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Two Novel Mutations in the *EYS* Gene Are Possible Major Causes of Autosomal Recessive Retinitis Pigmentosa in the Japanese Population

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Abstract

Retinitis pigmentosa (RP) is a highly heterogeneous genetic disease including autosomal recessive (ar), autosomal dominant (ad), and X-linked inheritance. Recently, arRP has been associated with mutations in *EYS* (Eyes shut homolog), which is a major causative gene for this disease. This study was conducted to determine the spectrum and frequency of *EYS* mutations in 100 Japanese arRP patients. To determine the prevalence of *EYS* mutations, all *EYS* exons were screened for mutations by polymerase chain reaction amplification, and sequence analysis was performed. We detected 67 sequence alterations in *EYS*, of which 21 were novel. Of these, 7 were very likely pathogenic mutations, 6 were possible pathogenic mutations, and 54 were predicted non-pathogenic sequence alterations. The minimum observed prevalence of distinct *EYS* mutations in our study was 18% (18/100, comprising 9 patients with 2 very likely pathogenic mutations and the remaining 9 with only one such mutation). Among these mutations, 2 novel truncating mutations, c.4957_4958insA (p.S1653KfsX2) and c.8868C>A (p.Y2956X), were identified in 16 patients and accounted for 57.1% (20/35 alleles) of the mutated alleles. Although these 2 truncating mutations were not detected in Japanese patients with adRP or Leber's congenital amaurosis, we detected them in Korean arRP patients. Similar to Japanese arRP results, the c.4957_4958insA mutation was more frequently detected than the c.8868C>A mutation. The 18% estimated prevalence of very likely pathogenic mutations in our study suggests a major involvement of *EYS* in the pathogenesis of arRP in the Japanese population. Mutation spectrum of *EYS* in 100 Japanese patients, including 13 distinct very likely and possible pathogenic mutations, was largely different from the previously reported spectrum in patients from non-Asian populations. Screening for c.4957_4958insA and c.8868C>A mutations in the *EYS* gene may therefore be very effective for the genetic testing and counseling of RP patients in Japan.

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Introduction

Retinitis pigmentosa (RP [MIM 268000]) is a highly heterogeneous genetic disease characterized by night blindness and visual field constriction leading to severe visual impairment. The disease appears with different modes of inheritance including autosomal recessive (ar), autosomal dominant (ad), and X-linked, and currently over half of cases are isolated in Japan.

To date, 53 causative genes and 7 loci of RP have been identified (http://www.sph.uth.tmc.edu/Retnet/), including the eyes shut homolog (*EYS*) gene encoding an ortholog of *Drosophila* spacemaker (spam), a protein essential for photoreceptor morphology. *EYS* spans over 2 Mb, making it one of the largest known genes expressed in the human eye [1,2]. *EYS* gene mutations, primarily truncating and some missense mutations, have been detected in arRP families of different ancestral origin and have reported to account for 5–16% of arRP [3–6]. Most gene mutations (e.g., *RHO*, *PRPH2*, *PRPF31*, *RP1*, and *IMPDH1*) have been found in Japanese patients with adRP, with few reports describing mutations in arRP [7,8]. Therefore, the genes causing arRP in most Japanese families have yet to be identified.

In this study, we screened all *EYS* gene exons in 100 unrelated Japanese RP patients. We found 2 novel truncating *EYS* gene mutations that were surprisingly related to 16% of Japanese arRP patients, but were not detected in Japanese patients with either adRP or Leber's congenital amaurosis (LCA [MIM204000], the earliest onset and most severe form of hereditary retinal dystrophy with several clinical features overlapping with those of RP). Additionally, these mutations were also detected in 9% of Korean arRP patients.

Methods

Patients and clinical evaluation

We screened all *EYS* gene exons in 100 unrelated Japanese RP patients with no systemic manifestations, excluding families with obvious autosomal dominant inheritance. Some pedigrees showed a pattern compatible with the recessive mode of inheritance; the other patients were considered isolated cases. In addition, 200 unrelated and non-RP Japanese individuals were screened as controls to evaluate the frequency of the mutations found in the patient samples. We also screened a part of *EYS* gene exons 26 and 44 in 19 unrelated Japanese adRP patients, 28 unrelated Japanese LCA patients, and 32 unrelated Korean arRP patients. The 19 Japanese adRP patients had already been screened for some principal adRP-causing genes, but the pathogenic mutations have not yet been detected. Examples of the screening list for adRP-causing genes and targeted exons include exon 3 and 4 in *RP1*; exon 1, 2, 3, 4, and 5 in *RHO*; exon 1, 2, and 3 in *PRPH2*; exon 2, 3, and 4 in *CRX*; exon 11 in *PRPF3*; exon 10, 11, and 12 in *IMPDH1*; exon 2 in *NRL*; exon 43 in *PRPF8*; exon 1 and 2 in *ROM1*; exon 5 and 6 in *RP9*; exon 2, 3, 5, 6, 7, 8, 11, and 12 in *PRPF31*; exon 11 and 15 in *SEMA4A*; exon 1 in *CA4*; exon 3 in *GUCA1B*; exon 3 in *SP4*; and exon 3 in *TOPORS*.

Japanese RP patients were examined either at the Department of Ophthalmology, Hamamatsu University Hospital in Hamamatsu (by YH), Department of Ophthalmology, Kobe City Medical Center General Hospital in Kobe (by MT), or Department of Ophthalmology, Nagoya University Hospital in Nagoya (by MK). Patients' origin varied widely, from the Tokyo to Osaka areas in Japan. Japanese LCA patients were examined at the Department of Ophthalmology and Laboratory of Cell Biology, National Center for Child Health and Development in Tokyo (by NA). LCA patients' origin varied widely, from all over Japan except the Okinawa islands. Meanwhile, Korean RP patients were examined at the Department of Ophthalmology, Kyungpook National University Hospital in Daegu (by ITK). The Korean patients' origin varied widely, from Daegu to Yeongju and Pohang areas in Gyeongsangbuk-do, Korea. A full ophthalmic examination was performed. Clinical diagnosis for RP was based on visual field, fundus examination, and electroretinogram findings, and clinical diagnosis for LCA was based on fundus examination and electroretinogram findings.

Ethics statements

This study was approved by the Institutional Review Board for Human Genetic and Genome Research at the 6 participating institutions (Hamamatsu University School of Medicine, RIKEN Center for Developmental Biology, Nagoya University Graduate School of Medicine, National Center for Child Health and Development, Chiba University Graduate School of Medicine, and Kyungpook National University Hospital), and its procedures conformed to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants before molecular genetic studies.

Mutation analysis

Genomic DNA in Japanese samples was extracted from the peripheral lymphocytes using standard procedures. In Korean samples, whole blood samples were collected on FTA cards (GE Healthcare). Blood samples were spotted onto the cards and air-dried for 1 h at room temperature. For polymerase chain reaction (PCR) amplification, a 1.2-mm disk was punched from a dried blood spot using a Harris micro-punch tool (GE Healthcare) and processed according to the manufacturer's instructions. PCR was performed using the KOD -Plus- ver. 2 PCR kit (Toyobo) with the primer sets described in Table S1 for 35 cycles of 98°C for 10 s, 60°C for 30 s, and 68°C for 1 min in an automated thermal cycler (GeneAmp PCR System 9700; Applied Biosystems). PCR products were purified with Wizard SV Gel and PCR Clean-up System (Promega) or treated with Exonuclease I and Antarctic Phosphatase (New England Biolabs). Direct sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI3100 autosequencer (Applied Biosystems). For Japanese arRP patients, all 44 exons, including 3 non-coding exons (exons 1–3) that cover the 5′ untranslated region and 41 coding exons (exons 4–44), were analyzed in both sense and antisense directions. For Japanese adRP and LCA patients, and Korean arRP patients, parts of exons 26 and 44 were analyzed (Table S1).

Assessment of pathogenicity

A sequence variant was considered pathogenic if it represented a truncating mutation (nonsense or frameshift), large-scale deletion mutation, or missense mutation affecting a conserved amino acid residue and did not appear in control samples (number of alleles studied ≤400) and/or in a public SNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/). Particularly, missense mutations were considered pathogenic if found together with a second variant, especially if it was truncating. As reference data, we employed 4 computational algorithms to evaluate the pathogenicity of missense mutations: SIFT (http://sift.jcvi.org/www/SIFT_seq_submit2.html), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/), PMut (http://mmb.pcb.ub.es/PMut/), and SNAP (http://rostlab.org/services/snap/).

Results

Mutation analysis

Mutation analysis of *EYS* in 100 unrelated Japanese patients revealed 7 very likely pathogenic mutations in 18 patients (18%). Of these 18 patients, a second mutant allele could not be detected in 9 patients. The very likely pathogenic mutations consisted of 3 truncating mutations, 1 deletion mutation, 2 missense mutations, and 1 previously described mutation (Fig. 1, Table 1, and Table 2). In addition, we also identified 6 possible pathogenic mutations in 8 separate patients (Table 1 and Table 2).

A novel truncating insertion, c.4957_4958insA, was detected in 12 patients and accounted for 15 of the 35 mutated alleles detected (42.9%) (Table 1 and Table 2). Three patients were homozygous for the c.4957_4958insA mutation, and the other 9 patients were heterozygous. Of the latter, 3 patients showed the second mutation while 6 did not. This insertion creates a frameshift mutation that predicts a premature stop at codon 1654 (p.S1653KfsX2). A novel truncating nonsense mutation c.8868C>A (p.Y2956X) was identified in 4 patients and accounted for 5 of the 35 mutated alleles detected (14.3%). Thus, these 2 novel truncating mutations were identified in 16 separate patients, resulting in a very high frequency of the 2 mutations in Japanese arRP patients.

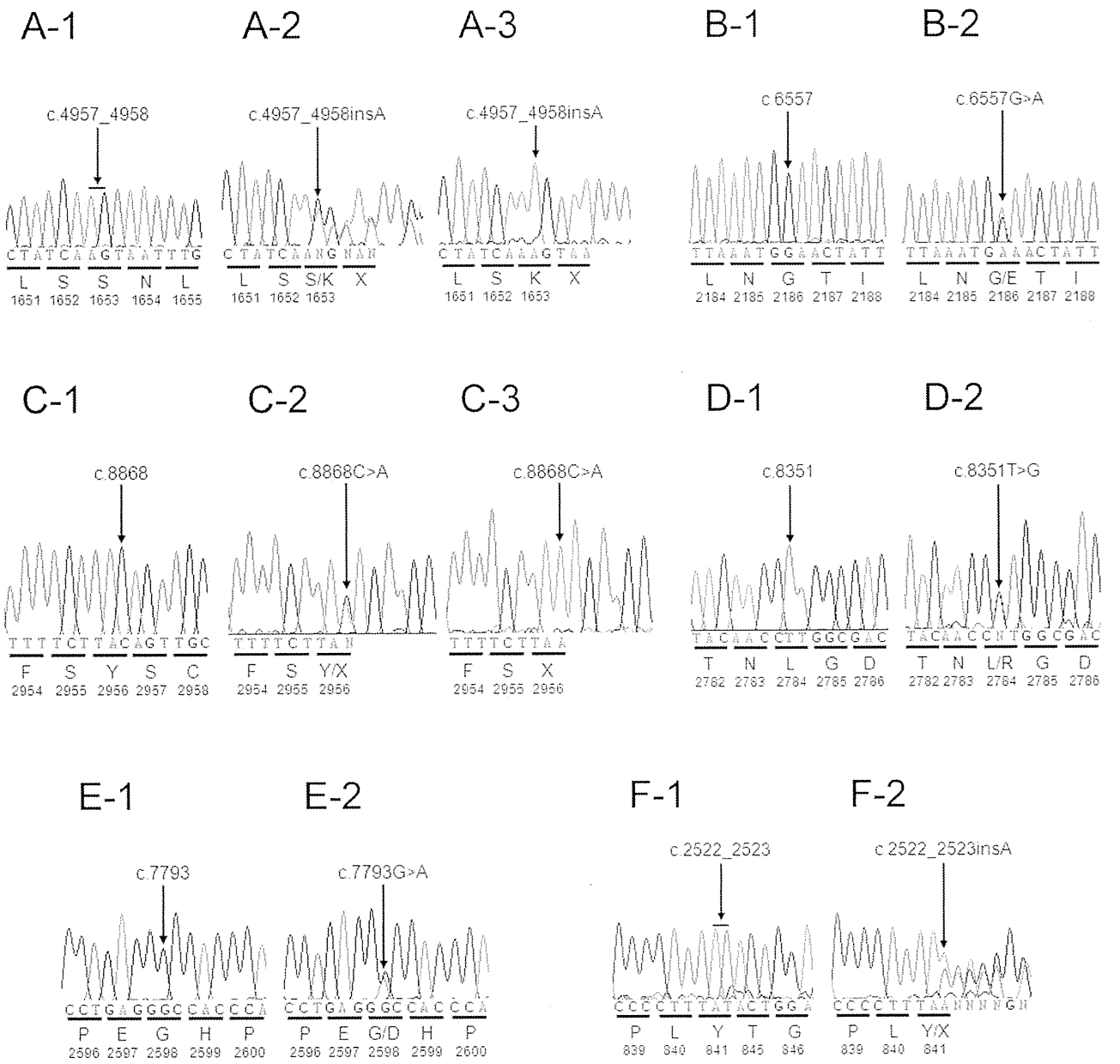

Figure 1. Electropherograms of the 6 likely pathogenic *EYS* mutations. Partial sequence of the *EYS* gene showing the normal control sequences (A-1 through F-1), heterozygous mutation sequences (A-2 through F-2), and homozygous mutation sequences (A-3 and C-3). Deduced amino acids are indicated under the sequence trace. The mutation location is indicated either by an arrow (for a nucleotide change) or a horizontal line (to show 2 nucleotides between which the insertion occurred). (A) c.4957_4958insA; p.S1653KfsX2 (Exon 26), (B) c.6557G>A; p.G2186E (Exon 32), (C) c.8868C>A; p.Y2956X (Exon 44), (D) c.8351T>G; p.L2784R (Exon 44), (E) c.7793G>A; p.G2598D (Exon 40), (F) c.2522_2523insA; p.Y841X (Exon 16). doi:10.1371/journal.pone.0031036.g001

Families with very likely pathogenic mutations and both alleles affected

Nine of the 18 patients bearing very likely pathogenic mutations appeared to have both alleles affected, suggesting that they received one mutated allele from each unaffected parent (Table 1 and Table 2). In 4 patients (RP3H, RP48K, RP56K, and RP81K), segregation analysis was performed, and the 2 pathogenic alleles were considered to be on different chromosomes (Fig. 2).

1. In RP3H, proband (II-6) was homozygous for c.4957_4958insA. The mutation co-segregated with the phenotype: the unaffected brother (II-4) demonstrated wild-type alleles, while the affected brother (II-5) was homozygous for the mutation.
2. In RP48K, proband (II-1) was homozygous for c.4957_4958insA. The unaffected brother (II-2) was heterozygous for the mutation.
3. In RP56K, proband (II-1) was compound heterozygous for c.4957_4958insA and missense mutation c.8351T>G (p.L2784R). The mutation co-segregated with the phenotype: the affected brother (II-2) also showed both mutations, while the unaffected brother (II-3) was heterozygous for c.4957_4958insA.

Table 1. Mutation spectrum of the *EYS* gene in Japanese families.

Family ID	Nucleotide change	Predicted effect	Domain[a]	Location in gene	Type of change	Reference
Families with very likely pathogenic mutations and both alleles affected						
RP3H[b]	c.4957_4958insA/ c.4957_4958insA	p.S1653KfsX2/ p.S1653KfsX2	Close to coiled-coil/ Close to coiled-coil	Exon 26/Exon 26	Homozygous	This study
RP48K[b]	c.4957_4958insA/ c.4957_4958insA	p.S1653KfsX2/ p.S1653KfsX2	Close to coiled-coil/ Close to coiled-coil	Exon 26/Exon 26	Homozygous	This study
RP54K	c.4957_4958insA/ c.4957_4958insA	p.S1653KfsX2/ p.S1653KfsX2	Close to coiled-coil/ Close to coiled-coil	Exon 26/Exon 26	Homozygous	This study
RP44K	c.4957_4958insA/ c.6557G>A	p.S1653KfsX2/ p.G2186E	Close to coiled-coil/ Laminin G	Exon 26/Exon 32	Heterozygous/ Heterozygous	This study/Abd El-Aziz et al., 2010; Littink et al., 2010; This study
RP56K[b]	c.4957_4958insA/ c.8351T>G	p.S1653KfsX2/ p.L2784R	Close to coiled-coil/ Laminin G	Exon 26/Exon 44	Compound Heterozygous	This study
RP87N	c.4957_4958insA/ c.7793G>A	p.S1653KfsX2/ p.G2598D	Close to coiled-coil/ Close to Laminin G	Exon 26/Exon 40	Heterozygous/ Heterozygous	This study
RP81K[b]	c.2522_2523insA/ c.6557G>A	p.Y841X/p.G2186E	EGF/Laminin G	Exon 16/Exon 32	Compound Heterozygous	This study/Abd El-Aziz et al., 2010; Littink et al., 2010; This study
RP21H	deletion exon32/ deletion exon32	p.D2142_S2191delinsG/ p.D2142_S2191delinsG	Laminin G/Laminin G	Exon 32/Exon 32	Homozygous	This study
RP35K	c.8868C>A/c.8868C>A	p.Y2956X/p.Y2956X	EGF/EGF	Exon 44/Exon 44	Homozygous	This study
Families with single very likely pathogenic mutations						
RP1H	c.4957_4958insA	p.S1653KfsX2	Close to coiled-coil	Exon 26	Heterozygous	This study
RP6H	c.4957_4958insA	p.S1653KfsX2	Close to coiled-coil	Exon 26	Heterozygous	This study
RP12H	c.4957_4958insA	p.S1653KfsX2	Close to coiled-coil	Exon 26	Heterozygous	This study
RP51K	c.4957_4958insA	p.S1653KfsX2	Close to coiled-coil	Exon 26	Heterozygous	This study
RP96H	c.4957_4958insA	p.S1653KfsX2	Close to coiled-coil	Exon 26	Heterozygous	This study
RP100N	c.4957_4958insA	p.S1653KfsX2	Close to coiled-coil	Exon 26	Heterozygous	This study
RP8H	c.8868C>A	p.Y2956X	EGF	Exon 44	Heterozygous	This study
RP25H	c.8868C>A	p.Y2956X	EGF	Exon 44	Heterozygous	This study
RP80K[b]	c.8868C>A	p.Y2956X	EGF	Exon 44	Heterozygous	This study
Families with single possible pathogenic mutations						
RP4H	c.9272T>C	p.I3091T	Laminin G	Exon 44	Heterozygous	This study
RP9H	c.8875C>A	p.L2959M	EGF	Exon 44	Heterozygous	This study
RP49K	c.9272T>C	p.I3091T	Laminin G	Exon 44	Heterozygous	This study
RP53K	c.5884A>G	p.T1962A	Laminin G	Exon 28	Heterozygous	This study
RP55K	c.9272T>C	p.I3091T	Laminin G	Exon 44	Heterozygous	This study
RP74K	c.5404C>T	p.L1802F	Close to Laminin G	Exon 26	Heterozygous	This study
RP79K	c.77G>A	p.R26Q	Close to signal peptide cleavage site	Exon 4	Heterozygous	This study
RP83K	c.2923T>C	p.C975R	EGF	Exon 19	Heterozygous	This study

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence FM209056, according to the nomenclature recommended by the Human Genome Variation Society (www.hgvs.org/mutnomen). The initiation codon is codon 1. None of these 13 mutations were found in the Japanese controls.

[a]EYS has a signal peptide, a putative coiled-coil, 29 EGF, and 5 Laminin G domains. See Fig. 3.

[b]Segregation analysis has been performed. See Fig. 2.

In RP56K and RP81K, 2 pathogenic alleles were considered to be on different chromosomes (compound heterozygous). See Fig. 2.

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4. In RP81K, proband (II-5) was compound heterozygous for truncating insertion c.2522_2523insA (p.Y841X) and missense mutation c.6557G>A (p.G2186E). This insertion results in premature termination of the encoded protein at codon 841 (p.Y841X). Missense mutation c.6557G>A has been previously reported as disease causing in one Korean/American and one Chinese patient [3,6]. The unaffected mother (I-2) was heterozygous for c.2522_2523insA, while the unaffected sister (II-6) was heterozygous for c.6557G>A.

For the other patients, segregation analysis could not be performed due to difficulties in collecting samples from the families of patients (Table 1). RP54K and RP35K were homozygous for truncating mutation c.4957_4958insA and c.8868C>A, respectively. RP21H was homozygous for deletion in exon 32, an in-frame deletion that results in the replacement of amino acids from D2142 to S2191 with G2142 (p.D2142_S2191delinsG) and disrupts the second laminin G domain (Fig. 3). RP44K and RP87N were heterozygous for truncating and missense mutations, c.4957_4958insA/c.6657G>A (p.G2186E) and

Table 2. Summary of the very likely and possible pathogenic mutations identified in 100 Japanese arRP patients.

							Allele frequency					Computational prediction[c]			
		Nucleotide change	Predicted effect	Location in gene	Domain[a]	Conservation in hu/o/m/ho/d/op/p/c/z/dr[b]	Control	Patient	Family ID	Reference	Species	SIFT	PolyPhen2 (HumDiv)	PMut	SNAP
Very likely pathogenic mutations	**Insertion**	c.2522_2523insA	p.Y841X	Exon 16	EGF	not applicable	0/400	1/200	RP81K	This study	Japanese				
		c.4957_4958insA	p.S1653KfsX2	Exon 26	Close to coiled-coil	not applicable	0/400	15/200	RP1H, RP3H, RP6H, RP12H, RP48K, RP51K, RP54K, RP44K, RP56K, RP87N, RP96H, RP100N	This study	Japanese				
	Nonsense	c.8868C>A	p.Y2956X	Exon 44	EGF	not applicable	0/400	5/200	RP8H, RP25H, RP35K, RP80K	This study	Japanese				
	Deletion	Deletion exon 32	p.D2142_S2191delinsG	Exon 32	Laminin G	not applicable	0/200[d]	2/200	RP21H	This study	Japanese				
	Missense	c.6557G>A	p.G2186E	Exon 32	Laminin G	G/G/G/G/G/-/-/-/-/-	0/400	2/200	RP44K, RP81K	Abd El-Aziz et al., 2010; Littink et al., 2010; This study	Chinese, South Korean/American, Japanese		Probably damaging	Pathological	Non-neutral
		c.7793G>A	p.G2598D	Exon 40	Close to Laminin G	G/G/G/-/-/-/-/G/I/T	0/400	1/200	RP87N	This study	Japanese		Probably damaging		Non-neutral
		c.8351T>G	p.L2784R	Exon 44	Laminin G	L/L/L/L/L/L/L/L/L/G	0/400	1/200	RP56K	This study	Japanese		Probably damaging		Non-neutral
Possible pathogenic mutations	**Missense**	c.77G>A	p.R26Q	Exon 4	Close to signal peptide cleavage site	R/R/R/K/K/-/-/-/-/-	0/400	1/200	RP79K	This study	Japanese	Affected protein function		Pathological	
		c.2923T>C	p.C975R	Exon 19	EGF	C/C/C/-/-/-/-/-/-/-	0/400	1/200	RP83K	This study	Japanese		Possibly damaging	Pathological	Non-neutral
		c.5404C>T	p.L1802F	Exon 26	Close to Laminin G	L/L/L/-/-/-/-/-/-/-	0/400	1/200	RP74K	This study	Japanese		Possibly damaging		
		c.5884A>G	p.T1962A	Exon 28	Laminin G	T/T/T/T/-/-/-/-/-/-	0/400	1/200	RP53K	This study	Japanese		Possibly damaging		
		c.8875C>A	p.L2959M	Exon 44	EGF	L/L/L/L/L/L/A/V/-/S	0/400	1/200	RP9H	This study	Japanese		Possibly damaging		
		c.9272T>C	p.I3091T	Exon 44	Laminin G	I/I/I/I/I/I/I/I/I/L	0/400	3/200	RP4H, RP49K, RP55K	This study	Japanese	Affected protein function	Probably damaging		

[a]EYS contains a signal peptide, a putative coiled-coil, 29 EGF, and 5 laminin G domains. See Fig. 3.

[b]hu/o/m/ho/d/op/p/c/z/dr denotes Human/Orangutan/Marmoset/Horse/Dog/Opossum/Platypus/Chicken/Zebrafish/Drosophila EYS orthologs, respectively. The hyphen (-) indicates that genomic sequence of corresponding region in the species was reported to be unknown [5].

[c]SIFT, PolyPhen2 (only the HumDiv data are shown), PMut, and SNAP were used as reference data to evaluate the pathogenicity of the missense mutations. c.77G>A, c.2923T>C, c.7793G>A, c.8351T>G, and c.9272T>C were predicted to be pathogenic by a number of different computational prediction programs. In addition, the c.6557G>A mutation, which had been previously reported as disease causing, was classified as pathogenic by the PolyPhen2, PMut, and SNAP programs.

[d]Homozygous exon 32 deletion mutation was not detected in 200 controls.

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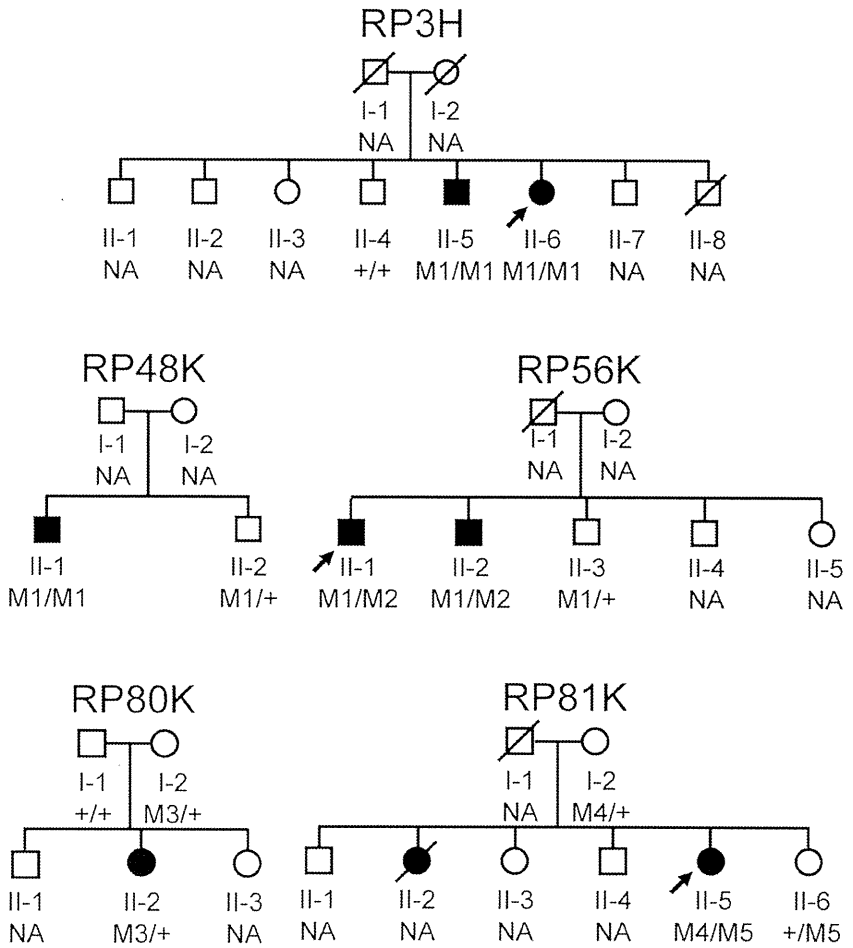

Figure 2. Pedigrees of the families that was available for mutation analysis. Below the individuals, genotypes are presented for either p.S1653KfsX2 (M1), p.L2784R (M2), p.Y2956X (M3), p.Y841X (M4), or p.G2186E (M5) detected to segregate with RP. M1/M1 represents homozygous mutation. M1/+ indicates heterozygous carriers, +/+ indicates individuals carrying 2 wild-type alleles, whereas M1/M2 represents individuals presenting both mutations as compound heterozygous. Square boxes indicate men, circles denote women, and affected individuals are pointed out by a black symbol. Slashed symbols indicate deceased individuals. The probands are indicated with an arrow. NA denotes unavailable DNA samples.
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c.4957_4958insA/c.7793T>G (p.G2598D), respectively. None of these 7 very likely pathogenic mutations were found in the Japanese controls.

Families with single novel very likely pathogenic mutations

The rest of the patients comprising the group with very likely pathogenic mutations presented only single truncating mutations (Table 1 and Table 2). RP1H, RP6H, RP12H, RP51H, RP96H, and RP100N were heterozygous for c.4957_4958insA. RP8H, RP25H, and RP80K were heterozygous for c.8868C>A. Segregation analysis was performed in patient RP80K. The unaffected father (I-1) demonstrated wild-type alleles, and the unaffected mother (I-2) was heterozygous for the mutation (Fig. 2). In RP96H, we found very likely pathogenic missense mutation c.8923T>C (p.F2975L), which was not detected in any of the 400 control alleles. However, as c.8923T>C has been described as rs79036642 in the dbSNP database, it was assigned to the group of possible non-pathogenic sequence alterations (Table 3).

Families with single novel possible pathogenic mutations

A group of patients with possible pathogenic mutations had only single missense mutations (Table 1 and Table 2). We report 6 novel missense mutations in 8 different patients (Table 1 and Table 2), none of which were identified in the 400 Japanese control alleles. All amino acid residues affected by these mutations were compared with those encoded by orthologous genes of various vertebrates (orangutan, marmoset, horse, dog, opossum, platypus, chicken, and zebrafish) and *Drosophila* and found to be highly conserved across species (Table 2). The novel missense mutation c.2923T>C (p.C975R) was predicted to be pathogenic by 3 different computational prediction programs (PolyPhen2, PMut, and SNAP) (Table 2). RP4H, RP49K, and RP55K were heterozygous for the same missense mutation c.9272T>C (p.I3091T), which was predicted to be pathogenic by SIFT and PolyPhen2 programs (Table 2). In addition, 54 possible non-pathogenic sequence alterations were found, of which 9 were previously unreported (Table 3).

Screening of the 2 truncating mutations

We focused on 2 truncating mutations, c.4957_4958insA in exon 26 and c.8868C>A in exon 44, which were identified in 16 separate Japanese arRP patients in this study. The frequency of the 2 mutations was very high in this Japanese arRP cohort. However, we did not detect the 2 mutations in 19 Japanese adRP

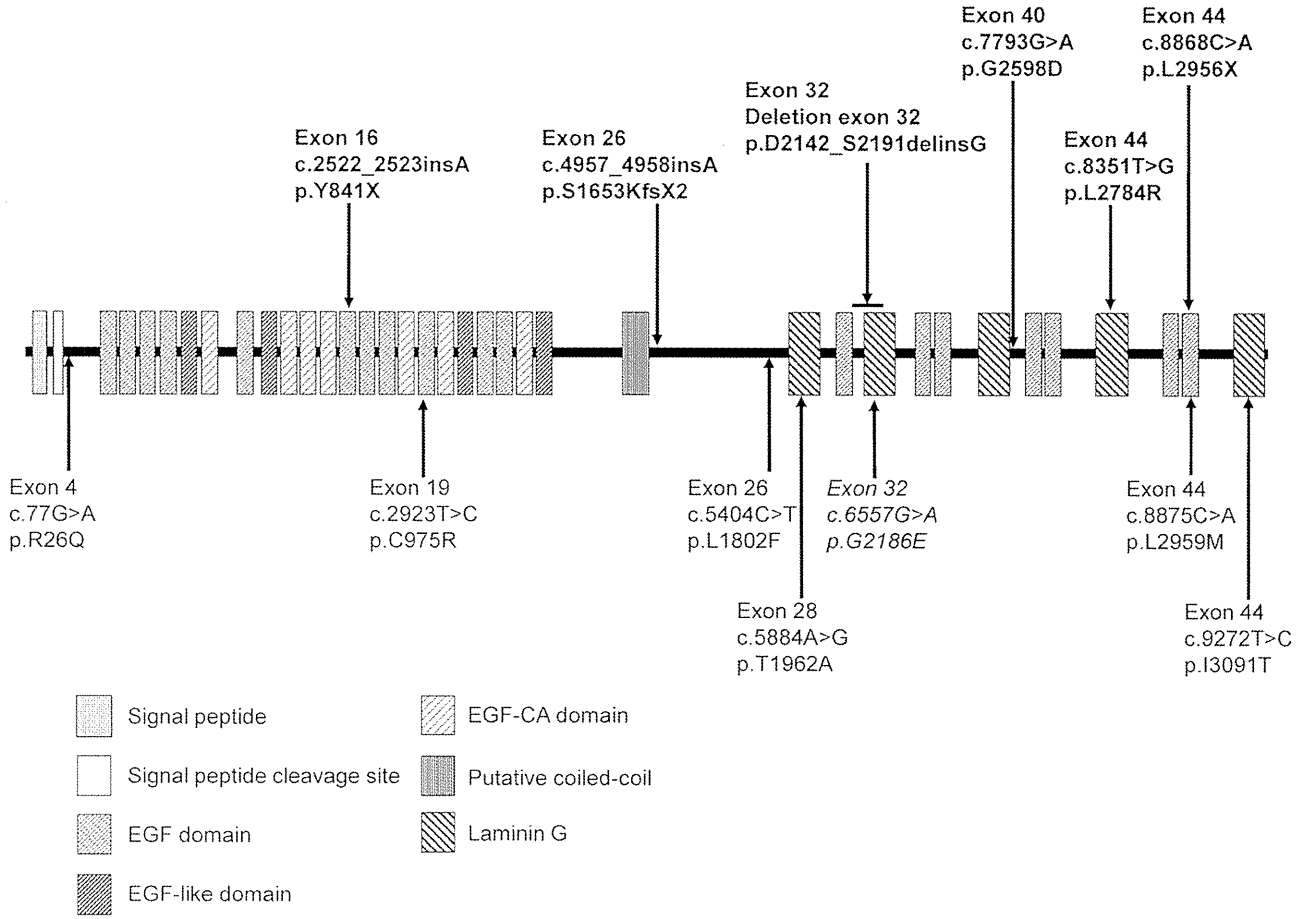

Figure 3. Predicted domain structure and distribution of identified *EYS* mutations. SMART (http://smart.embl-heidelberg.de/) and Pfam (http://pfam.sanger.ac.uk/) were used to search protein functional domains. A coiled-coil domain identified by Barragán et al. (2010) between the EGF-like domain and laminin G domain was also indicated. Novel very likely pathogenic mutations, novel possible pathogenic mutations, and a previously described mutation are shown in bold, normal, and italic type, respectively. Six out of 9 missense mutations were found in the EGF or laminin G domains. Furthermore, 7 were located in the latter half of the protein between the putative coiled-coil region and C-terminus.
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patients and 28 LCA patients who were recruited and screened to evaluate the frequency of the mutations. We also recruited 32 unrelated Korean arRP patients and screened for the 2 *EYS* gene mutations. The c.4957_4958insA mutation was detected in 2 patients and accounted for 3 of 64 Korean patient alleles (4.7%). One patient was homozygous and the other was heterozygous. The c.8868C>A mutation was identified in 1 patient and accounted for 1 of the 64 Korean patient alleles (1.6%).

Clinical findings

Nine Japanese patients with very likely pathogenic *EYS* gene mutations in both alleles, 9 Japanese patients with single very likely pathogenic changes, and a Korean patient with homozygous c.4957_4958insA mutation demonstrated classic RP with mostly night blindness as the initial symptom, followed by gradual constriction of the visual field. The fundus displayed bone spicules increasing in density with age and attenuated retinal vessels. Electroretinogram responses were not detectable, consistent with severe generalized rod-cone dysfunction. The remaining visual field determined using Goldmann kinetic perimetry with V-4 target ranged from approximately 10° to 60° of the central and inferior visual fields, respectively, in a 74-year-old woman (RP100N) to complete blindness in a 54-year-old man (RP21H). No remarkable clinical difference was observed between 9 patients with very likely pathogenic *EYS* gene mutations in both alleles and 9 patients with single very likely pathogenic changes.

Discussion

This study is the first to analyze mutations in the *EYS* gene among Japanese arRP patients. We detected 67 sequence alterations in the *EYS* gene, of which 21 were novel. Of these, 7 were very likely pathogenic mutations, 6 were possibly pathogenic mutations, and 54 were possible non-pathogenic sequence alterations (Table 1, Table 2, and Table 3).

Considering only the very likely pathogenic mutations, the minimum observed prevalence of distinct *EYS* gene mutations in our study is 18% (18/100, 9 patients with 2 very likely pathogenic mutations and 9 with only one such mutations). Additionally, if the possible pathogenic mutations are included in the prevalence estimation, prevalence increases to 26% (26/100, with 17 of 26 patients presenting single mutations). The estimated prevalence in our study may be extremely high compared with those in the previous studies [3–6]. Until recently, mutations in 34 genes have been associated with arRP (http://www.sph.uth.tmc.edu/Retnet/). The most frequently mutated gene is *USH2A*, accounting for

Table 3. Summary of the possible non-pathogenic sequence alterations in the *EYS* gene identified in this study.

Gene exon	Nucleotide change	Predicted effect	Conservation in hu/o/m/ho/d/op/p/c/z/dr[a]	Patient frequency	Control frequency	SNP ID	Reference
Exon 1	c.-500A>G			13/200		rs1490127	Abd El-Aziz et al., 2010
Exon 4	c.334G>A	p.V112I	V/I/I/I/I/I/-/-/-/E	1/200	0/192	rs112609906	
	c.359C>T	p.T120M	T/T/T/T/T/A/-/-/-/I	60/200		rs12193967	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.525_527delGGA	p.176delE	E/E/E/E/E/A/-/-/-/G	1/200	1/192		This study
Intron 5	c.863-23_863-22insTT			53/200		rs34154043	Abd El-Aziz et al., 2010
	c.863-23_863-22insTTT			44/200			This study
Exon 6	c.1005G>T	p.E335D	E/E/D/-/-/-/-/-/-/-	3/200		rs80095433	
Exon 7	c.1146T>C	p.N382N	N/N/T/-/-/-/-/-/-/-	97/200		rs974110	Audo et al., 2010; Abd El-Aziz et al., 2010
Intron 8	c.1300-3C>T			117/200		rs1936439	Audo et al., 2010; Abd El-Aziz et al., 2010
Exon 9	c.1382G>A	p.C461Y	C/C/Y/-/-/-/-/-/-/-	8/200	4/192	rs76754818	Littink et al., 2010
Intron 9	c.1599+96A>C			200/200		rs1502963	Abd El-Aziz et al., 2010
Intron 10	c.1600-38G>A			12/200		rs1502965	Abd El-Aziz et al., 2010
Exon 11	c.1712A>G	p.Q571R	Q/Q/Q/-/-/-/-/-/-/-	26/200		rs61753610	Audo I et al., 2010
Exon 12	c.1809C>T	p.V603V	V/V/V/-/-/-/-/-/-/-	178/200		rs9345601	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.1891G>A	p.G631S	G/S/E/C/C/-/-/-/-/-	178/200		rs9342464	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.1922A>T	p.E641V	E/E/E/E/E/-/-/-/-/-	18/200		rs17411795	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.1985G>T	p.R662M	R/R/R/S/S/-/-/-/-/-	8/200	3/96		This study
Intron 12	c.2023+6_2023+7insT			175/200		rs67504324	
	c.2024-14C>T			3/200		rs45628235	
Intron 15	c.2382-26C>G			106/200		rs9445437	
Exon 16	c.2490T>C	p.P830P	P/P/P/P/P/P/P/Q/P/-	2/200	1/392		This study
	c.2528G>A	p.G843E	G/G/G/G/G/G/G/A/G	16/200	9/192	rs74419361	
	c.2555T>C	p.L852P	L/P/P/-/S/P/S/P/-/E	106/200		rs9294631	Audo et al., 2010; Abd El-Aziz et al., 2010
Intron 18	c.2846+52_2846+53insTAAT			120/200		rs66504228	Abd El-Aziz et al., 2010
	c.2847-24C>T			178/200		rs7743515	
Exon 19	c.2980C>G	p.P994A	P/P/P/-/-/-/-/-/-/-	3/200	2/192		This study
Intron 22	c.3444-5C>T			69/200		rs9445051	Audo et al., 2010; Abd El-Aziz et al., 2010
Intron 23	c.3568+60delA			1/200			This study
Exon 25	c.3787A>G	p.I1263V	I/V/V/V/V/-/-/-/-/I	36/200		rs17404123	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.3809T>G	p.V1270G	V/V/V/V/V/-/-/-/-/P	1/200	1/192		This study
Intron 25	c.3877+17_22delAGATA			36/200			Barragán I et al., 2010
Exon 26	c.3906C>T	p.H1302H	H/H/H/H/H/-/-/-/-/S	10/200		rs12663916	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.3936A>G	p.T1312T	T/A/T/A/A/-/-/-/-/S	10/200		rs12662610	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.3973C>G	p.Q1325E	Q/E/K/K/K/-/-/-/-/S	12/200		rs12663622	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.4026C>T	p.S1342S	S/S/S/S/S/-/-/-/-/A	10/200		rs12663619	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.4081A>G	p.I1361V	I/I/T/V/V/-/-/-/-/S	12/200		rs17403955	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.4256T>C	p.L1419S	L/S/S/S/S/L/S/V/Q/V	137/200		rs624851	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.4352T>C	p.I1451T	I/T/T/K/K/-/-/-/-/T	13/200		rs62415828	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.4543C>T	p.R1515W	R/R/R/R/R/-/-/-/-/H	36/200		rs62415827	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.4549A>G	p.S1517G	S/G/D/T/T/-/-/-/-/H	36/200		rs62415826	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.4593G>A	p.E1531E	E/E/E/E/E/-/-/-/-/Q	36/200		rs62415825	Audo et al., 2010; Abd El-Aziz et al., 2010
	c.5244A>C	p.L1748F	L/L/L/L/L/-/-/-/-/F	8/200		rs57312007	Audo I et al., 2010; Littink et al., 2010
	c.5617C>G	p.L1873V	L/L/L/P/P/-/-/-/-/I	38/200		rs16895517	Audo I et al., 2010
Exon 27	c.5705A>T	p.N1902I	N/N/N/N/N/P/-/R/-/A	90/200		rs9353806	Audo et al., 2010; Abd El-Aziz et al., 2010
Intron 28	c.5928-35T>C			118/200		rs587278	Abd El-Aziz et al., 2010
Intron 29	c.6078+68A>G			81/200		rs36133910	Abd El-Aziz et al., 2010
	c.6079-4_6079-3delTC			87/200		rs35395170	Audo I et al., 2010
Intron 34	c.6834+61T>G			60/200		rs66502009	Abd El-Aziz et al., 2010

Table 3. Cont.

Gene exon	Nucleotide change	Predicted effect	Conservation in hu/o/m/ho/d/op/p/c/z/dr[a]	Patient frequency	Control frequency	SNP ID	Reference
Exon 35	c.6977G>A	p.R2326Q	R/R/R/L/L/L/L/L/I/L	95/200		rs4710457	Audo et al., 2010; Abd El-Aziz et al., 2010
Exon 37	c.7394C>G	p.T2465S	T/T/T/T/T/T/T/T/S/F	8/200	2/176		This study
Exon 39	c.7666A>T	p.S2556C	S/S/S/S/S/N/S/H/E/E	57/200		rs66462731	Audo et al., 2010; Abd El-Aziz et al., 2010; Barragán et al., 2010; Littink et al., 2010
Intron 41	c.8071+84T>G			53/200		rs4710257	Abd El-Aziz et al., 2010
Exon 44	c.8923T>C	p.F2975L	F/F/F/F/F/F/F/F/-/K	1/200	0/400	rs79036642	
	c.9300A>G	p.L3100L	L/L/L/L/L/L/L/L/V/I	4/200	2/192		This study

Fifty-four sequence alterations were identified in 100 patients. These alterations were predicted to be non-pathogenic for various reasons. Some have been reported as polymorphisms in previous reports. Newly identified alterations within the exons, except for c.334G>A and c.8923T>C, were also found in the control chromosome. The hyphen (-) indicates that genomic sequence of corresponding region in the species was reported to be unknown [5].

[a]hu/o/m/ho/d/op/p/c/z/dr denotes Human/Orangutan/Marmoset/Horse/Dog/Opossum/Platypus/Chicken/Zebrafish/Drosophila EYS orthologs, respectively.

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approximately 7% of arRP cases [9,10], whereas most other genes contribute to only 1% to 2% of arRP cases [11]. The estimated prevalence of very likely and possible pathogenic mutations of the *EYS* gene in our study was 26%, suggesting its major involvement in the pathogenesis of arRP in the Japanese population.

We found that 16% of Japanese arRP patients displayed at least one c.4957_4958insA or c.8868C>A mutation, which accounted for 57.1% (15+5/35) of the mutated alleles. Thus, these mutations seem to be frequent among Japanese arRP patients. Previous studies employing Indonesian, Pakistani, Chinese, Israeli, Spanish, French, British, Dutch, and Palestinian RP patient populations have not detected them [3–6,12–15]. Since the Japanese were divided into small semi-closed population groups among which intercommunication was quite less until the mid-20th century, obvious or latent consanguineous marriages were carried out more frequently, leading to relatively high inbreeding levels in those populations. The frequency of the c.4957_4958insA and c.8868C>A mutations may result from a founder effect like that of the 2299delG *USH2A* gene mutation, which accounts for 44% of disease alleles in Danish and Norwegian patients with Usher syndrome type II [16].

We detected 13 different very likely and possible pathogenic mutations. Three were truncating mutations and accounted for 60% (21/35) of mutated alleles. Likewise, previous studies reported that most pathogenic mutations were truncated type (nonsense, deletion, insertion, or splicing) [3–6,12–15]. Furthermore, c.6557G>A was the only mutation that was common between the Japanese and other populations. This mutation has been found in Korean/American and Chinese patients [3,6]. These results indicate that the *EYS* gene mutation spectrum among Japanese patients largely differs from that among the previously mentioned non-Asian populations. The Japanese and Korean mutation spectrum may resemble each other, but an accurate comparison could not be made, because further *EYS* gene analysis of Korean RP patients is required to clarify this possibility.

A second mutant allele could not be detected by direct sequencing in 17 of 26 patients in our study. Previous studies reported 7 of 10 [3] and 9 of 17 [5] patients with heterozygous *EYS* gene mutation, implying that this finding could be due to relatively large heterozygous deletions [15]. The second mutation in these families may also have been located within the gene regulatory elements or unknown exons including alternative splicing areas. Although rare, a single *EYS* mutation in combination with another mutation on a second gene could also explain this phenotype [3].

The c.4957_4958insA and c.8868C>A mutations were not detected in Japanese patients with adRP or with LCA. Abd El-Aziz et al. reported that *EYS* gene mutation screening did not reveal any pathogenic mutations in 95 British and Chinese adRP patients [3]. Bandah-Rosenfeld et al. reported that no mutation was found in 2 Oriental Jewish and Israeli Muslim LCA patients who had a large homozygous region harboring the *EYS* gene [12]. Although further analysis of all *EYS* gene exons is required, *EYS* gene mutations may not be detected in Japanese patients with adRP and LCA. The c.4957_4958insA and c.8868C>A mutations were also detected in Korean patients with arRP and accounted for 6.3% (4/64 alleles) of the disease alleles. Similar to Japanese arRP results, the c.4957_4958insA mutation was more frequently detected than the c.8868C>A mutation. The fact that both c.4957_4958insA and c.8868C>A mutations were also detected in Korean patients suggests the possibility that the mutations occurred in an ancient common ancestor and spread throughout East Asia.

RP is a highly heterogeneous disease, with a reported prevalence rate of 1 in 4,000–8,000 people in Japan. Given the population of Japan, approximately a 100 million, the number of patients with RP can be estimated to be 12,500–25,000. The relative frequencies of RP inheritance patterns in Japanese patients were estimated as 25.2% for autosomal recessive, 16.9% for autosomal dominant, 1.6% for X-linked, and 56.3% for simplex, indicating that most Japanese RP patients represent arRP or isolated cases [17]. Autosomal recessive and simplex cases account over 80% of RP cases in Japan (approximately 10,000–20,000 people). Our results indicate that c.4957_4958insA and c.8868C>A mutations are possibly present in 1,600–3,200 Japanese patients with RP. These 2 novel mutations will be very useful for genetic diagnosis and counseling, and analysis of the mutated proteins may be helpful in the development of effective therapies for RP in Japan and Korea.

In conclusion, mutation screening of the *EYS* gene in 100 Japanese patients revealed 13 different pathogenic mutations, confirming that the mutation spectrum in Japanese patients differs from the previously reported spectrum in patients of non-Asian populations. Among these 13 mutations, 2 truncating mutations, c.4957_4958insA and c.8868C>A, were detected in at least one mutated allele in 16% of Japanese arRP patients and may be the

most frequent mutations causing RP in the Japanese populations. Screening for c.4957_4958insA and c.8868C>A mutations in the *EYS* gene is, therefore, very effective for the genetic testing and counseling of RP patients in Japan. Further analysis is necessary to obtain a more precise mutation spectrum and to identify other frequent mutations in other East Asian populations.

Supporting Information

Table S1 PCR primer sequences for human *EYS*.
(DOC)

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Author Contributions

Conceived and designed the experiments: KH MT SY MK YH. Performed the experiments: KH CI YZ. Analyzed the data: KH CI. Contributed reagents/materials/analysis tools: MT DHP YH HN SU TY AH TF SN JPS ITK SY NA HT MS MK YH. Wrote the paper: KH SM YH.

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LETTER

A case of extraocular muscle swelling due to IgG4-related sclerosing disease

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A novel clinicopathological entity of IgG4-related sclerosing disease has recently been proposed that is characterized by infiltration of IgG4-positive plasma cells and T lymphocytes with fibrosis into various organs [1]. Mikulicz disease, characterized by symmetric bilateral swelling of the lacrimal and salivary glands, is considered a subtype of IgG4-related sclerosing disease [2]. We report a case of concurrent extraocular muscle swelling and Mikulicz disease.

Case report

A 68-year-old Japanese woman noticed swelling in her left eye in January 2005, and left palpebral edema developed in February 2008. She was referred to our hospital. She had a history of autoimmune pancreatitis (AIP).

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The eyes were normal bilaterally. Exophthalmos measured 13 mm in the right eye and 19 mm in the left eye. The Schirmer test results were 2.5 mm for the right eye and 3 mm for the left eye. The visual acuities were 1.5 in the right eye and 0.4 in the left. The critical flicker frequency values were 43 Hz in the right eye and 20 Hz in the left. Magnetic resonance imaging revealed swelling of the left extraocular muscles and of both lacrimal glands (Fig. 1a–c). Examination using the Hess chart showed slightly disturbed left supraduction. The patient reported slight diplopia on upward gaze.

Laboratory studies showed normal values for free thyroxine, free triiodothyronine, thyroid-stimulating hormone, cytoplasmic-antineutrophil cytoplasmic antibody, and angiotensin-converting enzyme, and negative results for autoantibodies related to collagen disease and Graves' ophthalmopathy (anti-SS-A/Ro, anti-SS-B/La, antinuclear, antidouble stranded DNA, antithyroglobulin, antithyroid peroxidase, thyrotropin-receptor, and thyroid-stimulating antibodies). However, the erythrocyte sedimentation rate was 89.6 mm (normal 3–15 mm), the serum IgG concentration was 3,609 mg/dl (normal 870–1,700 mg/dl), the serum IgG4 concentration was 2,170 mg/dl (normal 4.8–105 mg/dl), and the soluble interleukin-2 receptor concentration was 797 U/ml (normal 220–530 U/ml). Bilateral swelling of the submandibular glands was also found. Histopathological study of a biopsy specimen of the right submandibular gland revealed dense lymphoplasmacytic infiltration with mild fibrosis. Infiltrative lymphocytes and plasma cells appeared mature and without atypia (Fig. 2a). Immunostaining for IgG4 indicated intense IgG4-positive plasma cell infiltration (Fig. 2b), and the ratio of the IgG4-positive plasma cells/IgG-positive plasma cells was 78%. On the basis of the IgG4-positive plasma cell infiltration of the submandibular gland with a high

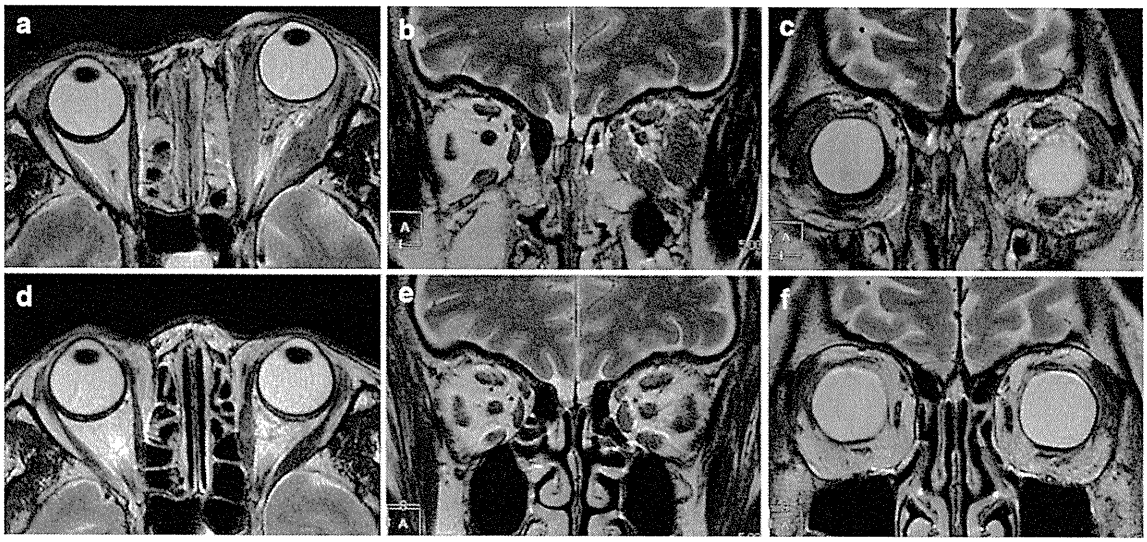

Fig. 1 Orbital T2-weighted MRIs. **a**, **b**, **c** The images showed swelling of the left extraocular muscles and of both lacrimal glands before therapy. **d**, **e**, **f** The swelling of the left extraocular muscles and both lacrimal glands had improved 2 months after treatment. MRIs: magnetic resonance images

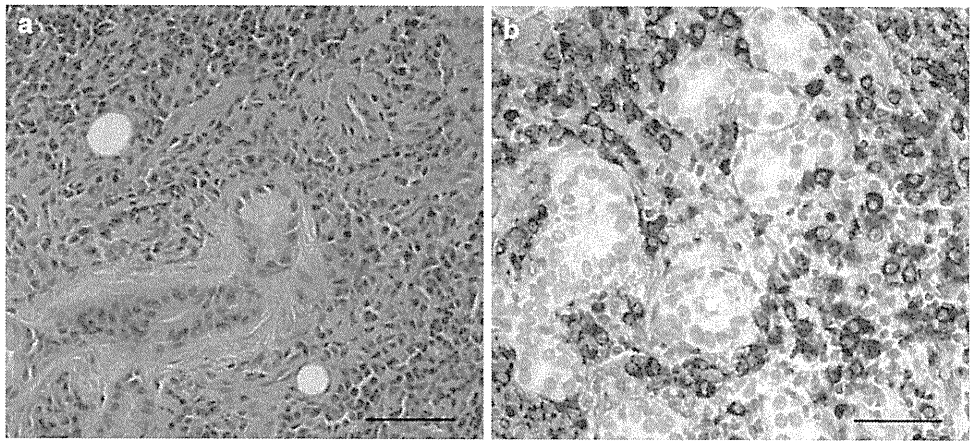

Fig. 2 Histopathologic and immunohistochemical findings of the right submandibular gland. **a** Dense, mature lymphoplasmacytic infiltration. *Bar* 50 μm. **b** IgG4 immunostaining showed numerous IgG4-positive plasma cells in the right submandibular gland. *Bar* 50 μm

serum IgG4 concentration, IgG4-related sclerosing disease was diagnosed and considered to be the cause of the extraocular muscle swelling.

Intravenous methylprednisolone 1,000 mg daily was administered for 3 days followed by oral prednisolone. In the left eye, the exophthalmos had decreased to 12 mm, and the visual acuity had improved to 1.5 and the critical flicker frequency values to 41 Hz 1 week after treatment. Magnetic resonance imaging showed improvement of the swelling of the left extraocular muscles and both lacrimal glands at 3 weeks and at 2 months after treatment (Fig. 1d–f). The serum IgG4 concentration had decreased to 607 mg/dl 4 weeks after treatment. The Schirmer test results were 12 mm in the right eye and 15 mm in the left eye 4 months after treatment. The left supraduction had also improved 5 months after treatment. The patient has not had a recurrence for 1 year.

Comments

On the basis of the IgG4-positive plasma cell infiltration of the submandibular gland with a high serum IgG4 concentration, the current patient was diagnosed as having IgG4-related sclerosing disease. A history of AIP, a type of IgG4-related sclerosing disease in the pancreas, also supported our diagnosis. To our knowledge, this is the first report to describe extraocular muscle swelling due to IgG4-related sclerosing disease. Moreover, Mikulicz disease was also diagnosed, which we believe was concurrent with the

extraocular muscle swelling. Therefore, measurement of the serum IgG4 concentration might be useful to investigate the cause of extraocular muscle swelling.

In the case presented here, not only the exocrine glands but also the extraocular muscles were involved, whereas many cases in which only the exocrine glands were involved have been reported. However, a case in which the retroperitoneum and mediastinum were involved was also reported [3], and therefore, IgG4-related sclelosing disease may involve organs other than exocrine glands.

Left ophthalmoplegia was slight in our patient, although left optic neuropathy occurred owing to severe muscle swelling. Given the histopathological finding that the fibrotic change in the submandibular gland was mild, we guess that the fibrotic change of the extraocular muscles could also have been mild. Therefore, the elongation and construction of the muscles were not severely disturbed.

Steroid therapy was rapidly effective in our case. Steroid therapy has also been effective for other organs affected by IgG4-related sclerosing disease [3–6]. In conclusion, IgG4-related sclerosing disease should be considered in the differential diagnosis of extraocular muscle swelling.

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上転障害以外に眼所見が乏しかった甲状腺眼症の2例

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要約 **目的**：上転障害を主な眼症状とする甲状腺眼症の2症例の報告。**症例と所見**：それぞれ65歳と67歳男性で，1例には1か月前から複視があり，左眼上転障害のみがあった。眼窩の磁気共鳴画像検査（MRI）で下直筋と内直筋の腫大があり，甲状腺眼症と診断された。他の1例は4年前に他医で右眼の動眼神経麻痺と診断されていた。右眼の上転障害のみがあり，MRIで右下直筋の腫大があり，甲状腺眼症と診断された。**結論**：甲状腺眼症には眼球運動障害が主症状である症例がある。眼球運動障害の原因精査では，MRIを含む眼窩の画像診断が重要である。

Two cases of thyroid-associated ophthalmopathy with impaired upward gaze as the chief ocular finding

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Abstract. **Purpose**：To report two cases of thyroid-associated ophthalmopathy with restricted upward gaze as the chief ocular manifestation. **Cases and Findings**：Both were males aged 65 and 67 years respectively. One case had diplopia since one month before with restricted upward gaze as the sole ocular symptom. Magnetic resonance imaging（MRI）showed swelling of medial and inferior rectus muscles, leading to the diagnosis of thyroid ophthalmopathy. The other case had been diagnosed with oculomotor palsy 4 years before. He showed restricted upward gaze as the sole ocular symptom. MRI showed swelling of right inferior rectus muscle. He was diagnosed with thyroid ophthalmopathy. **Conclusion**：These cases illustrate that impaired ocular movement may be the sole ocular finding in thyroid ophthalmopathy. Diagnostic imaging, including MRI of the orbit, may be crucial in the identification of the underlying cause.

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緒言

眼球運動障害の発症機序の1つとして，外眼筋の伸展制限があり，その代表的な原因疾患として甲状腺眼症が挙げられる。甲状腺眼症では炎症で生じた外眼筋の腫大・拘縮により，罹患筋の作用と反対方向の眼球運動障害が生じる[1]。一般的には，眼窩炎症により併発する眼球突出，眼瞼腫脹，結膜浮腫などの外眼部所見や甲状腺機能異常の既往が，その後の診断の重要な手がかりとなる。最終的には眼窩の磁気共鳴画像（magnetic resonance imaging：以下，MRI）やコンピュータ断層撮影（computed tomography：以下，CT）により外眼筋腫大を確認して診断を行う[2～4]。

今回筆者らは，甲状腺機能異常に対する治療歴がなく，片眼の上転障害以外の外眼部所見にも乏しく，眼窩MRI検査での下直筋腫大の所見をきっかけに，甲状腺眼症による眼球運動障害と診断した2例を経験したので報告する。

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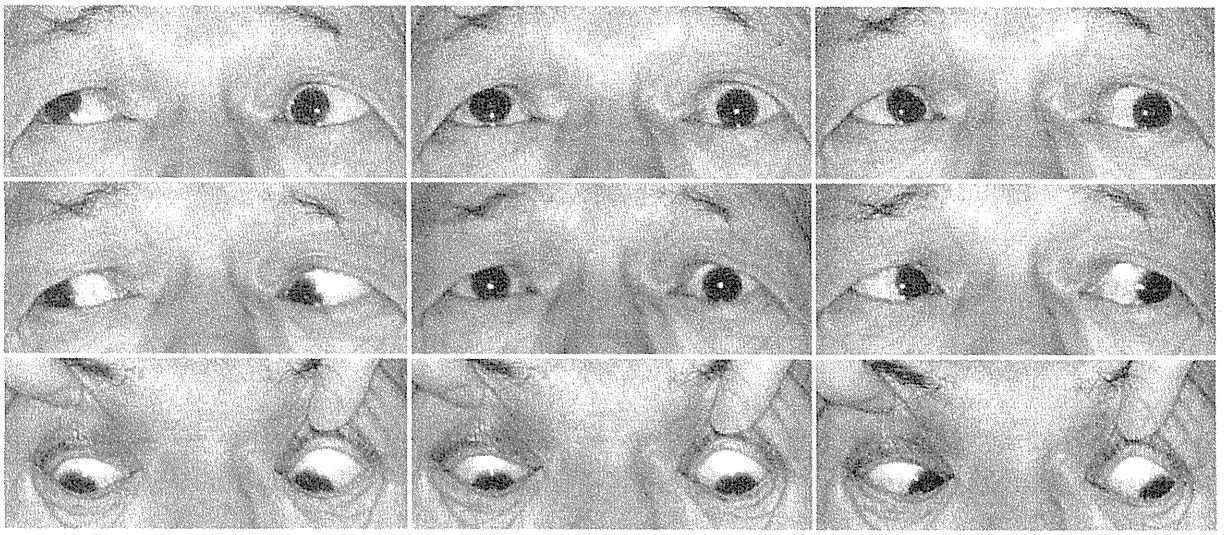

図 1　症例 1 の眼位，眼球運動
左眼下斜視とともに左眼上転障害を認めた。

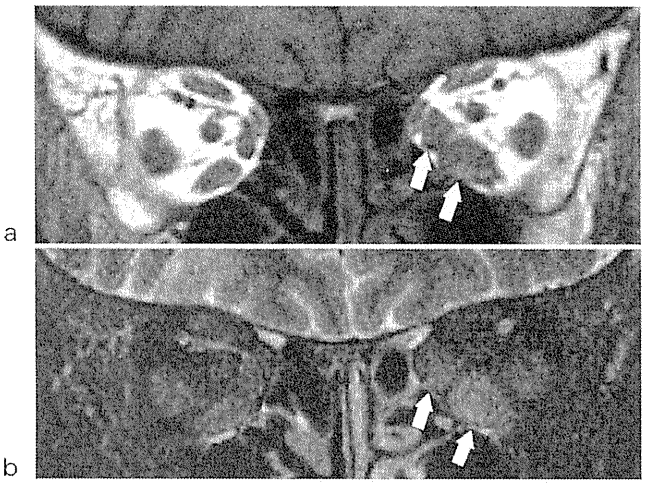

図 2　症例 1 の眼窩磁気共鳴画像（MRI）
a：T1 強調画像で左下直筋と内直筋の腫大を認めた。
b：STIR 画像で左下直筋と内直筋の高信号を認めた。

症例

［症例 1］

患者：65 歳，男性

主訴：複視

現病歴：2007 年 1 月頃から複視を自覚し，2 月に近医眼科を受診した。原因精査のため 2 月 21 日，当院神経内科を紹介され受診した。神経内科で左上転障害を指摘され，同日眼科にも受診した。

初診時所見：矯正視力は右（1.2），左（1.5），眼圧は右 19 mmHg，左 26 mmHg であった。前眼部，中間透光体，眼底に異常所見はみられなかった。眼位は左眼 10° の下斜視で，左眼上転障害を認めたが（図 1），外眼部の眼瞼，球結膜には炎症による発赤・腫脹はなかった。眼球突出度計測では右 11 mm，左 13 mm であった。

検査所見：頭部，眼窩 MRI を併施したところ，頭蓋内に異常はみられなかったが，眼窩 MRI で左下直筋と内直筋の腫大を認めた（図 2a）。short TI inversion recovery（以下，STIR）画像では，左下直筋，内直筋に高信号を認めた（図 2b）。しかし，上直筋には萎縮はなかった。その後，眼瞼皮膚弛緩のために初診時には気づかなかった左眼瞼後退を確認した。血液検査では，フリー T_3 7.5 pg/ml（基準値 2.0～4.9 pg/ml），フリー T_4 2.52 ng/dl（基準値 0.82～1.63 ng/dl），サイログロブリン 140 ng/ml（基準値 0～30 ng/ml），TSH レセプター抗体 18.5%（基準値 0～15%），甲状腺自己抗体 19.6 U/ml（基準値 0～0.3 U/ml），抗甲状腺ペルオキシダーゼ抗体 47.3 U/ml（基準値 0～0.3 U/ml）であり，甲状腺ホルモンと各甲状腺抗体が上昇しており，甲状腺眼症と診断した。

治療と経過：2007 年 4 月からステロイドパルス療法（メチルプレドニゾロン 1,000 mg×3 日間）3 クールと，放射線療法（総放射線量 20 Gy）を実施した。外眼筋の消炎を MRI で確認し，2007 年 12 月に，左下斜視に対して全身麻酔下で左下直筋後転術 5.5 mm を施行した。術中，左下直筋の牽引時に強い伸展制限を認めた。術後，眼位は

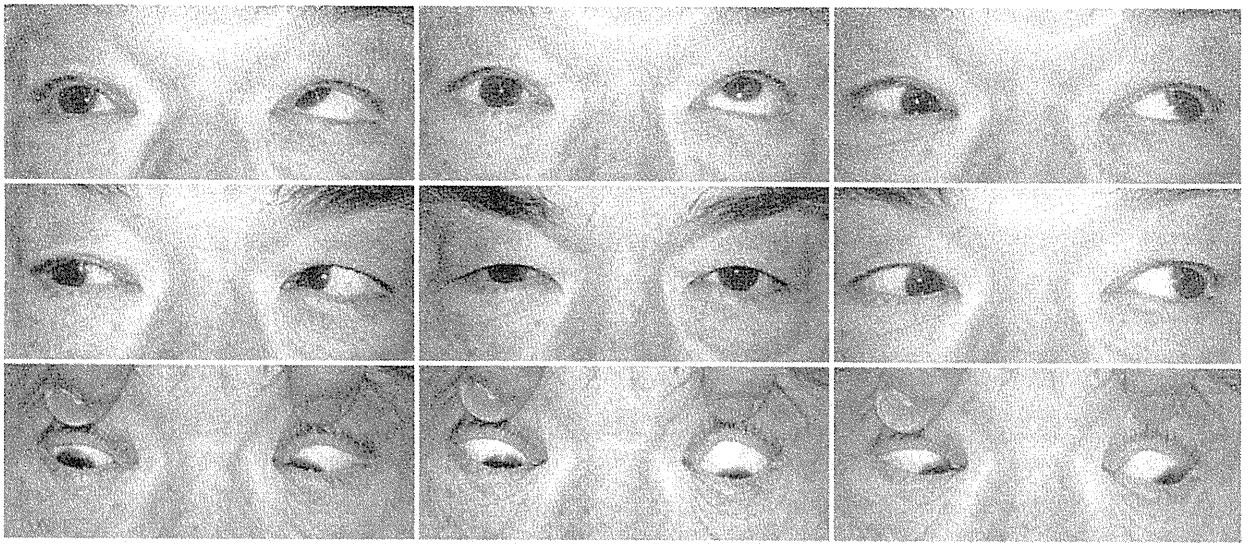

図 3　症例 2 の眼位，眼球運動
右眼下斜視とともに右眼上転障害を認めた。

ほぼ正位となり，左上転障害も改善し，上方 20°以下では複視が消失した。

［症例 2］

患者：69 歳，男性

主訴：複視

現病歴：2005 年 9 月，他院脳神経外科による下垂体腫瘍の手術前から複視を自覚していた。術後も改善は認めず，右動眼神経麻痺として経過観察されていた。2009 年 3 月 2 日近医眼科を受診し，3 月 27 日，精査加療目的で当科に紹介され受診した。

既往歴：61 歳で両眼白内障手術

初診時所見：矯正視力は右（0.6），左（1.0）で，眼圧は両眼とも 15 mmHg であり，眼球突出度は右 15 mm，左 14 mm であった。両眼とも偽水晶体眼で，眼底には両眼に黄斑上膜を認めた。眼位は右眼 12°の下斜視で，右眼上転障害が著明であり，正中を越えず，牽引試験では右眼上転方向で抵抗を認めた（図 3）。眼瞼，球結膜に炎症所見はなかった。

検査所見：眼窩 MRI で右下直筋の腫大を認めた（図 4a）が，STIR 法では外眼筋の信号は亢進していなかった（図 4b）。また，上直筋には明らかな萎縮はなかった。血液検査では，甲状腺刺激抗体が 183%（基準値 0～180%）と上限以上であったが，その他の甲状腺抗体，フリー T_3，T_4は正常値範囲内であった。外眼筋の MRI 所見と血液検査から陳旧性の甲状腺眼症と診断した。

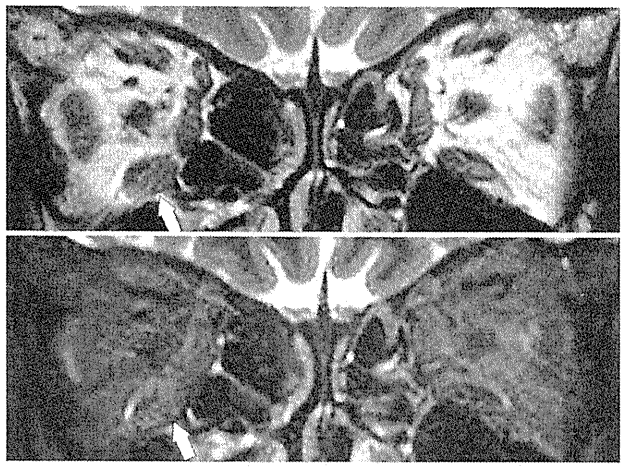

図 4　症例 2 の眼窩磁気共鳴画像（MRI）
a：T2 強調画像で右下直筋の腫大を認めた。
b：STIR 画像で右下直筋の信号亢進はなかった。

治療と経過：2009 年 7 月，全身麻酔下で右下直筋後転術 5 mm を実施した。術中，右下直筋の牽引時に強い伸展制限を認めた。術後右上転障害は残存したが，正面視での複視は消失した。

考按

今回の 2 症例は，いずれも片眼の上転障害を伴った甲状腺眼症症例で，他の眼所見に乏しく，眼窩画像診断による外眼筋腫大の所見がきっかけとなり，その後の血液検査所見とともに甲状腺眼症の診断に至った。海外でも同様の甲状腺眼症の

報告があり[5]，“pure eye muscle involvement”として，初診時に眼球運動障害以外に，甲状腺眼症の他の所見に乏しい7例の報告があり，そのうち6例は上下の複視を自覚しており，今回の症例と一致している。

一般に眼球運動障害の原因は神経原性障害が最も多く，帝京大学[6]と岡山大学[7]の報告ではいずれも神経原性が66%を占めている。一方，筋原性は帝京大学で5%，岡山大学では6%と頻度が低く，特に今回の2症例のように眼球運動障害以外の外眼部所見が乏しいか認められない場合は，神経原性病変の眼球運動障害と誤診される可能性がある。

筋原性眼球運動障害の中では，甲状腺眼症の占める割合が帝京大学の報告[6]では35%，岡山大学[7]では79%と施設により差があるが，いずれの施設でも最も多くを占めており，眼球運動障害の原因検索の際には本疾患の存在も念頭に置くべきである。さらに，村上ら[2]の報告では，甲状腺眼症での外眼筋の罹患率は下直筋が68%と最も高率であるため，今回の2症例のような上転障害例では，甲状腺眼症による下直筋の罹患の可能性があった。

今回の2症例のように，初診時に眼球運動障害の原因となる既往が明らかでない症例の精査には，MRIによる頭蓋内腫瘍や脳血管病変など頭部病変の有無の確認が重要であるが，その際眼窩MRIの併施による筋原性病変の有無の確認も必要と考える。症例1では初診時に，頭蓋内MRIとともに眼窩MRIも併施した。ただし，検査時間の制約もあり，眼窩MRIは冠状断のスピンエコー画像とSTIR画像のみにとどめたが，外眼筋の形態変化と炎症の存在を確認するには十分であった[3]。

一方，症例1では初診時から甲状腺眼症として着目すべきであった眼所見として，左眼瞼後退と左眼の高眼圧が挙げられる。甲状腺眼症の眼所見頻度に関するコホート研究によると[8]，眼瞼後退が90.8%で，眼球突出の62%や眼球運動障害の42.5%に比較しても最も頻度が高く，眼瞼後退は甲状腺眼症を診断する際の最も重要な所見であると述べており，症例1の眼瞼皮膚弛緩で当初確認が困難であった左眼瞼後退の所見の重要性を再認識した。

また，症例1での下斜視と上転障害を伴った左眼の高眼圧は，眼圧測定時に下斜視から正中に左眼球が上転した際に，下直筋の伸展制限により眼球が下直筋に圧迫されたためと考えた。古河ら[9]も下直筋の伸展制限の評価として，上転時の眼圧上昇の確認が有用であることを述べており，本症例の眼圧上昇も下直筋の伸展制限に起因する所見と考えられた。

甲状腺眼症による斜視，眼球運動障害での外眼筋手術は腫大筋の後転術が第一選択となり[10]，今回の2症例も，上方視での下直筋の伸展制限の軽減のために下直筋後転術を実施し，良好な眼位改善が得られ複視も軽減した。一方，麻痺性斜視では麻痺筋の強化術である短縮術が第一選択[11]であるため，甲状腺眼症での手術方針と異なる。したがって，眼球運動障害の治療方針の決定には，正確な原因精査が重要であることを再認識した。

最後に，今回の2症例を経験して，眼球運動障害の原因検索には神経原性だけでなく，筋原性病変の可能性を念頭に置くことを再認識した。初診時に眼球運動障害の原因が明らかでない場合，頭蓋内のみならず眼窩内の画像診断も併施することが重要であると考えた。

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Effect of vitamin C depletion on UVR-B induced cataract in SMP30/GNL knockout mice

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ABSTRACT

We investigated whether decreased vitamin C (VC) in a mouse model increases lens opacity (cataract) induced by in vivo exposure to ultraviolet radiation type B (UVR-B).

Senescence marker protein-30 (SMP30) knockout (KO) mice, which cannot synthesize VC due to genetic disruption of the gluconolactonase (GNL) gene, were divided into 2 groups: VC sufficient (VC (+)) and VC deficient (VC (−)). Starting at 1 month of age, these groups had free access to water containing 0.0375 and 1.5 g/L of VC, respectively. SMP30 KO VC (−), SMP30 KO VC (+), and wild-type (WT) mice, all 14 weeks of age, were unilaterally exposed in vivo to UVR-B (200 mW/cm^2) for 100 s twice a week for 3 weeks (total: 1200 mJ/cm^2). At 48 h after the last UVR-B exposure, cataract morphology was documented, and the ratio of cataract induction was quantified as the cataract area ratio (opacity area/anterior capsule).

UVR-B exposure induced cataract mainly at anterior sub-capsular in SMP30 KO VC (−), SMP30 KO VC (+), and WT mice. In SMP30 KO VC (−) lenses the opacities were more extensive than in SMP30 KO VC (+) or WT lenses (cataract area ratios: 59.3% ± 10% vs. 32.2% ± 11.7% or 29.0% ± 9.0%; $P < 0.01$).

In conclusion, VC depletion may increase the susceptibility to develop UVR-B induced cataracts in mice unable to endogenously produce VC.

1. Introduction

The most common cause of blindness worldwide is unoperated cataracts. The impact of this form of blindness is disproportionately felt in the developing world, and access to quality cataract surgery is a major factor in the growing numbers of unoperated cataracts and blind patients. Despite efforts to increase the availability of high quality surgery with acceptable visual outcomes, there is still a growing population of patients who are blind due to cataracts (McCarty, 2002).

Epidemiological studies have revealed ultraviolet radiation (UVR) (Taylor et al., 1988; Hiller et al., 1977), smoking (Christen et al., 1992; Kelly et al., 2005; Krishnaiah et al., 2005; Hankinson et al., 1992), insufficient vitamin C/E intake (Jacques and Chylack, 1991; Varma, 1991), and low blood beta-carotene levels to be risk factors for age-related cataract development in humans, and it has been increasingly recognized that oxidative stress is involved in the formation of cataracts by causing lens protein damage leading to aggregation of the protein into high molecular weight aggregates that scatter and block light (Truscott, 2005).

Oxidative stress arises when the production of reactive oxygen species (ROS) overwhelms the antioxidant defenses (Sies, 1991). Glutathione peroxidase, superoxide dismutase, and catalase are major enzymatic antioxidants, whereas vitamin C (VC), vitamin E, and glutathione are the main non-enzymatic antioxidants.

VC is water-soluble and scavenges ROS such as superoxide (Nishikimi, 1975), singlet oxygen (Bodannes and Chan, 1979), and hydroxyl radicals (Bielski et al., 1975). VC is quite high in aqueous humor, and its level is maintained by active transport from plasma across the blood aqueous barrier, to the posterior and then the anterior chamber of the eye. VC is thought to function in the aqueous humor to protect the lens from photo-oxidative stress, preventing lens protein damage, aggregation and consequent cataract formation due to ongoing light-catalyzed generation of various ROS (Varma et al., 1979, 1984).

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