Macular Degeneration Associated With Aberrant Expansion of Trinucleotide Repeat of the SCA7 Gene in 2 Japanese Families

Toshiaki Abe, MD; Takehide Tsuda, MD; Madoka Yoshida, MD; Yuko Wada, MD; Tetsuya Kano, MD; Yasuto Itoyama, MD; Makoto Tamai, MD

Objective: To evaluate the macular function of Japanese patients with a trinucleotide repeat expansion in the spinocerebellar ataxia type 7 (SCA7) gene.

Methods: Ophthalmic findings in patients whose DNA analysis revealed expanded alleles of the trinucleotide repeat in the SCA7 gene were evaluated.

Results: Trinucleotide repeat was expanded from 40 to 48 in affected patients (control subjects, 12 repeats). Affected patients were characterized by different degrees of visual acuity decrease (0.09-0.9), a tritan axis color vision, a coarse granular appearance of the macular region on scanning laser ophthalmoscopy, depression of multifocal electroretinograms, and macular degeneration. However, pigmentary changes were not observed in the retina. The trinucleotide repeat was longer and the onset of macular dysfunction was earlier in the younger generation. One patient in a family manifested decreased visual acuity 10 years preceding other neurologic signs.

Conclusions and Clinical Relevance: Patients with SCA7 mutations showed macular dysfunction or degeneration with expansion of CAG repeat in the SCA7 gene. However, the lesions were less pigmented than those previously reported. Patients also showed ophthalmologic anticipation, which has not been reported for the ocular changes in other patients who have trinucleotide repeat expansion of the responsible genes.

PATIENTS AND METHODS

PATIENTS AND TESTS

Family members from 2 pedigrees (Figure 1) whose members were identified as having an expanded allele of CAG repeat in the SCA7 gene were examined ophthalmologically at Tohoku University Hospital, Sendai, Japan. Ophthalmic examinations included best-corrected visual acuity, slitlamp biomicroscopy, visual field analysis, color vision testing, fundus examination, fluorescein angiography, scanning laser ophthalmoscopy (SLO), and electoretinography. Color vision was examined with 100-hue (Japan Focus, Tokyo, Japan) or panel D-15 (Luneau, Chartres, France) tests. Visual field analysis was performed using a Humphrey visual field analyzer with program 30-2 (Humphrey Systems, Dublin, Calif) or an Octopus 1-2-3 perimeter (Interzeag, Agschlieren, Switzerland) when possible.

Electoretinograms (ERGs) were recorded using the guidelines of the International Society for Clinical Electrophysiology of Vision.21 A standard white flash (20 J) was used to elicit the maximum responses of the rods and cones after 30 minutes of dark adaptation, a dim blue flash in the same dark-adapted condition was used to elicit rod-isolated responses, and a single white flash and a 30-Hz flicker stimulus of red light were used under light-adapted conditions to isolate cone responses. Multifocal ERGs22 (MAYO, Inazawa, Japan) were also recorded when possible.

We examined 70 control subjects genetically and ophthalmologically: 33 had trinucleotide repeat expansion in the responsible genes (18 with SCA1, 2 with SCA2, 7 with SCA3, 4 with DRPLA, and 2 with Huntington disease). 20 had ADCA and their DNA analysis revealed no responsible gene so far, and 17 had olivopontocerebellar atrophy or multiple system atrophy.

REPORT OF CASES

CASE S-I:2

A 75-year-old woman noticed a gait disturbance at age 50 years. Computed tomographic scans and magnetic resonance images showed atrophy in the cerebellar region. She also noticed decreased visual acuity at age 70 years. When she consulted us, her visual acuity was 0.4 OD with a refraction of −1.50 diopters (D) sphere and cylinder of −0.25 D X90 and 0.6 OS with a refraction of +1.00 D sphere. Pupillary reactions were relatively normal. Intraocular pressure was 12 mm Hg in both eyes. Nuclear sclerosis was observed. The D-15 test showed a tritan axis (Table). Other ophthalmic examinations could not be performed.

CASE S-II:3

A 51-year-old woman noticed a gait disturbance at age 40 years. Computed tomographic scans and magnetic resonance images showed atrophy in the cerebellar region. When she consulted us at age 51 years, her visual acuity was 0.7 OD with a refraction of −2.50 D sphere and a cylinder of −0.25 D X90 and 0.9 OS with a refraction of −1.75 D and a cylinder of −0.75 D X90. She had no eye complaints. Nystagmus was not detected, but dysmetria and convergence insufficiency were observed. Pupillary reactions were almost normal, and intraocular pressure was 16 mm Hg in both eyes. Slitlamp biomicroscopic examination showed that the media were clear and corneal endothelial cell density was normal. Results of fundoscopic and fluorescein angiographic examinations were almost normal. No pigmentation or halolike depigmentation was observed (Figure 3). A coarse granular appearance of the macular area was observed with a 633-nm wavelength laser of the SLO (Figure 4). Octopus visual field analysis showed mild depression of the threshold in the central 30° in both eyes. The 100-hue color vision test showed a high error score (731) and the D-15 test showed a tritan axis (Table). Results of ERGs were within the reference limits, although cone responses were at the lower limits of normal in our laboratory (Figure 5).24

DNA AND SEQUENCE ANALYSIS USING POLYMERASE CHAIN REACTION

DNA was extracted from peripheral blood lymphocyte samples (~20 mL) from family members and controls using a protocol described earlier.6 Informed consent was obtained from all participants.

Polymerase chain reaction (PCR) was carried out using a thermocycler (Perkin Elmer, Norwalk, Conn) in 50 µL of reaction mixture containing 1 µg of the patient's genomic DNA (described below); 20 µmol/L of each primer (forward: 5'-TGTTACATTGTGGACGGAAA-3' and reverse: 5'-CAGACTGCTCCAGCATCATT-3').20 200 µmol/L each of deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxythymidine triphosphate; 50-mmol/L potassium chloride; 10-mmol/L Tris hydrochloride (pH 8.3); 1.5-mmol/L magnesium chloride; 0.001% gelatin; and 2.5 U of Taq polymerase. We used 35 reaction cycles. The temperature settings for PCR were 94°C for 1 minute of denaturation, 38°C for 1 minute of annealing, and 72°C for 1 minute of polymerization.21 In each case, the amplified DNA was separated in 3% agarose gel (SeaKem: FMC BioProducts, Rockland, Me) containing homidium bromide, 0.05 µg/mL. To estimate the accuracy of the repeat numbers, we performed PCR with fluorescein isothiocyanate–labeled primer, as reported previously.6 Thus, a reversed primer with fluorescein isothiocyanate was used for PCR amplification. An aliquot of the product was electrophoresed on 6% denaturing polyacrylamide gel using an automated DNA sequencer (Pharmacia LKB ALF DNA sequencer; Pharmacia, Uppsala, Sweden) with a mixture of fluorescein isothiocyanate–labeled fragments (50-500 base pairs [bp] long) as a size marker (Sizer 50-500; Pharmacia). The PCR results showed that the expanded allele of the gene was observed in all of the affected family members examined and a member suspected to be an asymptomatic carrier. These alleles were cosegregated with the disease (Figure 1 and Figure 2). The size of the abnormal expansions differed in the patients (Figure 2, Table).

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CASE S-III:1

A 26-year-old man noticed a gait disturbance at age 21 years and a mild decrease of visual acuity at age 25 years. When he consulted us, his visual acuity was 0.6 OD with −2.0 to 0.75 D X90 and 0.5 OS with −1.75 to 0.75 D X90. The pupils were mildly dilated and the media were clear. No nystagmus was detected, but dysmetria and saccadic pursuit were observed. Intraocular pressures were normal, and the media were clear by biomicroscopic examination. Corneal endothelial cell density was normal. Results of funduscopic and fluorescein angiographic examinations were almost normal, although the temporal region of the optic disc showed some pallor and the macular area was somewhat mottled (Figure 3). A coarse granular appearance of the macular area was observed with a 633-nm wavelength laser of the SLO and was more prominent than in patient S-II:3 (Figure 4). No pigmentation or halolike lesion was observed in the fundus. The retinal vessels appeared normal, but a retinal tear was found at the 3-o’clock position in the left eye. Error score of the 100-hue test was 507, and the D-15 test showed a tritan axis (Table). Results of ERGs were within reference limits, although cone responses were at the lower limit for his age in our laboratory (Figure 5). Multifocal ERGs showed depression of the response amplitude, especially in the central 15° (Figure 6, A).

CASE S-III:2

A 22-year-old woman was healthy clinically and genetically. Her visual acuity was 1.2 OD with a myopia of −1.50 D sphere and 1.5 OS with a cylinder of −1.50 D X180. Funduscopic examinations showed tigroid fundi without retinal pigmentation. The results of all of the other examinations were normal.

CASE S-III:3

A 22-year-old woman showed no abnormal neurologic signs and had no complaints about her eyes. Her best-corrected visual acuity was 1.0 OD with −5.50 to 0.75 D X10 and 1.0 OS with a −5.25 to 1.0 D X180. Funduscopic examinations showed tigroid fundi without retinal pigmentation. The results of all of the other examinations were normal.

CASE T-II:3

A 46-year-old woman noticed decreased visual acuity at about age 30 years. Results of ophthalmologic examinations were normal at that time, and no neurologic abnormalities were observed. She noticed slurred speech and gait disturbance at age 40 years. Her visual acuity decreased

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**Ophthalmic Findings in Patients With Spinocerebellar Degeneration and Trinucleotide Repeat Expansion of the SCA7 Gene**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age, y/ Sex</th>
<th>Age at Onset, Ophthalmol/Cerebellar</th>
<th>Expanded/Normal Repeat</th>
<th>Best-Corrected Visual Acuity, OD/OS</th>
<th>Color Vision</th>
<th>ERG, R/L</th>
<th>Corneal Endothelial Cell Density, R/L, Cells/mm²†</th>
<th>Cerebellar CT/MRI Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-III:1</td>
<td>22/F</td>
<td>26/14/10</td>
<td>0.7/0.9</td>
<td>Tritan/tritan</td>
<td>ND</td>
<td>ND</td>
<td>N/A</td>
<td>Atrophy</td>
</tr>
<tr>
<td>S-III:2</td>
<td>22/F</td>
<td>–/–</td>
<td>1.2/1.5</td>
<td>Tritan/tritan</td>
<td>N/N</td>
<td>N/N</td>
<td>N/A</td>
<td>Atrophy</td>
</tr>
<tr>
<td>S-III:3</td>
<td>46/F</td>
<td>1.0/1.0</td>
<td>1.0/1.0</td>
<td>Tritan/tritan</td>
<td>507/507</td>
<td>N/N</td>
<td>N/A</td>
<td>Atrophy</td>
</tr>
<tr>
<td>T-II:3</td>
<td>50/40</td>
<td>47/10</td>
<td>0.6/0.5</td>
<td>Tritan/tritan</td>
<td>731/731</td>
<td>N/N</td>
<td>N/A</td>
<td>Atrophy</td>
</tr>
</tbody>
</table>

*SCA7 indicates spinocerebellar ataxia type 7; age at onset, ophthalmol/cerebellar; age at the first ophthalmologic and cerebellar signs in years; expanded/normal repeat, trinucleotide repeat number of expanded and normal alleles of the SCA7 gene; D-15, panel D-15 color discrimination test; R, right eye; L, left eye; 100h, 100-hue test of color vision with error scores; ERG, electroretinogram; CT, computed tomography; MRI, magnetic resonance imaging; ND, examination was not done; A, attenuated amplitude; S, subnormal amplitude; and N, normal amplitude.
†Normal corneal endothelial cell density is 2783 ± 380 cells/mm² in our institute.
‡Patient S-III:2, healthy clinically/genetically; patient S-III:3, healthy clinically/abnormally genetically (asymptomatic carrier).

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Figure 3. Fundus photographs of the right and left eyes, respectively, of family members. A and B, Patient S-II:3 is a 51-year-old woman. The white dots are artifacts. C and D, Patient S-III:1 is a 26-year-old man. E and F, Patient S-III:3 is a 22-year-old woman. Tigroid fundi without retinal pigmentation are observed. G and H, Patient T-II:3 is a 46-year-old woman.
gradually, and when she consulted us, her best-corrected visual acuity was 0.09 OD with $-1.50$ to $0.50$ D X180 and 0.09 OS with $-2.0$ to $0.50$ D X90. Gaze-evoked nystagmus was observed, and slow eye movement and dysmetria were seen in her eye movement. Intraocular pressures were 17 mm Hg OD and 16 mm Hg OS. The media were clear, and corneal endothelial cell density was within reference limits. Funduscopic and SLO examinations showed macular degeneration in both eyes without pigmentation (Figures 3 and 4). No halolike macular lesions were observed. The retinal arteries were attenuated and the optic disc was somewhat pale. Pigmentation was not found throughout the fundus. Octopus visual field analysis showed a relative central scotoma in both eyes. Results of the 100-hue test showed an extremely high error score (1491), and results of the D-15 test showed a tritan axis (Table). Electroretinographic recordings were extinguished for photopic and flicker ERGs. Single white flash and scotopic ERGs showed mild attenuation of each wave (Figure 5). Multifocal ERGs showed marked depression of peak response amplitude (20° from center) (Figure 6, B).

In general, macular dysfunction started earlier in the younger generation of patients than in the older patients. None of the control subjects showed macular dysfunction unless a mutation was present.

DNA analysis from peripheral blood samples showed expansion of the CAG repeat in one of the alleles of the SCA7 gene. The number of CAG repeats in patient S-I:2 was 40, in patient S-II:3 was 44, in patient S-III:1 was 47, in patient S-III:3 was 46, and in healthy family member S-III:2 was 12. The number of CAG repeats was 48 in patient T-II:3. The number of repeats was higher in the younger than the older generation. No expanded PCR product was observed in 140 chromosomes from 70 controls with use of the same primers.

Autosomal dominant cerebellar ataxia type II is classified as a progressive neuro-ophthalmologic disorder. It is al-
ways associated with pigmentary macular degeneration and with a variable degree of ophthalmoplegia, dementia, and extrapyramidal features. Ophthalmologic examinations have shown progressive bilateral visual loss, although the severity was different not only between families but among affected members in one family. Loss of the normal foveal reflex and a granular appearance of the macula were the first reported signs of a macular lesion. Later, coarse granular pigment or some pale areas with small pigment clumps were observed. Neetens and coworkers also reported pigmented macular lesions and halolike macular pigment or depigmented changes. Concomitantly, the macular pigmentary changes progressed to the periphery and led to secondary pigmentary retinopathy (inverse type) with marked decreased ERG amplitudes. A recent extensive linkage study in patients with ADCA II revealed that the responsible gene was linked to the short arm of chromosome 3 with the CAG repeat inside. The repeat was longer and the disease was more severe in the younger than the older patients. In this study, none of the controls showed macular dysfunction. However, the patients had abnormal color discrimination (tritan axis), decreased visual acuity, a coarse granular appearance of the macular area on SLO, depression of the multifocal ERG, and macular degeneration. These characteristics suggest that macular involvement is one of the first signs of intraocular abnormality in patients with SCA7, as previously reported. The repeat was longer and the disease was more severe in the younger than the older patients.

In this study, none of the controls showed macular dysfunction. However, the patients had abnormal color discrimination (tritan axis), decreased visual acuity, a coarse granular appearance of the macular area on SLO, depression of the multifocal ERG, and macular degeneration. These characteristics suggest that macular involvement is one of the first signs of intraocular abnormality in patients with SCA7, as previously reported. However, the macular findings in our Japanese patients were somewhat different from those reported earlier. Our patients, including the asymptomatic ones, did not show any pigmentation or halolike lesions at the macula during the early and even the advanced stage of the disease. In addition, none of the peripheral lesions of the retina showed pigmentary changes. This was true even though a coarse granular appearance was detected on SLO. Patient T-II:3, who had a 16-year history of decreased visual acuity, showed macular degeneration without pigmentary changes. She did, however, manifest mildly attenuated rod ERGs and attenuation of the retinal artery. Longer follow-up might be necessary to determine whether pigmentary changes will appear.

This difference in pigmentary changes might be due to race because this disorder was reported mainly in whites and North Africans. No other pedigree has been reported in Japan.

Decreased visual acuity due to optic atrophy, attenuation of the oscillatory potentials of the ERG, and decreased corneal endothelial cell density have been reported in patients with trinucleotide repeat expansion of the SCA1 gene. None of our patients showed this combination of ophthalmologic signs with trinucleotide repeat expansion of the SCA7 gene. In addition, none of our patients showed decreased corneal endothelial cell density, as found in patients with DRPLA.

As reported, patients with SCA7 are genetically homogeneous, and their condition is caused by a CAG repeat expansion in the SCA7 gene. Decreased visual acuity may distinguish SCA7 from other ADCAs, but other ophthalmologic tests may be necessary to distinguish it from ADCA because some patients with SCA7 may also demonstrate optic atrophy.

Patients with SCA1 or DRPLA also showed neurologic anticipation, and thus far, no ophthalmologic anticipation has been reported in our patients. However, the ophthalmologic signs start earlier and the macular function was more deteriorated in the younger than the older patients. Also, the trinucleotide repeat of the gene was longer in the younger than the older generations (Table). Patients with SCA7 showed potential ophthalmologic and genetic predisposition for neurologic anticipation.

Ophthalmologic abnormalities in patients with SCA1 were observed about 10 years after the onset of neurologic disorders and progressed slowly compared with other neurologic signs. These observations might also be true in pa-
tients with DRPLA.7 Pcd/pcd (Purkinje cell degeneration) mice, which have an autosomal recessive mutatation, showed almost complete loss of Purkinje cells by 5 weeks of age. Photoreceptor cells, on the other hand, degenerated by only 25% to 30% at 2 months of age.30 Degeneration of the cells in the retina may be slower than in the cerebellum in systemic neurodegenerative disease.

However, the decreased macular function may have preceded the neurologic disorders (cerebellar signs) in our patient (T-II:3) by about 10 years. This patient reported decreased visual acuity but not cerebellar signs may need at least 48 repeats of the expanded CAG repeat. The number of repeats of 48 was in our patient with initial visual acuity decrease and the patients with 47, 46, 44, and 40 repeats may also be true in our patients because the maximum number of repeats in our Japanese patients with SCA7 exhibited ophthalmologic signs earlier than neurologic signs, and the latency period between decreased visual acuity and the onset of cerebellar symptoms did not exceed 9 years (mean, 5 years). Furthermore, the mean size of the expanded CAG repeat was reportedly significantly greater in patients who started with decreased visual acuity than in those with cerebellar onset (average, 51 repeats in the former vs 47 repeats in the latter).30 These findings may also be true in our patients because the maximum number of repeats of 48 was in our patient with initial visual acuity decrease and the patients with 47, 46, 44, and 40 repeats had initial cerebellar signs. Patients who start with decreased visual acuity but not cerebellar signs may need at least 48 repeats in Japanese patients with SCA7.

Steinmann and coworkers36 reported that the normal SCA7 alleles carry 4 to 35 CAG repeats, whereas pathologic alleles carry more than 37 repeats. Our Japanese patients also showed more than 40 repeats, and the number of repeats necessary for the onset of neurologic signs might be approximately the same as that of whites and North Africans. The numbers of CAG repeats in our controls were 10 to 14, suggesting that the SCA7 protein (ataxin-7, a potential transcription factor15) may have an important role in cerebellar and macular function.

According to the results of a previous study,25 the histological findings of the retina in patients classified as Harding II (probably SCA7) showed complete absence of photoreceptors with hypertrophic and atrophic lesions in the retinal pigment epithelium. A few pigment spots were also observed on the surface of the retina.26 Although, histological examination was not performed in our Japanese family, the examination may also reveal a difference in pigmentary changes in the macular lesions. We plan to follow up the affected and nonaffected members of these 2 families.

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Reprints: Toshiaki Abe, MD, Department of Ophthalmology, Tohoku University School of Medicine, 1-1 Seiryomachi Aobaku Sendai, Miyagi 980-8574, Japan (e-mail: toshi@oph.med.tohoku.ac.jp).

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