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

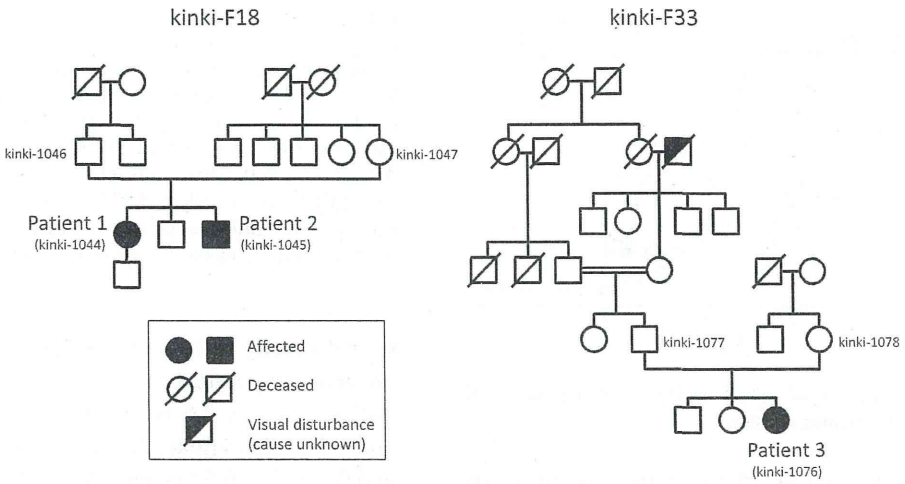


Fig. 1 Pedigrees of two unrelated families with Leber congenital amaurosis/early-onset retinal dystrophy (LCD/EORD) with *RDH12* mutation. Patients 1 and 2 were siblings (*left*, kinki-F18), and Patient 3 is from an unrelated family (*right*, kinki-F33). No consanguinity was reported between parents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

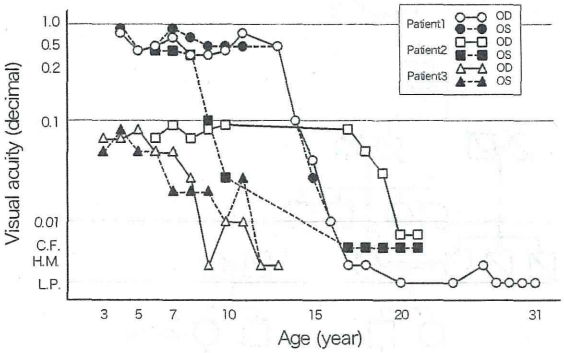


Fig. 2 Clinical course of visual acuity in each patient. *OD* oculus dexter, *OS* oculus sinister

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).

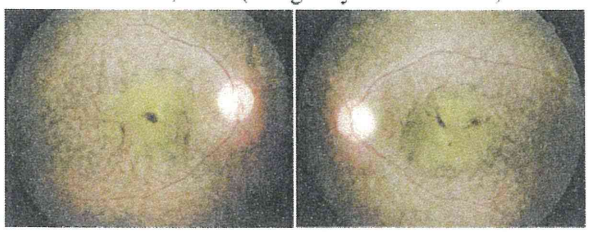
Fig. 3 Fundus photographs (*left*), optical coherence tomo-c graphic (OCT) and ultrasonographic images (*lower left*), Goldmann kinetic visual fields (*right*), and full-field ERGs (*bottom right*) of Patient 1. Fundus photographs and OCT images taken at age 27 and 30 years are blurred because of cataracts.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

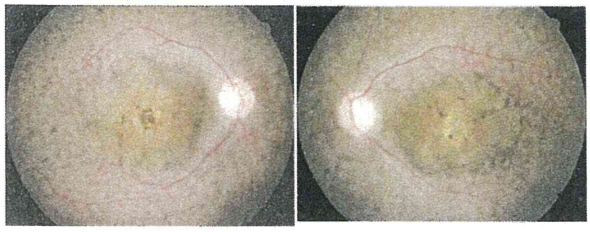
When the sequences of her whole exome were compared with the reference human genome (hs37d5), 940,138 mutations were found. We focused only on mutations that could change the amino-acid sequence and excluded common mutations by 1,000 genomes, HGVD [21], and our in-house database (see methods). As a result,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

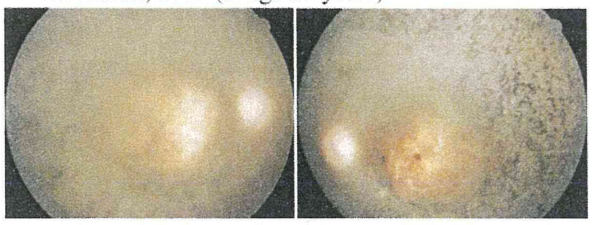


16th August, 1991 (at age 9 years 5 months)

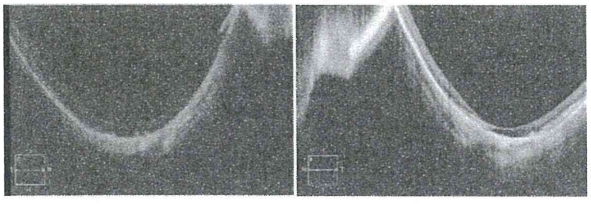


12th August, 1999 (at age 17 years 5 months)

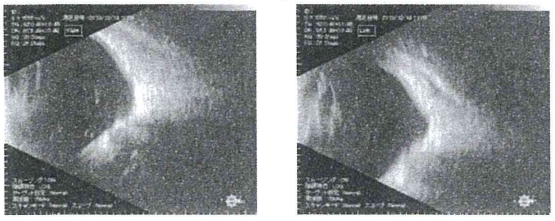
4th March, 2009 (at age 27 years)



24th September, 2012 (at age 30 years)

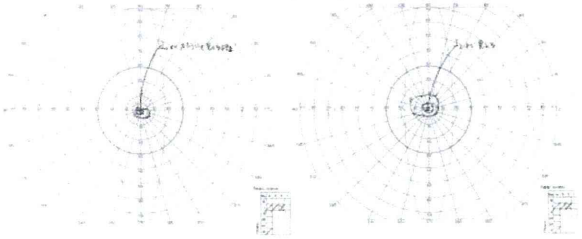


14th December, 2013 (at age 31 years)

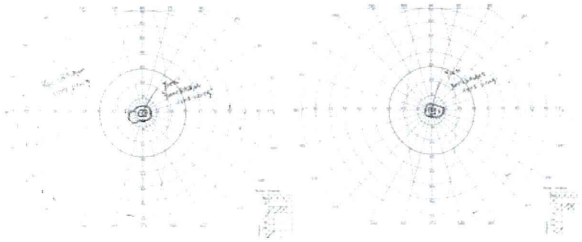


OS OD

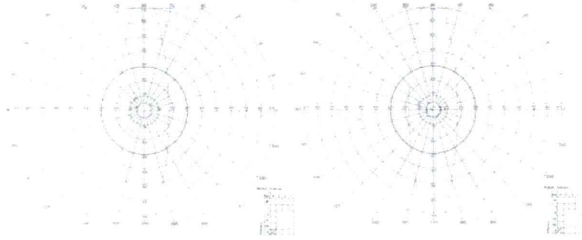
31st October, 1986 (at age 4 years 8 months)



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



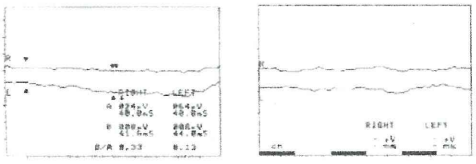
12th August, 1999 (at age 17 years 5 months)



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

Flash ERG Flicker ERG

OD
OS



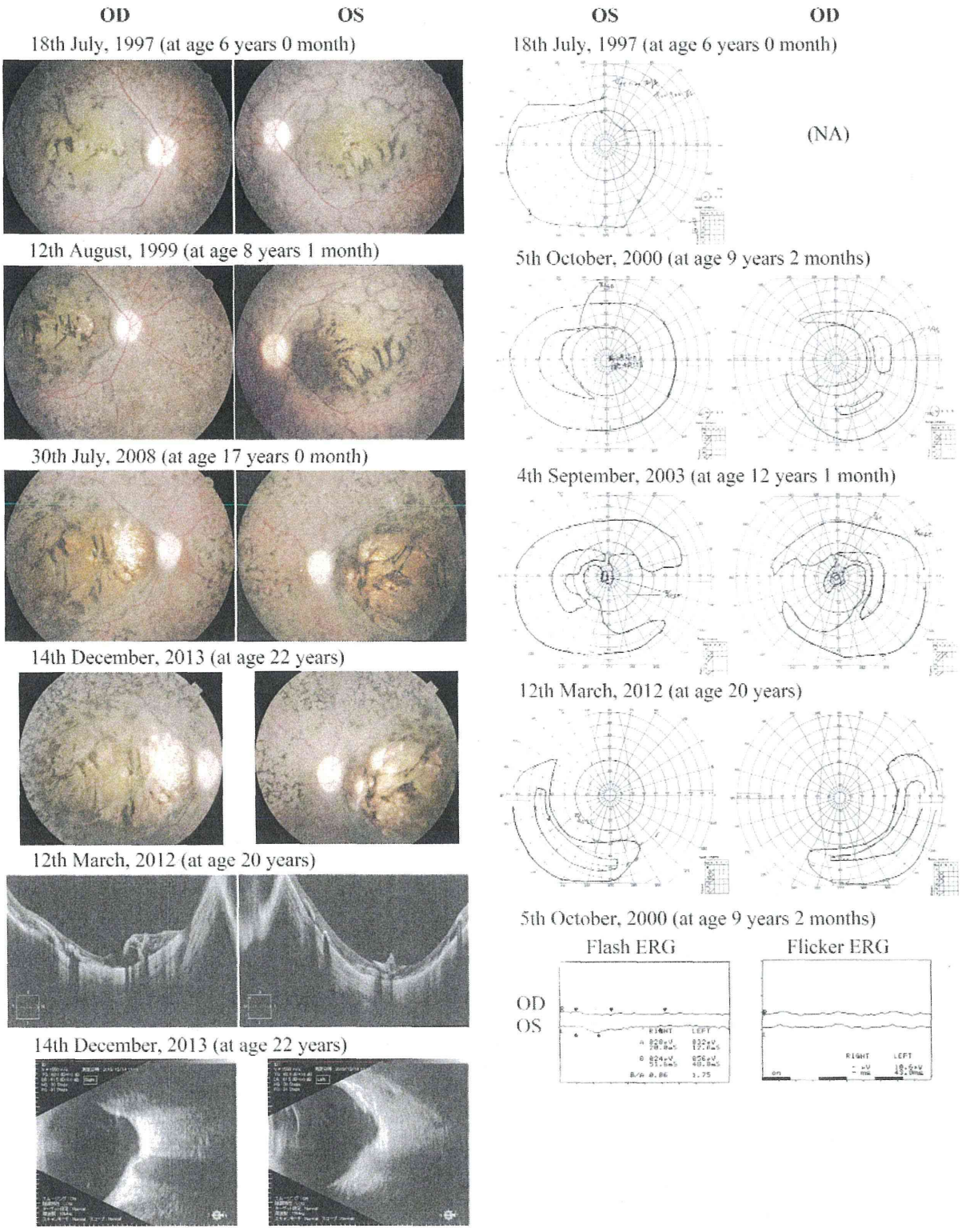


Fig. 4 Fundus photographs (*left*), OCT and ultrasonographic images (*lower left*), Goldmann kinetic visual fields (*right*), and full-field ERGs (*bottom right*) of Patient 2. Patient 2 was younger brother of Patient 1 (Figs. 1, 3). *NA* not available

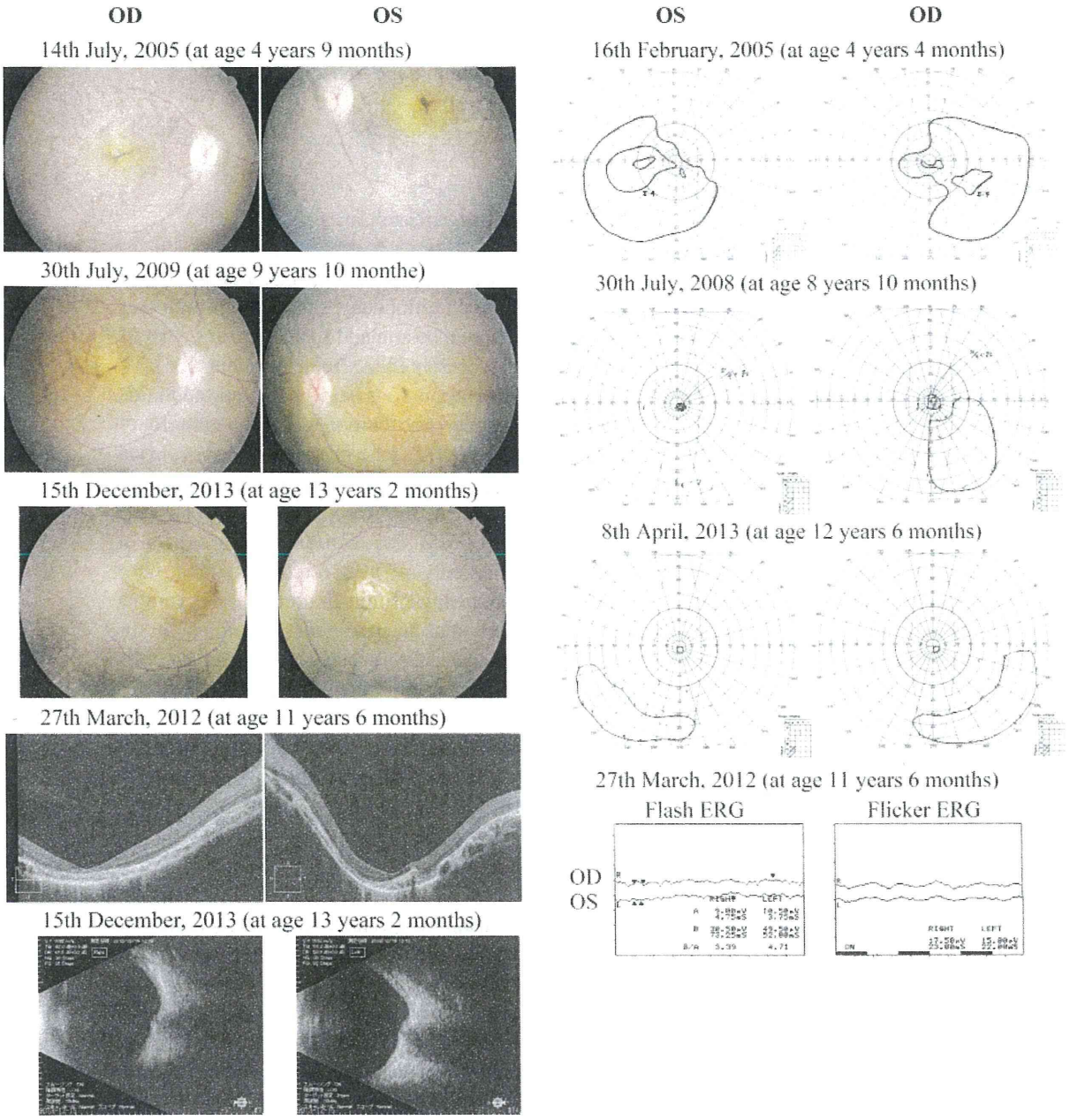


Fig. 5 Fundus photographs (*left*), OCT and ultrasonographic images (*lower left*), Goldmann kinetic visual fields (*right*), and full-field ERGs (*bottom right*) of Patient 3. Patient 3 was from unrelated family to that of Patients 1 and 2 (Fig. 1)

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.

References

1. Leber T (1869) Ueber Retinitis pigmentosa und angeborene Amaurose. Graefes Arch Klin Exp Ophthalmol 15:1–25
2. Franceschetti A, Dieterlé P (1954) Importance diagnostique et pronostique de l' électrorétinogramme (ERG) dans les dégénérescences tapéto-rétiniennes avec rétrécissement du champ visuel et héméralopie. Confin Neurol 14:184–186
3. Leber T (1916) Die Pigmentdegeneration der Netzhaut und die mit ihr verwandten Erkrankungen. In: Saemisch T, Elschnig A (eds) Graefe-Saemisch-Hess Handbuch der gesamten Augenheilkunde. Zweite Hälfte. Verlag von Wilherm Engelmann, Leipzig, pp 1076–1225
4. Weleber RG & Gregory-Evans K (2006) Leber congenital amaurosis. In: Hinton DR (ed), Ryan SJ (ed in chief) Retina, 4th edn, vol 1. Elsevier Inc., Philadelphia, pp 455–457
5. Weleber RG, Francis PJ, Trzupek KM & Beattie C (2014) Leber congenital amaurosis. In: Pagon RA (ed in chief), Adam MP, Bird TD, Dolan CR, Fong CT, Smith RJH, Stephens K (eds) GeneReviews™. NCBI Bookshelf. http://www.ncbi.nlm.nih.gov/books/NBK1298/. Accessed 12 Mar 2014
6. Daiger SP, The University of Texas Health Science Center (2014) RetNet™. Retinal information network. Updated 10 Mar 2014. https://sph.uth.edu/retnet. Accessed 12 March 2014
7. Jenecke AR, Thompson DA, Utermann G, Becker C, Hübner CA, Schmid E, McHenry CL, Nair AR, Rüschendorf F, Heckenlively J, Wissinger B, Nürnberg P, Gal A (2004) Mutations in *RDH12* encoding a photoreceptor cell retinol dehydrogenase cause childhood-onset severe retinal dystrophy. Nat Genet 36:850–854
8. Perrault I, Hanein S, Gerber S, Barbet F, Ducroq D, Dollfus H, Hamel C, Dufier JL, Munnich A, Kaplan J, Rozet JM (2004) Retinal dehydrogenase 12 (*RDH12*) mutations in Leber congenital amaurosis. Am J Hum Genet 75:639–646
9. Jacobson SG, Cideciyan AV, Aleman TS, Sumaroka A, Schwartz SB, Windsor EAM, Roman AJ, Heon E, Stone EM, Thompson DA (2007) *RDH12* and *RPE65*, visual cycle genes causing Leber congenital amaurosis, differ in disease expression. Invest Ophthalmol Vis Sci 48:332–338

10. Schuster A, Janecke AR, Wilke R, Schmid E, Thompson DA, Utermann G, Wissinger B, Zrenner E, Gal A (2007) The phenotype of early-onset retinal degeneration in persons with *RDH12* mutations. Invest Ophthalmol Vis Sci 48:1824–1831
11. Sun W, Gerth C, Maeda A, Lodowski DT, van der Kraak L, Saperstein DA, Héon E, Palczewski K (2007) Novel RDH12 mutations associated with Leber congenital amaurosis and cone-rod dystrophy: biochemical and clinical evaluations. Vision Res 47:2055–2066
12. Valverde D, Pereiro I, Vallespín E, Ayuso C, Borrego S, Baiget M (2009) Complexity of phenotype-genotype correlations in Spanish patients with *RDH12* mutations. Invest Ophthalmol Vis Sci 50:1065–1068
13. Benayoun L, Spiegel R, Auslender N, Abbasi AH, Rizel L, Hujeirat Y, Salama I, Garzozi HJ, Allon-Shalev S, Ben-Yosef T (2009) Genetic heterogeneity in two consanguineous families segregating early onset retinal degeneration: the pitfalls of homozygosity mapping. Am J Med Genet Part A 149A:650–656
14. Sodi A, Caputo R, Passerini I, Bacci GM, Menchini U (2010) Novel *RDH12* sequence variations in Leber congenital amaurosis. J AAPOS 14:349–351
15. Weleber RG, Michaelides M, Trzupek KM, Stover NB, Stone EM (2011) The phenotype of severe early childhood onset retinal dystrophy (SECORD) from mutation of *RPE65* and differentiation from Leber congenital amaurosis. Invest Ophthalmol Vis Sci 52:292–302
16. Mackay DS, Borman AD, Moradi P, Henderson RH, Li Z, Wright GA, Waseem N, Gandra M, Thompson DA, Bhattacharya SS, Holder GE, Webster AR, Moore AT (2011) *RDH12* retinopathy: novel mutations and phenotypic description. Mol Vis 17:2706–2716
17. Marmor MF, Fulton AB, Holder GE, Miyake Y, Brigell M, Bach M (2009) ISCEV Standard for full-field clinical electroretinography (2008 update). Doc Ophthalmol 118:69–77
18. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25:1754–1760
19. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA (2010) The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20:1297–1303
20. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM (2012) A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w^{1118}; *iso-2*; *iso-3*. Fly 6:80–92
21. Kyoto University (2014) Human genetic variation browser. http://www.genome.med.kyoto-u.ac.jp/SnpDB/. Accessed 12 Mar 2014
22. Adzhubei I, Jordan DM, Sunyaev SR (2013) Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet 76:7.20.1–7.20.41
23. National Institute of Genetics (NIG) (2014) Cell innovation program. http://cell-innovation.nig.ac.jp/index_en.html. Accessed 12 Mar 2014
24. Fingert JH, Oh K, Chung M, Scheetz TE, Andorf JL, Johnson RM, Sheffield VC, Stone EM (2008) Association of a novel mutation in the retinol dehydrogenase 12 (*RDH12*) gene with autosomal dominant retinitis pigmentosa. Arch Ophthalmol 126:1301–1307
25. Fulton AB, Hansen RM, Mayer DL (1996) Vision in Leber congenital amaurosis. Arch Ophthalmol 114:698–703
26. Walia S, Fishman GA, Jacobson SG, Aleman TS, Koenekoop RK, Traboulsi EI, Weleber RG, Pennesi ME, Héon E, Drack A, Lam BL, Allikmets R, Stone EM (2010) Visual acuity in patients with Leber's congenital amaurosis and early childhood-onset retinitis pigmentosa. Ophthalmology 117:1190–1198
27. Leighton DA, Harris R (1973) Retinal aplasia in association with macular coloboma, keratoconus and cataract. Clin Genet 4:270–274
28. Margolis S, Scher BM, Carr RE (1977) Macular colobomas in Leber's congenital amaurosis. Am J Ophthalmol 83:27–31
29. Heckenlively JR, Foxman SG, Parelhoff ES (1988) Retinal dystrophy and macular coloboma. Doc Ophthalmol 68:257–271
30. Mohamed MD, Topping NC, Jafri H, Raashed Y, McKibbin MA, Inglehearn CF (2003) Progression of phenotype in Leber's congenital amaurosis with a mutation at the LCA5 locus. Br J Ophthalmol 87:473–475
31. Nakamura M, Ito S, Miyake Y (2002) Novel de novo mutation in *CRX* gene in a Japanese patient with Leber congenital amaurosis. Am J Ophthalmol 134:465–467
32. Lotery AJ, Jacobson SG, Fishman GA, Weleber RG, Fulton AB, Namperumalsamy P, Héon E, Levin AV, Grover S, Rosenow JR, Kopp KK, Sheffield VC, Stone EM (2001) Mutations in the *CRB1* gene cause Leber congenital amaurosis. Arch Ophthalmol 119:415–420
33. Koenekoop RK, Wang H, Majewski J, Wang X, Lopez I, Ren H, Chen Y, Li Y, Fishman GA, Genead M, Schwartzentruber J, Solanki N, Traboulsi EI, Cheng J, Logan CV, McKibbin M, Hayward BE, Parry DA, Johnson CA, Nageeb M, FORGE Canada Consortium, Poulter JA, Mohamed MD, Jafri H, Rashid Y, Taylor GR, Keser V, Mardon G, Xu H, Inglehearn CF, Fu Q, Toomes C, Chen R (2012) Mutations in *NMNAT1* cause Leber congenital amaurosis and identify a new disease pathway for retinal degeneration. Nat Genet 44:1035–1039

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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.