Reduction of Inner Retinal Thickness in Patients with Autosomal Dominant Optic Atrophy Associated with OPA1 Mutations

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PURPOSE. To determine the morphologic changes in the retina in the macula and around the optic disc in patients with autosomal dominant optic atrophy (ADOA) associated with a mutation in the OPA1 gene.

METHODS. Cross-sectional images of the macular area of the retina were obtained by optical coherence tomography (OCT) in patients with ADOA who had a heterozygous mutation in the OPA1 gene. There were 15 eyes of eight patients from five families: four men and four women. The average age of the patients was 48.1 years. In the OCT images, the cross sections of the sensory retina were divided manually into four areas. The thickness of the overall sensory retina and the divided areas were measured at 1 and 2 mm on the temporal, nasal, superior, and inferior sides of the fovea as well as at the fovea. The thickness of the retinal nerve fiber layer (RNFL) around the optic discs was measured by taking circular scans (3.4 mm in diameter) centered on the optic disc. The results in the patients with ADOA were compared with those from 11 normal control subjects.

RESULTS. The overall thickness of the sensory retina in the macula area was significantly thinner in the patients with ADOA than in the control subjects at all points except the fovea (P < 0.0001). The RNFL in the macular area in the patients with ADOA was significantly thinner than that in control subjects at all points (P < 0.0001), especially at 1 mm from the fovea. The circumpapillary RNFL was significantly thinner at the temporal, superior, and inferior areas in patients with ADOA but not in the nasal area. The total cross-sectional area of the circumpapillary RNFL was significantly correlated with visual acuity. The thickness of the combined ganglion cell layer, inner plexiform layer, inner nuclear layer, and outer plexiform layer in the macular area was significantly thinner in the patients (P < 0.0056). The thickness of the outer nuclear layer and the photoreceptor inner segments and the thickness of the photoreceptor outer segments were not significantly different between the patients with ADOA and normal control subjects.

CONCLUSIONS. The RNFL and the layer including the ganglion cell layer are significantly thinner in patients with ADOA associated with an OPA1 gene mutation than in control subjects. The photoreceptor layers are not affected morphologically. The inner retina is the main area of the retina altered in ADOA. (Invest Ophthalmol Vis Sci. 2007;48:4079–4086) DOI: 10.1167/iovs.07-0024

Autosomal dominant optic atrophy (ADOA; MIM 165500; Mendelian Inheritance in Man) is a dominantly inherited disorder characterized by symmetrical optic atrophy, central visual impairment, and color vision defects. The prevalence of ADOA is estimated to be between 1:50,0002 and 1:10,000.3 The clinical severity of the disease varies considerably among patients, even in the same family,1–6 which would indicate an incomplete penetrance.7–10 The reduction of vision is usually not severe, and the visual impairment progresses very slowly with increasing age in most cases.11

In 2000, the OPA1 gene was identified as the causative gene of ADOA.12,15 Although ADOA is genetically heterogeneous and another uncloned causative gene, OPA4, has been reported,14 in most patients ADOA is considered to be caused by OPA1 gene mutations. Previous linkage analyses have demonstrated that most families with ADOA were linked to the OPA1 gene,11,15,16 and Delettre et al.17 detected OPA1 gene mutations in 17 of 19 unrelated cases with a reliable diagnosis of ADOA. To date, approximately 100 different mutations in the gene have been identified in 190 families12,13,17–27 and we have also found OPA1 gene mutations in 11 of 16 Japanese probands with typical clinical characteristics of ADOA.26

The OPA1 gene is expressed in all tissues examined, but most strongly in the retina and brain.13 The OPA1 protein is located in the mitochondria13,28 and is considered to be involved in the division and fusion processes of mitochondria.29 It is also considered to be essential in maintaining the structure of the mitochondrial network.6–30 Although the exact pathologic mechanism is unknown, an abnormality of the OPA1 protein may cause an abnormality of the mitochondria leading to insufficient energy support. This could then result in a dysfunction of axoplasmic transport in the papillomacular retinal nerve fibers and eventually to optic atrophy.31 The histopathologic changes in eyes obtained postmortem from two patients with ADOA have been reported. A decrease in the number of retinal ganglion cells and degenerative changes of residual ganglion cells mainly in the posterior pole were found. In addition, a decrease in the number of papillomacular retinal nerve fibers were observed.9,31 In the optic disc, the myelination of the nerve fibers decreased, and the density of collagen fibers increased.9 It was thus suggested that the retinal ganglion cells were first to degenerate, and the optic atrophy developed secondarily.6

Recent advances of optical coherence tomography (OCT) has enabled us to observe the morphology of the retina easily in patients. To understand the pathomorphologic changes of...
ADOA better, we developed a manual method to perform a structural layer-by-layer analysis of the retina using the OCT images (StratusOCT; Carl Zeiss Meditec, Inc., Dublin, CA). We used this technique to measure the thickness of each retinal layer in eight patients with ADOA with confirmed mutations in the \textit{OPA1} gene.

**Materials and Methods**

**Patients**

After an explanation of the purpose of the study and procedures to be used, informed consent was obtained from all patients before examination. The procedures used conformed to the tenets of the Declaration of Helsinki.

Fifteen eyes of eight patients of five families who had a heterozygous mutation in the \textit{OPA1} gene were studied. There were four men and four women with an average age of 48.1 years. The left eye of patient 169:III-3 was excluded because it had had a retinal detachment and had undergone surgery 11 years ago. The clinical and genetic data are presented in Table 1. Detailed information about the mutation and clinical features of the eight patients have been presented elsewhere.

\begin{table}[h]
\centering
\caption{\textit{OPA1} Mutation and Clinical Data in Study Patients}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Case & Sex & Age (y) & Visual Acuity (R/L) & Visual Field & Disc Appearance & Mutation \\
\hline
42:III-2 & F & 27 & 0.4/0.4 & Central scotoma & Normal & c.2708_2711delTTAG \\
92:III-2 & F & 49 & 0.06/0.06 & Central scotoma & Total atrophy & Ser545Arg \\
92:IV-4 & M & 24 & 0.04/0.05 & Central scotoma & Total atrophy & Ser545Arg \\
169:III-3 & M & 46 & 0.3/0.3 & Normal & Temporal pallor & c.2538insT \\
247:III-1 & M & 55 & 0.7/0.7 & Normal & Normal & Gin61Ter \\
247:III-3 & F & 51 & 0.01/0.01 & Concentric contraction & Total atrophy & Gin61Ter \\
247:II-2 & M & 83 & 0.3/0.3 & Blind spot enlargement & Normal & Gin61Ter \\
526:III-1 & M & 22 & 0.15/0.15 & Normal & Temporal pallor & c.2591insC \\
\hline
\end{tabular}
\end{table}

A fundus photograph of a representative patient (247:III-3) is shown in Figure 1A. Ten refraction- and age-matched normal individuals were analyzed as control subjects (16 eyes in 10 control subjects—4 men and 6 women—average age, 44.8 years).

**Layer-by-Layer Analyses of Macular Morphology**

Cross-sectional scans (5 mm vertically and horizontally, 512 A-scans) were made by OCT (StratusOCT; Carl Zeiss Meditec, Inc.) in the macular area centered on the fovea. The macula was scanned two or more times in the horizontal and vertical directions, and the best images were used. All these images had signal strengths greater than 5 (StratusOCT, ver. 4.0, Max 10; Carl Zeiss Meditec, Inc.) and were analyzed. In the end, 15 eyes of eight patients were examined for the analyses. The final average signal strength was $7.9 \pm 1.5$ (mean $\pm$ SD).

For the analysis, the raw images were exported to a personal computer and converted to Tiff images with image-analysis software (Photoshop, ver.7.0; Adobe Systems, Mountain View, CA). Then, the retinal images were aligned along the retinal pigment epithelium with custom-made software based on the public domain NIH Image program (ver. 1.62, developed by Wayne Rasband at the National Institutes of Health, Bethesda, MD; http://rsb.info.nih.gov/nih-image/). The intraretinal borders were sufficiently distinct to be distinguished, and were drawn manually by referring previous findings with the ultra-high-resolution OCT.

Representative OCT images of a patient with ADOA and a normal control subject are shown in Figure 2. The first line was drawn along the boundary between the vitreous and the retina (Fig. 2E, line 1). The second line was drawn between the most inner, bright retinal layer and the neighboring outer layer with lower brightness (Fig. 2E, line 2). The third line was drawn between the layer with less brightness and the next outer layer with the least brightness (Fig. 2E, line 3). The fourth line was placed on the bright line, which is reported to correspond to the boundary between the photoreceptor inner segments (ISs) and outer segments (OSs; Fig. 2E, line 4). The fifth line was drawn along the boundary of the OSs and retinal pigment epithelium (RPE; Fig. 2E, line 5).

From these retinal images with the manually drawn lines, the area between the first and the second lines corresponded to the cross section of the retinal nerve fiber layer (RNFL). The area between the second and the third lines included the ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), and outer plexiform layer (OPL). In this area, we could not clearly differentiate

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{(A) Fundus photograph of a representative 51 year-old female patient with ADOA (247:III-3). Temporal pallor of the optic disc can be seen. (B) Schematic drawing showing the scans and plots measured.}
\end{figure}
these four layers in the OCT images, and we refer to this area as "the middle layer" in this article. The area between the third and the fourth lines included the outer nuclear layer (ONL) and the photoreceptor ISs.32–34 It was not always possible to differentiate the border between ONL and ISs in the images. The area between the fourth and fifth lines corresponded to the photoreceptor OSs.32–34 The area between the first and the fifth lines corresponded to the overall sensory retina (Fig. 2E).

In each of the images, the thickness of the overall sensory retina and the four divided areas were measured at the fovea and at 1 and 2 mm on the temporal, nasal, superior, and inferior sides of the fovea (Fig. 1B). At the fovea where the RNFL is essentially nonexistent, the thickness of the overall sensory retina and the thickness of the photoreceptor OSs were measured. The thickness of each retinal layer was measured in the 8 patients with ADOA and was statistically compared with the corresponding layers in the 10 normal subjects, by unpaired $t$ tests with Bonferroni correction.

**Measurement of Circumpapillary RNFL Thickness**

The thickness of the RNFL was also measured around the optic discs. Circular scans (3.4 mm in diameter, 512 A-scans) centered on the disc were taken three times for each eye, and the best images were used. Images with a signal strength of <5 (Max 10 built-in software ver. 4.0; StratusOCT; Carl Zeiss Meditec, Inc.) were excluded, and images from the right eyes in patients 169:III-3 and 92:IV-4 were excluded. Thus, 13 eyes of 7 patients were examined for the analyses.

The raw images were exported using the built-in software and converted to Tiff images with image-analysis software (Photoshop, ver. 7.0; Adobe Systems) to obtain the maximum resolution. Then, the retinal images were aligned along the retinal pigment epithelium by using a custom-made software written in NIH Image, and the boundary between the vitreous and the sensory retina and the boundary between the RNFL and GCL were marked. Then, the circumference was divided into 12 segments, and the RNFL thickness of each segment was
### Table 2. Thickness of Each Layer in the Macula

<table>
<thead>
<tr>
<th>Layers</th>
<th>Superior</th>
<th>Inferior</th>
<th>Temporal</th>
<th>Nasal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 mm</td>
<td>1 mm</td>
<td>2 mm</td>
<td>1 mm</td>
</tr>
<tr>
<td><strong>RNFL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>44.8 ± 9.4*</td>
<td>33.0 ± 9.5*</td>
<td>35.2 ± 8.4*</td>
<td>43.2 ± 6.5*</td>
</tr>
<tr>
<td>Patients</td>
<td>5.2 ± 7.5</td>
<td>3.3 ± 4.9</td>
<td>2.1 ± 4.2</td>
<td>6.4 ± 7.9</td>
</tr>
<tr>
<td><strong>Middle layer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>136.0 ± 14.1†</td>
<td>175.6 ± 14.5†</td>
<td>190.9 ± 17.0†</td>
<td>135.1 ± 13.7†</td>
</tr>
<tr>
<td>Patients</td>
<td>122.1 ± 9.5</td>
<td>138.1 ± 14.4</td>
<td>146.8 ± 15.8</td>
<td>121.8 ± 10.3</td>
</tr>
<tr>
<td><strong>Outer nuclear layer and</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>photoreceptor inner</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>segment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>82.3 ± 7.1</td>
<td>92.5 ± 11.0</td>
<td>72.5 ± 14.5</td>
<td>64.2 ± 9.1</td>
</tr>
<tr>
<td>Patients</td>
<td>80.2 ± 7.6</td>
<td>91.4 ± 12.9</td>
<td>76.9 ± 14.9</td>
<td>71.1 ± 10.0</td>
</tr>
<tr>
<td><strong>Photoreceptor outer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>segment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>21.1 ± 3.6</td>
<td>22.7 ± 2.8</td>
<td>21.4 ± 3.1</td>
<td>18.7 ± 3.2</td>
</tr>
<tr>
<td>Patients</td>
<td>22.6 ± 4.5</td>
<td>22.7 ± 4.0</td>
<td>22.9 ± 2.9</td>
<td>18.4 ± 2.8</td>
</tr>
<tr>
<td><strong>Total retinal thickness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>284.2 ± 14.5†</td>
<td>323.8 ± 14.3†</td>
<td>320.0 ± 14.0†</td>
<td>261.3 ± 16.4†</td>
</tr>
<tr>
<td>Patients</td>
<td>230.1 ± 9.1</td>
<td>255.6 ± 10.8</td>
<td>248.6 ± 9.9</td>
<td>217.8 ± 8.5</td>
</tr>
</tbody>
</table>

Data are mean ± SD (μm).
* P < 0.0001.
† P < 0.00556.
averaged using another custom-made software written in NIH Image (Fig. 1B). We did not use the built-in software of the OCT system to measure the RNFL thickness of each segment, because the RNFL thickness was sometimes too thin to be measured appropriately by that software in patients with ADOA. The thickness of each area in the patients was statistically compared to that in the normal control subjects by using unpaired t-tests with Bonferroni correction.

The total cross-sectional area of the RNFL in the 3.4 mm circular scan was measured using the custom-made software, and its relationship with visual acuity was statistically examined by using the Mann-Whitney test.

**Measurement of Macular Volume**

The macular volume was calculated from six radial linear scans (scan length = 6 mm) centered on the fovea with the software included with the OCT and compared between the patients with ADOA and the normal control subjects by using unpaired t-tests. The correlation between the macular volume and visual acuity in the patients was calculated using Mann-Whitney tests.

**Reproducibility of the Manual Measurements**

We evaluated the reproducibility of the manual measurement. The images of the macular retina from the 8 patients with ADOA and 10 normal patients were analyzed by two medical doctors (YI, MI) using the method described earlier. Each doctor measured three times at intervals of at least 1 month, and the reproducibility was calculated.

**Statistical Analyses**

Unpaired t-tests with Bonferroni correction were used for statistical comparison between patients with ADOA, and normal control subjects to reduce type I error. For the Bonferroni correction, standard P < 0.05 was divided by number of tested points (e.g., 9 in the comparison at macula, 12 in the comparison at circumpapillary nerve fiber layer thickness). As a result, the adjusted probabilities for a significant difference were 0.00556 and 0.00417 for the macular and circumpapillary nerve fiber layer thickness comparison, respectively. The macular volume in the patients with ADOA was compared to that in the normal control subjects by unpaired t-tests with the statistical significance set at P < 0.05. The correlations between macular volume and visual acuity and that between the total cross-sectional area of the RNFL in the 3.4-mm circular scan and visual acuity were statistically examined by calculating Pearson correlation coefficients.

**RESULTS**

The results of the thickness of the overall sensory retina and the divided intraretinal layers in the macula area in the patients with ADOA and in the control subjects are shown in Table 2. They are also presented in Figure 3.

The RNFL in the macular area was significantly thinner in the patients with ADOA than in the control subjects at all points examined (P < 0.0001). It was very thin, especially 1 mm from the fovea, where it was not measurable (Table 2, Fig. 3A). The decrease in the RNFL thickness was detected in all patients, even in those with relatively good visual acuity.

The RNFL around the optic disc was significantly thinner in the patients with ADOA than in control subjects at the temporal, superior, and inferior areas of the disc but not at the nasal area (Table 3, Fig. 4). The results of circumpapillary RNFL thickness in normal individuals measured by the manual method were almost the same as those measured by the built-in software with the OCT (data not shown). They were also comparable to those of previously published data, which confirms the validity of our manual method (data not shown).

The thickness of the middle layer, including the GCL, IPL, INL, and OPL, in the macular area was also significantly thinner in the patients with ADOA than in the control subjects (P <
0.00556) at all eight points measured (Table 2, Fig. 3B). The thicknesses of the ONL, ISs, and OSs in the macular area of the patients with ADOA were not significantly different from those in the control subjects (Table 2, Figs. 3C, 3D).

The overall sensory retina in the macular area was significantly thinner in the patients with ADOA than in the control subjects ($P < 0.0001$) at all points measured except for the fovea, where the thickness in the patients with ADOA was not significantly different from that of the control subjects (Table 2, Fig. 3E).

The means and the standard deviations of macular volume in the patients with ADOA and normal control subjects were $5.5 \pm 0.2$ and $7.0 \pm 0.4$ mm$^3$, respectively, and this difference was significant ($P < 0.001$).

The visual acuity correlated significantly with the total cross-sectional area of the RNFL in the 3.4-mm circular scan around the optic disc (Pearson correlation coefficients; $r = 0.742$, $P = 0.004$; Fig. 5), but it did not correlate significantly with the macular volume ($P = 0.402$; data not shown).

The reproducibility of the measurement of the thickness of each retinal layer by the manual method was confirmed. Coefficient of variance ranged from 0.0% to 37.9% (5.9% ± 6.0%, mean ± SD) in operator 1 and from 0.0% to 40.1% (8.6% ± 7.6%) in operator 2.

**DISCUSSION**

Our results demonstrated a significant thinning of the RNFL in the papillomacular area in all patients with ADOA associated with ADOA. The Pearson correlation coefficient shows a significant correlation ($r = 0.742$, $P = 0.004$).
with OPA1 gene mutations. The RNFL was also significantly thinner in the temporal, superior, and inferior areas adjacent to the optic disc but not in the nasal area. These results are consistent with previous histopathologic observations that the papillomacular nerve fibers were selectively damaged in the posterior pole.9,31 The macular RNFL was markedly thin, not only in cases with severe clinical findings but also in one mild case with relatively good visual acuity with normal visual field and normal color vision (patient 247:III-1). This patient had not been aware of his disease and had been first diagnosed to have ADOA during an examination as a member of a family with a severely affected proband (247:III-3). The appearance of his optic discs was within the normal limit. These findings indicate that the RNFL thinning in the macular area probably preceded the early stage of the disease before the loss of visual acuity and visual field.

The clinical severity of ADOA differs considerably among patients, and the mild cases with normal appearing or only subtle temporal pallor of the optic disc with good vision would be hard to diagnose as having ADOA by ophthalmoscopy alone. Our results suggest that measurement of the RNFL thickness would be clinically helpful in diagnosing ADOA, especially in those with a mild clinical phenotype.

The total cross-sectional area of RNFL in the 3.4-mm circular scans around the optic disc, that was measurable in all patients including severe cases, correlated significantly with visual acuity. This indicates that the greater the thinning of the RNFL, the greater the reduction in visual acuity. The correlation between the macular volume and visual acuity was not significant, but this may be because the RNFL in the macula was very thin in all patients.

The middle layer of the retina including the ganglion cell layer, IPL, INL, and OPL was also thin in patients with ADOA, probably because of the loss of ganglion cells, and confirm a previous histopathologic study of ADOA that showed a reduced number of retinal ganglion cells and degeneration of the residual surviving cells, mainly in the posterior pole.9,31 However, because the resolution in the OCT is not sufficient to separate the ganglion cell layer and other midretinal layers (e.g., the IPL, INL, and OPL), we cannot conclude that the thinning was due to degenerative changes of only the ganglion cells. When the next generation OCT with higher resolution, such as ultra-high-resolution OCT, becomes available, the ganglion cell layer and the IPL and INL may be able to be differentiated, and their thickness may be analyzed.32–34

The thickness of the photoreceptor layers was not altered in all patients with ADOA which is consistent with the histopathologic observations in eyes obtained postmortem that there was no degeneration of the photoreceptor layer in patients with ADOA.9–31 In addition, the electroretinograms in patients with ADOA are, in general, normal.1,41–45 Thus, the photoreceptor layer is most likely not affected in this disease. The overall sensory retinal thickness at the fovea was normal in patients with ADOA probably because the RNFL and ganglion cell layer are essentially absent in this area.

The OCT findings in patients with ADOA are different from those in patients with degenerative retinal diseases. For example in patients with retinitis pigmentosa, a thinning of the photoreceptor layer is more prominent than that of the RNFL.44,45 and the overall sensory retinal thickness is thinner mainly in the peripheral atrophic retina. In addition, the morphology of the macular area is relatively well-preserved until the late stages. In a patient with retinitis punctata albescens, OCT examinations demonstrated a decrease in retinal thickness in the macular area, especially in the photoreceptor layer.46

Thus, the OCT images can be used to differentiate diseases affecting either the outer or the inner retina. Previous studies have reported that both the macular retinal thickness and the macular RNFL thickness are thinner in patients with glaucoma. This thinning is topographically associated with decreased localized visual sensitivity and usually vertical asymmetry, especially in the early stage.35–37 In our cases with ADOA, however, the RNFL or macular thinning was essentially vertically symmetric in all cases, suggesting that the reduction of the thickness was vertically symmetric from the early stage. This difference may be useful to differentiate ADOA from glaucoma.

In a recent OCT study of Leber’s hereditary optic neuropathy (LHON), it was reported that the RNFL was thicker in early LHON and severely thinned in atrophic LHON.38 With circular scan of the optic disc, the temporal fibers were the first and most severely affected and the nasal fibers seem to be partially spared, even in the late stage of the disease. Thus, ADOA shares similar pathomorphology with LHON, in that RNFL impairments occur relatively selectively in the macular area, perhaps because of the common pathogenesis of the diseases (i.e., mitochondrial abnormality). In contrast, in eyes with band atrophy caused by optic chiasm compression due to chiasmal tumors, the RNFL was thinned in all clock hours around optic disc, indicating a diffuse involvement of the RNFL in the retina.39

In conclusion, we have evaluated retinal morphology by OCT layer by layer in patients with ADOA associated with a mutation in the OPA1 gene. The thickness of RNFL and the middle layer including the GCL, IPL, INL, and OPL in the macular area were significantly thinner in the patients than those in normal control subjects, whereas the thickness of the photoreceptor layer was not significantly different. Circumpapillary RNFL thickness was significantly thinner at the temporal, superior, and inferior areas but not in the nasal area in patients with ADOA. These morphologic findings strongly confirm that the main pathologic changes in ADOA are located at the macular inner retina but not at the outer retina.

References
