Long-Term Characterization of Retinal Degeneration in *rd1* and *rd10* Mice Using Spectral Domain Optical Coherence Tomography

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PURPOSE. We characterize the in vivo changes over time in the retinal structure of wild-type mice alongside two lines of mice deficient in the β -subunit of phosphodiesterase (*rd1* and *rd10* mice) using spectral domain optical coherence tomography (SD-OCT).

METHODS. SD-OCT images were obtained using the BiopTigen spectral domain ophthalmic imaging system (SDOIS). Wild-type C57BL/6J, *rd1* and *rd10* mice ranging in age from P14 to P206 were sedated with 1% isoflurane. Horizontal and vertical linear scans through the optic nerve, and annular scans around the optic nerve were obtained.

RESULTS. SD-OCT imaging of wild-type mice demonstrated visibility of the inner segment/outer segment (IS/OS) junction, external limiting membrane (ELM), outer nuclear layer (ONL), and outer plexiform layer (OPL). At P14, most *rd10* mice exhibited normal SD-OCT profiles, but some displayed changes in the IS/OS junction. At the same time point, *rd1* mice had severe outer retinal degeneration. In *rd10* mice, imaging revealed loss of the IS/OS junction by P18, hyperreflective changes in the ONL at P20, hyperreflective vitreous opacities, and shallow separation of the neural retina from the RPE. Retinal separations were not observed in *rd1* mice. Segmentation analysis in wild-type mice demonstrated relatively little variability between animals, while in *rd10* and *rd1* mice there was a steady decline in outer retinal thickness. Histologic studies demonstrated correlation of retinal features with those seen on SD-OCT scans. Segmentation analysis provides a quantitative and reproducible method for measuring in vivo retinal changes in mice.

CONCLUSIONS. SD-OCT provides a non-invasive method of following long-term retinal changes in mice in vivo. Although *rd10* and *rd1* mice have mutations in the same gene, they

demonstrate significantly different features on SD-OCT. (*Invest Ophthalmol Vis Sci.* 2012;53:4644–4656) DOI:10.1167/iov.12-9611

Retinitis pigmentosa (RP) is a genetically heterogeneous disease that results in photoreceptor cell death, leading to constriction of visual fields, difficulties with night vision, and eventual loss of visual acuity. Approximately 5% of humans with RP have a mutation in the gene coding for the β -subunit of phosphodiesterase (PDE).¹ Efforts to understand the pathologic mechanisms underlying inherited retinal disease have used the *Pde6b^{rd1}* mouse (*rd1* mouse).²

In these animals, a spontaneous mutation in the gene for the β -subunit of PDE leads to a severe degeneration of photoreceptors starting at postnatal day 8 (P8) and progressing to complete loss of the rods by P20.^{2–7} The cones degenerate, but they do so at a slower rate. Although the *rd1* mouse has been a useful model for studying potential therapies, it has several limitations. The massive and rapid degeneration of photoreceptors leaves only a narrow window for treatment. Additionally, the onset of cell death overlaps with the final differentiation of the retina, making it difficult to distinguish between abnormal development and degeneration.

Another line of mice with a spontaneous mutation that offers hope as an improved model for a model of RP is the *Pde6b^{rd10}* (*rd10* mouse). This mutant line more accurately simulates the disease process seen in humans and offers a longer therapeutic window. The *rd10* mouse also harbors a mutation in the β -subunit of PDE, but its rod cells still express 40% of the endogenous level of this protein and demonstrate a slower onset of retinal degeneration.^{8,9} In *rd10* mice, rods begin to degenerate between P16 and P20, with maximum cell death occurring between P21 and P25 in a central to peripheral gradient.^{9–11} By P60, rods are no longer detectable and only cones remain.^{9–11}

Histologic studies of these mouse models have provided insight into the underlying pathology of inherited retinal degeneration. However, accurate quantitative measurements can be difficult due to the nonlinear artifacts induced by postmortem ischemia, fixation, and tissue processing.^{12,13} Spectral domain optical coherence tomography (SD-OCT) has been used effectively to analyze retinal structure in the mouse,^{14–25} and several studies have imaged *rd1* or *rd10* mice using time-domain OCT or SD-OCT.^{17,19,26,27} However, to our knowledge comprehensive, long-term SD-OCT studies have not been performed. We obtained SD-OCT images from these animals at ages ranging from eye opening (P14) into adulthood (P206). Our SD-OCT results provide quantitative measurements of inner and outer retinal thickness over time. Additionally, these scans revealed several unexpected and

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previously unreported results, including frequent *in vivo* separation of the retina from the retinal pigment epithelium in the *rd10* mouse, but not in the *rd1* mouse.

METHODS

Animals

Breeders were obtained from The Jackson Laboratory (Bar Harbor, ME); wild-type C57BL/6J (strain #000664, $n = 35$), *rd1* (strain #004766, $n = 39$), and *rd10* (strain #004297, $n = 44$) were used for this study. Mice

were housed in standard conditions under a 12/12-hour light-dark cycle. All experiments were approved by the Institutional Animal Care and Use Committee at OHSU, and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Imaging

Before imaging, the pupils of the mice were dilated with 1% tropicamide and 2.5% phenylephrine. Artificial tears were used throughout the procedure to maintain corneal clarity. Sedation was induced by 1% isoflurane delivered by a nose cone while the animal was seated in the Bioptigen AIM-RAS holder (Fig. 1A). SD-OCT images

FIGURE 1. (A) Setup for obtaining SD-OCT images from mice sedated with isoflurane. (B) En face image of a mouse eye obtained by OCT with relative locations of horizontal, vertical, and annular scans in yellow. (C) A typical horizontal linear SD-OCT image with layers identified as NFL, IPL, INL, OPL, ONL, ELM, IS/OS, and RPE. (D) Example of the results of segmentation of different retinal layers in a horizontal linear scan and groupings that were used for segmentation analysis (*white lines*). Retinal layers are represented with the following colors: NFL (*red*), IPL (*orange*), OPL (*green*), and choroid/sclera (*purple*). (E) Example of segmentation of the NFL in an annular scan to avoid distortions from inner retinal vessels.

FIGURE 2. (A) Horizontal linear SD-OCT scans from wild-type mice ages P15 through P145. Note the consistency of retinal thickness and visibility of all retinal layers at each time point. (B) Images from *rd10* mice from P14 to P145. Note the occurrence of retinal separations in the P62 and older animals. The overall retinal thickness decreased with time compared to wild-type. (C) Images from *rd1* mice from P14 to P145. These revealed a significantly thinned retina, and loss of the IS/OS and ELM.

were obtained using the Envisu R2200-HR SD-OCT device (Biophtigen, Durham, NC) with the reference arm placed at approximately 1185 mm. Horizontal and vertical linear scans (1.5 mm width, 1500 a-scans/b-scan $\times$ 60 frames/b-scan) were obtained first while centered on the optic nerve, then with the nerve displaced either temporally/nasally or superiorly/inferiorly (Fig. 1B). An annular scan of 0.8 mm was centered on the nerve (1500 a-scans/b-scan, 10 frames/b-scan).

Image Processing and Segmentation

SD-OCT scans were exported from InVivoVue as AVI files. These files were loaded into ImageJ (version 1.45; National Institutes of Health, Bethesda, MD) where they then were registered using the Stackreg plug-in and averaged as a z-stack. Central and displaced images were montaged using Adobe Photoshop CS5 (Adobe Systems Inc., San Jose, CA). A custom designed SD-OCT segmentation program built in IGOR Pro (IGOR Pro 6.12; WaveMetrics Inc., Lake Oswego, OR) was used to profile and measure the thickness of total retina and retinal layers represented in the SD-OCT images.^{28–30} The segmentation approach and strategy were comparable to the software (MATLAB based; MathWorks, Natick, MA) developed by Hood et al.³¹ Due to the presence of retinal vessels in linear scans, annular scans were used to measure the nerve fiber layer (NFL) thickness.

For segmentation analysis, we measured the thickness of the total retina (TR), the photoreceptor layer from Bruch's membrane to the inner nuclear layer (INL)/outer plexiform layer (OPL) interface (REC+), the inner retinal layer (IR) from the OPL to the vitreous border, and the retinal NFL (Fig. 1D). TR, REC+, and NFL thickness were plotted separately, and the data for a given retinal layer in each strain were averaged. To determine IR thickness, TR thickness was subtracted from REC+ thickness using Igor Pro's wave arithmetic plug-in. The resulting

waves then were plotted and averaged. For each point of the averaged wild-type mouse data, ± 2 SD were calculated and this range was shaded in gray.

Scatter Plots

TR and REC+ thickness averages (microns per degree) were determined for each segmented scan, calculating outward from -5° and 5° about the y-axis. The resulting average thicknesses were plotted against age (days) for all of the animals scanned and were grouped by strain. Best-fit equations were plotted for TR and REC+ for each data set.

Histology

Histologic sections were prepared in a manner similar to that of Knott et al.²⁵ Before enucleation, the superior edge of the eye was marked with a fine-tipped permanent marker. Eyes were placed immediately in 2.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer overnight at 4°C . The superior corneas were marked with coated sutures, at the location identified by the permanent marker, for orientation purposes. The eyes then were dehydrated in ethanol, propylene oxide, and epoxy resin. Samples were transferred to a dry mold and oriented such that the vascular pattern matched the vascular pattern seen on the en face image from the SD-OCT scan. The blocks were filled with embedding medium and polymerized. Sections were cut with a microtome to a thickness of 0.7 μm , stained with toluidine blue 1%, and viewed on a Leica DMI3000 B microscope (Leica Microsystems GmbH, Wetzlar, Germany) at $20\times$, and images were montaged with i2k Align (version 1.3.8; DualAlign LLC, Clifton Park, NY).

FIGURE 3. Mouse TR (A) and REC+ (B) thickness graphs of segmented horizontal SD-OCT scan data from P14 through P206 mice. Wild-type C57BL/6j (green), *rd10* (blue), and *rd1* (red) mice are compared. Raw and averaged data for each time point are shown. The gray plot indicates ± 2 SD of the wild-type averaged data.

RESULTS

Figure 1C depicts a typical image from a wild-type mouse demonstrating the ability to delineate multiple retinal layers, including the NFL, inner plexiform layer (IPL), INL, OPL, outer nuclear layer (ONL), external limiting membrane (ELM), the inner segment/outer segment (IS/OS) junction, and RPE. In some animals, a thin hypo-reflective layer between the NFL and IPL was visible, and presumably represents the ganglion cell layer; however, this layer could not be identified consistently. Figures 1D and 1E demonstrate linear and annular scans, respectively, after retinal layers were segmented. Due to the intrusion of blood vessels in the NFL in the linear scans, annular scans were used to measure the NFL (since the inner retinal vessels were more visible in cross section).

SD-OCT images from wild-type mice revealed normal retinal structures at the first imaging time point, P15 (Fig. 2A, arrow), although the retinal layers were slightly less distinguishable than those visible in images obtained a few days later at P18. From P18 to P145, there was little structural variation in the retinas of the wild-type mice. Even at P145, all retinal layers were visible, albeit slightly less discernible than those seen at younger ages. Images from *rd10* mice (Fig. 2B, arrow) at P14 revealed that approximately half of the eyes imaged had retinal morphologies that were quite comparable to those of the wild-

type mice, and demonstrated an intact ELM and IS/OS junction. The remaining half showed normal total retinal thickness, but poor visibility of the IS/OS junction. In contrast, images from *rd1* mice at P14 (Fig. 2C, arrow), revealed thinned retinas as well as loss of the IS/OS and ELM. Additionally, the ONL was quite thin and hyperreflective in some animals.

Figure 3 plots the topographic distribution of retinal thickness centered at the optic nerve from wild-type (green), *rd10* (blue), and *rd1* (red) mice. For the wild-type data, gray shading was used to indicate ± 2 SDs of the average. As can be observed from these graphs, wild-type animals maintain a relatively constant total retinal thickness across all time points, although a mild initial decline was noted. In contrast, *rd1* mice exhibit severe loss of retinal thickness at P14, which declines and settles around 100 μ m at approximately P62. There was no statistical difference between total retinal thickness in wild-type and *rd10* at P14. However, starting at P16, *rd10* mice demonstrate a slow and progressive decline in total retinal thickness, reaching a thickness similar to that in the *rd1* mouse around P103. Segmentation data from horizontal and vertical sections (Figs. 3A, 3B, S1A–C, and S2, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-9611/-/DCSupplemental>) did not reveal any significant asymmetries in retinal thickness between the temporal/nasal or inferior/superior retina. Further analysis of the changes of thickness in the REC+ layer and inner

FIGURE 3. Continued.

retinal layers (Figs. 3A, 3B, and S2, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-9611/-DCSupplemental>) demonstrated that the changes in total retinal thickness were due to a decrease primarily in outer retinal thickness rather than inner retinal thickness. Segmentation analysis also demonstrated decreased inner retinal thickness in wild-type, *rd10*, and *rd1* mice (Figs. S1–S3, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-9611/-DCSupplemental>). Scatter plots of average TR and REC+ thickness over time demonstrated considerable declines that were fit with exponential decay functions, with the exception of average REC+ thickness in wild-type mice, which was fit with a linear function (Figs. 9, S4, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-9611/-DCSupplemental>).

Some animals had hyperreflective opacities in the vitreous, which likely represent inflammatory cells (Figs. 2B, 4A). In *rd10* mice, these opacities were present at the first imaging time point, P14. They were most visible and numerous around P20, which occurs slightly before the timing of maximum cell death in this model.¹⁰ After P21, the number of vitreous opacities decreased with time. In contrast, *rd1* mice showed only mild vitreous opacities at the earliest time points (Figs 2C, 4B), which dissipated with time (data not shown).

By P18, the IS/OS junction had disappeared in all of the *rd10* mice that were imaged and the presence of vitreous opacities increased. Figures 5A and 5C show magnified images to highlight retinal changes over time in *rd10* mice. At P14,

retinal structures in *rd10* mice were comparable to wild-type, and the ELM and IS/OS junction were visible. At P16, the IS/OS junction was more difficult to visualize in most animals. By P18, many *rd10* mice demonstrated loss of the IS/OS junction with generalized retinal thinning, but the ELM still was intact. Around P20, the region of the ONL that was hypo-reflective became hyperreflective, and obscured visualization of the ELM and the border of the ONL and OPL (Fig. 2B). In one *rd10* mouse, this hyperreflective ONL was observed early, at P14, but in most animals it was not visible until P20 (Fig. 5C). By P22, the ONL returned to being hypo-reflective, but it was significantly thinner. In comparison to the *rd10* mice, the *rd1* mice demonstrated loss of the IS/OS junction in most mice at the earliest time of imaging (P14). At this time point, the ONL appeared hyperreflective, but it was difficult to assess due to the rapid cell loss and early disappearance of the ONL (Fig. 5B).

One of the most interesting features noted with SD-OCT imaging was the development of retinal separations from the RPE/choroid in *rd10* mice (Fig. 6, denoted by red arrowheads). Retinal separations were observed first at P27, but only in a minority of animals. By P64, almost all *rd10* mice demonstrated some degree of retinal separation. Retinal separations still were visible at the latest time point tested (P145), but in many cases the retina had appeared to settle back down onto the residual RPE/choroid. In a few animals, hyperreflective opacities were observed in the space that

FIGURE 4. (A) Examples of the hyperreflective vitreous opacities seen in *rd10* mice. Presumably these are inflammatory cells in the vitreous. Note the presence at the first imaging time point, P14. These opacities were visible most around P20, which correlates with the timing of maximum cell death in this model. After P20, the amount of cells decreases with time. (B) In contrast, *rd1* mice showed mild vitreous opacities at only the earliest time points.

FIGURE 5. (A) Magnified images to detail the retinal changes over time in *rd10* mice. At P14, retinal structures in *rd10* mice were comparable to wild-type (left) with the ELM and IS/OS junction visible. At P16, the IS/OS junction was more difficult to visualize in most *rd10* animals. By P18, many *rd10* mice demonstrated loss of the IS/OS junction, but the ELM was still intact. Also, note the generalized retinal thinning. Around P20, the region of the ONL, which was hypo-reflective, became hyperreflective, and obscured visualization of the ELM, and the border of the ONL and OPL. By P22, the ONL returned to its previously hypo-reflective state, but was significantly thinner. (B) In contrast to the *rd10* mice, most of the *rd1* mice demonstrated loss of the IS/OS junction at the earliest time of imaging (P14). At this time point, the ONL appeared hyperreflective, but it was difficult to assess since this layer had disappeared completely by P18. From P18 to P34, there was continued thinning of the retina in *rd1* mice. (C) Examples of *rd10* mice with hyperreflective ONLs. This transition was noted most often around P20, but there were exceptions, such as one animal shown that had these changes as early as P16.

separated the retina and RPE (Fig. 6, blue arrows). These might represent subretinal precipitates or inflammatory cells. One significant observation was that retinal separations were never seen in *rd1* mice.

Preliminary histologic studies correlate retinal features with those seen on SD-OCT scans (Fig. 7). In *rd10* mice at P14, there appears to be a normal number of cells in the ONL, and the IS/OS junction is visible on histology and SD-

FIGURE 6. Detailed examples of the retinal separation from the RPE observed in *rd10* mice (red arrowheads). Retinal separation was observed first at P27, but only in a minority of animals. By P64, almost all animals demonstrated some degree of retinal separation. Retinal separations still were visible at the latest time point tested (P145), but in many cases the retina had settled back down onto the RPE. A hyperreflective edge was visible on the scleral border of the detached retina (yellow arrows). This might represent a residual ELM. In a few animals, hyperreflective opacities were observed in the space that separated the retina and RPE (blue arrows). These might represent subretinal precipitates or inflammatory cells. Retinal separations were never observed in *rd1* mice.

FIGURE 7. (A) SD-OCT from an *rd10* mouse at P14 demonstrating intact retinal structures. (B) Histology correlating to the white box from the SD-OCT. This animal demonstrates a normal complement of 10–12 nuclei in the ONL, as well as visible inner and outer segments. (C) Magnified SD-OCT image from part (A) showing an intact ELM and IS/OS junction. (D) SD-OCT from an *rd10* mouse at P20 exhibiting thinning of the ONL, blurring of the ELM, and loss of the IS/OS junction. (E) Corresponding histologic image demonstrating an ONL with 5–6 cells and disorganized nuclei. Additionally, there is loss of the outer segments. (F) Magnified OCT image from part (D).

OCT (Figs. 7B, 7C). By P20, the SD-OCT demonstrates loss of the IS/OS junction, blurring of the ELM, and thinning of the ONL (Figs. 7E, 7F). Corresponding histology shows an ONL measuring approximately 5–6 cells as well as disruption and loss of the outer segments. Examples of retinal separations by SD-OCT in *rd10* mice at P51 and P64 are shown in Figures 8A, 8B, 8F, and 8G. Histology highlighting the same areas demonstrated separation of the remaining ONL from the underlying RPE. The RPE appears disrupted and absent in certain regions (Figs. 8H–8J). Some animals also demonstrated a splitting of the RPE from the underlying choroid (Figs. 8C–8E).

DISCUSSION

Histology long has been considered the gold standard for assessing and quantifying the rate of retinal degeneration in animal models, as well as for judging the efficacy of potential treatments. However, many disadvantages are associated with histologic processing. Because of the inherent need to sacrifice animals to obtain histologic data, an increase in the number of sequential time points in a long-term study greatly increases the total number of animals needed to conduct this type of analysis. Additionally, gathering histologic samples makes it impossible to perform such studies on the same animal over time. Another problem associated with histology is visible

artifacts, such as retinal separations, that may be induced by tissue processing. Quantitative measurements can be problematic due to the nonlinear artifacts induced by postmortem ischemia, fixation, or tissue processing.^{12,13} These difficulties have led most researchers to quantitate retinal thickness by counting individual cells rather than measuring absolute thickness. This approach is useful for quantifying layers, such as the ONL, but less helpful for non-cellular layers, such as the IPL.

The improved resolution of SD-OCT provides a means to measure and quantify retinal tissue *in vivo*, free of histologic artifacts. Although, SD-OCT has yet to achieve the resolution to resolve individual cells, distinct layers are visible clearly. Most are in agreement with the identities of the major layers visualized on the SD-OCT,^{12,13,32,33} although there has been a recent suggestion that the third highly reflective band in the outer retina arises from the ellipsoid body in the inner segment rather than the IS/OS junction.³⁴ The quantitative measurements with SD-OCT are not completely free from artifacts. For example, optical properties, such as the index of refraction, might vary between retinal layers that are rich in lipids, thereby altering the scale of thickness measurements.¹² Additionally, the logarithmic filtering that is applied to SD-OCT data can change the apparent thickness of different layers.³⁴ Further, without montaging, current SD-OCT imaging

FIGURE 8. (A) SD-OCT from an *rd10* mouse at P51 demonstrating retinal separations around the nerve. (B) Magnification of (A) denoting retinal separation (arrows). (C) Corresponding histologic section. (D, E) Magnified view adjacent to the nerve. (F) SD-OCT from an *rd10* mouse at P64 demonstrating retinal separations around the nerve. (G) Magnification of (F) denoting retinal separation (arrows). (H) Corresponding histologic section. (I, J) Magnified view adjacent to the nerve demonstrating retinal separation and focal loss of RPE in some areas.

is limited to approximately a 30-degree circle around the optic nerve.

SD-OCT has been used effectively to analyze the retinal structure of the mouse,^{14–25} and several studies have imaged *rd1* or *rd10* mice using Time-Domain OCT or SD-OCT,^{17,19,26,27} but to our knowledge comprehensive long-term SD-OCT studies have not been performed. Although, *rd10* and *rd1* mice have mutations in the same gene, they demonstrated several different features on SD-OCT. It long has been known that the rate of degeneration is much faster in the *rd1* mouse than in the *rd10* mouse.⁸ Our *in vivo* imaging reveals several new differences not appreciated before.

It has been shown that *rd1* mice demonstrate swelling in the mitochondria and the appearance of vacuolar inclusions in the inner segment at P8.^{35–40} Such swelling may result in the loss of the IS/OS junction if the ellipsoids are indeed the source of this signal. Our SD-OCT results demonstrated loss of the IS/OS junction in *rd1* mice at the earliest time point imaged (P14). The appearance of the SD-OCT images of the *rd10* mouse at P14 is consistent with previous reports that demonstrated normal histologic morphology and physiologic responses from bipolar cells.⁴¹ The *rd10* mouse never has a normal electroretinogram, indicating early photoreceptor dysfunction. However, SD-OCTs from most *rd10* mice at P14 were indistinguishable from those of wild-type mice,⁸ suggesting that SD-OCT may not be sensitive enough to pick up the

earliest signs of retinal dysfunction. Segmentation profiles revealed a slower and steady decline in retinal thickness of *rd10* mice compared to *rd1* mice without significant asymmetry between different regions of the retina. By P60, the average retinal thickness of the *rd10* mice was indistinguishable from the *rd1* mice; this is consistent with previous histologic reports showing that, at P60, almost no cells are left in the ONL of either line of mice.⁹ Segmentation analysis also demonstrates little change in inner retinal thickness over the course of five months, which also is consistent with previous histologic studies in the *rd10* mouse.⁹ Previous studies have shown decreased thickness in the inner retinal layers of the *rd1* mouse,^{42,43} and our data indicated a measurable decrease as well.

SD-OCT appears to be particularly useful for observing changes in the vitreous gel, which often is disrupted after histologic processing. One notable feature observed in the *rd10* and *rd1* mice was the presence of hyperreflective opacities in the vitreous. We suspect that these opacities represent inflammatory cells. Similar opacities on SD-OCT have been observed in a rodent model of experimental autoimmune uveoretinitis and cases of multifocal choroiditis.^{44,45} The *rd10* mice demonstrated numerous vitreous opacities peaking at P20, while *rd1* mice exhibited only a few at P15, which disappeared soon thereafter. This difference might be accounted for by the difference in timing of cell death between these

FIGURE 9. Scatter plot of average total retinal thicknesses of wild-type, *rd10*, and *rd1* mice across all time points. For each data set, a best-fit exponential decay function was plotted. The resulting exponential decay equations, coefficients of determination (R^2), and root-mean-square errors ($RMSE$) are indicated.

two models. Cell death peaks around P12 in the *rd1* mouse versus P25 in the *rd10* mouse.^{2,11} The size of these opacities ranges from approximately 6–30 μm in diameter. The average human lymphocyte is approximately 8 to 9 μm in diameter.⁴⁶ Thus, some of these opacities are consistent with single cells, while others likely represent clumps of cells or larger cells, such as macrophages.

Another curious feature that we observed using SD-OCT was the change in reflectivity of the ONL in *rd10* mice. The shift from a relatively hypo-reflective ONL to a hyperreflective ONL was most notable around P20. Many histologic changes occur in the degenerating retinas of *rd10* and *rd1* mice, including an up-regulation of glial fibrillary acidic protein (GFAP) in Müller cells,¹⁰ the appearance of pyknotic nuclei, the migration of macrophages into the ONL,⁴⁷ and mis-localization of the photoreceptor nuclei.¹¹ This up-regulation of GFAP occurs throughout the Müller cells, making it unlikely that this would result in a localized reflectivity change seen exclusively in the ONL. TUNEL staining first appears in *rd10* mice at P18,¹⁰ but peak cell death in the *rd10* mouse does not occur until around P25.^{11,12} A hyperreflective ONL was seen in some *rd10* mice as early as P16.^{10,11} Thus, the reflectivity change may not result from the presence of pyknotic nuclei, but rather from structural changes and cellular disorganization that occurs slightly before the terminal phase of cell apoptosis. At P20, there is displacement of the cone photoreceptor cell bodies in the ONL as well as mis-localization of rhodopsin throughout the rod cell body.¹¹ These changes could alter the optical properties of the ONL. The subsequent disappearance of this hyperreflectivity could result from the death and clearance of photoreceptor cell bodies. Due to the rapid cellular degeneration in the *rd1* mouse, it was difficult to assess whether the residual ONL demonstrated any relative hyper-reflectivity, but a few animals at P14 did appear to exhibit this phenomenon. Further histologic studies are underway to understand the source of the hyperreflective ONL seen in *rd1* mice at P14 and *rd10* mice at P18. Moreover, these studies will investigate what changes correlate exactly with the loss of the IS/OS junction and ELM on SD-OCT.

Perhaps one of the most interesting features of our study was the evidence of retinal separations observed in *rd10* mice. While artifactual retinal detachment commonly occurs after

histologic processing, we are unaware of any previous reports demonstrating these separations *in vivo*. Our histologic samples confirm that these separations often result from detachment of the retina from the underlying degenerated RPE. The sclerad boundary of the separated retina demonstrated a hyperreflective edge, which could represent a remnant ELM. A similar hyperreflective edge has been observed on SD-OCT in other rodent models of induced retinal detachment.²³ In some cases, the RPE appears to be splitting from the underlying choroid. It still is unclear if this reflects a histologic artifact, but separation of the RPE in *rd10* mice also has been observed by others (personal communication, Arlene Drack). Some animals demonstrated hyperreflective opacities in the subretinal space, which resembled inflammatory cells. Subretinal macrophages and microglia have been reported in models of experimental retinal detachment.^{48,49} We hypothesize that retinal separations result from a decreased pumping action of the RPE. However, it is unclear why retinal separations never were observed in *rd1* mice, which present with more extensive atrophy of RPE cells than *rd10* mice. One possibility is that the rapid degeneration the *rd1* mice experience results in early gliosis that renders the retina adherent to the underlying structures.

We used SD-OCT to demonstrate several new important features in *rd10* and *rd1* mice. With the use of the *rd10* mouse in therapeutic studies becoming more frequent, it is important to understand the limitations of this model. Retinal separations, such as those seen in our animals, are not a typical feature of RP seen in humans. Patients with RP can demonstrate cystoid macular edema, which can be treated with carbonic anhydrase inhibitors.^{50,51} The proposed mechanism of these drugs is an increased pumping action of the RPE. Therefore, it is tempting to speculate whether the retinal separations seen in *rd10* mice might be a model for cystoid macular edema. Future studies will analyze whether carbonic anhydrase inhibitors can decrease or prevent retinal separations in *rd10* mice.

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