A Comparison of Progressive Loss of the Ellipsoid Zone (EZ) Band in Autosomal Dominant and X-Linked Retinitis Pigmentosa

Cindy X. Cai,¹ Kirsten G. Locke,² Rithambara Ramachandran,³ David G. Birch,^{2,4} and Donald C. Hood^{3,5}

¹College of Physicians and Surgeons, Columbia University, New York, New York, United States

²Retina Foundation of the Southwest, Dallas, Texas, United States

³Department of Psychology, Columbia University, New York, New York, United States

⁴Department of Ophthalmology, University of Texas Southwestern Medical Center, Dallas, Texas, United States

⁵Department of Ophthalmology, Columbia University, New York, New York, United States

Correspondence: Donald C. Hood, 406 Schermerhorn, 1190 Amsterdam Avenue, New York, NY 10027, USA; dch3@columbia.edu.

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PURPOSE. In patients with retinitis pigmentosa (RP), the inner segment ellipsoid zone (EZ; also known as the inner segment/outer segment [IS/OS] border) is a marker of the usable visual field at a given point in time and of the progression of the disease over time. Here we compare the change in the width per year of the EZ band in patients with autosomal dominant (ad) and x-linked (xl) RP.

METHODS. Using optical coherence tomography (OCT), 9-mm horizontal and vertical line scans through the fovea were obtained for one eye of 26 xLRP patients and 33 adRP patients. Scans were repeated on average 2.0 years later (range, 0.6–4.8 years). Using a manual segmentation procedure, the EZ band was delineated and its horizontal width (HW) and vertical width (VW) were determined.

RESULTS. The adRP and xLRP patients had similar initial EZ HW (xLRP: $11.8 \pm 5.4^\circ$, adRP: $12.4 \pm 6.3^\circ$, $P = 0.69$) and VW (xLRP: $8.5 \pm 4.9^\circ$, adRP: $11.4 \pm 7.1^\circ$, $P = 0.09$). However, between visits the absolute loss and percent loss of the EZ width per year was significantly greater for xLRP than adRP for both HW (xLRP: $1.0 \pm 0.6^\circ/\text{y}$, $9.6 \pm 5.6\%/\text{y}$; adRP: $0.4 \pm 0.5^\circ/\text{y}$, $3.4 \pm 5.4\%/\text{y}$; $P < 0.001$) and VW (xLRP: $0.8 \pm 0.8^\circ/\text{y}$, $9.2 \pm 8.9\%/\text{y}$; adRP: $0.3 \pm 0.5^\circ/\text{y}$, $4.2 \pm 6.4\%/\text{y}$; $P < 0.01$). There was a weak correlation between the loss of EZ width per year and the initial width for xLRP ($r^2 = 0.17$, $P = 0.036$), but no correlation for adRP ($r^2 = 0.004$, $P = 0.73$). The test-retest difference of EZ HW was $0.2 \pm 0.5^\circ$.

CONCLUSIONS. The OCT data here support a faster rate of loss per year in the case of xLRP. (ClinicalTrials.gov number, NCT00100230.)

Keywords: retinitis pigmentosa, optical coherence tomography, progression

Retinitis pigmentosa (RP) describes a heterogeneous set of hereditary retinal dystrophies characterized by the progressive degeneration of rod and cone photoreceptor cells.^{1–3} The genetic basis of this disease is quite complex. At least 45 loci have been identified to be responsible for the disorder.⁴ It can be inherited as an autosomal-dominant (ad) condition (approximately 30%–40% of cases), autosomal-recessive condition (50%–60%), x-linked (xl) trait (5%–15%), and may even follow non-Mendelian inheritance patterns such as mitochondrial inheritance.² The xl disease is the most severe form of RP.⁵ These patients classically lose night vision within the first decade of life, experience reduced visual fields by the second decade, and finally suffer from severely decreased visual acuity by the fourth decade. By contrast, autosomal dominant forms are milder with reasonably good visual acuity often preserved until the sixth or seventh decade.^{5–8}

Most studies agree that xLRP starts earlier than adRP. However, some find that xLRP progresses faster than adRP thus contributing to its more severe phenotype, while others do not.^{8–11} Most of these studies used electroretinography (ERG)

or visual fields to follow disease progression; however, newer studies, suggest that optical coherence tomography (OCT) may be a more sensitive method to study RP.^{12–15}

One of the highly reflective structures in the outer retina that can be detected on OCT is the inner segment ellipsoid zone (EZ; also known as the inner segment/outer segment [IS/OS] line).^{16,17} This is an important anatomic landmark in RP as its disappearance marks the boundary between healthy and unhealthy retina.¹⁸ The point where this band disappears corresponds to the edge of the usable visual field. In particular, there are large changes in visual field sensitivities on either side of the edge of the EZ band.¹³ In addition, the presence of the EZ band has been found to correlate with visual acuity.^{19,20}

Recently, the EZ band has been proposed as a reliable marker of progression in RP, one that is more sensitive than currently used measures.^{12–14} Birch et al.¹² found that 95% of all test-retest differences were less than 0.43° with retest variability considerably less than values typically reported for full-field ERG cone flicker, kinetic perimetry, and static perimetry. Ramachandran et al.¹⁴ also found similar test-retest

TABLE. Demographics of the Individual xLRP and adRP Patients

ID Number	Age, y	Mutation	Sex	BCVA
xLRP				
3347	23	RPGR	M	1
5500	17	RPGR	M	0.67
6611	21	RPGR	M	0.67
6951	20	NA*	M	0.8
7617	13	RPGR	M	1
7669	17	RPGR	M	1
7715	16	RPGR	M	0.67
7765	14	RPGR	M	0.5
7811	14	RPGR	M	0.8
7846	20	RPGR	M	0.33
7938	17	RPGR	M	0.33
7956	13	RPGR	M	0.5
7993	13	RPGR	M	0.4
7994	17	RPGR	M	0.8
8011	31	RPGR	M	0.67
8017	16	RPGR	M	0.67
8083	15	RPGR	M	0.8
8124	20	RPGR	M	0.67
8157	13	RPGR	M	0.8
8271	12	RPGR	M	0.5
8272	22	RPGR	M	0.5
8280	30	RPGR	M	0.8
8367	16	RPGR	M	1
8553	21	RPGR	M	0.8
8555	14	RPGR	M	0.67
9208	15	RPGR	M	0.5
adRP				
333	56	RHO P23H	M	0.5
652	64	RHO P23H	F	0.63
2411	65	RHO P23H	M	0.33
2670	65	RHO C185A	M	0.67
2826	37	RHO P23H	F	0.8
3540	49	RHO P23H	F	1
4476	44	RHO P23H	F	0.8
4545	72	RHO P23H	F	0.67
4828	69	KLHL7	M	0.8
4829	62	KLHL7	M	1
5740	47	RHO P170A	F	1
5770	71	RHO P23H	M	0.5
5784	54	RHO P23H	F	0.8
6043	71	RHO P170A	F	1
6922	55	RHO P23H	M	1.2
6931	68	RHO A190A	F	1
6982	45	NA	M	0.63
7443	55	RHO T553C	M	1.2
7636	62	RHO P23H	F	0.5
7989	64	NA	F	1.2
8038	48	NA	F	0.3
8126	15	IMPDH1	M	0.63
8583	67	RP1	M	0.63
8794	59	NA	F	1.2
8898	17	PRPF31	M	0.63
9207	61	PRPH2	F	0.8
9715	49	RHO P23H	M	1.2
10083	60	PRPF31	M	0.5
10196	58	RHO G64X	F	0.33
10258	62	NA	M	0.63
10467	47	PRPF3	F	0.63
10468	55	PRPF3	F	0.63
10842	43	RHO P23H	F	0.5

* NA, not available.

Information is provided regarding age of patients (in years), the specific mutation (NA when not available), sex, and best correct visual acuity (BCVA).

differences and that the EZ was a more sensitive marker of change than other parameters derived from OCT, such as thickness. Here, we measure the edge of the EZ band in patients examined over a 2-year period to test the hypothesis that the rate of xLRP disease progression is faster than that of adRP.

METHODS

Subjects

Twenty-six xLRP patients (mean, 17.7 years; range, 12–31 years) and 33 adRP patients (mean, 55.0 years; range, 15–72 years) were included in the study.¹² Because of the inclusion requirements, which included regions with and without a discernible EZ band within the central 30° of the retina, the adRP sample was significantly older than the xLRP sample ($t = 13.3$; $P < 0.0001$). The xLRP patients were selected from a larger group of patients involved in a double-blind treatment trial (docosahexaenoic acid [DHA] versus placebo; clinicaltrials.gov NCT00100230).²³ The adRP patients were selected from a preexisting database of patients followed clinically at the Retina Foundation of the Southwest. The xLRP patients were from pedigrees where males were more severely affected than females and obligate carriers showed minimal or mild disease. The adRP families had at least three consecutive generations affected. All patients were screened for mutations, which were found in almost all cases. Of the 26 patients with xLRP, 25 had an RPGR mutation. Of the 33 adRP patients, 19 had a RHO mutation, 1 IMPDH1, 2 KLHL7, 1 PRPH2, 2 PRPF3, 2 PRPF31, and 1 RP1. The demographic information regarding these patients is located in the Table. Cystoid macular edema (CME), if present, was mild and in general did not affect the EZ band. Only 1 adRP patient had a break in the EZ band in the macular region due to CME, but this break did not obscure the edge where the IS/OS met the RPE. Additionally, the patients had to have at least one repeat visit at least 7 months later. One eye was chosen from each patient for analysis. When multiple scans were available, the one with the clearest EZ bands was chosen.

Frequency Domain OCT Measurements

Nine-millimeter horizontal and vertical line scans (HLS and VLS, respectively) through the fovea, taken with Spectralis HRA+OCT (Heidelberg Engineering, Vista, CA, USA) using the eye-tracking feature (ART) and an average of 100 images, were obtained for one eye of each patient at two visits. The length of follow-up was similar in the two groups (xLRP: mean 2.0 years; range, 1.9–2.3 years; adRP: mean 2.0 years; range, 0.6–4.8 years). One of the 26 xLRP patients did not have VLSs. Four of the 33 adRP patients did not have a VLS, and one did not have VLSs meeting study criteria (i.e., the EZ was not discernible).

All scans had a quality index of greater than 25 dB (range, 0–40 dB). Using a manual segmentation procedure,^{18,21} two boundaries were identified: the proximal edge of the retinal pigment epithelium (pRPE) located adjacent to the photoreceptor outer segments and the EZ band, labeled in magenta and green, respectively, in Figure 1. For all scans, the nasal and temporal edges of the EZ band were defined as the locations where the EZ band met the pRPE. The width of the EZ band was defined as the distance between these two locations and designated the horizontal width (HW) when obtained from the HLS or vertical width (VW) from VLS (Fig. 1). This retinal distance is represented in degrees assuming a conversion of 0.289 mm/degree. The rate of annual loss was estimated for

FIGURE 1. Horizontal midline frequency domain optical coherence tomography scan through the fovea with two borders manually segmented. The HW of the EZ band is defined as the distance between the nasal and temporal end points (i.e., where the middle of the EZ band merges with the pRPE).

each patient by dividing the change between the initial and final visits by the number of years between visits.

In order to test the repeat reliability of measuring the EZ width, the HW from a subset of adRP patients with Pro23His mutations in the rhodopsin protein ($n = 13$) was measured on two separate occasions by the same grader using the same scans. The grader was not allowed to consult the first set of measurements. A Bland-Altman analysis was performed to assess repeatability.

Two-tailed *t*-tests were used to test the hypothesis that adRP and xLRP did not differ from each other. For Pearson correlations, a two-tailed *P* value was generated to evaluate significance.

RESULTS

At the initial visit, the xLRP and adRP patients had similar EZ band HW (xLRP: $11.8 \pm 5.4^\circ$, adRP: $12.4 \pm 6.3^\circ$, $P = 0.69$) and

VW (xLRP: $8.5 \pm 4.9^\circ$, adRP: $11.4 \pm 7.1^\circ$, $P = 0.09$), as expected given the inclusion criteria. The initial HW and VW showed a strong correlation in both the xLRP and adRP groups (xLRP $r^2 = 0.91$, $P < 0.0001$; adRP $r^2 = 0.91$, $P < 0.0001$; Fig. 2).

Figure 3 shows the HLS and VLS from a pair of sample xLRP and adRP eyes both with an initial EZ HW of 10° . At higher magnification (insets), the edges of the EZ band at the initial and follow-up visits are marked in dotted yellow and solid yellow, respectively. For most eyes, the edges of the EZ band move closer to the fovea at the follow-up visit consistent with the known shrinking of the EZ over time, although this was not always the case. For example, the VLS of patient B (lower right panel in Fig. 3) demonstrated a slightly wider EZ band at the later visit. In particular, 26 of the 26 (100%) xLRP eyes and 24 of the 33 (73%) adRP eyes demonstrated a loss of HW, while 23 of the 25 (92%) xLRP eyes and 22 of 28 (79%) adRP eyes showed a loss of VW. Thus, a greater percentage of xLRP eyes showed loss when compared to adRP, and the loss in HW was similar to the loss in VW in both genetic groups.

FIGURE 2. Scatter plots of the initial horizontal and vertical EZ widths for the adRP (blue) and xLRP (red) patients. Individuals are plotted as open circles, and the solid line is the best fitting linear line. The correlation for the two groups is as follows: xLRP $r^2 = 0.89$, $P < 0.0001$; adRP $r^2 = 0.91$, $P < 0.0001$.

FIGURE 3. The horizontal and vertical scans from an xIRP and adRP patient both with similar initial horizontal EZ width. Insets show higher magnification of the EZ band with endings marked. Dashed vertical yellow line indicates the end of the EZ band at the initial visit, while the solid yellow line is for the last visit. The percent loss of the EZ width per year for each patient is located in the upper left hand corner of each panel.

In most cases, xIRP patients demonstrated a greater degree of loss of EZ width compared to adRP with the same initial EZ HW. This can be seen qualitatively as a wider difference between the dotted and solid green lines in patient A (xIRP) compared to patient B (adRP) in Figure 3. In Figure 4, the percent of EZ width loss per year for each patient is plotted as

open circles, where the data for the patients' scans in Figure 3 are coded in color (see figure caption). The estimated annual loss of HW over the mean 2-year follow-up was significantly greater ($P < 0.001$) for xIRP ($1.0 \pm 0.6\%/y$) than for adRP ($0.4 \pm 0.5\%/y$) patients, as was the percent loss per year ($P < 0.001$), $9.6 \pm 5.6\%/y$ (xIRP) vs. $3.4 \pm 5.4\%/y$ (adRP; Fig. 4).

FIGURE 4. Loss per year (in percent) of the EZ band horizontal (a) and vertical (b) widths. Individuals are represented as open circles and the mean $\pm$ standard error as solid squares. The patients whose scans are shown in Figure 3 are represented as solid circles, Patient A as dark purple, and patient B as light purple.

FIGURE 5. Bland-Altman plot showing the difference in EZ width from repeat analysis as a function of mean EZ band width. Mean difference in EZ width (solid black line) and 95% limits of agreement (black dashed line) are shown. Difference of 0° is shown as a red dashed line.

Loss of VW showed a similar pattern where degree loss per year was significantly ($P = 0.02$) greater in xLRP ($0.8 \pm 0.8^\circ$) than adRP ($0.3 \pm 0.5^\circ$) in addition to the percent loss per year (xLRP: $9.2 \pm 8.9\%$; range, adRP $4.2 \pm 6.4\%/y$, $P = 0.02$; Fig. 4). The percent loss per year of the HW was not statistically different from the VW in either the xLRP or adRP groups ($P = 0.28$ and $P = 0.91$, respectively).

There was no correlation between the loss of the EZ HW per year and the initial EZ HW for either xLRP ($r^2 = 0.026$, $P = 0.41$) or adRP ($r^2 = 0.004$, $P = 0.73$). There was a weak correlation between the loss of the EZ VW and initial EZ VW for xLRP ($r^2 = 0.17$, $P = 0.036$) but no correlation for adRP ($r^2 = 0.014$, $P = 0.54$).

The repeat reliability of the EZ width was tested through an analysis of the HLSs of a subset of adRP patients on two separate occasions (see Methods). We chose patients with Pro23His for this purpose because they represented the largest genetic subgroup in the adRP database. In any case, the test-retest variability was comparable to that found in our previous studies.^{12,14} The mean difference between widths was 0.2° and 95% of all values fell within $\pm 0.9^\circ$. These differences were independent of the average EZ width as shown in the Bland-Altman plot in Figure 5.

DISCUSSION

Given the similar initial EZ widths and length of follow-up between adRP and xLRP, the OCT data suggest a faster rate of progression in xLRP ($9.4\%/y$) compared to adRP ($3.8\%/y$). Assuming a roughly circular region of preserved retina and estimating the diameter as the average of the EZ HW and VW for each individual, there was, on average, a decrease in the preserved retinal area of $19.2\%/y$ for xLRP and $7.9\%/y$ for adRP. The xLRP eyes lost functioning retinal area at a 2.5 times faster

rate than did the adRP eyes. This is a slightly larger difference than the ratio of visual field loss reported by Sandberg et al.⁸ who found a 4.7% loss of visual field area for xl RPGR mutations compared to 2.9% for the ad RHO mutation. Birch et al.⁹ also found a similar difference in loss of rod specific visual field area, 4.4% for xLRP and 2.0% for adRP.⁹ Our results are within the range of changes in visual field area reported by others without regard to genetic subtypes: 4.6%/y by Berson et al.²² and 14% to 17%/y by Massof et al.¹⁰

On the other hand, Massof et al.¹⁰ found no difference in the visual field change between the xLRP ($n = 12$) and adRP ($n = 28$) groups. Both followed a first-order exponential decay model with statistically similar time constants, although as expected the xLRP patients started to lose field area at an earlier age. According to their model, if we catch adRP and xLRP at equivalent points along the common exponential decay curve and follow them for the same amount of time, we should find the same percent decrease per year in the two populations. In our study, we followed the two populations from equivalent points, as they had similar starting EZ widths, and followed both populations for 2 years, and yet found a difference. There are two methodological differences between our studies. First, they used data from visual fields and we used OCT scans. Second, they excluded patients whose fields had either increased or remained stable over the course of their study, while we did not exclude those patients.

One of the strengths of this study is that the OCTs were obtained from patients recruited for prospective studies. Thus it avoids the limitation of retrospective studies, which can contain a selection bias toward evaluation of only those patients who return because they believe their visual function is deteriorating.²² On the other hand, the fact that the xLRP patients were part of a study to see if nutritional DHA supplement might slow progression raises a concern. However, if DHA did slow progression, then this should decrease the

differences we find between the xLRP and adRP groups and not affect our main point of a difference in rates. In any case, there did not appear to be a difference in progression of placebo and treatment groups, at least as measured with the ERG.²³

There are, however, limitations to the EZ width method. It requires that the patients have a discernible EZ bands within the scan area. In addition, current technology restricts the scans to the central 30° of retina. These two factors preclude the tracking of patients with less advanced disease, as well as those with very advanced disease.

In any case, by tracking the loss of EZ width as a marker of disease progression, we find that xLRP progresses at a faster rate than adRP.

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