Reduction of Oscillatory Potentials and Photopic Negative Response in Patients with Autosomal Dominant Optic Atrophy with OPA1 Mutations

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PURPOSE. To study the electroretinographic (ERG) findings in patients with autosomal dominant optic atrophy (ADOA) with OPA1 mutations.

METHODS. Eight ADOA patients (age range: 24–55 years; mean, 41 years) with OPA1 mutations were studied. In addition to routine ophthalmological tests, full-field ERGs including the rod response, mixed rod-cone response, oscillatory potentials (OPs), single-flash cone response, and photopic negative response (PhNR) were recorded and compared with those from 25 age-matched controls. The correlation between the ERG data and averaged retinal nerve fiber layer (RNFL) thickness around the optic disk measured by optical coherent tomography, mean deviation of the static perimetry (Humphrey 30-2 program), or corrected visual acuity was also examined.

RESULTS. Amplitudes of the PhNR and OPs, both of which are believed to originate from inner retinal layers, were significantly smaller in ADOA patients than in control subjects (P < 0.01). Amplitudes of other ERG components were not statistically different in the two groups. OP amplitude was inversely correlated with the patient’s age. The RNFL was thinner and the retinal sensitivities obtained by static perimetry were lower in ADOA patients, but these values were not correlated with the amplitude of PhNR or OPs.

CONCLUSIONS. These results suggested that there are functional impairments not only in the ganglion cell layer but also in the inner nuclear and plexiform layers, including the amacrine cells of ADOA patients with OPA1 mutations. (Invest Ophthalmol Vis Sci. 2007;48:820–824) DOI:10.1167/iovs.06-0845

Autosomal dominant optic atrophy (ADOA) is the most common form of hereditary optic neuropathy. This disease is characterized by symmetrical bilateral optic atrophy associated with a decrease of visual acuity and color vision defect for blue hues. Visual impairments usually progress slowly, and phenotypic severity varies considerably among patients even within the same family. Histopathologic studies of donor eyes of patients with ADOA suggest that the fundamental pathologic condition is a degeneration of the retinal ganglion cells leading to optic atrophy.

ADOA is genetically heterogeneous, and mutations of the OPA1 gene are one of the causative genetic alterations. The OPA1 protein is a mitochondrial dynamin-related guanosine triphosphatase (GTPase) located in the mitochondrial inner membrane space mainly anchored to the cristae of the inner membrane. This protein is considered to be involved in mitochondrial fusion and in maintenance of the mitochondrial genome and network.

It was shown that the OPA1 gene is ubiquitously expressed in several tissues but is most abundant in the retina and brain. Recent immunohistochemical studies in rat and mice retinas showed that the OPA1 protein was expressed predominantly in ganglion cell layer but was also expressed in the inner plexiform layer, the inner nuclear layer including the amacrine cells, and the outer plexiform layer. It is generally believed that full-field ERG findings in patients with ADOA are normal. However, Holder et al. reported that some ADOA patients had a reduction of the P50 component of the pattern ERGs thought to originate distal to the retinal ganglion cells. Because of the results of these immunohistochemical and physiological studies, we thought that a more comprehensive functional examination with the use of electroretinography should be conducted on ADOA patients with OPA1 mutations.

We show here that the amplitudes of the photopic negative response (PhNR) and the oscillatory potential (OP), each of which is thought to originate from the inner retinal layer, were significantly reduced in the ADOA patients. Interestingly, the reduction of OPs was inversely correlated with patients’ ages. These results indicated that the functions not only of the ganglion cell layer but also of the inner nuclear and inner plexiform layers are altered in the human retina with OPA1 mutations.

Patients and Methods

Patients

Among our patients with OPA1 gene mutations, eight Japanese patients (five men and three women) from six families underwent electroretinographic examination and were recruited for this study. Detailed information on the OPA1 gene mutations and clinical characteristics in the eight patients have been reported. All the patients had typical characteristics of ADOA; one patient with an apparently atypical OPA1 gene mutation associated with a negative ERG finding was excluded from this study. The protocol of the study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Nagoya University. Informed consent was obtained from all patients after full explanation of this study.

Clinical Examination

Ophthalmic examination included best-corrected visual acuity, slit lamp biomicroscopy, indirect ophthalmoscopy, fundus photography,
visual field testing by kinetic and static perimetry, color vision testing with Farnsworth panel D-15 plates, retinal nerve fiber layer (RNFL) thickness analysis, and full-field electroretinography. Static perimetry was performed using the standard 30-2 program (target; Humphrey Field Analyzer; Carl Zeiss Meditec, Dublin, CA), and the mean visual field sensitivity (dB) within 30° borders of the visual field was determined. RNFL thickness was measured by optical coherence tomography (OCT-3000; Carl Zeiss Meditec) by calculating the mean RNFL thickness from 512 points around the optic disk.

Electroretinograms

Pupils were fully dilated with a combination of 0.5% tropicamide and 0.5% phentolamine hydrochloride. Corneas were anesthetized by topical 0.4% oxybuprocaine hydrochloride before contact lens electrodes were inserted. Full-field electroretinograms (ERGs) were recorded with a Burian-Allen bipolar contact lens electrode (Hansen Ophthalmic Development Laboratories, Iowa City, IA) and Ganzfeld ERG recording system (model GS2000; LACE, Pisa, Italy). A time constant of 0.1 second and a 500-Hz high-cut filter were used.

After 30 minutes of dark adaptation, the rod response was recorded with a dim blue light at an intensity of $5.2 \times 10^{-3}$ cd·s/m². A mixed rod-cone maximal ERG was elicited by a white flash at an intensity of $44.2$ cd·s/m². After 10 minutes of light adaptation, a single-flash cone ERG was elicited by a white stimulus of $1.9$ cd·s/m² on a white background of $18$ cd·s/m².

Methods used to measure the amplitudes of the OPs and photopic negative response (PhNR) are shown in the insets of Figures 1 and 2, respectively. OP amplitudes were calculated by adding the first four positive wavelets on the ascending limb of the b-wave (Fig. 1, inset). The amplitude of the PhNR was measured from the baseline to the first negative trough after the b-wave of the single-flash cone ERG (Fig. 2, inset).

RESULTS

Clinical Findings

Clinical characteristics of the patients with OPA1 mutations are summarized in Table 1. Visual acuities of the eight patients ranged from 0.7 to 0.01. Changes in the optic disks were symmetrical in all patients. Three patients had temporal pallor only, and in one it was subtle. The other four patients had diffuse atrophy of the optic disk. Visual field tests by Goldmann kinetic perimetry showed central scotoma in three patients (patients 1–3), concentric constriction in one patient (patient 4), and diffuse constriction in the other four patients (patients 5–8).
TABLE 2. Clinical Characteristics and OPA1 Mutations in Patients with ADOA

<table>
<thead>
<tr>
<th>Patient/Age/Sex</th>
<th>Family</th>
<th>Visual Acuity (OD/OS)</th>
<th>Test Eye</th>
<th>Disk Appearance</th>
<th>Humphrey Visual Field MD (dB)</th>
<th>RNFL Thickness (μm)</th>
<th>OPA1 Mutation</th>
<th>PhNR (μV)</th>
<th>OPs (μV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/24/M</td>
<td>92</td>
<td>0.04/0.05</td>
<td>OD</td>
<td>DA</td>
<td>−20.60</td>
<td>29.9*</td>
<td>p.S545R</td>
<td>17.3*</td>
<td>144.9</td>
</tr>
<tr>
<td>2/28/F</td>
<td>667</td>
<td>0.06/0.1</td>
<td>OD</td>
<td>TP</td>
<td>−8.30</td>
<td>71.6</td>
<td>p.R38X</td>
<td>19.3*</td>
<td>13.8*</td>
</tr>
<tr>
<td>3/27/F</td>
<td>42</td>
<td>0.4/0.4</td>
<td>OD</td>
<td>SP</td>
<td>−3.15</td>
<td>58.7*</td>
<td>c.2708_2711delTTAG</td>
<td>20.0*</td>
<td>96.6</td>
</tr>
<tr>
<td>4/46/M</td>
<td>169</td>
<td>0.3/0.3</td>
<td>OD</td>
<td>DA</td>
<td>−5.15</td>
<td>64.3*</td>
<td>c.2538insT</td>
<td>20.7*</td>
<td>62.1*</td>
</tr>
<tr>
<td>5/50/M</td>
<td>169</td>
<td>0.3/0.3</td>
<td>OD</td>
<td>DA</td>
<td>−10.34</td>
<td>52.9*</td>
<td>c.2538insT</td>
<td>22.4</td>
<td>55.2*</td>
</tr>
<tr>
<td>6/51/F</td>
<td>247</td>
<td>0.01/0.01</td>
<td>OD</td>
<td>DA</td>
<td>Not recorded</td>
<td>44.7*</td>
<td>p.Q61X</td>
<td>20.7*</td>
<td>41.4*</td>
</tr>
<tr>
<td>7/55/M</td>
<td>247</td>
<td>0.7/0.7</td>
<td>OD</td>
<td>TP</td>
<td>−2.04</td>
<td>66.9*</td>
<td>p.Q61X</td>
<td>25.2</td>
<td>62.1*</td>
</tr>
<tr>
<td>8/22/M</td>
<td>526</td>
<td>0.15/0.15</td>
<td>OD</td>
<td>TP</td>
<td>−3.90</td>
<td>68.9</td>
<td>c.2591insC</td>
<td>22.4</td>
<td>75.9</td>
</tr>
</tbody>
</table>

RNFL thickness for control subjects was 102.0 ± 16.2 μm (range, 67.8–123.0 μm). DA, diffuse atrophy; TP, temporal pallor; SP, subtle temporal pallor; MD, mean deviation.
* Lower than normal range (see also Figs. 3, 4).

6), and normal fields in four patients (patients 4, 5, 7, 8). Humphrey static perimetry showed reduced sensitivity of different degrees; mean deviation within 30° borders of the visual field ranged from −2.04 to −20.60 dB. Reliable results of static perimetry could not be obtained from one patient (patient 6) because of unstable fixation. Color vision tests using panel D-15 plates showed normal results for two patients (patients 3, 7), a minor error for one patient (patient 8), blue-yellow defects in four patients (patients 1, 2, 4, 5), and failure with no specific axis (patient 6).

The average RNFL thickness around the optic disk was 57.2 ± 16.2 μm, which was approximately 56% that of control subjects of 102.0 ± 16.2 μm (Table 1). More details about the OCT findings in ADOA patients will be presented elsewhere.

Full-Field ERGs

Rod-cone mixed maximal ERGs and single-flash cone ERGs recorded from one representative control subject and eight ADOA patients are shown in Figures 1 and 2, respectively. Mean ± SD of each ERG component for the eight ADOA patients and 25 age-matched control subjects are shown in Table 2. Differences in the amplitudes of the rod ERG b-wave, the a- and b-waves of the mixed maximal ERG, and the a- and b-wave of single-flash cone ERG were not significant in the two groups, though the average amplitudes of the a- and b-waves of the mixed maximal ERG and the a- and b-wave of single-flash cone ERG were smaller in ADOA patients than in controls.

OPs were normal in one patient (patient 1) but were clearly reduced for the other seven patients and were nearly undetectable for five patients (patients 2, 5–8; Fig. 1). The mean amplitude of OPs for ADOA patients was 69.0 ± 39.0 μV, which was approximately half that for the control subjects (155.1 ± 65.4 μV). In five of eight patients (patients 2, 4–7; Fig. 3), the amplitude of the OPs was smaller than the lower limit for control subjects.

Because the amplitudes of the a- and b-waves were slightly smaller for ADOA patients than for control subjects, we were uncertain whether the reduction in OPs resulted from an overall reduction in ERG amplitude. To examine the relationship between the OPs and the a- and b-waves, we calculated a ratio of the amplitudes of OPs to the b-wave. The mean OPs/b-wave ratio was significantly lower in ADOA patients, indicating that the reduction in the amplitude of the OPs cannot be explained by a reduction in the overall ERG (P = 0.001; Table 2).

The reduction in the amplitude of the OPs was also dependent on the age of the patients. The reduction in the amplitudes of the OPs as a function of age for 25 age-matched controls and eight ADOA patients are shown in Figure 3. In the control subjects, no significant correlation was observed between OP amplitude and age (r = 0.08; P = 0.75). In contrast, a significant inverse correlation was observed between OP amplitude and age (r = −0.78; P = 0.02) for the ADOA patients. It was also noted that for patients older than 40, OP amplitude was smaller than the lowest amplitude of the control subjects (Fig. 3).

The PhNR was also significantly smaller for ADOA patients (21.0 ± 2.4 μV) than for control subjects (34.3 ± 9.0 μV; P < 0.001; Fig. 2; Table 2). The amplitude of the PhNR in five of the eight ADOA patients was lower than the lower limit of normal in control subjects (patients 1–4, 6; Fig. 4). We also calculated the ratio of the amplitudes of PhNR to the b-wave, and this ratio was also significantly reduced for the ADOA group (P = 0.01; Table 2).

Table 2. Amplitude of Each ERG Component for Control Subjects and ADOA Patients with OPA1 Mutations

<table>
<thead>
<tr>
<th>Rod ERG</th>
<th>Mixed Rod-Cone Maximal ERG</th>
<th>Cone ERG</th>
<th>PhNR (PhNR/b-Wave)</th>
</tr>
</thead>
<tbody>
<tr>
<td>b-Wave</td>
<td>a-Wave</td>
<td>b-Wave</td>
<td>(OP/b-Wave)</td>
</tr>
<tr>
<td>Control</td>
<td>132.2 ± 37.8</td>
<td>322.6 ± 66.4</td>
<td>441.0 ± 104.5</td>
</tr>
<tr>
<td>Patient</td>
<td>133.3 ± 47.4</td>
<td>295.0 ± 55.5</td>
<td>432.1 ± 88.4</td>
</tr>
<tr>
<td>P</td>
<td>0.785</td>
<td>0.312</td>
<td>0.950</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. Mann-Whitney U test was used for statistical comparison. n = 25 control subjects; n = 8 patients.
No significant correlation was observed between PhNR amplitude and age for control subjects ($r = -0.32; P = 0.09$) and for ADOA patients ($r = -0.41; P = 0.32$).

**Correlation between ERG Amplitudes and RNFL Thickness or Psychophysical Measurements**

Mean RNFL thickness around the optic disk, visual acuity, and mean deviation of static perimetry were not significantly correlated with PhNR and OP amplitudes.

**DISCUSSION**

It has generally been thought that full-field ERGs are normal in patients with ADOA because the primary abnormality of ADOA is the degeneration of ganglion cells. Thus, Gräne et al. examined the different components of the full-field ERGs in ADOA patients with OPA1 mutations and reported that they were within the normal range for rod and cone components. Unfortunately, they did not analyze the ERG components that originate from inner retinal layers. In 1999, Holder et al. reported that the N95 component of the pattern ERG, which is thought to originate from retinal ganglion cells, was lower than the normal limit in many ADOA patients and supported the idea that the fundamental abnormality of ADOA lies in the retinal ganglion cells.

In our analysis of eight ADOA patients with OPA1 mutations, we found that PhNR amplitudes were significantly reduced. PhNR is a negative component of the photopic ERG seen after the b-wave, and it is thought to originate mainly from the activity of ganglion cells and their axons. Thus, it is reduced in the eyes of monkeys with experimentally induced glaucoma. In clinical studies, a selective reduction of the PhNR has been reported in patients with glaucoma and optic nerve diseases. Thus, it is not surprising to find that the mean PhNR amplitude in ADOA patients was approximately two thirds that of control subjects and that the amplitudes of the PhNR in five of eight patients were lower than the lower limit of normal in control subjects. These results support the idea that PhNR can be a useful objective indicator of the function of ganglion cells and their axons.

The most interesting finding in this study was the severe reduction in OP amplitude in ADOA patients. Thus, the mean OP amplitude in patients was less than half that in control subjects, and OP amplitude in four of eight patients was smaller than the lower limit of normal in control subjects. In addition, the strong inverse correlation between OP amplitude and age suggested progressive dysfunction of retinal neurons/circuits that gave rise to the OPs. The origin of OPs has not been definitively determined, but OPs are generally thought to originate from feedback neural pathways in the inner retina, especially around the inner plexiform layer. The cellular origin of OPs is thought to be mainly amacrine cells, though ganglion cells and bipolar cells may contribute to some parts of the OPs. Our results strongly suggested that the OPA1 gene is required not only for ganglion cell functioning but also for inner nuclear and plexiform layers—including amacrine cell—functioning. However, it is uncertain whether OPA1 is directly related to the function of amacrine cells, where the OPA1 gene is expressed, or whether the dysfunction of amacrine cells is secondary to ganglion cell degeneration.

Our results also indicated that the generators of the PhNR are affected severely in younger patients but that OP generators decrease slowly and progressively with age. Although the mechanism causing the gradual amplitude reduction of OPs is still undetermined, these different effects of the disease on the OPs and PhNRs may help to determine disease stage or severity.

A limitation of this study was that our patients were only relatively older patients—the youngest patient was 22—and therefore we could not analyze retinal function at earlier stages. Another limitation was that we did not record the ERGs from the same patient at different ages and thus could not state definitively the progressive nature of ADOA. Finally, our data did not differentiate whether the amplitude reduction of PhNR and OPs was specific to patients with OPA1 mutations or more generally to optic atrophy. Further studies are needed to clarify the functional characteristics of the human retina arising from OPA1 mutations.

**References**


