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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, et al.

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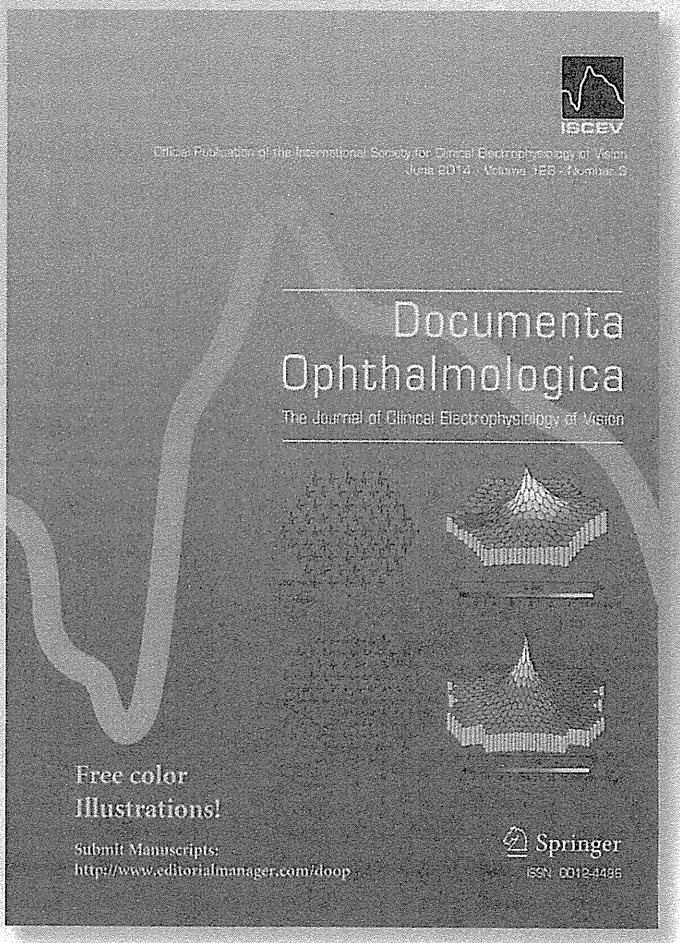

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CLINICAL CASE REPORT

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.

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

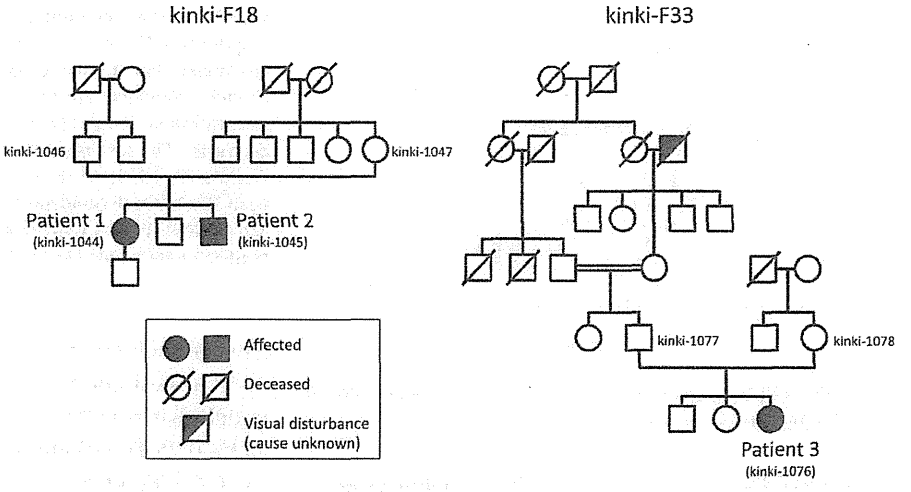


Fig. 1 Pedigrees of two unrelated families with Leber congenital amaurosis/early-onset retinal dystrophy (LCD/EORD) with *RDH12* mutation. Patients 1 and 2 were siblings (*left*, kinki-F18), and Patient 3 is from an unrelated family (*right*, kinki-F33). No consanguinity was reported between parents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

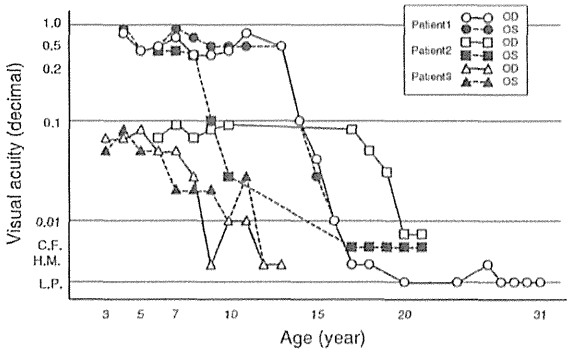


Fig. 2 Clinical course of visual acuity in each patient. *OD* oculus dexter, *OS* oculus sinister

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).

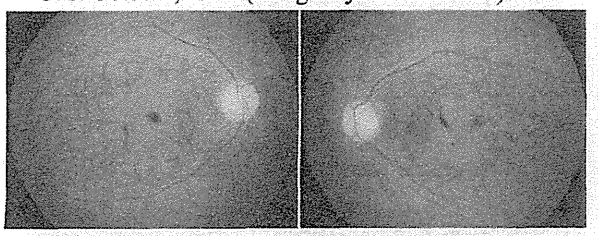
Fig. 3 Fundus photographs (*left*), optical coherence tomographic (OCT) and ultrasonographic images (*lower left*), Goldmann kinetic visual fields (*right*), and full-field ERGs (*bottom right*) of Patient 1. Fundus photographs and OCT images taken at age 27 and 30 years are blurred because of cataracts.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

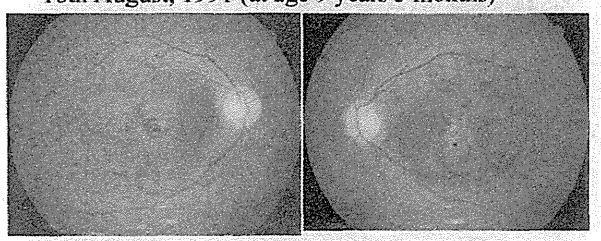
When the sequences of her whole exome were compared with the reference human genome (hs37d5), 940,138 mutations were found. We focused only on mutations that could change the amino-acid sequence and excluded common mutations by 1,000 genomes, HGVD [21], and our in-house database (see methods). As a result,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

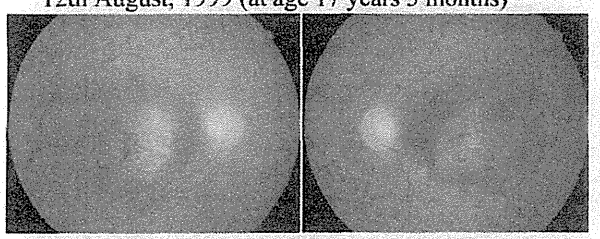
31st October, 1986 (at age 4 years 8 months)



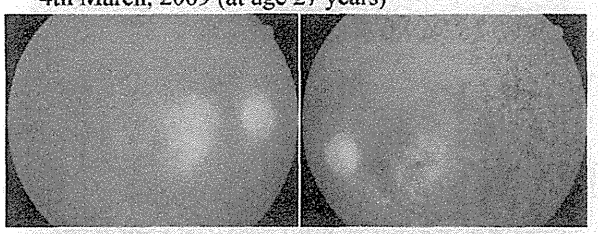
16th August, 1991 (at age 9 years 5 months)



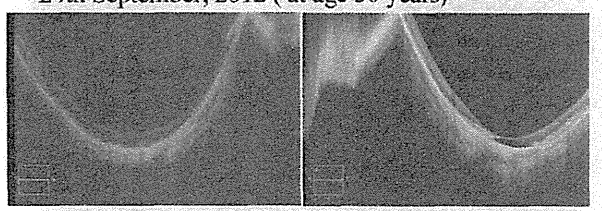
12th August, 1999 (at age 17 years 5 months)



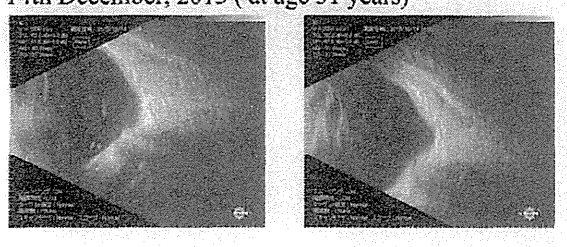
4th March, 2009 (at age 27 years)



24th September, 2012 (at age 30 years)

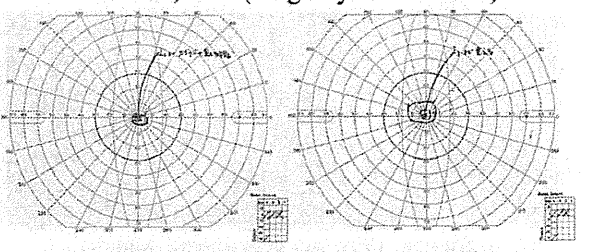


14th December, 2013 (at age 31 years)

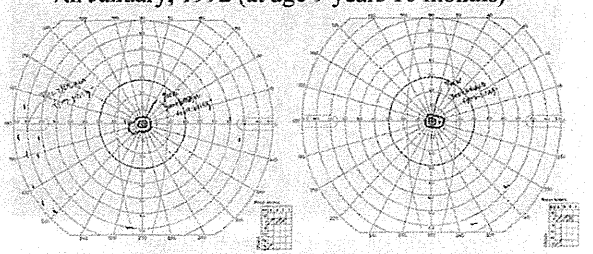


OS OD

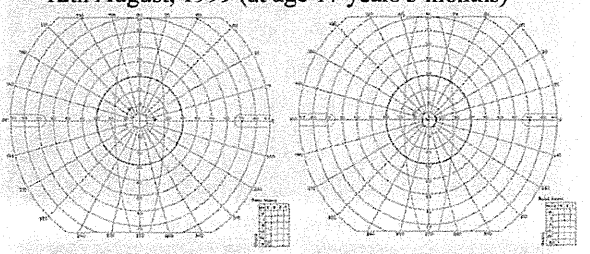
31st October, 1986 (at age 4 years 8 months)



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



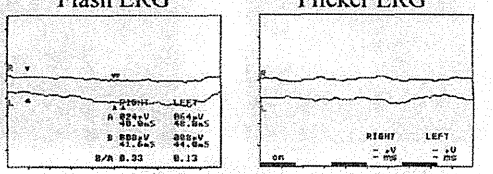
12th August, 1999 (at age 17 years 5 months)



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

Flash ERG Flicker ERG

OD
OS



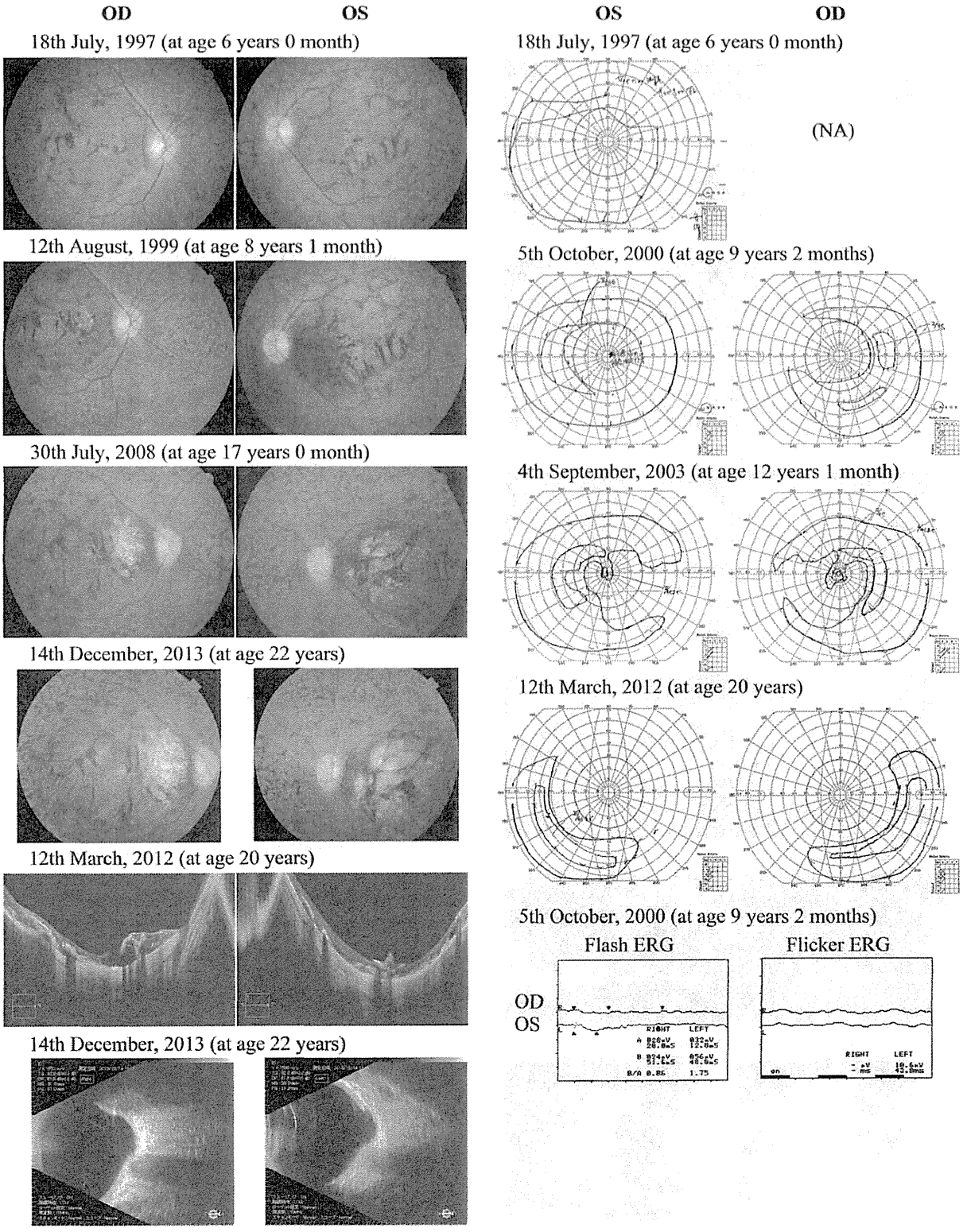


Fig. 4 Fundus photographs (*left*), OCT and ultrasonographic images (*lower left*), Goldmann kinetic visual fields (*right*), and full-field ERGs (*bottom right*) of Patient 2. Patient 2 was younger brother of Patient 1 (Figs. 1, 3). *NA* not available

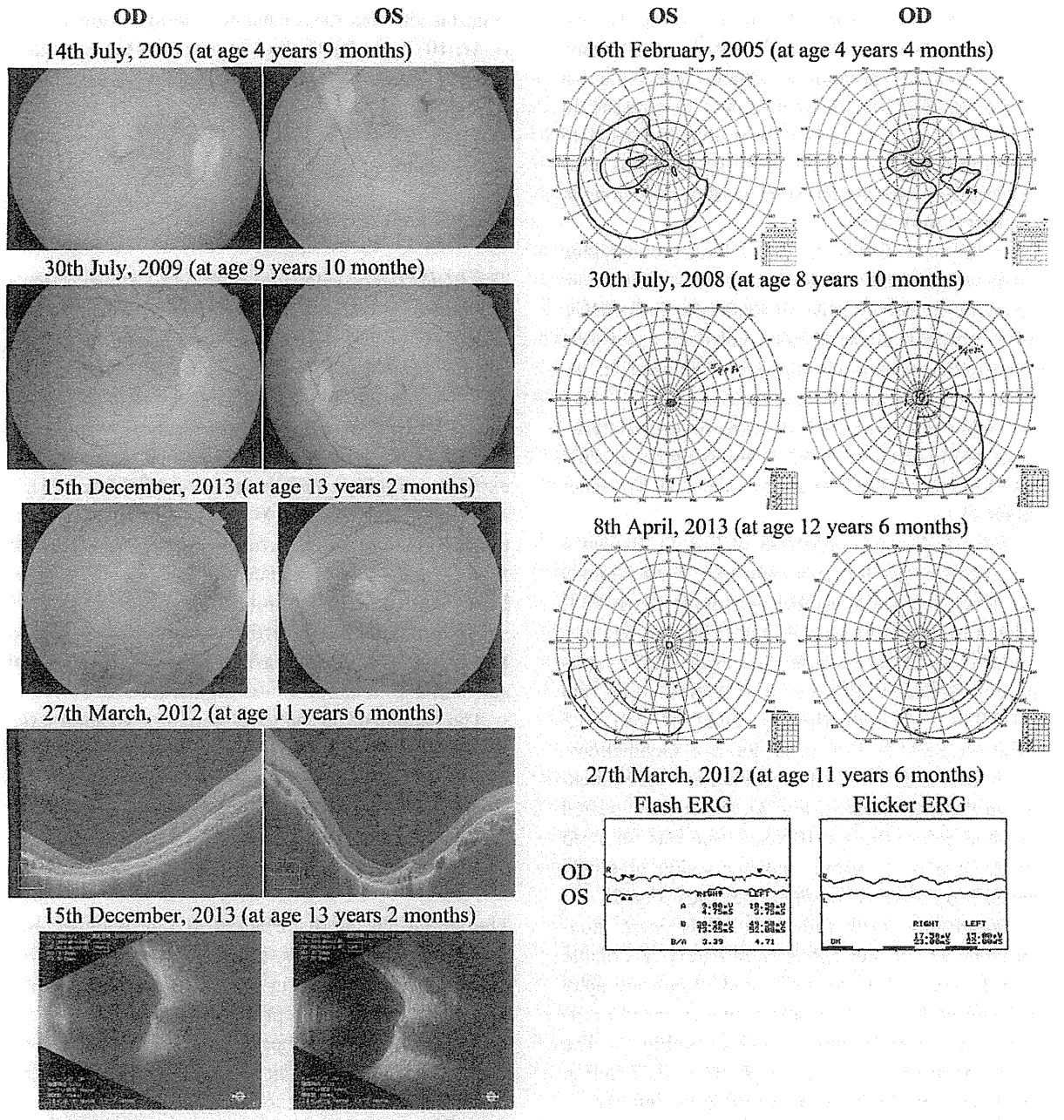


Fig. 5 Fundus photographs (*left*), OCT and ultrasonographic images (*lower left*), Goldmann kinetic visual fields (*right*), and full-field ERGs (*bottom right*) of Patient 3. Patient 3 was from unrelated family to that of Patients 1 and 2 (Fig. 1)

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