Retinal Nerve Fiber Layer Defects in RP Patients

Saloni Walia, Gerald A. Fishman, Deepak P. Edward, and Martin Lindeman

PURPOSE. To determine, with the use of optical coherence tomography (OCT), the peripapillary retinal nerve fiber layer (RNFL) thickness among patients with retinitis pigmentosa (RP) of different degrees of disease severity.

METHODS. One eye in each of 25 RP patients was included. All patients underwent complete ocular examination, including the measurement of intraocular pressure, corneal thickness, and detailed fundus examination. Visual fields were evaluated by Goldmann perimetry. An RNFL thickness protocol was used to acquire circular scans of 3.46 mm in diameter around the optic nerve. For each eye, RNFL thickness was studied in the temporal (316°–45°), superior (46°–135°), nasal (136°–225°), and inferior (226°–315°) quadrants. Three smaller segments were also measured within each quadrant, all automatically calculated by the existing OCT software. The severity of damage to the peripapillary nerve fiber layer was compared with the clinical appearance of the optic disc, severity of field loss, and mode of inheritance for RP.

RESULTS. The mean age of patients included in the study was 48.6 years (range, 23–73 years). Of the 25 patients examined, 10 had normal thinning of the peripapillary RNFL in 2 or more segments, and 7 of those had normal thinning in at least 1 quadrant. The number of patients with abnormal thinning of the RNFL was considerably greater in those with clinically observed moderately severe or severe pallor of the optic nerve. For each patient, RNFL thickness was measured within each quadrant, all automatically calculated by the existing OCT software. The severity of damage to the peripapillary nerve fiber layer was compared with the clinical appearance of the optic disc, severity of field loss, and mode of inheritance for RP.

CONCLUSIONS. Patients with retinitis pigmentosa may have a measurable degree of RNFL thinning as determined by OCT. These observations could have an impact on future treatment strategies and imply that patients considered for various treatment options would benefit by an evaluation of nerve fiber layer thickness. (Invest Ophtalmol Vis Sci. 2007;48: 4748–4752) DOI:10.1167/iovs.07-0404

Retinitis pigmentosa (RP) is a group of hereditary retinal diseases that feature degeneration of rod and cone photoreceptors. It is a significant cause of inherited blindness. The worldwide prevalence of RP is approximately 1 in 4000, with a total of more than 1 million persons affected. The features of RP include night blindness, progressive loss of peripheral visual fields, reduced or nondetectable electroretinogram (ERG) amplitudes, and characteristic pigmentary degenerative changes of the retina.

Histopathologic studies of eyes with RP have documented a reduced number of rods and cones and shortened or severely distorted outer segments in the remaining photoreceptors. It has been hypothesized that early outer retinal damage in RP may lead to transneuronal changes causing ganglion cell loss. Stone et al. have documented a correlation between photoreceptor loss and some transneuronal ganglion cell death. Santos et al. have also shown significant loss of cells in the outer nuclear and ganglion cell layers but better preservation of cells in the inner nuclear layer in patients with moderate to severe RP. Li et al. have attributed the loss of inner retinal neurons to the narrowing and occlusion of the vascular lamina, not just to transneuronal degeneration. Other authors have concluded that the loss of ganglion cells is seen only in advanced stages of RP and that the pallor of the optic nerve can be attributed to glial tissue proliferation and around the optic nerve. However, Flannery et al. did not find a significant loss of nerve fibers in the optic nerve of an RP patient compared with normal findings. Thus, these studies indicate that photoreceptor loss may be accompanied by some loss of ganglion cells in patients with RP.

Recent therapeutic modalities, such as gene therapy and retinal stem cell transplantation, are aimed at restoring or preserving photoreceptor function. In patients with RP, these can be successful only if there is some preservation of inner retinal layers. Optical coherence tomography (OCT) has now emerged as a noninvasive modality to study the in vivo retinal architecture. It is reproducible and reliable in measuring peripapillary retinal nerve fiber layer (RNFL) thickness. In this study, we used OCT-3 to measure the peripapillary RNFL in RP patients with various degrees of disease severity to further evaluate possible ganglion cell loss in these patients.

METHODS

The study was conducted in the Department of Ophthalmology at the University of Illinois at Chicago. It was approved by the institutional review board and was performed in accordance with tenets of the Declaration of Helsinki.

A representative sample of patients, older than 18 years and with a known diagnosis of RP and a spectrum of disease severity, was asked to participate in the study. Patients were randomly selected and enrolled based on their willingness to undergo OCT testing. The diagnosis of RP was based on history of night blindness, impairment of peripheral visual fields, reduction of electroretinogram rod and cone amplitudes, and presence of characteristic fundus pigmentary changes. Exclusion criteria used for screening included visual acuity less than 20/80 on a Snellen acuity chart (with one exception), refractive error of more than ± 6.0 diopters spherical or ± 2.0 diopters cylindrical correction, inability to maintain steady fixation, media opacity sufficient to hinder an OCT examination, previous history of glaucoma or intraocular pressure higher than 21 mm Hg, excessively large or small size of the optic nerve on clinical examination, and diabetic retinopathy. Twenty-six patients were included in the study, all of whom provided informed consent. One patient was subsequently excluded because of nerve fiber layer drusen that resulted in inaccurate measurement of the nerve fiber layer thickness value on OCT testing. Of

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the 25 patients included, 1 had visual acuity of less than 20/80 (20/400) but could hold steady fixation using the external fixation target on the OCT and, hence, was asked to participate in the study.

All patients underwent complete eye examination, including best-corrected visual acuity, slit lamp biomicroscopy, 2 intraocular pressure readings at 5-minute intervals, fundus stereophotography, and corneal pachymetry. In the participating patients, the best-corrected visual acuity was determined by the Early Treatment of Diabetic Retinopathy Study chart (The Lighthouse, Long Island City, NY). The better eye was then dilated in each patient using 2.5% phenylephrine and 1% tropicamide, and detailed fundus examination was performed.

RNFL thickness was measured with a third-generation OCT machine (StratusOCT, software version 4.0.1; Carl Zeiss Meditec, Inc., Dublin, CA). The fast RNFL thickness protocol, consisting of 256 individual A-scans along a circular scan path, was used. Internal fixation was used in all patients except one, who required external fixation, as already discussed. The optic disc was then centered within the scan circumference. The software automatically used a scan diameter of 3.46 mm and performed 3 successive scans, with a total acquisition time of 1.92 seconds. Scans were only accepted if they had a signal intensity of 5 or more and were free of artifacts. The average of the 3 scans in each acquisition was automatically calculated by the OCT software. To increase the reliability of the test, each patient underwent testing 3 times, with the third acquisition the repeat sequence of the second one. In 14 patients, all 3 acquisitions were found to be reproducible in the number of abnormal quadrants and, hence, were used for analysis. In the remaining 11 patients, 2 of the 3 acquisitions were reproducible and were used for the purpose of analysis.

For each eye, we studied RNFL thickness in the temporal (316°–45°), superior (46°–135°), nasal (136°–225°), and inferior (226°–315°) quadrants. We also measured 3 smaller segments within each quadrant, all automatically calculated with the use of existing OCT software. The data thus obtained were compared to the normative database already incorporated in the StratusOCT unit. The RNFL was considered abnormally thin if its value was below the 5th percentile of the age-related normal value and thicker if it was above the 95th percentile. Abnormal thinning of the RNFL in at least 1 quadrant, or abnormal thinning of the RNFL in at least 2 smaller segments, present consistently in at least 2 acquisitions, was considered significant and therefore abnormal.

Goldmann perimetry (Haag-Streit AG, Koeniz, Switzerland) using target sizes V4e, III4e, and II4e was performed to measure the kinetic visual field. The area of visual field for each target size was then measured by a planimetric calculation in square inches and was converted to square millimeters.

One of the authors (DPE), who was masked to the findings of the nerve fiber layer measurements, reviewed the stereo photographs of the optic disc of each patient for any evidence of glaucomatous damage. Factors that were considered in designating an optic disc as glaucomatous included a large cup-to-disc ratio greater than 0.7 that did not follow the ISNT rule,24,25 presence of polar notching, increase in sloping of the cup margins, and baring of blood vessels on the disc surface.

An unpaired t-test was used to calculate the 2-tailed P value to test for significance between 2 means. P < 0.5 was considered statistically significant.

**RESULTS**

The average age of patients in the study was 48.6 years (range, 23–73 years); 11 were women and 14 were men. Based on ethnicity, our study group was composed of 21 Caucasian persons (84%), 2 Hispanic persons (8%), 1 black person (4%), and 1 Asian person (4%).

Of the 25 patients examined, 10 (40%) had abnormal thinning of the nerve fiber layer in 2 or more segments (Fig. 1A). Of these 10 patients, 7 also showed abnormal RNFL thinning in 1 or more quadrants (Figs. 1B, 1C).

![Example of abnormal thinning of the RNFL in 2 segments, with normal quadrants (A), abnormality in 3 quadrants (B), and in segments and quadrants (C).](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933441/)
RNFL was seen in the nasal, superior, and inferior quadrants. The temporal quadrant was thicker than normal in 7 of these 10 patients and within the normal range in 3 patients.

Among the 15 patients who did not show any thinning of the nerve fiber layer, 2 had thicker RNFL in all 4 quadrants, 2 in 2 quadrants (superior and inferior), and 6 in just the temporal quadrant.

Average best-corrected visual acuity was 0.22 logMAR units (range, −0.06 to 1.3, equivalent to 20/16 to 20/400 on a Snellen acuity chart). Visual acuity of 22 patients could be corrected to 0.40 logMAR units (20/50 on a Snellen acuity chart) or better. Of these 22 patients, 9 patients (40.9%) had abnormal thinning of the RNFL in 2 or more segments; among these 9 patients, 6 (66.7%) also had abnormal thinning of the RNFL in 1 more quadrants on OCT testing.

The average of the 2 intraocular pressure measurements for each patient was normal (range, 12–20 mm Hg). The average pachymetry value was 554.2 μm (range, 482–596 μm).

RNFL thickness was compared between patients who had a normal-appearing or very mild pallor of the nerve head with those who had moderately severe to severe pallor of the optic disc, as judged qualitatively on clinical examination by 2 authors (GAF, SW). Results are presented in Table 1. The proportion of patients with abnormal RNFL thinning and no or mild disc pallor was found to be considerably lower than the proportion with moderately severe or severe pallor and abnormal RNFL thinning.

On examination of the optic disc for glaucomatous damage, it was observed that 16 patients had no evidence of such damage, 4 had increased sloping of the neuroretinal rim in 1 quadrant though it was within the range considered normal, 2 had borderline findings with increased cup size and some thinning of the neuroretinal rim, and 3 were thought to have findings characteristic of glaucomatous damage. All 3 had marked increases in the cup-to-disc ratio, 2 of the 3 had haring of the blood vessels on the disc, and 2 had pale notching of the optic cup. RNFL thinning was seen in 7 of the 20 patients (35%), with no apparent glaucomatous damage to the optic disc, and in 2 of 5 (66.7%) with suspicious and in 1 of 2 (50%) with borderline glaucomatous damage. Table 2 compares glaucomatous and nonglaucomatous-appearing optic disc findings with RNFL thickness.

Table 3 compares the mean and range of planimetry values for each of the 3 target sizes—4°e, III4e, and II4e—used in perimetry, in patients without and with abnormal RNFL thinning. No significant difference was found in the area of the visual field between patients with or without RNFL thinning for all 3 test targets.

On the basis of pedigree analysis, 6 patients had autosomal dominant (average age, 52.8 years), 4 had autosomal recessive (average age, 39 years), 11 had isolated (average age, 54.3 years), and 3 had X-linked recessive (average age, 34.3 years) inheritance; the inheritance pattern of 1 patient (age, 42 years) could not be determined because the patient was adopted. The number of patients with thinning of the nerve fiber layer was 2 of 3 for the X-linked group, 2 of 6 for the autosomal dominant, 1 of 4 for the autosomal recessive, and 5 of 11 for the isolated category.

The temporal quadrant (316°–45°) of the parapapillary nerve fiber layer region either was spared or was found to be thicker than normal in all patients; the other 3 quadrants were affected equally, and no predilection for abnormal thinning of the RNFL was found in any quadrant. The average nerve fiber layer thickness for all 25 RP patients and those with normal RNFL was 97 ± 19.7 μm and 96.5 ± 19.8 μm, respectively, in the temporal quadrant, 125.2 ± 29.46 and 141.1 ± 21.6 μm, respectively, in the superior quadrant, 75.8 ± 22.2 μm and 88.2 ± 17.7 μm, respectively, in the nasal quadrant, and 125.2 ± 30.1 μm and 141.4 ± 23.6 μm, respectively, in the inferior quadrant.

**DISCUSSION**

Results of our study show that 40% of all participating RP patients had some thinning of the peripapillary RNFL as measured by OCT. Various studies in the past have shown a reduced number of ganglion cells in RP patient eyes compared with a control group. Some have attributed this loss to trans-synaptic neuronal damage when ganglion cells lose neuronal input because of photoreceptor cell degeneration.6–8,26 Others have implicated diminished blood flow to the inner retinal layers,9 or hypothesized a direct effect on ganglion cells from the gene defect responsible for photoreceptor cell death.8 However, all previous studies were performed on postmortem eyes. The present study is the only one that has measured RNFL defects as an indicator for ganglion cell loss in vivo in RP patients.

This study also shows that RNFL thinning by OCT in RP patients may be present in patients with a normal appearance of the optic disc on clinical examination. Nevertheless, increasing pallor of the optic nerve may be equated with a higher probability of nerve fiber layer defect and, hence, ganglion cell loss. RNFL damage may also be present in patients without clinically significant loss of visual acuity.

Of the 25 patients who participated in the study, 20 had no evidence of optic disc changes thought to be caused by glaucoma. Seven of these 20 patients had abnormal RNFL thinning, further confirming that ganglion cell loss and nerve fiber layer loss may occur in RP patients in the absence of clinically apparent glaucoma. Three patients in our study group had optic discs with possible glaucomatous damage; however, all 3 patients had intraocular pressures within the normal range. They also had optic discs of normal size, and none of them was a high myope. Two of these 3 patients had RNFL defects, as detected by the OCT. Because of known disc changes, including mainly waxy disc pallor and significant visual field impairment known to occur in RP patients, the presence of optic disc

<table>
<thead>
<tr>
<th>Disc Pallor</th>
<th>Patients with Abnormal Segments</th>
<th>Patients with Abnormal Quadrant(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal or mild</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Moderate or severe</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

**Table 2. Comparison of Glaucoumatous and Nonglaucoumatous- Appearing Optic Disc Findings with RNFL Thinning**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Patients with Abnormal Segments</th>
<th>Patients with Abnormal Quadrant(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glaucoumatous</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Borderline</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Nonglaucoumatous</td>
<td>20</td>
<td>7</td>
</tr>
</tbody>
</table>
findings—enlarged cup-to-disc ratio, disc notching, sloping, bariring of vessels in 3 of our patients—could not unambiguously be interpreted as definitively representing glaucomatous changes. Nevertheless, it is relevant that in our cohort of 25 RP patients, 40% showed RNFL thinning.

Visual field loss in RP patients has been attributed to the loss of photoreceptors and has traditionally been the method of monitoring disease progression. We could not find any correlation between total area of the remaining visual field and nerve fiber layer defect for any of the 3 targets (V4e, III4e, II4e) used for examination. Nevertheless, our findings do not preclude the possibility that thinning of the nerve fiber layer might have contributed to the visual field impairment in our patients.

Analysis of ganglion cell layer counts in a study by Humayun et al. showed significantly more counts in patients with autosomal dominant RP than in those with X-linked RP. These findings are consistent with the OCT findings among our RP patients with different genetic subtypes. The proportion of patients with abnormal RNFL thinning was maximal for the X-linked group and measurably less for the autosomal dominant RP group. Although this correlates with the more severe disease seen in patients with X-linked RP, because of the small number of patients in each genetic subtype in our cohort, we did not think reporting a statistical analysis would be meaningful.

Maximum loss of photoreceptors in RP patients has been reported in the inferonasal quadrant. However, it is unknown whether this translates to significantly greater loss of the retinal ganglion cell layer in any one quadrant. In our study, we found that in different patients, superior, inferior, and nasal quadrants were all equally affected with thinning of the RNFL, whereas the temporal quadrant was not found to be thin in any of our patients. Moreover, the average RNFL thickness of the temporal quadrant in our study was greater than what has been reported in the literature. The reasons behind this change are unclear. It is possible that in our RP patients, glial cell proliferation was more abundant temporal to the disc than in other quadrants and might have contributed to the increase in thickness as measured by the OCT. The potential impact of cystoid macular edema would also have to be considered. We had no OCT measurements of the macula for 5 patients. Five patients had cystoid macular edema; in 2 patients, RNFL was thickened in the temporal quadrant, and in 1 patient all 4 quadrants were thicker than normal. Ten other patients also had thickened temporal quadrants but did not have cystoid macular edema.

One of the factors that might influence RNFL thickness measured by OCT is the axial length of the eye. We did not correct for this factor in our study. However, we only included patients with a low refractive error in whom a normal or close to normal axial length might be assumed. Although we did not measure the optic disc area, none of the patients had clinically apparent optic discs that were judged to be other than normal in size.

In summary, this study confirms thinning of the RNFL in patients with RP, as determined by OCT. Treatment strategies aimed at restoring or preserving photoreceptor function will likely only or mainly be useful in patients who have some preservation of their inner retinal layers. Our findings support the conclusion that patients considered for these treatment options should also be considered for RNFL thickness measurements.

### References

11. Gartner S, Henkind P. Pathology of retinitis pigmentosa. *Ophthal-

### Table 3. Comparison of Visual Field Area in Patients with and without RNFL Thinning

<table>
<thead>
<tr>
<th>Quadrant(s)</th>
<th>Without Thinning in Segments</th>
<th>Abnormal Thinning in Segments</th>
<th>Without Thinning in Quadrant(s)</th>
<th>Abnormal Thinning in Quadrant(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V4e</td>
<td>Mean (mm²) 9591.1 P 0.33</td>
<td>7162.4</td>
<td>9378.3 P 0.31</td>
<td>6668.6</td>
</tr>
<tr>
<td></td>
<td>Range (mm²) 271–18672.0</td>
<td>348.4–21072.2</td>
<td>271–21072.2</td>
<td>348.4–12871.7</td>
</tr>
<tr>
<td>III4e</td>
<td>Mean (mm²) 3469.5 P 0.81</td>
<td>3895.7</td>
<td>4010</td>
<td>2688.6</td>
</tr>
<tr>
<td></td>
<td>Range (mm²) 135.5–8536</td>
<td>180.7–19194.7</td>
<td>135.5–19194.7</td>
<td>180.7–8168.2</td>
</tr>
<tr>
<td>II4e</td>
<td>Mean (mm²) 1799.0 P 0.77</td>
<td>2153.0</td>
<td>2254.6</td>
<td>1090.4</td>
</tr>
<tr>
<td></td>
<td>Range (mm²) 45.2–5981</td>
<td>83.9–13658.9</td>
<td>45.2–13658.9</td>
<td>90.3–4935.8</td>
</tr>
</tbody>
</table>

P values are based on the Student’s t-test.


