

# Clinical Characteristics of Neuronal Intranuclear Inclusion Disease-Related Retinopathy With CGG Repeat Expansions in the *NOTCH2NLC* Gene

Natsuko Nakamura,<sup>1,2</sup> Kazushige Tsunoda,<sup>2</sup> Akihiko Mitsutake,<sup>3</sup> Shota Shibata,<sup>3</sup> Tatsuo Mano,<sup>3</sup> Yu Nagashima,<sup>3</sup> Hiroyuki Ishiura,<sup>3</sup> Atsushi Iwata,<sup>3</sup> Tatsushi Toda,<sup>3</sup> Shoji Tsuji,<sup>3-5</sup> and Hiromasa Sawamura<sup>1</sup>

<sup>1</sup>Department of Ophthalmology, The University of Tokyo, Tokyo, Japan

<sup>2</sup>Division of Vision Research, National Institute of Sensory Organs, National Hospital Organization Tokyo Medical Center, Tokyo, Japan

<sup>3</sup>Department of Neurology, The University of Tokyo, Tokyo, Japan

<sup>4</sup>Department of Molecular Neurology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

<sup>5</sup>Institute of Medical Genomics, International University of Health and Welfare, Chiba, Japan

Correspondence: Natsuko Nakamura, Department of Ophthalmology, The University of Tokyo, 7-3-1 Hongo Bunkyo-ku, Tokyo 113-8655, Japan; [nakamura-ntk@umin.ac.jp](mailto:nakamura-ntk@umin.ac.jp).

Received: January 29, 2020

Accepted: August 12, 2020

Published: September 15, 2020

Citation: Nakamura N, Tsunoda K, Mitsutake A, et al. Clinical characteristics of neuronal intranuclear inclusion disease-related retinopathy with CGG repeat expansions in the *NOTCH2NLC* gene. *Invest Ophthalmol Vis Sci.* 2020;61(11):27. <https://doi.org/10.1167/iovs.61.11.27>

**PURPOSE.** To report the ocular characteristics of neuronal intranuclear inclusion disease (NIID)-related retinopathy with expansion of the CGG repeats in the *NOTCH2NLC* gene.

**METHODS.** Seven patients from six families (aged 66–81 years) diagnosed with adult-onset NIID were studied. Ophthalmologic examinations, including the best-corrected visual acuity (BCVA), Goldmann perimetry, fundus photography, fundus autofluorescence (FAF) imaging, optical coherence tomography (OCT), and full-field electroretinography (ERGs), were performed. The expansion of the CGG repeats in the *NOTCH2NLC* gene was determined.

**RESULTS.** All patients had an expansion of the CGG repeats (length approximately from 330–520 bp) in the *NOTCH2NLC* gene. The most common symptoms of the five symptomatic cases were reduced BCVA and night blindness. The other two cases did not have any ocular symptoms. The decimal BCVA varied from 0.15 to 1.2. Goldmann perimetry was constricted in all four cases tested; physiological blind spot was enlarged in two of the cases. The FAF images showed an absence of autofluorescence (AF) around the optic disc in all cases and also showed mild hypo-AF or extinguished AF in the midperiphery. In all cases, the OCT images showed an absence of the ellipsoid zone of the photoreceptors in the peripapillary region, and hyperreflective dots were also present between the retinal ganglion cell layer and outer nuclear layer. The macular region was involved in the late stage of the retinopathy. The full-field ERGs showed rod-cone dysfunction.

**CONCLUSIONS.** Patients with adult-onset NIID with CGG repeats expansions in the *NOTCH2NLC* gene had similar ophthalmologic features, including rod-cone dysfunction with progressive retinal degeneration in the peripapillary and midperipheral regions. The primary site is most likely the photoreceptors. Because the ocular symptoms are often overlooked due to dementia and occasionally precede the onset of dementia, detailed ophthalmological examinations are important for the early diagnosis of NIID-related retinopathy.

**Keywords:** neuronal intranuclear inclusion disease, retinal dystrophy, CGG repeat, *NOTCH2NLC/NBPF19* gene, dementia

The expansion of trinucleotide repeats in the DNA is a major cause of neurologic and neuromuscular disorders,<sup>1-4</sup> and some of these cases have been known to cause retinal disorders, that is, macular degeneration in spinocerebellar ataxia types 1 and 7<sup>5-7</sup> and pigmentary changes in myotonic dystrophy type 1.<sup>8</sup> Neuronal intranuclear inclusion disease (NIID) is a slowly progressive neurodegenerative disorder characterized by eosinophilia and ubiquitin-positive intranuclear inclusions in various organs.<sup>9,10</sup> The onset of NIID varies from infancy to old age, and there are

three forms based on the age of onset: infantile, juvenile, and adult. Two-thirds of patients with NIID are said to be the infantile or juvenile forms.<sup>9</sup> Various clinical manifestations have been reported.<sup>9,10</sup> In adult-onset NIID, dementia is the main symptom in sporadic cases and elderly familial cases.<sup>10</sup> In relatively young adult familial cases, muscle weakness is the main symptom.<sup>10</sup> Other symptoms include peripheral neuropathy that develops at various ages.<sup>9,10</sup>

NIID is also known to have several ophthalmologic manifestations, such as miosis, night blindness, and reduced

electroretinographic (ERG) responses, and retinal degenerative changes with onset at various ages.<sup>11–15</sup> Adult-onset NIID is often accompanied by miosis, and part of infantile- or juvenile-onset NIID is reported to be associated with nystagmus and eye movement disorders.<sup>11</sup> Although there are few reports on the retinal findings including the ERG findings, ERG abnormalities have been reported in all age groups. However, detailed phenotypic and genetic characteristics of NIID retinopathy have not been investigated in detail.

Very recently, an expansion of the CGG repeats in the 5' UTR of the *NOTCH2NLC* (previously called *NBPF19*) gene has been determined to be the cause of adult-onset NIID.<sup>16–18</sup>

The purpose of this study was to determine the ophthalmologic characteristics of this newly identified retinal disorder, NIID-related retinopathy with expansions of CGG repeats in the *NOTCH2NLC* gene. To accomplish this, we have performed comprehensive ophthalmologic examinations on seven patients from six families who were neurologically diagnosed with NIID and found to have expanded CGG repeats in the *NOTCH2NLC* gene. We shall show that all of the patients had retinal degeneration in the peripapillary and midperipheral regions by multimodal imaging. The full-field ERGs showed rod-cone dysfunction, with electronegative configuration to bright flash scotopic stimuli (10 cd·s/m<sup>2</sup>) in two cases. The results of multimodal imaging and full-field ERGs indicated that the primary site of the lesions was the photoreceptors, but synaptic or inner retinal dysfunction may also be involved.

## PATIENTS AND METHODS

The procedures used adhered to the tenets of the Declaration of Helsinki and were approved by the Ethics Committee of The University of Tokyo, Tokyo, Japan. An informed consent was received from all patients for the examinations (reference: G1396).

### Participants

We studied the medical records of nine patients who were diagnosed with adult-onset NIID by the Department of Neurology of the University of Tokyo between June 2009 and November 2018. They were referred to the Department of Ophthalmology for the screening of visual involvement. The diagnostic criteria of adult-onset NIID were presence of dementia, characteristic magnetic resonance imaging findings (e.g., high-intensity lesions in the corticomedullary junction in the brain diffusion-weighted images), and/or eosinophilic- and ubiquitin-positive intranuclear inclusions by skin biopsy.<sup>17</sup> Two of the nine patients could not undergo fundus examinations due to severe dementia and were excluded from this study. In the end, seven patients underwent the comprehensive ophthalmologic examinations.

### Clinical Investigations

A detailed medical history including the visual symptoms and the onset of the symptoms was obtained from the patients and their family members. We defined the beginning of NIID as the onset of dementia because it was the most frequent sign of adult-onset NIID.<sup>10</sup> For patients whose ocular symptoms could not be precisely documented due to

dementia, we questioned the family members about when and what kinds of ocular symptoms appeared in the patients.

Comprehensive ophthalmologic examinations were performed, including measurements of the decimal best-corrected visual acuity (BCVA), ophthalmoscopy, Goldmann perimetry, fundus photography (Optos 200Tx; Optos, Dunfermline, UK), fundus autofluorescence (FAF; Optos 200Tx [Optos] and HRA 2 [Heidelberg Engineering, Heidelberg, Germany]), spectral-domain optical coherence tomography (OCT; Spectralis; Heidelberg Engineering), and full-field ERG (LE4000; Tomey Corporation, Aichi, Japan). The ERGs were recorded based on the International Society of Clinical Electrophysiology and Vision standard protocol to evaluate the retinal function under both scotopic and photopic conditions.<sup>19</sup>

### Genetic Analyses

All seven patients with NIID were evaluated by repeat-primed PCR analysis to confirm the expanded CGG repeats in the *NOTCH2NLC* gene. To determine the number of the expanded repeats, Southern blot hybridization analysis of the cDNAs was performed in all seven patients. Some of the clinical findings in all seven patients were reported earlier.<sup>17</sup>

## RESULTS

### Clinical Findings of Patients

The clinical characteristics and the results of ocular examinations are presented in Tables 1 and 2. There were four women and three men (Table 1). Four patients (patients 3, 4, 5, and 6) had family history compatible with an autosomal dominant mode of inheritance, and three patients (patients 1, 2, and 7) were sporadic. Patient 4 was the aunt of patient 5.

The median age at the time of examination was 75 years with a range of 66 to 81 years. The median age of the onset of visual symptoms was 66 years with a range of 63 to 75 years, and the median interval from the onset of visual symptoms to the last examination was 7 years with a range of 0 to 12 years.

All patients had ocular abnormalities in both eyes. Five patients had ocular symptoms: night blindness (3/7, 42.9%), reduced visual acuity (3/7, 42.9%), and photophobia (1/7, 14.3%). Patient 5 and patient 7 had ocular symptoms before developing dementia. Patient 1 and patient 2 did not have any ocular symptoms.

The pupillary diameters of all patients were <2.0 mm. The decimal BCVA varied from 0.15 to 1.2.

Three patients had mild senile cataract in both eyes, and four patients had undergone intraocular lens implantation in both eyes before our examinations (Table 1). Patient 7 had band keratopathy in both eyes of unknown origin. Patient 6 had diabetic mellitus but did not have diabetic retinopathy. The other patients did not have any systemic disorders that could have affected the visual function other than NIID.

Visual field testing was performed on four patients by Goldmann perimetry. A concentric constriction of the I-4 isopter was detected in all four cases, and the other isopters also showed significant constrictions. An enlargement of the physiological blind spot was detected in patients 4 and 7 (Fig. 1).

Ophthalmoscopic examinations showed that all 14 eyes had chorioretinal atrophy (CRA) in the peripapillary regions

TABLE 1. Summary of Clinical Characteristics in Seven Patients With NIID

Patient ID	Family (Patient) ID in the Previous Report <sup>17</sup>	Age at Examination (y)	Sex	Family History	CGG Repeat Expansion in <i>NOTCH2NLC</i>	CGG Repeat Length (bp)	Age at Visual Symptom (y)	Visual Symptoms	Miosis	Decimal BCVA at Last Visit		
										OD	OS	Other Ocular Disease
1	F10983*	75	M	—	+	330	—	None	OU	0.6	0.9	Mild cataract, OU
2	F11596*	66	F	—	+	400	—	None	OU	1.2	1.2	Mild cataract, OU
3	F10709*	73	F	+	+	520	75	Reduced visual acuity	OU	0.5	0.7	IOL, OU
4	F6321 (II-4)	79	F	+	+	350	66	Night blindness	OU	0.2	0.2	IOL, OU
5	F6321 (II-5)	75	M	+	+	340	66	Night blindness	OU	1.0	0.8	IOL, OU
6	F11393 (II-4)	73	F	+	+	370	75	Reduced visual acuity	OU	0.3	0.15	Mild cataract, OU
7	F9468*	81	M	—	+	~500 (smear)	63	Reduced visual acuity Night blindness Photophobia	OU	0.5	0.4	Band keratopathy, OU IOL, OU

IOL, intraocular lens.

\* Patient ID was not provided in the previous report.<sup>17</sup>

TABLE 2. Summary of Fundusoscopic Appearance, FAF, OCT, and Full-Field ERG in Seven Patients With NIID

Patient ID	Fundusoscopic Appearance				FAF		OCT		
	Peripapillary CRA	RPE Abnormalities in Periphery	White Dots	Extinguished AF in the Posterior Pole	Peripheral Hypo-AF	Hyper- and Hypo-AF Spots in the Posterior Pole	Peripapillary Region	Fovea EZ	Full-Field ERG
1	+	+	+	+	+	—	Extinguished EZ Preserved RPE	Normal	Rod-cone dysfunction
2	+	+	+	+	—	+	Extinguished EZ Preserved RPE	Normal	Rod-cone dysfunction
3	+	+	+	+	—	+	Extinguished EZ RPE atrophy NE	Normal	NE
4	+	+	—	+	NE	+	Extinguished EZ RPE atrophy	NE	Rod-cone dysfunction
5	+	+	+	+	+	+	Extinguished EZ RPE atrophy	Normal	Rod-cone dysfunction
6	+	+	+	+	+	+	Extinguished EZ RPE atrophy	Normal	Rod-cone dysfunction
7	+	+	+	+	+	+	Extinguished EZ RPE atrophy Choroidal atrophy	Disrupted	Extinguished

NE, not examined.

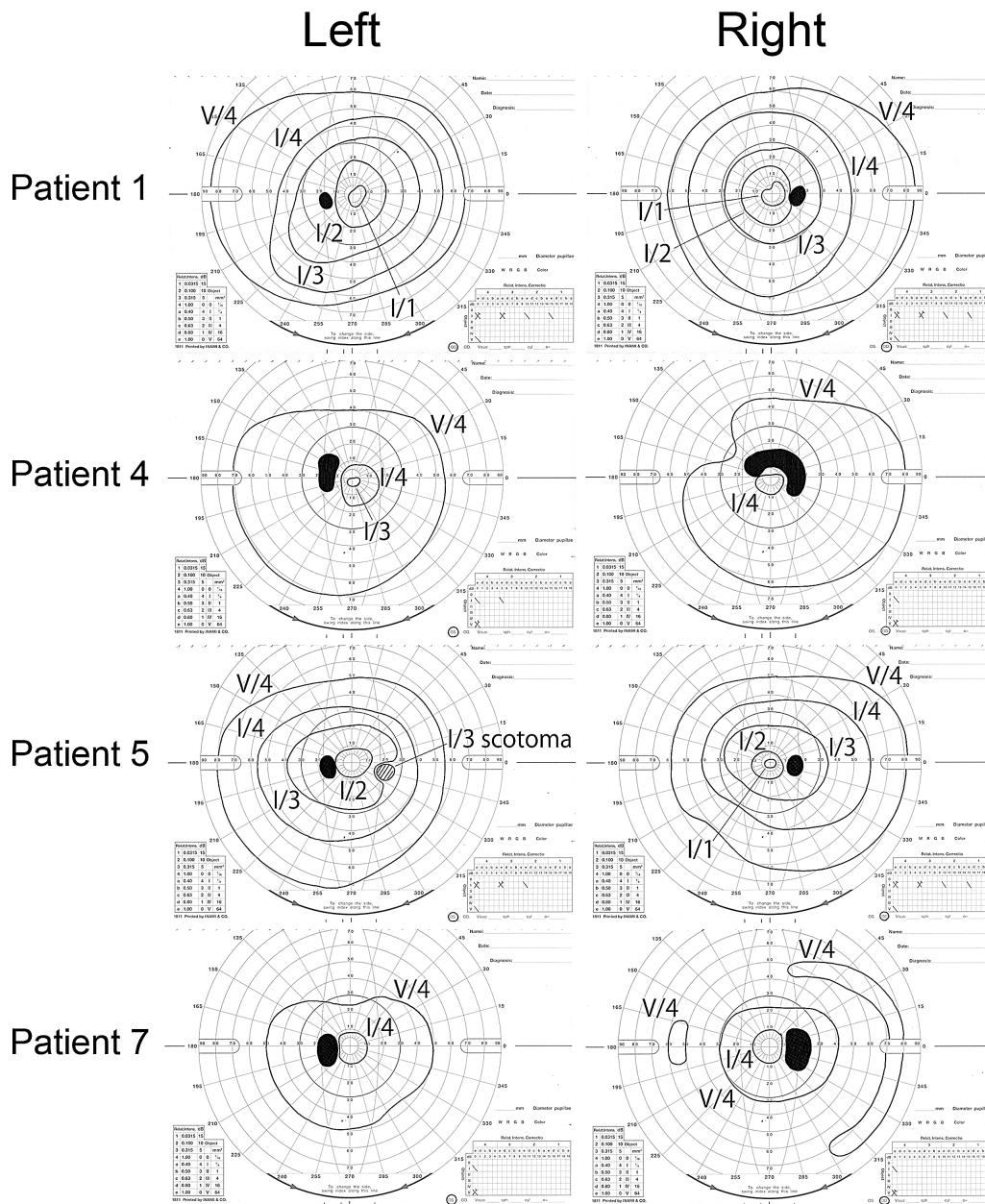


FIGURE 1. Results of Goldmann perimetry of four cases of NIID-related retinopathy with CGG repeat expansions in the *NOTCH2NL1C* gene. Concentric contraction of the I-4 isopter is present in all four cases. The physiological blind spot is also enlarged in patients 4 and 7.

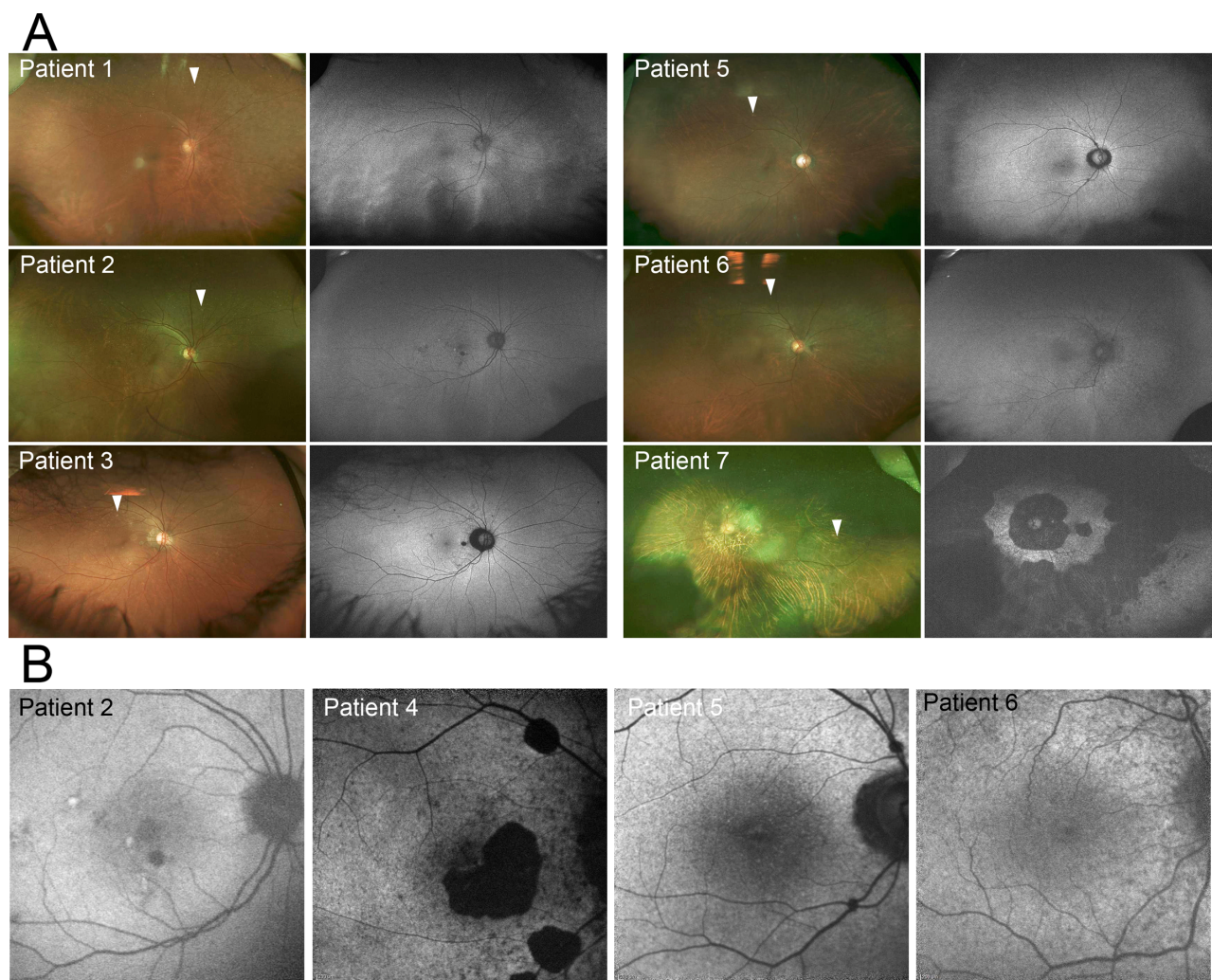
(Fig. 2, Table 2). The region of peripapillary CRA was less than 1.0-disk diameter in all patients except patient 7. The CRA extended to the macula in patient 7. In the peripheral retina, all patients showed abnormalities in the color of the retinal pigment epithelium (RPE) and diffuse CRA in patient 7. Fine white dots were detected in the posterior pole or peripheral regions in all cases except patient 4.

FAF imaging did not detect any autofluorescence (AF) around the optic disk in all cases (Fig. 2, Table 2). The regions of extinguished AF corresponded to the regions of the CRA in the ophthalmoscopic images. In the mid-periphery, mild hypo-AF was observed along the equator in patients 1, 5, and 6 and absent AF in patient 7. In patient 7, the regions of extinguished AF corresponded with the scalloped regions due to the CRA in the ophthalmoscopic

images. There were hyper- and hypo-AF spots in the posterior polar region in all cases except in patient 1.

The spectral-domain OCT images showed abnormalities in the outer retina in all patients (Fig. 3, Table 2). In the peripapillary region, the ellipsoid zone (EZ) of the photoreceptors was absent in all cases. The extent of the absence of the EZ varied among the cases (region between two yellow arrows in Fig. 3), and the EZ in patient 7 was not detected in the entire posterior pole.

The foveal microstructures were relatively well preserved in all cases. Both the EZ and interdigitation zone (IZ) of the photoreceptors were well preserved at the fovea in patients 1, 2, and 3, but only a disrupted EZ was observed at the fovea in patient 7. The RPE and choroid were especially thin in the entire posterior pole in patient 7.



**FIGURE 2.** Fundus photographs and FAF images of patients with NIID. (A) Ultra-widefield photographs and FAF images. CRA in the peripapillary regions was observed in all cases. In patient 7, the CRA extended to close to the macula. All patients showed abnormalities of the color of the RPE in the peripheral regions. In patient 7, diffuse CRA in the peripheral region was observed. Fine white dots were detected in all the cases except in patient 4 either in the posterior pole or peripheral regions (*white arrowhead*). Widefield FAF images show absence of AF around the optic disk in all cases. In the midperiphery, mild-hypo AF was observed along the equator in patients 1, 5, and 6 and diffuse absence of AF in patient 7. (B) Posterior FAF images showed hyper- and hypo-AF spots in the posterior polar region.

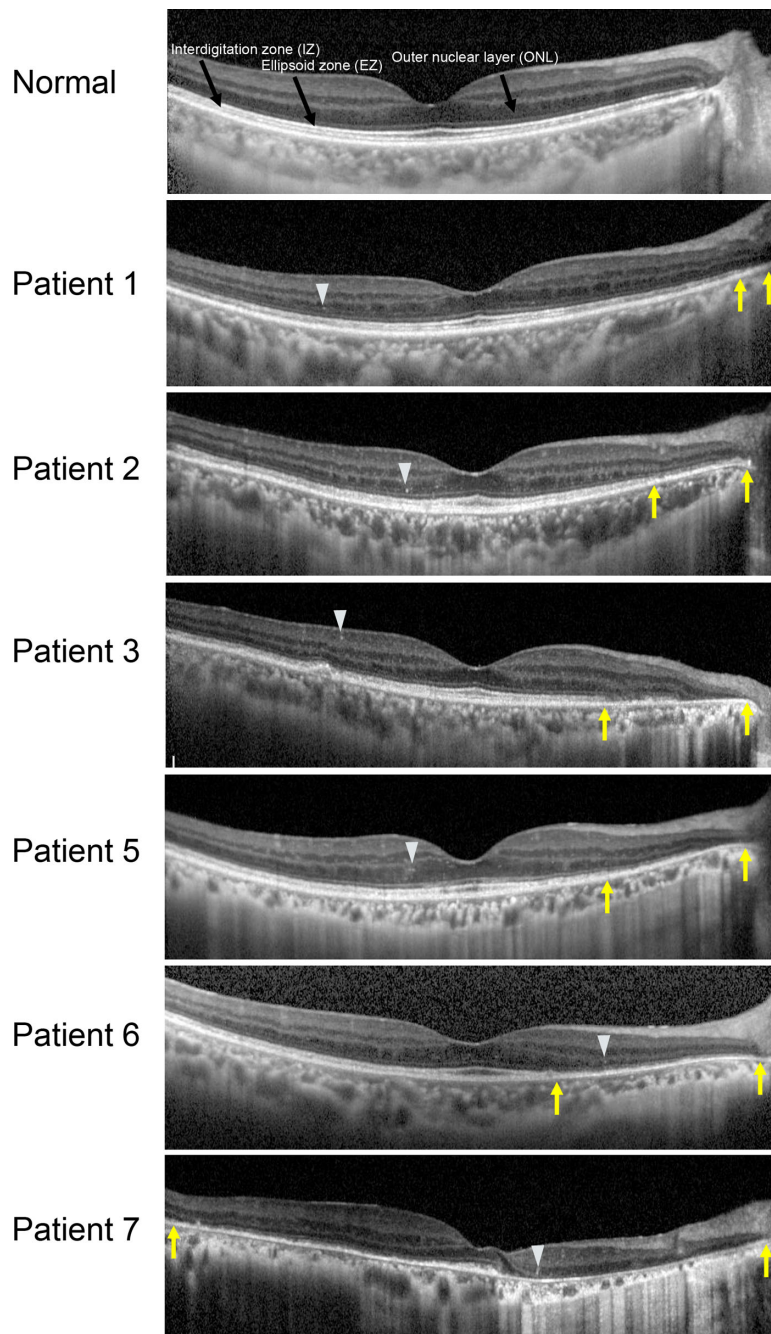
The inner retinal structures—namely, the nerve fiber layer (NFL) and retinal ganglion cell layer (GCL)—were preserved, but high-reflectivity fine spots were observed between the GCL and outer nuclear layer (ONL) in all cases (white arrowheads in Fig. 3).

The scotopic full-field ERGs were severely reduced or extinguished in all cases (Fig. 4). The amplitudes of the b-wave of the dark-adapted 0.01 ERG and the a- and b-waves of the dark-adapted 10.0 ERG were reduced in patients 1 through 6, except for patient 3, whose ERG had not been performed. The b-/a-wave ratio in patient 1 was slightly lower but not a negative waveform (b/a ratio: 1.17) than the same age group. Two patients (patients 4 and 5) had a slightly electronegative waveform (b/a ratio: 0.79 and 0.94, respectively). The results of the analyses of the b/a ratio are shown in Supplementary Table S1. The amplitudes of the a- and b-waves of the light-adapted 3.0 ERG were moderately reduced in patients 1, 2, and 5 and severely reduced in patients 4 and 6. The amplitudes of the flicker ERG were reduced in patients 1 through 5. In patient 7,

all dark-adapted and light-adapted responses were extinguished. Thus, the ERGs indicated a rod-cone dysfunction in all the cases except for patient 7 who had extinguished scotopic and photopic ERGs.

#### CGG Repeat Expansion in the *NOTCH2NLC* Gene

All seven patients were confirmed to have CGG repeat expansions in the *NOTCH2NLC* gene by repeat-primed PCR analysis. The Southern blot hybridization analysis of seven patients revealed that the length of expanded repeats ranged from approximately 330 to 520 bp, corresponding to 110 to 173 repeat units (Table 1). Patient 7 showed a smearing pattern in the Southern blot analysis with the highest signal intensity located at 500 bp (corresponding to 167 repeat units). This smearing indicated marked somatic instability of the expanded repeats, which made it difficult to determine the exact repeat length in this patient.



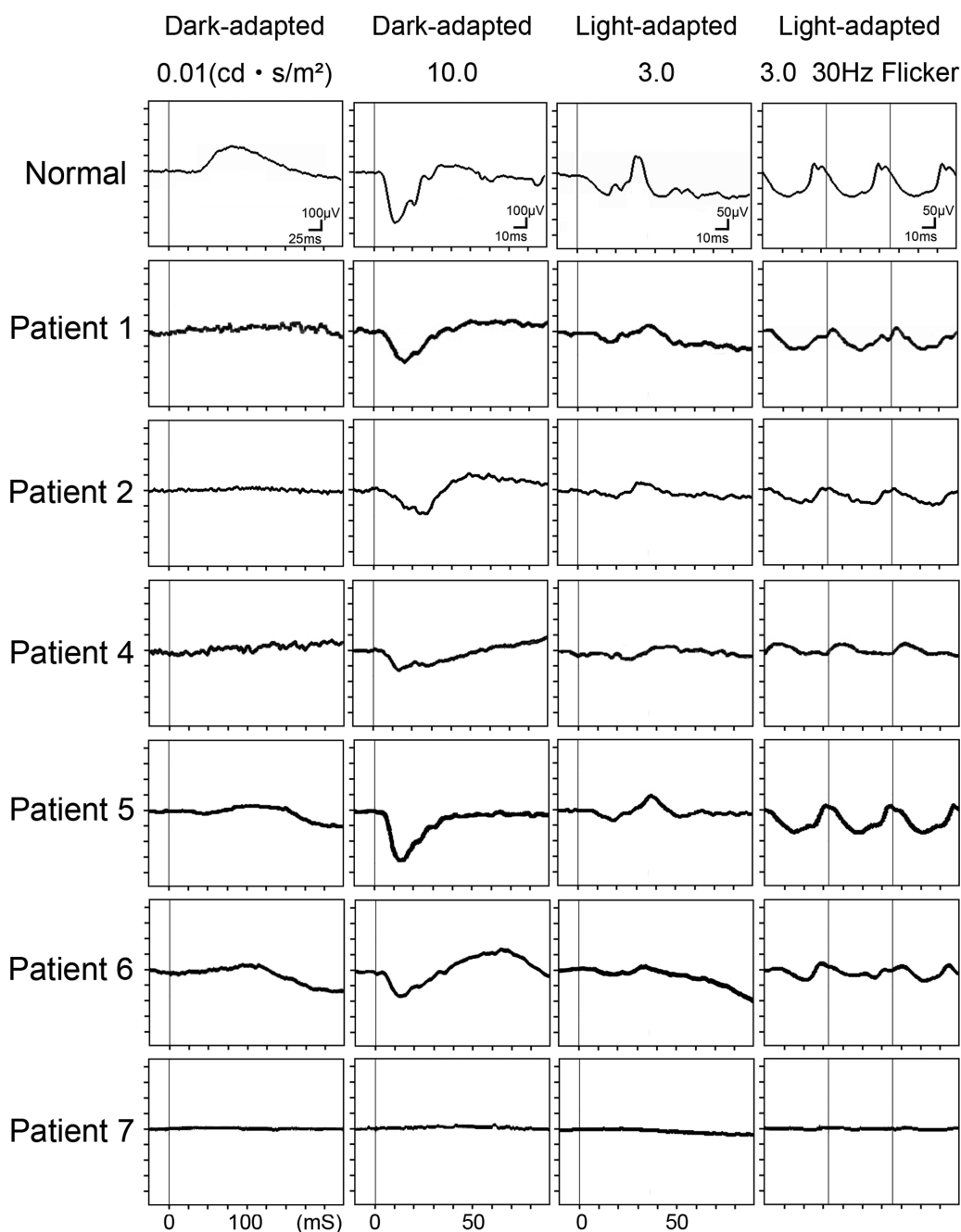
**FIGURE 3.** OCT images of six patients. The EZ is absent in the peripapillary regions in all cases. The regions of absent EZ are shown by two *yellow arrows*, and their size varies among the cases. In patient 7, the EZ is absent in the entire posterior pole region. The foveal EZ was relatively preserved in all the cases. A thinning of the ONL is present in the peripapillary region in all cases except patient 1. A thinning of the RPE and choroid is observed in the peripapillary region in patients 3, 4, and 6 and in the entire posterior pole region in patient 7. The inner retinal structures (i.e., NFL and retinal GCL) are normally preserved, and high-reflectivity fine spots can be seen between the GCL and ONL in all cases (*white arrowheads*).

## DISCUSSION

This is the first report on the ophthalmologic characteristics of adult-onset NIID with expansions of the CGG repeats in the *NOTCH2NLC* gene.<sup>9,10,20</sup> All of the cases were neurologically diagnosed with NIID, and all had ocular abnormalities. The symptoms, retinal findings of the multimodal images, and ERGs were similar. We have named this disorder NIID-related retinopathy.

## Ocular Symptoms

All but two patients complained of night blindness and/or reduced visual acuity in their seventh or eighth decade of life. There were only two patients, patients 5 and 7, who noticed their visual disturbance before they were diagnosed with NIID. Because the initial sign of adult-onset NIID is dementia,<sup>10</sup> the exact age of the onset of the ocular symptoms was difficult to determine accurately. The



**FIGURE 4.** Full-field ERGs recorded under scotopic and photopic conditions. The scotopic ERGs are severely reduced or extinguished in all cases. The amplitudes of the a-wave and b-wave of the dark-adapted 10.0 and light-adapted 3.0 ERGs are reduced with prolonged implicit times. All the responses were nonrecordable in patient 7.

relatively well-preserved foveal microstructures in all cases (Fig. 3) is probably the reason why the patients did not notice any symptoms until their photoreceptor function in the fovea was severely impaired. However, patient 6 had severely reduced visual acuity compared to the relatively well-maintained foveal structure in the OCT images. Patients with adult-onset NIID have dementia, so there is also a gap between the results of the subjective and objective examinations. Yamada et al.<sup>15</sup> reported on a patient with adult-onset NIID who had no ocular symptoms despite a rod-cone dysfunction of the ERGs. All of our patients had moderate to severe rod-cone dysfunction, and only two did not complain

of any ocular symptoms. Thus, it is important in individuals with adult-onset NIID to investigate whether they have night blindness or constricted visual field and to perform fundus examinations.

### Retinal Abnormalities

All patients had similar abnormalities in the multimodal retinal images, namely, CRA in the peripapillary region although the CRA of patient 6 was slight. The RPE abnormalities in the midperiphery were slight to severe in all seven patients and fine white dots in the posterior pole and midperipheral



regions in six of seven patients. Considering the distribution patterns of the affected regions in the individual cases, it is possible that the CRA appeared in the peripapillary region in the early stage, which then expanded centrifugally toward the macula (Figs. 2 and 3). Moreover, the concentric contraction of the visual fields (Fig. 1), mild hypo-AF in the midperipheral regions (Fig. 2), and rod-cone dysfunction of the ERGs (Fig. 4) indicated that an early retinal dysfunction and degeneration in NIID-related retinopathy may appear first in the midperiphery. Taken together, both peripapillary and midperipheral retinal regions could be the initial sites of the defects in NIID-related retinopathy, although this needs to be confirmed by longitudinal studies. Patient 7 was examined 18 years after the onset of visual symptoms, and this case had the longest follow-up period. The case with an expanded CRA in the peripapillary, midperipheral, and macular regions is thought to be a representative case of advanced-stage NIID-related retinopathy (Figs. 2 and 3).

The OCT images showed that the outer retinal structures—namely, the EZ, IZ, and RPE—were disrupted in all cases (Fig. 3). Six OCT images are shown consecutively according to the size of lesions (yellow arrows in Fig. 3). These images indicated that the early photoreceptor abnormalities appeared in the EZ and IZ, followed by the RPE and choroid. This pattern indicates that the photoreceptor outer segments are possibly the primary site of the defects in NIID-related retinopathy.

The FAF findings in patient 7 and the reduced full-field ERGs in all patients suggested a widespread degeneration was present in the midperiphery even at an early stage. Yamada et al.<sup>13</sup> reported that the ERGs recorded from a patient with NIID were the electronegative-type ERGs, which suggested inner retinal dysfunction. Electronegative-type ERGs were observed in two of our cases (Fig. 4). It was not possible to identify abnormalities in the peripheral region by OCT, but we cannot exclude the possibility that the inner retina was the primary site in this disorder.

Another feature of the OCT images was the fine, highly reflectivity spots that appeared between the GCL and ONL layers (white arrowhead in Fig. 3). These fine white dots were also observed ophthalmoscopically (Fig. 2). Fine hyper-reflective spots in the OCT images may be seen in normal eyes, but those observed in our cases were observed more clearly. These findings may be specific to NIID, but a correspondence of these spots in the OCT images and ophthalmoscopic images could not be confirmed. Further investigations are needed to determine the underlying pathologic mechanism for these white spots in the retina.

### Histologic Studies

The histopathologic studies of the retina of a patient with juvenile-onset NIID were reported by Haltia et al.<sup>11</sup> They showed a loss of the NFL and GCL, and many of the remaining retinal ganglion cells had intranuclear inclusion bodies. On the other hand, there were no intranuclear inclusion bodies in the bipolar cells, photoreceptors, and RPE cells. These findings are not consistent with our suggestion that the photoreceptors were the primary site of the pathology of the adult-onset NIID. However, their patient did not have adult-onset NIID retinopathy, and the genetic cause of their case was not determined. Considering that intranuclear inclusion bodies in fragile X tremor/ataxia syndrome are formed by repeat-associated non-AUG-initiated translation (RAN translation) of CGG repeats in *FMRI*,<sup>21</sup> the intranu-

clear inclusion bodies in NIID are presumably formed by a similar mechanism.

There can be a negative correlation between the frequency of neuronal inclusions and neuronal loss, as reported by Takahashi-Fujigasaki.<sup>9</sup> Thus, the relationship between the presence of neuronal inclusion bodies and inner/outer retinal function is not completely understood, and further investigations, including retinal histopathologic studies of adult-onset NIID eyes, are needed to resolve these conflicting observations.

### Role of Noncoding CGG Repeat Expansions in *NOTCH2NLC* Gene and Possibility of Similar Repeat Expansions in Other Retinal Diseases

The function of the *NOTCH2NLC* gene has not been definitively determined, and the mechanism of how the CGG repeat expansion mutations affect neuronal and retinal function remains unknown. A possible hypothesis is that a gain-of-toxic function of the CGG repeats plays an important role in the pathogenesis of NIID, rather than haploinsufficiency of the *NOTCH2NLC* gene.<sup>17,22</sup> In some repeat expansion diseases such as CAG repeat disorders, the lengths of the expanded repeats are significantly correlated with the age at onset and severity. Only a few studies have been performed for NIID, but one study has reported that there was no significant correlation between the lengths of the CGG repeats and the age at onset or severity of the disease.<sup>16</sup>

Hayashi et al.<sup>23</sup> reported one patient with early onset NIID who had CGG repeat expansions in the *NOTCH2NLC* gene. The patient was a 49-year-old woman with an initial symptom of childhood night blindness, and the main symptom was muscle weakness. The number of her repeat expansion in the gene was 434 and this was not so different from our patients, and the part of phenotypes were similar in that the outer retinal layer was atrophied and the inner retinal layer was preserved. However, the other signs and symptoms, the age of onset, the main symptoms, and the rod response with prolonged latency despite maintained amplitude were different. It is not known why such phenotypic variations are present.

In patients 5 and 7, the visual reduction began before the onset of dementia. Thus, patients with chorioretinal degeneration should be evaluated for expanded CGG repeats in *NOTCH2NLC* even though they do not have dementia. If the gain-of-toxic function of expanded CGG repeats plays an essential role in the pathogenesis of NIID-related retinopathy, repeat expansion mutations with similar motifs in other genes could possibly cause similar retinal phenotypes. Thus, the results of this study also suggest the importance of searching for repeat expansion mutations in hereditary retinal degenerations of unknown cause.

### Limitations

There are limitations in this study. We could not follow each patient for a long duration due to the poor prognosis in cases with adult-onset NIID. In addition, the ocular symptoms were not accurately determined due to the dementia. To accurately determine the phenotypic features and etiology of NIID-related retinopathy, systematic longitudinal studies incorporating electrophysiologic and multimodal imaging assessments in a larger cohort will be needed.

In conclusion, we report for the first time, the detailed clinical characteristics of adult-onset NIID-related retinopathy with CGG repeat expansions in the *NOTCH2NLC* gene. Although the effect of the gene mutation on retinal function was not determined, these findings will facilitate understanding the etiology and clinical diagnosis of this rare form of progressive neurodegenerative disease.

### Acknowledgments

The authors thank the patients and their families for participation in this study. They also thank Professor Emeritus Duco Hamasaki of the Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami, FL, for critical discussions and editing our manuscript.

Supported by grants from Japan Society for the Promotion of Science (JSPS) KAKENHI (JP20K09767) and Takeda Science Foundation (HS), the Japan Agency for Medical Research and Development (JP18kk0205001 and JP19ek0109279) (ST), and JSPS KAKENHI (JP15k20941, JP17H05085, and JP20H03588) (HI).

Disclosure: **N. Nakamura**, Santen (F) and Novartis (F); **K. Tsunoda**, Astellas (F), Novartis (F), Santen (F), and Senju (F); **A. Mitsutake**, None; **S. Shibata**, None; **T. Mano**, None; **Y. Nagashima**, None; **H. Ishiura**, None; **A. Iwata**, None; **T. Toda**, None; **S. Tsuji**, None; **H. Sawamura**, Santen (F), Alcon Japan (F), KOWA (F), and Novartis (F)

### References

1. Thdcr G. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell*. 1993;72:971–983.
2. Verkerk AJ, Pieretti M, Sutcliffe JS, et al. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell*. 1991;65:905–914.
3. Orr HT, Chung MY, Banfi S, et al. Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1. *Nat Genet*. 1993;4:221–226.
4. Brook JD, McCurrach ME, Harley HG, et al. Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3' end of a transcript encoding a protein kinase family member. *Cell*. 1992;68:799–808.
5. Abe T, Abe K, Aoki M, Itoyama Y, Tamai M. Ocular changes in patients with spinocerebellar degeneration and repeated trinucleotide expansion of spinocerebellar ataxia type 1 gene. *Arch Ophthalmol*. 1997;115:231–236.
6. Abe T, Tsuda T, Yoshida M, et al. Macular degeneration associated with aberrant expansion of trinucleotide repeat of the SCA7 gene in 2 Japanese families. *Arch Ophthalmol*. 2000;118:1415–1421.
7. Hirose A, Katagiri S, Hayashi T, et al. Progress of macular atrophy during 30 months' follow-up in a patient with spinocerebellar ataxia type1 (SCA1) [published online ahead of print July 9, 2020]. *Doc Ophthalmol*, doi:10.1007/s10633-020-09782-z.
8. Kimizuka Y, Kiyosawa M, Tamai M, Takase S. Retinal changes in myotonic dystrophy: clinical and follow-up evaluation. *Retina*. 1993;13:129–135.
9. Takahashi-Fujigasaki J. Neuronal intranuclear hyaline inclusion disease. *Neuropathology*. 2003;23:351–359.
10. Sone J, Mori K, Inagaki T, et al. Clinicopathological features of adult-onset neuronal intranuclear inclusion disease. *Brain*. 2016;139:3170–3186.
11. Haltia M, Tarkkanen A, Somer H, Palo J, Karli H. Neuronal intranuclear inclusion disease: clinical ophthalmological features and ophthalmic pathology. *Acta Ophthalmol (Copenh)*. 1986;64:637–643.
12. Arrindell EL, Trobe JD, Sieving PA, Barnett JL. Pupillary and electroretinographic abnormalities in a family with neuronal intranuclear hyaline inclusion disease. *Arch Ophthalmol*. 1991;109:373–378.
13. Yamada W, Takekoshi A, Ishida K, et al. Case of adult-onset neuronal intranuclear hyaline inclusion disease with negative electroretinogram. *Doc Ophthalmol*. 2017;134:221–226.
14. Omoto S, Hayashi T, Matsuno H, et al. Neuronal intranuclear hyaline inclusion disease presenting with childhood-onset night blindness associated with progressive retinal dystrophy. *J Neurol Sci*. 2018;388:84–86.
15. Yamaguchi N, Mano T, Ohtomo R, et al. An autopsy case of familial neuronal intranuclear inclusion disease with dementia and neuropathy. *Intern Med*. 2018;57:3459–3462.
16. Tian Y, Wang JL, Huang W, et al. Expansion of human-specific GGC repeat in neuronal intranuclear inclusion disease-related disorders. *Am J Hum Genet*. 2019;105:166–176.
17. Ishiura H, Shibata S, Yoshimura J, et al. Noncoding CGG repeat expansions in neuronal intranuclear inclusion disease, oculopharyngodistal myopathy and an overlapping disease. *Nat Genet*. 2019;51:1222–1232.
18. Sone J, Mitsuhashi S, Fujita A, et al. Long-read sequencing identifies GGC repeat expansions in NOTCH2NLC associated with neuronal intranuclear inclusion disease. *Nat Genet*. 2019;51:1215–1221.
19. McCulloch DL, Marmor MF, Brigell MG, et al. Erratum to: ISCEV Standard for full-field clinical electroretinography (2015 update). *Doc Ophthalmol*. 2015;131:81–83.
20. Takahashi-Fujigasaki J, Nakano Y, Uchino A, et al. Adult-onset neuronal intranuclear hyaline inclusion disease is not rare in older adults. *Geriatr Gerontol Int*. 2016;16:51–56.
21. Todd PK, Oh SY, Krans A, et al. CGG repeat-associated translation mediates neurodegeneration in fragile X tremor ataxia syndrome. *Neuron*. 2013;78:440–455.
22. Fiddes IT, Lodewijk GA, Mooring M, et al. Human-specific NOTCH2NLC genes affect notch signaling and cortical neurogenesis. *Cell*. 2018;173:1356–1369.e1322.
23. Hayashi T, Katagiri S, Mizobuchi K, et al. Heterozygous GGC repeat expansion of NOTCH2NLC in a patient with neuronal intranuclear inclusion disease and progressive retinal dystrophy. *Ophthalmic Genet*. 2020;41:93–95.