

# Light Damage in *Abca4* and *Rpe65<sup>rd12</sup>* Mice

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**PURPOSE.** Bisretinoids form in photoreceptor cells and accumulate in retinal pigment epithelium (RPE) as lipofuscin. To examine the role of these fluorophores as mediators of retinal light damage, we studied the propensity for light damage in mutant mice having elevated lipofuscin due to deficiency in the ATP-binding cassette (ABC) transporter *Abca4* (*Abca4*<sup>-/-</sup> mice) and in mice devoid of lipofuscin owing to absence of Rpe65 (*Rpe65<sup>rd12</sup>*).

**METHODS.** *Abca4*<sup>-/-</sup>, *Rpe65<sup>rd12</sup>*, and wild-type mice were exposed to 430-nm light to produce a localized lesion in the superior hemisphere of retina. Bisretinoids of RPE lipofuscin were measured by HPLC. In histologic sections, outer nuclear layer (ONL) thickness was measured as an indicator of photoreceptor cell degeneration, and RPE nuclei were counted.

**RESULTS.** As shown previously, A2E levels were increased in *Abca4*<sup>-/-</sup> mice. These mice also sustained light damage-associated ONL thinning that was more pronounced than in age-matched wild-type mice; the ONL thinning was also greater in 5-month versus 2-month-old mice. Numbers of RPE nuclei were reduced in light-stressed mice, with the reduction being greater in the *Abca4*<sup>-/-</sup> than wild-type mice. In *Rpe65<sup>rd12</sup>* mice bisretinoid compounds of RPE lipofuscin were not detected chromatographically and light damage-associated ONL thinning was not observed.

**CONCLUSIONS.** *Abca4*<sup>-/-</sup> mice that accumulate RPE lipofuscin at increased levels were more susceptible to retinal light damage than wild-type mice. This finding, together with results showing that *Rpe65<sup>rd12</sup>* mice did not accumulate lipofuscin and did not sustain retinal light damage, indicates that the bisretinoids of retinal lipofuscin are contributors to retinal light damage.

**Keywords:** *Abca4*, bisretinoid, light, lipofuscin, retina, retinal degeneration, retinal pigment epithelium

It is well known that the retina is subject to photochemical damage when exposed to prolonged periods of light of increased intensity.<sup>1,2</sup> Light damage to both photoreceptor cells and retinal pigment epithelial (RPE) cells has been observed. However, the mechanisms that lead to the damage and which irradiance levels can be considered safe are not completely understood. Conclusions as to whether photoreceptor cells or RPE are damaged first and which cells are affected more severely have varied. For instance, the loss of photoreceptor cells is greatest when intense light exposure begins during the normal nighttime phase of the diurnal cycle.<sup>3,4</sup> The death of both cell types occurs by means of programmed apoptotic processes.<sup>5,6</sup> Two action spectra for photochemical damage to the retina have been described: one adheres to the absorption spectrum of rhodopsin and has been recorded by using long exposures (>24 hours) at low irradiances,<sup>7,8</sup> while the other exhibits decreasing thresholds at shorter wavelengths and is associated with high irradiances and shorter exposure periods.<sup>9,10</sup>

Light-induced retinal injury is linked to the availability of the 11-*cis*-retinal chromophore of visual pigment. This feature is evidenced by the protection against light damage provided by conditions that block or slow the regeneration of 11-*cis*-retinal. These conditions include null mutations in Rpe65<sup>11</sup> and lecithin:retinol acyltransferase<sup>12</sup>; amino acid variants in Rpe65<sup>13,14</sup>; systemic delivery of 13-*cis*-retinoic acid<sup>15</sup>; and

halothane anesthesia that interferes with 11-*cis*-retinal binding to the opsin molecule.<sup>16</sup> On the other hand, an increase in rhodopsin content in rod outer segments increases light damage susceptibility.<sup>17</sup> Studies<sup>18</sup> also indicate that the superior hemisphere of rat retina may be more sensitive to phototoxicity because of greater concentrations of rhodopsin.

Although light exposure triggers photoreceptor cell apoptosis only after photo-isomerization of visual pigment, phototransduction may not be necessary.<sup>6,12,19</sup> Products of bleaching such as all-*trans*-retinal ( $\lambda_{\max} \sim 380$  nm),<sup>6,12</sup> or metarhodopsin II<sup>20,21</sup> have been suggested as the damaging agent. An extensive bleach of rhodopsin can potentially release  $\sim 3$  mM all-*trans*-retinal,<sup>21</sup> yet a single bleaching event does not induce photoreceptor cell death in mice.<sup>6</sup> Moreover, all-*trans*-retinal has an absorbance maximum of 380 nm and only little absorbance above 450 nm<sup>22</sup>; at these longer wavelengths retinal photodamage readily occurs.

Since repetitive photon absorption is required to elicit retinal damage, photoproducts formed after sustained activation of rhodopsin could accumulate and act as photosensitizers causing cellular damage.<sup>7,23</sup> Potential initiators that fit this description are the lipofuscin fluorophores of retina. The vitamin A aldehyde-derived fluorophores of this lipofuscin form in photoreceptor cells as byproducts of photon absorbance by the 11-*cis*-retinal chromophore of visual pigment; this origin is consistent with evidence coupling retinal light damage

to activation of visual pigment. Under conditions of dark-rearing, the bisretinoids of lipofuscin probably also form from reactions of 11-*cis*-retinal.<sup>24</sup> After being transferred to RPE when shed outer segments are phagocytosed, these fluorophores subsequently accumulate as lipofuscin in the lysosomal compartment of RPE. Significantly, native lipofuscin in the RPE exhibits an excitation spectrum with a range of approximately 425 to 580 nm and a peak at approximately 480 nm.<sup>25</sup> Individual bisretinoid fluorophores that have been identified exhibit excitation maxima at 435, 439, 443, 490, and 510 nm.<sup>26–29</sup> These relatively long excitation wavelengths are enabled by carbon-carbon double bonds that form extended conjugation systems in the bisretinoid molecules. Lipofuscin isolated from RPE cells can serve as a photo-inducible generator of singlet oxygen and superoxide anion and in the presence of light and RPE lipofuscin, lipid undergoes peroxidation.<sup>30–33</sup> Retinal pigment epithelium cells that contain lipofuscin or one of its constituents, A2E, are subject to mitochondrial and nuclear DNA damage and a loss of viability.<sup>34–38</sup>

To examine the role of RPE lipofuscin as a mediator of light damage, we studied the susceptibility to light damage of wild-type mice as compared to mice burdened with elevated RPE lipofuscin due to a null mutation in the ATP-binding cassette (ABC) transporter *Abca4* (*Abca4*<sup>−/−</sup> mice). We also studied mice that do not synthesize lipofuscin owing to deficiency in Rpe65 (*Rpe65*<sup>rd12</sup>).

## METHODS

### Mice

Albino *Abca4/Abcr* null mutant mice and *Abca4/Abcr* wild-type mice (homozygous for Rpe65-Leu450), were generated and genotyped by PCR amplification of tail DNA.<sup>39,40</sup> For genotyping of the murine Rpe65 variant, digestion of the 545-bp product with *Muoi* restriction enzyme (New England Biolabs, Ipswich, MA) indicated the Leu450 variant if 180- and 365-bp fragments were generated. *Rpe65*<sup>rd12</sup> mice and C57BL/6J mice (homozygous for Rpe65-Met450) were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were raised under 12-hour on-off cyclic lighting with in-cage illuminance of 30 to 80 lux. All procedures involving animals adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The proposed research involving animals has been approved by the Institutional Animal Care and Use Committee.

### High-Performance Liquid Chromatography

A2E was measured by HPLC as we have described previously.<sup>39,41</sup> Briefly, murine eyecups were homogenized, extracted in chloroform/methanol (2:1) and after drying, the extract was redissolved in 50% methanolic chloroform containing 0.1% trifluoroacetic acid (TFA) and analyzed by reverse phase HPLC (Alliance System; Waters Corp., Milford, MA) using a dC18 column. Gradients of water and acetonitrile with 0.1% TFA were used for the mobile phase. Chromatographic peak areas were determined by using Empower software (Waters Corp.), and molar quantity per eye was calculated by using external standards of synthesized compounds, the structures of which have been confirmed.<sup>27,29,42</sup>

### Light Exposure

Mice were anesthetized with 80 mg/kg ketamine and 5 mg/kg xylazine, anesthesia that does not interfere with light-damage models.<sup>43</sup> Pupils of right eyes were dilated with phenylephrine hydrochloride (2.5%; Alcon Laboratories, Fort Worth, TX) and

cyclopentolate hydrochloride (0.5%, Alcon Laboratories), and ViscoTears Liquid Gel (2 mg/g; Novartis Pharmaceuticals, North Ryde, NSW, Australia) was applied to the surface of the cornea to maintain hydration and clarity. The cornea was positioned at a constant distance from the light source and to eliminate light scatter at the corneal surface, a thin glass plate (diameter, 7.5 mm; thickness, 0.4 mm) was placed on the cornea; light lost by absorption was <10%. Light intensity was measured with a Newport optical power meter (model 840; Newport Corporation, Irvine, CA). Light (430 ± 20 nm) was delivered to the superior hemisphere of the retina from a mercury arc lamp at an intensity of 50 mW/cm<sup>2</sup> for 30 minutes.<sup>43</sup> Throughout the period of illumination the operator carefully observed the positioning so as to re-adjust if shifts in the position occurred. Right eyes were light exposed and left eyes served as unexposed controls.

### Histometric Analysis

Seven days after light exposure, eyes were fixed in 4% paraformaldehyde for 24 hours at room temperature. Hematoxylin-eosin-stained, sagittal, 6-mm paraffin sections were prepared. For measurement of outer nuclear layer (ONL) thickness, three sections through the optic nerve head (ONH) of each eye were imaged digitally (Leica Microsystems, Leica, Buffalo Grove, IL) with an ×10 objective, and ONL thickness was measured at 200-μm intervals from the ONH to a position 2.4 mm superior and inferior to the ONH along the vertical meridian. Outer nuclear layer width in pixels was converted to micrometers (1 pixel = 0.92 μm) and data from the three sections were averaged. For groups of *Abca4*<sup>−/−</sup> and *Abca4*<sup>+/+</sup> mice at each age, mean ONL thickness at each position along the vertical meridian was plotted as a function of eccentricity from the ONH.<sup>44,45</sup> Outer nuclear layer area was calculated as the sum of the ONL thicknesses in superior hemiretina (ONH to 1.0 mm) with multiplication by the measurement interval of 0.2 mm.

To count RPE nuclei, three sections through the ONH were imaged with the ×40 objective. Nuclei were counted within 200-μm intervals beginning at the edge of the ONH and continuing superiorly along the vertical axis. Data from the three sections were averaged to obtain mean values for each measurement interval. Mean nuclei numbers within the 0.2-mm measurement intervals were plotted as a function of distance from ONH to 1.6 mm superiorly. Counts were also summed to obtain total numbers of RPE.

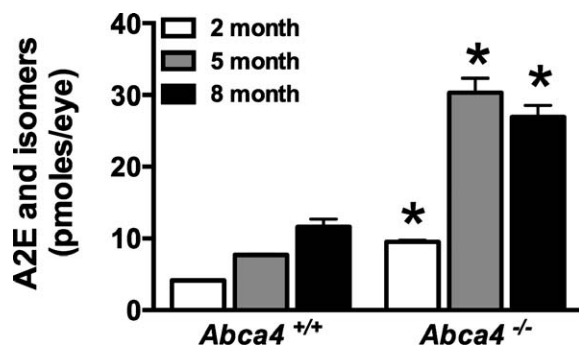
### Statistical Analysis

Significance was assessed at the 0.05 level (Prism 6; GraphPad Software, Inc., La Jolla, CA) by using statistical tests as indicated.

## RESULTS

### HPLC Quantitation of A2E

As formerly reported,<sup>20,39,46</sup> deficiency in *Abca4* was associated with substantial accumulations of lipofuscin pigments in the RPE (Fig. 1). We quantified A2E, a bisretinoid compound of RPE lipofuscin, by integrating HPLC peaks and normalizing to a calibration curve. A2E levels in the *Abca4*<sup>−/−</sup> mice were 3.8-fold greater than in wild-type mice at 5 months of age. However, the pattern of change in the two groups of mice was different. Specifically, while A2E increased steadily from 2 to 8 months of age in the *Abca4*<sup>+/+</sup> mice, the amounts in *Abca4*<sup>−/−</sup> mice rose between 2 and 5 months of age and then leveled off at 8 months of age (Fig. 1).



**FIGURE 1.** HPLC quantitation of A2E in eyes of *Abca4*<sup>+/+</sup> and *Abca4*<sup>-/-</sup> mice, age 2 to 8 months. A2E, its minor isomers, and isoA2E were measured separately and summed. Values are the mean  $\pm$  SEM (2–5 samples per mean, 4–8 eyes per sample). \* $P < 0.01$  when age-matched *Abca4*<sup>+/+</sup> and *Abca4*<sup>-/-</sup> mice were compared by unpaired two-tailed *t*-test.

### ONL Thickness

Consistent with previous work,<sup>43</sup> our light damage model yielded a lesion in superior central retina; the lesion size was  $\sim 800$   $\mu\text{m}$  along the vertical meridian. The measurement of ONL is an established approach to assessing photoreceptor cell integrity. Thus, to detect the light-induced lesion, we measured and plotted ONL thicknesses as a function of distance superior and inferior to the optic nerve head in the vertical plane (Fig. 2). In wild-type (*Abca4*<sup>+/+</sup>) mice at 2 months of age, no ONL thinning was observed in superior hemiretina of light-stressed (right) eyes after 1 week (Fig. 2A). Conversely, ONL width was reduced in superior retina of light-exposed eyes of *Abca4* null mutant mice, with the differences at the 0.4- and 0.6-mm positions being statistically significant ( $P < 0.05$ ; one-way ANOVA and Sidak's multiple comparison test; Fig. 2B). For mice at 5 months of age, 430-nm light induced a superior lesion in both the *Abca4*<sup>+/+</sup> (0.6 mm, right versus left;  $P > 0.05$ ) and *Abca4*<sup>-/-</sup> (0.2–0.8 mm, right versus left;  $P < 0.05$ , one-way ANOVA and Sidak's multiple comparison test) hemiretina, although visual inspection revealed that ONL thinning was more pronounced in the *Abca4* null mutant versus wild-type mice (Figs. 2C, 2D, respectively). The lesions in the 8-month-old *Abca4*<sup>-/-</sup> and *Abca4*<sup>+/+</sup> superior hemiretinae (Figs. 2E, 2F) were also distinct. However, although the difference between exposed and unexposed retinas in *Abca4*<sup>-/-</sup> mice was significant (0.2–0.6 mm, right versus left;  $P < 0.05$ , one-way ANOVA and Sidak's multiple comparison test), the plots of ONL thickness revealed that the lesions in the *Abca4*<sup>-/-</sup> mice were less pronounced at 8 months of age than at age 5 months. In this regard it was also apparent that even in the absence of experimental light damage (Fig. 2F, left eyes), ONL width was reduced in the 8-month-old *Abca4*<sup>-/-</sup> mice; this observation has been previously reported.<sup>40,47,48</sup> Thus, in the case of the 8-month-old *Abca4*<sup>-/-</sup> mice, experimental light exposure was delivered on a background of genetically induced photoreceptor cell degeneration.

For further quantitation, we also calculated ONL area by summing ONL thicknesses in superior hemiretina (0.2–1.0 mm) and multiplying by the measurement interval of 0.2 mm (Fig. 3A). For the *Abca4*<sup>+/+</sup> mice exposed to 430-nm light, ONL area was not reduced at 2 months of age. However, at 5 and 8 months of age, ONL area was decreased by 9% ( $P < 0.05$ ) and 15% ( $P < 0.05$ ), respectively, when compared to the control (left eyes, paired *t*-test). In the *Abca4*<sup>-/-</sup> mice, ONL area in light-exposed eyes (right) was decreased by 19% even at 2 months of age ( $P > 0.05$ , right versus left, paired *t*-test), and at 5 months of age, the reduction reached 47% ( $P < 0.05$ , left

versus right eyes, paired *t*-test). The reduction in ONL area associated with light stress was also significantly greater in the *Abca4*<sup>-/-</sup> versus *Abca4*<sup>+/+</sup> mice at 2 and 5 months of age when compared to wild type ( $P < 0.05$ , unpaired *t*-test). In addition, an effect of age was observed, with the light-induced ONL thinning in *Abca4*<sup>-/-</sup> mice being greater at 5 months than at 2 months and thinning in *Abca4*<sup>+/+</sup> mice being greater at 8 months than at 2 months ( $P < 0.05$ , one-way ANOVA and Tukey's multiple comparison test). Shown in Figure 3B are representative images of hematoxylin-eosin-stained superior retinae from *Abca4*<sup>+/+</sup> and *Abca4*<sup>-/-</sup> mice (age 5 months). In the *Abca4*<sup>-/-</sup> mice, ONL thickness was severely reduced as compared to wild type.

Counting of RPE nuclei within superior hemiretina also revealed a loss of RPE in light-stressed *Abca4*<sup>+/+</sup> and *Abca4*<sup>-/-</sup> mice, the reduction being greater with the null mutation (Figs. 4A–F). For comparative purposes we calculated the sum of the nuclei counted within the RPE monolayer from the ONH to a distance 1.0 mm superiorly (Fig. 4G). In the wild-type eyes, RPE cell numbers in light-stressed (right) eyes were reduced by 20% to 26% (ages 2–8 months when compared to left control;  $P > 0.05$ , paired *t*-test), while in *Abca4*<sup>-/-</sup> mice the reduction ranged from 59% to 78% ( $P < 0.05$ , paired *t*-test). At 2 and 8 months of age, the reduction in RPE nuclei in the *Abca4*<sup>-/-</sup> mice was more pronounced than the reduction in the *Abca4*<sup>+/+</sup> mice ( $P < 0.05$ , unpaired *t*-test).

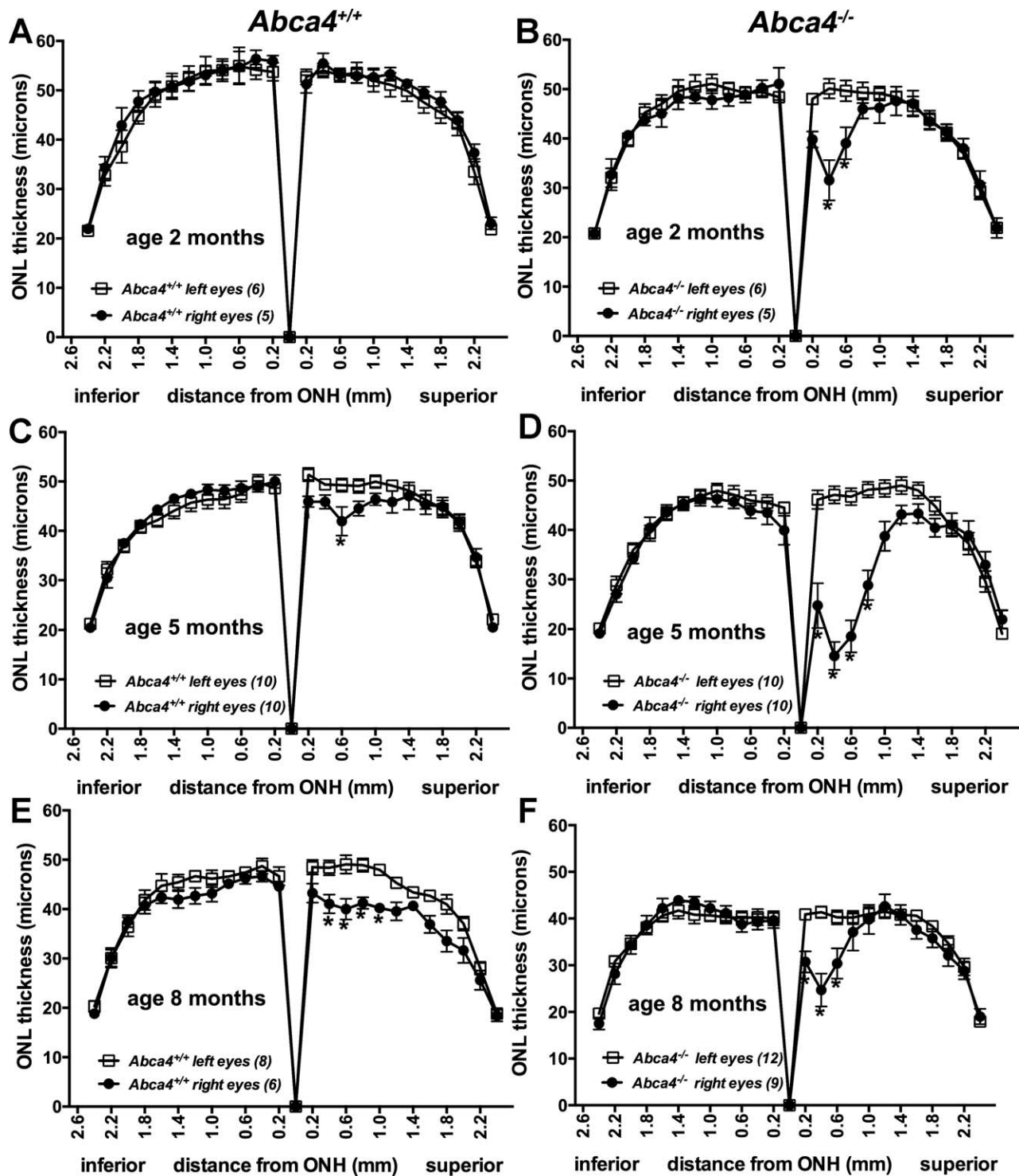
### Light Damage and the *Rpe65*<sup>rd12</sup> Mouse

The *Rpe65*<sup>rd12</sup> mouse is characterized by loss of function of Rpe65 due to a naturally occurring severe truncation of the protein together with nonsense-mediated mRNA degradation.<sup>49</sup> It has previously been reported that Rpe65 null mutation prevents an accumulation of the RPE fluorescence attributable to lipofuscin.<sup>50</sup> Consistent with the latter observation, we found that bisretinoids of lipofuscin were not detected in *Rpe65*<sup>rd12</sup> mice (Fig. 5). The bisretinoids examined chromatographically (Fig. 5A) included A2E, A2E isomers, A2-DHP-PE (dihydropyridine-phosphatidylethanolamine), and atRAL dimer-PE (all-*trans*-retinal dimer phosphatidylethanolamine). Rpe65 deficiency is also reported to confer a resistance to light damage.<sup>11</sup> Thus, we tested our light damage model under conditions of a null mutation in Rpe65 by submitting *Rpe65*<sup>rd12</sup> mice (5 months of age) to  $430 \pm 20$  nm light (50 mW/cm<sup>2</sup> for 30 minutes). There was no difference in ONL thickness in light-stressed (right) compared to non-light-stressed (left) eyes of *Rpe65*<sup>rd12</sup> mice ( $P > 0.05$ , 0.2–2.0 mm, right versus left eyes;  $P < 0.05$ , one-way ANOVA and Sidak's multiple comparison test; Fig. 5B). Thinning was, however, observed owing to the Rpe65 mutation. Specifically, when non-light-stressed *Rpe65*<sup>rd12</sup> mice (age 5 months) were compared to C57BL/6J mice (age 6 months), there was a noticeable decrease in ONL thickness (Fig. 5B). This change was consistent with previous reports indicating that mice homozygous for the *Rpe65*<sup>rd12</sup> mutation undergo thinning of ONL after 3 months of age, with a 50% reduction in rows of ONL nuclei being measurable in the *Rpe65*<sup>rd12</sup> mutants by 7 months of age.<sup>49,51</sup>

### DISCUSSION

Here we reported that a mutant mouse line (*Abca4*<sup>-/-</sup>) that accumulates RPE lipofuscin at amplified levels is more susceptible to short-wavelength light damage than wild-type mice. A2E, one of the components of lipofuscin, was 3.8-fold more abundant at 5 months of age in *Abca4*<sup>-/-</sup> mice than wild-type mice. In 5-month-old light-stressed *Abca4*<sup>-/-</sup> mice, ONL

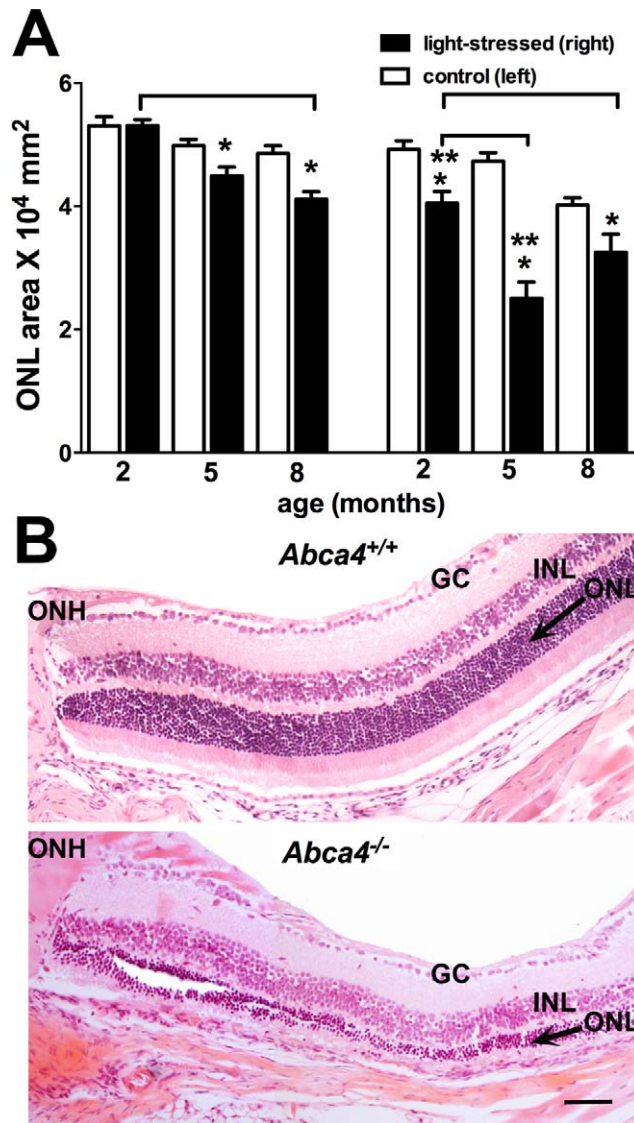




**FIGURE 2.** ONL thicknesses measured in *Abca4*<sup>+/+</sup> (A, C, E) and *Abca4*<sup>-/-</sup> (B, D, F) mice, both light-stressed (right) and control (non-light-stressed; left) eyes of 2-, 5-, and 8-month-old mice 7 days after light exposure. Values are mean ± SEM of numbers of eyes presented in parentheses. \**P* < 0.05 when right eyes were compared to left eyes by one-way ANOVA and Sidak's multiple comparison test. The lesions present as areas of reduced ONL thickness in superior retinae.

area (0.2–1.0 mm from ONH), a measure of photoreceptor cell viability, was reduced by 47% relative to control fellow eyes, while the light-induced reduction in the wild-type eyes was only 10% at this age. Moreover, A2E amounts increased with age (2 vs. 5 months) in the *Abca4*<sup>-/-</sup> and wild-type (2, 5, and 8 months) mice, as did the severity of light damage. We observed

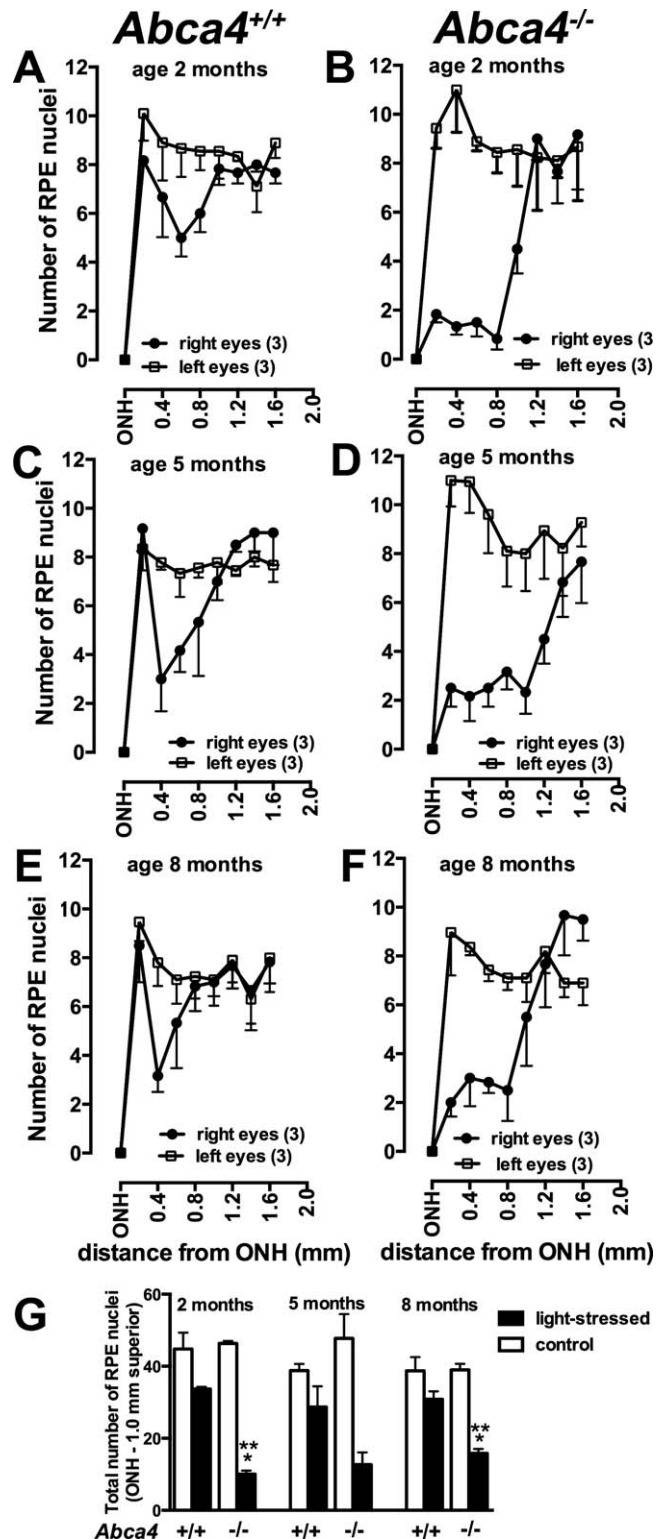
that light-induced damage was more pronounced in central retina than the periphery. In this regard it is significant that the level of A2E, just one of the bisretinoids of lipofuscin, is also higher centrally in the mouse.<sup>52</sup> These findings are consistent with a role for RPE lipofuscin in mediating light damage. A functional 11-*cis*-retinal chromophore is necessary to elicit



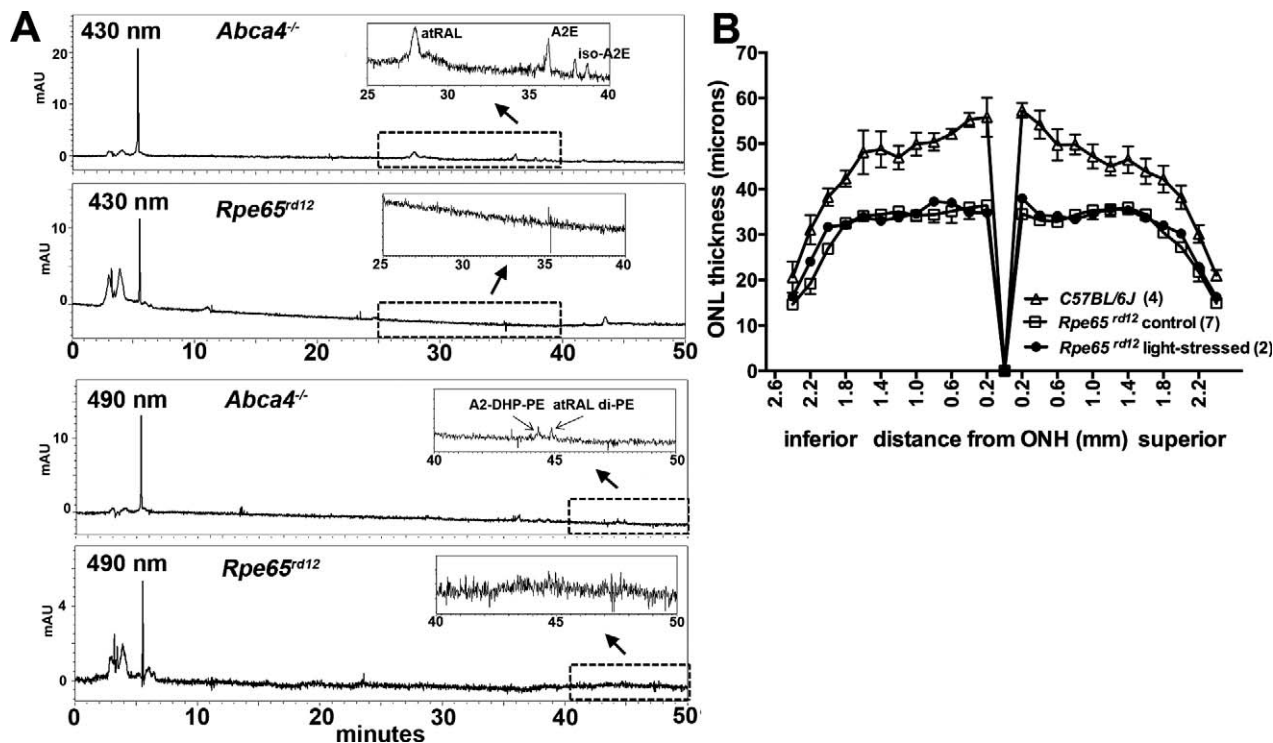
**FIGURE 3.** ONL thinning in light-stressed *Abca4*<sup>-/-</sup> and *Abca4*<sup>+/+</sup> mice. (A) ONL areas in superior hemiretinae of right (light-stressed) and left (nonstressed control) eyes 1 week after light irradiation. ONL area was calculated by summing thickness measurements (0.2–1.0 mm from ONH) and multiplying by 0.2 mm. Values are means ± SEM of 5 to 11 mice. \**P* < 0.05, right eyes as compared to fellow left eyes (paired *t*-test); \*\**P* < 0.05, right eyes of *Abca4*<sup>-/-</sup> mice as compared to age-matched right eyes of *Abca4*<sup>+/+</sup> mice (unpaired *t*-test, *P* < 0.05); bracket indicates *P* < 0.05, in *Abca4*<sup>+/+</sup> mice, age 2 vs. 8 months, and in *Abca4*<sup>-/-</sup> mice, 2 vs. 5 months of age (ANOVA and Tukey's multiple comparison test). (B) Representative light micrographs of superior hemiretina of right eyes of 5-month-old *Abca4*<sup>+/+</sup> (upper) and *Abca4*<sup>-/-</sup> (lower) mice 1 week after short-wavelength light exposure. Scale bar: 50 μm. GC, ganglion cell layer; INL, inner nuclear layer.

retinal light damage,<sup>11,12</sup> but a direct effect of the chromophore cannot explain these results, since rhodopsin and 11-*cis*-retinal levels are not different in *Abca4*<sup>-/-</sup> versus wild-type mice.<sup>20</sup>

Whereas *Abca4* deficiency increases bisretinoid formation and light damage, we showed that an absence of Rpe65 activity abrogates bisretinoid formation (Fig. 5) and, as has been reported previously,<sup>11</sup> the mice are correspondingly refractory to light damage (Fig. 5). Rpe65 activity is rate limiting in the visual cycle and the failure to generate 11-*cis*-retinal owing to Rpe65 deficiency is the cause of photoreceptor cell degener-



**FIGURE 4.** Effect of light stress on RPE cells in superior retina of *Abca4*<sup>+/+</sup> and *Abca4*<sup>-/-</sup> mice. (A–F) Numbers of RPE cells (mean ± SEM) counted within 0.2-mm intervals and plotted as function of distance from ONH in superior hemiretina at 2, 5, and 8 months of age in *Abca4*<sup>+/+</sup> (A, C, E) and *Abca4*<sup>-/-</sup> mice (B, D, F). (G) Total numbers of RPE cells from ONH to 1.0 mm along vertical axis in superior hemisphere of light-stressed (right) and control non-light-stressed eyes of *Abca4*<sup>+/+</sup> and *Abca4*<sup>-/-</sup> mice at 2, 5, and 8 months of age (mean ± SEM; means are based on three values). \**P* < 0.05 as compared to fellow control eye; \*\**P* < 0.05 light-stressed *Abca4*<sup>+/+</sup> versus *Abca4*<sup>-/-</sup> mice; unpaired *t*-test.



**FIGURE 5.** Light stress in *Rpe65<sup>rd12</sup>* mice. (A) Reverse phase HPLC chromatograms with UV-visible detection at 430 and 490 nm. The bisretinoids A2E, A2-DHP-PE, and atRAL dimer-PE are detected in eyes of *Abca4<sup>-/-</sup>* mice (age 6 months; four eyes pooled/sample) but are not detected in *Rpe65<sup>rd12</sup>* mice (age 8 months; eight eyes pooled/sample). (B) Measurement of ONL in light-exposed (right) and control (left) eyes of *Rpe65<sup>rd12</sup>* mice, age 5 months. Thicknesses are plotted as distance from ONH. C57BL/6J mice (6 months) served as wild type. Values are mean  $\pm$  SEM of numbers of eyes presented in parentheses.

ation beginning at 3 months of age.<sup>49,51</sup> The underlying photoreceptor cell degeneration in *Rpe65<sup>rd12</sup>* mice is not likely to have obscured the effects of experimental light damage if it had occurred: reduced ONL width in the *Abca4<sup>-/-</sup>* mouse was measurable even when light stress was delivered on a background of gene-induced ONL thinning (i.e., age 8 months). While 11-*cis*-retinal is not detected in *Rpe65<sup>-/-</sup>* mice, a small light response can be elicited from isorhodopsin, the latter forming from low levels of 9-*cis*-retinal<sup>53</sup>; nevertheless, this level of photosensitivity is not sufficient to detect bisretinoid formation (Fig. 5). It is significant that even a reduction in Rpe65 activity, as in the case of the Rpe65 variant at amino acid 450 (leucine to methionine), is associated with reduced lipofuscin accumulation<sup>39</sup> and correspondingly, with diminished sensitivity to light damage.<sup>13,14</sup>

We note that the effects of genetically induced photoreceptor cell degeneration combined with experimental light damage were not additive in the *Abca4<sup>-/-</sup>* model. This observation can be made by inspecting ONL thinning in the light-stressed *Abca4<sup>-/-</sup>* mice at 5 and 8 months of age (Fig. 2). Specifically, the reduction in ONL thickness attributable to experimental light damage when light stress was delivered on a background of genetically induced photoreceptor cell degeneration (age 8 months) was less pronounced than at a younger age (age 5 months) preceding the onset of genetically induced photoreceptor cell death. Among the explanations for this effect could be the possibility that the two cell-death stimuli share a common downstream molecular pathway or that the underlying genetically induced photoreceptor cell degeneration elicits cell survival mechanisms that partially protect against subsequent light damage.

Various chromophores in RPE have been suggested to mediate short-wavelength light damage, including riboflavin,

the mitochondrial enzyme cytochrome c oxidase, and melanin.<sup>23,54-58</sup> However, riboflavin and cytochrome c oxidase are not expected to increase with a null mutation in *Abca4* or with age, and these molecules would not be modulated by *Rpe65* knockout. Ocular melanin can be excluded as a damaging agent, since pigmented and albino animals are equally susceptible to retinal light damage.<sup>56,59</sup> In fact, ocular melanin decreases retinal irradiance, thereby lengthening the time to retinal damage. In another model, the *Rdb8<sup>-/-</sup>Rdb12<sup>-/-</sup>Abca4<sup>-/-</sup>* mouse, A2E levels were also many-fold greater than in wild type, and photoreceptor cell degeneration 7 days after a 30-minute white light exposure (10,000 lux)<sup>60</sup> was more pronounced than in wild-type retinas. In this case, however, the damaging agent was assumed to be all-*trans*-retinal.

While most studies of light damage address photoreceptor cell loss, the site of origin of light damage in primates may be photoreceptor outer segments and/or RPE cells; indeed, some investigators have reported the greatest damage in RPE cells.<sup>9,61-65</sup> In the work presented here, we observed light-induced loss of both RPE and photoreceptor cells. The light stress-induced reduction of RPE numbers was greater with the *Abca4<sup>-/-</sup>* null mutation than in wild type; however, for reasons unknown to us, we were unable to detect an age-related increase in light stress-induced RPE loss in the wild-type and null mutant mice. We cannot draw conclusions as to whether damage was initiated in photoreceptor cells and RPE independently or whether for instance, the primary injury resided in RPE cells, with the loss of photoreceptor cells following owing to the dependence of photoreceptor cells on RPE functioning. The formation of the pigments of RPE lipofuscin begins in photoreceptor outer segments with transfer to the RPE when these cells phagocytose packets of distal outer segment membrane.<sup>25</sup> Accordingly, bisretinoids are present in both



photoreceptor cells and RPE, although owing to outer segment shedding, bisretinoids in photoreceptor cells are kept to a minimum and instead accumulate in RPE. We found that in both *Abca4*<sup>+/+</sup> and *Abca4*<sup>-/-</sup> mice, the severity of the acute light-induced ONL thinning was greater in older mice. Since the bisretinoids of lipofuscin accumulate in RPE cells with age, the age-related increase in retinal light damage we observed may point to injury that is initiated in RPE cells. In general, whether damage occurs primarily in the RPE versus photoreceptor cells, or both, may depend on the paradigm under which light is delivered. For instance, since long-duration light-damage models could allow for regeneration of visual pigment and repeated bleaching, formation of bisretinoids in photoreceptor cells may be promoted under these conditions, thus increasing the likelihood of primary photoreceptor cell damage. It is reported that photoreceptor cell damage is initiated distally in the outer segment and subsequently progresses to include the entire length of the outer segment.<sup>18,66</sup> While this observation may be explained by apical-basal differences in the lipid composition of the outer segment membrane,<sup>2</sup> another factor could be that apically situated more aged discs contain higher levels of the bisretinoid compounds that become the lipofuscin of RPE.

Photo-oxidative mechanisms are clearly involved in retinal light damage, since antioxidants can protect against photic damage, and increased oxygen availability lowers the threshold.<sup>61,67-69</sup> Inhibition of NADPH oxidase activity also suppresses rod photoreceptor light-induced degeneration.<sup>70</sup> A role for antioxidants in protection against light damage is significant since bisretinoids are well known to be capable of short-wavelength light-induced singlet oxygen generation, with the latter reacting back at carbon-carbon double bonds and thereby photo-oxidizing the bisretinoid.<sup>71</sup> Interestingly, TEMPOL-H, a compound having antioxidant properties, has been shown both to inhibit A2E photo-oxidation elicited by short-wavelength light exposure<sup>72</sup> and to protect against light-induced damage to both photoreceptor cells and RPE.<sup>73,74</sup> Of additional note, the increase in retinal proteins modified by the reactive aldehyde 4-hydroxynonenal, a product of polyunsaturated fatty acid oxidation, is a feature of retinal light damage in rats<sup>45</sup> and of cultured RPE cells that have accumulated A2E and are irradiated with short-wavelength light.<sup>75</sup>

The photo-oxidation and photodegradation of bisretinoid injure cells<sup>36,38</sup> and can be visualized as photobleaching, a light-induced decline in fluorescence.<sup>76</sup> Evidence that lipofuscin photobleaching is linked to RPE cell death in vivo has been provided by observations made during imaging of the RPE monolayer by fluorescence adaptive optics scanning light ophthalmoscopy in nonhuman primates. At irradiance levels below American National Standards Institute safety standards an abrupt autofluorescence bleaching of RPE lipofuscin is detected.<sup>77,78</sup> Depending on the retinal irradiance, the immediate decrease in the magnitude of lipofuscin autofluorescence emission either recovers ( $\leq 210$  J/cm<sup>2</sup>) after a few days or progresses to structural disruption of the RPE mosaic ( $\geq 247$  J/cm<sup>2</sup>), consistent with RPE photodamage. This photo-injury is observed at both 488- and 568-nm exposures and it occurs even when photoreceptor cells appear normal.

We have previously demonstrated that albino *Abca4*<sup>-/-</sup> mice undergo photoreceptor cell loss that can readily be detected at 8 months of age and that is not observed in age-matched wild-type mice. This genetically induced ONL thinning is more pronounced in superior hemisphere of retina<sup>40,47</sup> and, given our present findings, may be linked to ambient light exposure. Our present findings indicate that light is likely a critical mediator of bisretinoid toxicity. Accordingly, it has been suggested that patients with macular ABCA4 disease should be protected from unnecessary light exposure.<sup>79</sup> The

present findings are also significant since the progression of retinal degeneration in some forms of retinitis pigmentosa (RP) may be aggravated by light exposure.<sup>80</sup> Light-mediated perturbation has also been demonstrated in animal models of RP, including those carrying mutations in rhodopsin.<sup>81-84</sup> Thus, perhaps small-molecule therapeutics that combat overproduction of bisretinoid formation could aid in preserving photoreceptor cells in some forms of RP, until other forms of therapy (e.g., gene therapy) can be delivered. While epidemiologic studies<sup>85-88</sup> have reported conflicting views regarding a role for light exposure in AMD pathogenesis, the European Eye Study<sup>89</sup> has concluded that there is a significant association between light exposure and AMD in subjects with a low intake of antioxidants.

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### References

- Hunter JJ, Morgan JL, Merigan WH, Sliney DH, Sparrow JR, Williams DR. The susceptibility of the retina to photochemical damage from visible light. *Prog Retin Eye Res.* 2012;31:28-42.
- Organisciak DT, Vaughan DK. Retinal light damage: mechanisms and protection. *Prog Retin Eye Res.* 2010;29:113-134.
- Organisciak DT, Darrow RA, Barsalou L, Kutty RK, Wiggert B. Circadian-dependent retinal light damage in rats. *Invest Ophthalmol Vis Sci.* 2000;41:3694-3701.
- Beatrice J, Wenzel A, Reme CE, Grimm C. Increased light damage susceptibility at night does not correlate with RPE65 levels and rhodopsin regeneration in rats. *Exp Eye Res.* 2003;76:695-700.
- Hafezi F, Marti A, Munz K, Reme CE. Light-induced apoptosis: differential timing in the retina and pigment epithelium. *Exp Eye Res.* 1997;64:963-970.
- Wenzel A, Grimm C, Samardzija M, Reme CE. Molecular mechanisms of light-induced photoreceptor apoptosis and neuroprotection for retinal degeneration. *Prog Retin Eye Res.* 2005;24:275-306.
- Noell WK, Walker VS, Kang BS, Berman S. Retinal damage by light in rats. *Invest Ophthalmol.* 1966;5:450-473.
- Williams TP, Howell WL. Action spectrum of retinal light damage in albino rats. *Invest Ophthalmol Vis Sci.* 1983;24:285-287.
- Ham WT, Mueller HA. Retinal sensitivity to damage from short wavelength light. *Nature.* 1976;260:153-154.
- van Norren D, Gorgels TGME. The action spectrum of photochemical damage to the retina: a review of monochromatic threshold data. *Photochem Photobiol.* 2011;87:747-753.
- Grimm C, Wenzel A, Hafezi F, Yu S, Redmond TM, Reme CE. Protection of Rpe65-deficient mice identifies rhodopsin as a mediator of light-induced retinal degeneration. *Nat Genet.* 2000;25:63-66.
- Maeda A, Maeda T, Golczak M, et al. Involvement of all-trans-retinal in acute light-induced retinopathy of mice. *J Biol Chem.* 2009;284:15173-15183.
- Danciger M, Matthes MT, Yasamura D, et al. A QTL on distal chromosome 3 that influences the severity of light-induced damage to mouse photoreceptors. *Mamm Genome.* 2000;11:422-427.

14. Wenzel A, Reme CE, Williams TP, Hafezi F, Grimm C. The Rpe65 Leu450Met variation increases retinal resistance against light-induced degeneration by slowing rhodopsin regeneration. *J Neurosci.* 2001;21:53-58.
15. Sieving PA, Chaudhry P, Kondo M, et al. Inhibition of the visual cycle in vivo by 13-cis retinoic acid protects from light damage and provides a mechanism for night blindness in isotretinoin therapy. *Proc Natl Acad Sci U S A.* 2001;98:1835-1840.
16. Keller GA, Grimm C, Wenzel A, Hafezi F, Reme CE. Protective effects of halothane anesthesia on retinal light damage: inhibition of metabolic rhodopsin regeneration. *Invest Ophthalmol Vis Sci.* 2001;42:476-480.
17. Rapp LM, Tolman BL, Koutz CA, Thum LA. Predisposing factors to light-induced photoreceptor cell damage: retinal changes in maturing rats. *Exp Eye Res.* 1990;51:177-184.
18. Vaughan DK, Nemke JL, Fliesler SJ. Evidence for a circadian rhythm of susceptibility to retinal light damage. *Photochem Photobiol.* 2002;75:547-553.
19. Hao W, Wenzel A, Obin MS, et al. Evidence for two apoptotic pathways in light-induced retinal degeneration. *Nat Genet.* 2002;32:254-260.
20. Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH. Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice. *Cell.* 1999;98:13-23.
21. Lamb TD, Pugh EN. Dark adaptation and the retinoid cycle of vision. *Prog Retin Eye Res.* 2004;23:307-380.
22. Becker RS, Inuzuka K, Balke DE. Comprehensive investigation of the spectroscopy and photochemistry of retinals, I: theoretical and experimental considerations of absorption spectra. *J Am Chem Soc.* 1971;93:38-42.
23. Mellerio J. Light effects on the retina. In: Albert DM, Jakobiec FA, eds. *Principles and Practice of Ophthalmology: Basic Science.* Philadelphia, PA: WB Saunders Co; 1994;1326-1345.
24. Boyer NP, Higbee D, Currin MB, et al. Lipofuscin and N-retinylidene-N-retinylethanolamine (A2E) accumulate in the retinal pigment epithelium in the absence of light exposure: their origin is 11-cis retinal. *J Biol Chem.* 2012;287:22276-22286.
25. Sparrow JR, Boulton M. RPE lipofuscin and its role in retinal photobiology. *Exp Eye Res.* 2005;80:595-606.
26. Yamamoto K, Yoon KD, Ueda K, Hashimoto M, Sparrow JR. A novel bisretinoid of retina is an adduct on glycerophosphoethanolamine. *Invest Ophthalmol Vis Sci.* 2011;52:9084-9090.
27. Parish CA, Hashimoto M, Nakanishi K, Dillon J, Sparrow JR. Isolation and one-step preparation of A2E and iso-A2E, fluorophores from human retinal pigment epithelium. *Proc Natl Acad Sci U S A.* 1998;95:14609-14613.
28. Kim SR, Jang YP, Jockusch S, Fishkin NE, Turro NJ, Sparrow JR. The all-trans-retinal dimer series of lipofuscin pigments in retinal pigment epithelial cells in a recessive Stargardt disease model. *Proc Natl Acad Sci U S A.* 2007;104:19273-19278.
29. Wu Y, Fishkin NE, Pande A, Pande J, Sparrow JR. Novel lipofuscin bisretinoids prominent in human retina and in a model of recessive Stargardt disease. *J Biol Chem.* 2009;284:20155-20166.
30. Gaillard ER, Atherton SJ, Eldred G, Dillon J. Photophysical studies on human retinal lipofuscin. *Photochem Photobiol.* 1995;61:448-453.
31. Rozanowska M, Jarvis-Evans J, Korytowski W, Boulton ME, Burke JM, Sarna T. Blue light-induced reactivity of retinal age pigment: in vitro generation of oxygen-reactive species. *J Biol Chem.* 1995;270:18825-18830.
32. Boulton M, Dontsov A, Jarvis-Evans J, Ostrovsky M, Sivistunenko D. Lipofuscin is a photoinducible free radical generator. *J Photochem Photobiol B.* 1993;19:201-204.
33. Wassell J, Davies S, Bardsley W, Boulton M. The photoreactivity of the retinal age pigment lipofuscin. *J Biol Chem.* 1999;274:23828-23832.
34. Godley BF, Shamsi FA, Liang FQ, Jarrett SG, Davies S, Boulton M. Blue light induces mitochondrial DNA damage and free radical production in epithelial cells. *J Biol Chem.* 2005;280:21061-21066.
35. Schutt F, Davies S, Kopitz J, Holz FG, Boulton ME. Photodamage to human RPE cells by A2-E, a retinoid component of lipofuscin. *Invest Ophthalmol Vis Sci.* 2000;41:2303-2308.
36. Sparrow JR, Cai B. Blue light-induced apoptosis of A2E-containing RPE: involvement of caspase-3 and protection by Bcl-2. *Invest Ophthalmol Vis Sci.* 2001;42:1356-1362.
37. Sparrow JR, Nakanishi K, Parish CA. The lipofuscin fluorophore A2E mediates blue light-induced damage to retinal pigmented epithelial cells. *Invest Ophthalmol Vis Sci.* 2000;41:1981-1989.
38. Sparrow JR, Vollmer-Snarr HR, Zhou J, et al. A2E-epoxides damage DNA in retinal pigment epithelial cells. Vitamin E and other antioxidants inhibit A2E-epoxide formation. *J Biol Chem.* 2003;278:18207-18213.
39. Kim SR, Fishkin N, Kong J, Nakanishi K, Allikmets R, Sparrow JR. The Rpe65 Leu450Met variant is associated with reduced levels of the RPE lipofuscin fluorophores A2E and iso-A2E. *Proc Natl Acad Sci U S A.* 2004;101:11668-11672.
40. Wu L, Nagasaki T, Sparrow JR. Photoreceptor cell degeneration in *Abcr*<sup>-/-</sup> mice. *Adv Exp Med Biol.* 2010;664:533-539.
41. Kim SR, Nakanishi K, Itagaki Y, Sparrow JR. Photooxidation of A2-PE, a photoreceptor outer segment fluorophore, and protection by lutein and zeaxanthin. *Exp Eye Res.* 2006;82:828-839.
42. Fishkin N, Sparrow JR, Allikmets R, Nakanishi K. Isolation and characterization of a retinal pigment epithelial cell fluorophore: an all-trans-retinal dimer conjugate. *Proc Natl Acad Sci U S A.* 2005;102:7091-7096.
43. Grimm C, Wenzel A, Williams TP, Rol PO, Hafezi F, Reme CE. Rhodopsin-mediated blue-light damage to the rat retina: effect of photoreversal of bleaching. *Invest Ophthalmol Vis Sci.* 2001;42:497-505.
44. Mittag TW, Bayer AU, LaVail MM. Light-induced retinal damage in mice carrying a mutated SOD1 gene. *Exp Eye Res.* 1999;69:677-683.
45. Tanito M, Elliot MH, Kotake Y, Anderson RE. Protein modifications by 4-hydroxynonenal and 4-hydroxyhexenal in light-exposed rat retina. *Invest Ophthalmol Vis Sci.* 2005;46:3859-3868.
46. Maeda A, Maeda T, Golczak M, Palczewski K. Retinopathy in mice induced by disrupted all-trans-retinal clearance. *J Biol Chem.* 2008;283:26684-26693.
47. Sparrow JR, Blonska A, Flynn E, et al. Quantitative fundus autofluorescence in mice: correlation with HPLC quantitation of RPE lipofuscin and measurement of retina outer nuclear layer thickness. *Invest Ophthalmol Vis Sci.* 2013;54:2812-2820.
48. Radu RA, Yuan Q, Hu J, et al. Accelerated accumulation of lipofuscin pigments in the RPE of a mouse model for ABCA4-mediated retinal dystrophies following Vitamin A supplementation. *Invest Ophthalmol Vis Sci.* 2008;49:3821-3829.
49. Pang JJ, Chang B, Hawes NL, et al. Retinal degeneration 12 (rd12): a new, spontaneously arising mouse model for human Leber congenital amaurosis (LCA). *Mol Vis.* 2005;11:152-162.
50. Katz ML, Redmond TM. Effect of Rpe65 knockout on accumulation of lipofuscin fluorophores in the retinal pigment epithelium. *Invest Ophthalmol Vis Sci.* 2001;42:3023-3030.



51. Maeda T, Maeda A, Casadesus G, Palczewski K, Margaron P. Evaluation of 9-cis-retinyl acetate therapy in Rpe65<sup>-/-</sup> mice. *Invest Ophthalmol Vis Sci.* 2009;50:4368-4378.
52. Grey AC, Crouch RK, Koutalos Y, Schey KL, Ablonczy Z. Spatial localization of A2E in the retinal pigment epithelium. *Invest Ophthalmol Vis Sci.* 2011;52:3926-3933.
53. Fan J, Rohrer B, Frederick JM, Bachr W, Crouch RK. Rpe65<sup>-/-</sup> and Lrat<sup>-/-</sup> mice: comparable models of Leber congenital amaurosis. *Invest Ophthalmol Vis Sci.* 2008;49:2384-2389.
54. Noell WK. Possible mechanisms of photoreceptor damage by light in mammalian eyes. *Vis Res.* 1980;20:1163-1171.
55. Reme CE, Hafezi F, Marti A, Munz K, Reinboth JJ. *Light Damage to Retina and Retinal Pigment Epithelium.* New York, NY: Oxford University Press; 1998;68-85.
56. Organisciak DT, Winkler BS. Retinal light damage: practical and theoretical considerations. *Prog Retin Eye Res.* 1994;13:1-29.
57. Boulton M. *Melanin and the Retinal Pigment Epithelium.* New York, NY: Oxford University Press; 1998.
58. Pautler EL, Morita M, Beezley D. Hemoprotein(s) mediate blue light damage in the retinal pigment epithelium. *Photochem Photobiol.* 1990;51:599-605.
59. Hoppeler T, Hendrickson P, Dietrich C, Reme CE. Morphology and time-course of defined photochemical lesions in the rabbit retina. *Curr Eye Res.* 1988;7:849-860.
60. Maeda A, Golczak M, Maeda T, Palczewski K. Limited roles of Rdh8, Rdh12, and Abca4 in all-trans-retinal clearance in mouse retina. *Invest Ophthalmol Vis Sci.* 2009;50:5435-5443.
61. Ham WT, Mueller HA, Ruffolo JJ, et al. Basic mechanisms underlying the production of photochemical lesions in the mammalian retina. *Curr Eye Res.* 1984;3:165-174.
62. Ham WTJ, Allen RG, Feeney-Burns L, et al. The involvement of the retinal pigment epithelium. In: Waxler M, Hitchins VM, eds. *CRC Optical Radiation and Visual Health.* Boca Raton, FL: CRC Press, Inc; 1986;43-67.
63. Friedman E, Kuwabara T. The retinal pigment epithelium, IV: the damaging effects of radiant energy. *Arch Ophthalmol.* 1968;80:265-279.
64. Busch EM, Gorgels TGMF, Roberts JE, van Norren D. The effects of two stereoisomers of N-acetylcysteine on photochemical damage by UVA and blue light in rat retina. *Photochem Photobiol.* 1999;70:353-358.
65. Ham WT, Ruffolo JJ, Mueller HA, Clarke AM, Moon ME. Histologic analysis of photochemical lesions produced in rhesus retina by short-wavelength light. *Invest Ophthalmol Vis Sci.* 1978;17:1029-1035.
66. Bush RA, Remé CE, Malnoë A. Light damage in the rat retina: the effect of dietary deprivation of N-3 fatty acids on acute structural alterations. *Exp Eye Res.* 1991;53:741-752.
67. Tanito M, Kaidzu S, Anderson RE. Protective effects of soft acrylic yellow filter against blue light-induced retinal damage in rats. *Exp Eye Res.* 2006;83:1493-1504.
68. Organisciak DT, Darrow RM, Jiang YI, Marak GE, Blanks JC. Protection by dimethylthiourea against retinal light damage in rats. *Invest Ophthalmol Vis Sci.* 1992;33:1599-1609.
69. Tomita H, Kotake Y, Anderson RE. Mechanism of protection from light-induced retinal degeneration by the synthetic antioxidant phenyl-N-tert-butyl nitron. *Invest Ophthalmol Vis Sci.* 2005;46:427-434.
70. Haruta M, Bush RA, Kjellstrom S, et al. Depleting Rac1 in mouse rod photoreceptors protects them from photo-oxidative stress without affecting their structure or function. *Proc Natl Acad Sci U S A.* 2009;2009:9397-9402.
71. Sparrow JR, Gregory-Roberts E, Yamamoto K, et al. The bisretinoids of retinal pigment epithelium. *Prog Retin Eye Res.* 2012;31:121-135.
72. Zhou J, Jang YP, Chang S, Sparrow JR. OT-674 suppresses photooxidative processes initiated by an RPE lipofuscin fluorophore. *Photochem Photobiol.* 2008;84:75-80.
73. Tanito M, Li F, Elliot MH, Dittmar M, Anderson RE. Protective effect of TEMPOL derivatives against light-induced retinal damage in rats. *Invest Ophthalmol Vis Sci.* 2007;48:1900-1905.
74. Tanito M, Li F, Anderson RE. Protection of retinal pigment epithelium by OT-551 and its metabolite TEMPOL-H against light-induced damage in rats. *Exp Eye Res.* 2010;91:111-114.
75. Zhou J, Cai B, Jang YP, Pachydaki S, Schmidt AM, Sparrow JR. Mechanisms for the induction of HNE-MDA- and AGE-adducts, RAGE and VEGF in retinal pigment epithelial cells. *Exp Eye Res.* 2005;80:567-580.
76. Yamamoto K, Zhou J, Hunter JJ, Williams DR, Sparrow JR. Toward an understanding of bisretinoid autofluorescence bleaching and recovery. *Invest Ophthalmol Vis Sci.* 2012;53:3536-3544.
77. Morgan JL, Dubra A, Wolfe R, Merigan WH, Williams DR. In vivo autofluorescence imaging of the human and macaque retinal pigment epithelial cell mosaic. *Invest Ophthalmol Vis Sci.* 2009;50:1350-1359.
78. Morgan JL, Hunter JJ, Masella B, et al. Light-induced retinal changes observed with high-resolution autofluorescence imaging of the retinal pigment epithelium. *Invest Ophthalmol Vis Sci.* 2008;49:3715-3729.
79. Cideciyan AV, Swider M, Aleman TS, Roman MI, Sumaroka A, Schwartz SB. Reduced-illumination autofluorescence imaging in ABCA4-associated retinal degenerations. *J Opt Soc Am A Opt Image Sci Vis.* 2007;24:1457-1467.
80. Cideciyan AV, Hood DC, Huang YZ, et al. Disease sequence from mutant rhodopsin allele to rod and cone photoreceptor degeneration in man. *Proc Natl Acad Sci U S A.* 1998;95:7103-7108.
81. Naash ML, Peachey NS, Li Z-Y, et al. Light-induced acceleration of photoreceptor degeneration in transgenic mice expressing mutant rhodopsin. *Invest Ophthalmol Vis Sci.* 1996;37:775-782.
82. Wang M, Lam TT, Tso MO, Naash MI. Expression of a mutant opsin gene increases the susceptibility of the retina to light damage. *Vis Neurosci.* 1997;14:55-62.
83. Cideciyan AV, Jacobson SG, Aleman TS, et al. In vivo dynamics of retinal injury and repair in the rhodopsin mutant dog model of human retinitis pigmentosa. *Proc Natl Acad Sci U S A.* 2005;102:5233-5238.
84. Organisciak DT, Darrow RM, Barsalou L, Kutty RK, Wiggert B. Susceptibility to retinal light damage in transgenic rats with rhodopsin mutations. *Invest Ophthalmol Vis Sci.* 2003;44:486-492.
85. Tomany SC, Cruickshanks KJ, Klein R, Klein BEK, Knudtson MD. Sunlight and the 10-year incidence of age-related maculopathy: The Beaver Dam Eye Study. *Arch Ophthalmol.* 2004;122:750-757.
86. McCarty CA, Mukesh BN, Fu CL, Mitchell P, Wang JJ, Taylor HR. Risk factors for age-related maculopathy: the Visual Impairment Project. *Arch Ophthalmol.* 2001;119:1455-1462.
87. Hyman LG, Lilienfeld AM, Ferris FL, Fine SL. Senile macular degeneration: a case-control study. *Am J Epidemiol.* 1983;118:213-227.
88. Delcourt C, Carriere I, Ponton-Sanchez A, Soureyre S, Lacroix A, Papoz L. Light exposure and the risk of age-related macular degeneration. *Arch Ophthalmol.* 2001;119:1463-1468.
89. Fletcher AE, Bentham GC, Agnew M, et al. Sunlight exposure, antioxidants, and age-related macular degeneration. *Arch Ophthalmol.* 2008;126:1396-1403.