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CLINICAL CASE REPORT

Novel nonsense and splice site mutations in *CRB1* gene in two Japanese patients with early-onset retinal dystrophy

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Abstract
Purpose To report novel mutations in the *CRB1* gene in two patients with early-onset retinal dystrophy (EORD) and the longitudinal clinical course of EORD.
Patients and methods The patients were two unrelated Japanese children. Standard ophthalmic examinations including perimetry, electroretinography, and optical coherence tomography were performed on both patients. Whole exomes of the patients and their nonsymptomatic parents were analyzed using a next-generation sequence (NGS) technique.
Results *Patient 1* was noted to have esotropia and hyperopia at age 3. His decimal best-corrected visual acuity (BCVA) was 0.6 OD and 0.3 OS at age 6 with de-pigmentation of the retinal pigment epithelium (RPE). At age 19, his central vision was still preserved; however, numerous pigment granules were present in the retina. NGS analysis revealed a p.R632X nonsense and c.652 + 1_652 + 4delGTAA splice site mutations in the *CRB1* gene. *Patient 2* was noted to have hyperopia at age 3. His decimal BCVA at age 6 was 0.3 OD and 0.4 OS with de-pigmented RPE. The degree of retinal pigmentation was increased but his BCVA was good until the age of 14 years. NGS analysis revealed c.652 + 1_652 + 4delGTAA and c.652 + 1_652 + 2insT splice site mutations in the *CRB1* gene.
Conclusions The phenotypes of these novel mutations for EORD are typical of *CRB1*-associated EORD (LCA8). They were slowly progressive until the second decade of life.

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Keywords Leber congenital amaurosis · Early-onset retinal dystrophy · *CRB1* · Optical coherence tomography · Electroretinography · Visual fields · Japanese

Introduction

The early-onset retinal dystrophies (EORDs) are a milder form of Leber congenital amaurosis (LCA) [1, 2], and EORDs and LCA are considered to be clinically similar diseases (LCA/EORD). The onset of the signs and symptoms of LCA/EORD is usually from birth through the first decade of life [1, 2]. The appearance of the fundus of eyes with LCA/EORD varies from normal, flecked retina to diffuse pigmentary retinal degeneration [3].

To date, 17 causative genes have been reported for LCA/EORD [4, 5]. Among them, the *CRB1* (crumbs homolog 1) gene, which is located on chromosome 1q31.3, was reported to be causative for LCA/EORD in 2001 (LCA8) [6]; the prevalence of LCA/EORD is estimated to be 10 % [6]. The phenotypes of *CRB1*-associated retinopathy varies from LCA/EORD [6–9], retinitis pigmentosa [10–12], para-arterior preserved retinal dystrophy [7, 9], and retinal telangiectasia with exudation (Coats-like vasculopathy) [11]. The inheritance pattern is usually autosomal recessive [6–12].

We report the clinical course and findings of genetic examinations of two unrelated Japanese patients with EORD.

Patients and methods

The patients were 2 unrelated Japanese children. Standard ophthalmic examinations including perimetry, electroretinography (ERG), and optical coherence tomography (OCT) were performed on each patient. Full-field ERGs were recorded according to the guidelines of International Society for Clinical Electrophysiology of Vision (ISCEV) [13] with a contact-lens electrode with embedded white light-emitting diodes (LEDs; EW-102; Mayo Corporation, Inazawa, Japan). The LEDs were controlled by a driver (WLS-20; Mayo Corporation, Inazawa, Japan), and the responses were amplified with a bioamplifier (MEB-5504; Nihon Kohden, Tokyo, Japan). The OCT image was obtained by Cirrus^{TM} HD-OCT version 5.1 (Carl Zeiss Meditec, Dublin, CA, USA).

Genetic investigations of the whole exome were performed with the next-generation sequencing (NGS) technique. Details of the genetic investigation have been described [14].

The research protocol was approved by the Ethics Review Board of the Kinki University Faculty of Medicine in November 2011 and conformed to the tenets of the Declaration of Helsinki of the World Medical Association. The genetic analyses were performed after obtaining a signed informed consent form from all of the parents of the patients.

Results

A summary of the clinical findings is presented in Figs. 1, 2 and 3.

Patient 1 (F64-kinki 1136)

Patient 1 was 6-year-old boy at the initial visit to our clinic. He had night blindness from his infancy and was noted to have esotropia at age 3. His parents were nonconsanguineous. His decimal best-corrected visual acuity (BCVA) at age 6 was 0.5 with +5.0 diopters sphere (DS) OD and 0.3 with +7.0 DS OS. The refractive errors were obtained with cycloplegia. He had no nystagmus. Ophthalmoscopy revealed de-pigmentation of the retinal pigment epithelium (RPE) in the mid-periphery (Fig. 1). The ERGs were reduced, and perimetry revealed scotomas in the mid-periphery (Figs. 1, 3). He was observed for 13 years, and his BCVA at age 19 was 0.8 OD and 0.1 OS. During the follow-up period, numerous clumped pigments appeared in the mid-periphery of the retina (Fig. 1). The scotomas gradually enlarged but the ERGs decreased only slightly (Figs. 1, 3). OCT revealed a thickened and disorganized retina in both eyes (Fig. 1).

NGS on his whole exome revealed 1,650,353 mutations when compared with the reference human genome. Among them, 455 mutations were selected which could change the amino acid sequence after an exclusion of common mutations. They were filtered, and 7 genes were selected as causal candidates. Finally, *CRB1* was selected to be the disease-causing gene because the other genes

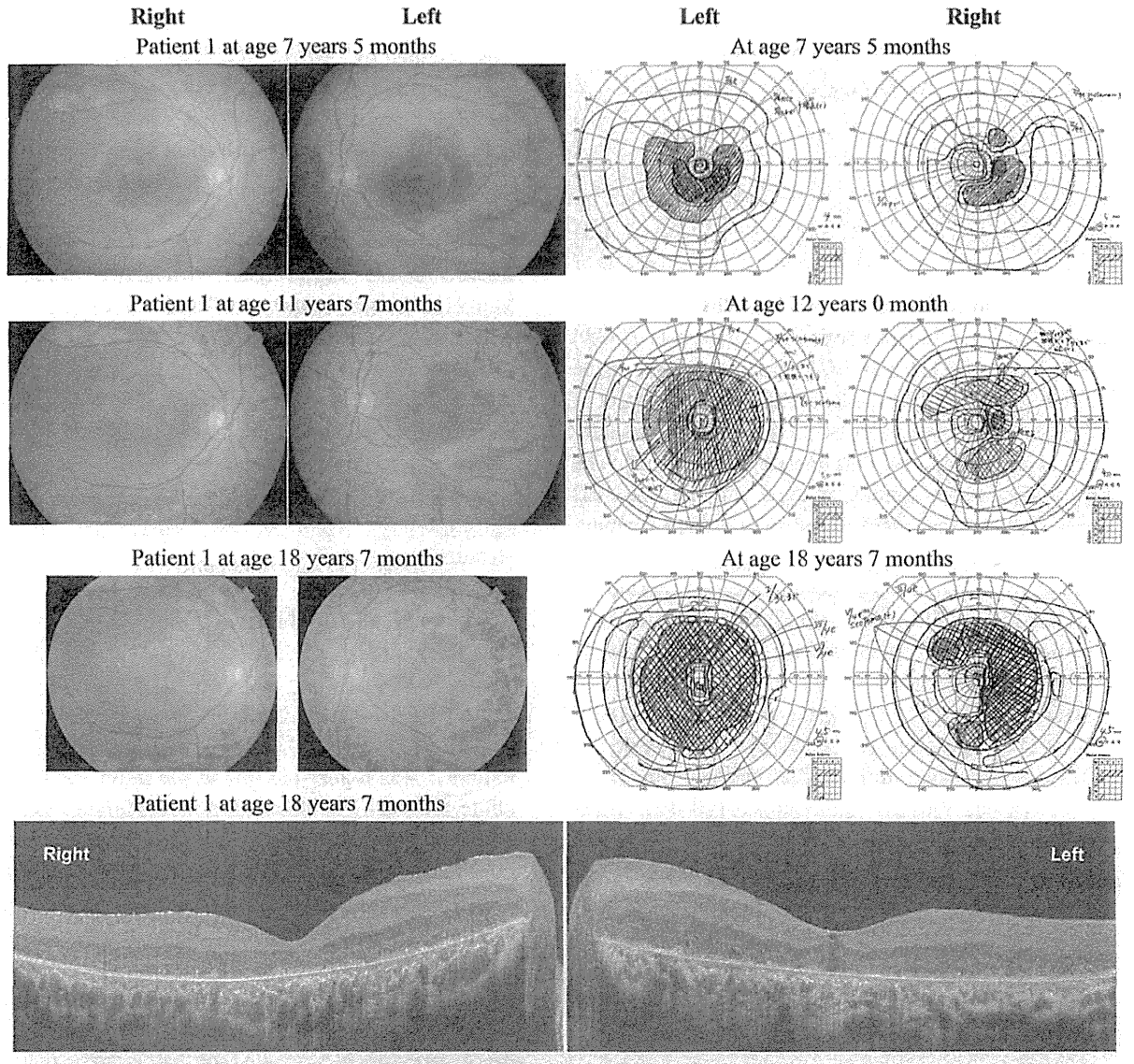

Fig. 1 Fundus photographs (*left column*), Goldmann kinetic visual fields (*right column*), and *horizontal* section of optical coherence tomographic (OCT) image (*bottom*) of Patient 1. The fundi of the two eyes show a preservation of the para-arteriolar retinal pigment epithelium (RPE). During the follow-up period, numerous clumps of pigments appeared in the mid-periphery and *yellowish* mottling of the macula became gradually apparent (*left column*). The OCT images revealed thickened and disorganized retinae (*bottom*). Length of the OCT scanning is 9 mm

were not registered in the RetNet™ database [4] as causative for inherited retinal diseases.

Genetic analysis revealed compound heterozygous mutations, p.R632X nonsense mutation, and c.652 + 1_652 + 4delGTAA splice site mutation in the *CRB1* gene. Genetic analysis of his father revealed heterozygous p.R632X nonsense mutation and that of his mother revealed heterozygous c.652 + 1_652 + 4delGTAA splice site mutation.

Patient 2 (F84-kinki 1194)

Patient 2 was a 6-year-old boy from a family unrelated to Patient 1. He had night blindness from his infancy and was noted to be hyperopic at age 3. His parents were nonconsanguineous. His BCVA at age 6 was 0.3 OD and 0.3 OS. His cycloplegic refractive errors were +3.0 DS and −1.5 D cylinder (DC) ax180° in the right eye and +3.75 DS and −2.5 DC ax180° in the left eye.

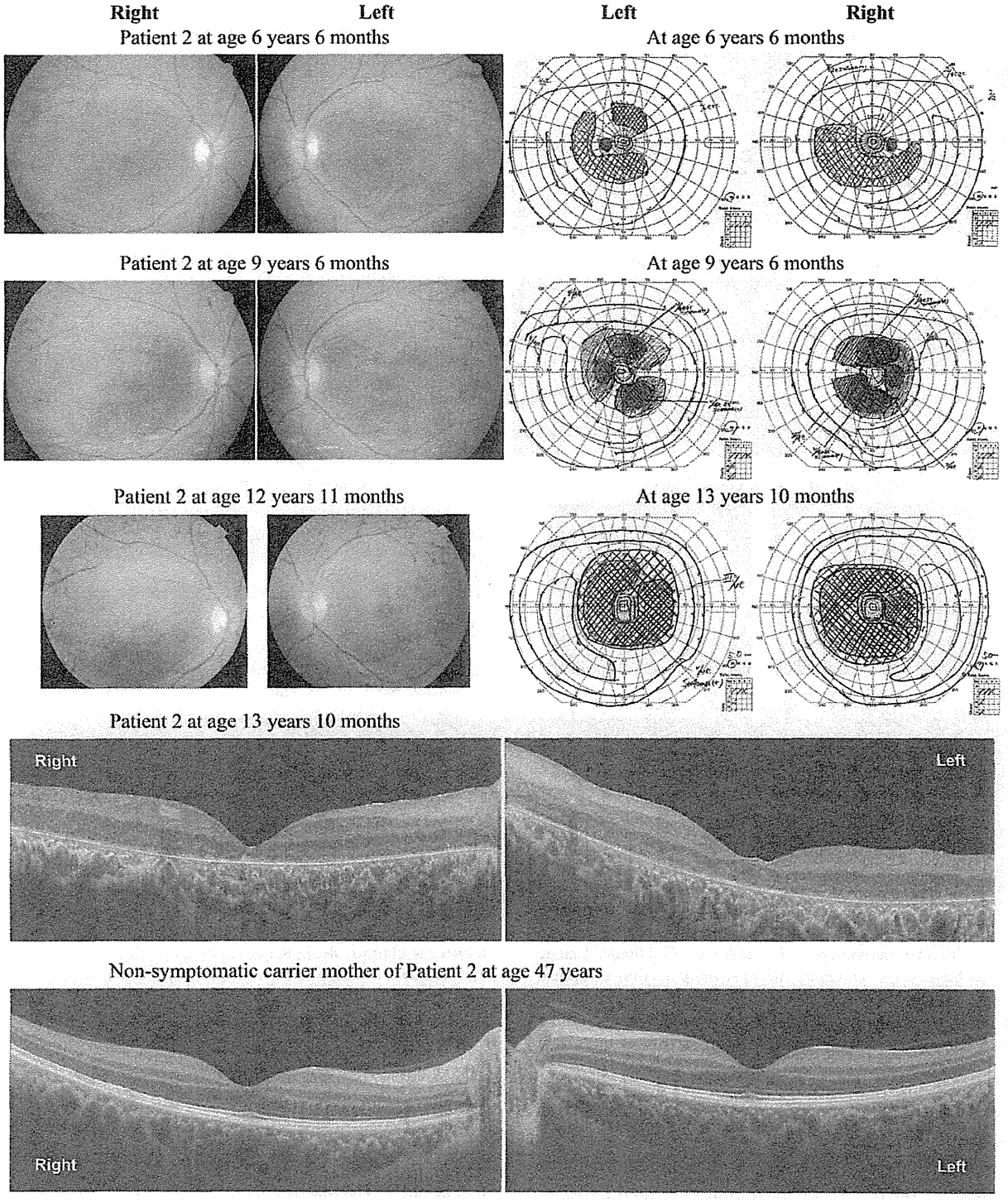

Fig. 2 Fundus photographs (*left column*), Goldmann kinetic visual fields (*right column*), and *horizontal* section of OCT image (*lower*) in Patient 2. The phenotype and clinical course are similar to those in Patient 1; however, Patient 2 has bone-spicule pigmentation. The OCT images of nonsymptomatic carrier mother are presented at the *bottom*; they are normally laminated. Length of the OCT scanning is 9 mm

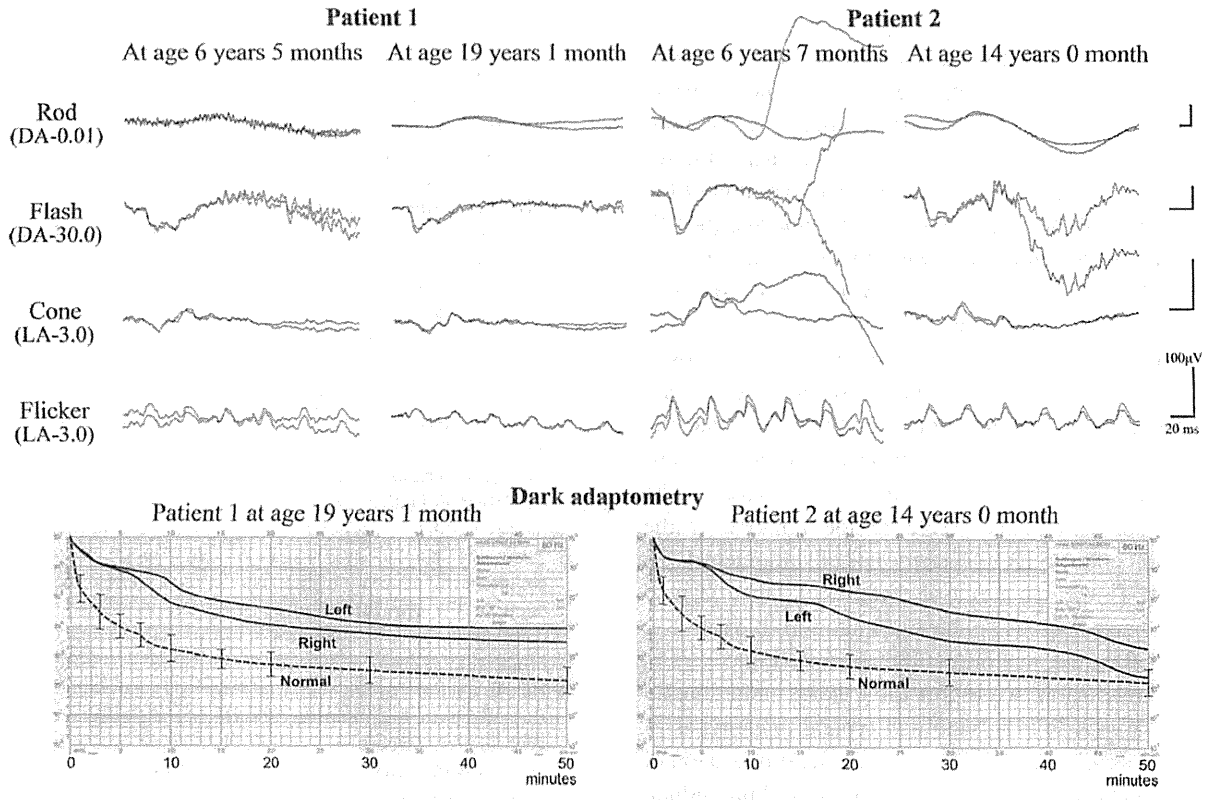

Fig. 3 Results of full-field electroretinography (ERG) (*upper*) and dark adaptometry (*lower*) in Patients 1 and 2. ERG responses from both eyes are superimposed. ERGs were not reduced much during the clinical follow-up. *Dashed lines* and *vertical bars* are the means ± standard deviations of normal controls. *DA* dark-adapted, *LA* light-adapted

He had no nystagmus. His phenotype and clinical course were similar to those in Patient 1; however, Patient 2 had bone-spicule pigmentation (Figs. 2, 3). He was observed for 8 years, and his BCVA at age 14 was 0.3 OD and 0.4 OS.

NGS analysis on his whole exome revealed 1,555,508 mutations compared with the reference human genome. Among them, 447 mutations remained as candidates after exclusion of common mutations. They were filtered, and six genes were selected as causal candidates. Finally, *CRB1* was selected as causative for his disease because the other genes were not registered in the RetNet^{TM} database [4].

Thus, genetic analysis revealed compound heterozygous mutations and c.652 + 1_652 + 4delGTAA and c.652 + 1_652 + 2insT splice site mutations in the *CRB1* gene. Genetic analysis of his father revealed heterozygous c.652 + 1_652 + 4delGTAA and that of his mother revealed heterozygous c.652 + 1_652 + 2insT splice site mutation. It was confirmed that his brother who had normal eyesight did not have these mutations.

Discussion

The three mutations in our patients, namely the p.R632X nonsense mutation, and the c.652 + 1_652 + 4delGTAA and c.652 + 1_652 + 2insT splice site mutations in the *CRB1* gene have not been reported as causative for retinal dystrophy. The first nonsense mutation is located in exon 6, and the latter two splice site mutations are in exon 2 of the 12 exons in the *CRB1* gene [15]. Thus, the first nonsense mutation truncates the protein to approximately one-half, and the latter two splice site mutations truncate the protein to 1/5 of the original size. Thus, they will lead to a loss of almost the entire molecule of the CRB1 protein.

Against expectations from these mutations, the phenotypes of our patients were not severe. They did

not have nystagmus, and they had relatively good daytime vision until the second decade of life. ERGs were recordable until the ages of 19 and 14 years (Fig. 3), and amplitudes were comparable to those reported [9]. These findings suggest that other unknown factors may influence the severity and variation of the phenotype of the *CRB1*-associated retinopathy [6–12].

A thickened and coarsely laminated retina was seen in the OCT images (Figs. 1, 2), which is characteristic of *CRB1*-associated retinopathy [9, 16]. This OCT finding is very similar to that reported by Jacobson et al. [16]. They reported that the disorganized retina was similar to an immature normal retina, and the disorganization resulted from an abnormal development caused by nonfunctioning CRB1 protein. The CRB1 protein plays a role in the polarity of the photoreceptor cells during retinal development [15]. The OCT image in the carrier mother of Patient 2, who had a heterozygous splice site mutation in exon 2 of the *CRB1* gene, had normal lamination (Fig. 2).

In conclusion, we report three novel mutations in patients with EORD, who had typical phenotypes of *CRB1*-associated EORD. Although the progression of their retinal dysfunction was slow, more longitudinal observations are needed because Henderson et al. [9] reported that vision in *CRB1*-associated patients declined to counting finger or worse in their thirties.

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Letter to the Editor

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Macular Electroretinogram in Stargardt's Disease/ Fundus Flavimaculatus

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Dear Editor

We appreciate the comments by Gundogan et al. [1] on our paper entitled 'Multifocal electroretinograms in Stargardt's disease/fundus flavimaculatus', published in *Ophthalmologica* [2]. They asked us to present more details on the full-field electroretinograms (ERGs) and also to calculate the correlation between the visual amplitudes of the multifocal ERGs' central response, the visual acuity and age for each type of Stargardt's disease/fundus flavimaculatus (SFF) [3].

For full-field ERGs, Itabashi et al. [4] from our laboratory published a detailed report on the retinal function in patients with SFF using the same ERG recording system as was used in our paper [fig. 7 in 4]. The cone responses (photopic b-wave) were significantly reduced in SFF, and the retinal function was the worst in SFF type 3E [4]. The full-field and multifocal ERG findings in our patients [2] are in good agreement with the results presented in the paper by Itabashi et al. [4].

We have also calculated the correlation between the central multifocal ERGs' responses and the best-corrected visual acuity (BCVA) for each type of SFF (fig. 1). As shown in figure 1a, c, no significant correlation was found between the BCVA and the

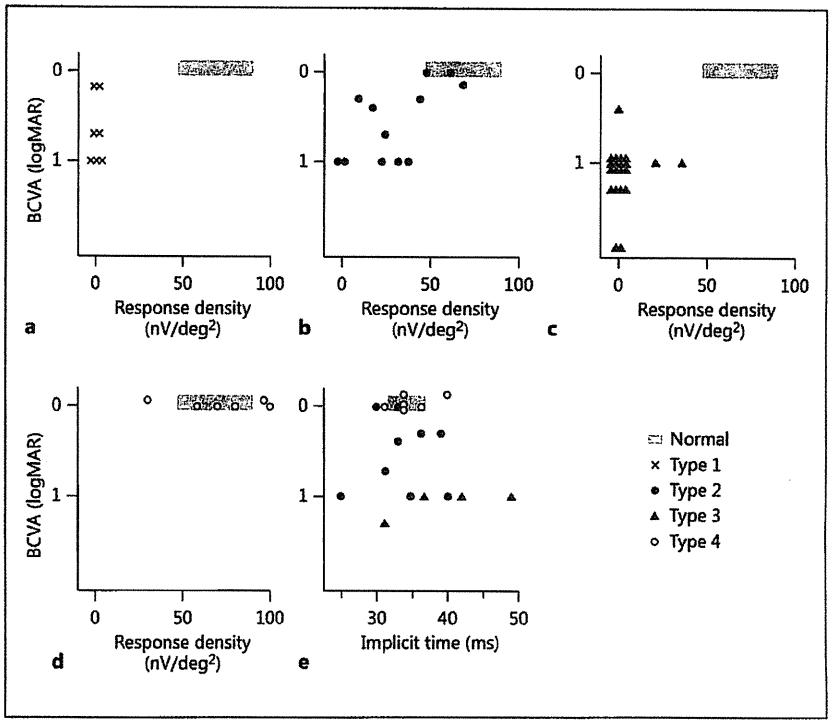

Fig. 1. Correlation between BCVA and P1 [5] of the multifocal ERGs' central response in each SFF type [3]. **a–d** Types 1–4. **e** Implicit time.

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central multifocal ERGs' responses for SFF types 1 and 3, because the responses were at noise level. In eyes with SFF type 2, the response densities of P1 [5] of the multifocal ERGs' central response appeared to be associated with the BCVA, but we did not perform any statistical analysis because of the small number of eyes. In eyes with SFF type 4, both the BCVA and the multifocal ERGs' central responses were well preserved. The implicit times of P1 of the multifocal ERGs' central response were not significantly correlated with the BCVA (fig. 1e). As shown previously [table 1 in 2], the BCVA and the multifocal ERGs' responses appear to depend more on the fundus appearance including SFF type than on age. However, Itabashi et al. [4] reported, for each patient, that the BCVA in eyes with SFF decreased with increasing age, and 25% of the patients over 40 years became legally blind.

We conclude that analyzing the ERGs is essential in evaluating the retinal function in eyes with SFF, and the amplitudes of the ERGs are well correlated with the type of SFF. Again, we thank Gundogan and colleagues for their interest in our paper.

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CLINICAL CASE REPORT

OAT mutations and clinical features in two Japanese brothers with gyrate atrophy of the choroid and retina

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Abstract
Background Gyrate atrophy (GA) of the choroid and retina is an extremely rare inherited chorioretinal dystrophy. Ornithine aminotransferase (*OAT*) gene mutations are identified in patients with GA. The purpose of this study was to report a novel deletion mutation of the *OAT* gene and describe clinical features of two brothers with GA in a Japanese family.
Methods We performed ophthalmic examinations, including best-corrected visual acuity, slit-lamp biomicroscopy, dilated funduscopy, fundus autofluorescence imaging, optical coherence tomography, visual field testing, and full-field electroretinography (ERG). Serum ornithine concentrations and *OAT* activities were analyzed. Mutation screening of the *OAT* gene was performed using Sanger sequencing.
Results Both brothers had compound heterozygous mutations (p.K169DfsX10 and p.R426X), one of which was novel. Their unaffected parents carried one of the mutations heterozygously. An arginine-restricted diet was started in the younger brother at the age of 2 years, while the diet was not initiated in the older brother until the age of 6 years. After more than 15 years of follow-up, the dietary treatment seemed to slow the progression of the chorioretinal lesions in the younger brother. However, when compared at the same age, the younger brother had more reduced ERG amplitudes and constricted visual fields than his older brother.
Conclusions We identified a novel frameshift mutation (p.K169DfsX10) in the *OAT* gene. While an early arginine-restricted dietary treatment suppressed the fundus changes of GA to some degree in the younger brother, the efficacy of suppressing the progression of visual function loss could not be clearly determined.

Keywords Inherited retinal disease · Gyrate atrophy · OAT · Mutation · Arginine-restricted diet

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Introduction

Gyrate atrophy of the choroid and retina (GA) is an extremely rare autosomal recessive chorioretinal

dystrophy. The ornithine aminotransferase (*OAT*) gene, localized on chromosome 10q26, is known as a causative gene of GA [1–3].

Ocular manifestations include constricted visual fields, elevated dark adaptation thresholds, myopia, cataracts, vitreous changes, a characteristic pattern of chorioretinal atrophy, and macular changes [4–11]. With regard to the general clinical course of GA, sharply demarcated circular patches of chorioretinal atrophy appear in the periphery of the retina in the first decade of life [12]. In addition, there have been several reports of macular changes with GA in the first or second decade of life despite the presence of preserved visual acuity [11, 13–17].

In GA patients, the ornithine concentrations in serum, urine, cerebrospinal fluid, and aqueous humor are 10–20 times higher than those observed in normal subjects [18]. In terms of treatment, use of an arginine-restricted diet limits the source of ornithine, which can lead to reducing serum ornithine levels to nearly normal levels [19]. Many studies report that use of an arginine-restricted diet [20–27] or a low-protein diet [28] may slow the progression of chorioretinal lesions and visual loss in GA. In addition, although several patients with GA have been shown to be responsive to vitamin B6 supplementation and have reduced serum ornithine concentrations, most GA patients are not responsive to vitamin B6 supplementation [29, 30].

OAT mutations were first identified in patients with GA in 1988 [31–33]. To date, 65 *OAT* mutations have been identified according to the Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/). While there have been several reports of GA in the Japanese population [29, 34–40], only ten *OAT* mutations have been positively identified in Japanese patients [38–40].

In this study, we identified compound heterozygous mutations of the *OAT* gene in two Japanese brothers with GA, with one of the mutations a novel deletion mutation. This report also describes the clinical features of the two brothers and evaluates the effect of reductions in the serum ornithine level on the retinal appearance and function.

Patients and methods

The protocol of this study was approved by the Institutional Review Board of The Jikei University School of Medicine. The protocol adhered to the tenets of the Declaration of Helsinki, and informed consent was obtained from all participants.

Clinical studies

The study was conducted in one Japanese family (Family, JU#0213) with GA. The patients examined were two brothers (II-1: older brother, II-2: younger brother) (Fig. 1a), with both undergoing ophthalmic examinations that included decimal best-corrected visual acuity (BCVA), slit-lamp biomicroscopy, dilated funduscopy, fundus autofluorescence imaging (FAI) (Spectralis HRA; Heidelberg Engineering, Heidelberg, Germany), and time-domain and spectral-domain optical coherence tomography (TD-OCT [OCT3 Stratus; Carl Zeiss Meditec AG, Dublin, CA, USA] and SD-OCT [Cirrus HD-OCT; Carl Zeiss Meditec AG]). The older brother (II-1) additionally underwent fluorescein angiography (FA). Full-field electroretinography (ERG) was performed in accordance with the protocols of the International Society for Clinical Electrophysiology of Vision. The procedure and conditions have been previously reported in detail [41]. Visual fields were tested using kinetic perimetry (examined by Goldmann perimeter (GP); Haag Streit, Bern, Switzerland).

Analysis of ornithine concentration and OAT activity

Diet adherence was monitored by self-reporting and measurement of serum ornithine concentrations during patient follow-up visits to the Department of Pediatrics. The *OAT* activities for both patient II-1 and his parents were measured using cultured skin fibroblasts as per the previously published method [42].

Molecular genetic studies

Blood samples were obtained from four members of the same family (two affected brothers and their parents). Genomic DNA was extracted from peripheral blood leukocytes using a blood DNA isolation kit (Puregene; Gentra Systems, Minneapolis, MN, USA), which was used as the template for the polymerase chain reaction (PCR) when amplifying the *OAT* gene. All primers were produced by Sigma-Aldrich (Tokyo, Japan). All coding exons (exons 3–11) of the *OAT*

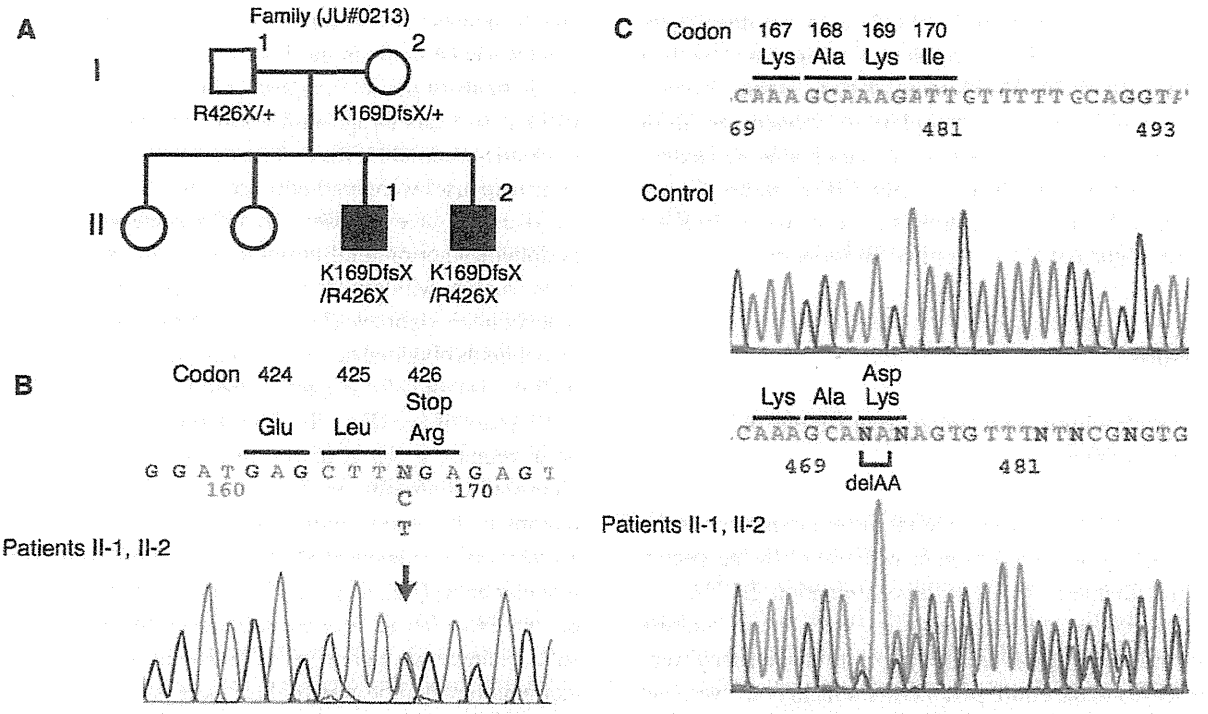

Fig. 1 Pedigree of the Japanese family (Family, JU#0213) and the sequencing results of the *OAT* gene in patients II-1 and II-2 and in the control. **a** Unaffected family members (males, *open squares*; females, *open circles*) and affected members (males, *solid squares*). **b** Partial nucleotide sequences of exon 11 in patients II-1 and II-2. A single-nucleotide mutation (c.1276C>T) results in the substitution of a stop codon (TGA) for arginine (CGA) at amino acid position 426 (p.R426X). **c** Partial nucleotide sequences of exon 5 in a control, and in patients II-1 and II-2. A deletion mutation (c.504_505delAA) results in the substitution of aspartic acid (GAT) for lysine (AAG) at amino acid position 169 and a frame shift that leads to a premature termination codon at ten amino acid residues downstream from the mutation (p.K169DfsX10)

Table 1 Polymerase chain reaction primers used to amplify the coding exons (exons 3–11) of the *OAT* gene

Region to amplify	Primer name	Sequence (5′–3′)	Annealing temperature
Exon 3 (406 bp)	OAT-3F	AGGCATAAGCCAAGGATTCTC	62°
	OAT-3R	TTAACCATGTCTGCAATATACAC	
Exons 4 & 5 (652 bp)	OAT-4F	TAGGCATTCAGAGGGCTTGC	62°
	OAT-5R	ACTCCAGGGCTCAAAGACTC	
Exon 6 (376 bp)	OAT-6F	TTGAGTCAAACTTCTTGTGGTG	62°
	OAT-6R	ACTAATTGATCGCTACTGAGAAC	
Exon 7 (272 bp)	OAT-7F	ATATGTGTGGTATATGCTTTCAG	62°
	OAT-7R	AGCCCATTCAGCCTCATCAC	
Exon 8 (429 bp)	OAT-8F	GAGGGCACATCAGAATTACAC	62°
	OAT-8R	GTAAGTGGGTCACACACTGG	
Exon 9 (362 bp)	OAT-9F	TGCTTAGTAGAATGCTTAGTGC	62°
	OAT-9R	AATCCAGTCTACTAGGCCAAG	
Exon 10 (393 bp)	OAT-10F	AAAGCAAGACTCTGAGCTAGTG	62°
	OAT-10R	CCAAGTGTATTTTAGGTCTTCC	
Exon 11 (330 bp)	OAT-11F	TGCCCATACATACGGCAAGG	62°
	OAT-11R	TCATGGGAGTGGAATGTGCC	

gene were amplified by PCR using primer pairs (Table 1). The PCR products were purified with a QIAquick PCR Purification Kit (Qiagen, Tokyo, Japan) and used as the template for sequencing. Both strands were sequenced on an automated sequencer (3730*xl* DNA Analyzer; Applied Biosystems, Foster City, CA, USA) through the use of a BigDye Terminator Kit V3.1 (Applied Biosystems).

Results

Ophthalmology clinical course in patient II-1 (older brother)

Patient II-1 underwent ophthalmic examinations at the age of 6 years and 4 months because of the suspected night blindness. At his initial examination, BCVA was 1.0 (with −2.50 diopters) in both eyes. Sharply demarcated circular areas of chorioretinal atrophy were observed in the entire peripheral retina in both eyes, and he had an extremely high serum ornithine concentration of 1,041 μmol/l (normal range 47–72 μmol/l). He was subsequently diagnosed with GA at the age of 6 years and 5 months. At 7 years of age, GP showed constricted visual fields in the I-4 isopters with preserved visual fields of the V-4 isopters in both eyes (Fig. 2a). ERG at 10 years of age showed that the rod responses were non-recordable, while the standard-combined and cone responses were markedly reduced (Fig. 3a). FA at 11 years of age showed hypofluorescence in the peripheral chorioretinal atrophic areas in both of his eyes, and a window defect was observed near the macula in his right eye (Fig. 4a). Comparisons with the visual fields obtained at 7 years of age (Fig. 2a) showed both the I-4 and V-4 isopters became more constricted at 17 years of age (Fig. 2b). Photographic montages of both retinas at 18 years of age showed extensive chorioretinal atrophy, with retinal pigment epithelium clumps in the entire periphery (Fig. 4b). TD-OCT at 18 years of age showed slight cystic changes in both macular areas (Fig. 4c). At 23 years of age, his BCVA decreased to 0.6 in both eyes. Mild posterior subcapsular cataracts were observed in both eyes. Funduscopic examination demonstrated that there was a definite posterior expansion of the chorioretinal atrophy. FAI showed a loss of autofluorescence that corresponded to the chorioretinal atrophic areas, and there was ring-

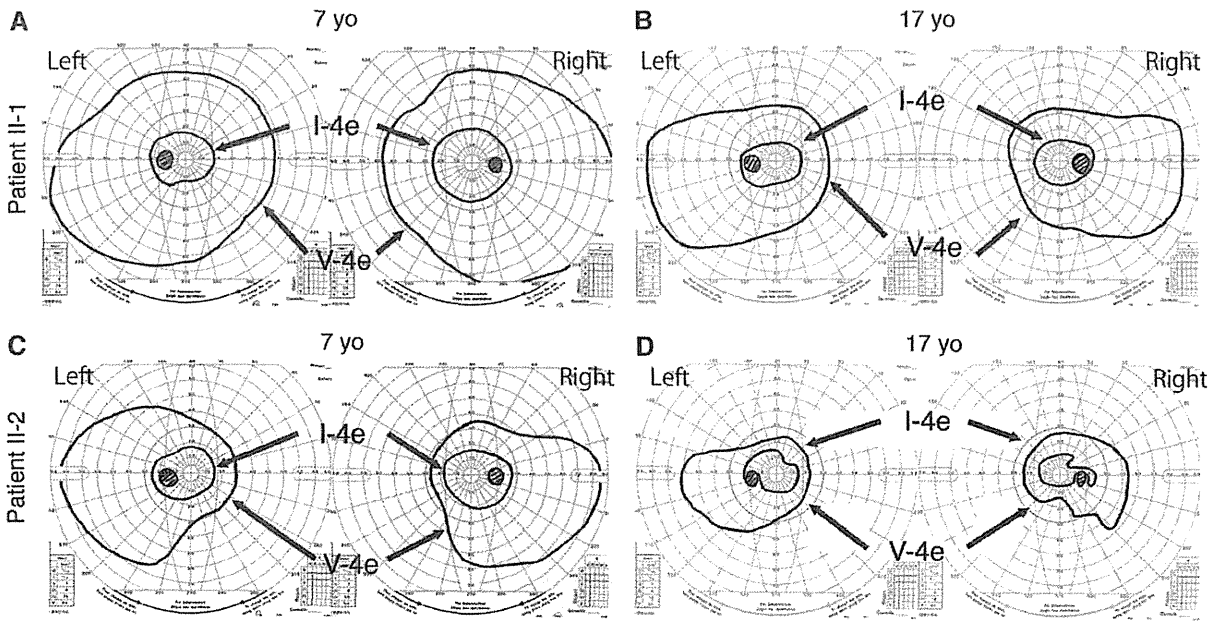

Fig. 2 Visual fields with Goldmann kinetic perimetry (GP) in patients II-1 and II-2. **a** GP of patient II-1 at 7 years of age shows constricted visual fields in the I-4 isopters with preserved visual fields of the V-4 isopters in both eyes. **b** GP of patient II-1 at 17 years of age shows additional constricted visual fields in both the I-4 and V-4 isopters as compared to the results obtained at 7 years of age. **c** GP of patient II-2 at 7 years of age shows constricted visual fields in the I-4 and V-4 isopters. **d** GP of patient II-2 at 17 years of age shows additional constricted visual fields in both the I-4 and V-4 isopters as compared to the results obtained at 7 years of age

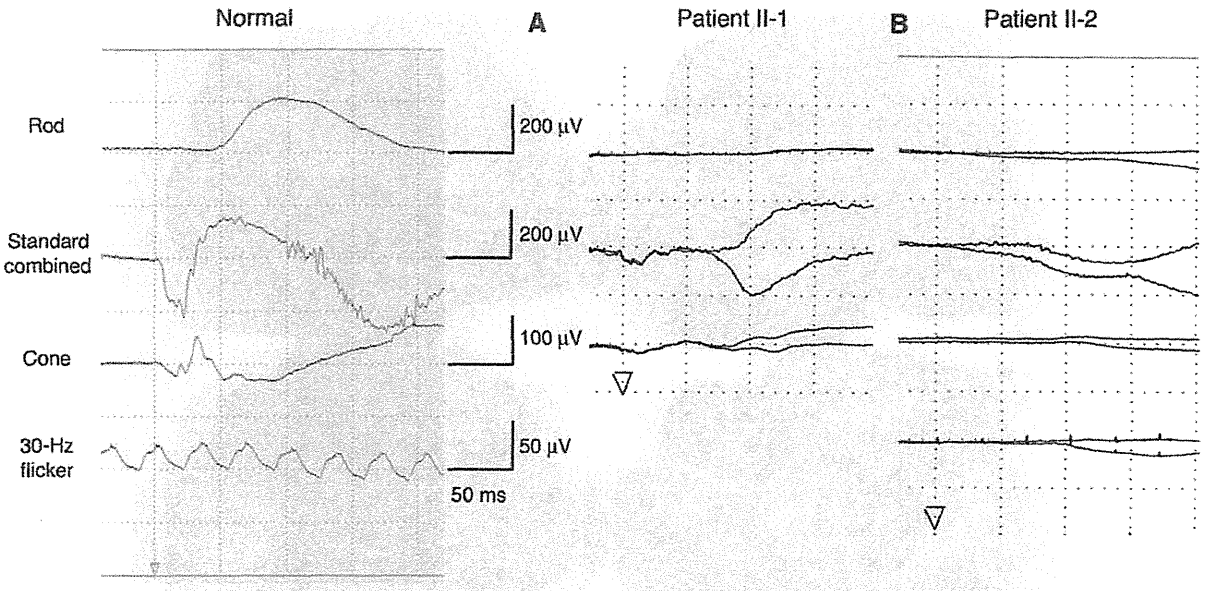

Fig. 3 Full-field electroretinograms (ERGs). **a** The ERGs at 10 years of age show that the rod responses were non-recordable and that the standard-combined and cone responses are markedly reduced. **b** At 10 years of age, rod, standard-combined, photopic, and 30-Hz flicker ERGs were non-recordable

shaped hyperautofluorescence around the macula in both eyes (Fig. 5a). SD-OCT indicated that there were moderate cystoid spaces within the inner nucleus layer (INL) and marked thinning of the outer nuclear layer (ONL) with a few smaller cysts in both eyes. In addition, there was marked splitting of the INL in the temporal area of his right eye (Fig. 5b).

Ophthalmology clinical course for patient II-2 (younger brother)

Patient II-2 was first examined at the age of 6 months. His serum ornithine concentration was 145 μmol/l, and there was no ophthalmic examination performed. At the age of 2 years and 8 months, his serum ornithine concentration was 952 μmol/l. Fundus examination showed there was retinal degeneration in the superior peripheral area in both eyes. He was diagnosed with GA. At 4 years of age, the BCVA was 1.0 in his right eye and 1.2 in his left eye. GP at 7 years of age showed constricted visual fields of the I-4 and V-4 isopters (Fig. 2c). At 10 years of age, rod, standard-combined, cone, and 30-Hz flicker ERGs were non-recordable (Fig. 3b). Although photographic montages at 12 years of age demonstrated that there was retinal degeneration similar to retinitis pigmentosa in the entire periphery of both eyes (Fig. 4d), chorioretinal atrophy at the superior retinal arcade was observed in the right eye (Fig. 4d). TD-OCT performed at 12 years of age showed macular thinning (Fig. 4e). At 17 years of age, his BCVA was 0.7 in the right eye and 0.9 in the left eye. There was no subcapsular cataract in either eye. Funduscopic examination indicated that there was a definite posterior expansion of the chorioretinal atrophy. FAI also showed there was a loss of autofluorescence in the peripheral chorioretinal atrophic areas and ring-shaped hyperautofluorescence around the macula in both eyes (Fig. 5c). SD-OCT showed there were moderate cystoid spaces within the INL and an abnormally thin ONL with a few smaller cysts in both eyes. In addition, there was marked splitting of the INL in the temporal area of his left eye (Fig. 5d). Compared to the findings obtained at 7 years of age (Fig. 2c), the visual fields of both the I-4 and V-4 isopters became more constricted at 17 years of age (Fig. 2d).

Pediatric clinical course for patient II-1

In order to determine the possible treatments, patient II-1 was tested for *OAT* activity using skin

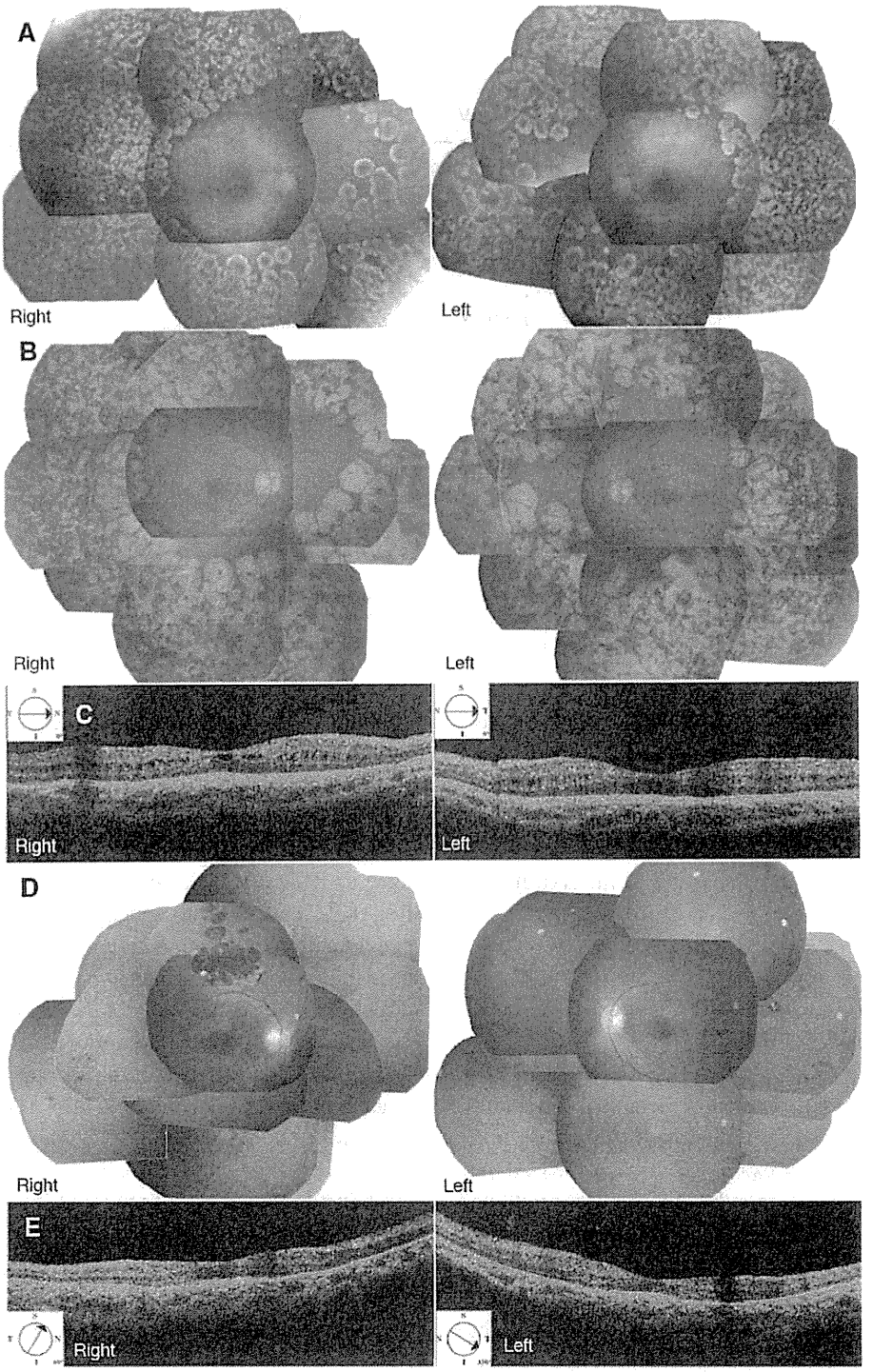

Fig. 4 Fluorescein angiography (FA), photographic montages, and time-domain optical coherence tomography (TD-OCT) of patients II-1 and II-2. **a** FA of patient II-1 at 11 years of age shows hypofluorescence in the peripheral chorioretinal atrophic areas in both eyes and slight hyperfluorescence near the macula in his right eye. **b** Photographic montages of both retinas of patient II-1 at 18 years of age show extensive chorioretinal atrophy with retinal pigment epithelium clumps in the entire periphery. **c** TD-OCT of patient II-1 at 18 years of age shows slight cystic changes in both macular areas. **d** Although photographic montages of patient II-2 at 12 years of age show retinal degeneration similar to retinitis pigmentosa in the entire periphery of both eyes, there is chorioretinal atrophy at the superior retinal arcade in the right eye. **e** TD-OCT of patient II-2 at 12 years of age shows macular thinning

fibroblasts at 6 years of age (Table 2). Concentrations of 20 or 400 μM of pyridoxal phosphate (an activated form of vitamin B6) resulted in a markedly lower *OAT* activity in the patient as compared to his father (I-1), his mother (I-2), and a control (Table 2). Moreover, *OAT* activities in patient II-1 were extremely reduced as compared to the levels in both his parents and the control. Vitamin B6 supplementation also proved to be ineffective in this patient. In fact, after patient II-1 received vitamin B6 supplementation, there was no reduction in the serum ornithine concentrations. Therefore, the patient was prescribed an arginine-restricted diet at the age of 6 years and 5 months, with follow-ups conducted several times each year. His serum ornithine concentrations repeatedly increased and then decreased. Moreover, even after being placed on the arginine-restricted diet, his serum ornithine concentrations continued to be above the normal range (Fig. 6).

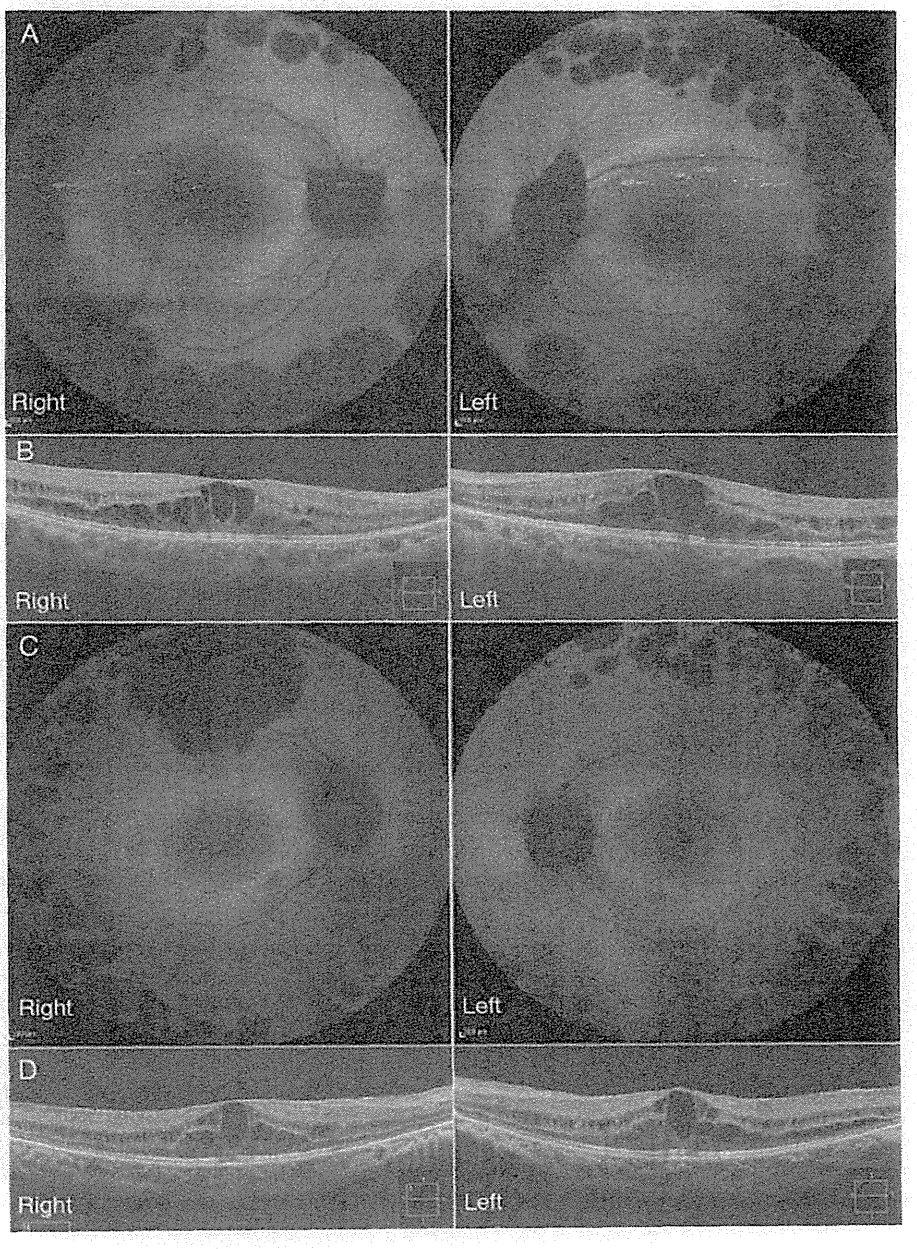

Fig. 5 Fundus autofluorescence imaging (FAI) and spectral-domain optical coherence tomography (SD-OCT) of patients II-1 (at 23 years of age) and II-2 (at 17 years of age). **a** FAI of patient II-1 shows loss of autofluorescence in the chorioretinal atrophic areas and ring-shaped hyperautofluorescence around the macula in both eyes. **b** Horizontal SD-OCT images through the fovea of patient II-1 show moderate cystoid spaces within the inner nucleus layer (INL) and an abnormally thin outer nuclear layer (ONL) with a few smaller cysts in both eyes. There is also marked splitting of the INL in the temporal area of his right eye. **c** FAI of patient II-2 shows loss of autofluorescence in the chorioretinal atrophic areas and ring-shaped hyperautofluorescence around the macula in both eyes. **d** Horizontal SD-OCT images through the fovea of patient II-2 show moderate cystoid spaces within the INL and an abnormally thin ONL with a few smaller cysts in both eyes. There is also marked splitting of the INL in the temporal area of his left eye

Table 2 Ornithine aminotransferase activity (nmoles/h/mg)

	PLP (20 μM)	PLP (400 μM)
Patient II-1 (at 6-year old)	9.71	13.4
Father (I-1)	170	175
Mother (I-2)	261	257
Control	210	Not measured

PLP pyridoxal phosphate

Pediatric clinical course for patient II-2

From the time the patient was initially placed on an arginine-restricted diet at the age of 2 years and 8 months and until he was 9 years of age, his serum ornithine concentrations were well controlled (Fig. 6). However, after 9 years of age, his serum ornithine concentrations began to increase and remained above the normal range (Fig. 6).

Molecular genetic findings

Mutation analysis revealed that the affected brothers (II-1 and II-2) had compound heterozygous mutations, c.504_505delAA (exon 5) and c.1276C>T (exon 11). The single-nucleotide mutation (c.1276C>T) results in a stop codon at the amino acid position 426 (p.R426X), whereas the deletion mutation (c.504_505delAA) causes the substitution of aspartic acid (GAT) for lysine (AAG) at position 169 and a frame shift that leads to a premature termination codon (PTC) at ten amino acid residues downstream from the mutation (p.K169DfsX10). Although the nonsense mutation (p.R426X) has been found in Japanese GA patients [29, 43], the truncating mutation (p.K169DfsX10) has never been reported. Both the patients' father (I-1) and mother (I-2) carried the heterozygous mutation, p.R426X and p.K169DfsX10, respectively. However, the novel mutation (p.K169DfsX10) has not been

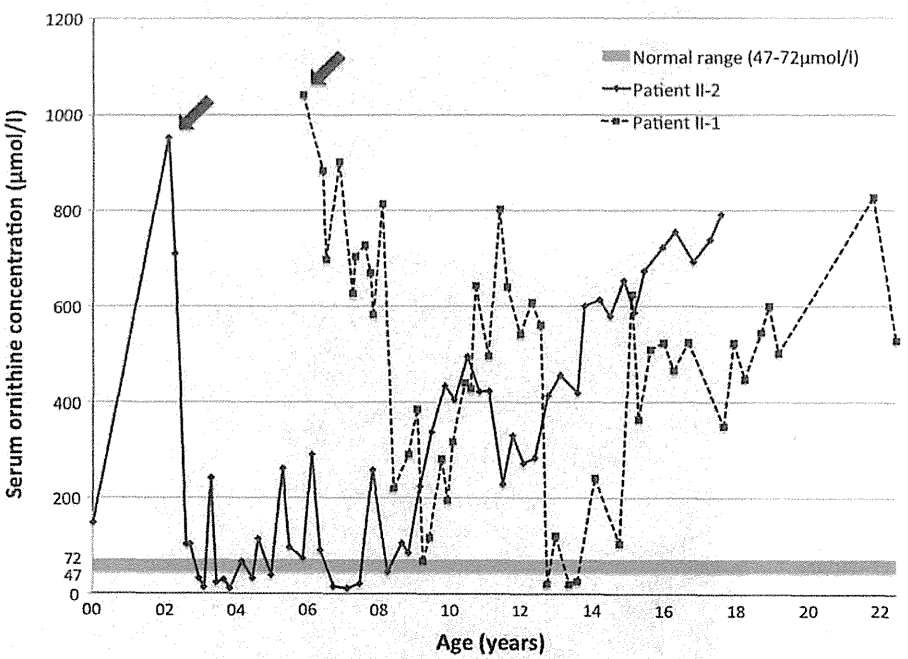

Fig. 6 Serum ornithine concentrations for patients II-1 and II-2. Broken line indicates serum ornithine concentrations for patient II-1. The concentration shows repeated increases and decreases. Despite the arginine-restricted dietary treatment, serum ornithine concentrations are above the normal range during the follow-up period. Solid line indicates serum ornithine concentrations for patient II-2. After beginning the dietary treatment at the age of 2 years and 8 months and up until 9 years of age, the serum concentrations are well controlled. However, once the patient reached 9 years of age, the concentrations are no longer well controlled and continued to be above the normal range. The *two arrows* indicate the beginning of the dietary treatment in both patients. The gray area shows the normal range of serum ornithine concentrations (47–72 μmol/l)

reported in the Single Nucleotide Polymorphism Database (dbSNP; http://www.ncbi.nlm.nih.gov/projects/SNP/), the 1,000 genomes database (http://browser.1000genomes.org/index.html), the NHLBI Sequencing Project/Exome Variant Server Database (EVS; http://evs.gs.washington.edu/EVS/), or in the HGMD.

Discussion

This study reports clinical and genetic features of two brothers with GA (patients II-1 and II-2) who had compound heterozygous truncating mutations, one of which was a novel mutation (c.504_505delAA, p.K169DfsX10). Mutations in the *OAT* gene are scattered over 9 coding exons, with some of the mutations able to generate truncating mutations that result in PTC. A mechanism called nonsense-mediated mRNA decay (NMD) is known to specifically degrade mRNA transcripts with PTC. Analysis of transcripts with truncating mutations (or PTC) has demonstrated that the amounts of OAT mRNA were markedly reduced in segments of the mRNA encoded by exon 10 or by more upstream exons as compared with those encoded by the last exon (exon 11) [43, 44]. More specifically, mRNA transcripts with c.192_193delAG (exon 4), p.Y209X (exon 6), p.Y299X (exon 8), c.952delG (exon 9), and c.1031delA (exon 10) mutations have been shown to lead to abnormally low levels compared with normal levels, whereas the amounts of mRNA transcripts with p.R396X (exon 11), p.G401X (exon 11), and p.R426X (exon 11) mutations are close to normal levels [43, 44]. Our current patients were found to have the compound heterozygous truncating mutations, p.K169DfsX10 (exon 5) and p.R426X (exon 11). Therefore, it is more likely that the mRNA transcript with the p.K169Dfs X10 mutation would be unstable and degraded by the NMD mechanism.

Currently, genotype–phenotype correlations in GA have yet to be clearly defined [45]. Phenotypes in Japanese GA patients with the *OAT* gene mutations (Table 3) are indeed markedly variable. For example, the age at the time of diagnosis ranges from 2 to 35 years of age, the BCVA ranges from 0.03 to 1.2, and the ERG findings range from subnormal to non-recordable. Therefore, we focused on the phenotype of a Japanese patient who have had the homozygous

Table 3 Ophthalmic and genetic findings in Japanese patients with gyrate atrophy of the retina and choroid and reported *OAT* mutations

Patient number	Patient ID in source publication, gender	Diagnosed age	Mutations		BCVA at first exam		ERG	Visual fields	Notes	References
			Allele 1	Allele 2	Right	Left				
1	Patient 1, M	23	E125X	N326K	0.1	0.08	Non-Recordable	Constricted	Disk pale	[39]
2	Patient 2, F	6	H319Y	H319Y	0.25	0.25	Almost Extinct	Constricted	Disk slightly pale	[39]
3	Patient 3, F	24	G142E	G142E	0.1	0.1	Non-Recordable	Constricted	R-VH	[39]
4	Patient 4, M	17	T181 M	T181M	0.03	0.6	Subnormal	Constricted		[39]
5	Patient 5, M	8	R426X	R426X	0.4	0.4	Subnormal	Constricted		[39]
6	Patient 6, M	5	D195Y	R271 K	0.3	0.3	Subnormal	ND		[39]
7	Patient 7, ND	24	R271K	R271K	ND	ND	ND	ND		[39]
8	Patient, M	35	Q90E	Q90E	ND	ND	ND	ND		[38]
9	Patient, M	7	G237D	G237D	ND	ND	ND	ND	L-RD	[40]
10	II-1 (JU#0213), M	6	K169DfsX10	R426X	1.0	1.0	Severely Reduced	Constricted		Current study
11	II-2 (JU#0213), M	2	K169DfsX10	R426X	1.0	1.2	Non-Recordable	Constricted		Current study

BCVA decimal best-corrected visual acuity, *ERG* electroretinograms, *M* male, *F* female, *ND* not described; *VH* vitreous hemorrhage, *RD* retinal detachment

p.R426X mutation [29, 43], which is the same as one of the compound heterozygous mutations that was found in our current patients. Ophthalmological evaluations that have been previously performed in the Japanese patient with the homozygous p.R426X mutation showed that there were subnormal ERG responses at the age of 8 [29, 43]. On the other hand, the ERG findings for our current patients revealed that the cone responses were extremely reduced or absent and that the rod responses were also absent at the age of 10. These ERG findings demonstrated that the retinal function of our patients may have been more severely damaged than that of the previously reported patient with the homozygous p.R426X mutation. This suggests that the p.R426X (in exon 11) is likely a less severe mutation than p.K169DfsX10 (in exon 5).

With regard to the clinical courses between affected siblings with GA, Kaiser-Kupfer et al. [25] analyzed six pairs of affected siblings and reported that there were strikingly similar phenotypes in the affected members of the same pair of siblings. However, variations in the severity of the ERG amplitudes and cataract formation indicated that the phenotypes obviously differed between the pairs of siblings [25]. These findings strongly suggest that intrafamilial variation is less than interfamilial variation in GA patients, thereby showing that genetic heterogeneity plays a role in the phenotypic variation of GA. A previous study that compared two siblings with GA demonstrated that early implementation of an arginine-restricted dietary treatment in the younger of the two siblings appeared to slow the progression of the chorioretinal lesions and resulted in a unique fundus appearance that was similar to an early stage of retinitis pigmentosa [26]. This study also concluded that long-term substantial reduction of serum ornithine levels may slow the progressive loss of visual function to some extent [26]. In our current cases, the arginine-restricted diet of patient II-2 was started earlier and his serum ornithine concentrations were well controlled until 9 years of age. As a consequence, the fundus appearance of patient II-2 at 12 years of age was similar to that of retinitis pigmentosa and unlike that found in GA (Fig. 4d). However, between the ages of 9–17 years, the serum ornithine concentrations for patient II-2 were no longer well controlled, which suggests that adherence to the arginine-restricted diet might not have been adequate. The fundus appearance (data not shown) and FAI (Fig. 5c) of patient II-2 at 17 years of age were clearly worse than those for the patient at 12 years of age (Fig. 4d). On the other hand, even though the ornithine concentrations were well controlled in patient II-2 until he was 9 years old, patient II-2 had more decreased ERG amplitudes and constricted visual fields as compared to patient II-1 (Figs. 2 and 3). When taken together, although an arginine-restricted diet may slow the progression of the chorioretinal lesions of GA, as was distinctly seen in patient II-2, there were no clear differences noted between the two patients with regard to visual function.

In conclusion, we evaluated two patients with GA in a Japanese family who carry a novel frameshift mutation of the *OAT* gene. While an early arginine-restricted dietary treatment suppressed the degree of the peripheral fundus changes of GA, no differences in the retinal function were found between the patients.

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Conflict of interest The authors declared no conflict of interest about this study.

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