, Data curation. **Yasuhiro Ikeda:** Writing – review & editing, Data curation. **Mineo Kondo:** Writing – review & editing, Data curation. **Kazushige Tsunoda:** Writing – review & editing, Data curation. **Yoshihiro Hotta:** Writing – review & editing, Data curation. **Tadashi Nakano:** Writing – review & editing.

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