別紙４

平成２５年度　　研究成果の刊行に関する一覧表（氏名　篠田啓）

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
篠田啓	眼循環と網膜疾患の関与	石田晋	Retina Medicine	先進医学社.	東京	2013	43-49

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Matsumoto CS, Shinoda K, Matsumoto H, Funada H, Mizota A.	Comparison of flash effect during pattern visually evoked potentials in different driving frequency Liquid Crystal Display monitors.	*Ophthalmic Res*	In press		
Gocho K, Kikuchi S, Kabuto T, Kameya S, Shinoda K, Mizota A, Yamaki K, Takahashi K.	High-resolution en face images of microcystic macular edema in patients with autosomal dominant optic atrophy.	*J Ophthalmol, BioMed Research International*	Epub Nov 28.		2013
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別紙４

平成２５年度　　研究成果の刊行に関する一覧表（氏名　國吉一樹）

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
國吉一樹	Q&A 眼科診療ピットフォール	下村嘉一・松本長太	Q&A 眼科診療ピットフォール	金芳堂	京都市	2013	79-90
國吉一樹	ERG の有用性	白神史雄	専門医のための眼科診療クオリファイ 16 糖尿病合併症	中山書店	東京都	2013	37-40

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Kuniyoshi K, Sakuramoto H, Nakao Y, Matsumoto C, Shimomura Y	Two types of acute zonal occult outer retinopathy differentiated by dark- and light-adapted perimetry	Japanese Journal of Ophthalmology	58	17187	2014
Kuniyoshi K, Sugioka K, Sakuramoto H, Kusaka S, Wada N, Shimomura Y	Intravitreal injection of bevacizumab for retinopathy of prematurity	Japanese Journal of Ophthalmology		In press	2014
Kuniyoshi K, Terasaki H, Arai M, Hirose T	Multifocal electroretinograms in Stargardt's disease/fundus flavimaculatus	Ophthalmologica		In press	2014
櫻本宏之，國吉一樹	電気生理学的検査. 全視野 ERG	眼科	56	65-75	2014
Kuniyoshi K, Hayashi T, Sakuramoto H, Nakao A, Sato T, Utsumi T, Tsuneoka H, Shimomura Y.	Novel mutations in enhanced S-cone syndrom	Ophthalmology	120	431 e1-6	2013
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國吉一樹	抗アクアポリン４抗体陽性視神経炎	臨床眼科　臨時増刊号	67	188-193	2013

別紙４

平成２５年度　研究成果の刊行に関する一覧表　（氏名　林 孝彰）

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
林　孝彰	網膜色素変性	伊藤利之、江藤文夫、木村彰男	今日のリハビリテーション指針	医学書院	東京	2013	374-377
林　孝彰	全色盲	伊藤利之、江藤文夫、木村彰男	今日のリハビリテーション指針	医学書院	東京	2013	382-383

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発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Shibata A, Ohkuma Y, Hayashi T, Tsuneoka H.	Efficacy of reduced-fluence photodynamic therapy for serous retinal pigment epithelial detachment with choroidal hyperpermeability	Clin Ophthalmol	7	2123-2126	2013
Katagiri S, Yoshitake K, Akahori M, Hayashi T, Furuno M, Nishino J, Ikeo K, Tsuneoka H, Iwata T.	Whole-exome sequencing identifies a novel *ALMS1* mutation (Q2051X) in two Japanese bothers with Alström Syndrome	Mol Vis	19	2393-2406	2013
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Ohkuma Y, Hayashi T, Sakai T, Watanabe A, Yamada H, Akahori M, et al.	Retinal angiomatous proliferation associated with risk alleles of *ARMS2/HTRA1* gene polymorphisms in Japanese patients	Clin Ophthalmkol	8	143-148	2014
Katagiri S, Gekka T, Hayashi T, Ida H, Ohashi T, Eto Y, Tsuneoka H.	*OAT* mutations and clinical features in two Japanese brothers with gyrate atrophy of the choroid and retina	Doc Ophthalmol	128	137-148	2014
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別紙４

平成２５年度　　研究成果の刊行に関する一覧表（氏名　上野真治）

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
なし							

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Ueno S, Nishiguchi KM, Tanioka H, Enomoto A, Yamanouchi T, Kondo M, Yasuma TR, Yasuda S, Kuno N, Takahashi M, Terasaki H	Degeneration of retinal on bipolar cells induced by serum including autoantibody against TRPM1 in mouse model of paraneoplastic retinopathy.	PLoS One.	25	e81507	2013
Hibi N, **Ueno S**, Ito Y, Piao CH, Kondo M, Terasaki H.	Relationship between retinal layer thickness and focal macular electroretinogram components after epiretinal membrane surgery.	Investigative Ophthalmology and Visual Science.	1	7207-7214	2013
Ueno S, Koyasu T, Kominami T, Sakai T, Kondo M, Yasuda S, Terasaki H	Focal cone ERGs of rhodopsin Pro347Leu transgenic rabbits.	Vision Research	18	118-123	2013
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IV.　研究成果の刊行物・別刷

Doc Ophthalmol
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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 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

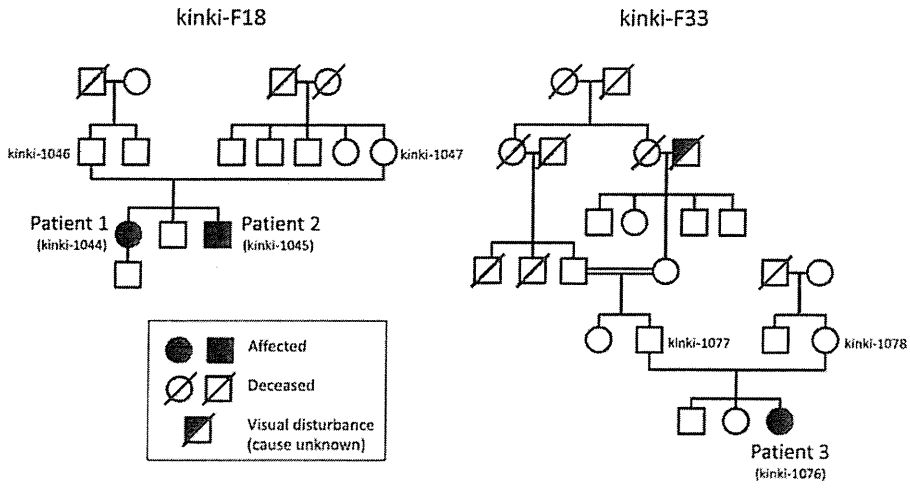


Fig. 1 Pedigrees of two unrelated families with Leber congenital amaurosis/early-onset retinal dystrophy (LCD/EORD) with *RDH12* mutation. Patients 1 and 2 were siblings (*left*, kinki-F18), and Patient 3 is from an unrelated family (*right*, kinki-F33). No consanguinity was reported between parents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

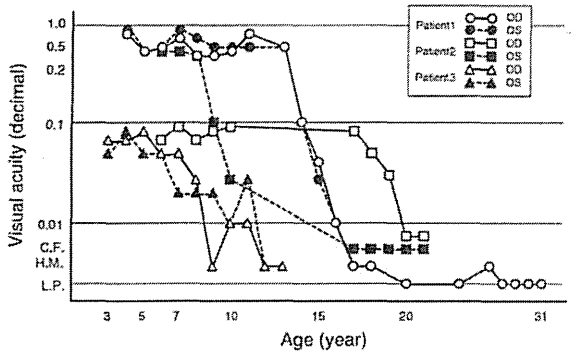


Fig. 2 Clinical course of visual acuity in each patient. *OD* oculus dexter, *OS* oculus sinister

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

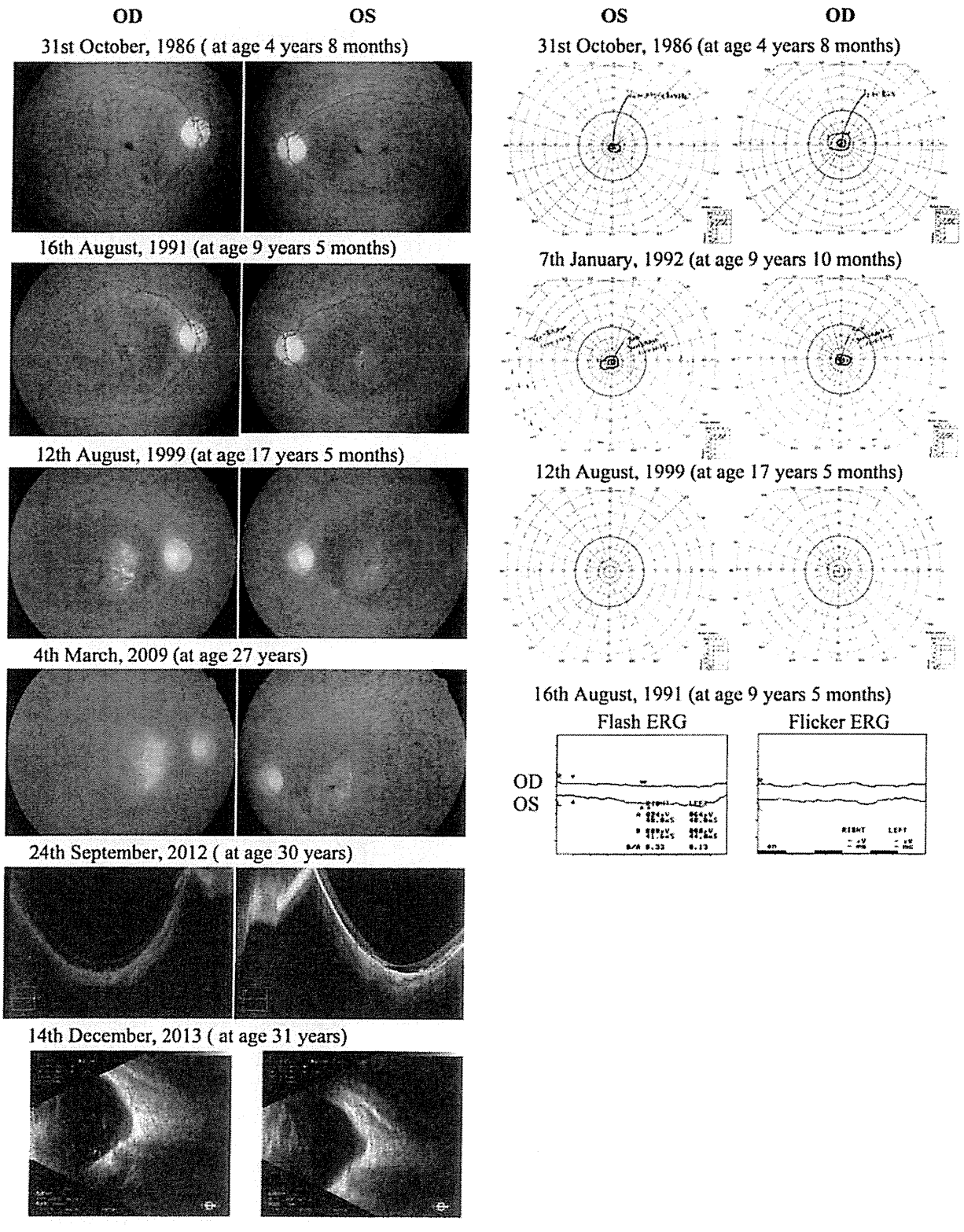
The clinical course of the visual acuity in the three patients is presented in Fig. 2. Summaries of the clinical findings are shown in Figs. 3, 4, and 5.

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

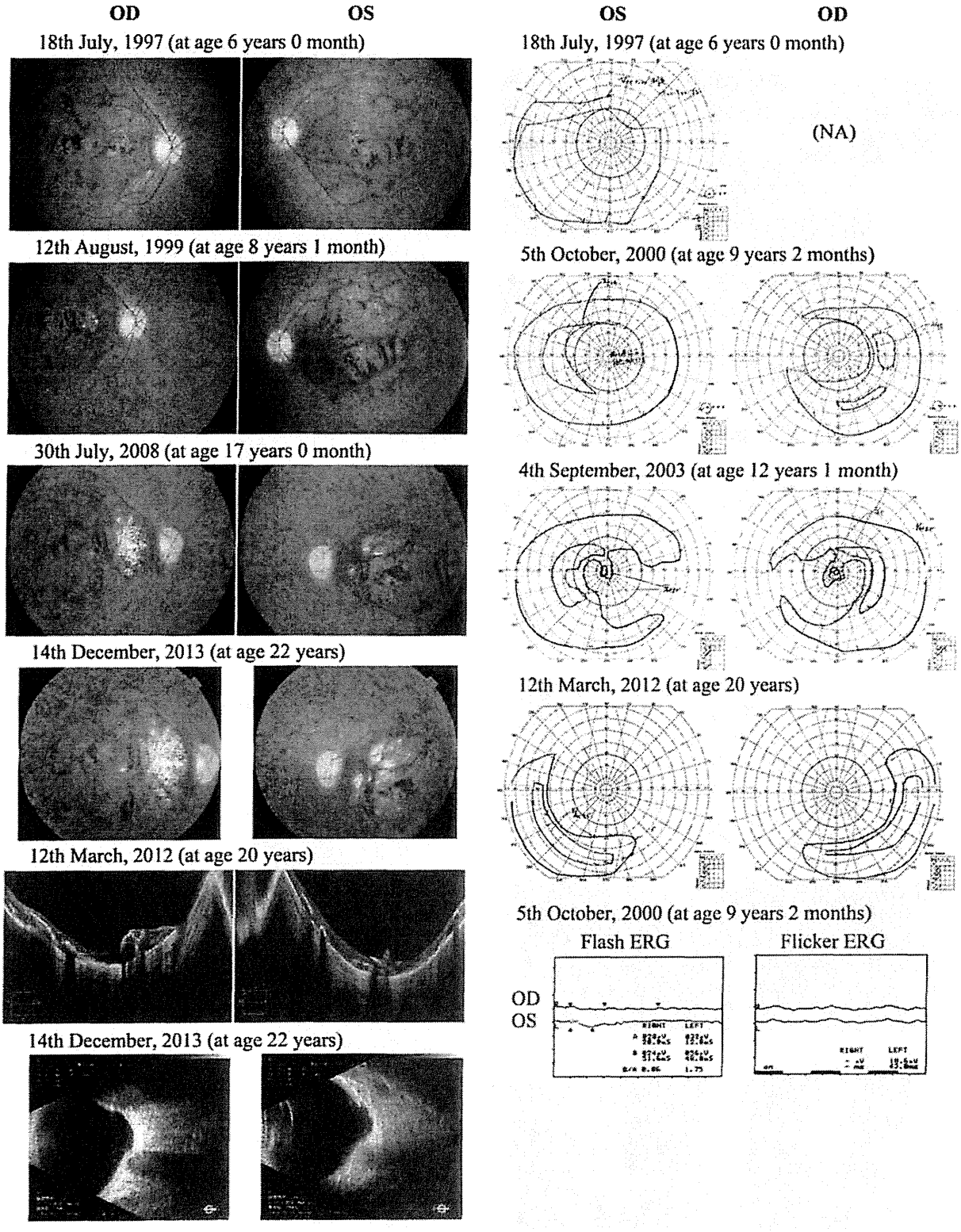


Fig. 4 Fundus photographs (*left*), OCT and ultrasonographic images (*lower left*), Goldmann kinetic visual fields (*right*), and full-field ERGs (*bottom right*) of Patient 2. Patient 2 was younger brother of Patient 1 (Figs. 1, 3). *NA* not available

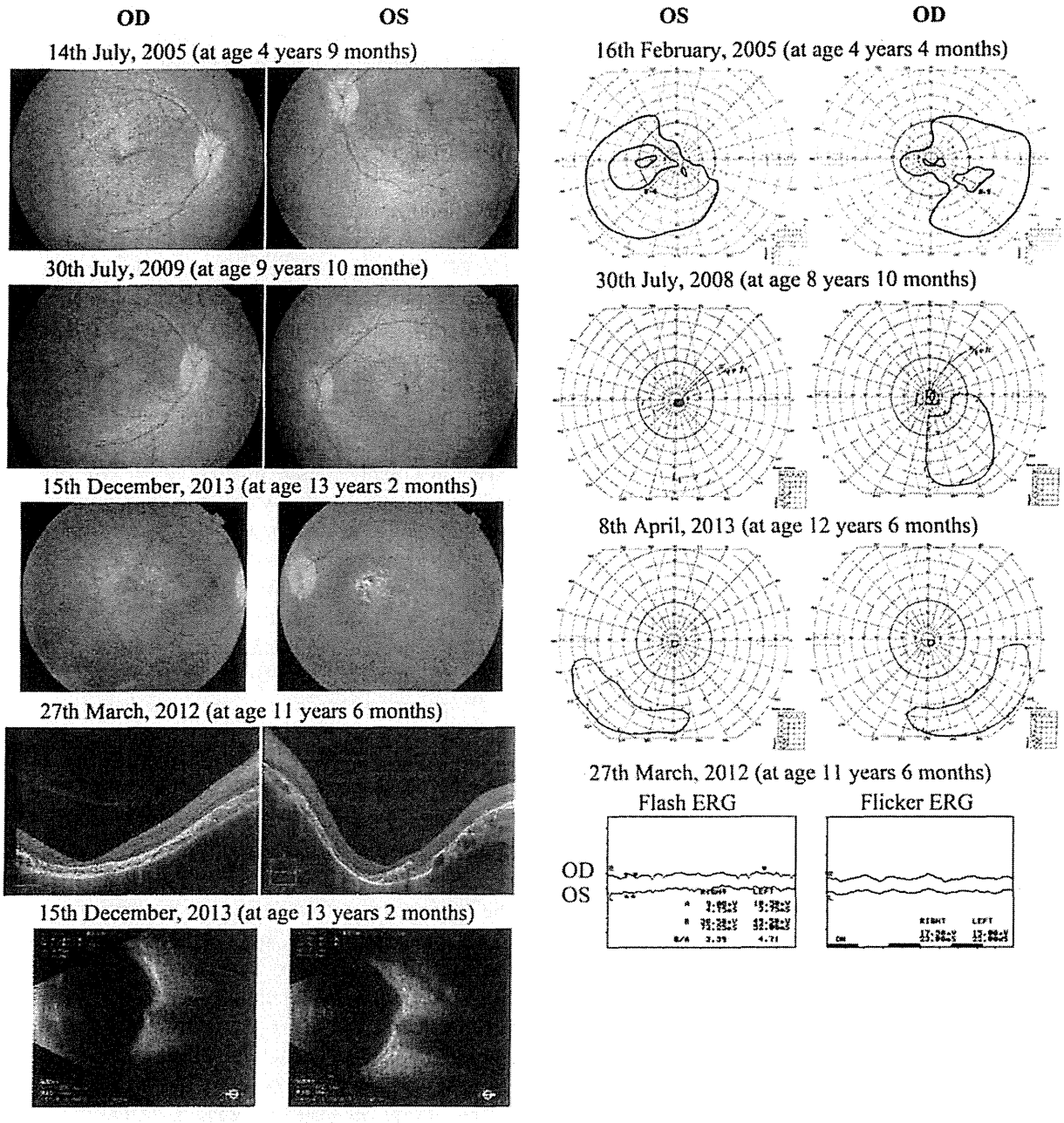


Fig. 5 Fundus photographs (*left*), OCT and ultrasonographic images (*lower left*), Goldmann kinetic visual fields (*right*), and full-field ERGs (*bottom right*) of Patient 3. Patient 3 was from unrelated family to that of Patients 1 and 2 (Fig. 1)

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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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.

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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

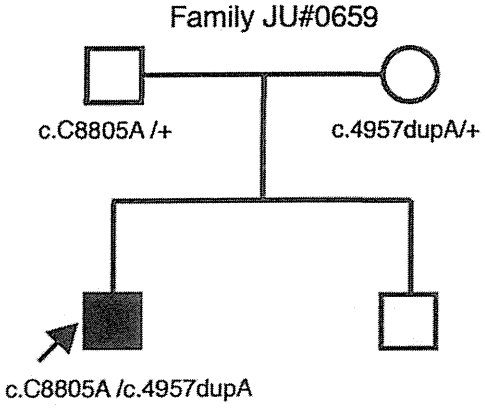

Fig. 1 Pedigree of a Japanese family. Unaffected family members (males, *open squares*; females, *open circles*) and an affected proband (male, *solid square*) are shown

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

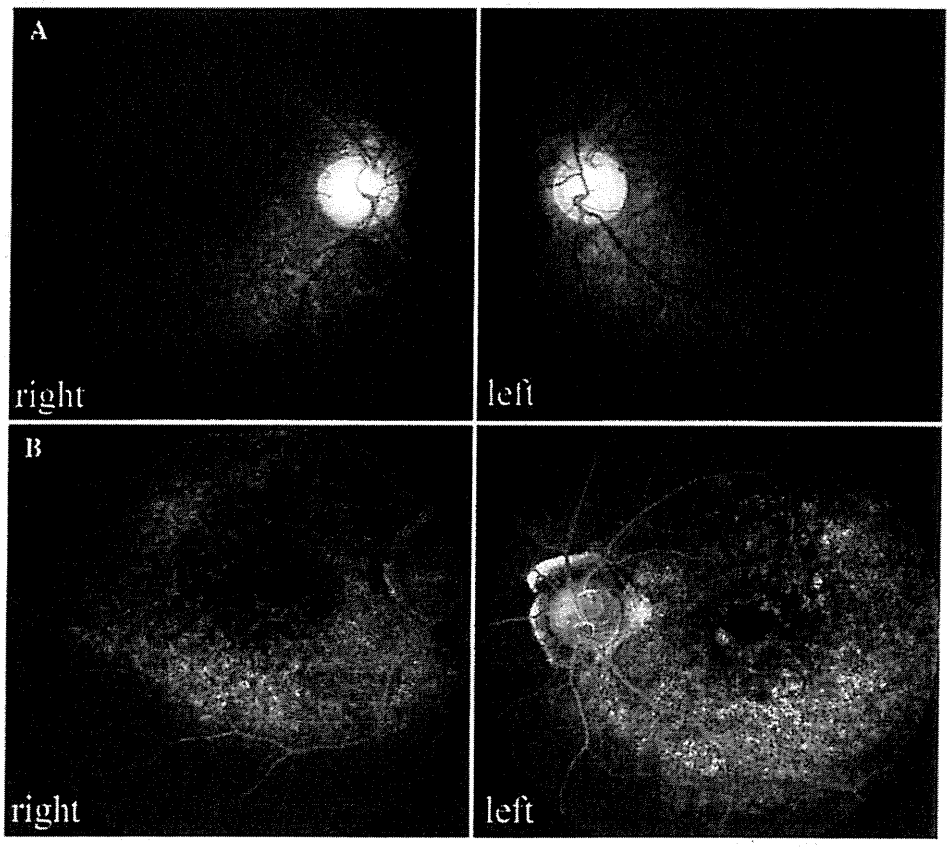

Fig. 2 Fundus photographs and fluorescein angiography images (FA) of the patient at the age of 31 years. **a** Fundus photographs show retinal degenerations within the vascular arcades in both eyes. **b** FA shows a hyperfluorescence pattern due to a window defect within the vascular arcades of both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

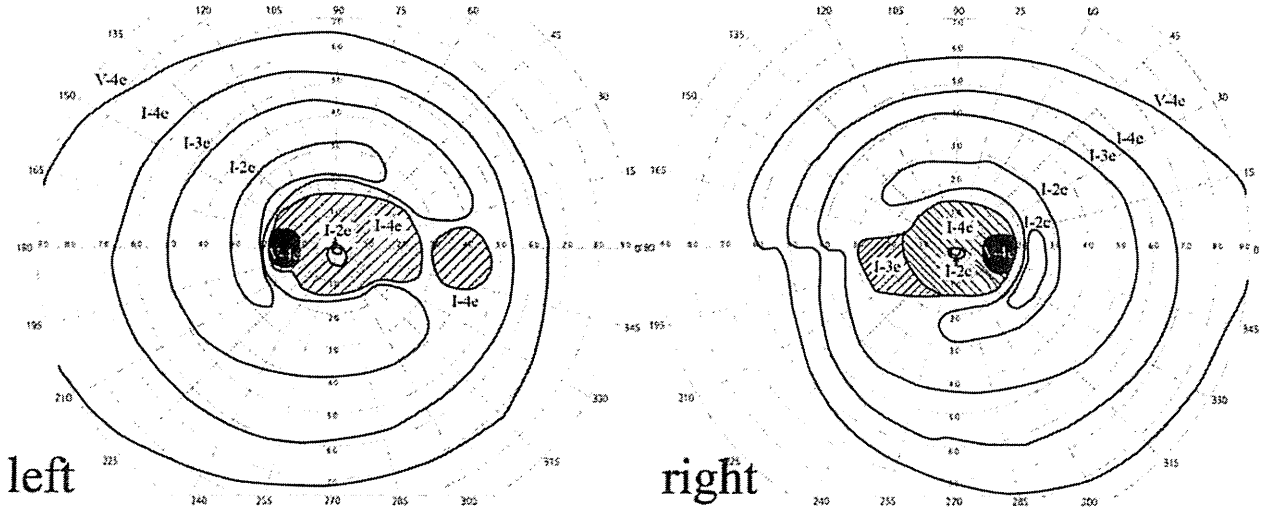

Fig. 3 Visual field testing using Goldmann kinetic perimetry (GP) at the age of 32 years. GP shows bilateral central scotomas of the I-3e and I-4e isopters with relative sparing of the center, but with preserved peripheral visual fields of the V-4e and I-4e isopters in both eyes

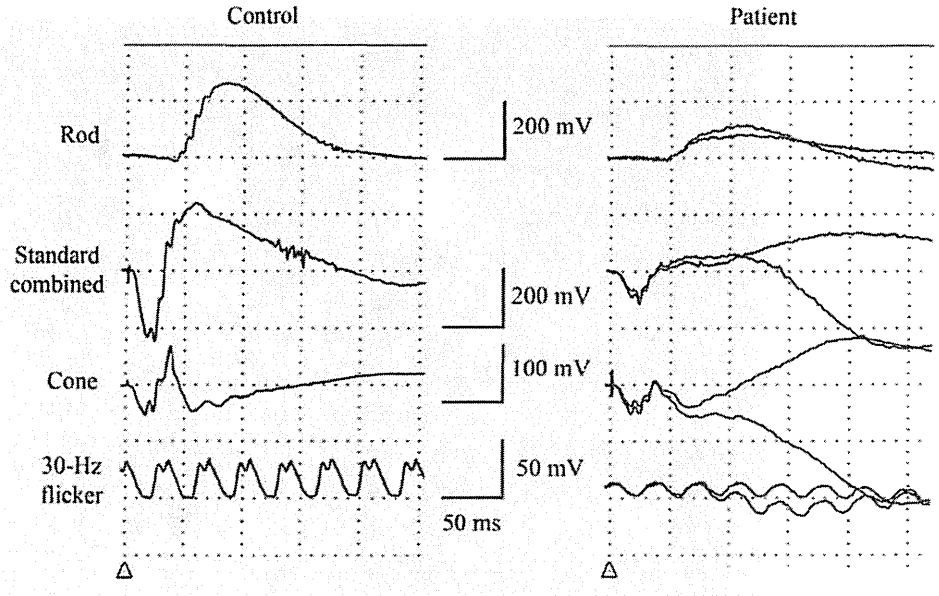

Fig. 4 Full-field electroretinography (ERG) at the age of 32 years. ERG shows that the rod, standard combined, cone, and 30-Hz flicker responses are bilaterally reduced to about one-third of those in a control, but the peak implicit time of each response is not delayed

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.

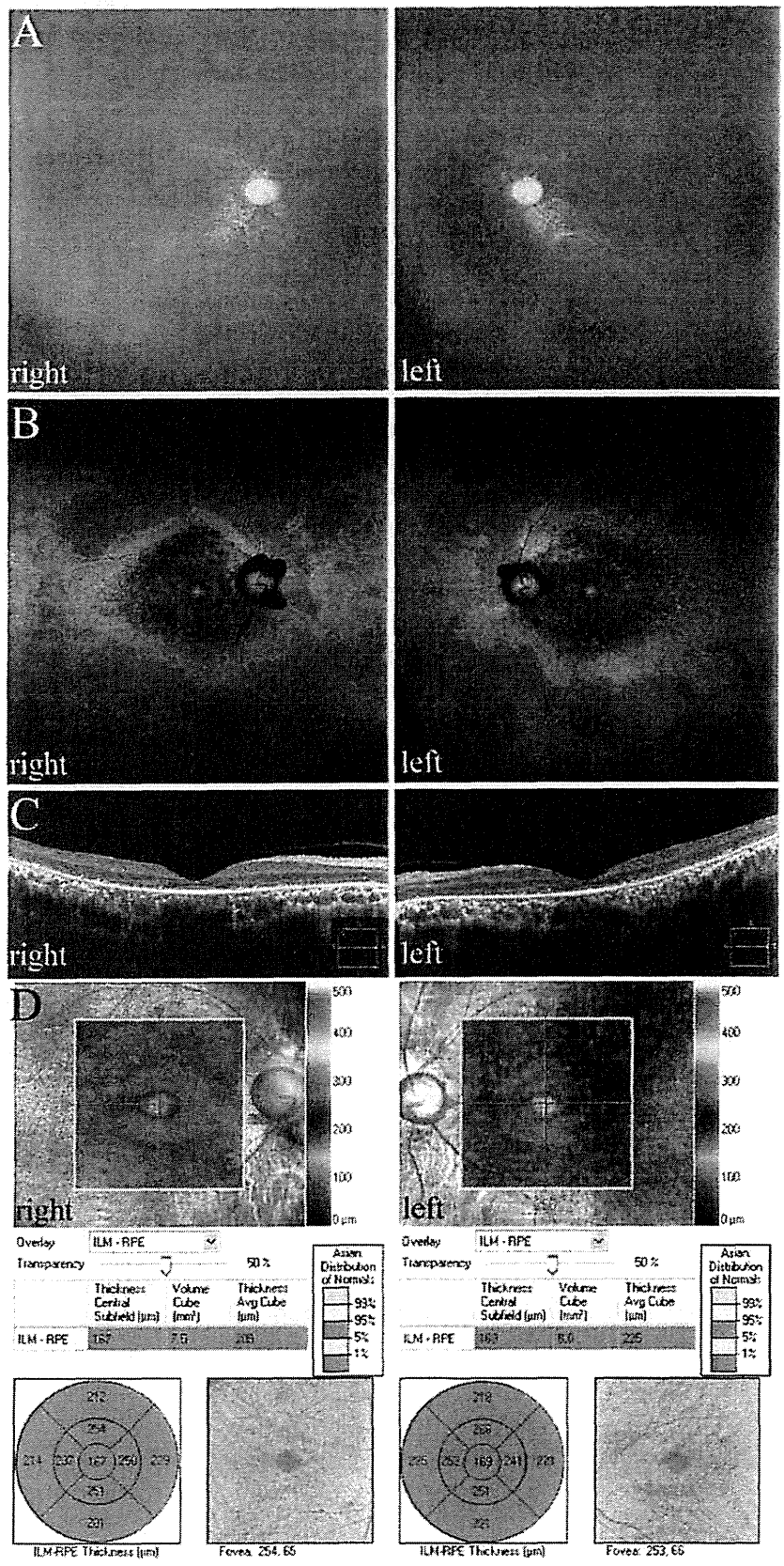

Fig. 5 Fundus photographs, fundus autofluorescence images (FAI), and optic coherence tomography images (OCT) at the age of 36 years. **a** Fundus photographs show retinal degenerations within the vascular arcades in both eyes, but relatively preserved mid-peripheral to peripheral retinal findings with no apparent attenuation of the retinal vessels. **b** FAI shows decreased autofluorescence within the vascular arcades but increased autofluorescence of the foveal area, and increased autofluorescence outside the vascular arcades in both eyes. **c** OCT (HD 5-line raster scan) shows retinal thinning with a visible foveal external limiting membrane line in both eyes. **d** OCT (Macular cube scan) shows entire macular thinning in both eyes