# Drusen-Associated Degeneration in the Retina

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**Purpose.** Drusen are variably sized extracellular deposits that form between the retinal pigmented epithelium (RPE) and Bruch's membrane. They are commonly found in aged eyes, however, numerous and/or confluent drusen are a significant risk factor for age-related macular degeneration. The purpose of this study was to investigate the impact of drusen on overlying cells of the retina.

**Methods.** Tissue containing retina and RPE/choroid was dissected from human donor eyes, embedded in agarose, and sectioned at 100 $\mu$m using a vibratome. Sections were immunostained with a panel of antibodies that labeled glial cells, first-, second-, and third-order retinal neurons and processed for confocal microscopy.

**Results.** Retinal cells that overlie both soft and hard drusen exhibited numerous structural and molecular abnormalities. Normally detectable only in the outer segments of rod photoreceptors, rod opsin immunolabeling was also observed in the inner segment, cell body, axon, and axon terminal of photoreceptors that overlie drusen. Labeling with this antibody also revealed the deflection and shortening of rod inner and outer segments. Cone photoreceptors displayed similar structural abnormalities, as well as a decrease in cone opsin immunoreactivity. Drusen-associated abnormalities in the synaptic terminals of photoreceptor cells were also observed. In addition, an increase in intermediate filament protein immunoreactivity (vimentin and glial fibrillary acidic protein) was observed within Müller glial cells in areas of retina overlying drusen. Both soft and hard drusen were associated with a similar spectrum of effects in both macular and extramacular regions. Second- and third-order neurons, including bipolar, horizontal, amacrine, and ganglion cells all appeared unaffected. The structural and molecular abnormalities observed in photoreceptors and Müller glial cells were confined to retinal regions directly overlying and immediately adjacent to drusen; more distant retinal regions appeared unperturbed. Remarkably, significant abnormalities were observed over small subclinical drusen.

**Conclusions.** Retinal cells overlying both soft and hard drusen exhibit structural and molecular abnormalities indicative of photoreceptor degeneration and Müller glial activation. These abnormalities resemble the degenerative effects common to many forms of retinal degeneration, but are confined to areas directly overlying drusen. This suggests that photoreceptor cell function is compromised as a consequence of drusen formation. (*Invest Ophthalmol Vis Sci.* 2003;44:4481–4488) DOI:10.1167/iovs.03-0436

Age-related macular degeneration (AMD) is a disease characterized by the progressive loss of central vision. It is currently the leading cause of irreversible blindness in the industrialized world, afflicting approximately 1 in 20 people over the age of 60 years.[1] The early stages of AMD are characterized by the presence of extracellular deposits, known as drusen, that form between the RPE and Bruch's membrane. It has been hypothesized that the progressive loss of vision associated with the nonexudative (dry) form of AMD is attributable to the accumulation of drusen.[2] As AMD progresses, drusen typically increase in size and number, eventually compromising the function of the RPE. RPE cells overlying and flanking drusen often exhibit classic morphologic signs of impending cell death.[3,4] Because the RPE is essential for the survival of photoreceptor cells, drusen-induced RPE degeneration has been hypothesized to account for the loss of photoreceptor cells observed in eyes diagnosed with AMD.[5]

Recent theories on drusen deposition and the progression of AMD focus on the role of inflammatory, as well as immune-mediated events.[6,7] The most compelling evidence in support of these hypotheses comes from the identification of complement-activating molecules and other immune mediators in drusen and the observation of monocyte-derived cellular processes within drusen. Studies suggest that cellular debris generated by local RPE cell degeneration acts as a chronic inflammatory stimulus and initiates drusen formation.[4,8] Age-related increases in drusen size are thought to result from the deposition of material derived from local cellular sources as well as plasma.

Clinically, drusen are divided into two main phenotypes, hard and soft. Hard drusen are nodular with well-defined borders whereas soft drusen are more irregular in shape without distinct borders. To date, no significant differences in the molecular composition of hard and soft drusen have been identified, suggesting that they may have a common origin.[6,9] In advanced stages of AMD, hard drusen can coalesce to form confluent plaques that are associated with the atrophy of large patches of RPE and macular regions of retina (geographic atrophy).[10] Large numbers of drusen or large confluent drusen are also associated with a significantly increased risk of developing choroidal neovascularization, characteristic of the exudative (wet) form of AMD.[11]

Little is known concerning the effects of drusen on the retina, or why they are a significant risk factor for vision loss. Histopathologic studies examining the retinas of eyes diagnosed with AMD have identified changes in intermediate filament expression in Müller glia[12–14] and in astrocytes.[12,15] In addition, Curcio et al.[5] reported a correlation between the presence of drusen and photoreceptor cell death in the retinas of eyes diagnosed with AMD. Medieros and Curcio[16] also

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**Table 1.** Primary Antibodies

| | | |
|---|---|---|
| Apolipoprotein E | Goat polyclonal | Chemicon, Temecula, CA |
| Calbindin D | Mouse monoclonal | Sigma, St. Louis, MO |
| CD44 | Rat monoclonal | CalBiochem, San Diego, CA |
| Cytochrome oxidase | Mouse monoclonal | Molecular Probes, Eugene, OR |
| GFAP | Rabbit polyclonal | DAKO, Carpenteria, CA |
| MWS-cone Opsin | Rabbit polyclonal | a gift from Dr. Jeremy Nathans |
| Neurofilament (70 + 200kDa) | Mouse monoclonal | Biomeda, Hayward, CA |
| PKC | Rabbit polyclonal | Biomolecular Research Labs, Plymouth, PA |
| Rod opsin (Rho4D2) | Mouse monoclonal | a gift from Dr. Robert Molday |
| Synaptophysin | Rabbit polyclonal | DAKO, Carpenteria, CA |
| Vimentin | Mouse monoclonal | DAKO, Carpenteria, CA |

demonstrated that ganglion cells are compromised in neovascular AMD, but not in nonexudative AMD. Most recently, Dunaief et al.[14] demonstrated that photoreceptors die via an apoptotic mechanism near sites of geographic atrophy in advanced AMD, and that this cell death might be mediated by the Fas/Fas ligand system. Thus, the effects of drusen on retinal neurons are largely uncharacterized and a causative role for drusen in photoreceptor cell death has not been established.

Drusen are considered to be of clinical importance when they exceed 63 $\mu$m in width,[17] yet no histopathologic data exist to correlate drusen size with pathologic impact on the overlying retina. Because drusen accumulate gradually over the life of an individual and increase in size with age, it is reasonable to propose that the effects of drusen on the retina might also be gradual and cumulative, with photoreceptor cell death being the final stage in a lengthy degenerative process. In this study we investigated the impact of drusen on cells of the neural retina. The results indicate that drusen are associated with significant degenerative changes in adjacent photoreceptor cells and that these changes are also associated with small subclinical drusen.

## Materials and Methods

### Tissue

Human donor eyes were provided by the Central Oregon Lions Eye Bank (Portland, OR). After removal of the anterior chamber, eyecups were immersed in 4.0% paraformaldehyde in 0.1 M sodium cacodylate buffer and shipped overnight on ice. Upon arrival eyes were rinsed and stored in 0.4% paraformaldehyde at 4°C. The postmortem intervals to fixation ranged from 1 to 7 hours. A total of 22 eyes from 22 donors were analyzed. Donor ages ranged from 49 to 86 years; one donor eye had a prior clinical diagnosis of exudative AMD.

Trephine punches (6 mm in diameter) containing retina, RPE, and choroid were taken from macular and extramacular regions of eyecups from 22 donors. The macula was defined as a 6-mm circular region centered on the fovea. Extramacular tissue was sampled from regions $>$ 5 mm from the edge of the macula at varying eccentricities. Soft and/or hard drusen were present in 16 of 22 donor eyes. Thirteen eyes contained only hard drusen; the others contained examples of both hard and soft drusen. Macular tissue from 8 of the eyes contained drusen, 5 contained variable numbers of hard drusen, and 3 contained both soft and hard drusen. Over 200 individual drusen were analyzed.

### Immunohistochemistry

Tissue was embedded in 5% agarose in 0.1 M PBS and sectioned at 100 $\mu$m using a vibratome (Technical Products International; Polysciences, Warrington, PA). Tissue sections were rinsed in PBS and blocked at 4°C for 6 hours in PBTA (PBS with 0.5% bovine serum albumin; Sigma, St. Louis, MO; 0.1% Triton X-100; Roche, Indianapolis, IN and 0.05% sodium azide; Sigma) containing 5% normal donkey serum (Jackson ImmunoResearch Laboratories, West Grove, PA). Sections were then incubated overnight at 4°C in primary antibodies (Table 1) diluted in PBTA, rinsed 3 times for 20 minutes in PBTA, and incubated overnight at 4°C in donkey anti-mouse, anti-rabbit, or anti-goat IgG secondary antibodies conjugated to Cy2, Cy3, or Cy5 fluorochromes (Jackson). Sections were then rinsed 3 times for 20 minutes in PBTA, mounted in 5% n-propyl gallate in glycerol, and examined on a laser scanning confocal microscope (BioRad 1024; BioRad, Hercules, CA). Optimal iris and gain functions were determined for each primary antibody and maintained constant during the examination of all sections labeled with that probe. Images were acquired with software (Lasersharp; BioRad); pseudo double-labeled images were generated by optimizing the Cy3 (red) channel to allow visualization of autofluorescence from lipofuscin pigment in RPE cells and Bruch's membrane.

Soft and hard drusen were stained with antibodies to apolipoprotein E[18] (Table 1) and identified using confocal microscopy. Drusen measurements were taken from z-series reconstructions using software (Lasersharp; BioRad). Both the lateral spread (width) and the retinal penetration (height) of individual drusen were measured. The reported numbers correspond to the widest and/or tallest aspect of individual drusen recorded in each z-series reconstruction.

## Results

### Drusen-Associated Retinal Abnormalities

In normal retina, immunohistochemical staining using antibodies specific for the rod photoreceptor protein opsin revealed intense rod outer-segment labeling, while inner segments, cell bodies, axons, and axon terminals were only faintly immunoreactive (Fig. 1A, normal). However, in regions of retina overlying drusen deposits, rod photoreceptors exhibited abnormal structure and opsin immunoreactivity patterns (Figs. 1A, 1B). In these regions, intense rod opsin immunolabeling was associated not only with rod outer segments, but also with rod inner segments, cell bodies, axons, and axon terminals. In many cases severely degenerate rod opsin-immunopositive cells were observed and the outer nuclear layer was severely disrupted (Fig. 1C). In addition to the abnormal distribution of rod opsin protein, rod inner and outer segments were physically deflected by underlying drusen and outer segments were much shorter than nearby outer segments not overlying drusen (Figs. 1A, 1C).

Cone photoreceptors overlying drusen exhibited similar abnormalities. Immunolabeling with antibodies specific for medium/long wavelength sensitive cone opsin (MWS-opsin) revealed that cone outer segments, like those of rods, are deflected and/or truncated when overlying drusen (Fig. 1D). In addition, there were typically fewer immunopositive cones directly over drusen. Additional structural changes in cones overlying drusen cannot be monitored using this marker be-

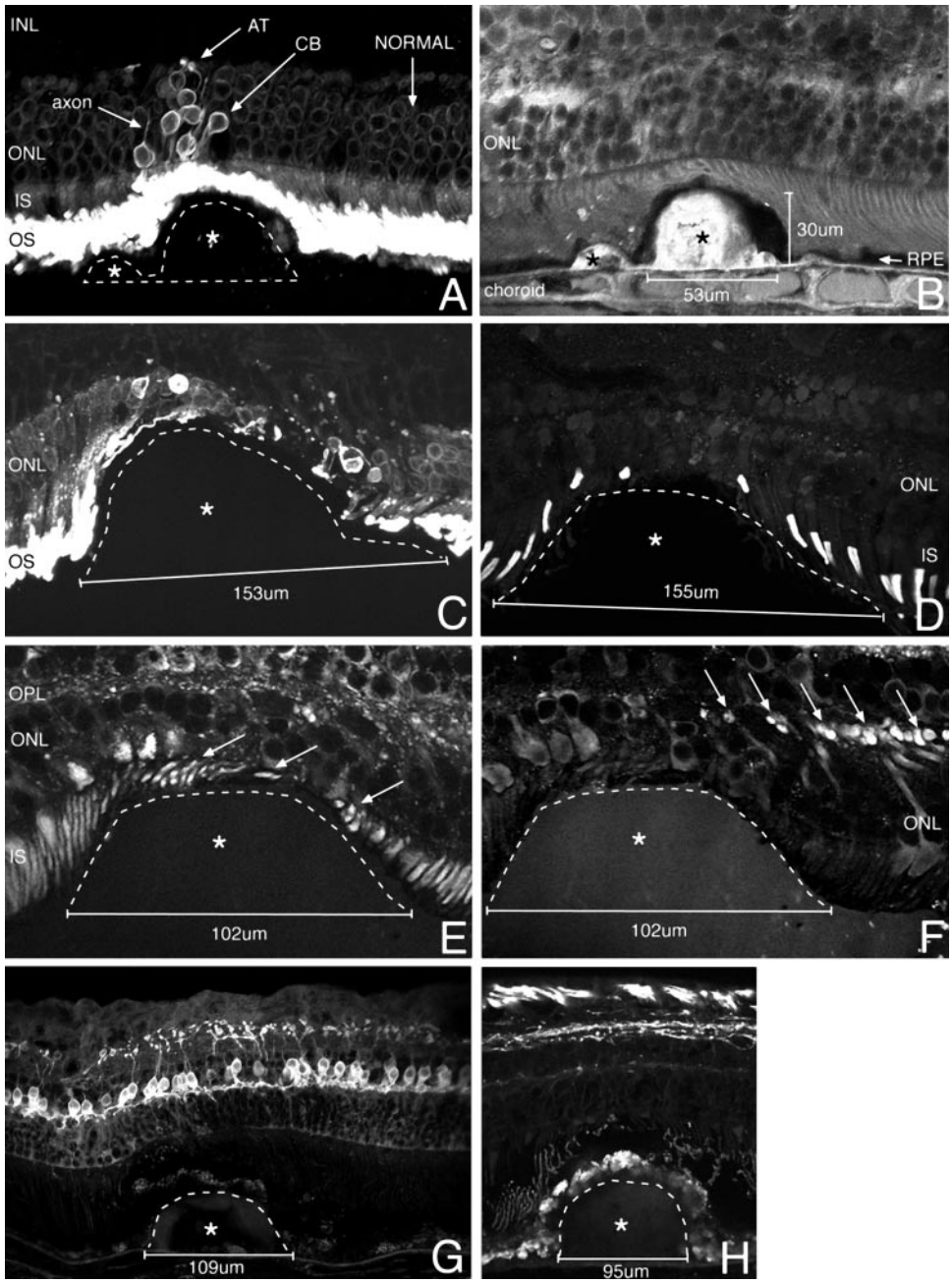


**FIGURE 1.** Drusen-associated photoreceptor changes in the retina. (**A**) Rod opsin immunoreactivity is intense in the outer segments (OS) of rod photoreceptors. The redistribution of opsin is observed in rod photoreceptor outer segments, inner segments (IS), cell bodies (CB), axons, and axon terminals (AT) that overlie drusen (*asterisks*). Nearby retina, not overlying drusen, appears normal. (**B**) Same section shown in (A) stained with antibodies to apolipoprotein E (ApoE) to label drusen (*asterisks*). The RPE is immunonegative but faint labeling within the retina can be detected. (**C**) A relatively large druse (*asterisks*) with a lateral spread of 153 $\mu$m is associated with extensive degeneration in rod photoreceptors. Rod opsin immunoreactivity demonstrates redistribution of opsin as well as a decreased number of cells in the overlying ONL. (**D**) MWS-cone opsin immunoreactivity demonstrates a decrease in cone outer segment length and in the number of immunoreactive outer segments over drusen (*asterisk*). (**E**) Cytochrome oxidase immunoreactivity in rod and cone IS demonstrates a decrease in IS length and a structural alteration (*arrows*) in photoreceptors that overlie drusen (*asterisk*). The thickness of the ONL is also reduced over drusen. (**F**) The number of synaptophysin-immunopositive rod and cone axon terminals (*arrows*) is significantly reduced in retina overlying drusen (*asterisk*). (**G**) PKC immunoreactivty in rod bipolar cells is not significantly altered in cells that overlie drusen (*asterisk*). (**H**) Neurofilament immunoreactivty in ganglion cell dendrites and axons appears normal in retina overlying drusen (*asterisk*). Lipofuscin autofluorescence can also be detected in the RPE in this image. Note: In all images drusen boundaries were determined by ApoE immunoreactivity (**A**, **B**). INL, inner nuclear layer; ONL, outer nuclear layer; OPL, outer plexiform layer.

cause opsin redistribution to cone cell bodies, axons or axon terminals is rarely observed.

Antibodies against cytochrome oxidase label the mitochondria-rich rod and cone inner segments and demonstrated that they are shortened and that their structure is often altered in photoreceptors that overlie drusen (Fig. 1E). In many cases rod and cone inner segments are deflected as much as 90° from their normal orientation by underlying drusen. In some areas impacted by drusen, neither rod nor cone inner segments were detectable.

Additional drusen-associated changes in photoreceptors were demonstrated by immunostaining with antibodies against synaptic vesicle proteins. Immunolabeling with antibodies against synaptophysin is normally restricted to rod and cone axon terminals within the outer plexiform layer (Fig. 1F, arrows). When drusen are present, however, synaptophysin immunolabeling is often absent (Fig. 1F), suggesting that synaptic architecture was altered and synaptic transmission in photoreceptors overlying drusen may be compromised.

Drusen-associated abnormalities appeared to be limited to photoreceptor cells. Antibodies that label horizontal, bipolar, amacrine, and ganglion cells did not reveal significant alterations in the cellular, dendritic, or axonal morphology of these second- and third-order neurons (Figs. 1G, 1H; anti-calbindin D labeling of horizontal and amacrine cells not shown). Even in areas overlying relatively large drusen in which regions of the outer nuclear layer have degenerated (Fig. 1G), the dendritic and axonal arborizations of bipolar cells appeared normal. Studies have reported that ganglion cells overlying regions of photoreceptor degeneration stemming from retinal detachment upregulate neurofilament proteins.[19] We did not observe any significant changes in neurofilament immunoreactivity in

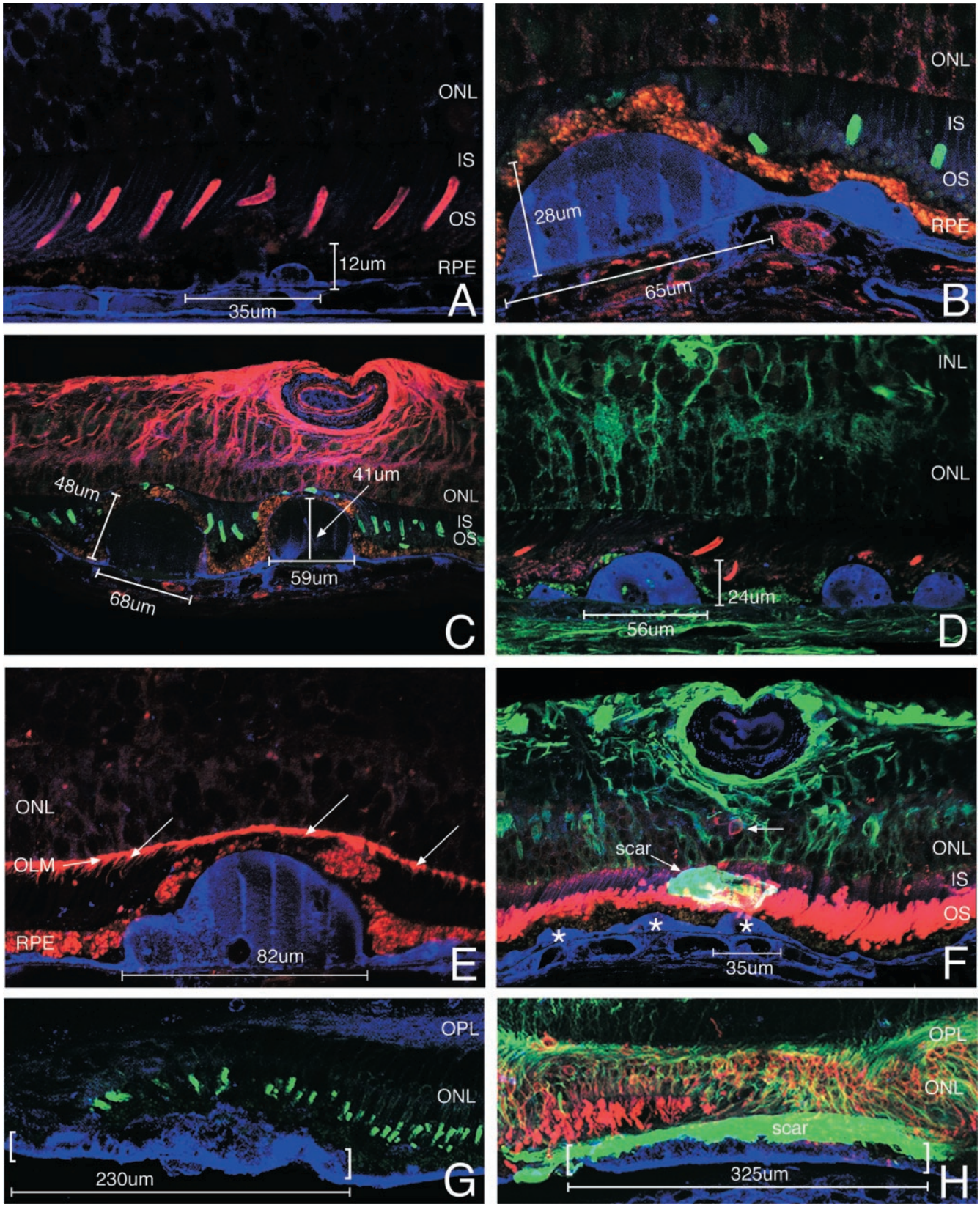


**Figure 2.** Drusen-associated photoreceptor changes in the retina. (**A**) MWS-cone opsin immunoreactivity (*red*) demonstrates the physicial deflection of a cone outer segment (OS) that overlies three small drusen. ApoE immunoreactivity (*blue*) labels drusen, as well as the choroid and portions of the retina. Faint lipofuscin autofluorescence within the RPE can also be detected (*red*). (**B**) Severe disruption of MWS-cone opsin immunoreactive OS (*green*) is associated with large drusen (compare with Fig. 2A). Faint vimentin immunoreactivity (*red*) can be detected in the retina, RPE, and choroid. Autofluorescent lipofuscin granules are also detectable within the RPE in this image. (**C**) Severe disrpuption of cone OS

ganglion cells overlying drusen using the same antibodies (Fig. 1H).

### Drusen Size Correlates with Photoreceptor Changes

Changes in adjacent photoreceptor cells were observed wherever drusen were present. The extent of these changes is dependent on the size of underlying drusen. Even small, subclinical drusen (<63-$\mu$m lateral spread) can impact photoreceptor morphology, as evidenced by the deflection of both inner and outer segments and by decreased outer-segment length. For example, three small drusen (Fig. 2A) extend 35 $\mu$m laterally beneath the RPE and a maximum of 12 $\mu$m into the retina. Despite these small dimensions and minimal retinal penetration, a cone outer segment directly overlying these drusen is displaced. As the lateral spread of drusen increases, overlying photoreceptor inner and outer segments show more significant displacement and decreased length, and immunolabeled cone outer segments decrease in density (Fig. 1D). The outer segments of cone photoreceptors overlying drusen that extend laterally 56 to 68 $\mu$m beneath the RPE (Figs. 2B–D) show reduced antiopsin immunolabeling, a decrease in outer segment length, and in many cases, the outer nuclear layer overlying drusen of this size is noticeably thinner. Each of over 100 drusen of this size (>50 $\mu$m lateral spread) that were examined in tissue from 16 donors showed one or more of these effects on overlying rod and cone photoreceptors.

Changes in morphology, protein distribution, and protein expression in photoreceptor cells that overlie drusen are not dependent solely on the extent of their lateral spread beneath the RPE, but also on the extent of protrusion into the retina. Due to the variable shape and size of drusen, drusen lateral spread is not always indicative of the extent to which they protrude into the retina. For example the druse in Figure 2C, which extends laterally approximately 59 $\mu$m beneath the RPE, protrudes approximately 41 $\mu$m into the retina. The druse in Figure 2D extends a similar distance beneath the RPE (56 $\mu$m) but only protrudes 24 $\mu$m into the retina. Significant structural defects were observed overlying both drusen, however, cone inner and outer segments appeared more adversely affected over more invasive drusen.

### Müller Glial Cells Overlying Drusen Increase the Expression of Intermediate Filaments

A previous study has shown that retinal glial cells overlying drusen often increase the expression of intermediate filament glial fibrillary acidic protein (GFAP).[13] Data collected in this study confirm these observations. Antibodies against GFAP, and also vimentin, revealed an elevated level of intermediate filament immunoreactivity in most aged retinas, and particularly in those regions of retina overlying drusen (Figs. 2C, red; 2D, green). Furthermore, the upregulation of intermediate filament expression was often observed in regions displaying abnormal rod opsin expression (Figs. 2F, 2H) and cone opsin immunolabeling (Figs. 2C, 2D). Within the outer nuclear layer, Müller glial cell processes are intensely immunoreactive as they weave around rod and cone cell bodies, eventually reaching the outer-limiting membrane (OLM). This glial response does not appear to extend into the interphotoreceptor matrix. Even in those retinal regions adjacent to large drusen that are associated with abnormal photoreceptor inner and outer segments, CD44 immunostaining shows normal Müller glial cell microvilli immunolabeling patterns (Fig. 2E, arrows). In rare instances, Müller glial microvilli did upregulate intermediate filament expression in this retinal compartment, and formed large retinal scars over drusen (Fig. 2F).

### Soft and Hard Drusen Exert Similar Effects

Immunohistochemical staining with antibodies specific for ApoE was used to identify both the shape and size of drusen.[18] Hard drusen are nearly hemispherical deposits with sharply delineated, smooth borders (Figs. 2A-F). Soft drusen, however, are more amorphous, with multiple peaks and poorly defined borders (Figs. 2G, 2H). Immunolabeling revealed that the same photoreceptor abnormalities were observed overlying both soft and hard drusen. Cone photoreceptors overlying large soft drusen are distorted and there are fewer labeled cells than when compared with adjacent normal retina (Fig. 2G). The effects associated with both hard and soft drusen appeared to be related to the distance that they extend into the retina. Hard drusen typically extend further into the retina than do soft drusen, thus more severe photoreceptor abnormalities are typically observed over hard drusen. Soft drusen, however, typically have a much larger lateral spread than hard drusen. Thus photoreceptor abnormalities over soft drusen are not typically as severe, but they extend over a much larger retinal area. When observed, large glial scars are more frequently associated with soft drusen than with hard drusen (Fig. 2H).

### Macular versus Extramacular Drusen

Drusen within the macula are a significant risk factor for the development of AMD.[20,21] During the course of this study, retina overlying both macular and extra-macular drusen were examined and no significant differences were observed. Rod opsin immunoreactivity is present throughout rod photoreceptors that overly both soft and hard drusen in the macula (Fig. 3A). MWS-cone opsin immunoreactivity revealed a decrease in the density of labeled outer segments overlying drusen within the macula, as well as abnormal outer-segment morphology (Figs. 2G, 3B). Similar Müller glial cell alterations were also observed overlying drusen in both retinal regions (data not shown).

## Discussion

Although overall drusen "load" has been shown to be positively correlated with photoreceptor cell death in eyes diag-

(*green*) length and density occurs overlying two drusen (ApoE, *blue*). Adjacent Müller glial cells show increased vimentin immunoreactivity (*red*). (**D**) Intermediate filament expression in Müller cell processes (vimentin, *green*) in the inner (INL) and outer nuclear layers (ONL) increases over drusen. MWS-opsin immunoreactivity labels the few remaining cone outer segments (*red*) and ApoE (*blue*) labels drusen. (**E**) Müller glial cell microvilli (*arrows*; CD44, *red*) rarely exhibit detectable changes within the interphotoreceptor space, even in the presence of very large drusen. RPE autofluorescence is also *red* in this image and drusen are labeled with anti-apoE (*blue*). (**F**) A large glial scar (GFAP, *green*) has formed among rod opsin-immunoreactive inner segments (IS) and OS (*red*) overlying drusen deposits (*asterisks, blue*). Opsin immunoreactivity is also present in rod cell bodies overlying this glial scar (*arrow*). (**G**) ApoE immunostaining (*blue*) reveals the fuzzy borders and amorphous shape of soft drusen in the macula (*brackets*). ApoE immunoreactivity is also present in Müller glial processes within the outer plexiform layer (OPL) of the retina. MWS-cone opsin immunolabeling (*green*) over soft drusen is less frequent and more disorganized compared with adjacent regions not overlying soft drusen. (**H**) A large glial scar (GFAP, *green*) has formed over ApoE-immunoreactive soft drusen (*blue, brackets*). Opsin antibodies (*red*) intensely label rod cell bodies, axons, and axon terminal in this region of the retina.

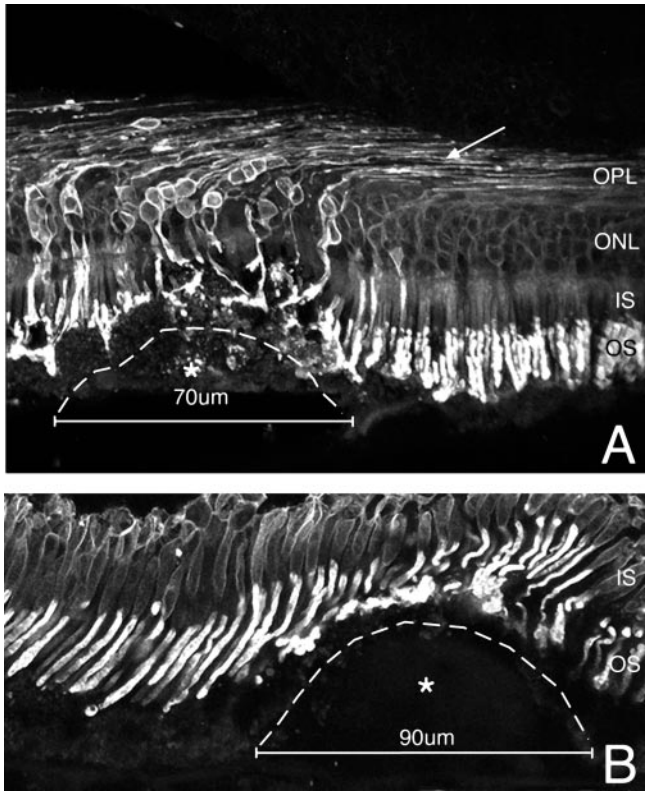


**FIGURE 3.** Drusen-associated retinal changes within the macula. (**A**) Hard drusen (*asterisk*) within the macula are associated with outer segment (OS) disruption and opsin redistribution in overlying rod photoreceptors (compare with Fig. 1A). Note the deflection of long-rod opsin immunopositive axons (*arrow*) in the outer plexiform layer (OPL) within the macula. (**B**) MWS-opsin immunoreactive cone OS are severely disrupted when overlying drusen (*asterisk*) in the macula. IS, inner segment.

nosed with AMD,[5] very little is known about the specific effects of drusen on overlying retinal cells. This study represents a first attempt to characterize the specific effects of drusen on retinal neurons. We demonstrate that a number of localized changes occur in photoreceptor cells that overlie soft as well as hard drusen. These include changes in cellular protein distribution, protein expression, and morphology. These changes are similar in macular and extramacular regions of the retina and are associated with even very small subclinical drusen. The extent of the abnormalities in the retina increase as drusen size increases, often including the loss of outer segments, a reduction in the thickness of the outer nuclear layer (ONL), and changes in synaptic cytoarchitecture over large drusen. We found that both the distance drusen protrude into the retina, as well as their lateral spread beneath the RPE, affect the severity of these changes. These effects occur to varying degrees over every drusen with a lateral spread greater than 50 $\mu$m that we observed over the course of this study (and often over considerably smaller drusen as well). Only in rare circumstances were retinal changes observed to extend laterally past the borders of individual drusen.

Our results suggest that the cellular and molecular changes observed in photoreceptors overlying drusen are degenerative in nature and may eventually lead to cell death. Since the progression of AMD is gradual and occurs over many years, it is likely that these changes represent part of a continuum between a healthy photoreceptor and a dying one. The redistribution of rod opsin, the decrease in cone opsin expression, the loss of synapse-related machinery, as well as other structural alterations in photoreceptors are also observed to precede cell death in other models of retinal degeneration, such as retinal detachment.[22] It is not known whether photoreceptor cells are able to function properly while in this compromised state, however, reports indicate that individuals diagnosed with early age-related maculopathy (defined by one or more drusen $> 63$ $\mu$m, focal RPE hyperpigmentation, or both) have detectable visual deficits.[23]

Both soft and hard drusen have been implicated as causative in the progression of AMD.[17] In this study, we observed similar drusen-associated effects in retina overlying both types of drusen. In a limited number of eyes ($n = 3$), glial scars were found overlying drusen, most commonly over soft drusen. The scars were not associated with histologic evidence of neovascularization, although, the possibility that their development was influenced by earlier neovascular events cannot be excluded.[2] Because subretinal scars have been correlated with substantial photoreceptor cell death,[24] their predilection for forming over soft drusen could underlie the correlation between soft drusen and an increased risk of developing AMD. Because soft drusen typically extend greater lateral distances beneath the RPE than hard drusen, it is also possible that glial scar formation is more highly correlated with this aspect of drusen size as opposed to drusen phenotype.

There are several nonmutually exclusive explanations for the observed changes in photoreceptors associated with drusen. First, it is possible that the displacement of photoreceptors by encroaching drusen damages their structural integrity. Mere displacement of photoreceptor outer segments might be sufficient to render them dysfunctional or, in extreme cases, cause cell death. Smith et al.[25] demonstrated a correlation between early age-related macular changes associated with AMD and a perceived Stiles-Crawford effect, suggesting that altered photoreceptor architecture can lead to visual deficits. A correlation has also been made between macular drusen and abnormal contrast sensitivity in the central visual field,[26] although earlier studies failed to detect drusen-associated changes.[27] Furthermore, genetic mutations that lead to a compromise in the structural integrity of outer segments, such as those that occur in the retinal degeneration slow (RDS) mouse, have been demonstrated to lead to apoptotic photoreceptor cell death.[28] Thus, it is logical to hypothesize that defects in the synaptic architecture within photoreceptor terminals are detrimental to photoreceptor cell function.

Secondly, it is possible that drusen impair the normal exchange of ions and metabolites between the choroidal blood supply and photoreceptors by establishing a physical barrier to diffusion. Experiments using the feline model of retinal detachment, in which the retina and the RPE/choroid are surgically separated, demonstrate impaired diffusion of oxygen from the choroidal blood supply to the retina[29] resulting in massive photoreceptor cell death.[22] When oxygen supplementation is provided, fewer photoreceptors die.[30–33] It is certainly conceivable that drusen constitute a similar, but more localized diffusional barrier.

Thirdly, it is possible that drusen indirectly affect photoreceptors by compromising the function of RPE cells. Primary RPE pathology is regarded by many investigators as causal in AMD. Many of the changes noted in drusen-associated RPE cells are indicative of injury that often precedes cell death.[34–37] Rod and cone photoreceptor cells of the retina are among the most metabolically active cells in the body[38] and require the functional support of the RPE for the exchange of metabolites necessary for the regeneration of photopigment, the structural support of photoreceptor outer segments, and the phagocyto-

sis of shed photoreceptor disc membranes.[39] Because the retina and the RPE maintain such an intimate structural and functional relationship, a viable RPE is a prerequisite for normal photoreceptor cell function and necessary for the maintenance of the retinal microenvironment. Mutations that impair vital RPE functions, such as phagocytosis of shed outer-segment disc membranes, are known to result in photoreceptor cell death.[28]

Finally, it is possible that drusen contain molecules that have direct cytotoxic effects on photoreceptor cells. Studies investigating the composition of drusen suggest that they contain molecules derived from multiple sources including RPE, choroid, and plasma.[6] Many of the proteins identified in drusen are functionally related to the process of inflammation or its aftermath.[4,40] Drusen also contain oxidatively modified proteins,[41] cholesterol,[42] apolipoproteins,[18] and other poorly characterized lipid and carbohydrate moieties. Most recently, amyloid beta has been identified in substructural components of drusen.[43] Studies have shown that the direct injection of amyloid beta into the vitreous cavity of eyes induces a significant amount of photoreceptor cell death as well as Müller glial cell hypertrophy.[44,45] Thus, it is quite possible that drusen accumulate molecules that are toxic to overlying photoreceptors.

Most likely, the degenerative effects exerted by drusen on the retina are due to a combination of factors. Drusen-associated physical and molecular insults to photoreceptors, combined with a newly created barrier to the diffusion of essential metabolites and a lack of support from degenerating RPE could all be responsible for severely destabilizing these cells and eventually leading to their demise. Future research aimed at testing these individual hypotheses will shed light on the mechanisms involved in drusen-associated photoreceptor degeneration and disease progression in AMD.

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