Functional Principal Component Analysis Reveals Discriminating Categories of Retinal Pigment Epithelial Morphology in Mice

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Retinal pigment epithelium (RPE), a multifunctional cell monolayer that separates the retinal photoreceptors from the choroid, is crucial in maintaining photoreceptor cells. RPE regulates the flow of nutrients and oxygen into the retina, removes waste byproducts from the retina, phagocytizes shed discs from photoreceptors, absorbs stray light that passes through the retina, and regulates the osmolarity of the eye. The RPE sheet functions as a barrier to maintain gradients and protects photoreceptor cells and inner neurons from the immune system and from external assaults from infectious agents. To preserve the integrity of the barrier, when an epithelial cell dies, the apoptotic cell is extruded from an intact sheet through a p115RhoGEF-mediated process that maintains the barrier. However, disruptions in RPE occur with age and in several ocular disorders, including age-related macular degeneration and retinitis pigmentosa.

The biomechanical forces that organize RPE and other epithelial cell–cell contacts include adhesion, tension, and contraction. An actin-myosin cytoskeleton contributes contractile forces that lead to regular polygonal (mostly hexagonal) shapes. One of many molecules that provides a high-quality outline of the RPE cell border is zonula occludens-1 (ZO-1), a tight junction protein. Trafficking and movement of proteins that are part of the adhesion, tension, and contraction mechanisms lead to changes in the force balance. Additionally, the death of RPE cells, because of retinal disease progression, bystander effects, and mechanical stress from the presence of drusen-like substances, flecks, or the like, must contribute to the changes in force balance.

Consequently, we hypothesize that remodeling of RPE cell shape causes alterations in cell packing and a rearrangement of the patterns and tiling of the RPE sheet. We hypothesize that the RPE morphology changes differently with age and disease progression. This study established the relationship between RPE morphology and the phenotypic degeneration of the retina specified by rd10.

We studied the morphometric properties of RPE sheets from C57BL/6J and rd10 mice on a C57BL/6J background. Here, C57BL/6J mice are considered wild-type (WT) mice and were used to study the effect of aging in the RPE cell morphology, distinct from disease. rd10 mice were used to assess effects of photoreceptor loss and aging on RPE cell morphology. This strain has a missense mutation in PDE6B, the gene encoding the beta subunit of rod phosphodiesterase.
Discriminating Categories of RPE in Mice

Methods

Mice

B6.CXB1-Pde6b rd10/J (rd10) mice and C57BL/6J (WT) mice were obtained from Jackson Laboratories, Inc. (Bar Harbor, ME). The original rd10 mouse lesion was discovered in a C57BL/6J mouse, and, thus, rd10 is fully congenic with C57BL/6J. Mice were housed in Emory University Division of Animal Resources managed facilities according to Association for Research in Vision and Ophthalmology (ARVO) guidelines and as approved by the Emory University IACUC. Mice were maintained on a 12-hour light:12-hour dark cycle at an approximate light level of 50 to 100 lux in the cages during daytime. Mice were offered rodent chow (product 5001; Purina, St. Louis, MO) and water ad libitum. The mice were examined at ages ranging from postnatal day (P)30 until approximately 2 years of age.

RPE Flatmount Technique

Mice were euthanized with CO₂ in accordance with Emory IACUC guidelines and ARVO guidelines for treatment of animals. One eye from each mouse was marked on the superior side with a fine point, permanent ink pen (Sharpie, Oak Brook, IL), enucleated, fixed for 10 min in 10% neutral buffered formalin (Fisher, Pittsburgh, PA), and washed three times with PBS (catalog no. 46-013-CM; Cellgro, Manassas, VA). Excess tissue was removed from the outside of the globe. No

Table. Sample Group Characteristics for WT and rd10 Young and Old Mice

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>WT Young</th>
<th>WT Old</th>
<th>rd10 Young</th>
<th>rd10 Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age range ≤P61</td>
<td>≥P180</td>
<td>≤P61</td>
<td>≥P100</td>
<td></td>
</tr>
<tr>
<td>No. of mice</td>
<td>27</td>
<td>23</td>
<td>27</td>
<td>34</td>
</tr>
<tr>
<td>Average age ± SD</td>
<td>44.6 ± 13.0</td>
<td>464.7 ± 256.1</td>
<td>49.6 ± 10.2</td>
<td>243.3 ± 214.5</td>
</tr>
<tr>
<td>Average cell area ± SD</td>
<td>247.5 ± 12.1</td>
<td>268.4 ± 10.9</td>
<td>247.4 ± 9.5</td>
<td>264.6 ± 16.0</td>
</tr>
<tr>
<td>Mode cell area ± SD</td>
<td>195.9 ± 7.1</td>
<td>193.7 ± 5.2</td>
<td>201.6 ± 8.4</td>
<td>190.7 ± 8.4</td>
</tr>
<tr>
<td>Average aspect ratio ± SD</td>
<td>0.74 ± 0.01</td>
<td>0.73 ± 0.01</td>
<td>0.70 ± 0.02</td>
<td>0.62 ± 0.03</td>
</tr>
<tr>
<td>Mode aspect ratio ± SD</td>
<td>0.78 ± 0.01</td>
<td>0.79 ± 0.01</td>
<td>0.74 ± 0.02</td>
<td>0.63 ± 0.05</td>
</tr>
<tr>
<td>Highest peak (x,y,z)</td>
<td>(198,0.78,0.015)</td>
<td>(198,0.78,0.012)</td>
<td>(210,0.73,0.014)</td>
<td>(180,0.64,0.009)</td>
</tr>
<tr>
<td>X-Y area including 95% of cells</td>
<td>162.9</td>
<td>211.0</td>
<td>207.6</td>
<td>294.5</td>
</tr>
<tr>
<td>Area at z = 4e-04</td>
<td>204.0</td>
<td>248.4</td>
<td>253.7</td>
<td>333.5</td>
</tr>
<tr>
<td>Age breakdown per major group:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P50 n = 10</td>
<td>P180 n = 8</td>
<td>P50 n = 3</td>
<td>P100 n = 13</td>
<td></td>
</tr>
<tr>
<td>P45 n = 8</td>
<td>P330 n = 4</td>
<td>P45 n = 13</td>
<td>P180 n = 13</td>
<td></td>
</tr>
<tr>
<td>independent mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P60 n = 4</td>
<td>P720 n = 6</td>
<td>P60 n = 6</td>
<td>P330 n = 3</td>
<td></td>
</tr>
<tr>
<td>P61 n = 5</td>
<td>P722 n = 5</td>
<td>P61 n = 5</td>
<td>P725 n = 2</td>
<td></td>
</tr>
<tr>
<td>Collapsed data from P60.5; n = 9</td>
<td>P721; n = 11</td>
<td>P60.5; n = 11</td>
<td>P727.5 n = 5</td>
<td></td>
</tr>
<tr>
<td>(summed P60)</td>
<td>(summed P720)</td>
<td>(summed P60)</td>
<td>(summed P723)</td>
<td></td>
</tr>
<tr>
<td>and P61)</td>
<td>and P722)</td>
<td>and P61)</td>
<td>and P732)</td>
<td></td>
</tr>
</tbody>
</table>

Area at z = 4e-04, the area of the contour where z = 4 × 10⁻⁴ from the plots given in Figures 6E–H; highest peak, x, y, and z coordinates from Figure 6 of the mode for each major group; mode, the value at the most abundantly represented cell area or aspect ratio; SD, standard deviation; X-Y area including 95% of the cells, area of the contour that includes 95% of the cells. These statistics suggest that WT young cells were normal in size and shape; WT old were normal in shape but abnormal in size; rd10 young were abnormal in shape but normal in size; and rd10 old were abnormal in size and shape. The larger X-Y areas indicate increased size and shape variability of RPE cells in rd10 and old mice compared to WT young mice.
Figure 1. RPE flatmount images. Cell boundaries were stained green with anti-ZO-1, and cell nuclei were stained red with propidium iodide. (A) Merged image of whole-RPE flatmount of an rd10 mouse at P100 is shown. Scale bars and sizes are indicated on the images. Highly compressed, elongated, and tortuous cells in the midperiphery are indicated by the four white arrowheads. (B) Region of RPE flatmount of a WT mouse at P45 is shown. A patch of approximately 795 cells is illustrated. Most cells were regular in shape and consistent in size. (C) RPE is shown in the midperipheral region of an rd10 mouse eye at P100. A transition zone that separated compressed cells from large and variably sized cells posterior to the...
incision was made in the cornea before or during fixation. The incision was made only after the initial fixative was removed and replaced with PBS. Flatmounting was done by making four radial cuts from the center of the cornea back toward the optic nerve. A drop of Dulbecco calcium- and magnesium-free PBS was placed on the eye to keep it moist. The flaps were peeled away from the lens, and the lens was removed. The iris and retina were removed using forceps. Tension from the sclera was relieved by making cuts halfway through each flap at the ciliary body/cornea margin and small cuts through the ciliary body.

ZO-1 Staining

The RPE flatmounts were blocked with Hanks’ balanced salt solution HBSS (SH30588.01; HyClone, Logan, UT) containing 0.1% (v/v) Triton X-100 and 1% (w/v) bovine serum albumin (antibody buffer) for 30 minutes. Immunostaining with a 1:100 dilution of rabbit anti-ZO-1 antibody (catalog no. 61-7300; Invitrogen, Carlsbad, CA) in antibody buffer was conducted for approximately 16 hours at room temperature. The flatmounts were washed 5 times with HBSS plus 0.1% (v/v) Triton X-100 (wash buffer) for 2 minutes and then stained for 1 hour with Oregon Green-conjugated goat anti-rabbit immunoglobulin G secondary antibody (catalog no. O11038; Invitrogen) in antibody buffer and then washed three times with wash buffer. The flatmounts were mounted with Vectashield hardset (catalog no. H-1400; Vector Laboratories, Burlingame, CA) and allowed to harden overnight.

Imaging

Imaging of the flatmounts was performed with a confocal imaging system (model C1; Nikon) using argon laser excitation at 488 nm. Confocal images were digitally merged (Adobe Photoshop CS2; Adobe, San Jose, CA). Selected representative regions of equal size (181 x 266 pixels; 225 x 331 μm) were cropped from the merged flatmount image from areas devoid of dissection artifacts. As many selected representative regions as possible were taken from each image (45–60 selected representative regions per image). Twenty-one morphometric measurements for every cell identified in the selected representative region, including cell location, cell area, perimeter, eccentricity, aspect ratio, form factor, and number of neighbors, were calculated using CellProfiler. Statistical Analysis

The RPE images and the quantitative data output from CellProfiler analysis were examined, and cell area and cell shape (measured by aspect ratio) were chosen as two key features to discriminate between the genotypes and age

![Figure 2](image-url)
Discriminating Categories of RPE in Mice

FIGURE 3. Principal component score plots of cell area are shown for all 4 genotype and age groups. Black squares are WT eyes, and red triangles are rd10 eyes. The y-axis on each plot (A–C) is a different first principal component because the dataset from which it was derived was different in each plot; the same applies to each y-axis; each second PC score axis is different in the three plots. (A) The plot of the first two principal components for WT eyes is shown. This panel shows the clear separation WT young from WT old based only on cell area. (B) The rd10 mice are resolved, old mice from young, but not as well as in the WT mice. (C) Data are derived from all 4 groups; old are resolved from young but do not clearly resolve the four distinct groups (WT young, WT old, rd10 young, rd10 old) based solely on cell area. Panel (C) illustrates the central point of this experiment, that old can be separated from young, but not WT young from rd10 young.

groups. The classification rule was constructed using the following four steps:

1. Density functions were generated for the area and shape data, respectively, from each eye, using a penalized likelihood method. A joint density function of the area and the aspect ratio was estimated by the kernel method for each eye based on its area and shape data.

2. A newly proposed FPCA method was applied to the collection of joint density functions for all eyes to perform dimension reduction.\(^{30}\) The first four principal components were chosen, and the corresponding principal component scores were calculated for each eye. Therefore, we obtained a 111 x 4 matrix of PC scores.

3. Three popular classification methods were selected and applied to the matrix of PC scores obtained in step 2: linear determinant analysis (LDA), quadratic determinant analysis (QDA), and support vector machine (SVM).

4. The leave-one-out cross-validation method was used to determine the accuracy of the classification results; the PC scores of one eye image were selected as the test data, and the PC scores of the remaining eye images were used as training data to construct the classification rule applied to the test data. This process was repeated such that each RPE image was used once as the test data.

RESULTS

We assessed a total of 111 flatmounts, including 50 from WT and 61 from rd10 mice. For each genotype, we sampled a wide age span, from P30, P45, P60, P100, P180, and P330 to over P\(^{720}\). The Table lists the sample sizes and average ages of the mice in each of the four major groups.

Figure 1 sample images show the differences between the RPE sheet morphology of WT mouse eyes and that of rd10 mouse eyes. Figure 1A is a merged image of a whole RPE flatmount from an rd10 mouse at P100; it illustrates the flatmounting of the RPE sheet. This panel gives an overview of the entire sheet and shows the important areas of the RPE sheet that need to be analyzed and the overall characteristics of the different types of alterations to the RPE sheet depending on location on the sheet. The green borders of RPE cells stained with anti-ZO-1 can be recognized. The entire back of the eye was a uniform sheet with the exception of artifacts such as knife marks or scratches of the RPE monolayer. Brightly stained corneal endothelium was highlighted in bright orange. We routinely removed the superior tab of the cornea to mark that quadrant and to orient the flatmounts.

Just inner to the corneal endothelium, the remnants of the ciliary body in bright green were identified. Just inside the ciliary epithelium, a ring of 8 to 10 cells of a loosely knit group of RPE cells in a transitional zone at the far periphery of the RPE sheet was seen. Inside the transitional zone, the cells were packed in regular hexagonal and pentagonal patterns, with RPE cells a little smaller than in the transitional zone. The optic nerve head was present in the center of the preparation. The retina was detached from this point, and frequently there was a small dissection artifact from which the RPE cells were missing. Underlying the RPE was the choroid, which could be readily observed if the RPE cells were stretched thinly, or if there was an artifact or scratch to the RPE sheet that exposed the choroid. Figure 1B (from normal WT RPE at P45) shows a patch of 795 RPE cells with 1176 nuclei (Fig. 1B shows 48% of the RPE cells had two or more nuclei and the remainder were mononucleate). These counts excluded any cells on the edge or incomplete cells. Most of the RPE cells were regular, with four or six sides, and the sides were all of approximately the same length. Thus, the cells were isotropic, and few if any carried internal vertices with angles greater than 145° (i.e., most of the angles were close to 108° in the pentagons and 120° in hexagons). In the case of changes to the RPE caused by bystander or collateral damage from the rd10 mutation, expressed in the rod photoreceptor cell, many irregularities in the shape of RPE cells began to develop (Figs. 1A, 1C). Cells lost their regular hexagonal shape, became elongated, and the size of the cells became more variable in comparison to WT RPE cells of the same age. Figure 1C (same magnification as Fig. 1B) shows 844 complete cells; nuclei were not counted due to variations in the plane of focus that did not allow counting nuclei. This is unavoidable, as this reflects underlying pathology of the RPE layer in the rd10 mice, which is fatter and thicker in the bulge. The demonstration of the bulge is one of the purposes of presenting this image. There appeared to be
versus ‡

not normal (Shapiro-Wilk test, data not shown): a peak

young (grouped the density distributions according to age as either
extracted the first four principal components by FPCA. We
plotted the density distributions (which contain all data) and
median may not explain the most variability in the data, we
area between WT and rd10 eyes at the same ages. Because the
occurred between 60 and 180 days. In the rd10 mice (Fig. 2C),
2-year life span of the mouse. The greatest area change
approximately 10% to 15% in WT mice over the approximately
approximately six to eight mice per age group (Table) were
mately 55,000 RPE cells total per eye) and sample sizes of
Approximately 10,000 cells per mouse eye (of the approxi-
RPE cell during the aging of both WT and rd10 mice.

a distinct bulging ring or bull's-eye pattern of highly
compressed or elongated cells at the midperiphery in the
sheet, which was noticeable to the human observer (Fig. 1A).
Generally, parts of the RPE sheet more peripheral to the ring
did not change at this time point in the rd10 mice. Large cells
with more than six sides were found posterior to the ring of
compressed cells in the rd10 sheets (Figs. 1A, 1C).

Figure 2 summarize metrics and statistics of changes in the
RPE cell during the aging of both WT and rd10 mice.
Approximately 10,000 cells per mouse eye (of the approxi-
ately 55,000 RPE cells total per eye) and sample sizes of
approximately six to eight mice per age group (Table) were
analyzed. The median cell area, en face (Fig. 2A), the general
trend was that with age, the cell size gradually increased by
approximately 10% to 15% in WT mice over the approximately
2-year life span of the mouse. The greatest area change
occurred between 60 and 180 days. In the rd10 mice (Fig. 2C),
cell areas trended upward with increasing age as well.
However, there were no obvious differences in median cell
area between WT and rd10 eyes at the same ages. Because the
median may not explain the most variability in the data, we
plotted the density distributions (which contain all data) and
extracted the first four principal components by FPCA. We
grouped the density distributions according to age as either
young (≥P61) or old (≥P180 for WT or ≥P100 for rd10) based
on the aforementioned greatest cell area change, the generally
accepted notions of juvenile versus adult mouse ages, and
arbitrary partitioning of two age groups. After the cell area
density distributions were plotted (Fig. 2B, WT; Fig. 2D, rd10),
qualitative properties of younger versus older mice (≤P61
versus ≥P180) could be seen. The density distributions were
not normal (Shapiro-Wilk test, data not shown): a peak
(“mode”) at approximately 200 μm² was observed in both
WT and rd10 mice. Distinguishing the older from the younger
mice, the major peak at approximately 200 decreased in
magnitude with age, and a tail of larger sized cells was
appreciated in both WT and old rd10 mice. However, the
distribution of older cells was different in comparing rd10 and
WT mice. The WT mice had fewer cells over 400 μm² (Fig. 2B)
with a distinct shoulder at approximately 380 μm². The rd10
older mice lacked the shoulder at 380 μm² and had a larger and
longer tail with more cells evident at 600 μm² (Fig. 2D) than
the WT mice.

When principal components were extracted from the area
density distributions, there was a clear resolution of the young
(black squares) from old (red triangles) RPE cells in both WT
(Fig. 3A) and rd10 (Fig. 3B). Figure 3C combines the joint data
for cell area from genotypes (both WT and rd10) and age
(young versus old) from the density distributions. Figure 3C
shows the first two PC scores plotted on the x- and y-axes. The
separation between the solid symbols and the open symbols
was clear, indicating that the young (≤61 days, solid symbols)
and the old (open symbols) could be clearly discriminated.
However, the two colors, black and red, were more mixed,
indicating that the discrimination between the two genotypes
(black for WT, red for rd10) was not distinct, indicating that the
two genotypes were not well discriminated solely by cell area.

In Figure 4, the aspect ratio of the RPE cell was evaluated,
much as the RPE cell area in Figures 2 and 3. The aspect ratio
is defined as the ratio of the minor axis length to the major axis
length, calculated as the lengths of the major (minor) axis of an
ellipse that has the same normalized second central moments
as the cell region. The major and minor axes lengths were
the output of CellProfiler.31 When the aspect ratio is equal to 1, the

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**Figure 4.** Analysis of RPE cell aspect ratio. (A) Boxplots of median cell aspect ratio (dimensionless) is shown as a function of age for WT eyes. Boxplots display the five-number summaries of the aspect ratio: smallest aspect ratio, lower quartile (25%), median, upper quartile (75%), and largest observed aspect ratio. There was no significant change in aspect ratio relative to age, indicating that baseline cell shape change in age was very small in WT mice. (B) Density plots are shown for each WT eye. The mode is at an aspect ratio of approximately 0.8 for both young and old WT. (C) Boxplots of median cell aspect ratios are shown as a function of age for rd10 eyes. (D) Densities were plotted for each rd10 eye. The mode is at an aspect ratio of approximately 0.8 for young rd10 and approximately 0.6 for old rd10. Black lines and symbols are for eyes ≤P61 and red for ≥P100 (rd10) or P180 (WT) eyes. Numbers of mice per age group and genotype are given in the Table.
DISCUSSION

In this study, we showed that novel statistical analyses using multivariate FPCA and tools for the classification of size and shape of RPE morphology allowed discrimination of RPE genotypes and age. The use of the joint density distributions from roughly 10,000 RPE cells per mouse and FPCA instead of a simple median were key to deriving discriminatory power.

We drew two conclusions: (1) there are distinctive bystander effects on the RPE cells of the rd10 mutant that are obvious in flatmount preparations; and (2) the effects include characteristic patterns and changes in tiling that can be readily recognized at early stages; these changes can be quantified quite precisely, and we can distinguish these quantitative pathological changes in rd10 eyes from normal aging changes.

We calculated the first 4 PC scores for both genotypes using two-dimensional, joint area and shape measurements. These four PC scores from 111 eyes form a matrix of size $111 \times 4$, which we used to construct classification rules. We applied each of the three classification methods, LDA, QDA, and SVM, which we used to construct classification rules. We applied each of the three classification methods, LDA, QDA, and SVM, which we used to construct classification rules. We applied each of the three classification methods, LDA, QDA, and SVM, which we used to construct classification rules. We applied each of the three classification methods, LDA, QDA, and SVM, which we used to construct classification rules.
FIGURE 6. Joint cell area and shape density distributions: WT young eyes (A), WT old eyes (B), rd10 young eyes (C), and rd10 old eyes (D). The height of the peaks indicates the density of cells. The colors show that WT young eyes had the sharpest and most concentrated peak of cell size aspect ratio distribution. The three other mountain plots (B–D) were blunted in height, had shoulders, and were broadly dispersed in combinations of size and shape. (E–H) Contours through the mountains illustrate the wider distribution and variation in combined cell size and shape (e.g., [E] WT young; [F] WT old; [G] rd10 young; and [H] rd10 old) of the effects of increasing age and affected genotype.
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It remains to be determined whether this morphometrics approach can be generalized: could this approach distinguish rd10 from rd1, rd8, or other animal retinal degenerations, or AMD from Stargardt or other human retinal disease at young ages in a masked study? The present study provides a proof-of-principle and a set of tools to answer these questions in the future.

Here, we found that, qualitatively, RPE cells in a WT mouse eye (Fig. 1B) had rather homogeneous sizes and nearly hexagonal packing. The cells appeared isotropic, with no obvious elongation or orientation bias. In contrast, RPE cells in the rd10 eyes looked more irregular, and variability increased with age (Fig. 1).11,32,33 Thus, qualitatively, as RPE in rd10 mice were greatly affected compared to those in WT mice, we sought to develop quantitative discriminators among the four groups (WT young, WT old, rd10 young, and rd10 old). Three tests were conducted to find accurate quantitative discriminators (cell area alone versus shape alone versus area-shape jointly).

First, in Figures 2 and 3, the cell area density curves and their first and second PC scores showed distinct features that resolved young from old WT RPE cells. To a lesser extent, young and old rd10 cells were resolved, but the four groups (WT young, WT old, rd10 young, and rd10 old) were not well resolved based only on RPE cell area.

Second, to test a measure of RPE cell shape, we chose aspect ratio. The smaller the aspect ratio, the more elongated the cell. Beyond P30, at each age tested, WT always had a greater median aspect ratio than rd10 (Figs. 4A, 4C). The density distributions and FPCA of the aspect ratio (Figs. 4, 5) showed the clear discrimination between two genotypes despite the confounding factor of age. Although we felt that further refinement of our analysis would achieve better discrimination among the four groups.

Third, to discriminate among four genotype-age groups (WT young, WT old, rd10 young, rd10 old), it was necessary to use a joint area-shape dataset. Three-dimensional density plots (Figs. 6A−D) and their corresponding contour plots (Figs. 6E−H) showed (1) a tight and narrow size-shape density distribution of the WT young RPE cells; (2) the WT old RPE cells developed a shoulder extending to larger cell areas but remained confined in aspect ratio; (3) the young rd10 had area ranges similar to those of young WT eyes but greater variability and ranges in aspect ratio; (4) the old rd10, on the other hand, varied widely in both aspect ratio and area. Thus, qualitatively, these combined size-shape plots suggested good discrimination of each group from the other three. This was borne out by FPCA analyses with the first two PC scores as shown in Figure 7 and a clear visualization of the separation among the four sample groups. This led to a clear quantitative discrimination based on the first four PC scores for each group of the joint area-shape measurements. This reduced the morphometric data from each flat mount to four PC scores. Each classification method had an accuracy of >96%, that is, given a single RPE sheet for which the area and aspect ratio density distributions are known, the odds are <4% that we would misclassify its age and genotype.

This method (using joint density distributions, FPCA, and classification) might be generally applicable to RPE morphometric data, such that different categories of RPE could be distinguished despite the age factor. Discrimination between normal subjects and patients with mutations that affect the RPE at an early age would be important diagnostic and prognostic goals, if the approach described here could be applied to highly novel noninvasive human RPE sheet imaging techniques (Rossi E, et al. IOVS 2013;54:ARVO E-Abstract 6282).

Here, we focused on the rd10 model. The RPE cells are bystanders to the rd10 degeneration that begins within photoreceptors.11,16,54 Here we showed that collateral damage to the RPE sheet occurred after the initial genetic insult to the photoreceptors. It is not yet apparent how the collateral changes occurred, but there ought to be severe oxidative stress in the RPE cells during the degeneration of photoreceptors and the remodeling of the neural retina.35 We found alterations in RPE cell shape and size, which resembled known stimulation of migration and meandering following the death of neighboring cells. We previously found regional differences in the rd10 RPE sheet as the mice aged and the disease progressed.11,55 Also we found differences in morphology, comparing the posterior RPE with far periphery regardless of age, genotype, or species. Analyzing by zone, region, quadrant, or ring, we might expect even more robust differences that may discriminate better than the whole-sheet analyses (without considering cell location) as we did here. We plan to test this hypothesis in the future.

In summary, we are now able to discriminate between age (~P61 versus ≥P180) and genotype (WT versus rd10) of the mouse by RPE morphometry in the present proof-of-principle study. This result is exciting because in the future we might be able to consider RPE morphology as a diagnostic and prognostic tool. Validation on human samples is an essential next step. Non-invasive imaging of the living human eye may soon provide comparable morphometric data for the human RPE (Rossi E, et al. IOVS 2013;54:ARVO E-Abstract 6282). In conclusion, we compared RPE characteristics of young and old rd10 and WT mice, and we found that principal component analysis distinguished between age and pathology.

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References

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