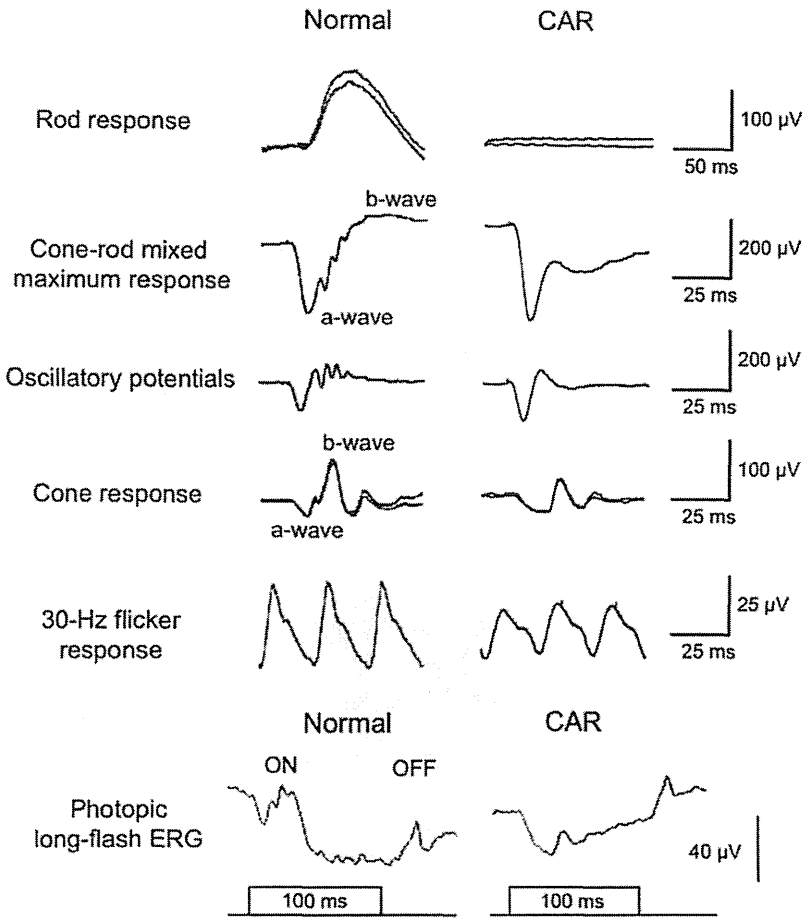

Figure 2. Full-field ERG recordings. The rod response was recorded with a blue light at an intensity of 5.2×10^{-3} cd-s/m^2 after 30 minutes of dark-adaptation. The cone-rod mixed maximum response was elicited by a white flash at an intensity of 44.2 cd-s/m^2. The oscillatory potentials were recorded with a white flash at an intensity of 44.2 cd-s/m^2 using a band-pass filter of 50–1000 Hz. The cone response and a 30 Hz flicker response were elicited by a white stimulus of 4 cd-s/m^2 and 0.9 cd-s/m^2, respectively, on a blue background of 30 cd/m^2. Photopic long-flash ERG responses were also elicited by long-duration flashes of 100 ms using a densely-packed array of white LEDs of 200 cd/m^2 on a white background of 30 cd/m^2.
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To examine whether the serum from the CAR patient recognized retinal bipolar cells, we carried out an immunohistochemical analysis on monkey and mouse retinas. We first performed immunohistochemistry on the retina of a 3-year-old rhesus monkey (*Macaca mulata*) and on the retina of a one-month-old C57/B6 mouse using the serum of the CAR patient, however, we did not obtain a significant staining signal above background (data not shown). We then concentrated the serum by IgG purification followed by filter spin column centrifugation and performed immunohistochemistry on the monkey retina using the concentrated serum (Fig. 3D–G). We observed a significant immunolabeling on the INL in the monkey retina (Fig. 3D, F) whereas the normal serum did not give a significant labeling (Fig. 3E, G). The antibodies immunolabeled both the bipolar side and amacrine side of the INL. Since most of the cells residing on the outer side of the INL are ON bipolar cells, at least some of the stained cells are ON bipolar cells. It should be noted some of the staining signals show a spotted pattern in the outer plexiform layer (Fig. 3F) as is observed in TRPM1 or mGluR6 immunostaining on the mouse retina [13], suggesting that the CAR patient serum recognizes the bipolar dendritic tips where some of the TRPM1 protein localizes.

Western blot analysis of the sera from MAR patients

Since the functional defect in the retina of MAR patients is known to be due to abnormal signal transmission between photoreceptors and ON bipolar cells [8,9], we then investigated whether or not autoantibodies to TRPM1 were also present in the sera of MAR patients. We obtained the sera of 26 MAR patients from two hospitals in Japan (Chiba University Hospital and Iwate Medical University Hospital) and Ocular Immunology Laboratory in the USA (Casey Eye Institute). We found that the sera from patients #8 and #23 exhibited a significant immunoreactive band against TRPM1-transfected cell lysates by Western blot analysis (Fig. 4A and B). The control serum showed no significant immune response against the TRPM1-transfected cell lysates (Fig. 3C). These results suggest that the sera from some MAR patients contain autoantibodies against TRPM1. Due to the limited volume of sera from the MAR patients, we could not try immunostaining on the monkey or the mouse retina using the serum from the patients #8 and #23.

MAR patient #8, was a 76-year-old man with a history of skin melanoma. He had ring scotomas and abnormal ERGs indicating that he had MAR. The other patient, MAR #23, was a 57-year-

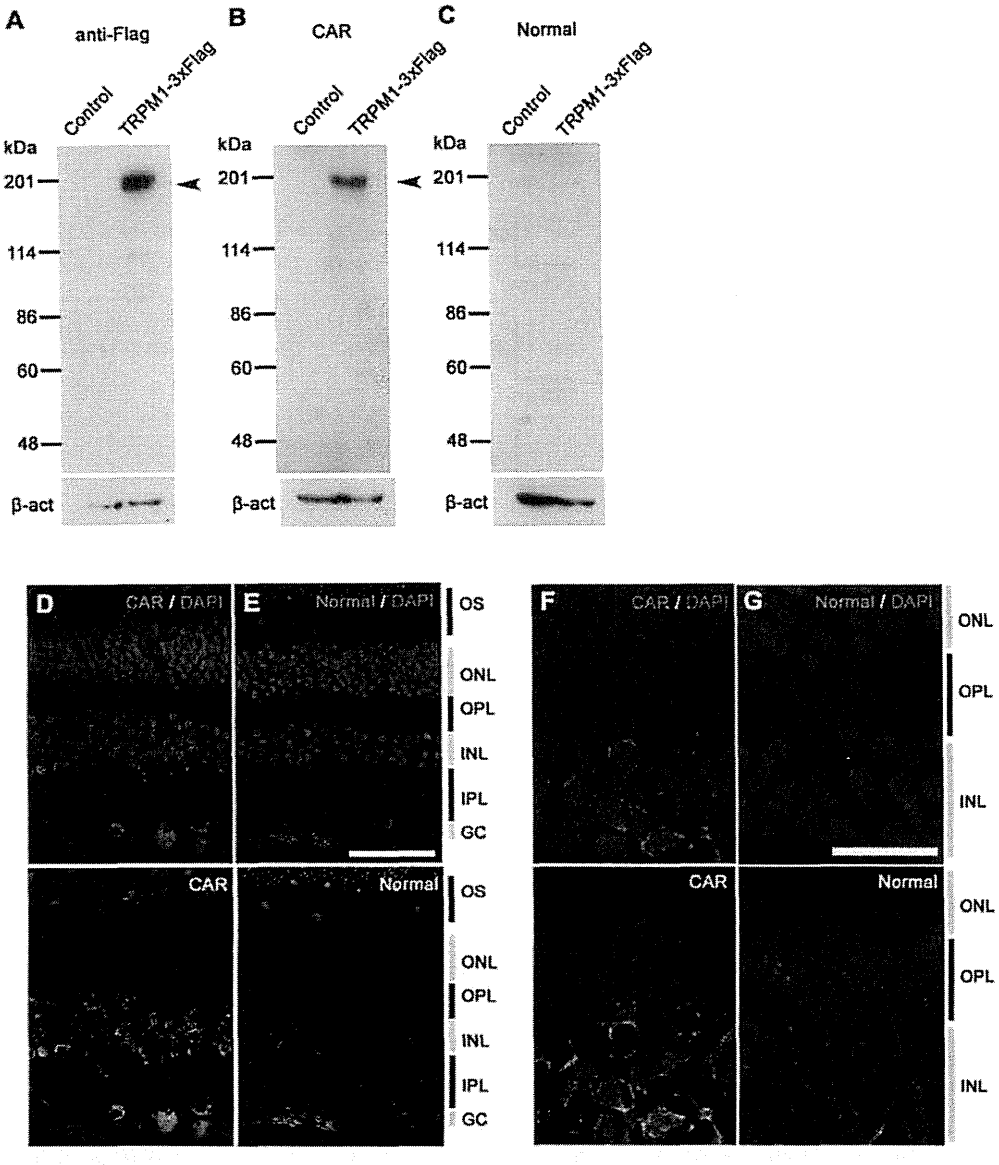

Figure 3. Immunostaining and Western blot analysis of human TRPM1 using serum from the CAR patient. (A–C) Immunoblots of the transfected cell lysates using an antibody against Flag tag (A), serum from CAR patient (B), and control serum (C). Arrowheads indicate the TRPM1-3xFlag protein bands. HEK293T cells were transfected with the pCAGGS or pCAGGS-human TRPM1-3xFlag plasmid, and cells were harvested after 48 hrs. β-actin (β-act) was used for a loading control. (D–G) Confocal images of a three-year-old rhesus monkey retina immunostained with the concentrated serum from the CAR patient (D, F) or the concentrated normal serum (E, G). Cell nuclei are visualized with DAPI. CAR patient serum presented signals on INL cells and the inner part of the OPL (D, F). Scale bar = 50 μm in (E) and 20 μm in (G).
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old man with poor night vision, abnormal scotopic ERGs and abnormal color vision. He had a history of skin melanoma and thyroid cancer. There was no other clinical information available on these two patients because these sera were obtained from other institutes several years before without detailed clinical information.

Discussion

PR, including MAR and CAR, presents visual disorders associated with systemic cancer. Antibodies against retinal cells and proteins have been detected in the sera of patients with PR suggesting an autoimmune basis for the etiology of the PR. The autoantibodies identified so far include rhodopsin, retinal transducin alpha and beta, recoverin, S-arrestin, α-enolase, carbonic anhydrase II, and heat shock protein-60 which reside abundantly in photoreceptors [1–10,16]. MAR and CAR can cause bipolar cell dysfunction [7–12]. The results of the ERG [8,9] and immunohistochemistry [7] studies suggested that the main target of MAR are retinal ON bipolar cells in both the rod and cone pathways. However, autoantibodies specifically reacting with a bipolar cell antigen had not been identified in the sera of patients with PR, including those with CAR and MAR. In the current study, we identified autoantibodies against TRPM1, a component of the ON bipolar cell transduction channel negatively regulated by Goα in the mGluR6 signaling pathway [13–15], in the

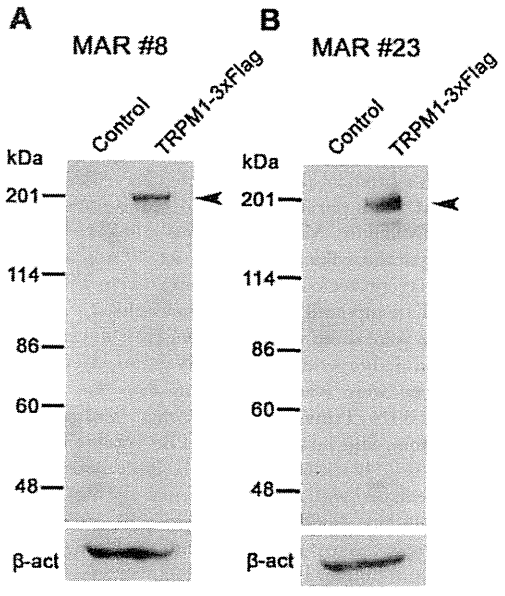

Figure 4. Western blot analysis of human TRPM1 using sera from the MAR patients. (A, B) Immunoblots of the transfected cell lysates using sera from MAR patient #8 (A) and MAR patient #23 (B). HEK293T cells were transfected with pCAGGS or pCAGGS-human TRPM1-3xFlag plasmid, and cells were harvested after 48 hrs. Arrowheads indicate the TRPM1-3xFlag protein bands. β-actin (β-act) was used for a loading control.
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sera of one CAR patient and two MAR patients. The CAR patient exhibited a dysfunction of ON bipolar cells, and to our knowledge, this is the first report on an autoantibody against a bipolar cell antigen in the serum of PR patients affecting the ON bipolar cell function.

Previously, we isolated a mouse *TRPM1-L* cDNA corresponding to the human long form of *TRPM1*, and found that the TRPM1-L protein is developmentally localized at the tips of the ON bipolar dendrites co-localizing with mGluR6, but not on OFF bipolar cells [13,14]. The *TRPM1* null mutant mouse completely loses the ON bipolar cell photoresponses to light, indicating that TRPM1 plays a critical role in the synaptic transmission from photoreceptors to ON-bipolar cells [13,15]. In addition, we demonstrated using a CHO cell reconstitution system that TRPM1-L is a nonselective cation channel which is negatively regulated by Goα downstream of the mGluR6 signaling cascade in ON bipolar cells [13]. Recently, four groups including ours independently reported that mutations of human *TRPM1* are associated with the complete-type of congenital stationary night blindness (cCSNB), an inherited human retinal disease [17–20]. cCSNB is a non-progressive retinal disease characterized by congenital night blindness with a moderate decrease in visual acuity and myopia [21-24]. Previous ERG studies have suggested that the defect in cCSNB patients lies in the signal transmission from photoreceptors to ON bipolar cells in both the rod and cone pathways [25–28]. We have identified five different mutations in our three cCSNB patients, and have shown that these mutations lead to either abnormal TRPM1 protein production or mislocalization of the TRPM1 protein in bipolar cell dendrites [17]. These results suggest that TRPM1 plays a critical role in mediating the photoresponses of ON bipolar cells in humans as well. Based on these findings, we hypothesize that the ectopic expression of TRPM1 in tumor cells of some CAR and MAR patients may result in aberrant production of autoantibodies to TRPM1 through B-lymphocytic responses [29–32]. These antibodies may react to the TRPM1 protein in retinal ON bipolar cells resulting in dysfunction of the TRPM1 transduction cation channel downstream of the mGluR6 signaling cascade. However, we could not confirm whether TRPM1 is expressed in the tumor cells of the three PR patients examined in this study [29] because tumor samples were not available.

Another question regarding the disease mechanism underlying PR is whether the binding of TRPM1 autoantibody to bipolar cells results in the cell death or dysfunction of bipolar cells. As far as we examined the retinal structure of the CAR patient using a spectral domain optical coherence tomography (SD-OCT) retinal imaging device, the structure of the retinal bipolar cell layer appeared to be well preserved even three months after the onset of symptoms (Fig.1D). This suggests that the autoantibodies reacting to TRPM1 cause dysfunction of the ON bipolar transduction pathway rather than bipolar cell death. However, further studies are needed to clarify the exact disease mechanism.

In the sera of MAR patients, several types of autoantibodies against retinal proteins have been reported, including the 22 kDa neuronal antigen GNB1, rhodopsin, S-arrestin, and aldolase-A and -C [10,16,33,34]. We initially considered that TRPM1 might be a major MAR target antigen, because TRPM1 is exclusively expressed in retinal ON bipolar cells. However, autoantibodies against TRPM1 were detected in only two out of 26 MAR patients' sera (7.7%, Fig. 4A, B). We tested whether the sera of one CAR patient and 26 MAR patients recognized human mGluR6, which is specifically expressed in ON bipolar cells, however, none of the sera exhibited a significant band in Western blot analysis (data not shown). Thus, antigens other than TRPM1 or mGluR6 may be involved in the pathogenesis of a large proportion of MAR.

Immunohistochemical analyses using the serum of the CAR patient showed labeling in the inner nuclear layer and outer plexiform layer of the adult rhesus monkey retina (Fig. 3D–G), where the bipolar cell bodies and dendrites reside, respectively. This immunostaining pattern is somewhat similar to our previous immunostaining results on the mouse retina with specific antibody against mouse TRPM1-L, which corresponds to the human TRPM1 long form [13]. Other labeling was also observed in the amacrine cells and ganglion cells. The reason for the immunoreactivity with these cells is uncertain, however, it may be due to the presence of other autoantibodies against amacrine cell and ganglion cell antigens. Lu *et al.* reported the presence of various different autoantibodies in the serum of a single PR patient [10]. If this is the case, it may explain why our CAR patient displayed severely reduced visual sensitivities in the visual field tests (Fig. 1A) unlike cCSNB patients with TRPM1 mutations[17].

It should be noted that we did not confirm whether there are any autoantibodies against TRPM1 in the sera of normal subjects by using a large number of samples. However, this possibility is thought to be low, because Shimazaki *et al.* reported that the molecular weights of the IgGs with observed anti-retinal reactivity in 92 normal sera were smaller than 148 kDa, which is smaller than the TRPM1 molecular weight of ~200 kDa, although relatively high molecular weight reactivity was not intensively investigated [35].

One limitation of the current study is that we could not obtain detailed information on the two MAR patients, MAR #8 and #23, associated with the TRPM1 autoantibody. We confirmed that these two patients had skin melanomas accompanying the visual disturbances, but could not obtain a more detailed clinical history or data on visual acuity, visual field, or ERGs because these sera were sent from different hospitals several years ago. Thus, we do not know whether these two MAR patients really had retinal ON bipolar cell dysfunction. Further prospective studies of the TRPM1 autoantibodies in large numbers of MAR patients are needed.

In conclusion, our study suggests that TRPM1 may be one of the causative antigens responsible for PR associated with ON bipolar cell dysfunction.

Note added in proof

During the course of revision process of this manuscript, Dhingra *et al.* (J. Neurosci. 31, 3962–3967, 2011) independently reported the presence of autoantibodies against TRPM1 in two MAR patients. Our study reports on autoantibodies against TRPM1 in CAR serum in addition to MAR sera.

Materials and Methods

Subjects

The Nagoya University Hospital Ethics Review Board approved this study (approval ID 1131). Of the PR patients that were examined in the Nagoya University Hospital, one PR patient with lung cancer and ON bipolar cell dysfunction was studied in detail. The examinations included routine ophthalmological and electrophysiological tests. In addition, immunohistochemical and Western blot analyses were performed using the serum of this patient. The procedures used conformed to the tenets of the Declaration of Helsinki of the World Medical Association. A written informed consent was obtained from the patient after he was provided with sufficient information on the procedures to be used.

We also obtained sera of 26 patients with MAR from two hospitals in Japan (Chiba University Hospital and Iwate Medical University Hospital) and Ocular Immunology Laboratory in the USA (Casey Eye Institute) for Western blot analysis.

Ophthalmologic examinations

The ophthalmologic examination included best-corrected visual acuity, biomicroscopy, ophthalmoscopy, fundus photography, fluorescein angiography, static perimetry, and spectral-domain optical coherence tomography (SD-OCT). Static visual fields were obtained with the Humphrey 30-2 program (Carl Zeiss, Dublin, USA), and the results are shown in gray scale. SD-OCT was performed with a 9-mm horizontal scan through the midline with 50 averages (Spectralis HRA+OCT; Heidelberg Engineering, Vista, CA).

Electroretinograms (ERG)

Full-field ERGs were elicited with a Ganzfeld dome and recorded with a Burian-Allen bipolar contact lens electrode. The ground electrode was attached to the ipsilateral ear.

After 30 minutes of dark-adaptation, a rod response was elicited with a blue light at an intensity of 5.2×10^{-3} cd-s/m^2. A cone-rod mixed maximum response was elicited by a white flash at an intensity of 44.2 cd-s/m^2. A cone response and a 30 Hz flicker response were elicited by a white stimulus of 4 cd-s/m^2 and 0.9 cd-s/m^2, respectively, on a blue background of 30 cd/m^2. Full-field cone ERGs were also elicited by long-duration flashes of 100 ms using a densely packed array of white LEDs. The array was positioned at the top of the Ganzfeld dome and covered by a diffuser. The stimulus intensity and background illumination measured in the dome was 200 cd/m^2 and 30 cd/m^2, respectively. Responses were amplified by 10K and the band pass was set to 0.3 to 1000 Hz. The data were digitized at 4.3 kHz, and 5 to 20 responses were averaged (Neuropack, Nihonkohden, Tokyo, Japan).

Immunohistochemistry

For immunohistochemistry, patient and normal sera (300 µl) were purified using the Melon Gel IgG purification kit according to the manufacturer's protocol (Pierce Biotechnology, Rockford, IL) to remove IgM, and purified sera were concentrated by Amicon Ultra 100 (Millipore, MA). The rhesus monkey eye cup was fixed with 4% paraformaldehyde in PBS for 30 min at 4°C. The samples were cryoprotected with 30% sucrose in PBS and embedded in OCT compound (Sakura Finetechnical, Tokyo, Japan). These tissues were sliced with a Microm HM 560 cryostat microtome (Microm Laborgeräte GmbH, Walldorf, Germany) into 14 µm. Sections were washed twice in PBS for 5 min, permeabilized with 0.1% Triton-X100/PBS, then washed with PBS 3 times for 5 min, and incubated with PBS containing 4% donkey serum for 1 hr to block samples. For the immunoreaction, the samples were incubated with a purified normal or CAR serum (1:300) diluted in blocking buffer at 4°C overnight. After PBS-washing, these samples were incubated with a DyLight-488 conjugated donkey anti-human IgG (H+L) (1:400) as a secondary antibody (Jackson Immunoresearch Laboratories) at room temperature for 1 hr and washed with PBS.

Transfection and Western blot analyses

HEK293T cells were cultured in D-MEM containing 10% fetal bovine serum (FBS; Nissui, Tokyo, Japan). These cells were grown under 5% carbon dioxide at 37°C. The calcium phosphate method was used to transfect the cells. Transfected cells were incubated at 37°C for 48 hrs, and then harvested for further analysis. The proteins extracted from the cells were separated by SDS–PAGE on a 7.5% precast gel (ATTO, Tokyo, Japan), and then transferred to a polyvinylidene difluoride membrane using the Invitrogen iBlot system (Invitrogen, Carlsbad, CA, USA). The membrane was incubated with primary antibodies, mouse anti-Flag (1:1,000; Sigma, St Louis, MO), sera from patients (1:100), normal human serum (1:100), or mouse anti-β-actin (1:5,000; Sigma). The membrane was then incubated with a horseradish peroxidase-conjugated goat anti-mouse IgG (1:10,000; Zymed Laboratories, San Francisco, CA) or donkey anti-human IgG (1:10,000; Jackson Immuno Research Laboratories, West Grove, PA) as secondary antibodies. The bands were developed using Chemi-Lumi One L (Nacalai Tesque, Kyoto, Japan).

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Author Contributions

Conceived and designed the experiments: MK TF. Performed the experiments: MK RS SU YN NH. Analyzed the data: MK RS SU TF. Contributed reagents/materials/analysis tools: MK SU HO SY SM HT GA. Wrote the paper: MK TF. Supervised the project: MK HT TF.

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CASE REPORT

Oguchi disease masked by retinitis pigmentosa

Hiroko Sonoyama · Kei Shinoda · Chie Ishigami · Yumi Tada · Hidenao Ideta · Ryuichi Ideta · Masayo Takahashi · Yozo Miyake

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Abstract The purpose of this study was to report a patient with Oguchi disease whose ophthalmological characteristics were masked by retinitis pigmentosa (RP). The method used in this study was case report. A 53-year-old man had a progressive decrease in his visual acuity and was diagnosed with RP because of night blindness, fundoscopic findings, ring scotoma, and extinguished single-flash electroretinograms (ERGs). However, a faint golden-yellowish reflex of the retina prompted us to make a more detailed examination of the fundus after a long period of dark adaptation, ERGs, and genetic analysis. Examinations showed the Mizuo-Nakamura phenomenon, relative intact photopic ERGs, and a *SAG* mutation, and the patient was diagnosed with RP associated with Oguchi disease. When RP accompanies Oguchi disease, the clinical characteristics of Oguchi disease might be masked. In such a situation, the correct diagnosis is difficult. However, careful analysis of clinical findings will suggest Oguchi disease, which can be confirmed by molecular genetics.

Keywords Oguchi disease · Mizuo-Nakamura phenomenon · SAG1 gene · Retinitis pigmentosa

H. Sonoyama · H. Ideta · R. Ideta · Y. Miyake
Ideta Eye Hospital, Kumamoto, Japan

K. Shinoda (✉)
Department of Ophthalmology, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan
e-mail: shinodak@med.teikyo-u.ac.jp

C. Ishigami · Y. Tada · M. Takahashi
RIKEN Center for Developmental Biology, Kobe, Japan

Y. Miyake
Aichi Medical University, Aichi, Japan

Introduction

Oguchi disease, first described [1] in 1907, is a rare autosomal recessive form of congenital stationary night blindness. It is characterized by a yellowish-gold fundus that disappears after prolonged dark adaptation, the Mizuo-Nakamura phenomenon. In general, the visual acuity, visual fields, and color vision are normal in patients with Oguchi disease [2]. The full-field rod electroretinograms (ERGs) after 30 min of dark adaptation are non-recordable but the cone and 30-Hz flicker ERGs are essentially normal. The a-wave of the mixed rod–cone ERGs elicited by a bright flash is significantly reduced, the b-wave is non-detectable, and the oscillatory potentials are well preserved [2, 3].

There are two genes that cause Oguchi disease: the G protein-coupled receptor kinase 1 gene (*GRK1*; MIM, 180381) and the *S* antigen gene (*SAG*; MIM, 181031) [4, 5]. There is evidence that Oguchi disease and retinitis pigmentosa (RP) can coexist in the same family or even in the same individual [6–8]. Only little information is available on genetically confirmed

cases of Oguchi disease with concomitant RP-like fundus appearance [9–13]. We report a case of genetically confirmed Oguchi disease that had typical characteristics of RP.

Case report

A 53-year-old man who had night blindness since his childhood was diagnosed with RP in an eye clinic in his neighborhood. He was referred to the Ideta Eye Clinic for further examination in 2008. His visual acuity had been 1.5 OU without correction but decreased in his thirty's. He had no family history, and his parents were not consanguineous. On examination, his best-corrected decimal visual acuity was 0.4 OD and 0.2 OS. Ophthalmoscopy showed diffuse changes in the retinal pigment epithelium (RPE), depigmented spots along the arcade vessels that extended to the mid-peripheral region, and bone corpuscle-like deposits (Fig. 1). Neither optic disk pallor nor atrophy was found, and a narrowing of retinal vessels was minimal. Cystoid macular edema (CME) was present, and a well-demarcated golden-yellowish reflex with Mizuo-Nakamura phenomenon was observed in the inferior peripheral area (Fig. 1). Fluorescein angiography (FA) showed window defects and granular hyperfluorescence corresponding to the RPE changes and pooling of the dye in the CME (Fig. 1). No special FA changes were observed in the area of golden-yellowish reflex. A ring scotoma and decreased sensitivity in the central area were detected

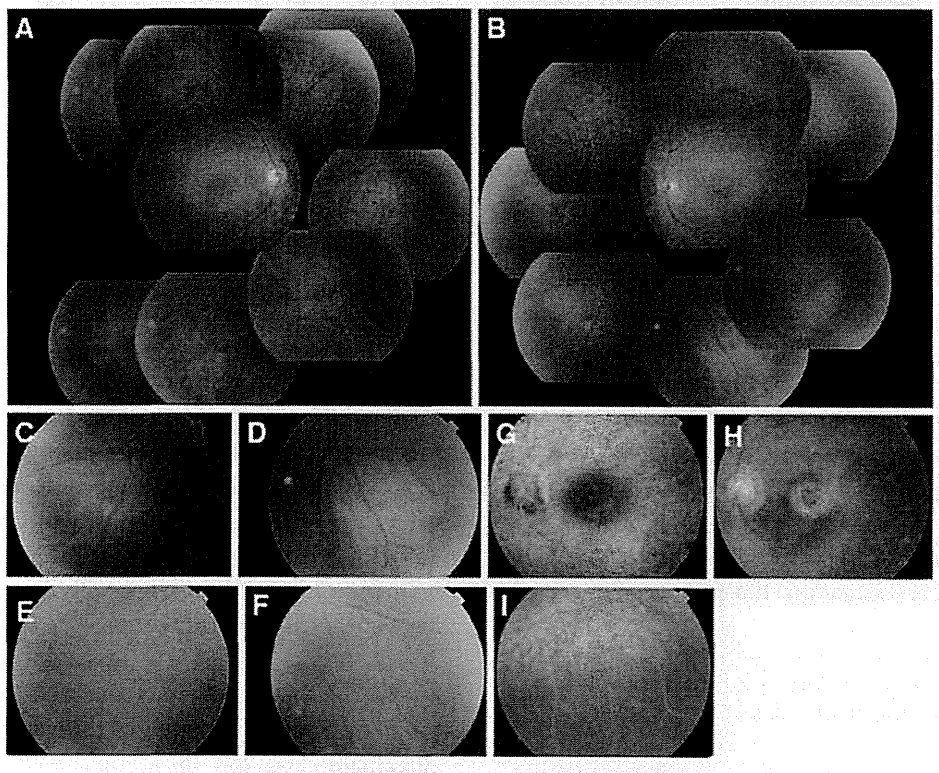

Fig. 1 Fundus photographs and fluorescein angiograms of a patient with Oguchi disease. **a**, **b** Fundus photographs of the *right* (**a**) and *left* (**b**) eyes showing diffuse changes in the retinal pigment epithelium, depigmented spots along the arcade vessels extending to the mid-peripheral region, and bone corpuscle-like deposits. Neither optic disk pallor nor atrophy can be seen, and the narrowing of retinal vessels was minimal. **c**, **d** The inferior peripheral fundus photographs of the *left* eye showing well-demarcated *golden-yellowish* reflex. **e**, **f** The inferior peripheral fundus photographs of the *left* eye corresponding to **c** and **d**, respectively, after 3 h of dark adaptation. The *golden-yellowish* reflex disappeared, suggesting Mizuo-Nakamura phenomenon. **g**–**i** Fluorescein angiograms of the *left* eye. Fundus photographs of the posterior pole at early phase (**g**) and late phase (**h**) showing window defect and granular hyperfluorescence corresponding to the changes in the retinal pigment epithelium and pooling of the dye at the cystoid macular edema. No special findings can be seen at the area of *golden-yellowish* reflex (**i**)

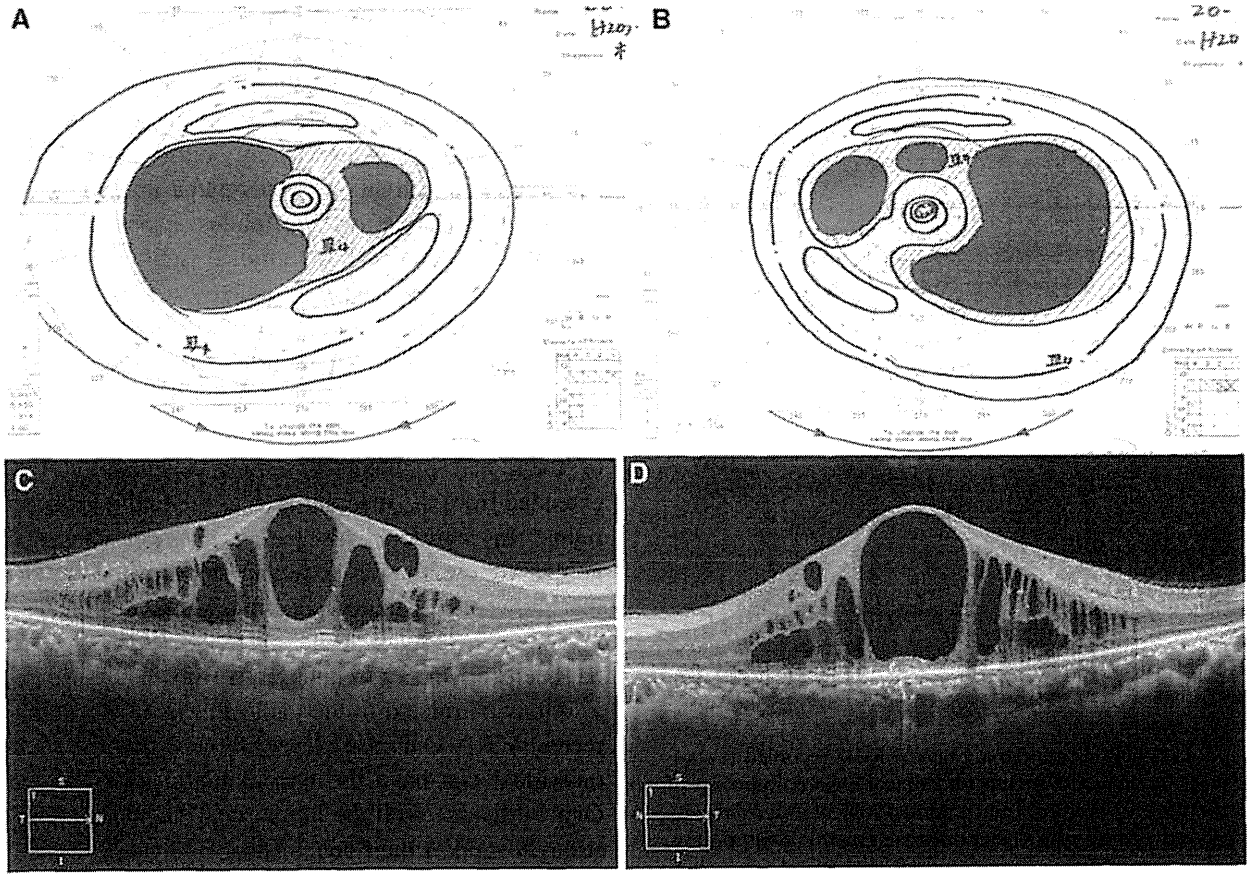

Fig. 2 Goldmann visual fields and optical coherence tomographic (OCT) images of a patient with Oguchi disease. **a**, **b** Visual fields of the *left* (**a**) and *right* (**b**) eyes showing ring scotomas and decreased sensitivity in the central area. **c**, **d** OCT (Cirrus HD-OCT) images of the *right* (**c**) and *left* (**d**) eyes showing severe cystoid macular edema and the border of the photoreceptor inner and outer segments (IS/OS *line*) were partially indiscernible or discontinued. These findings were more marked in the *left* eye

by Goldmann perimetry (Fig. 2). Optical coherence tomography (OCT Cirrus HD-OCT) showed severe CME, and the border of the photoreceptor inner and outer segments (IS/OS line) was not clear and where it was seen it was discontinuous. The changes were more apparent in the left eye (Fig. 2).

Full-field ERGs were recorded according to the International Society for Clinical Electrophysiology of Vision (ISCEV) protocol, and the scotopic (rod) ERGs and single-flash mixed rod–cone ERGs after 30 min of dark adaptation were abolished. The amplitudes of the photopic cone responses were approximately 50% of that of normal subjects, while the 30-Hz flicker ERG was relatively well preserved (Fig. 3). The mixed rod–cone ERG did not change even after 3 and 6 h of dark adaptation. The multifocal ERGs were diffusely reduced in the right eye and almost extinguished throughout the recording area in the left eye.

A diagnosis of RP seemed to be correct, but we detected some subtle signs which suggested Oguchi disease and we performed further genetic studies.

Molecular genetic studies

The research protocol was approved by the Ethics Review Board of Ideta Eye Clinic. The protocol adhered to the tenets of the Declaration of Helsinki, and an informed consent was obtained from the patient.

The coding regions of *SAG/arrestin* and *GRK1/RHOK* were amplified by polymerase chain reaction and were analyzed using direct sequencing in both directions. The coding regions of 34 genes responsible for autosomal dominant RP were also analyzed. A homozygous mutation of c.926delA (N309TfX320)

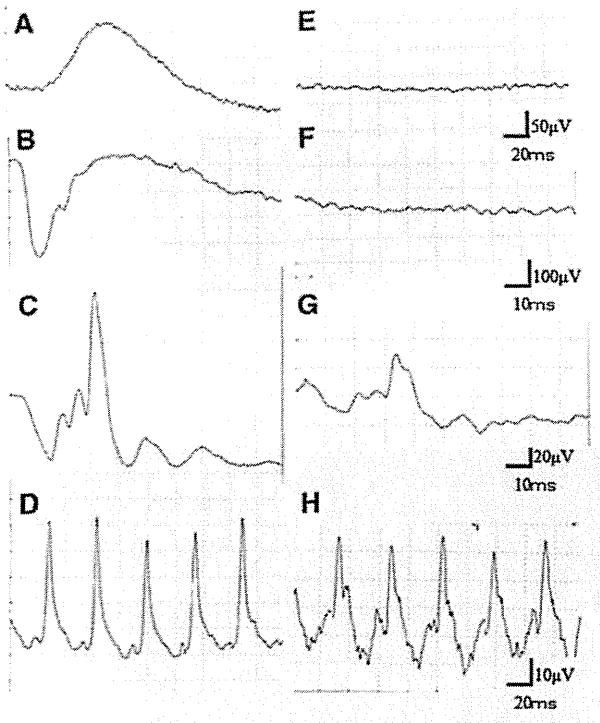

Fig. 3 Full-field electroretinograms (ERGs) recorded according to the International Society for Clinical Electrophysiology of Vision (ISCEV) protocol from a normal subject (*left column* a–d) and our patient with Oguchi disease (*right column* e–f). e, f The scotopic ERG (e) and single-flash ERG (f) are extinguished. g The cone (g) responses are reduced by approximately 50% of that of normal subject (c). h The 30-Hz flicker ERG is relatively preserved

was identified in exon 11 of the *SAG* gene. No other mutations were found in any other coding regions.

Discussion

Our case was initially diagnosed as typical RP from the progressive, diffuse RPE atrophy associated with bone corpuscle-like deposits, bilateral CME, ring scotoma, extinguished mixed rod–cone ERG, and no improvement in ERG after prolonged dark adaptation. However, faint localized areas of golden-yellowish reflex of the fundus attracted our attention. Further examination revealed several signs characteristic of Oguchi disease: viz., night blindness since his childhood, Mizuo-Nakamura phenomenon, slight or no optic disk atrophy, slight narrowing of the retinal vessel, and recordable cone ERGs, which were masked by typical features of RP. A Pubmed search extracted only 5 cases of genetically confirmed Oguchi disease with RP-like fundus appearance (Table 1) [9–13]. All of these cases except that of Nakazawa [12] were easily diagnosed as Oguchi disease from the golden-colored fundus with the Mizuo-Nakamura phenomenon, and all had only sectoral chorioretinal atrophy and/or pigmentation. Our case had a typical RP fundus appearance, but careful examination of the clinical findings strongly suggested Oguchi disease, which was proven by molecular genetics.

Nakazawa et al. [12] reported three cases of RP with a 1147delA mutation in the arrestin gene. All of their cases had partial chorioretinal atrophy, and one of them had pigmentary retinal degeneration that extended from the macular area to the mid-peripheral retina. In addition, this patient had an abnormal golden-yellow fundus reflex in the peripheral area that showed the Mizuo-Nakamura phenomenon.

They concluded that the arrestin 1147delA, which has been known as a frequent cause of Oguchi disease, also may be related to the pathogenesis of autosomal recessive RP. Our case clearly showed that the same individual can have the typical findings of RP and Oguchi disease with the mutation. Thus, our findings strongly support their conclusion.

The golden-yellowish reflex is occasionally seen in young normal individuals especially in the peripheral area. Our case suggests that such retinal reflexes may be a subtle sign of Oguchi disease, and checking for the presence of the Mizuo-Nakamura phenomenon might be of great help in the diagnosis. The pathology or mechanism of the Mizuo-Nakamura phenomenon has not been fully determined. No golden-yellowish reflex was observed at the area with RPE degeneration in our case. This suggests that such reflex and Mizuo-Nakamura phenomenon may be related to RPE change. Further careful attention on the retinal area of golden-yellowish reflex will be important.

CME is known to be associated with RP, but no case of Oguchi disease with CME has been reported. Its presence in our case also hampered our diagnosis of Oguchi disease.

Our ERG findings showed that the b-wave of the photopic cone ERG was present but was almost extinguished in the mixed ERGs. One explanation for this is that the b-wave of the mixed ERGs was greatly reduced by the photopic hill phenomenon i.e., the amplitude of the b-wave increases with increasing

Table 1 Previous reports on coexistent Oguchi disease and retinitis pigmentosa in a same family or same individual associated with *SAG* mutation

No.	Author	Year	Ref.	Mutation	Gender (age)	
					Oguchi disease	RP
Same family						
F1	Yoshii et al.	1998	[17]	N309 (1-bp del) homo	M (45), F (42)	M (52)
F2	Nakazawa et al.	1998	[12]	N309 (1-bp del) homo	F (55)	M (58)
F3	Maw et al.	1998	[9]	R193X homo	M (28)*	M (18)*

						Fundus findings	Mizuo-Nakamura phenomenon	Visual field defect	Bright flash ERG	Scotopic rod ERG	Photopic cone ERG
Same individual											
C1	Nakamachi et al.	1994	[13]	N309 (1-bp del) homo	M (54)	Typical Oguchi disease with sectoral RP	(+)	(+)	Mildly diminished a-wave and extinguished b-wave	Extinguished b-wave	n.m.
C2	Nakazawa et al.	1997	[10]	N309 (1-bp del) homo	F (58)	Typical Oguchi disease with sectoral RP	(+)	(+)	Diminished a-wave and extinguished b-wave	Extinguished	well preserved
C3	Nakazawa et al.	1998	[12]	N309 (1-bp del) homo	M (35)	Typical RP with sectral Oguchi disease	(+)	n.m.	Severely diminished a-wave and extinguished b-wave	n.m.	n.m.
C4	Maw et al.	1998	[9]	R193X homo	M (18)	Typical Oguchi disease OD and typical RP OS	n.m.	n.m.	(OD) white flash elicited a negative wave and the response to blue flash was extinguished	(OS) extinguished	(OS) extinguished
C5	Hayashi et al.	2011	[11]	N309 (1-bp del) homo	M (63)	Typical Oguchi disease with Sectoral RP	(+)	(+)	Diminished a-wave and extinguished b-wave	Extinguished	Diminished
C6	The current case			N309 (1-bp del) homo	M (53)	Typical RP with sectral Oguchi disease	(+)	(+)	Almost extinguished	Extinguished	Diminished

F2 and C3, F3 and C4 are same reports

M male, *F* female, *RP* retinitis pigmentosa, *n.m.* not mentioned

* They are two of three siblings, and the elder showed typical Oguchi OU and the younger showed Oguchi OD and RP OS

stimulus intensities at lower intensities, reaches a plateau, and then decreases at higher stimulus intensities [14]. Because rod responses are extinguished in Oguchi disease, the mixed ERGs might be affected by this phenomenon. In our case, the cone responses were also reduced due to progression of the disease process. Therefore, the b-wave of the mixed ERGs appeared to be almost extinguished. These ERG findings have not been reported even in cases with a coexistence of Oguchi disease and RP (Table 1). Therefore, further examinations in such cases will be helpful to clarify the reason of the discrepancy between the b-waves of the photopic ERGs and the mixed ERGs.

Mutations in the *SAG* gene are known to cause Oguchi disease in the Japanese [10, 11, 15, 16]. It has also been reported that *SAG* may cause RP [16–18]. In fact, several reports suggest that Oguchi disease and RP can coexist in the same family or even in the same individual [9–13, 17] (Table 1). Four mutations in the arrestin gene, R175X, R193X, N309T, and R292X, have been reported [9–12], and their phenotypes varied from typical Oguchi disease to that of RP. These non-sense or frameshift mutations with premature termination of protein translation may cause different protein products leading to dysfunction in the visual cycle, which results in the phenotypic variations. But it is still not known why the same mutation region of arrestin has such phenotypic variations [18].

In addition to the phenotypic heterogeneity of Oguchi disease, Oguchi patients can develop RP at an advanced stage [19]. The first case of Oguchi disease was reported to develop typical RP later in life [20]. In addition, atypical cases of Oguchi disease with this mutation had progressive visual field defects, reduced cone ERGs, and regional chorioretinal atrophy [9–11, 13]. In such cases, fundoscopic and electroretinographic features of Oguchi disease would be masked and careful attention on the very slight alterations is important. It is also necessary to follow patients with Oguchi disease carefully for a longer period keeping in mind the possibility of RP development.

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Conflict of interest None.

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VITRECTOMY FOR PROLIFERATIVE RETINOPATHY IN PATIENT WITH ADVANCED DUCHENNE MUSCULAR DYSTROPHY

Keitetsu So, MD,*† Kei Shinoda, MD, PhD,† Emiko Watanabe, MD, PhD,†
Toru Mashiko, MD,* Atsushi Mizota, MD, PhD†

Purpose: Retinal vascular abnormalities are rare in patients with Duchenne muscular dystrophy. We present a patient with Duchenne muscular dystrophy who developed severe proliferative retinopathy for which vitrectomy was successfully performed in one eye.

Method: Case presentation. A 23-year-old Japanese man with Duchenne muscular dystrophy complicated by cardiac and respiratory insufficiency had reduced vision in both eyes. His best-corrected visual acuity was 0.01 in the right eye and hand movements in the left eye. Ophthalmoscopy showed vitreous hemorrhage and proliferative tissue attached to the optic disk bilaterally. Ultrasound echography showed tractional retinal detachment in the left eye.

Results: Because general anesthesia was considered to be a high risk, vitrectomy, lensectomy, neovascular membrane removal, endolaser photocoagulation, and silicone oil injection were performed under local anesthesia on the right eye. After removal of the silicone oil and intraocular lens implantation, the best-corrected visual acuity was 0.8 in the right eye. Vitrectomy was performed on the left eye, but the retina could not be attached.

Conclusion: The etiology of the proliferative retinopathy in our case is not known. Because early treatment has the potential to improve and maintain vision, we recommend periodic fundus examinations in patients with Duchenne muscular dystrophy.

RETINAL CASES & BRIEF REPORTS X:1–3, 2011

AQ : 1

*From the *Department of Ophthalmology, Teikyo University School of Medicine, University Hospital Mizonokuchi, Kawasaki, Japan; and †Department of Ophthalmology, Teikyo University School of Medicine, University Hospital Itabashi, Tokyo, Japan.*

Duchenne muscular dystrophy (DMD) is the most common form of X-linked recessive neuromuscular disorder,[1–3] with an incidence of 1 in 3,500 live-born male infants. The major clinical manifestation of DMD usually appears around 3 years to 5 years of age and is progressive. By age 12, most patients become wheelchair-dependent and develop fixed contractures and scoliosis. By age 16 to 18, patients are predisposed to serious, sometimes fatal, pulmonary infections.

Abnormal retinal neurotransmission is associated with a mutation in the dystrophin gene,[4–6] and retinal vascular abnormalities have also been reported in few cases of DMD.[7,8] We have examined a patient with DMD who developed severe proliferative retinopathy in both eyes, and retinal reattachment was achieved by vitreous surgery in one eye but was not achieved in the other eye.

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Reprint requests: Kei Shinoda, MD, Department of Ophthalmology, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo,173-8605, Japan; e-mail: shinodak@med.teikyo-u.ac.jp

Case Report

A 23-year-old Japanese man was referred to us in November 2008 for the diagnosis and treatment of decreased vision of 4 weeks duration in both eyes. The patient had DMD, which was

complicated by cardiac and respiratory insufficiency requiring ventilation assistance. He had no family history of ocular disease or trauma, and his best-corrected visual acuity was 0.01 in the right eye and hand movements in the left eye. The intraocular pressure was not measured. The anterior segment examination with a handheld slit lamp was unremarkable, except for mild cataracts in both eyes. Ophthalmoscopy showed proliferative tissue on the disks and condensed vitreous hemorrhage around the posterior poles bilaterally, but the peripheral retina appeared to be attached
F1 (Figure 1). Ultrasound echography showed a tent-shaped vitreous cortex that was attached to the posterior retina in the right eye. The
F2 posterior retina was also detached in the left eye (Figure 2).

The single-flash full-field electroretinogram consisted of a reduced *b* wave with preserved *a* wave. The amplitude of the *a* wave was larger than that of the *b* wave in the right eye, making a negative-type electroretinogram. An electroretinogram was not recorded from the left eye (Figure 2).

Blood tests showed 2.56×10^6 μL of erythrocytes, 8.2 g/dL of hemoglobin, 60.8 mg/dL of blood urea nitrogen, and 0.4 mg/dL of creatinine. The leukocytes and C-reactive protein were within the normal range. The cardiac ejection fraction was 52% of the predicted level. The patient had no history of diabetes mellitus or hypertension. Because of the cardiac and respiratory insufficiency, we recommended vitreous surgery on the right eye under local anesthesia, and permission was obtained from the patient and family for the surgical procedures. Pars plana vitrectomy and pars plana lensectomy combined with laser photocoagulation and silicone oil injection were successfully performed on the right eye in December. After 3 months, the silicon oil was removed and an intraocular lens was implanted. The best-corrected visual acuity improved to 0.8 in the right eye, and this vision was maintained for 12 months. The right fundus photograph taken 1 year after the last
F3 surgical procedure is shown in Figure 3. The primary ophthalmologist, who referred this patient, performed pars plana vitrectomy on the left eye under local anesthesia 1 month after the first surgery on the right eye but failed to reattach the retina. The best-corrected visual acuity remained at hand movements in the left eye.

Discussion

The mutation causing DMD is the gene encoding dystrophin, a 427-kDa protein expressed in photoreceptor terminals and around retinal vessels.[2,3] Most DMD patients have reduced *b*-wave amplitudes with preserved *a* wave under scotopic conditions.[4] Thus, the electroretinogram has the negative-type pattern, as was found in our patient and reported for other patients with DMD. It is believed that the deficiency of dystrophin leads to abnormal transmission between the photoreceptors and subsequent neural cells resulting in the reduced *b* waves.[5]

The pathogenesis of the severe proliferative retinopathy with neovascularization is unknown. A search of PubMed extracted only two studies that reported on the association of proliferative retinopathy and DMD.[7,8] Louie et al[7] stated that they believed that cardiopulmonary compromise was the primary contributor to the development of retinal neovascularization. Ober et al[8] presented a case of DMD with cardiomyopathy and anemia. They hypothesized that their patient with chronic anemia and cardiovascular compromise had surpassed the threshold beyond which retinal neovascularization might be stimulated. Neither cardiopulmonary compromise nor the absence of dystrophin usually leads to retinal neovascularization. The location of dystrophin around retinal vessels of DMD patients suggests that they may be more susceptible to retinal hypoxemia, which can then lead to retinal neovascularization. Because we examined only one patient, we cannot generalize our finding of retinal neovascularization in patients with DMD. However, clinician should be aware that proliferative retinopathy can be associated with retinal vascular complications in patients with DMD. All three cases reported to date, our case and the two cases from the literature,[7,8] involved patients in the very late stages of DMD, and an early surgery may have improved the success rate.

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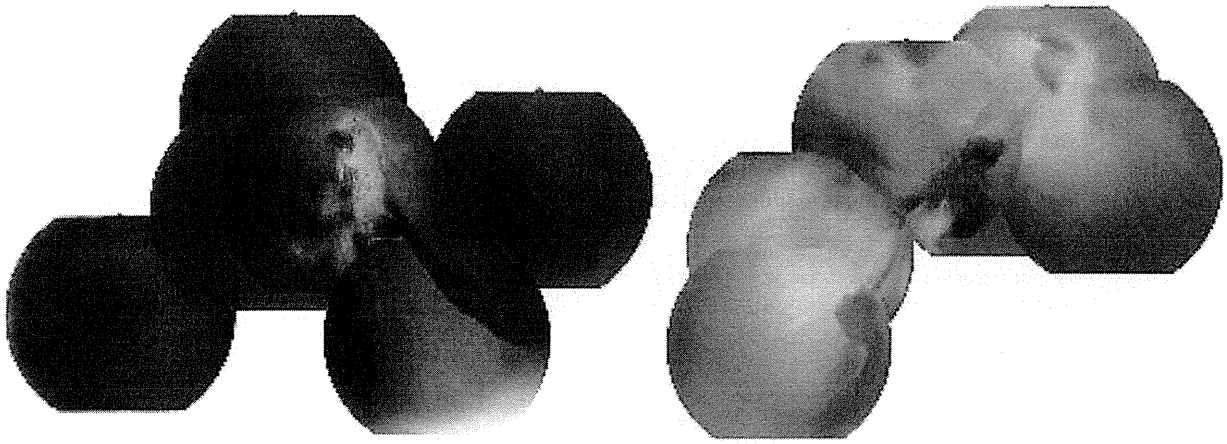

Fig. 1. Fundus photograph of the right (left) and left (right) eyes showing that the optic disks and maculas are partially obscured by hemorrhage.

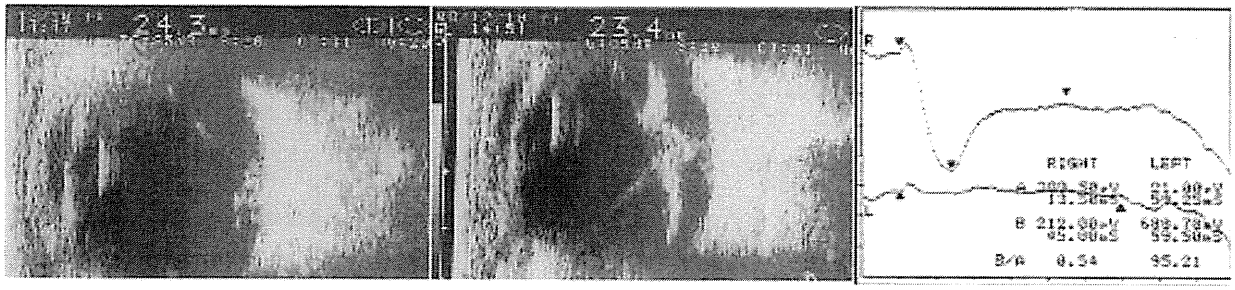

Fig. 2. Ultrasound echograms of the right (left) and left (middle) eyes showing vitreoretinal adhesions in the posterior pole. In the left eye, proliferative tissue and tractional retinal detachment can be seen. Single-flash electroretinogram (right) showing attenuated *b* wave and normal *a* wave resulting in a negative-type electroretinogram in the right eye and no response in the left eye.

Pars plana vitrectomy was effective in the right eye, whereas the vitreous hemorrhage and tractional retinal detachment prevented proper treatment such as photocoagulation or surgery on the left eye. Considering that an early treatment has the potential to improve the prognosis, periodic fundus examination is warranted in patients with DMD.

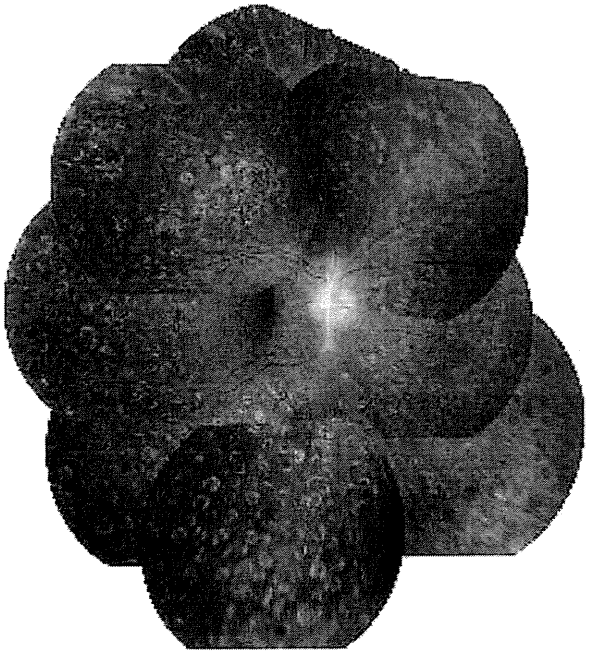

Fig. 3. Fundus photograph of the right eye taken 1 year after the last surgical procedure.

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Key words: Duchenne muscular dystrophy, proliferative retinopathy, pars plana vitrectomy, dystrophin.

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SIMULTANEOUS BILATERAL CENTRAL RETINAL ARTERY OCCLUSION IN CHURG–STRAUSS SYNDROME

Yuko Akiyama, MD,* Kei Shinoda, MD, PhD,* Emiko Watanabe, MD, PhD,* Toru Mashiko, MD,† Atsushi Mizota, MD, PhD*

Purpose: Retinal vascular abnormalities are rare in patients with Churg–Strauss syndrome. We present the findings in a patient with Churg–Strauss syndrome who developed bilateral central retinal artery occlusion simultaneously.

AQ : 1 **Methods:** Case report.

Results: A 68-year-old Japanese man developed acute bilateral vision decrease to counting finger in the right eye and hand movements in the left eye. Ophthalmoscopic and angiographic examinations revealed a central retinal artery occlusion with choroidal circulatory disturbances in both the eyes. The patient had bronchial asthma, hypereosinophilia, radiographically determined migratory pulmonary opacities, and paranasal sinus abnormalities, thus fulfilling the American College of Rheumatology criteria for Churg–Strauss syndrome. Antineutrophil cytoplasmic antibody was absent. High-dose steroid therapy was used, but after 6 weeks, his visual acuity in the right eye did not improve, and the vision in the left eye was no light perception. Later, vitreous hemorrhage was developed in the left eye followed by retinal detachment associated with proliferative retinopathy.

Conclusion: Bilateral central retinal artery occlusion can occur in patients with antineutrophil cytoplasmic antibody–negative Churg–Strauss syndrome. The cause of the central retinal artery occlusion is not known, but consideration for prophylactic steroid therapy may be recommended in antineutrophil cytoplasmic antibody–negative cases to prevent potential visual loss.

RETINAL CASES & BRIEF REPORTS X:1–5, 2011

*From the *Department of Ophthalmology, Teikyo University School of Medicine, University Hospital Itabashi, Tokyo, Japan; and †Department of Ophthalmology, Teikyo University School of Medicine, University Hospital Mizonokuchi, Kawasaki, Japan.*

Churg–Strauss Syndrome (CSS) is an uncommon systemic disease characterized by asthma, hypereosinophilia, and vasculitis of different organs.[1] Ocular involvements are infrequent and include conjunctival nodules, panuveitis, orbital myositis, ischemic optic neuropathy, and retinal artery and vein occlusion.[2–5] There has recently been a rapidly growing literature concerning retinal vascular complications in CSS.[4–12] We present a patient with CSS who developed simultaneous bilateral central retinal artery occlusion (CRAO).

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Reprint requests: Kei Shinoda, MD, Department of Ophthalmology, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo,173-8605, Japan; e-mail: shinodak@med.teikyo-u.ac.jp

Case Report

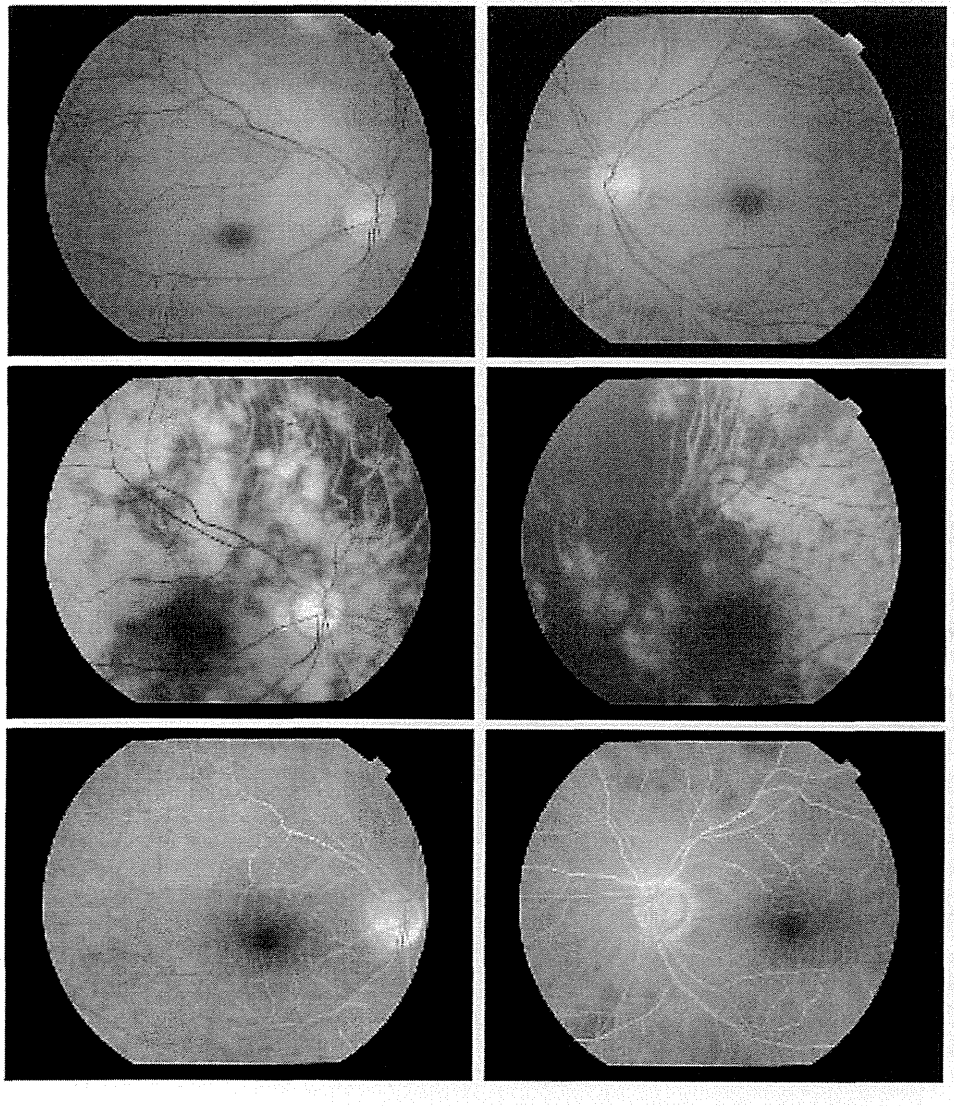
A 68-year-old Japanese man was seen by an internist for his regular appointment for chronic asthma in October 2008. Because his laboratory data showed marked elevations of leukocyte count (17,850 per mm^3) and eosinophils (10,000 per mm^3; 56.0%), he was referred and admitted in the department of internal medicine in our hospital. Neurologic examinations on admission were unremarkable. Laboratory data showed 17,000 per cubic millimeter of leukocytes with eosinophilia of 3.8×10^9 per liter (17%), an erythrocyte sedimentation rate of 42 mm at 1 hour, and a C-reactive protein of 7.27 mg/dL (reference value <0.5 mg/dL). The results from blood tests for renal, hepatic, and pancreatic function were normal. Urinalysis showed no abnormalities. Blood coagulation

tests were within normal limits. There was a marked elevation of rheumatoid factor 50.1 IU/mL (reference value <20 IU/mL). Tests for antinuclear antibodies, antineutrophil cytoplasmic antibodies (ANCA), the lupus anticoagulant, anticardiolipin antibodies, and circulating immune complexes were negative. The patient did not have a history of diabetes mellitus or hypertension. A computed tomography scan and a magnetic resonance imaging scan of the brain showed no abnormalities except for sinusitis. Radiographic examinations of the chest showed multiple migratory opacities in both bronchi.

An acute vision reduction was bilaterally developed in the patient that was thought to be CSS by the systemic signs and symptoms, and he was referred to the Ophthalmology Department. He had no history of ocular disease or trauma, and his best-corrected visual acuity was counting finger in the right eye and hand movement in the left eye. The intraocular pressure was 12 mmHg in both the eyes. The anterior segment examination was unremarkable except for mild cataracts in both the eyes.

Ophthalmoscopy showed marked retinal whitening, a cherry red spot, and attenuated retinal arteries without an embolus in both the eyes (Figure 1). Fluorescein angiography showed a marked delay in choroidal and retinal artery filling, and the arteriovenous transit time was increased. There were signs of possible reestablishment of the retinal circulation in both the eyes. There also appeared to be choroidal circulatory compromises in both the eyes with patchy filling delay of the choroidal vessels (Figure 1). F1

A diagnosis of CSS with the retinal vascular involvement was made. Intravenous methylprednisolone, 1 g/day, for 3 days was administrated twice. Thereafter, oral prednisolone was started from 60 mg/day and was gradually tapered and kept at 27.5 mg/day. The eosinophil count normalized together with a decline in the erythrocyte sedimentation rate and C-reactive protein. However, 4 weeks later, the visual acuity in the right eye had not improved and decreased to no light perception in the left eye. After 6 months, the left eye developed dense vitreous hemorrhage (Figure 2). The intraocular pressure was 16 mmHg in both the eyes, and rubeosis was not observed in both the eyes. Ophthalmoscopy showed a pale optic disk in the right eye and dense vitreous hemorrhage in the left eye. Ultrasound echography showed a detached retina and proliferative retinopathy in the left eye (Figure 2). F2



C O L O R

Fig. 1. Fundus photographs and fluorescein angiograms on the day after the onset of central retinal artery occlusion. A and B, Fundus photographs showing cherry red spot in both the eyes. C and D, Fluorescein angiograms at the early phase showing marked delay in retinal artery filling and choroidal circulatory compromise in both the eyes. E and F, Fluorescein angiograms at the arteriovenous phase showing delayed arteriovenous transit time and possible reestablishment of the retinal circulation in both the eyes.

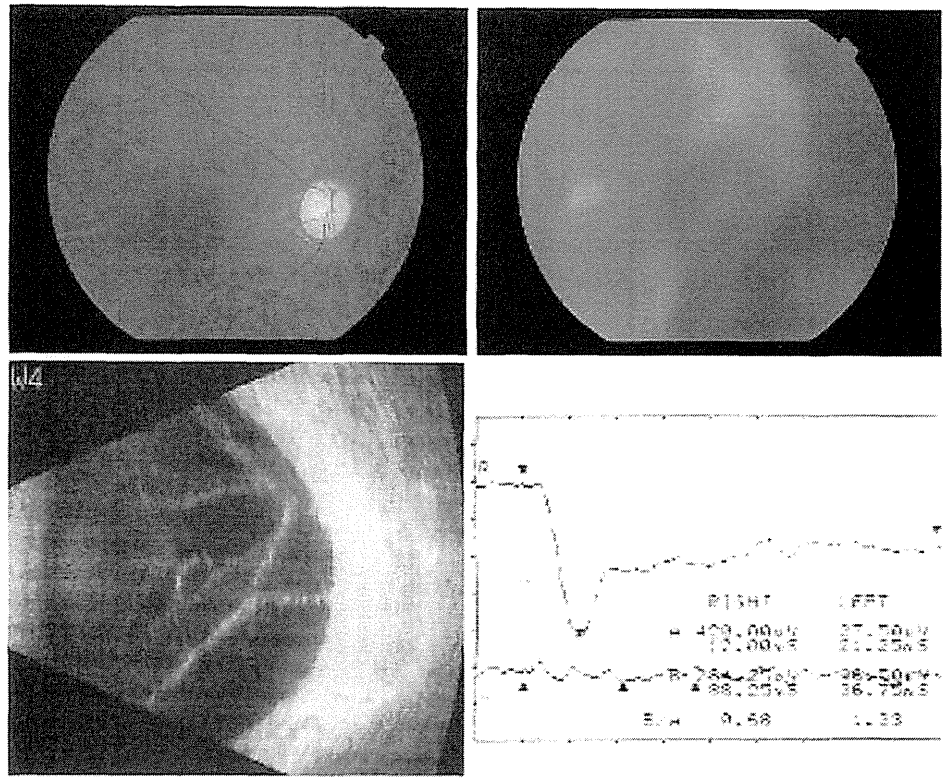


Fig. 2. Ultrasound echography and electroretinography 6 months after the onset. Left: A B-mode ultrasound photograph showing a detached retina in association with proliferative retinopathy in the left eye. Right: Single-flash, full-field electroretinogram consists of a reduced *b* wave with preservation of the *a* wave, leading to a negative-type electroretinogram, in the right eye and was nonrecordable from the left eye.

C O L O R

The single-flash full-field electroretinogram consisted of a reduced *b* wave with preserved *a* wave. The amplitude of the *a* wave was larger than that of the *b* wave in the right eye, making a negative-type electroretinogram. An electroretinogram could not be recorded from the left eye (Figure 2).

Discussion

Our patient was diagnosed with CSS because he had bronchial asthma, hypereosinophilia, radiographically detected migratory pulmonary opacities, and paranasal sinus abnormalities. These are four of the six diagnostic criteria for CSS proposed by the American College of Rheumatology in 1990.[1]

Acute blindness has been rarely described in cases of CSS, and a CRAO in cases of CSS has been reported in only a few cases (Table 1).[4,7–12] Our case showed not only retinal but also choroidal circulatory alterations. These findings suggest that the occlusion was probable in the ophthalmic artery. Udono et al[4] reported a similar case of CSS showing bilateral CRAO, although the CRAO developed in the left eye first and 6 days later in the right eye. Their case also demonstrated some choroidal circulatory compromise in both the eyes. To the best of our knowledge, the development of CRAO simultaneously in both the eyes has not been reported.

Several possible causes for the CRAO were considered. First, an increased thrombotic propensity because of eosinophilia may have caused the artery thrombosis.[12,13] Second, the inflammatory process of CSS vasculitis may involve the central retinal artery.[10] Finally, positive antiphospholipid serology hypercoagulable state, such as positive antiphospholipid serology[12] or high fibrin degradation products,[4] could be associated with the thrombotic process. In our case, no hypercoagulable state was observed, and neither retinal arterial emboli nor vasculitides were observed ophthalmoscopically or by fluorescein angiography as in most cases of CRAO reported (Table 1).[4,7,10] Because the underlying syndrome is usually manifested through vasculitis-induced organ damage, it cannot be denied that the retinal vascular occlusion might be associated directly or through attack on the posterior ciliary arteries with vasculitis. Bilateral ophthalmic arterial occlusion suggests that a relatively more central site is responsible in our case. However, it was not possible to determine a direct inflammatory infiltration or thrombotic event of the ophthalmic artery, and the exact pathophysiology is unknown.

The ocular manifestations in cases of CSS can be classified into two groups: the pseudotumor type (orbital inflammatory syndrome) and the ischemic vasculitis type.[2] The presence of ANCA is

Table 1. Previous Reports on Central Retinal Artery Occlusion Associated with Churg–Strauss Syndrome

Number	Author	Year	Reference Number	Age	Gender	Laterality	TIA	Retinal Findings: Embolus	Retinal Findings: Vasculitis	Retinal Findings: ANCA	Complication: Ocular	Complication: Systemic	Steroid	Visual Acuity: Initial	Visual Acuity: Final
1	Granata et al	2001	[11]	48	M	R	NM	NM	NM	+	Papilledema	—	80 mg/day	NM	No recovery
2	Udono et al	2003	[4]	68	M	R	NM.	—	NM	+	Choroidal circulatory compromise	High fibrin degradation products	HD	NLP	NLP
						L		—	NM		Choroidal circulatory compromise			NLP	NLP
3	Hoffman et al	2005	[12]	54	F	L	NM.	—	—	—	—	Moderate titer of anticardiolipin IgM	75 mg/day	LP	Improved peripheral vision
4	Hamann et al	2006	[6]	42	M	R	Yes	NM	NM	—	CRVO, NVG*	Hyperhomo-cysteinemia	50 mg/day	HM	No recovery
5	Türkçüoğlu et al	2007	[7]	44	F	L	Yes	—	NM	—	Cataract	—	HD	HM	HM
6	Skrapari et al	2008	[10]	50	F	L	NM	—	—	—	—	—	HD	LP	CF
7	De Salvo et al	2009	[9]	55	M	L	NM	NM	NM	NM	BRVO*, hypertensive retinopathy	—	75 mg/day	LP	LP

Visual acuity of the case reported by Granata et al is not clear. They reported just "vision loss."

*NVG was a late complication and BRVO was a preceded complication.

M, male; F, female; R, right; L, left; TIA, transient ischemic attack; NM, not mentioned; CRVO, central retinal vein occlusion; NVG, neovascular glaucoma, BRVO, branch retinal vein occlusion; :HD, high-dose therapy; NLP, no light perception; LP, light perception, HM, hand movement; CF, counting finger.

characteristic of the ischemic vasculitis type, which typically has a sudden onset of vision loss, a quiet-looking eye, and an absence of orbital imaging abnormalities. However, ANCA was negative in our patient whose clinical findings were more like those with the ischemic vasculitis type. In addition, there have been several cases of CSS that developed CRAO, but the ANCA score was negative (Table 1).[7,8,10,12] Retinal artery occlusion may not always occur as a characteristic manifestation in the ANCA-positive CSS patients. However, clinician should be aware that CRAO can develop in patients with CSS. Prophylactic steroid therapy should be considered even in ANCA-negative cases to prevent potential visual loss.

Key words: Churg–Strauss syndrome, ocular ischemia, central retinal artery occlusion, ocular artery occlusion.

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