thelium in the macula in all four cases, and granular change in the macula in Case 4. The visual acuity in Cases 1 and 2 did not change during the follow-up period, but the acuity in Cases 3 and 4 gradually decreased. Photoreceptor abnormalities in optical coherence tomography were found in all the cases, but were more severe in Cases 3 and 4.

Conclusion: The long-term courses in Japanese patients were variable. The OCT was helpful in evaluating the disease progression.

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Key words: Cone dystrophy, *KCNV2*, Electroretinogram (ERG), Optical coherence tomography (OCT), Long-term follow-up

I 緒 言

「杆体反応の増強を伴う錐体ジストロフィ」は1983年Gourasらにより最初に報告された，比較的まれな常染色体劣性遺伝の錐体機能不全である[1]．主な症状は視力低下，色覚異常，羞明，夜盲，幼少時の眼振などである．異常所見は乳児期から存在するものと考えられるが，視力低下，色覚異常，羞明などの自覚症状は幼児期以降に顕性化することが多い．一般に羞明の自覚は強いが，夜盲は後期になるまで出現しないことが多いともいわれている[2]．全視野網膜電図(ERG)においては杆体b波の振幅が光刺激の増強に伴って著しく増大するという特徴がみられ，本疾患の名称の由来となっている．2006年にはWuらにより，本疾患の原因遺伝子として*KCNV2*が同定された[3]．このため最近では本疾患を*KCNV2*-related retinopathy(*KCNV2*関連網膜症)と呼ぶ傾向にある[4]．

我が国では1986年にYagasakiら[5]，1993年にKatoら[6]，2005年にTanimotoら[7]が本疾患について論文報告をしているが，眼底に異常所見が乏しい症例が多く診断が困難なためか，その報告数は非常に少ない．近年欧米を中心に，*KCNV2*遺伝子の新たな変異が相次いで報告されている[3,4,8)-13)]．1アミノ酸置換から大きな領域の欠損まで，発症に関与すると思われる50種類以上の多型が報告されているが，遺伝子型と表現型の対応関係はいまだに明らかにされていない．しかし日本人患者の遺伝子変異や長期経過に関する報告はまだなく，詳細な長期経過については海外においても報告が少ない．

本論文では，「杆体反応の増強を伴う錐体ジストロフィ」と確定診断した日本人患者3家系4名について，視力の長期経過とともに光干渉断層計(optical coherence tomography：OCT)，眼底自発蛍光(fundus autofluorescence：FAF)などの検査所見を含めた臨床像を紹介する．

II 症 例

症例1：24歳，女性(症例2の姉)．

主訴：視力低下，夜盲．

既往歴：特になし．

家族歴：次弟(症例2)に同様の眼症状が生じている．長弟には幼少時からの視力不良および外斜視があるが，知的障害を伴っており詳細な眼科的検査が困難であった．

発症および経過：就学時健診で両眼の視力不良を指摘され9歳時に帝京大学病院眼科を受診した．矯正視力は右(0.7× −1.25 D⊂cyl −2.00 D Ax 160°)，左(0.7× −2.25 D⊂cyl −1.25 D Ax 10°)．両眼の前眼部および中間透光体に異常なく，眼底は黄斑部の網膜色素上皮に軽度の色調不整がみられる以外は概ね正常であった．Goldmann視野検査(Goldmann perimetery：GP)，頭部computed tomography(CT)はいずれも正常であった．9歳時のbright flash ERGは本疾患に特徴的な矩形a波を示していたが，当時本疾患の認知度が低かったためERGは正常と判定されていた．このため非器質性(心因性)視力障害を疑われ精神科を受診したが，特に異常は認められず，11歳まで経過観察されていた．14歳時より平和眼科にて経過観察を行った．同院初診時の矯正視力は右(0.9× −3.50 D⊂cyl −2.75 D Ax 180°)，左(0.9× −3.75 D⊂cyl −2.00 D Ax 15°)．色覚検査では，東京医科大学式色覚検査表(TMC表)と石原式色覚検査表のすべての表が読めず，また，パネルD-15色相配列テストはpassであった．前眼部，中間透光体に異常はみられず，眼底所見はほぼ正常であった．18歳時に精査目的にて東京医療センターを受診．初診時の矯正視力は右(0.6× −3.75 D⊂cyl −2.50 D Ax 175°)，左(0.5× −4.25 D⊂cyl −1.50 D Ax 5°)．前眼部および中間透光体に異常なく，眼底は黄斑部の網膜色素上皮に軽度の色調不整がみられる以外は概ね正常であった．International Society for Clinical Electrophysiology of Vision(ISCEV) standard[14]に基づいて全視野ERGを実施したところ，杆体反応(Dark-adapted 0.01)におけるb波の頂点潜時延長，最大応答(Dark-adapted 30.0)における矩形a波とb波の振幅増強，錐体反応(Light-adapted 3.0)におけるa波およびb波の著明な振幅低下を認め，「杆体反応の増強を伴う錐体ジストロフィ」と診断した．最終矯正視力は24歳時で右(0.6× −4.25 D⊂cyl −2.75 D Ax 175°)，左(0.7× −5.50 D⊂cyl −2.00 D Ax 175°)である．

なお，夜盲は初期から自覚していたが，羞明の自覚はないとのことだった．ただし眼科外来における各検査時には，光に対してひどくまぶしがる様子をみせていた．

原著論文 / 症例報告

10年以上の長期観察を行った杆体反応の増強を伴う錐体ジストロフィの4例

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要　約

背　景：「杆体反応の増強を伴う錐体ジストロフィ」については国内での報告が少なく，また長期経過についての記載はない．今回，*KCNV2*遺伝子変異を有する日本人患者について，10年～15年間の長期経過観察を行ったので報告する．

症　例：対象は3家系4例，2例が姉弟(症例1：24歳女性，症例2：17歳男性)，2例は孤発例(症例3：17歳女性，症例4：21歳女性)である．全症例で本疾患に特徴的な網膜電図が得られ，黄斑部の色調に軽度の不整を認めた．症例4では経過中に黄斑部に顆粒状変化が生じた．視力は症例1，2では大きな変化はなかったが，症例3，4では経過観察期間中に次第に低下した．光干渉断層計(OCT)では中心窩における視細胞層の異常を全例で認め，特に視力低下の生じた症例3，4で顕著であった．

結　論：本疾患の長期的な経過には症例による違いがみられた．進行度の評価にはOCTが有用であった．(日眼会誌 117：629-640，2013)

キーワード：錐体ジストロフィ，*KCNV2*，網膜電図，光干渉断層計，長期経過

Original Article / Case Report

Long-term Observation over Ten Years of Four Cases of Cone Dystrophy with Supernormal Rod Electroretinogram

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Abstract

Background: 'Cone dystrophy with a supernormal rod electroretinogram (ERG)' is rare form of cone dystrophy, and no longitudinal description of the disease course has been reported in a Japanese population. Here, we describe long-term courses of 10 to 15 years in four Japanese patients with mutations in the *KCNV2* gene.

Cases: Four patients from three families were recruited. Two were siblings (Case 1, 24 y/o women ; Case 2, 17 y/o man), and two were sporadic cases (Case 3, 17 y/o women ; Case 4, 21 y/o women). All the patients presented with characteristic ERG findings. There were minimal abnormalities in fundus appearance : slight mottling of retinal pigment epi-

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roles in targeting ALMS1 to the centrosomes and ciliary basal bodies [44]. In our patients, if the truncated protein caused by the mutation (p.Q2051X) is expressed in the retina, the protein would not contain the two regions important for targeting (residues 2,261–2,602 and residues 3,176–4,169) or the putative leucine-zipper and ALMS motifs. Therefore, this truncated mutation would cause loss of function of ALMS1, resulting in the AS phenotype. Although genotype–phenotype correlations are not clear among AS patients with *ALMS1* mutations [45,46], patients with mutations in exon 8 are reported to have delayed and milder renal complications compared with those with mutations in exons 10 and 16 [13]. In our patients, the p.Q2051X mutation was present in exon 8, explaining normal renal function.

The syndromic disorder AS is often misdiagnosed as LCA, ACHM, or other ciliopathies [11,20,21], so the identification of diagnostic mutations is important. Also, early diagnosis may improve longevity and long-term quality of life. By the whole-exome sequencing analysis technique, we were able to comprehensively determine the disease-causing gene mutation by using the fewest samples possible from the pedigree and analyzing all exon sequences in a relatively short time. Because of the autosomal recessive inheritance pattern, the parents and two affected brothers were enough to narrow down the candidate genes. Consequently, we identified a single causative gene mutation (p.Q2051X of *ALMS1*). Whole-exome sequence analysis should play an important role in future diagnostics for AS.

In conclusion, there has been no report of any AS patient with an *ALMS1* mutation in the Japanese population, probably because AS is an extremely rare inherited disease. We identified a novel *ALMS1* mutation in two brothers of a consanguineous family and examined their clinical features in detail. Our results indicate the presence of different mutations in AS between Japanese and other populations.

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Table 3. DNA sequence statistics

Family members	Read length (bp)	Number of reads	Mapping rate (%)	Mean depth (fold)	Coverage (%)
II-2 (younger brother)	101	47,724,724	99.4	46.6	88.5
II-1 (elder brother)	101	68,584,852	99.4	59.6	86.1
I-1 (father)	101	57,103,807	99.3	69.3	88.5
I-2 (mother)	101	59,776,264	99.3	56.8	86
Average	101	58,297,412	99.3	58.1	87.3

or anatomic changes of the retina [14,18,21,40] have been reported. For instance, a study of the pathology of the retina of a 2-year-old girl with AS showed hypocellularity of the ganglionic cell layer, the inner nuclear layer, and the outer nuclear layer (ONL) in addition to an absence of rod and cone outer segments and disruption of retinal pigment epithelium [18,21]; a study of a 42-year-old female with AS revealed severe reduction of all retinal layers containing a complete lack of photoreceptors and deposits of melanin pigments in the inner nuclear layer [14]; and OCT findings of a 5-year-old boy with AS showed only a slight thinning of the central retina [40]. In our patients, OCT findings showed marked retinal thinning (Figure 5A,B). The retinal layers of patient II-2 could not be distinguished because of marked retinal thinning (Figure 5C).

A study using retinal sections of *Alms1* knockout (*Alms1*$^{-/-}$) mice showed loss of the cell bodies in the ONL, shortening of the inner and outer segments, and incorrect localization of rhodopsin to the ONL [7]. The mislocalization of rhodopsin in the *Alms1*$^{-/-}$ mice indicates a defective rhodopsin transport system through the photoreceptor-connecting cilium [7]. The connecting cilium, damaged by loss of function of ALMS1, modifies the outer segments of the photoreceptors. Therefore, it has been suggested that defective protein transport across the connecting cilium is the probable cause of early onset severe retinal degeneration in AS patients [10]. We consider that the marked retinal thinning (Figure 5) and loss of retinal function (Figure 2) observed in our patients are due to a defective transport system across the photoreceptor-connecting cilium, resulting from the homozygous truncated mutation (p.Q2051X).

Variability in the phenotypic expression of AS is observed within sets of affected siblings [14,41–43]. Most patients with AS eventually develop T2DM, although there is wide variability in the age of onset [14]. Here, patient II-1 showed T2DM, but patient II-2 exhibited hyperinsulinemia, a predictor of T2DM (Table 2), suggesting that he might develop T2DM in the future. In addition, patient II-2 showed subclinical hypothyroidism, whereas patient II-1 did not exhibit hypothyroidism (Table 2). Hypothyroidism or subclinical hypothyroidism is reported to exist in approximately 20% of AS patients [14,19]. Most clinical features, such as retinal degeneration, hepatic dysfunction, hyperlipidemia, hypogonadism, short stature, and wide feet, were common features of the affected brothers (Table 1, Table 2); however, slight phenotypic differences in terms of glucose tolerance and thyroid function were observed between them.

ALMS1 protein has several notable sequence features, including an extensive tandem repeat domain (34×47 amino acid approximate tandem repeat, residues 538–2,199), a putative leucine-zipper motif (residues 2,480–2,501), and an ALMS motif (residues 4,035–4,167). Although the precise roles of the above domain and motifs are unknown, it is suggested that two regions of ALMS1—a relatively small internal region (residues 2,261–2,602) and a larger C-terminal region (residues 3,176–4,169)—play important

Table 4. Number of mutations after each filtering step

Filtering step	Number of mutations
1. Raw single-nucleotide variants plus *insertion–deletion* polymorphisms	3,506,741
2. Mutations capable of changing amino acid sequence	19,574
3. Mutations filtering by the snpEff score and existing at a frequency of less than 1% in 1000 genomes	3,685
4. Mutations filtering by the pattern of inheritance	17
5. Mutations expressed in retina, confirmed by SAGE database[a]	9
6. Mutations narrowed down using BIOBASE Biologic Database[b] and RetNet database[c]	1

[a]SAGE: serial analysis of gene expression;[b]BIOBASE Biologic Database (EyeSAGE); [c]RetNet database.

Bio-information and blood test results	Normal range	Patient II-1	Patient II-2
Thyroid stimulating hormone (mIU/l)	0.34–4.04	3.06	**4.67**
Anti-thyroid peroxidase antibody (U/ml)	0.0–15	10	10
Anti-thyroglobulin antibody (U/ml)	0.0–27	11	<10
Thyroglobulin (ng/ml)	0.0–32.7	6.1	29.4
Thyroid stimulating hormone receptor antibody (IU/ml)	<1.0	0.9	<1.0
Parathyroid hormone (pg/ml)	10–65	26	20
Luteinizing hormone (mIU/ml)	1.7–8.6	**13.2**	**9.1**
Follicle stimulating hormone (mIU/ml)	1.5–12.4	**30.7**	**20.3**
Estradiol (pg/ml)	14.0–43.9	18.7	**10.6**
Total testosterone (ng/ml)	250.0–1100.0	**90.7**	**53.5**
Prolactin (ng/ml)	4.3–13.7	3.7	2.5
Cortisol (mg/dl)	4.0–18.3	8.1	10.6
Insulin (μU/ml)	0.0–13.0	9.2	**63.6**
Growth hormone (ng/ml)	0–2.47	0.3	0.27

Bold type indicates values outside the normal range

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Table 2. Bio-information and biochemical assessment

Bio-information and blood test results	Normal range	Patient II-1	Patient II-2
Bio-information			
Weight (kg)		60	52
Height (m)		1.52	1.55
Body mass index (kg/m^2)	18.5–25	**25.96**	21.6
Biochemical assessment			
Fasting blood glucose (mg/dl)	65–109	**247**	77
Hemoglobin A1c (%)	4.6–6.2	**12.5**	6.0
Urea (mg/dl)	8–20	16	10
Creatinine (mg/dl)	0.50–1.10	0.99	0.63
Uric acid (mg/dl)	3.1–6.9	4.2	3.6
Sodium (mmol/l)	136–146	140	141
Potassium (mmol/l)	3.6–4.8	4.1	4.3
Chloride (mmol/l)	98–109	100	104
Calcium (mg/dl)	8.6–10.2	**10.3**	10.2
Aspartate aminotransferase (U/l)	10–33	**76**	**81**
Alanine aminotransferase (U/l)	6–35	**99**	**241**
Gamma glutamyl transpeptidase (U/l)	12–65	**134**	**135**
Alkaline phosphatase (U/l)	96–300	285	**319**
Low density lipoprotein-cholesterol (mg/dl)	70–139	120	**282**
Total cholesterol (mg/dl)	120–219	211	**441**
Triglycerides (mg/dl)	30–149	**309**	**761**
Albumin/globulin (g/dl)	3.5–5.2	5.0	5.1
Hemogram			
White blood cells (10^3/ml)	3.3–8.6	4.7	6.4
Red blood cells (10^6/ml)	4.10–5.50	5.00	4.88
Hemoglobin (g/dl)	13.5–16.5	14.0	14.3
Hematocrit (%)	40.0–50.0	42.3	42.7
Mean corpuscular volume (fl)	83.0–101.0	84.6	87.5
Platelets (10^3/ml)	150–350	166	251
Erythrocyte sedimentation rate (mm/h)	2–10	21	19
Hormones and autoantibodies			
Free T3 (pg/ml)	2.36–5.00	**2.34**	2.47
Free T4 (pmol/l)	0.88–1.67	1.33	**0.79**

Table 1. Clinical characteristics

Clinical findings	Patient II-1	Patient II-2	Percentage (%) in 182 cases[a]
Low vision	+	+	100
Subcapsular cataracts	+	+	32
Hearing loss	–	–	88
Cardiomyopathy	–	–	62
Type 2 diabetes mellitus	+	–	68
Childhood obesity	+	–	98
Hyperinsulinemia	–	+	92
Short stature	+	+	98
Hypertriglyceridemia	+	+	52
Hypothyroidism	–	+ (subclinical)	17
Hypogonadism	+	+	78
Elevated hepatic enzyme levels	+	+	92
Renal insufficiency	–	–	50
Renal hypertension	–	–	30
Pulmonary symptoms	–	–	52
Asthma	+ (childhood)	+ (childhood)	19
Muscle weakness	–	–	29
Global development delay	–	–	49
Wide feet	+	+	ND
Abnormal digits	–	–	ND
Scoliosis	–	–	ND
Alopecia	–	–	ND

ND, not described. [a]The cases are cited [13].

progress known phenotype with syndromic disorders. Finally, *ALMS1* was speculated to be the disease-causing gene. The *ALMS1* sequence was compared with the NCBI reference sequence of the *ALMS1* transcript (GenBank NM_015120.4).

As a result, in the two affected brothers we identified a novel single-nucleotide substitution at position 6151 (c.6151C>T in exon 8) that causes a premature termination codon at amino acid 2051 (p.Q2051X) of the *ALMS1* gene resulting in a truncated protein. Both brothers were homozygous for the mutant allele, whereas the unaffected parents were heterozygous carriers of the allele, also reconfirmed by Sanger sequencing. The novel *ALMS1* mutation (p.Q2051X) was not found in any of 100 Japanese individuals without ocular disease in the Single Nucleotide Polymorphism Database or in the Human Gene Mutation Database.

DISCUSSION

To date, no patient with *ALMS1*-associated AS has been reported in the Japanese population. Here, we identified a novel *ALMS1* mutation (p.Q2051X) in two Japanese brothers with AS.

Marshall et al. advocate criteria for the diagnosis of AS [19]. In patients over the age of 15, it is necessary to fulfill "two major and two minor criteria" or "one major and four minor criteria" [19]. Our brother patients exhibited two major (*ALMIS1* mutation and loss of vision, such as legal blindness) and more than four minor criteria (obesity, insulin resistance, and/or T2DM, hepatic dysfunction, short stature, and hypogonadism). Also, the phenotypic expression of AS is differentiated from BBS characterized by later onset retinal dystrophy, polydactyly, central obesity, learning disabilities, hypogonadism, and renal anomalies [38].

In patients with AS, phenotypic variability in disease severity and retinal function assessed by electroretinographic and visual-field testing [39] and variability in pathological

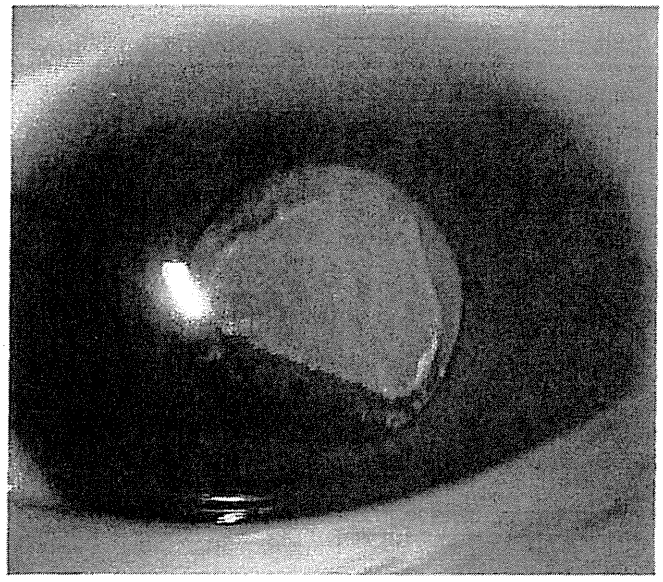

Figure 6. Anterior segment of the right eye in patient II-1. A severe cortical and anterior subcapsular cataract is present at the age of 29 years.

Systemic features except ocular findings: Systemic examinations were performed for patient II-1 at the age of 29 and patient II-2 at the age of 23. Both patients had hepatic dysfunction, hyperlipidemia, hypogonadism, short stature, and flat feet, and neither patient had hearing loss, renal failure, abnormal digits, history of developmental delay, mental retardation, scoliosis, hypertension, or alopecia. Obesity was present in patient II-1 only. Patient II-1 had T2DM, whereas patient II-2 showed hyperinsulinemia. Subclinical hypothyroidism was diagnosed in patient II-2 only. Recurrent pulmonary infections were not observed, and chest X-rays showed neither fibrotic infiltrations nor cardiac dilation in either patient. Infantile asthma was experienced by both patients. Electrocardiogram analysis showed no arrhythmia in either patient. Summaries of the clinical features, bio-information, and detailed laboratory data are presented in Table 1 and Table 2. Collectively, the phenotypes of the brothers were consistent with those described for AS.

Exome sequencing analysis and identification of a gene mutation: We performed whole-exome sequencing of the two affected brothers and their parents by using the Agilent SureSelect Human All Exon kit followed by Illumina HiSeq 2000 platforms. Sequences of average length 11.8 Gb were generated from 101-bp paired-end sequences. After eliminating reads from PCR duplicates by discarding reads with duplicated start sites, we achieved 58-fold depth and 87% coverage in Refseq annotated regions (Table 3). When the sequences were compared with the reference human genome (hs37d5), 3,506,741 mutations were found in the two brothers and their parents (Table 4). To distinguish potentially causal mutations from other mutations, we focused only on mutations that could change the amino acid sequence (19,574 mutations), such as nonsynonymous mutations, splice acceptor and donor site mutations, and INDELs. We also assumed the frequency of the mutations responsible for AS is likely to be under 1%. After filtering with snpEff score and frequency criteria, we filtered the remaining 3,685 mutations by using the pattern of inheritance and identified 17 gene mutations as causal candidates. Among these mutations, nine mutations were found homozygous in the HECT domain containing E3 ubiquitin protein ligase 3 (*HECTD3*), the vitrin (*VIT*), the protein kinase domain containing, cytoplasmic (*PKDCC*), the ATP-binding cassette, sub-family G (*WHITE*), member 8 (*ABCG8*), the leucine-rich pentatricopeptide repeat containing (*LRPPRC*), the G protein-coupled receptor 75 (*GPR75*), the notochord homeobox (*NOTO*), the matrix-remodelling associated 5 (*MXRA5*), and the *ALMS1* genes. Eight mutations were found as compound heterozygous mutations within the PERP, TP53 apoptosis effector (*PERP*), the transforming, acidic coiled-coil containing protein 2 (*TACC2*), the zinc finger protein, FOG family member 1 (*ZFPM1*), and the lipoxygenase homology domains 1 (*LOXHD1*) genes. No de novo mutations were found. To determine the causative gene, we investigated SAGE (EyeSAGE) database to determine if the candidate genes are expressed in the retina. Nine candidate mutations were identified within *VIT*, *LRPPRC*, *PERP*, *TACC2*, *ZFPM1*, and *ALMS1* genes. These nine candidate genes were further reduced by the BIOBASE Biologic Database and RetNet to determine which of the candidate genes would be likely to

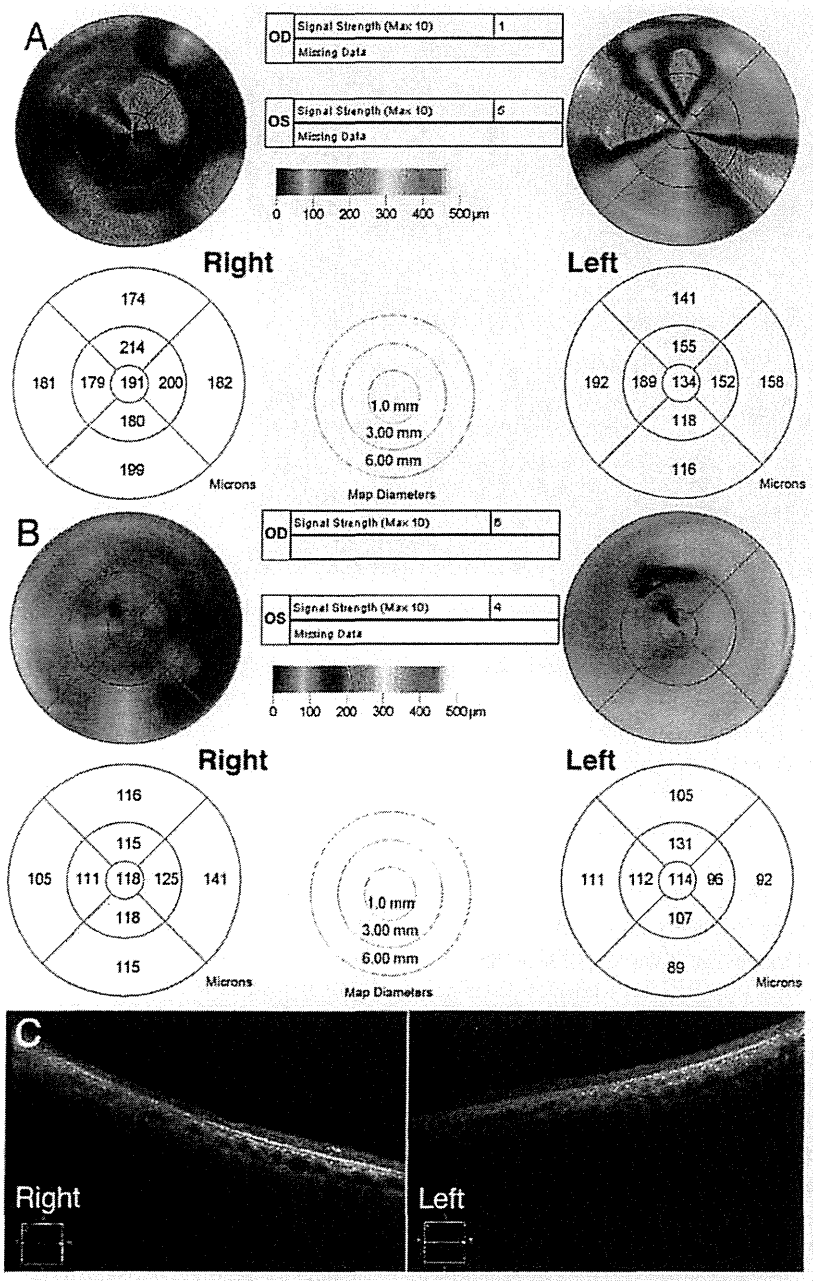

Figure 5. Optic coherence tomography findings. A and B: Time domain optic coherence tomography (OCT; retinal mapping) of patient II-1 at the age of 22 years (A) and II-2 at the age of 16 years (B) show total macular thinning in both eyes. C: Spectral-domain OCT (HD-5-line raster) of patient II-2 at the age of 23 years, showing marked macular thinning with indistinguishable retinal layers in the macular areas of both eyes.

the age of 8 years showed retinal degeneration with attenuated vessels from the arcade artery to the periphery in both eyes (Figure 4B). TD-OCT at the age of 16 years showed total macular thinning in both eyes (Figure 5B). At the age of 23 years, his BCVA was LP in both eyes, the intraocular pressure was within the normal range in both eyes, posterior subcapsular cataracts were present in both eyes, and SD-OCT showed total macular thinning with indistinguishable retinal layers in both eyes (Figure 5C).

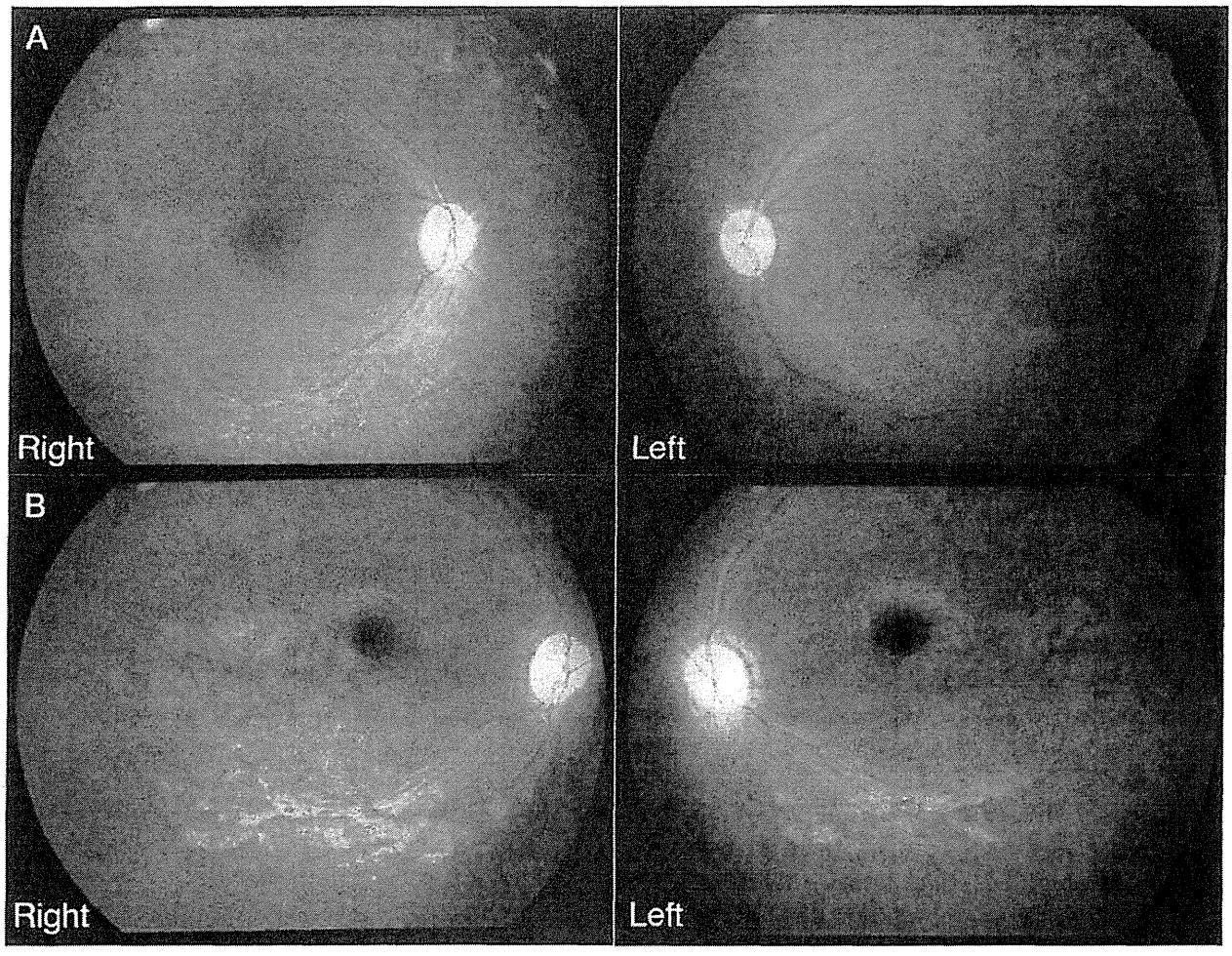

Figure 4. Fundus photographs of patients II-1 and II-2. **A** and **B**: Fundus photographs of patient II-1 at the age of 14 years (**A**) and patient II-2 at the age of 8 years (**B**) show retinal degeneration with attenuated vessels in the posterior poles of both eyes.

combined, photopic, or 30-Hz flicker responses in either eye (Figure 2). GP analysis at the age of 11 years showed markedly constricted visual fields in V-4e and I-4e isopters of both eyes (Figure 3A). The fundus photographs at the age of 14 years showed retinal degeneration with attenuated vessels from the arcade to the periphery in both eyes (Figure 4A). GP analysis at the age of 16 years showed more marked constricted visual fields of V-4e and I-4e isopters in both eyes than those observed at the age of 11 years (Figure 3B); a similar analysis at the age of 22 years showed a small visual field of V-4e isopter in the right eye and no visual field in the left eye (Figure 3C). TD-OCT at the age of 22 years showed total macular thinning in both eyes (Figure 5A). At the age of 29 years, his BCVA was light perception (LP) in the right eye and no light perception in the left eye. Intraocular pressure in each eye was within the normal range. He had severe cortical and subcapsular cataracts in the right (Figure 6) and left eyes, and the fundi were not visible due to these cataracts.

Ophthalmologic findings for patient II-2: Patient II-2, the younger of the two brothers, visited our hospital at the age of 2 years and 6 months with the main complaint of poor visual acuity and photophobia. At the age of 3 years, his BCVA was 0.01 (+1.50 dpt) in the right eye and 0.01 (+1.50 dpt) in the left eye. Fundus examination showed retinal degeneration with slight attenuation of peripheral vessels. At the age of 4 years, he failed the Ishihara test. At the age of 6 years, the panel D-15 test showed irregular arrangements along no particular axis, and the GP could not be measured well because of low visual acuity and nystagmus. The ERG at the age of 7 years showed no rod, standard combined, photopic, or 30-Hz flicker responses in either eye (Figure 2). The fundus examination at

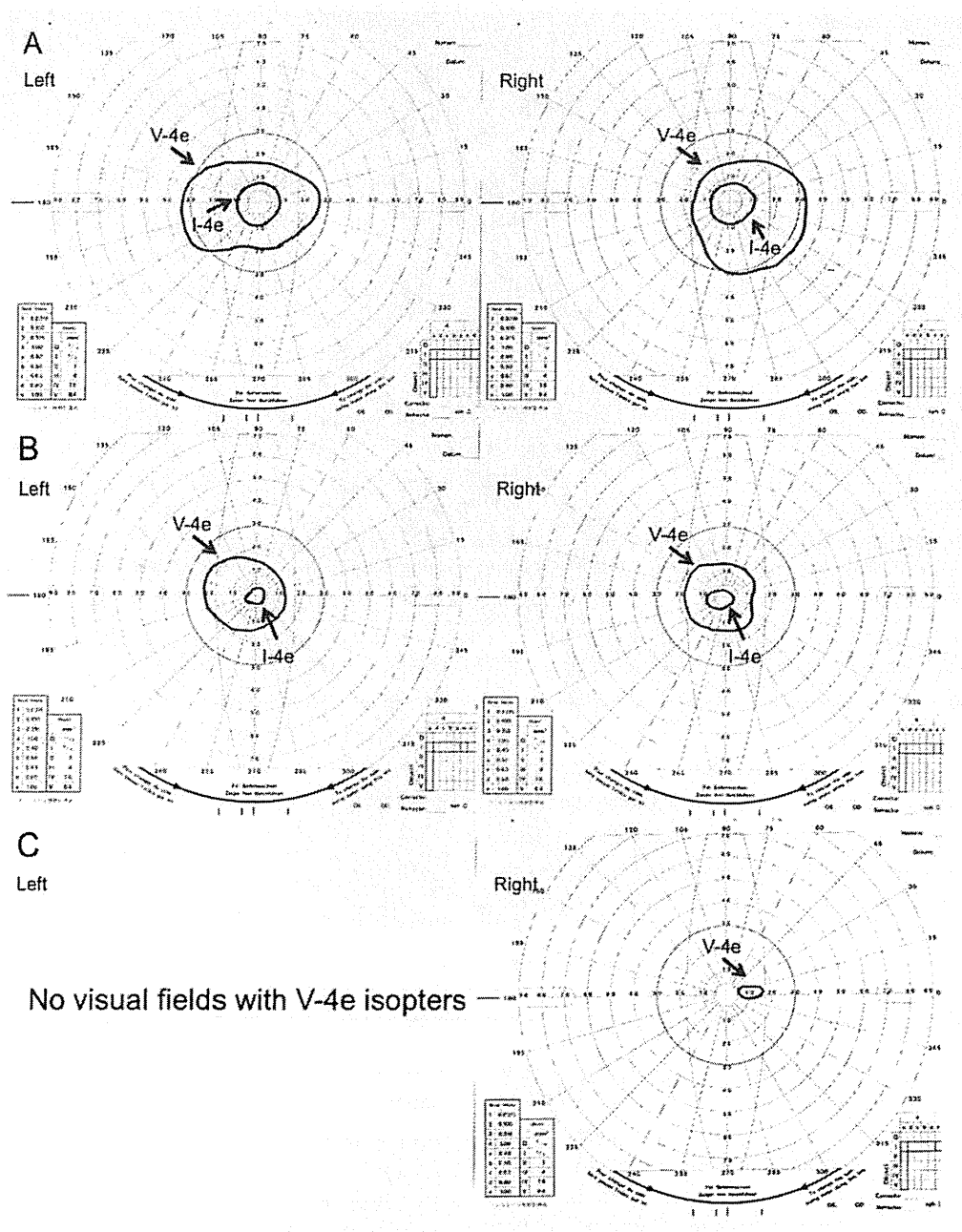

Figure 3. Visual fields assayed by Goldmann perimetry in patient II-1. A–C: Visual fields at the age of 11 years (A), at the age of 16 years (B), and at the age of 22 years (C). Markedly constricted visual fields (V-4e and I-4e isopters) are observed in both eyes, and the visual fields become constricted as the patient ages.

RESULTS

Ophthalmologic findings for patient II-1: Patient II-1, the elder of the two brothers, was referred to our hospital at the age of 7 years and 4 months for the assessment of poor visual acuity from infancy. His BCVA was 0.04 (with +2.00 diopter [dpt], cylinder [cyl] –1.00 dpt axis [Ax] 180°) in the right eye and 0.06 (with +2.00 dpt, cyl –1.00 dpt Ax 180°) in the left eye. Fundus examination showed slight retinal degeneration in both eyes. At the age of 9 years, the patient recognized only the first plate in the Ishihara test for color vision, the panel D-15 test for color vision showed irregular arrangements along no particular axis, and the ERG showed no standard

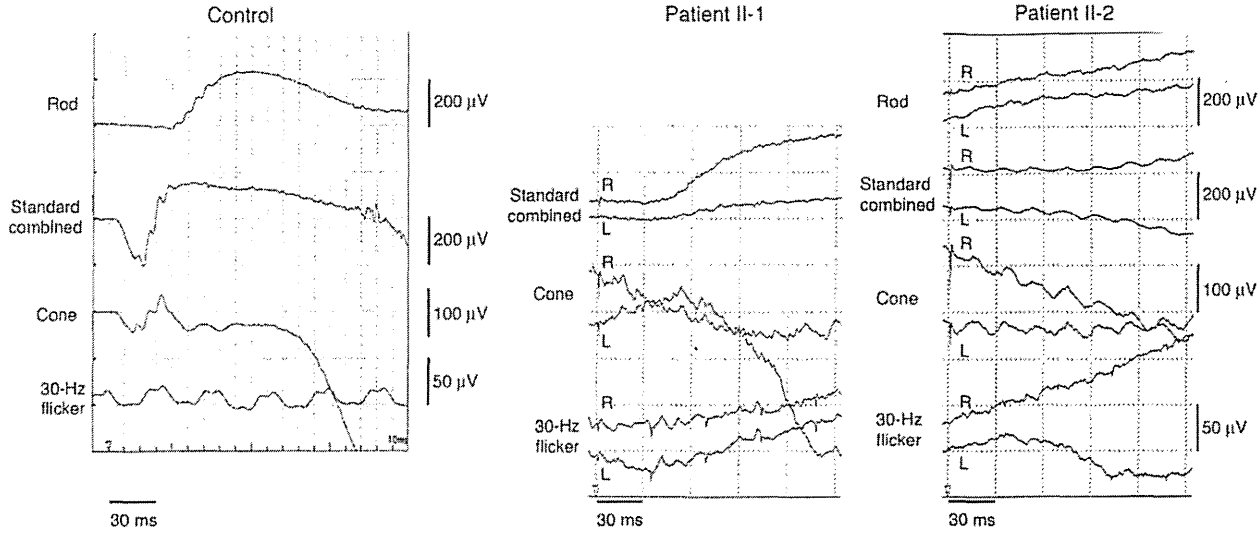

Figure 2. Full-field electroretinogram. The electroretinograms (ERGs; patient II-1) at the age of 9 years, showing no standard combined, photopic, or 30-Hz flicker responses in either eye. The ERGs (patient II-2) at the age of 7 years, showing no rod, standard combined, photopic, or 30-Hz flicker responses in either eye.

Clinical Electrophysiology of Vision. The procedure and conditions for ERG recording have been detailed previously [33].

Fasting venous blood samples were analyzed for glucose, lipid, lipoprotein, and hemogram levels and renal, liver, and thyroid function tests. In addition, hemoglobin A_{1c}, insulin, anti-thyroid peroxidase, anti-thyroglobulin antibodies, cortisol, luteinizing hormone, follicle stimulating hormone, testosterone, estradiol, prolactin, parathyroid hormone, and thyroid receptor antibody levels were examined. Chest X-rays and electrocardiograms were also performed.

DNA preparation and exome sequencing analysis: We obtained venous blood samples from the affected brothers and their unaffected parents. Genomic DNA was extracted from the blood samples by using a Gentra Puregene Blood kit (Qiagen, Tokyo, Japan) and sheared with a Covaris Ultrasonicator (Covaris, Woburn, MA). Construction of paired-end sequence libraries and exome capture were performed by using the Agilent Bravo automated liquid-handling platform with SureSelect XT Human All Exon kit V4 + UTRs kit (Agilent Technologies, Santa Clara, CA) according to the manufacturer's instructions. Enriched libraries were sequenced by using in Illumina HiSeq2000 sequencer (San Diego, CA), according to the manufacturer's instructions for 100-bp paired-end sequencing. Reads were mapped to the reference human genome (1000 genomes phase 2 reference, hs37d5) with Burrows–Wheeler Aligner software version 0.6.2 [34]. Duplicated reads were then removed by Picard MarkDuplicates module version 1.62, and mapped reads around insertion–deletion polymorphisms (INDELs) were realigned by using the Genome Analysis Toolkit (GATK) version 2.1–13 [35]. Base-quality scores were recalibrated by using GATK. Calling of mutations was performed by using the GATK UnifiedGenotyper module, and called single-nucleotide variants and INDELs were annotated by using snpEff software version 3.0 [36]. The mutations were annotated with the snpEff score ("HIGH," "MODERATE," or "LOW") and with the allele frequency in the 1000 genomes database. 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 of less than 1% in the 1000 genome database were analyzed further. We also used new variations, which were not found in the in-house database of seven people exome data 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 [37], which predicts the effect on the structure and function of the protein. The above exome analysis pipeline is available at Cell Innovation.

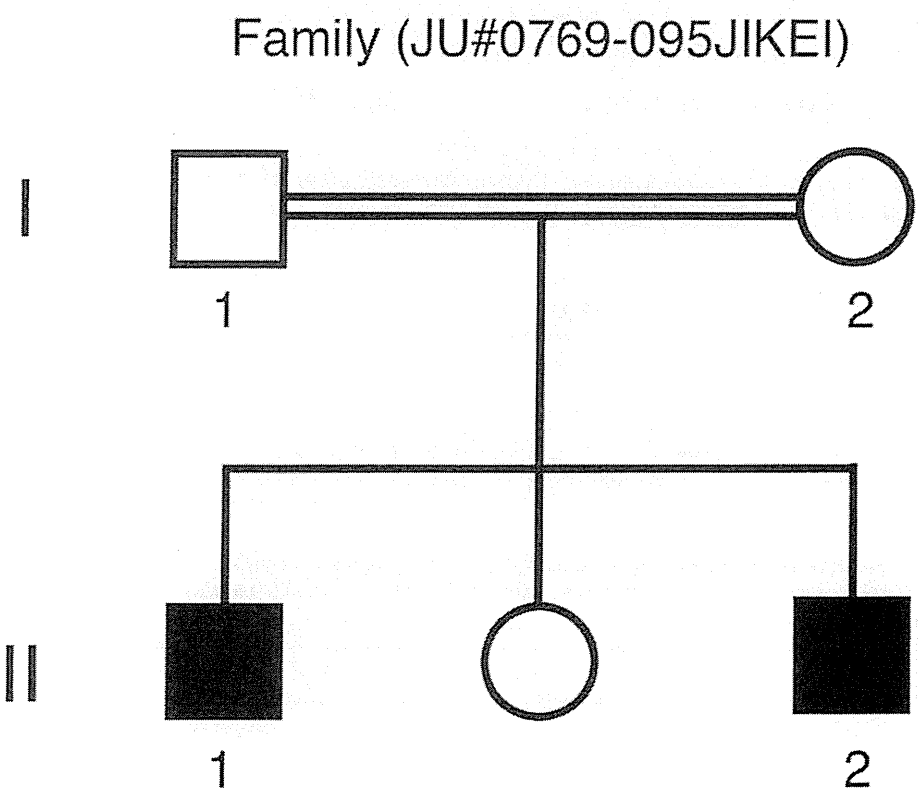

Figure 1. A consanguineous family (JU#0769–095JIKEI) with Alström syndrome. Two affected brothers (II-1 and II-2) with Alström syndrome and their unaffected parents are depicted.

show progressive retinal degeneration, with 90% becoming totally blind by the age of 16 years [19] and all becoming blind eventually [14,19]. Due to severe retinal degeneration and visual impairment during the first months of life, AS is often confused with congenital retinal degenerations, such as Leber congenital amaurosis (LCA) and congenital achromatopsia (ACHM) [20,21]. There are several reports of Japanese patients with AS [22–24]; however, there has been no report identifying any *ALMS1* mutation associated with AS in the Japanese population.

Recently, the development of next-generation sequencing technology has facilitated biologic and biochemical research by enabling the broad analysis of genomes [25–28]. The whole genome of an individual can now be sequenced at great depth, and genomic capture technology can be used to isolate sequences of interest [29–32].

Here, we used whole-exome sequencing to identify a novel *ALMS1* mutation in two Japanese brothers with AS. We also examined the clinical features of the two brothers in detail.

METHODS

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

Clinical studies: The study was conducted in one consanguineous Japanese family (JU#0769–095JIKEI) with AS (Figure 1). The parents were second cousins. The clinical history was taken in detail, and the following ophthalmic examinations were performed: decimal best-corrected visual acuity (BCVA), slit-lamp and fundus examinations, and time-domain optical coherence tomography (TD-OCT; OCT3 Stratus; Carl Zeiss Meditec AG, Dublin, CA) or spectral-domain OCT (SD-OCT; Cirrus HD-OCT; Carl Zeiss Meditec AG). In color-vision tests, we used the Ishihara test (38-plate edition) and the Farnsworth Panel D-15 (Panel D-15). Visual-field testing by kinetic perimetry was conducted by using the Goldmann perimeter (GP; Haag Streit, Bern, Switzerland). Full-field electroretinography (ERG) was performed according to the protocols of the International Society for

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Whole-exome sequencing identifies a novel *ALMS1* mutation (p.Q2051X) in two Japanese brothers with Alström syndrome

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Purpose: No mutations associated with Alström syndrome (AS), a rare autosomal recessive disease, have been reported in the Japanese population. The purpose of this study was to investigate the genetic and clinical features of two brothers with AS in a consanguineous Japanese family.

Methods: Whole-exome sequencing analysis was performed on two brothers with AS and their unaffected parents. We performed a complete ophthalmic examination, including decimal best-corrected visual acuity, slit-lamp and funduscopic examination, visual-field and color-vision testing, full-field electroretinography, and optical coherence tomography. Fasting blood tests and systemic examinations were also performed.

Results: A novel mutation (c.6151C>T in exon 8) in the Alström syndrome 1 (*ALMS1*) gene that causes a premature termination codon at amino acid 2051 (p.Q2051X), was identified in the homozygous state in the affected brothers and in the heterozygous state in the parents. The ophthalmologic findings for both brothers revealed infantile-onset severe retinal degeneration and visual impairment, marked macular thinning, and severe cataracts. Systemic findings showed hepatic dysfunction, hyperlipidemia, hypogonadism, short stature, and wide feet in both brothers, whereas hearing loss, renal failure, abnormal digits, history of developmental delay, scoliosis, hypertension, and alopecia were not observed in either brother. The older brother exhibited type 2 diabetic mellitus and obesity, whereas the younger brother had hyperinsulinemia and subclinical hypothyroidism.

Conclusions: A novel *ALMS1* mutation was identified by using whole-exome sequencing analysis, which is useful not only to identify a disease causing mutation but also to exclude other gene mutations. Although characteristic ophthalmologic findings and most systemic findings were similar between the brothers, the brothers differed slightly in terms of glucose tolerance and thyroid function.

Alström syndrome (AS; OMIM: 203800) is a rare and autosomal recessive hereditary disease with an estimated prevalence of less than 0.001% [1,2]. AS is caused by mutations in the *ALMS1* gene, which is located on chromosome 2p13 [3,4]. ALMS1 is localized to centrosomes and ciliary basal bodies [5,6] and has been implicated in the function, formation, and maintenance of primary cilia [5,7–9]. Dysfunction of primary cilia caused by mutations in genes such as *ALMS1* leads to a multitude of human monogenic disorders known as ciliopathies [10,11]; these include plural systemic diseases, such as AS, Usher syndrome, Bardet–Biedl syndrome (BBS), Senior–Løken syndrome, Joubert syndrome, Meckel–Gruber syndrome, and orofaciodigital syndrome 1 [11,12]. The majority of *ALMS1* mutations are nonsense and frameshift variations (primarily clustered in exons 8, 10, and 16) that are predicted to cause truncated proteins [3,4,13]. In the photoreceptors, *ALMS1* mutations lead to defective function of the connecting cilium.

AS is characterized by a wide spectrum of disorders, such as early onset severe retinal degeneration, obesity from childhood, hyperinsulinemia, type 2 diabetic mellitus (T2DM), hepatic dysfunction, heart failure, sensory hearing loss, and renal failure [14]. Other manifestations include acanthosis nigricans, alopecia, hypogonadism, hypothyroidism, hyperlipidemia, short stature, and scoliosis [15,16]. In most cases of AS, cone–rod degeneration in the first decade, normal intelligence, and no polydactyly serve as a differential diagnosis of BBS, which exhibits similar clinical findings to AS [17].

Almost all patients with AS show nystagmus and severe photophobia from infancy [14,18]. Visual impairment is usually seen at an age younger than 1 year [18]. Although the rate of progression of vision loss is variable, all patients

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Whole-exome sequencing analysis disclosed that our patient had the *EYS* mutations, which demonstrates that the *EYS* mutations can be responsible for both the arCRD and the arRP phenotypes. Interestingly, mutations in the *ABCA4* [12–14], *CERKL* [15–18], and *C8orf37* [19, 20] genes have also been reported to be disease-causing mutations of both the arCRD and arRP phenotypes. With regard to the *ABCA4* gene mutations, the degree of functional damage caused by the various *ABCA4* mutation types can underlie the different degeneration patterns, for example, Stargardt disease (a type of macular dystrophy), arCRD or arRP [12–14]. The majority of patients with *CERKL* mutations exhibit arCRD [17, 18] and less frequently arRP [15, 16]. This is consistent with the fact that the CERKL protein is predominantly expressed in the cone photoreceptors [21]. In addition, different *C8orf37* mutations can cause either the arCRD or arRP phenotypes, which is consistent with the fact that the C8orf37 protein is expressed in both the rod and cone photoreceptors [19, 20]. However, this does not explain the pattern of the photoreceptor degeneration. On the other hand, the compound heterozygous *EYS* mutations (p.Y2935X and p.S1653KfsX2) that were found in our patient have also been reported in an arRP patient [7]. Although it is not understood why the same compound heterozygous mutations would underlie either the arCRD or arRP phenotypes, this finding suggests there is the presence of different modifier alleles between the arCRD or arRP patients with the compound heterozygous *EYS* mutations. Even so, our whole-exome sequencing analysis did not demonstrate any compound heterozygous or homozygous mutations in other 206 retinal disease-associated genes published in the RetNet database.

In conclusion, we demonstrated that *EYS* mutations are the cause of not only arRP but also arCRD. Further investigations will need to be undertaken in order to clarify the prevalence of *EYS* mutations among arCRD patients, and to determine the genotype–phenotype correlations between the arCRD and *EYS* mutations.

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Conflict of interest The authors declare there are no conflicts of interest for this study.

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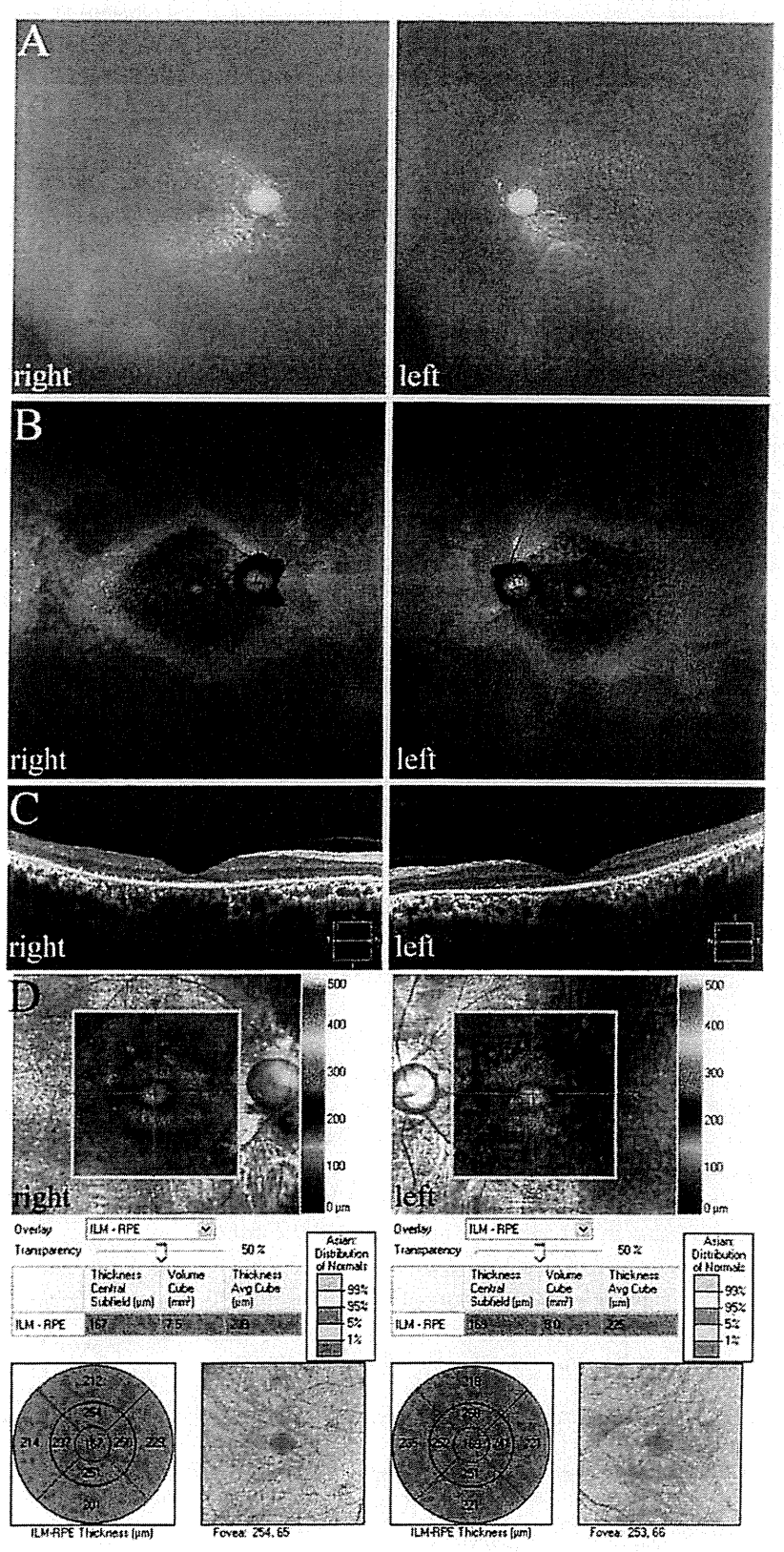

Fig. 5 Fundus photographs, fundus autofluorescence images (FAI), and optic coherence tomography images (OCT) at the age of 36 years. **a** Fundus photographs show retinal degenerations within the vascular arcades in both eyes, but relatively preserved mid-peripheral to peripheral retinal findings with 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

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Molecular characteristics of four Japanese cases with *KCNV2* retinopathy: Report of novel disease-causing variants

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Purpose: To describe the molecular characteristics of four Japanese patients with cone dystrophy with supernormal rod responses (CDSRR).

Methods: Four individuals with a clinical and electrophysiological diagnosis of CDSRR were ascertained. The pathognomonic findings of the full-field electroretinograms (ERGs) included a decrease in the rod responses, a square-shaped a-wave, an excessive increase in the b-wave in the bright flash responses, and decreased cone-derived responses. Mutational screening of the coding regions and flanking intronic sequences of the potassium channel, subfamily V, member 2 (*KCNV2*) gene was performed with bidirectional sequencing. The segregation of each allele was confirmed by screening other family members. Subsequent in silico analyses of the mutational consequences for protein function were performed.

Results: There were two siblings from one family and one case in each of the two families. One family had a consanguineous marriage. Mutational screening revealed compound heterozygosity for the two alleles, p.C177R and p.G461R, in three patients, and homozygosity for complex alleles, p.R27H and p.R206P, in one patient from the consanguineous family. There were three putative novel variants, p.R27H, p.C177R, and p.R206P. The four variants in the families with *KCNV2* were highly conserved in other species. In silico analyses predicted that all of the missense variants would alter protein function.

Conclusions: Biallelic disease-causing variants were identified in four Japanese patients with CDSRR suggesting that the pathognomonic electrophysiological features are helpful in making a molecular diagnosis of *KCNV2*. Three novel variants were identified, and we conclude that there may be a distinct spectrum of *KCNV2* alleles in the Japanese population.

Patients with cone dystrophy and supernormal rod electroretinograms (ERGs) were first reported in 1983, and the abnormality in the ERGs indicated a progressive degeneration of the cone photoreceptors associated with unique rod system abnormalities [1]. More detailed characteristics of this rare, autosomal recessive condition were reported in later studies, and the disease was named cone dystrophy with supernormal rod responses (CDSRR; MIM #610356) [2-8].

Most cases with CDSRR typically present in the first two decades of life with reduced visual acuity, abnormal color vision, and photophobia [8-11]. Night blindness is a later feature of the disorder [8]. The fundus appearance is variable, with some having a normal peripheral retina and a range of macular abnormalities [8-10]. The pattern of the autofluorescence (AF) images is also variable: Young cases have either a normal pattern or small parafoveal ring enhancements, while older cases have a narrow high-signal annulus that can encircle a central atrophic area of the retinal pigment epithelium (RPE) [6,12]. Recently, spectral domain optical coherence tomography (SD-OCT) and adaptive optics scanning laser ophthalmoscope (AOSLO) studies have described morphological changes of the fovea even at the early stages [10,13,14].

The electrophysiological findings are pathognomonic of CDSRR, and they assist in its early diagnosis [3,5,8-12,15-17]. The light-adapted ERGs are usually delayed and decreased in keeping with a generalized cone system dysfunction. There is also a unique rod system abnormality; the dark-adapted ERGs elicited by dim flashes are markedly decreased and delayed, and increasing the flash intensity results in an excessive increase in the b-wave amplitude accompanied by a shortening of the peak time of the b-wave [8,9,11]. A square-shaped a-wave trough of the dark-adapted bright flash ERGs is also a characteristic feature of this disorder [9,11].

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