A Comparison of Methods for Tracking Progression in X-Linked Retinitis Pigmentosa Using Frequency Domain OCT

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Received: 7 September 2013

Accepted: 21 October 2013

Published: 11 December 2013

Keywords: retinitis pigmentosa; optical coherence tomography; progression

Citation: Ramachandran R, Zhou L, Locke KG, Birch DG, Hood DC. A comparison of methods for tracking progression in x-linked retinitis pigmentosa using frequency domain OCT. *Trans Vis Sci Tech.* 2013;2(7):5. <http://tvstjournal.org/doi/full/10.1167/tvst.2.7.5>, doi:10.1167/tvst.2.7.5

Purpose: To compare the efficacy of frequency domain optical coherence tomography (fdOCT)-derived outer retinal measures in tracking disease progression in x-linked (xl) retinitis pigmentosa (RP) patients.

Methods: Macular volume scans and line scans (Spectralis) were obtained from 27 xLRP patients (15.3 ± 6.4 years) at two visits approximately 2 years apart. Changes in average outer retinal layer thicknesses across the volume scan were compared to changes detected by measures derived from the edge of the inner segment ellipsoid zone (EZ) band, that is, where the EZ band (also known as inner segment/outer segment border) disappears. Repeatability was tested on an independent set of 18 RP patients (43.5 ± 18.0 years).

Results: Average outer segment (OS) and outer nuclear layer (ONL) thickness showed marginally significant annual changes ($P < 0.05$), while total receptor (TR) thickness showed a greater change ($P < 0.01$). All measures derived from the edge of the EZ band significantly decreased ($P < 0.01$). Mean $\pm$ SD for test-retest differences in horizontal widths was 0.01 ± 0.06 mm.

Conclusions: Measures of the EZ band are more effective in detecting disease progression than are thickness measures. Given the similar effectiveness of line and volume scans, manually marking the EZ band edge on vertical and/or horizontal line scans can be useful in tracking progression.

Translational Relevance: Because disease progression in RP can be relatively slow, annual changes can be difficult to monitor during the course of a clinical trial. Here we suggest a quick, effective, and reliable method for detecting subtle changes.

Introduction

Retinitis pigmentosa (RP) is a heterodegenerative disorder that typically starts with midperipheral vision loss and can lead to complete blindness. Because the progression of visual loss is relatively slow in patients with RP, detecting change during the course of a typical clinical trial presents a challenge. Frequency domain optical coherence tomography (fdOCT) offers a possible method for following disease progression in clinical studies of RP. With fdOCT, anatomically distinct layers of the outer retina can be visualized.^{1,2} Various aspects of the

outer retina, as seen on the fdOCT, have been quantitatively measured in eyes with RP. A number of studies have reported a decrease in thickness of the total receptor, outer nuclear layer (ONL), and/or outer segment (OS) due to RP.^{3–13} Further, the thickness of these layers in the transition zone between healthy and severely affected retina provides a possible model of disease progression.^{9–11} The earliest change seen in the transition zone with fdOCT is a decrease in the thickness of the OS layer, followed by decreases in the ONL.^{10,11} With further loss in vision, the OS region disappears completely. This disappearance of the OS region does not occur until 9 to 10 dB of sensitivity has been lost as

measured with static automated perimetry.^{8,12,13} However, the point at which the OS region disappears is a marker of the edge of the usable visual field,¹² meaning the location at which visual sensitivity shows a precipitous drop. Most important, change in the location at which the OS layer is no longer present appears to be an excellent measure of progression of visual loss in patients with RP.^{12,13}

However, the OS is less than 40 μm ^{4,8} thick and thus measures of its thickness are variable. Fortunately, it is not necessary to measure OS thickness in order to estimate the point on the retina where it disappears. One can simply measure the point at which the inner segment ellipsoid zone (EZ) is no longer present. The EZ band (also known as inner segment [IS]/OS band) is thought by some² to be due to light scattered by the mitochondria of the ellipsoid region of the IS, and by others¹⁴ to be due to the change in refractive indices of the IS and OS. In either case, by convention, the OS thickness is measured between the EZ band and the proximal border of the retinal pigment epithelium (RPE). Thus, when the EZ band disappears (i.e., is no longer discernible from the RPE border), the OS thickness is by definition zero.

While previous studies have shown that the EZ band can be used to track annual progression,^{12,13} it is not clear if this is the best OCT measure. The fdOCT volume scans offer a number of possible measures of the thickness and volume of the OS layer and ONL. Here we compare various outer retinal measures derived from fdOCT line and volume scans in a group of x-linked (xl) RP patients who were followed over time.

Methods

Subjects

Twenty-seven patients with x-linked retinitis pigmentosa (xLRP) (age 15.3 ± 6.4 years; range 7–27 years) were included in this study. They were selected from a larger group of patients involved in an ongoing double-blind treatment trial (docosahexaenoic acid versus placebo; clinicaltrials.gov NCT00100230). To be included, the central 30° of the visual field had to contain regions both with and without an EZ band.¹³ Twenty-eight patients met these inclusion criteria,¹³ but one was omitted due to the poor quality of the fdOCT macular volume scan. Cystoid macular edema, if present, was mild and did not affect the EZ band.

All procedures adhered to the Declaration of

Helsinki and were approved by the Institutional Review Board at the University of Texas Southwestern Medical Center. Informed consent was obtained from all participants.

Frequency Domain OCT Measurements

The fdOCT images (Spectralis HRA+OCT; Heidelberg, Germany) from one eye per patient were obtained in sessions approximately 2 years apart. Volume scans and averaged (100 scans) horizontal and vertical line scans along the horizontal and vertical meridian were acquired at both visits.

Volume Scans

For each volume scan, all 31 individual b-scans were segmented using an automated program¹⁵ and manually hand corrected as needed.³ The volume scan was centered at the fovea, and automated eye tracking (ART) was used to align multiple scans. Scans from subsequent visits were captured with the aid of automatic registration to align scan placement. Five borders were segmented (Fig. 1): (1) border between the inner nuclear layer (INL) and outer plexiform layer (OPL); (2) outer limiting membrane (OLM); (3) EZ band; (4) proximal edge of the retinal pigment epithelium (pRPE); and (5) Bruch's membrane (BM)/choroid boundary. From these five boundaries, the thickness of four layers could be calculated: (1) ONL: between INL/OPL and OLM; (2) OS: between EZ and pRPE; (3) RPE: between pRPE and BM/choroid; and (4) total receptor plus RPE (total receptor [TR] thickness) between INL/RPE and BM/choroid. By taking the global average thickness of each layer across the volume scan at both the initial and final visits, four measures of progression (OS, ONL, RPE, and TR thickness) were derived from the volume scans.

The disappearance of the EZ band corresponds to the location where the OS thickness (the distance between the EZ band and the pRPE) goes to zero. Each b-scan of the volume was segmented as described above, and the disappearance of the EZ band (i.e., the "EZ edge") was marked on each scan (red arrow in Fig. 1). The EZ contour was derived by interpolating between the EZ edge points throughout the volume scan.¹²

From the EZ contour and volume scans, four measures were calculated: (1) OS volume within the EZ contour; (2) area within the EZ contour; (3) EZ band horizontal width (EZ HW); and (4) EZ band vertical width (EZ VW). Horizontal width was defined as the distance between the nasal and

Figure 1. Horizontal midline fdOCT scan of the nasal retina with five borders manually segmented.

temporal edges of the EZ band across the center b-scan, while vertical width was calculated as the distance between the superior and inferior edges of the EZ band on the b-scans in which it was discernible.

Line Scans

The final two parameters studied were the EZ HW and EZ VW derived from the horizontal line and vertical line scan, respectively. Widths on the line scans were determined by marking the nasal and temporal EZ edges by hand, without manual or automated segmentation of the entire EZ band. In order to test for repeat reliability of EZ HW, the EZ edges were marked on two scans obtained from two sessions on the same day for an independent sample of 18 RP patients¹³ (age 43.5 ± 18.0 years) of mixed genetic types, and a Bland-Altman analysis was performed. For all EZ-derived measures, annual loss was defined as the change between the initial and final visits divided by the number of years between visits.

For all measures, two-tailed *t*-tests were used to test the hypothesis that the annual change was not significantly different from zero.

Results

Average Thickness Measurements

Figure 2 shows the thickness of the OS, ONL, and TR regions across the volume scan for a healthy control and at the initial and final visits for two xLRP patients. The two patients were chosen as representative of the xLRP population studied. All scans were oriented as if they were from the right eye. The color

bar on the right indicates thickness (mm) across the volume scan, with thinner and thicker regions presented as cooler (blue) and warmer colors (red), respectively.

As expected, the xLRP patients showed a loss of OS, ONL, and TR thickness. As illustrated in Figure 2A, the OS was missing (dark blue) except in the central region. Similarly, the ONL (Fig. 2B) and TR thicknesses (Fig. 2C) are noticeably thinner than in controls in the periphery. Changes between the initial and the final visit are subtle, although patient 2 has a clear constriction of the OS layer, and both patients have a constriction of the TR layer.

In order to quantify the changes between the initial and final visits, annual thickness changes were calculated for all 27 patients (Table 1). The annual changes for each patient are plotted as open circles in Figure 3, where patients 1 and 2 from Figure 2 are represented as filled green and red circles, respectively. The boundaries of the box plots indicate the 25th, 50th, and 75th percentile, and the thick black bar is the median. A value of 0 μm (horizontal dashed line) represents no change in average thickness, while negative values indicate a loss between visits. The change in OS thickness was small, but significant ($P = 0.013$), with 24 of the 27 patients exhibiting OS loss. The change in ONL thickness just met a 5% significance level ($P = 0.044$), with 22 patients showing a loss. Total receptor thickness showed the largest annual change. While this change was statistically significant ($P = 0.006$), only 20 of 27 patients showed a decrease in thickness. Finally, there was no significant change in RPE thickness. The median change was close to 0 μm , and only 12 patients exhibited thinning (Table 1).

Figure 2. Pseudo-color thickness maps for fOCT volume scans in one control (left column) and two representative patients (columns 2–5). Difference in thicknesses between the initial visit (columns 2 and 4) and final visit (columns 3 and 5) are illustrated for the outer segment layer (A), outer nuclear layer (B), and total receptor layer (C). Color legend given on the right in millimeters.

Measured Based Upon EZ Contour and Volume Scan

Figure 4 shows the OS thickness maps for two patients (patients 3 and 4) with EZ contours (white) superimposed. As evidenced in Figure 4, at both time points there was a central region preserved where the

OS is the thickest. It thins out into the periphery until it reaches 0 mm at the EZ contour. Annual decreases in OS volume and OS area within the EZ contour, EZ HW (dotted red line in inset), and EZ VW (dashed red line in inset) were calculated for each individual.

The mean $\pm$ SD annual changes are provided in Table 2. Figure 5 shows box plots similar to the global

Table 1. Average Decrease in Thickness per Year

Layer	Mean $\pm$ SD, μ m	No. of Eyes Showing Loss	<i>t</i> Statistic	<i>P</i> Value
OS	0.39 $\pm$ 0.77	24	2.7	0.013*
ONL	0.89 $\pm$ 2.2	22	2.1	0.044*
TR	1.9 $\pm$ 2.5	20	3.9	6.0 $\times 10^{-3}$ **
RPE	0.085 $\pm$ 1.8	12	0.25	0.80

* $P < 0.05$, ** $P < 0.01$.

Figure 3. Box plots showing the distribution of losses in annual global average thickness for the OS, ONL, and TR thickness. Individual thickness losses are plotted as *circles*, including patient 1 (*green circle*) and patient 2 (*red circle*) from [Figure 2](#). *Black lines* indicate median values. Significance levels are illustrated with *stars* above the box plot (* $P < 0.05$, ** $P < 0.01$).

Figure 4. Pseudo-color thickness maps for fdOCT volume scans in two patients. Ellipsoid zone contour superimposed onto outer segment thickness map. *Inset:* Expanded view of the EZ contour (*white border*) with the EZ horizontal width (HW; *dotted line*) and vertical width (VW; *dashed line*) marked.

Table 2. Average Decrease in EZ Contour-Derived Measures per Year

Measure	Mean $\pm$ SD	No. of Eyes Showing Loss	t Statistic	P Value
OS volume	$1.2 \times 10^7 \pm 2.1 \times 10^7 \mu\text{m}^3$	23/27	2.9	0.0062**
Area	$6.4 \times 10^5 \pm 6.5 \times 10^5 \mu\text{m}^2$	26/27	5.2	<0.0001**
EZ HW	$210 \pm 340 \mu\text{m}$	21/27	3.2	0.0036**
EZ VW	$140 \pm 140 \mu\text{m}$	19/27	5.1	<0.0001**

** $P < 0.01$.

average measurements in Figure 3. The two patients in Figure 4 are shown as filled green and red circles. Although these two patients started with similar areas at the first visit, little progression was seen for patient 3 (green circle), while patient 4 (red circle) exhibited clear progression between visits. The annual changes in OS volume ($P = 0.0062$), EZ contour area ($P < 0.0001$), EZ HW ($P = 0.0036$), and EZ VW ($P < 0.0001$) were all significant. The area within the contour was the most robust measurement, showing loss in 26 of 27 patients.

EZ Measures From High-Quality Line Scan

The results above suggest that the measures of EZ HW and VW are as good as or better than any of the metrics derived from using the entire volume scan. Line scans provide these same two measures (EZ HW and EZ VW) on, in general, higher-quality images. In

addition, the vertical scan has the added advantage of providing a more precise measurement of EZ VW without the need to interpolate between b-scans to estimate the edge of the EZ band.

By marking the temporal/nasal and superior/inferior EZ edges on both line scans, a loss in EZ width was measured in 26 of 27 patients on the horizontal line scan and in 24 of 25 patients on the vertical line scan. (A vertical line scan was not available for one patient, and a second did not have an EZ band on the vertical line scan on either visit.) These data are presented as box plots in Figure 6 and as mean $\pm$ SD in Table 3. The changes in both EZ HW and EZ VW are highly significant, but not significantly different from each other. Further, there was an approximately equal annual rate of change in the horizontal and vertical directions.

To assess the repeat reliability of the EZ HW

Figure 5. Box plots showing the distribution of annual losses in OS volume, EZ contour area, EZ HW, and EZ VW. Individual losses are plotted as circles, including patient 3 (green circle) and patient 4 (red circle) from Figure 4. Black lines indicate median values. Significance levels are illustrated with stars above the box plot. All measures met the $P < 0.01$ significance level (indicated by two stars).

Figure 6. Box plots showing the distribution of annual losses in EZ HW and VW measured from high-quality line scans. Individual losses are plotted as circles. Black lines indicate median values. Significance levels are illustrated with stars above the box plot. All measures met the $P < 0.01$ significance level (indicated by two stars).

measure, the EZ edges were marked on two scans obtained from the same session for an independent sample of 18 RP patients (see Methods). As seen in Figure 7, test-retest differences were independent of the average EZ HW, and the mean difference between widths calculated at the same testing session was 10 μm . The 95% limits of agreement (dashed lines) fell within $\pm 127 \mu\text{m}$. Mean annual constriction of the EZ band in the xLRP population tested was 270 μm , which is well outside the 95% limits of confidence of test-retest differences.

Discussion

Our findings confirm previous reports^{12,13} that a change in the EZ width is a good measure of progression in patients with RP. While a priori it seemed reasonable that global thickness measures might provide a better metric, only TR thickness showed an average annual loss that was significant at the $P < 0.01$ level, and losses were seen in only 20 of

Figure 7. Difference in EZ width from repeat scans as a function of mean EZ width. Mean difference in EZ width (dark black dashed line) and 95% limits of agreement (light black dashed line) are shown. Difference of 0 μm is shown as a red dashed line.

the 27 patients studied. In general, metrics derived from the EZ contour did better. In retrospect, this is not surprising. According to the transition zone model of RP disease progression,¹⁰ the OS thickness within the EZ contour changes relatively little with progression. Therefore, the significant reduction in the OS volume within the area confined by the EZ contour was driven by the inward movement of the EZ edge rather than by the changes in thickness of the OS region within this area. In fact, the area within the contour was able to pick up losses in the same patients as the OS volume measure.

For clinical purposes, measuring the location of the EZ edge on horizontal or vertical line scans is the most practical measure. One can measure the EZ width by simply marking two points on the line scan, without the need for segmentation algorithms. In principle, one might expect that the change in area measured within the EZ contour on a volume scan might be a better metric as it utilizes information from the entire volume scan. However, the EZ HW and

Table 3. Change in High-Quality Line Scan Measures

Measure	Mean $\pm$ SD, μm	No. of Eyes Showing Loss	<i>t</i> Statistic	<i>P</i> Value
EZ HW	270 $\pm$ 390	26/27	3.6	0.0013**
EZ VW	280 $\pm$ 230	24/25	6.0	<0.0001**

** $P < 0.01$.

VW from the line scans performed just as well. Because of the symmetric nature of the disease, progression can be detected on just the horizontal or the vertical scan. Additionally, image quality on the line scans is typically higher than on the volume scan, due to the use of more extensive averaging of individual b-scans. Finally, this method of marking two points on a line scan has good repeat reliability (Fig. 7). The patients for this analysis are a subset of patients reported by Birch et al.,¹³ who found a mean test–retest difference of 23 μm and 95% limits of agreement within 124 μm , close to the values we obtained. That is, without segmentation of the entire EZ band as in their study, similar repeat reliability of the EZ width was obtained.

There are limitations to the proposed EZ width method. First, this method is not useful in cases of extreme RP, when the EZ is no longer present. The EZ band cannot be measured in these cases, and the OCT protocol is difficult to administer because these patients often have poor fixation. Second, due to the current limitations of the OCT, loss of retinal function beyond approximately 30° cannot be easily measured. Third, both rod and cone photoreceptors need to be affected before the EZ band disappears.¹⁶ If rod receptor loss precedes cone death, as in Massof and Finkelstein's¹⁷ "type 1 RP," the EZ band will track cone function, not rod function. This is not necessarily a disadvantage, as traditional visual fields also track cone function. Fourth, it remains to be determined if this method has clinical utility in treatment trials that aim to recover vision, as we do not know the extent to which recovery of the EZ band tracks recovery of visual sensitivity. Finally, the same person marked all the EZ edges and corrected all the volume scans in this study. We know from previous work,¹⁸ that individuals differ in terms of how they segment lines and presumably mark the edge of the EZ band. However, these individual differences can be minimized with the use of a few pages of instructions on how to deal with ambiguous cases. Whether one uses corrected volume scans or marks by hand the end of the EZ band, a simple manual should be used to maximize consistency across segmenters, as well as across different centers.

In summary, we suggest that the EZ width, as measured by hand on fdOCT high-quality horizontal and vertical line scans, provides a sensitive, easy, and reliable method of tracking disease progression in patients with RP. With a set of guidelines for marking the EZ edge, volume scans and segmentation algorithms may not be necessary.

Acknowledgments

The authors thank Xian Zhang, Ali Raza, and David Rhee for their help.

This study was supported by grant R01-EY-09076 from the National Eye Institute and by grant FDR-02543 from Foundation Fighting Blindness.

Disclosure: **R. Ramachandran**, None; **L. Zhou**, None; **K.G. Locke**, None; **D.G. Birch**, None; **D.C. Hood**, Topcon, Inc. (F, C)

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