Objective: To describe retinal and optic disc atrophy and a progressive decrease of visual function in 2 Japanese brothers. Both had a mutation in the CACNA1F gene, the causative gene of incomplete congenital stationary night blindness (CSNB).

Methods: We studied observational case reports and performed comprehensive ophthalmologic examinations including best-corrected visual acuity, biomicroscopy, ophthalmoscopy, fundus photography, and electroretinography. Genomic DNA was extracted from the peripheral blood, and all 48 exons of the CACNA1F gene were directly sequenced.

Results: The 2 brothers had retinal and optic disc atrophy and a progressive reduction of visual acuity with increasing age. Although these clinical features are not typical of previous patients with incomplete CSNB, both patients had an in-frame mutation with deletion and insertion in exon 4 of the CACNA1F gene. In both patients, the bright-flash, mixed rod-cone electroretinogram had a negative configuration, a characteristic of incomplete CSNB. However, the full-field scotopic and photopic electroretinograms were nonrecordable, indicating severe, diffuse retinal malfunction, which is not typical in incomplete CSNB.

Conclusion: These findings indicate that a mutation of the CACNA1F gene may be associated with retinal and optic disc atrophy with a progressive decline of visual function.

Clinical Relevance: In patients with retinal and optic disc atrophy associated with negative-type electroretinograms, a CACNA1F gene mutation should be considered.

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We describe 2 brothers with a CACNA1F mutation whose clinical findings were unlike those reported for patients with incomplete CSNB. They both had retinal atrophy with attenuated retinal vessels, marked optic disc atrophy, and progressive decline of visual function. They also differed from the usual patient with incomplete CSNB by showing nonrecordable full-field scotopic and photopic ERG responses. These results indicate a phenotypic heterogeneity in CACNA1F mutations.

METHODS

SUBJECTS

This study conformed to the tenets of the Declaration of Helsinki, and informed consent was obtained from the subjects after an explanation of the study’s purpose. We examined 2 Japanese brothers who had retinal and optic disc atrophy as well as negative-type bright-flash ERGs. They had received follow-up in the Department of Ophthalmology of Nagoya University (Nagoya, Japan). Because their clinical findings did not correspond to a known retinal disease pattern, we examined candidate genes for retinal degeneration using molecular genetic analyses. The CACNA1F gene, the causative gene of incomplete CSNB, and the XLR1 gene, the causative gene of retinoschisis, were examined because patients with such diseases show negative-type ERGs. The known causative genes for optic atrophy (e.g., the OPA1 gene, which causes dominant optic disc atrophy, and mitochondrial DNA, which causes Leber hereditary optic neuropathy) were also examined.

DNA ANALYSIS

Genomic DNA was extracted from leukocytes of the peripheral blood. Exons 1 through 48 of the CACNA1F gene were amplified by polymerase chain reaction (PCR) using the DNA Thermal Cycler 9700 (PerkinElmer Applied Biosystems, Foster City, Calif). Primers were purchased (Life Technologies Oriental, Inc, Tokyo, Japan) according to previously reported sequences.1 For all exons, 200 ng of genomic DNA was amplified in a 50-µL reaction with 0.5 µM of each primer and 0.2 mM each of deoxyribonucleoside triphosphate and DNA polymerase (AmpliTaq Gold; PerkinElmer Applied Biosystems). The PCR conditions were as follows: 5 minutes at 94°C, 35 cycles at 94°C for 30 seconds followed by 30 seconds at 58°C, and 45 seconds at 72°C with a final extension step at 72°C for 7 minutes.

The PCR products were purified using a purification kit (High Pure PCR Purification Kit; Boehringer Mannheim, GmbH, Mannheim, Germany) and then directly sequenced with a DNA sequencing kit (Dye Terminator Cycle Sequencing Ready Reaction Kit, PerkinElmer Applied Biosystems) and an automated DNA sequencer (model 373; Applied Biosystems, Foster City). Primers for the sequence reactions were the same as for the PCR reaction. To search for polymorphisms, exon 4 of the CACNA1F gene in 100 alleles from unrelated healthy Japanese individuals (34 men and 33 women) was directly sequenced.

For analysis of the XLR1 gene, the OPA1 gene, and mitochondrial DNA, primers were purchased as described in previous studies11-16 and were used for direct genomic sequencing.

CLINICAL AND ELECTROPHYSIOLOGICAL EXAMINATIONS

The clinical characteristics of the patients were evaluated using a comprehensive ophthalmologic examination including BCVA, refraction, biomicroscopy, ophthalmoscopy, fundus photography, Goldmann kinetic visual field examination, and ERGs.

Standardized full-field ERGs were elicited by Ganzfeld stimuli after pupillary dilation with 0.5% tropicamide and 0.5% phenylephrine hydrochloride with 30 minutes of dark adaptation. The rod ERGs were elicited by a blue stimulus at an intensity of 5.2 × 10⁻³ candela (cd)/m² per second. The mixed rod-cone single-flash (bright-white) ERGs were elicited by a white stimulus at an intensity of 44.2 cd/m² per second. The cone ERGs and 30-Hz flicker ERGs were elicited by a white stimulus at an intensity of 4 cd/m² per second and 0.9 cd/m² per second, respectively, on a white background of 68-cd/m² luminance.

Our system for recording focal cone macular ERGs with infrared television fundus monitoring has previously been described in detail.15,16 After the patient’s pupils were fully dilated, focal ERGs were recorded using 3-Hz rectangular stimuli with equal light and dark periods. The stimulus spot size was either 5°, 10°, or 15° in diameter and centered on the fovea. A time constant of 0.03 second was used for recording a-waves and b-waves, and a signal processor was used to average the 512 responses.

RESULTS

In the 2 brothers (case 1: IV-5 and case 2: IV-2 in Figure 1), we found a 4-base pair deletion between nucleotides 271 and 274 with an insertion of an abnormal 34-base pair sequence in exon 4 of the CACNA1F gene. This led to a substitution of serine and alanine residues in codons 91 and 92, respectively, for 12 unusual residues (Figure 2). The same mutation has already been reported in a Japanese fam-

Figure 1. Pedigree of the family with optic disc atrophy and severe retinal dysfunction showing affected (solid symbols) and unaffected (open symbols) members. Individuals whose DNA was tested are indicated with an X. Squares indicate men; circles, women; and slash through symbol, deceased.

Figure 2. Nucleotide sequences of the CACNA1F gene using sense primers in the 2 patients. Bars indicate the positions of the mutations. In cases 1 and 2, a hemizygous 4-base pair deletion at nucleotide 271 to 274 with a replacement by an abnormal 34-base pair sequence causes amino acid substitution of Ser91 and Ala92 with 12 unusual residues in exon 4.
ily with incomplete CSNB. Two of the sons of the patient in case 2, V-1 and V-2 in Figure 1, had no ocular disorders and showed no mutation in the gene. The mutation found in the 2 brothers was not present in 100 control alleles. No mutations in the XLRS1 or OPA1 gene or the mitochondrial DNA were found in these siblings.

REPORT OF CASES

Case 1

A 56-year-old Japanese man (IV-5 in Figure 1) had reduced near and distance visual acuity. His BCVA was 20/200 OD and 20/300 OS with refractive errors of −2.25 −1.00 × 30° OD and −3.50 −0.50 × 170° OS. He stated that his BCVA had been 20/30 OD and 20/40 OS when he was a schoolboy. At age 23 years, he had undergone a cervical sympathectomy to improve the blood circulation to the eye, either because his retinal vessels were attenuated or because he was suspected to have Leber hereditary optic neuropathy. Despite the surgery, his corrected visual acuity had decreased to 20/60 OD and 20/100 OS when he was next tested at age 30 years.

His parents were first cousins, and an eye disease had probably affected his late maternal grandfather (II-3 in Figure 1) because he was reported to have poor vision.

A fundus examination revealed marked optic disc atrophy and attenuated retinal vessels in both eyes, and diffuse pigmented atrophy was observed in both retinas (Figure 3A and B). The anterior segments and intraocular pressure were normal. Goldmann kinetic perimetry disclosed a relative central scotoma in both eyes with the I-4e target (Figure 4A).

The patient failed all of the Ishihara color plate tests and showed strong blue-yellow and red-green color defects with the AO H-R-R pseudoisochromatic plates, Farnsworth D-15 panel test, Lanthony New Color Test, and Farnsworth-Munsell 100-hue test without any particular axis of errors. He stated that he had been diagnosed as having a mild red-green color vision abnormality when he was a schoolboy.

Goldmann-Weekers dark adaptometry (Haag Streit, Koniz, Switzerland) of the right eye showed a biphasic curve with an elevation of the final normal rod threshold by about 1.0 log unit.
Full-field scotopic and photopic ERGs were significantly reduced, and the 30-Hz flicker ERGs were at noise level (Figure 5). The bright-flash, mixed rod-cone ERG had a negative configuration with loss of the oscillatory potentials (Figure 5). The a-waves and b-waves of the focal cone macular ERGs were reduced to noise level for the 5°, 10°, and 15° stimuli, indicating a significant dysfunction in the macular areas of both eyes (Figure 6).

When the patient was seen at age 72 years, cataract surgery had been performed on both eyes without any complications, but his BCVA was reduced to 20/500 OD and 20/2000 OS. Goldmann kinetic perimetry revealed a pericentral scotoma in the right eye and a centrocecal scotoma in the left eye with the III-4e and IV-4e targets, respectively (Figure 4B).

Case 2

The 64-year-old brother of the patient in case 1 was first examined in our hospital because of reduced vision. His BCVA was 20/500 OD and no light perception OS with high myopic refractive errors of −15.5 −3.00 × 180° OD and −12.75 sphere OS. A significant nystagmus was present. A mild cataract was noted in the right eye, and his left eye was aphakic. The intraocular pressure was within normal limits. According to his recollection, his BCVA had been 20/40 OU when he was a schoolboy. Cataract surgery had been performed on both eyes without any complications, but his BCVA had been 20/40 OU when he was a schoolboy. Cataract surgery had been performed on his left eye for an unknown reason when he was 15 years old, and afterward he had lost his vision in that eye. The BCVA in his right eye had gradually declined to 20/60 OD when he was a high school student, and it was 20/100 OD at age 20 years.

A fundus examination revealed optic disc atrophy and retinal vessel attenuation in both eyes (Figure 3C and D). Severe chorioretinal atrophy was present in the left eye (Figure 3D). Goldmann kinetic perimetry revealed a general constriction of the visual field to the V-4e target in the right eye and no recognition to the II-4e target in all fields (Figure 4C). The patient complained of severe loss of color vision and could not perform any of the color vision tests.

Full-field ERGs of his right eye were recorded when he was 70 years old. The bright-flash ERGs had a negative configuration with absent oscillatory potentials. The a-wave amplitude was reduced. The scotopic, photopic, and 30-Hz flicker ERG responses were nonrecordable, indicating a significant diffuse retinal malfunction (Figure 5).

During the follow-up period the cataract in the right eye became prominent, and the patient underwent cataract surgery at age 75 years. The surgery was successful without any complications; however, his corrected visual acuity gradually declined and became counting fingers by age 78 years.

COMMENT

Although these 2 brothers had a mutation in the CACNA1F gene, their clinical features did not correspond with those of previous patients with incomplete CSNB. First, both patients had retinal atrophy with attenuated retinal vessels, whereas eyes with incomplete CSNB have essentially normal fundi except for myopic changes. Second, in incomplete CSNB, the optic disc is normal except for a tilted disc and/or temporal pallor in some cases. Both brothers had marked optic disc atrophy, which has not been reported in earlier cases of incomplete CSNB. Third, they showed a progressive decline of visual function resulting in poor, uncorrectable visual acuity, whereas the clinical course of incomplete CSNB is stationary with mildly reduced visual acuity. The stationary clinical course in incomplete CSNB was seen earlier in 2 patients from a single family. A 10-year-old boy and his 75-year-old maternal grandfather had similar clinical features with comparable ERG responses despite the difference in their ages.

The ERG findings were also atypical of incomplete CSNB. Although the negative-type bright-flash ERGs were similar to those in patients with incomplete CSNB, the a-wave amplitudes were smaller and the oscillatory poten-
tials were absent. The scotopic ERG responses were also highly reduced, indicating severe loss of rod function; in incomplete CSNB, some rod function is preserved, with mildly reduced but clearly detectable scotopic ERG responses. In addition, the photopic and 30-Hz flicker ERG responses appeared to be more severely depressed in our patients than in those who have typical incomplete CSNB. These results indicate that retinal function in our patients deteriorated far more severely than in patients with typical incomplete CSNB.

These clinical and ERG findings indicate that phenotypic variations including a progressive clinical course and severe deterioration of visual function can be induced by CACNA1F gene mutations. The possibility of phenotypic variations was also suggested from the findings in another patient with a CACNA1F gene mutation. That patient had symmetrical retinal atrophy and relative scotomas around the inferior vascular arcades in both eyes. These observations are similar to those with different gene mutations that cause other types of CSNB and are associated with retinal degeneration. For example, an arrestin gene mutation that causes Oguchi disease was reportedly associated with retinitis pigmentosa, and the 11-cis-retinol dehydrogenase gene mutations that cause fundus albipunctatus are sometimes associated with cone dystrophy or macular dystrophy.

It is still unclear whether the clinical phenotypes of these siblings depended only on CACNA1F gene defects. In fact, the same CACNA1F mutation has been detected in other patients with typical clinical and ERG findings of incomplete CSNB. One possibility may be that a mutation or polymorphism in another gene worked synergistically with the CACNA1F gene mutation to induce the additional pathologic changes in these 2 brothers. If so, that gene remains to be determined. Also, we cannot completely exclude environmental factors before or after birth. However, even in such cases, we suggest that the CACNA1F mutation was the important factor in the severe retinal dysfunction and optic atrophy. Although most patients with CACNA1F gene mutations exhibit in-

![Figure 5](http://archophth.jamanetwork.com/pdfaccess.ashx?url=/data/journals/ophth/9909/) Results of full-field electroretinograms (ERGs) recorded after 30 minutes of dark adaptation in a healthy subject, a patient with typical incomplete congenital stationary night blindness (CSNB), and cases 1 and 2. The rod ERG amplitudes are significantly reduced or nonrecordable in cases 1 and 2, whereas they are mildly reduced but clearly recordable with typical incomplete CSNB. The cone ERG amplitudes are more significantly reduced in cases 1 and 2 than with typical incomplete CSNB. In both cases, amplitude results of the bright-flash, rod-cone mixed ERG amplitudes are the negative type, as in typical incomplete CSNB; however, the oscillatory potentials are absent in our cases, whereas they are present in typical incomplete CSNB. Arrowheads indicate the stimulus onset.

![Figure 6](http://archophth.jamanetwork.com/pdfaccess.ashx?url=/data/journals/ophth/9909/) Focal macular cone electroretinograms recorded with 5°, 10°, and 15° stimuli from the macular area in the right eye of a healthy subject and in case 1. The amplitudes in case 1 are significantly reduced. a indicates a-wave; b, b-wave.
complete CSNB with a stationary clinical course and normal fundi, some cases of CACNA1F mutations are associated with retinal or optic disc atrophy and progressive visual loss. In patients with retinal and optic disc atrophy associated with negative-type ERGs, a CACNA1F mutation should be considered. The features of additional cases must be gathered to understand the clinical features and genotype-phenotype correlation in CACNA1F mutations.

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REFERENCES