2. 学会誌・雑誌等における論文掲載

掲載した論文(発表題目)	発表者氏名	発表した場所 (学会誌・雑誌等名)	発表した時期	国内・外の別
Longitudinal clinical course of three Japanese patients with Leber congenital amaurosis/early onset retinal dystrophy with RDH12 mutation	Kazuki Kuniyoshi, Hiroyuki Sakuramoto, Kazutoshi Yoshitake, Kosuke Abe, Kazuho Ikeo, Masaaki Furuno, Kazushige Tsunoda, Shunji Kusaka, Yoshikazu Shimomura, Takeshi Iwata	Documenta Ophthalmologica	June 2014	国外
Clinical and Molecular Characteristics of Childhood-Onset Stargardt Disease	Fujinami K, Zernant J, Chana RK, Wright GA, Tsunoda K, Ozawa Y, Tsubota K, Robson AG, Holder GE, Allikmets R, Michaelides M, Moore AT.	Ophthalmology	2014 Oct	国外
Novel nonsense and splice site mutations in CRB1 gene in two Japanese patients with early-onset retinal dystrophy	Kazuki Kuniyoshi, Kazuho Ikeo, Hiroyuki Sakuramoto, Masaaki Furuno, Kazutoshi Yoshitake, Yoshikazu Hatsukawa, Akira Nakao, Kazushige Tsunoda, Shunji Kusaka, Yoshikazu Shimomura, Takeshi Iwata	Documenta Ophthalmologica	2014 Oct	国外
Whole exome analysis identifies frequent CNGA1 mutations in Japanese population with autosomal recessive retinitis pigmentosa	Katagiri S, Akahori M, Sergeev Y, Yoshitake K, Ikeo K, Furuno M, Hayashi T, Kondo M, Ueno S, Tsunoda K, Shinoda K, Kuniyoshi K, Tsurusaki Y, Matsumoto N, Tsuneoka H, Iwata T.	PLoS One.	2014 Sep	国外
Clinical course of focal choroidal excavation in Vogt-Koyanagi-Harada disease.	Nishikawa Y, Fujinami K, Watanabe K, Noda T, Tsunoda K, Akiyama K.	Clin Ophthalmol	2014 Dec	国外
Occult macular dystrophy	Miyake Y and Tsunoda K	Japanese Journal of Ophthalr	2015, in press	国外
Congenital achromatopsia and macular atrophy caused by a novel recessive PDE6C mutation (p.E591K)	Satoshi Katagiri, Takaaki Hayashi, Kazutoshi Yoshitake, Yuri Sergeev, Masakazu Akahori, Masaaki Furuno, Jo Nishino, Kazuho Ikeo, Kazushige Tsunoda, Hiroshi Tsuneoka, and Takeshi Iwata	Ophthalmic Genetics,	2015	国外

Fundus auofluorescence imaging in patient with juvenile form of galactosialidosis	Risa Yamazaki, Kazushige Tsunoda, Kaoru Fujinami, Toru Noda, Kazuo Tsubota	Ophthalmic Surgery Lasers & Imaging Retina	May/June 2014	国外
'Association of retinal artery and other inner retinal structures with distribution of tapetal-like reflex in Oguchi's disease'	Yu Kato, Kazushige Tsunoda, Kaoru Fujinami, Takeshi Iwata, Masamichi Saga, Yoshihisa Oguchi	2015, in press	2015, in press	国外
(総説)				
「黄斑ジストロフィ(三宅病を含めて)」	角田和繁、藤波芳	眼科	2014	国内
「黄斑ジストロフィとERG」	角田和繁	オクリスタ	2014	国内
「オカルト黄斑ジストロフィ(三宅病)のOCT所見」	角田和繁	「オカルト黄斑ジストロフィ(三宅病)のOCT所見」	2014	国内
「黄斑部局所ERG」	藤波芳、中村奈津子、角田和繁	眼科	2014.4	国内
「Functional OCT」	鈴木航、角田和繁、谷藤学	臨床眼科	2014.10.30	国内
「卵黄様黄斑ジストロフィ」	角田和繁	眼科 2015年臨時増刊号	2015年	国内
(書籍)				
「Chapter 10. Fundus Autofluorescence in occult macular dystrophy」		Lippincott		国外
「網膜電図(ERG)」	角田和繁	眼科臨床クローズアップ	2014年4月10日	国内
「網膜遺伝性疾患」	角田和繁	眼科グラフィック		国内
「オカルト黄斑ジストロフィ	角田和繁	新版 どうとる？どう読む？ERG」		国内

(注１) 発表者氏名は、連名による発表の場合には、筆頭者を先頭にして全員を記載すること。

(注２) 本様式はexcel形式にて作成し、甲が求める場合は別途電子データを納入すること。

Ⅳ. 研究成果の刊行物・別刷

Doc Ophthalmol
DOI 10.1007/s10633-014-9436-z

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.

A part of this paper was presented at the 8th congress of Asia Pacific Vitreo-retina Society in Nagoya, Japan, on December 7, 2013.

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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 paraarteriolar preservation of the retinal pigment epithelium, *BBS* Bardet-Biedl syndrome, *JBTS* Joubert syndrome, *SLSN* Senior-Loken syndrome, *MKS* Meckel syndrome, *SVD* snowflake vitreoretinal degeneration

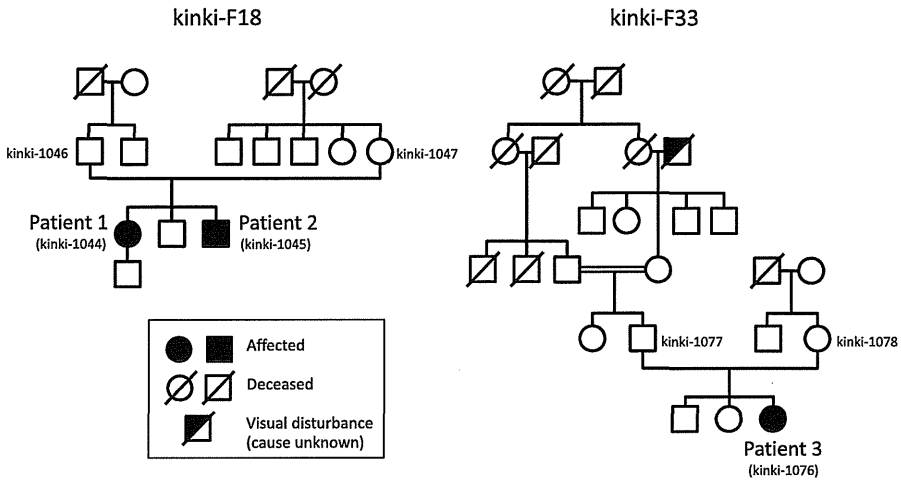


Fig. 1 Pedigrees of two unrelated families with Leber congenital amaurosis/early-onset retinal dystrophy (LCD/EORD) with *RDH12* mutation. Patients 1 and 2 were siblings (*left*, kinki-F18), and Patient 3 is from an unrelated family (*right*, kinki-F33). No consanguinity was reported between parents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

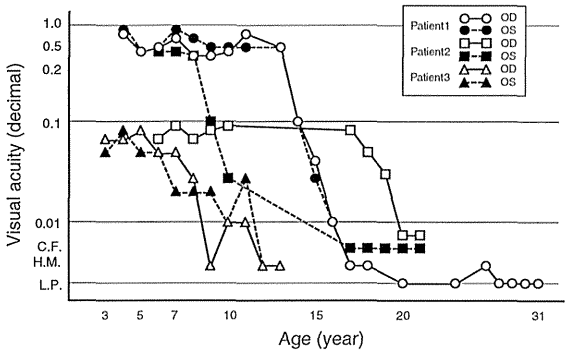


Fig. 2 Clinical course of visual acuity in each patient. *OD* oculus dexter, *OS* oculus sinister

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).

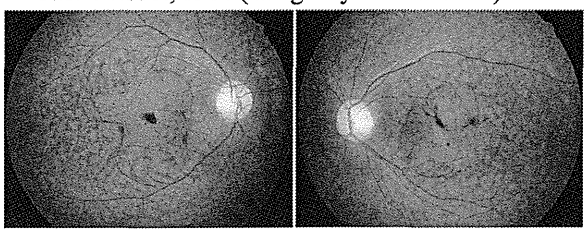
Fig. 3 Fundus photographs (*left*), optical coherence tomographic (OCT) and ultrasonographic images (*lower left*), Goldmann kinetic visual fields (*right*), and full-field ERGs (*bottom right*) of Patient 1. Fundus photographs and OCT images taken at age 27 and 30 years are blurred because of cataracts. 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.

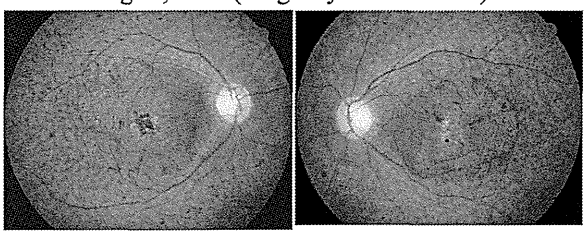
When the sequences of her whole exome were compared with the reference human genome (hs37d5), 940,138 mutations were found. We focused only on mutations that could change the amino-acid sequence and excluded common mutations by 1,000 genomes, HGVD [21], and our in-house database (see methods). As a result, 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**

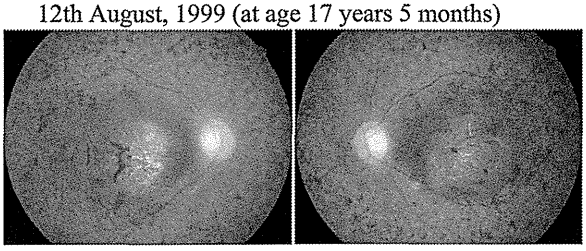
31st October, 1986 (at age 4 years 8 months)



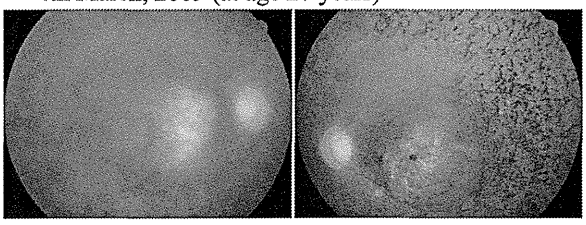
16th August, 1991 (at age 9 years 5 months)



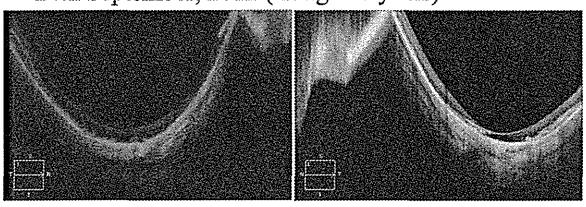
12th August, 1999 (at age 17 years 5 months)



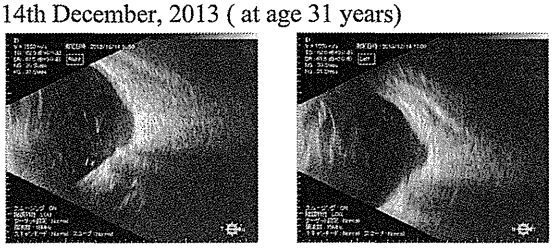
4th March, 2009 (at age 27 years)



24th September, 2012 (at age 30 years)

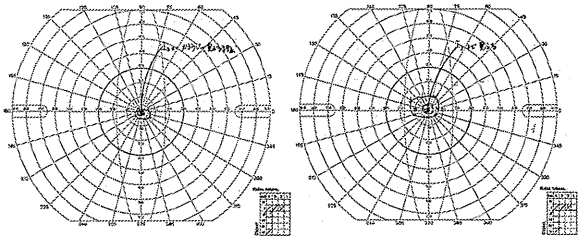


14th December, 2013 (at age 31 years)

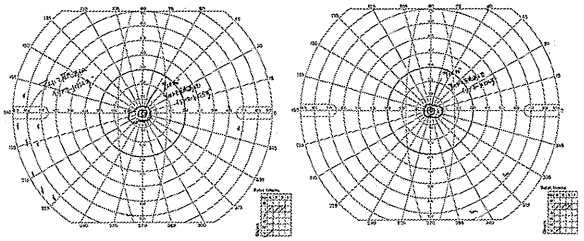


OS **OD**

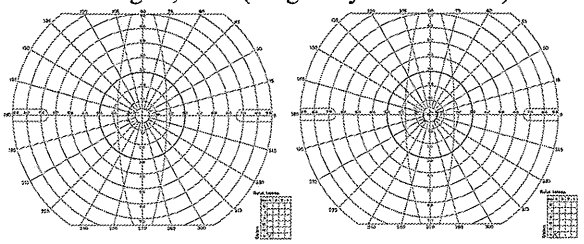
31st October, 1986 (at age 4 years 8 months)



7th January, 1992 (at age 9 years 10 months)



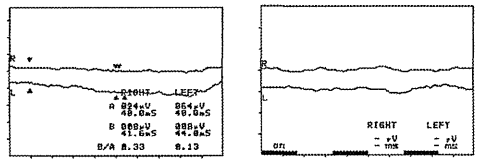
12th August, 1999 (at age 17 years 5 months)



16th August, 1991 (at age 9 years 5 months)

Flash ERG Flicker ERG

OD
OS



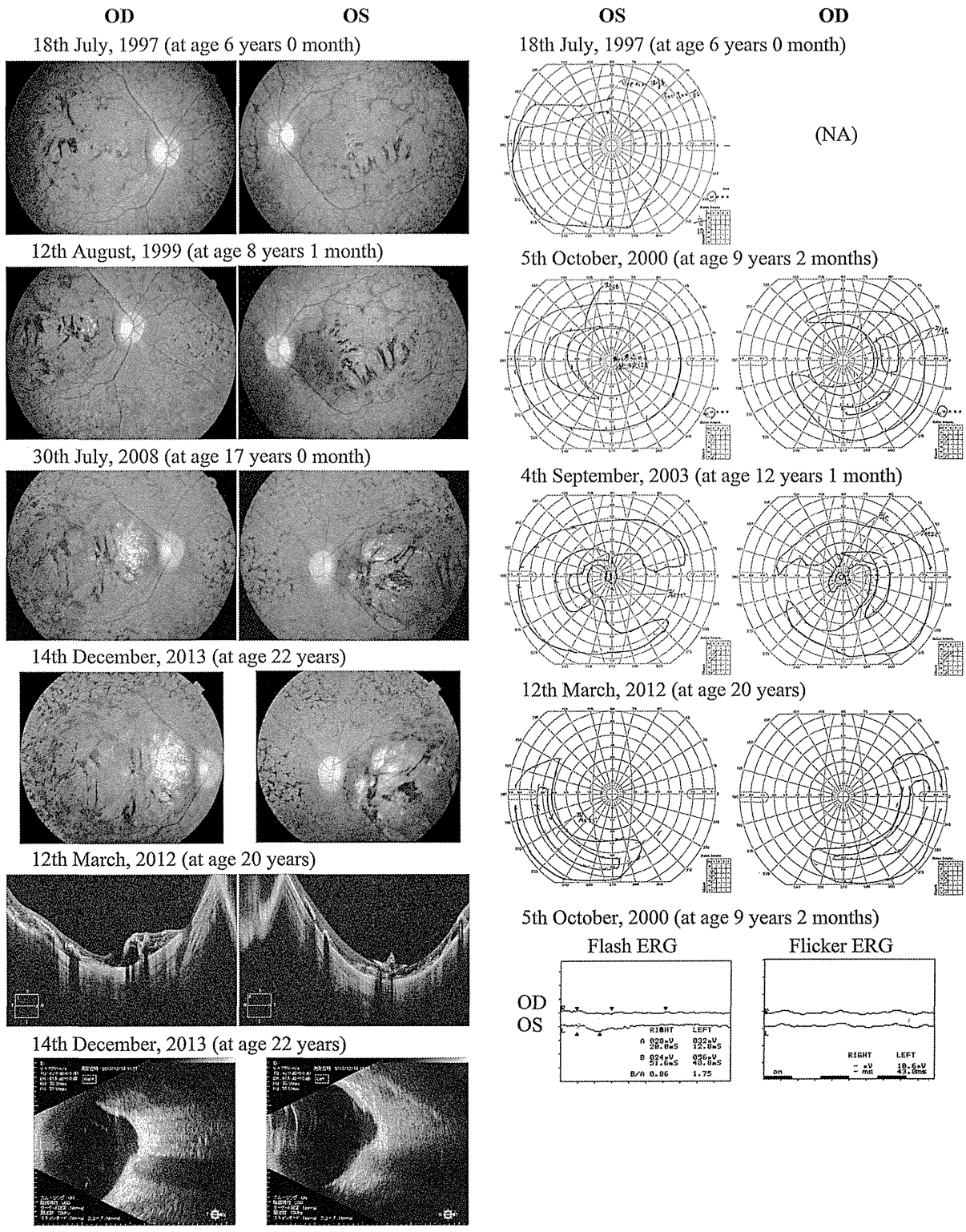


Fig. 4 Fundus photographs (*left*), OCT and ultrasonographic images (*lower left*), Goldmann kinetic visual fields (*right*), and full-field ERGs (*bottom right*) of Patient 2. Patient 2 was younger brother of Patient 1 (Figs. 1, 3). *NA* not available

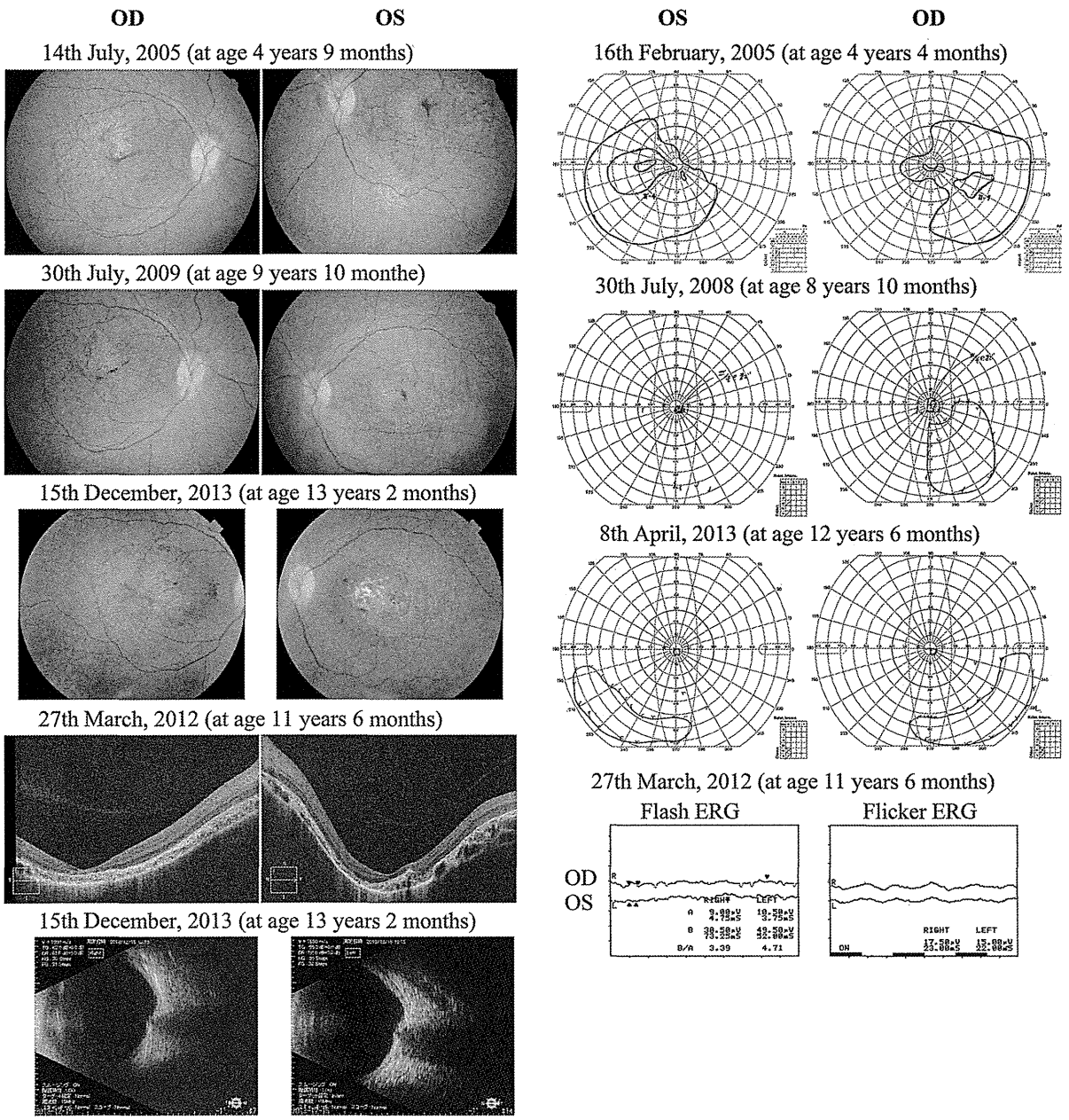


Fig. 5 Fundus photographs (*left*), OCT and ultrasonographic images (*lower left*), Goldmann kinetic visual fields (*right*), and full-field ERGs (*bottom right*) of Patient 3. Patient 3 was from unrelated family to that of Patients 1 and 2 (Fig. 1)

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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Chapter 10L. Fundus Autofluorescence in occult macular dystrophy

Kazushige Tsunoda

Occult macular dystrophy (OMD) was first described by Miyake et al. to be a dominantly inherited macular dystrophy without visible fundus abnormalities.[1] Main symptoms are slowly progressive deterioration of visual acuity and central visual field in both eyes. Some patients complain of photophobia as in other macular dystrophies. The age at the onset is very variable from the first to seventh decade of life, but the majority of the patients notice symptoms in the second to fourth decade.[2] The visual acuity does not become worse than 0.1 (6/60 Snellen equivalent) and the peripheral vision remains well preserved even in the final stage.

Because patients have normal appearing fundus, normal fundus fluorescein angiography (FFA), normal indocyanine-green angiography (ICGA) and normal full-field electroretinogram (ERG) during the entire course of the disease, they are often misdiagnosed as optic neuropathy, amblyopia and non-organic visual loss. The key for the diagnosis is to prove selective dysfunction at the macula by focal-macular or multifocal ERG.[1,3] Recently, spectral-domain OCT and fundus autofluorescence (AF) has become a strong tool for the detection of early photoreceptor changes in OMD.[2]

Although, the OMD was originally reported as dominantly inherited macular dystrophy, there have been number of sporadic cases which were electrophysiologically diagnosed as OMD. Recently, mutations in the *RP1L1* gene were found responsible in dominantly inherited cases [4], and the cases with mutations have been reported to share the same clinical features, especially on

OCT images.[2] Because the etiology of OMD has not been completely clarified, the AF findings presented in this chapter will refer only to OMD related to mutations in the *RP1L1* gene.

MOLECULAR BASIS AND PATHOLOGY

In 2011, linkage analysis in two dominant families revealed that mutations in the *RP1L1* gene located in the short arm of chromosome 8 were responsible for OMD.[4] A number of cases of OMD with the *RP1L* gene mutations have been reported[4-8]; all of them heterozygous missense mutations, the most common one is p.Arg45Trp in exon 2.

The *RP1L1* gene was originally cloned as a gene derived from common ancestors as a retinitis pigmentosa 1 (*RP1)* gene, which is responsible for 5-10% of autosomal dominant retinitis pigmentosa (RP) worldwide, on the same chromosome 8 (see also Chapter 10A).[9-13] An immunohistochemical study on cynomolgus monkeys showed that *RP1L1* was expressed in rod and cone photoreceptors, and *RP1L1* is thought to play important roles in the morphogenesis of the photoreceptors.[9,14] Heterozygous *RP1L1* knock-out mice were reported to be normal while homozygous knock-out mice develop subtle retinal degeneration.[14] However, the RP1L1 protein has a very low degree of overall sequence identity (39%) between humans and mice compared to the average values of sequence similarity observed between humans and mice proteins. The cellular mechanisms to explain why only the macular region is impaired in the OMD in human remains unsolved.

DIAGNOSTIC TECHNIQUES

The family history of a dominant disease is sometimes overlooked because the visual acuity varies from 0.1 (6/60 Snellen equivalent) to normal; there are patients with good visual acuity

who show reduced foveal sensitivity in the automated visual field analyzer and reduced macular responses in the multifocal ERG, focal macular ERG and pattern ERG.[1-3]

Fundus fluorescein angiography and indocyanine green angiography

The fundus appearance, FFA and ICGA remain normal even in cases with long duration such as 30 to 50 years **(Fig. 1)**.[1-3] In some cases with long duration, round-shaped very weak staining at the fovea may be present in FFA.

Electrophysiology

Electrophysiological tests are the key for the diagnosis of OMD. Both rod- and cone-induced responses are normal in the full-field ERG, however, focal macular ERG , multifocal ERGs or pattern ERG can detect very early macular dysfunction in the OMD **(Fig. 2)**. The amplitudes of cone-induced response in the full-field ERG may be borderline or slightly reduced in some cases with *RP1L1* mutation,[6] where the region of dysfunctional retina expands over the macula toward the periphery. These cases may be better referred to as central cone dystrophy, however, the fundus appearance is quite normal.

Optical Coherence Tomography

The OCT, together with focal macular or multifocal ERGs, is a strong tool to diagnose OMD.[2] The most prominent features on OCT are the abnormalities of the two lines at the macula, corresponding to the ellipsoid of photoreceptor inner segment (ISe) and cone outer segment tip (COST) **(Fig 2)**. The ISe line at the fovea look thickened and blurred in the early stage, and disrupted or disappeared in the later stage. The COST line cannot be clearly observed in the macular area even in the early stage. In the peri-macular regions which have normal visual

function, all of the outer retinal structures remain normal. In cases with longer duration like over 30 years, both the photoreceptor and outer nuclear layer become thinned at the macula, however, the layer for retinal pigment epithelium remains unchanged.

Fundus Autofluorescence

In patients with OMD, AF images are generally normal in the entire posterior pole region **(Fig 1)**. However, some cases (~50%) may demonstrate a round-shaped area with increased AF signal at the fovea.[15] The round-shaped area of increased AF is very faint in some cases **(Fig 2)** and apparent in others **(Fig 3).** This implies that the primary lesion of OMD is the photoreceptor rather than the RPE. The relationship between duration of the disease and intensity of the increased AF signal has not been confirmed.

Infrared Reflectance

In fundus infrared reflectance images (λ = 830 nm), round-shaped hyporeflectance centered on the fovea was commonly seen in patients with *RP1L1* mutation. [7]

Differential Diagnosis

Due to the normal fundus appearances and normal full-field ERGs, OMD is often misdiagnosed as optic neuropathy of unknown origin, amblyopia and non-organic visual loss. There are also cases with senile cataract, which can be later diagnosed as OMD due to unexpectedly low visual acuity following cataract surgery.

SUMMARY

The AF images of the OMD with *RP1L1* mutations are unspecific as compared with other macular dystrophies. AF can be normal or abnormal on OMD. If normal, AF will help the clinician to differentiate OMD from other macular dystrophies, which most often have distinctive patterns of AF. If abnormal, AF will point towards a macular/retinal disease that requires further evaluation with other diagnostic tools, such as focal-macular / multifocal ERG, OCT and genetic analysis.

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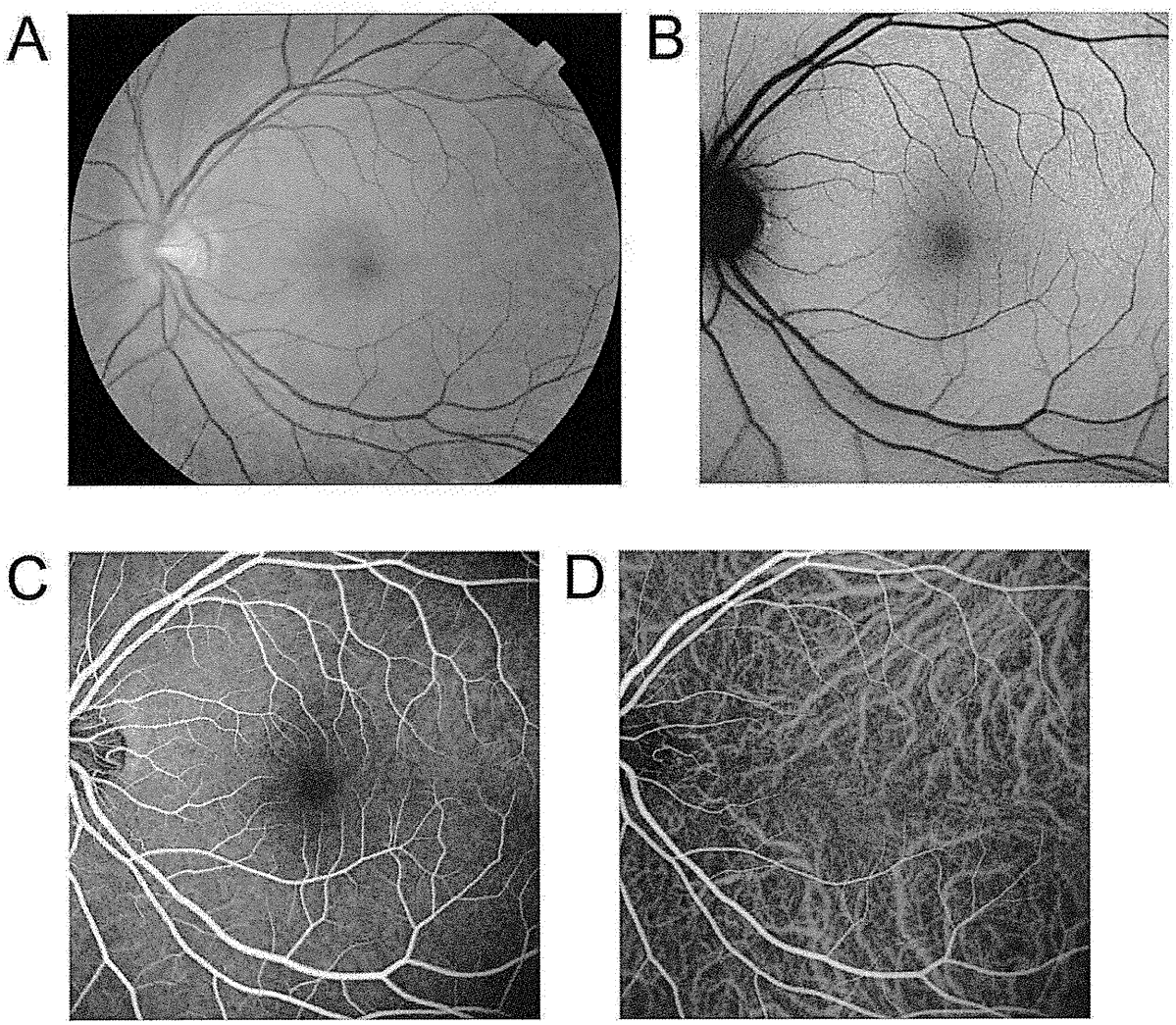

FIGURE 1. Color fundus photograph **(A)**, fundus autofluorescence (AF) image **(B)**, fluorescein angiography **(C)** and indocyanine-green angiography (IA) **(D)** from a patient with OMD (32 year-old female. Best-corrected visual acuity; 0.3(OD), 0.4(OS). *RP1L1* p.Arg45Trp Heterozygous).

There are no abnormalities in all the images.

All the AF images in this chapter were recorded with 488 nm wavelength using a barrier filter for the detection of emitted light above 500 nm (HRA2 ; Heidelberg Engineering).