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CLINICAL CASE REPORT

# Longitudinal clinical course of three Japanese patients with Leber congenital amaurosis/early-onset retinal dystrophy with *RDH12* mutation

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## Abstract

*Purpose* To report the longitudinal clinical course of three Japanese patients from two families with Leber congenital amaurosis/early-onset retinal dystrophy (LCA/EORD), and the results of next-generation DNA sequences on them.

*Patients and methods* The patients were three Japanese children: a 4-year-old girl, a 6-year-old boy, and a 3-year-old girl. Patients 1 and 2 were siblings, and patient 3 was from an unrelated family. Standard ophthalmic examinations including perimetry, electroretinography, optical coherence tomography, and ultrasonography were performed on each patient. The patients were observed for 28, 16, and 10 years. Whole exomes of the patients and their non-symptomatic parents were analyzed using a next-generation sequence technique.

*Results* The decimal visual acuity varied between 0.07 and 0.6 at the initial visit and decreased to counting finger to hand motion in their teens. Funduscopy showed diffuse retinal and macular degeneration. During the follow-up period, a posterior staphyloma developed and the macular area became atrophic. Patient 1 developed cataracts in her early twenties. Genetic analysis revealed a homozygous A126V substitution in the *RDH12* gene in all patients.

*Conclusions* The three patients with LCA/EORD had a progressive decrease of their vision with the formation of a posterior staphyloma. This is the first report of Japanese patients with LCA/EORD with a *RDH12* mutation.

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**Keywords** Leber congenital amaurosis · Early-onset retinal dystrophy · *RDH12* · Macular dystrophy · Posterior staphyloma · Electroretinogram · Next-generation sequence analysis

## Introduction

Leber congenital amaurosis (LCA) is the most severe form of early-onset retinal dystrophy and was first reported by Theodor Leber in 1869 [1]. He reported blind infants who had nystagmus and no pupillary light reflexes, and their fundus was initially normal and progressed to pigmentary retinal dystrophy [1]. For the diagnosis of LCA, it is necessary to show the presence of searching nystagmus, absence of pupillary light reflexes, and non-recordable electroretinograms (ERGs) [2]. Leber also described milder forms of this disease [3], which is now referred to as early-onset severe retinal dystrophy (EOSRD), severe early-childhood-onset retinal dystrophy (SECORD), or early-onset retinal dystrophy (EORD). The appearance of the fundus of LCA/EORD varies widely, including normal fundus appearance, flecked retina, diffuse pigmentary retinal degeneration, and macular coloboma/posterior staphyloma. In addition, keratoconus and cataract can be present in these patients [4].

Most cases of LCA/EORD have an autosomal recessive inheritance pattern. To date, 17 causative genes have been identified for LCA/EORD (LCA1-17, Table 1) [5, 6]. Since *RDH12* was reported as a causative gene for LCA/EORD in 2004 [7, 8], several studies have reported on the phenotype of LCA/EORD patients with a *RDH12* mutation [9–16]. These studies reported a progressive reduction in vision leading to legal blindness in young adulthood, and the presence of diffuse retinal degeneration with macular degeneration and cataract formation [7–16]. However, the longitudinal clinical course of cases of LCA/EORD with the *RDH12* mutation has not been reported.

**Table 1** Genes reported as causative for Leber congenital amaurosis or early-onset retinal dystrophy (LCA/EORD) [5,6]

| Phenotype | Name | Loci | Year reported in LCA/EORD | Note |
|---|---|---|---|---|
| LCA1 (ar) | *GUCY2D* | 17q13.1 | 1996 | CORD6 (ad) |
| LCA2 (ar) | *RPE65* | 1q31.3-2 | 1997 | keratoconus, RP20 (ar) |
| LCA3 (ar) | *SPATA7* | 14q31.3 | 2009 | |
| LCA4 (ar) | *AIPL1* | 17p13.2 | 2000 | macular degeneration, juvenile CRD (ad) |
| LCA5 (ar) | *LCA5* | 6q14.1 | 2003 | coloboma |
| LCA6 (ar) | *RPGRIP1* | 14q11.2 | 2001 | CORD13 (ar) |
| LCA7 (ad/ar) | *CRX* | 19q13.32 | 1998 | coloboma, CORD2 (ad) |
| LCA8 (ar) | *CRB1* | 1q31.3 | 2001 | coloboma, PPRPE (ar), RP12 (ar) |
| LCA9 (ar) | *NMNAT1* | 1q36.22 | 2012 | coloboma |
| LCA10 (ar) | *CEP290* | 12q21.32 | 2006 | BBS14 (ar), JBTS5 (ar), SLSN6 (ar), MKS4 (ar) |
| LCA11 (ad) | *IMPDH1* | 7q32.1 | 2006 | RP10 (ad) |
| LCA12 (ar) | *RD3* | 1q32.3 | 2006 | |
| LCA13 (ar) | *RDH12* | 14q24.1 | 2004 | maculopathy, RP53 (ad) |
| LCA14 (ar) | *LRAT* | 4q32.1 | 2001 | |
| LCA15 (ar) | *TULP1* | 6q21.31 | 2004 | maculopathy, RP14 (ar) |
| LCA16 (ar) | *KCNJ13* | 2q37.1 | 2011 | SVD (ad) |
| LCA17 (ar) | *GDF6* | 8q22.1 | 2013 | |

*ar* autosomal recessive, *ad* autosomal dominant, *CORD* and *CRD* cone-rod dystrophy, *RP* retinitis pigmentosa, *PPRPE* RP with parafoveolar preservation of the retinal pigment epithelium, *BBS* Bardet-Biedl syndrome, *JBTS* Joubert syndrome, *SLSN* Senior-Loken syndrome, *MKS* Meckel syndrome, *SVD* snowflake vitreoretinal degeneration

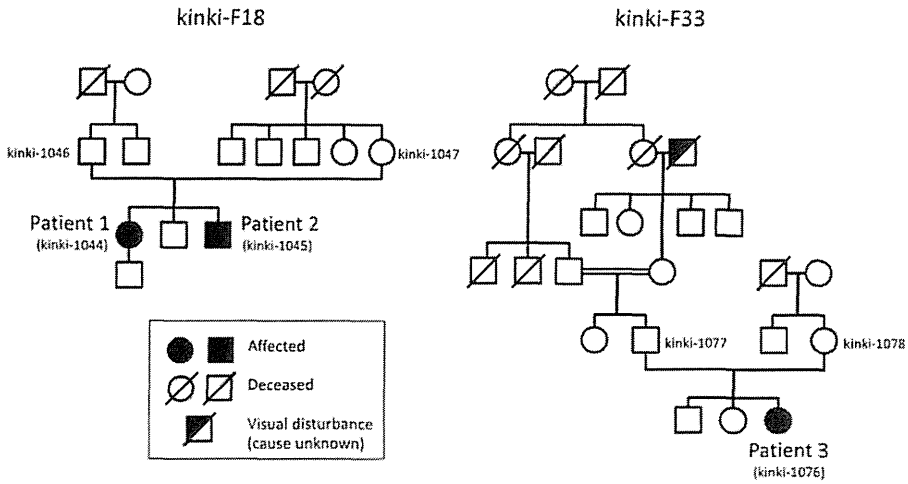



**Fig. 1** Pedigrees of two unrelated families with Leber congenital amaurosis/early-onset retinal dystrophy (LCD/EORD) with *RDH12* mutation. Patients 1 and 2 were siblings (*left*, kinki-F18), and Patient 3 is from an unrelated family (*right*, kinki-F33). No consanguinity was reported between parents of the patients

We report the 10- to 28-year continuous course of three Japanese patients with LCA/EORD, and the results of next-generation sequence analyses on them.

## Patients and methods

The patients were three Japanese individuals from two unrelated families (Fig. 1). Patients 1 and 2 were siblings (kinki-F18), and Patient 3 was a member of another unrelated family (kinki-F33; Fig. 1).

The research protocol was approved by the Ethics Review Board of the Kinki University Faculty of Medicine in November 2011, and the procedures conformed to the tenets of the Declaration of Helsinki. The genetic analysis was performed after obtaining a signed informed consent form from all patients and/or their parents.

### *Clinical studies*

The ophthalmic examinations consisted of measurements of the visual acuity, slit-lamp biomicroscopy, ophthalmoscopy, Goldmann kinetic perimetry, full-field ERGs, optical coherence tomography (OCT), and ultrasonography. ERG recordings were performed according to the guideline of the International Society for Clinical Electrophysiology of Vision (ISCEV Standard, 2008 update) [17]. OCT was performed with the Cirrus™ HD-OCT version 5.1 (Carl Zeiss Meditec, Dublin, CA, USA). All clinical tests were performed in the Kinki University Hospital, and all patients were examined yearly from the initial visit to year 2013.

### *DNA preparation and exome sequencing analysis*

The genetic analyses were performed in 2013. We obtained venous blood samples from the patients and their non-symptomatic parents in the Kinki University Hospital. The blood samples were sent to the Division of Molecular and Cellular Biology in National Institute of Sensory Organs of the National Hospital Organization Tokyo Medical Center, and genomic DNA was extracted from the blood samples using Gentra Puregene Blood Kit (Qiagen, Tokyo, Japan). The purified genomic DNA was sent to RIKEN or Macrogen Japan (Tokyo, Japan) and shared with Covaris Ultrasonicator™ (Covaris, Woburn, MA, USA). Construction of paired-end sequence libraries and exome capture were performed using the Agilent Bravo Automated Liquid Handling Platform with SureSelect XT Human All Exon V4 + UTRs kit (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. Enriched libraries were sequenced with the Illumina HiSeq 2000 sequencer (San Diego, CA, USA), according to the manufacturer's instructions for 100-bp paired-end sequencing. The results of the sequence analysis were sent to Laboratory of DNA Data Analysis in National Institute of Genetics and analyzed. Reads were mapped to the reference human genome (1,000 genomes, phase 2 reference, hs37d5) with the Burrows-Wheeler Aligner software, version 0.6.2 [18]. Duplicated reads were then removed by Picard MarkDuplicates module version 1.62, and mapped reads around insertion–deletion polymorphisms (INDELs) were realigned using the Genome Analysis

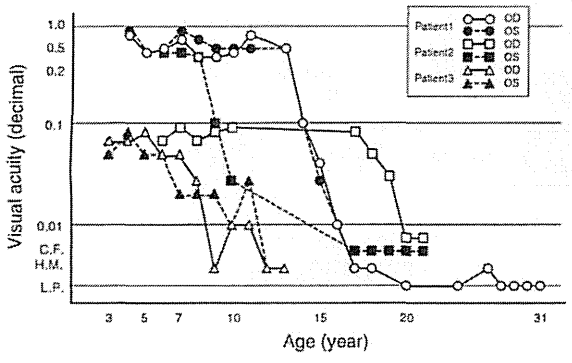



**Fig. 2** Clinical course of visual acuity in each patient. *OD* oculus dexter, *OS* oculus sinister

Toolkit (GATK) version 2.7-4 [19]. Base-quality scores were recalibrated using GATK. The calling of mutations was performed using the GATK UnifiedGenotyper module, and the called single-nucleotide variants and INDELs were annotated with the snpEff software, version 3.3 [20]. The mutations were annotated with the snpEff score ("HIGH," "MODERATE," or "LOW") and with the allele frequency in the 1,000 genomes database and Human Genetic Variation Browser (HGVD) [21]. The mutations were then filtered so that only those with "HIGH" or "MODERATE" snpEff scores indicating that the amino acid sequence would be functionally affected, and a frequency <1 % in the 1,000 genomes database and HGVD were further analyzed. We also used new variations, which were not found in the in-house database of exome data of seven people with control individuals without ocular diseases. Mutations were classified by hereditary information into homozygous recessive, heterozygous recessive, and de novo mutations in the family members. Filtered mutations were scored with PolyPhen software version 2.2.2 [22], which predicts the effect on the structure and function of the protein. This exome analysis pipeline is available at Management and Analysis System for Enormous Reads (Maser) [23].

## Results

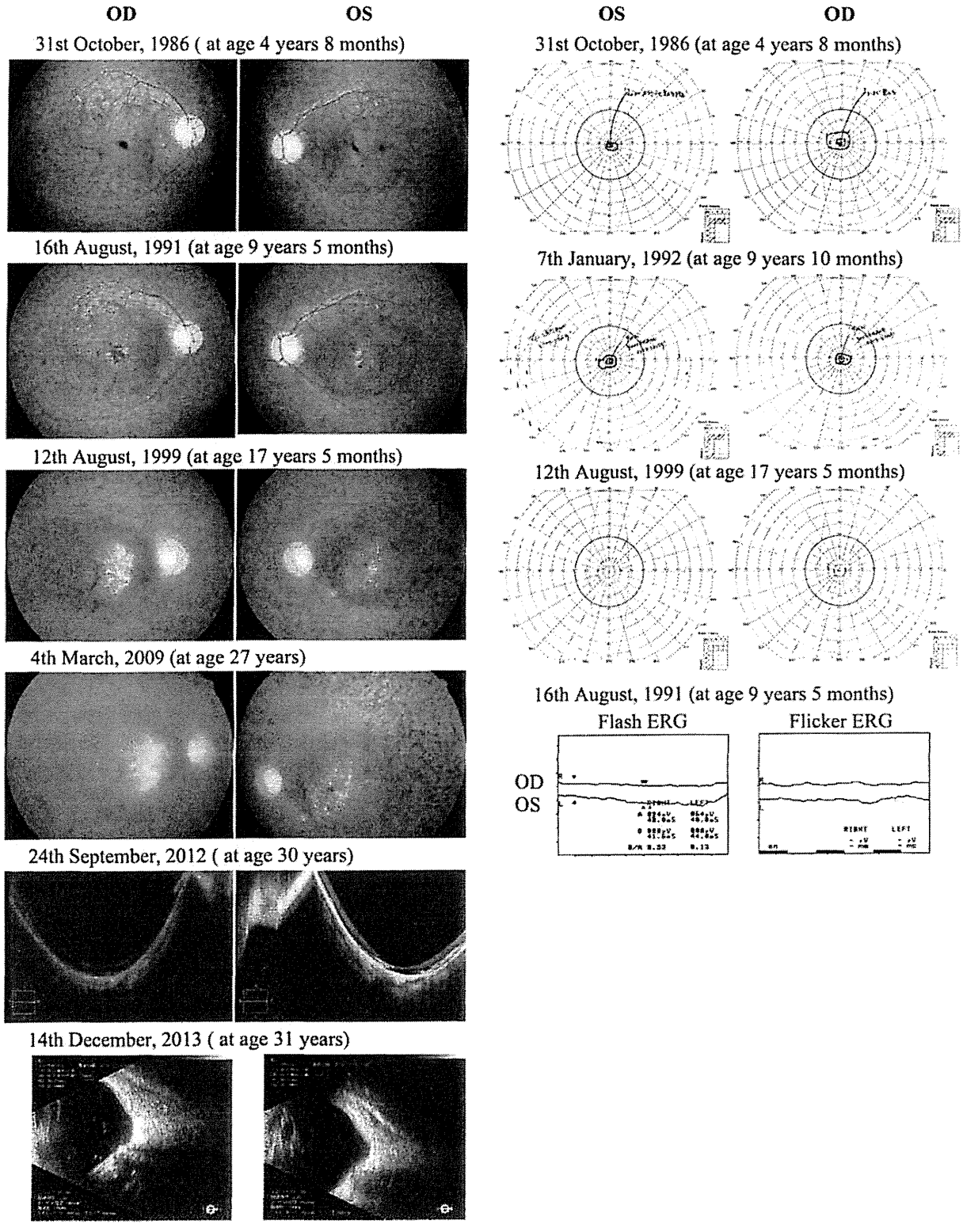
The clinical course of the visual acuity in the three patients is presented in Fig. 2. Summaries of the clinical findings are shown in Figs. 3, 4, and 5.

*Patient 1* (Fig. 3, kinki-1044 in Fig. 1): Patient 1 was a girl who was 4-year old when we first examined her in 1986. Her parents reported that she seemed to have difficulty in the dark from the age of 3 years. Her decimal best-corrected visual acuity (BCVA) at the initial visit was 0.6 with +1.25 diopter sphere (DS) and −0.75 D cylinder (DC) ax 160° in the right eye and 0.6 with +0.5 DS and −0.25 DC ax 20° in the left eye. Her visual fields were severely constricted, and ophthalmoscopy showed diffuse retinal degeneration with macular degeneration (Fig. 3). Her fundi appeared reticulated before the age 10 years. Her vision markedly decreased in her middle teens resulting in hand motion vision at age 17 years (Fig. 2). At this age, the macular degeneration appeared atrophic and a posterior staphyloma was present in both eyes (Fig. 3). A posterior subcapsular cataract was noticed when she was 23-year old. She is now 31-year old, and her vision is light perception in both eyes (Fig. 2).

**Fig. 3** Fundus photographs (*left*), optical coherence tomographic (OCT) and ultrasonographic images (*lower left*), Goldmann kinetic visual fields (*right*), and full-field ERGs (*bottom right*) of Patient 1. Fundus photographs and OCT images taken at age 27 and 30 years are blurred because of cataracts. The last perimetric examination was performed on August 12, 1999, when no isopter could not be detected. The flash ERGs are dark-adapted 30.0 ERG with 20-J xenon strobe, and the flicker ERG was recorded using red light-emitting diode built-in contact lens electrode (Tomey, Nagoya, Japan)

Single-bright flash full-field ERGs recorded at age 9 years were non-recordable, and the flicker ERGs were barely recordable (Fig. 3). OCT and ultrasonography performed at 30 and 31 years of age showed deep excavation and a thinning of the retina at the posterior pole of both eyes (Fig. 3). The axial length at age 31 years was 22.72 ± 0.05 mm in the right eye and 21.20 ± 0.09 mm in the left eye.

When the sequences of her whole exome were compared with the reference human genome (hs37d5), 940,138 mutations were found. We focused only on mutations that could change the amino-acid sequence and excluded common mutations by 1,000 genomes, HGVD [21], and our in-house database (see methods). As a result, 467 mutations remained as candidate mutations. We filtered the remaining mutations by using the pattern of inheritance (homozygous recessive, heterozygous recessive, or de novo mutation) with her parents and her brother (Patient 2) and found only 2 genes as causal candidates. Finally, *RDH12*, which was the only one of the genes registered in the RetNet database of genes and the loci causing inherited retinal diseases [6], was assumed to be the


OD
OS
31st October, 1986 ( at age 4 years 8 months)
16th August, 1991 (at age 9 years 5 months)
12th August, 1999 (at age 17 years 5 months)
4th March, 2009 (at age 27 years)
24th September, 2012 ( at age 30 years)
14th December, 2013 ( at age 31 years)
OS
OD
31st October, 1986 (at age 4 years 8 months)
7th January, 1992 (at age 9 years 10 months)
12th August, 1999 (at age 17 years 5 months)
16th August, 1991 (at age 9 years 5 months)
Flash ERG
Flicker ERG
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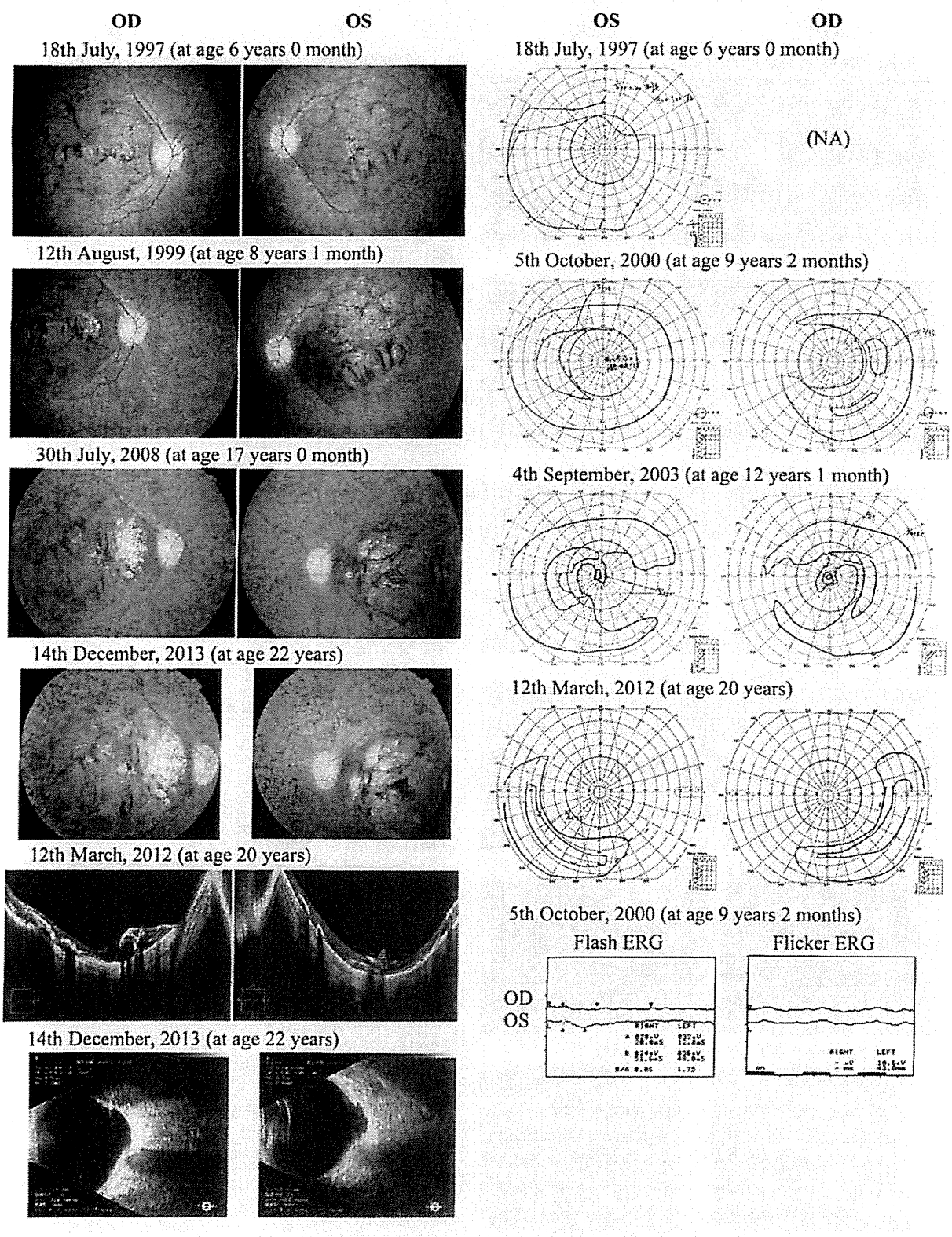


**Fig. 4** Fundus photographs (*left*), OCT and ultrasonographic images (*lower left*), Goldmann kinetic visual fields (*right*), and full-field ERGs (*bottom right*) of Patient 2. Patient 2 was younger brother of Patient 1 (Figs. 1, 3). *NA* not available

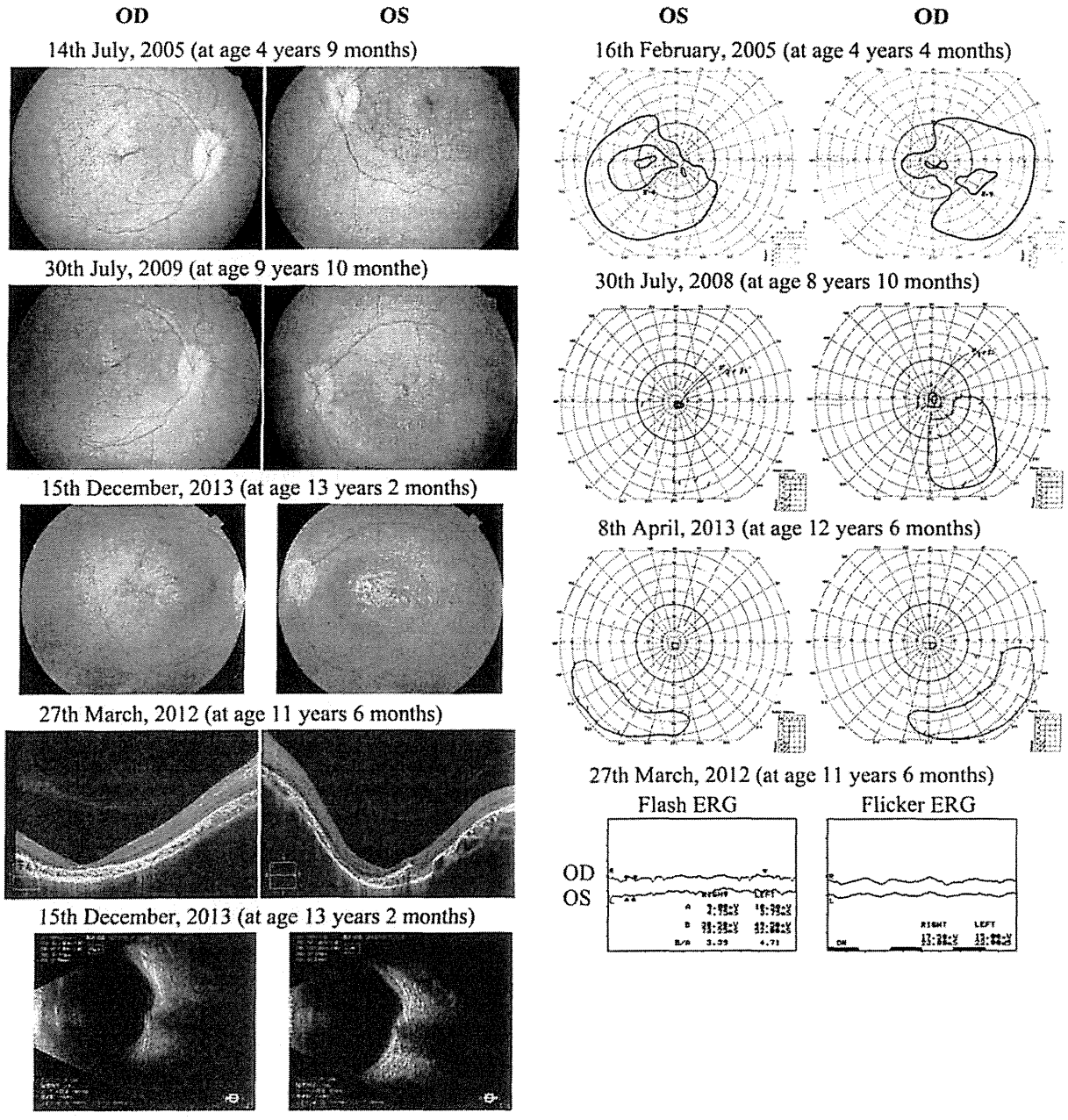


Fig. 5 Fundus photographs (*left*), OCT and ultrasonographic images (*lower left*), Goldmann kinetic visual fields (*right*), and full-field ERGs (*bottom right*) of Patient 3. Patient 3 was from unrelated family to that of Patients 1 and 2 (Fig. 1)

disease-causing gene. Then, genetic analysis revealed a homozygous c.377C>T transition in exon 4 resulting in an alanine126 to valine substitution (A126V) in the *RDH12* gene. Genetic analyses of her non-symptomatic parents (kinki-1046 and 1047, Fig. 1) revealed a heterozygous A126V substitution in the *RDH12* gene.

*Patient 2* (Fig. 4, kinki-1045 in Fig. 1): Patient 2 was a boy who was 6-year old when we first examined him in 1997. He was the younger brother of Patient 1 (Fig. 1). He visited our clinic because his parents noticed he was having visual difficulties since age 5 years. His decimal visual acuity was 0.07 in his right eye. The vision was uncorrectable, and his left BCVA was 0.4 with 0 DS and −1.5 DC ax 160°. Ophthalmoscopy showed diffuse retinal degeneration, but it was especially severe in the macula which was similar

to that of his older sister, Patient 1 (Fig. 3, 4). The fundi appeared reticulated before the age 10 years. The macular degeneration gradually spread, and a posterior staphyloma developed and progressed in both eyes (Fig. 4). His central vision decreased to hand motion in his late teens (Fig. 2). He is now 22-year old, and he still has some peripheral vision but no cataracts in both eyes.

The full-field ERGs, OCT, and ultrasonographic findings were similar to those of his older sister (Patient 1), namely, non-recordable single-bright flash ERGs, barely recordable flicker ERGs, and deep excavation and thin retina at the posterior pole of both eyes (Fig. 4). The axial length at age 22 years was 23.82 ± 0.05 mm in the right eye and 24.06 ± 0.02 mm in the left eye.

Genetic analysis revealed a homozygous A126V substitution in *RDH12* gene, the same as his sister (Patient 1).

*Patient 3* (Fig. 5, kinki-1076 in Fig. 1): Patient 3 was a girl who was 3-year old when we first examined her in 2004. She was a member of a family (kinki-F33) unrelated to that of Patients 1 and 2 (Fig. 1). She was brought to our clinic because of esotropia and nystagmus. Her decimal BCVA was 0.07 with +6.0 DS and −1.0 DC ax 115° in the right eye and 0.07 with +5.5 DS and −1.5 DC ax 175° in the left eye. Ophthalmoscopy showed diffuse retinal degeneration with pigmentation in the macular area (Fig. 5). Her fundi appeared reticulated before the age 10 years. She was followed until the age of 13 years, and her vision gradually decreased to light perception in both eyes (Fig. 2).

Single-bright flash full-field ERGs were non-recordable, and flicker ERGs were barely recordable at age 11 years (Fig. 5). OCT and ultrasonography performed at 11 and 13 years of age revealed excavation of the posterior pole of both eyes (Fig. 5). The axial length at age 13 years was 20.92 ± 0.37 mm in the right eye and 21.22 ± 0.93 mm in the left eye.

When the sequence of her whole exome was compared with the reference human genome (hs37d5), 1,488,313 mutations were found. After excluding common mutations, 406 mutations remained. We filtered the remaining mutations by the pattern of inheritance with her parents and found 16 genes as causal candidates. Finally, they were compared to that of Patients 1 and 2, and only *RDH12* was shared between three patients. As a result, genetic analysis showed a homozygous c.377C>T transition in exon 4 resulting in alanine126 to valine substitution (A126V) in the *RDH12* gene. Genetic analyses on her non-symptomatic parents (kinki-1077 & 1078, Fig. 1) showed heterozygous A126V substitution in the *RDH12* gene.

## Discussion

### ERG findings in carrier relatives

The *RDH12* gene is located at 14q 24.1 and encodes a photoreceptor cell retinol dehydrogenase. Mutation of the *RDH12* gene is estimated to account for <4 % of all autosomal recessive LCA/EORD patients [5, 8]. To date, 16 different mutations have been reported in this gene [6]; however, the homozygous substitution of A126V in the *RDH12* gene has never been reported except in a highly consanguineous Arabic family [13] and our patients. In the Arabic family, a non-symptomatic relative who was a heterozygous carrier of A126V had markedly reduced rod ERGs, and the cone ERGs were at the lower limits of normal [13]. Another study reported that heterozygous mutations in the *RDH12* gene can cause a late-onset, relatively mild autosomal dominant retinitis pigmentosa [24].

The parents of our patients were non-symptomatic, and their fundi were normal. The rod and cone ERGs performed on three of them (kinki-1047, kinki-1077, and kinki-1078 in Fig. 1) were normal.

### Clinical course of visual acuity

The initial visual disturbance in our patients was noticed at age 2–5 years, and there was a progressive decrease thereafter (Fig. 2). Their central vision decreased to light perception in the teens. Patients 2 and 3 maintained some peripheral vision at age 22 and 13 years although Patient 1 lost vision in the entire visual field at age 17 years (Figs. 3, 4, 5).

The vision in patients with LCA/EORD was investigated by Fulton et al. [25] and Walia et al. [26]. Walia et al. [26] related the vision of patients with LCA/EORD to their causative genes and reported that LCA/EORD caused by *RPE65* (LCA2), *CRB1* (LCA8), and *RDH12* (LCA13) mutations led to a wide variations in visual disturbances, whereas LCA/EORD caused by *GUCY2D* (LCA1), *AIPL1* (LCA4), *RPGRIP1* (LCA6), and *CRX* (LCA7) gene mutations had severe visual disturbances which began in the first year of life. Other studies on LCA/EORD associated

with *RDH12* mutations reported an initial vision reduction occurring between birth to 20 years with most of them at age 3–7 years [7–16].

These results are consistent with our patients who had decreased vision at age 2–5 years and loss of their central vision in their teens (Fig. 2).

### Coloboma/posterior staphyloma and LCA/EORD

The fundus of our three patients appeared similar; namely, they showed diffuse retinal degeneration and macular atrophy (Figs. 3, 4, 5). The fundi also had a reticulated appearance (Figs. 3, 4, 5). These findings are similar to the phenotype reported for *RDH12*-associated LCA/EORD [7–16].

In our patients, the macular degeneration progressed to atrophic macula with the formation of a posterior staphyloma which resembled a coloboma (Figs. 3, 4, 5). The relationships between LCA and macular coloboma have been discussed in several papers [27–29], before the causative genes for LCA/EORD were discovered. Recently, a macular coloboma/posterior staphyloma was reported in patients with *LCA5* (LCA5) [30], *CRX* (LCA7) [31], *CRB1* (LCA8) [32], *NMNAT1* (LCA9) [33], and *RDH12* (LCA13) mutations [7, 9–11, 14, 16]. A relationship between LCA/EORD and the macular coloboma/posterior staphyloma is still unknown. Single-gene mutation cannot explain the formation of a macular coloboma/posterior staphyloma because they are present in cases of LCA/EORD associated with several different causative genes.

In our patients, the reticulated appearance of the fundus was present in early childhood, and it became less apparent after the formation of the posterior staphyloma. Whether the reticulated appearance was related to the development of the staphyloma was not determined.

One limitation of this study is the small number of the patients. In addition, a more detailed screened investigation of the phenotypes and genotypes of patients with LCA/EORD is needed to confirm our results.

In conclusion, we report the longitudinal clinical course of three patients in two families with LCA/EORD who had homozygous A126V substitution in the *RDH12* gene. All of the patients had a progressive retinal degeneration and posterior staphyloma, and impairment of the central vision. This is the first report of Japanese patients with LCA/EORD which was caused by *RDH12* gene mutation.

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**Conflict of interest** All authors have no commercial interests related to this research.

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CLINICAL CASE REPORT

# Autosomal recessive cone–rod dystrophy associated with compound heterozygous mutations in the *EYS* gene

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**Abstract**
*Background* *EYS* mutations have been identified only in patients with autosomal recessive retinitis pigmentosa (arRP). This study was conducted to describe clinical and genetic features of a Japanese patient with autosomal recessive cone–rod dystrophy (arCRD) and *EYS* mutations.
*Methods* We performed complete ophthalmic examinations including full-field electroretinography (ERG). Genetic analysis using whole-exome sequencing and Sanger sequencing was performed to identify the disease-causing mutation in a 31-year-old male patient.
*Results* At the initial visit, the patient's decimal best-corrected visual acuity (BCVA) was 0.9 and 0.6 in his right and left eyes, respectively. Funduscopy indicated retinal degenerations were predominantly affected within the vascular arcades and preserved retinal vessels in the mid-periphery in both eyes. Visual field testing showed there were relative central scotomas and preserved peripheral visual fields in both eyes. ERG indicated there was a decreased pattern for both the rod and cone responses. At the age of 36 years, his BCVA decreased to 0.2 in both eyes. Optical coherence tomography showed marked retinal thinning of the macular regions in both eyes. Genetic analysis identified compound heterozygous truncating mutations (p.Y2935X and p.S1653KfsX2) in the *EYS* gene. His unaffected parents were heterozygous for each mutation.
*Conclusions* Our results demonstrated that *EYS* mutations can be the cause of not only arRP but also arCRD. Our findings extend the phenotypic spectrum of patients with *EYS* mutations.

**Keywords** *EYS* gene · Whole-exome sequencing · Genetics · Retinitis pigmentosa · Cone–rod dystrophy

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## Introduction

The eyes shut homolog (*EYS*) gene (Online Mendelian Inheritance in Man: *612424), largest gene known to be expressed in the human eye, spanning more than 2 Mb within the *RP25* locus (6q12). The human EYS protein is a homolog of the *Drosophila* eyes shut/spacemaker (eys) protein, which is an extracellular matrix protein essential for photoreceptor development and morphology of the insect eye.

In 2008, mutations in the *EYS* gene were reported in patients with autosomal recessive retinitis pigmentosa (arRP) [1, 2]. The *EYS* mutations, which have been shown to be predominantly truncating mutations, have been described in patients with different ethnic origins and account for 5–16 % of arRP [3–7]. Thus, disruption of the EYS function has been identified as a frequent cause of arRP worldwide. With the exception for arRP, to date there have been no *EYS* mutations reported for any other phenotype.

Here, we report clinical and genetic features of a patient with autosomal recessive cone–rod dystrophy (arCRD) associated with compound heterozygous *EYS* mutations.

## Case report

A 31-year-old male patient (JU#0659) was referred to our hospital with a complaint of loss of visual acuity. He first reported a decreased visual acuity at the age of 29 years. Family history indicated that his parents had no previous reports of any ocular symptoms (Fig. 1). At his initial examination, decimal best-corrected visual acuity (BCVA) was 0.9 [with −6.00 diopter (dpt), cylinder (cyl) −1.25 dpt axis (Ax) 130°] in his right eye and 0.6 (with −4.50 dpt, cyl −0.75 dpt Ax 180°) in his left eye. Anterior segment examination showed no remarkable findings. Intraocular pressures were 16 mmHg in the right and 15 mmHg in the left eye. Funduscopy showed retinal degenerations within the vascular arcade in both eyes (Fig. 2a). Neither retinal degeneration nor attenuation of retinal vessels

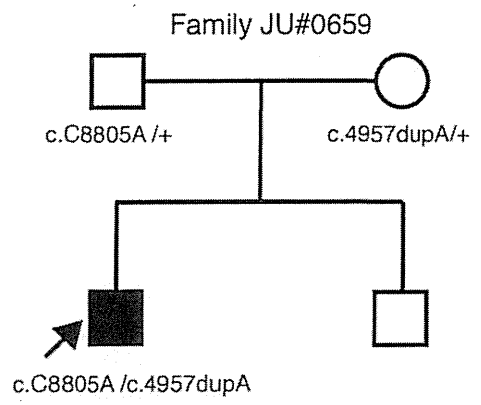


**Fig. 1** Pedigree of a Japanese family. Unaffected family members (males, *open squares*; females, *open circles*) and an affected proband (male, *solid square*) are shown

was observed in the periphery. Fluorescein angiography (VISUCAM NM/FA; Carl Zeiss Meditec AG, Dublin, CA, USA) showed a hyperfluorescence pattern due to a window defect within the vascular arcades of both eyes (Fig. 2b). At the age of 32 years, visual field testing using Goldmann kinetic perimetry (GP; Haag-Streit, Bern, Switzerland) showed bilateral central scotomas of the I-3e and I-4e isopters with relative sparing of the center, but preserved peripheral visual fields of the V-4e and I-4e isopters in both eyes (Fig. 3). Full-field electroretinography (ERG) was performed according to the protocols of the International Society for Clinical Electrophysiology of Vision. The procedure and conditions for ERG recording have been reported previously [8]. The ERG showed the rod, standard combined, cone, and 30-Hz flicker responses were bilaterally reduced to about one-third of those in a control, but the peak implicit time of each response was not delayed (Fig. 4).

At the age of 36 years, his BCVA decreased to 0.2 in both eyes. Funduscopic images using the Optos 200Tx imaging system (Optos PLC, Dunfermline, United Kingdom) showed retinal degenerations within the vascular arcades in both eyes, but relatively preserved mid-peripheral to peripheral retinal findings with no apparent attenuation of the retinal vessels (Fig. 5a). Fundus autofluorescence imaging (FAI) (Optos PLC) showed decreased autofluorescence within the vascular arcades but increased autofluorescence of the foveal area, and increased autofluorescence outside the vascular arcades in both eyes (Fig. 5b). Optical coherence tomography (OCT) (Cirrus HD-OCT; Carl Zeiss Meditec AG) showed retinal thinning with a visible foveal external limiting membrane line (Fig. 5c), and entire macular thinning in both eyes (Fig. 5d).

To identify disease-causing gene mutations, we performed whole-exome sequencing analysis as per a previously described method [9]. The obtained sequence data in the patient were compared with reference human genome sequences. Initially, we focused on only variants that could change the amino acid sequence. Subsequently, we filtered the remaining variants based on the criteria that a frequency of mutation was less than 1 % in the 1000-genome database (http://www.1000genomes.org). Finally, we screened variants residing within 207 retinal disease-associated genes published in the November 15, 2013

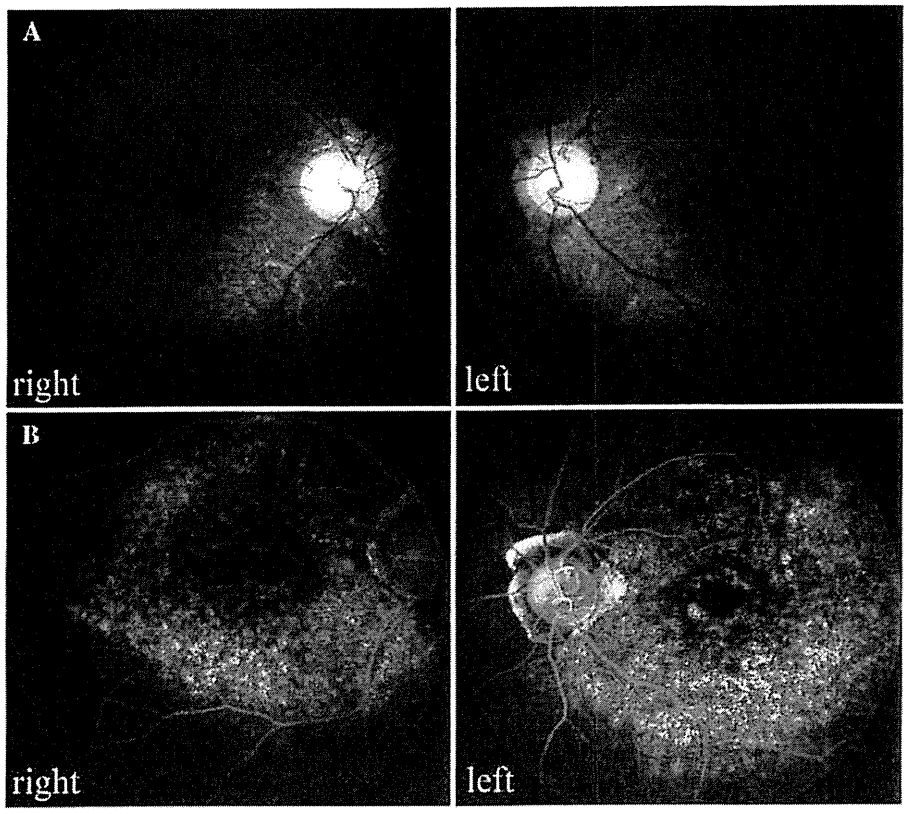


**Fig. 2** Fundus photographs and fluorescein angiography images (FA) of the patient at the age of 31 years. **a** Fundus photographs show retinal degenerations within the vascular arcades in both eyes. **b** FA shows a hyperfluorescence pattern due to a window defect within the vascular arcades of both eyes

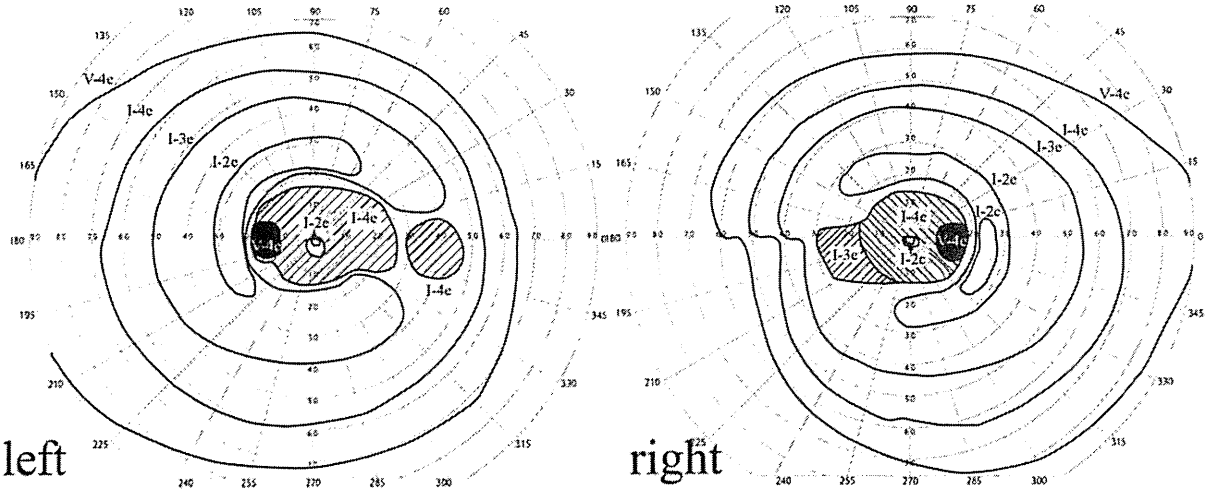


**Fig. 3** Visual field testing using Goldmann kinetic perimetry (GP) at the age of 32 years. GP shows bilateral central scotomas of the I-3e and I-4e isopters with relative sparing of the center, but with preserved peripheral visual fields of the V-4e and I-4e isopters in both eyes

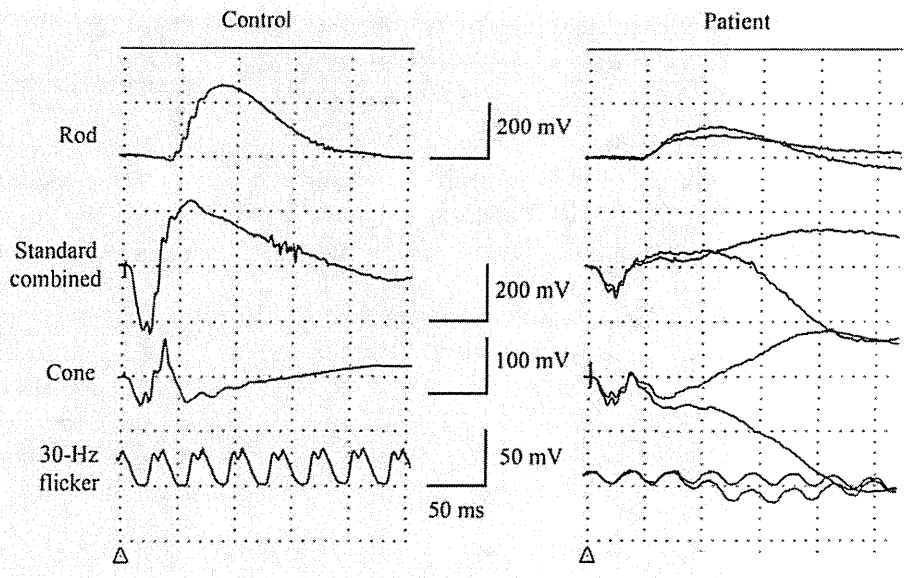


**Fig. 4** Full-field electroretinography (ERG) at the age of 32 years. ERG shows that the rod, standard combined, cone, and 30-Hz flicker responses are bilaterally reduced to about one-third of those in a control, but the peak implicit time of each response is not delayed

RetNet database (https://sph.uth.edu/retnet/). Based on the obtained data, known *EYS* mutations were identified in a compound heterozygous state as disease-causing mutations. In the other 206 genes, there were no mutations found in compound heterozygous or homozygous states. The identified *EYS* gene mutations were c.C8805A and c.4957dupA, which result in the truncating mutations p.Y2935X and p.S1653KfsX2, respectively. The findings were confirmed by Sanger sequencing. The patient's unaffected parents were heterozygous for each mutation. The compound heterozygous mutations (p.Y2935X and p.S1653KfsX2) have been previously reported as a cause of arRP [7]. The accession number of the *EYS* mRNA reference sequence we used was NM_001142800.1 from the National Center for Biotechnology Information.

## Discussion

In this report, whole-exome sequencing analysis identified *EYS* mutations in a Japanese patient with arCRD. *EYS* mutations have previously only been reported in patients with arRP [1, 2]. This is the first report that describes arCRD associated with compound heterozygous mutations in the *EYS* gene.

Previous studies have revealed that the EYS protein is expressed specifically in the human retina and is localized in the outer segment of the photoreceptor layers of the porcine retina [1, 2]. Although the function and structure of human EYS protein remain unclear, it has been suggested to be essential for photoreceptor morphogenesis [2]. In fact, *EYS* mutations give rise to RP phenotypes with thinning of the outer retinal layers [5, 7], which results from the degeneration of both the rod and cone photoreceptors. Clinical features of patients with *EYS* mutations include a typical form of RP that is characterized by a progressive constricted visual field, bone pigmentations and attenuation of the retinal vessels [3–5]. The pattern of the ERG in RP patients shows nonrecordable or markedly decreased responses [3–5]. On the other hand, our patient exhibited retinal degenerations that were predominantly seen within the vascular arcades (Fig. 5a, b), central scotomas and preserved peripheral visual fields (Fig. 3), and decreased responses in the both rod and cone ERG (Fig. 4) in both eyes. Generally, CRD exhibits several features such as decreased central vision, a predominant degeneration of the macular region as compared with the mid-peripheral region, and decreased amplitudes in the cone ERG that are equal to or worse than the decreased rod ERG amplitudes [10, 11]. These characteristics of CRD were clearly consistent with the phenotype of our patient. Taken together, these findings indicated that the patient diagnosis was arCRD and not arRP.

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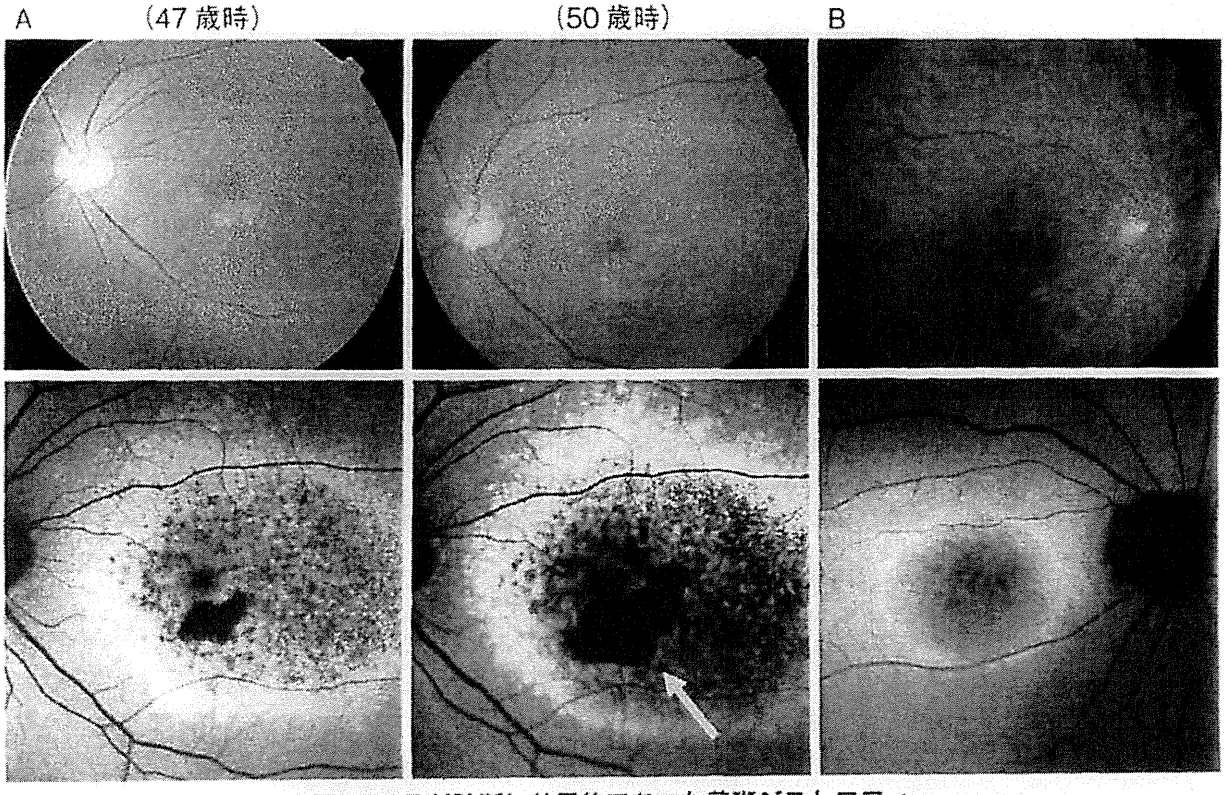


**図 4　AF が診断に効果的であった黄斑ジストロフィ**

A：50 歳女性。47 歳時に中心視野の一部が欠けていることに気が付いた。50 歳時の矯正視力は右 1.2，左 0.9。眼底写真では黄斑部の耳側を中心にびまん性の色素上皮萎縮を認める。AF では，過蛍光，低蛍光の混在した変性領域が黄斑部を取り囲み，変性周囲には輪状の過蛍光領域が認められる。中心窩の下方は萎縮により自発蛍光が消失している。AF を用いると，中心窩に隣接した萎縮部が経過観察中に拡大していることが明瞭に観察される(矢印)。
B：62 歳女性。30 歳頃から両眼の視力が徐々に低下し，他院で視神経障害と診断されていた。矯正視力は右 0.3，左 0.3。眼底写真では明らかな黄斑変性はみられないが，AF では黄斑部に低蛍光領域，およびその周囲に境界明瞭な輪状過蛍光を認め，視力低下の原因が黄斑ジストロフィであることが判明した。

斑ジストロフィを分別できるほど特異的なものではない。

しかしそれでも，黄斑ジストロフィの診断に AF を用いることの意義は大きい。ひとつは疾患の経時的変化を比較することで，AF を用いると検眼鏡的検査では困難な病変の空間的変化を詳細に捉えることができる(**図 4A**)。また眼底がほぼ正常に見える患者においても AF によって黄斑部の病変が明らかになることがあり，黄斑部病変のスクリーニングとしての意義は大きい(**図 4B**)。

## おわりに

「黄斑ジストロフィと自発蛍光」と題して，代表的な 2 つの疾患，スターガルト病とベスト病についてそれぞれの AF 所見を紹介した。黄斑ジストロフィの画像診断には，従来より FA が重要な役割を担ってきたが，網脈絡膜の血管異常が主病変でない場合には，むしろ AF のほうが診断に有用であることも多い。今後，黄斑ジストロフィを含めた網膜疾患に対して，眼科診療における AF 撮影の価値はますます高まっていくものと思われる。

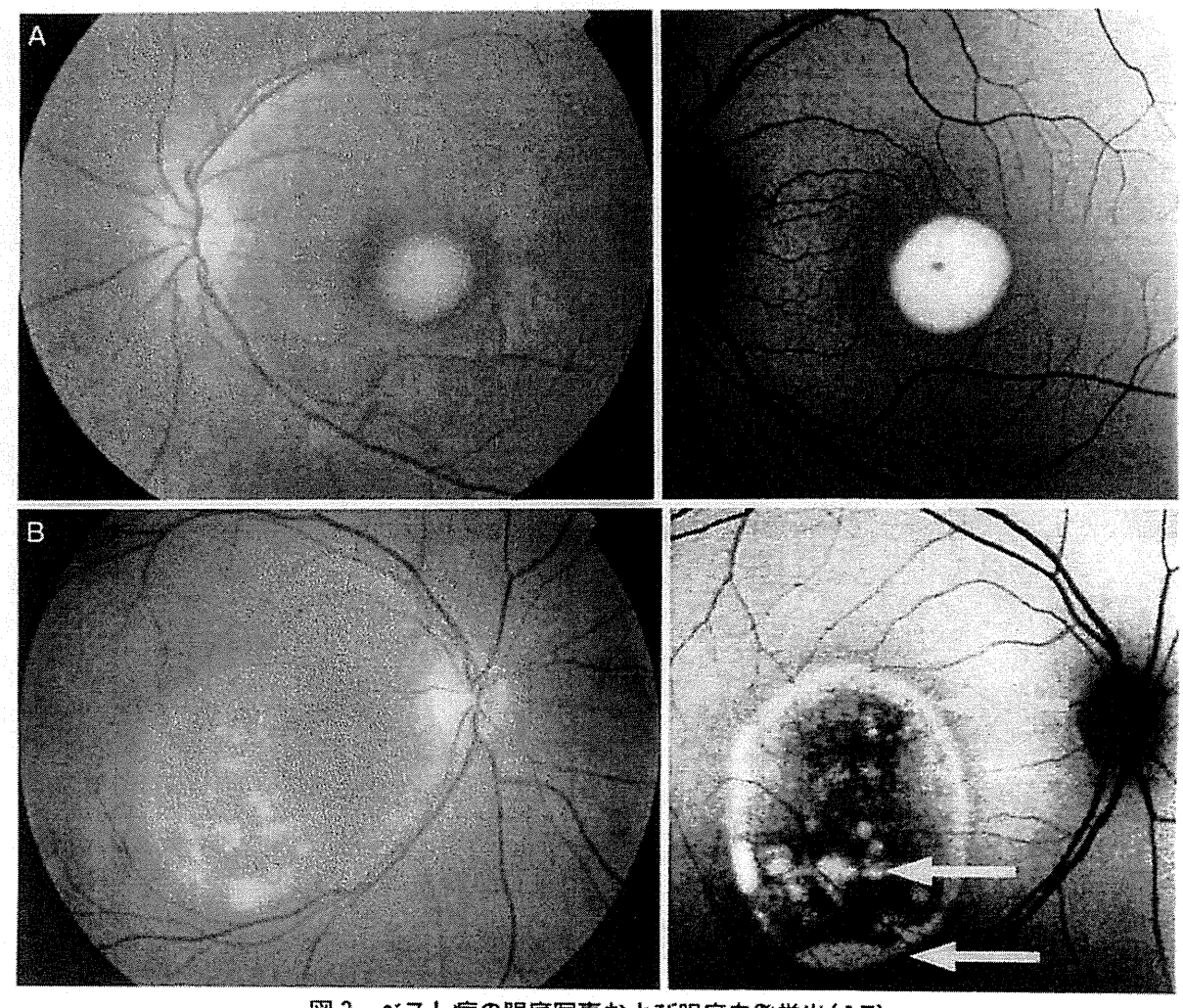


**図3　ベスト病の眼底写真および眼底自発蛍光(AF)**

A：卵黄期の8歳男児。矯正視力，右1.0，左1.0。
眼底写真では黄斑部に卵黄様物質の沈着を認める(左)。AFでは卵黄様物質の部位に一致した過蛍光を認める(右)。
B：炒り卵期の45歳男性。矯正視力，右0.3，左0.15。
眼底写真では黄斑部の楕円形病変部と，その内部に散在する網膜下沈着物を多数認める(左)。AFでは沈着物，および楕円形病変部の辺縁に過蛍光を認める(右，矢印)。

常を呈し，EOGは重度の異常を呈する。

なお，成人期に発症するタイプもしばしばみられ，成人発症卵黄様黄斑変性症(adult vitelliform macular dystrophy)と呼ばれている。眼底所見はベスト病に類似しているがEOGが正常であることが多く，機能的にはベスト病とは異なる病態生理をもつ疾患であると認識されている[B17]。

AFについては，黄色部病変(リポフスチン様物質)の存在に一致して過蛍光を呈する(**図3**)　黄色部が不鮮明であっても，AFの過蛍光は病変部の辺縁に明瞭に観察することができる(**図3B**)。さらに病期が進行して網膜色素上皮が萎縮した場合，その部位は低蛍光となる。

## III. その他の黄斑ジストロフィ

黄斑ジストロフィの原因は「はじめに」で述べたように多様であり，特徴的な一部の疾患を除いて，検眼鏡的所見やAF所見のみで遺伝学的な原因を類推することは困難である。すなわちAFでみられる異常所見は，一般に各種の黄

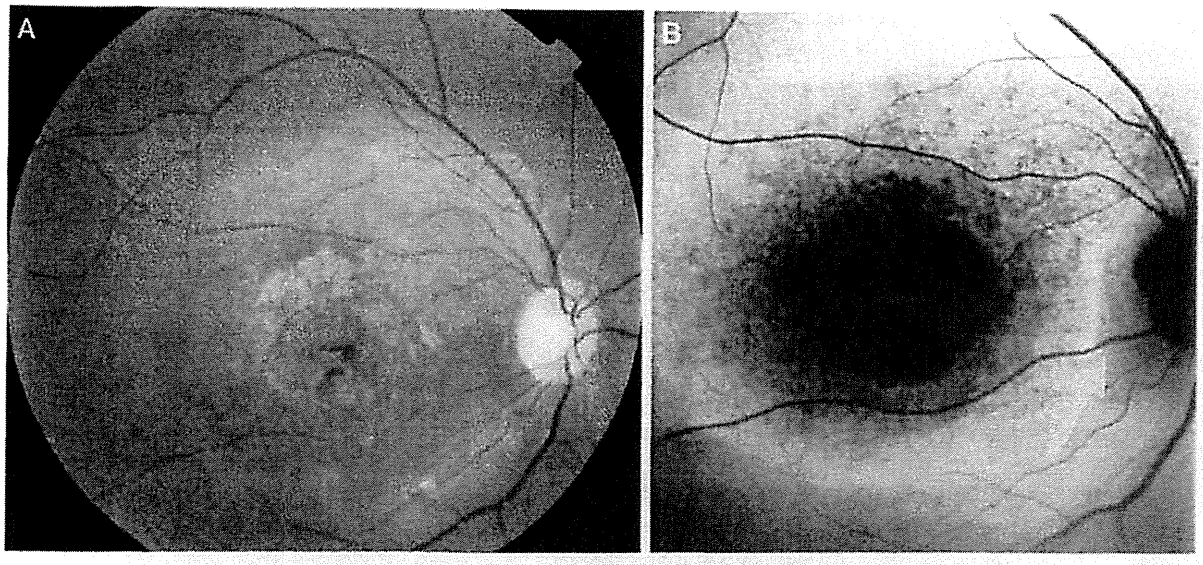


**図2 スターガルト病(11歳，女児)の眼底写真および眼底自発蛍光(AF)**
A：眼底写真。後極部に広範囲の変性を認める。特に黄斑部では萎縮が進行している。
B：AF。後極部変性部位の外周に輪状の過蛍光を認める。黄斑部では萎縮により自発蛍光が消失している。また，視神経乳頭周囲には輪状の正常領域(peripapillary sparing)を認める(矢印)。
この患者は7歳時に視力低下を自覚している。11歳時の矯正視力は右0.08，左0.15であった。遺伝子検査では，*ABCA4*遺伝子に複合ヘテロ変異を認めた。

蛍光を認める[9,10](**図1**)。また，進行に伴うfleckの増加，ならびに黄斑部変性領域の拡大は，検眼鏡的検査やOCTよりも，AFを用いることでより明瞭に観察できる。さらに，スターガルト病に特徴的とされるperipapillary sparingの所見も，AFを用いると容易に観察が可能となる[11]。Peripapillary sparingとは視神経乳頭周囲部分の感覚網膜，網膜色素上皮の構造，機能が局所的に温存される所見を示し，後極部網膜全域に病変が広がっている症例においても，乳頭周囲のAF所見が正常に見える(**図2**，矢印)。

## II. ベスト病(Best disease)

卵黄状黄斑ジストロフィ(vitelliform macular dystrophy)とも呼ばれる，常染色体優性遺伝の黄斑ジストロフィである。眼底所見が特徴的で，黄斑部に「卵黄様」と呼ばれる黄色円形病変を認める[12]。学童期に視力低下を主訴に発症することが多いが，眼底所見の割に視力低下が軽度の症例もある。全視野ERGは正常であるが，眼球電図(EOG)では基礎電位の低下，Arden比の低下が顕著にみられる[13]。原因遺伝子として*BEST1*が同定されている[14]。*BEST1*は主に網膜色素上皮の基底膜に存在する蛋白質，ベストロフィン(bestrophin)をコードしており[15]，網膜色素上皮の機能障害によりEOGの顕著な異常が認められると考えられている。

黄斑部にみられる黄色物質は網膜色素上皮のリポフスチン様物質と考えられており[16]，年齢とともに眼底像が変化する。眼底にほとんど異常を認めない前卵黄期，眼底に卵黄様物質が沈着する卵黄期(**図3A**)，卵黄が崩れて下方に貯留する偽膿瘍期，黄色斑がまだらになる炒り卵期(**図3B**)，黄斑部に萎縮性変化をきたす萎縮期の5期を経るとされている[12]。

また最近では，同じ*BEST1*遺伝子異常による常染色体劣性遺伝のベストロフィノパチー(autosomal recessive bestrophinopathy：ARB)という疾患概念が報告されている[17]。ARBでは全視野ERGは杆体系，錐体系ともに軽度異

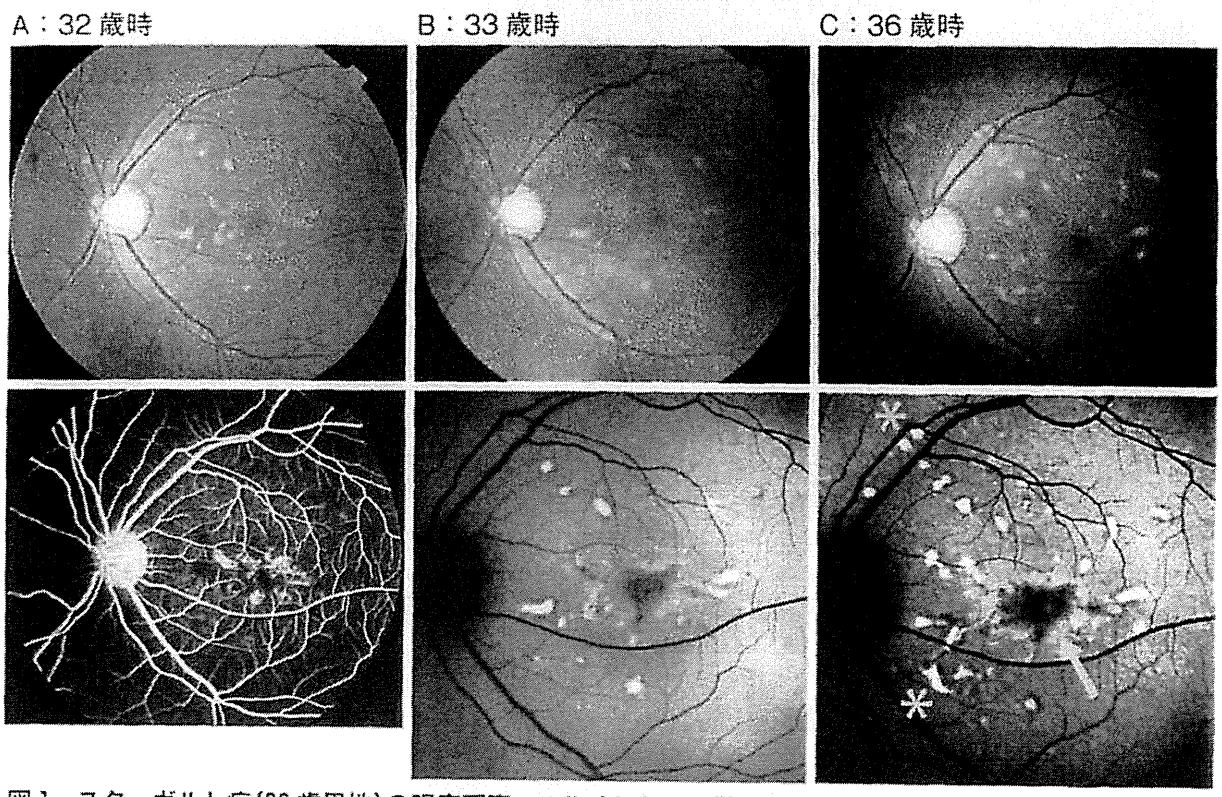


**図1　スターガルト病(36 歳男性)の眼底写真，フルオレセイン蛍光眼底造影(FA)および眼底自発蛍光(AF)**
A：32 歳時の眼底写真(上)および FA。黄斑部の萎縮と，その周囲に散在する黄色斑(fleck)を認める。FA では dark choroid と黄斑萎縮に一致した window defect による過蛍光を認める。
B, C：同患者の 33 歳時および 36 歳時の眼底写真(上)および AF(下)。AF では，黄色斑に一致した過蛍光および黄斑萎縮に一致した低蛍光を認める。背景の輝度は全体的に高い。33 歳時と 36 歳時で AF 所見を比較すると，黄斑萎縮部の拡大(矢印)および黄色斑の新たな出現(＊)が明瞭に観察される。
この患者は 30 歳時に初めて視力低下を自覚している。36 歳時の矯正視力は右 0.5，左 0.4 であった。遺伝子検査では，*ABCA4* 遺伝子に 2 つの異なる変異を認めた。

## I. スターガルト病(Stargardt disease)

比較的頻度の高い常染色体劣性遺伝の黄斑ジストロフィである。若年者に発症する黄斑部の感覚網膜，網膜色素上皮(retinal pigment epithelium：RPE)の萎縮病変，その周囲に散在する多発性黄色斑(fleck)を特徴とする疾患である[2,3]。かつては黄斑萎縮が軽度で fleck を顕著に認める黄色斑眼底(fundus flavimaculatus)とは別疾患と考えられていたが，後に両者とも原因遺伝子が *ABCA4* で同一の遺伝子異常であることが確認され[4,5]，現在では同一疾患と考えられている。10 歳代までに自覚する両眼の視力低下，中心暗点などを主訴に来院することが多い。発症年齢が 20 歳以上であれば視力予後が比較的良いとされる[6]。FA ではリポフスチンの蓄積により背景蛍光がブロックされる低蛍光所見(dark choroid)が約半数の症例にみられるほか[7]，黄斑萎縮に一致した window defect による過蛍光，fleck 部分での過蛍光が特徴的所見である。全視野 ERG 所見は，錐体系，杆体系ともに正常のものから，錐体系にのみ異常のみられるもの，錐体系，杆体系ともに傷害されるものなど，重症度によってさまざまである[8]。

AF 所見については，典型例では背景過蛍光，黄斑萎縮部位の低蛍光，fleck 部に一致した異常

特　集
眼底自発蛍光と網膜臨床

# 3. 黄斑ジストロフィと自発蛍光

## — Fundus autofluorescence imaging in macular dystrophy —

角田和繁* 藤波　芳*

## はじめに

黄斑ジストロフィは両眼性，進行性の機能障害を網膜黄斑部にきたす疾患の総称であり，多くのものが遺伝性と考えられている。さまざまな臨床所見を呈するため，臨床診断にあたっては検眼鏡的所見，フルオレセイン蛍光眼底造影(fluorescein angiography：FA)，光干渉断層計(optical coherence tomography：OCT)，網膜電図(ERG)，眼電位図(EOG)などの電気生理学的検査を含む眼科的検査を包括的に行う必要がある[1]。眼底自発蛍光(fundus autofluorescence：AF)は主に網膜色素上皮細胞内のリポフスチンに由来し，その輝度の変化により網膜色素上皮のさまざまな病態が画像に反映される。AFはFAとは異なり非侵襲的であるため，近年は黄斑ジストロフィの診断においてもその利用価値が高く認められている。

近年黄斑ジストロフィを呈する疾患については，特徴的な眼底所見による分類よりも分子遺伝学的な病因分類が重要視されてきている。黄斑ジストロフィの関連遺伝子としては，*ABCA4*(スターガルト病)，*BEST1*(ベスト病)，*RS1*(X染色体性若年網膜分離症)，*RP1L1*(オカルト黄斑ジストロフィ：三宅病)，*PRPH2*，*PROM1*，*CRX*，*GUCY2D*，*GUCA1A*，*RPGR*，*KCNV2*，*RDH5*等，多くの遺伝子が報告されている。

AFは網膜の異常を検出するための感度は非常に高いものの，上記の疾患群を分類するための特異度は決して高くない。このためAFを黄斑ジストロフィの診断に用いる目的は，一般に黄斑部に異常があるか否かの判定(スクリーニング)，病巣の空間的広がりの把握，進行程度の評価などが中心となる。

黄斑ジストロフィにおけるAF読影の基本は，他の疾患と大きな違いはない。すなわち，初期の病変においては過蛍光となり，網膜色素上皮機能の低下とともに自発蛍光が低下し，完全な萎縮に至ると蛍光は消失する。特に進行期においては正常領域と病的領域の境界が過蛍光となり，黄斑部を取り囲む輪状の高輝度病変を呈することが多い。

本稿では，黄斑ジストロフィのなかから，特にスターガルト病，ベスト病を取り上げ，AF画像を提示する。またそれ以外に，AFが診断に有効である黄斑ジストロフィ症例について紹介する。

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