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Antecedents of Soft Drusen, the Specific Deposits of Age-Related Macular Degeneration, in the Biology of Human Macula

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AMD pathobiology was irreversibly changed by the recent discovery of extracellular cholesterol-containing deposits in the subretinal space, between the photoreceptors and retinal pigment epithelium (RPE), called subretinal drusenoid deposits (SDDs). SDDs strikingly mirror the topography of rod photoreceptors in human macula, raising the question of whether an equivalent process results in a deposition related to foveal cones. Herein we propose that AMD's pathognomonic lesion—soft drusen and basal linear deposit (BLinD, same material, diffusely distributed)—is the leading candidate. Epidemiologic, clinical, and histologic data suggest that these deposits are most abundant in the central macula, under the fovea. Strong evidence presented in a companion article supports the idea that the dominant ultrastructural component is large apolipoprotein B,E–containing lipoproteins, constitutively secreted by RPE. Lipoprotein fatty acids are dominated by linoleate (implicating diet) rather than docosahexaenoate (implicating photoreceptors); we seek within the retina cellular relationships and dietary drivers to explain soft druse topography. The delivery of xanthophyll pigments to highly evolved and numerous Müller cells in the human fovea, through RPE, is one strong candidate, because Müller cells are the main reservoir of these pigments, which replenish from diet. We propose that the evolution of neuroglial relations and xanthophyll delivery that underlie exquisite human foveal vision came with a price, that is, soft drusen and sequela, long after our reproductive years.

Keywords: age-related macular degeneration, drusen, lipoproteins, cholesterol, retinal pigment epithelium, Bruch's membrane, macula, fovea, Müller cells, xanthophyll pigment

Age-related macular degeneration (AMD) is a major cause of legal blindness in the elderly, approachable through multidisciplinary research involving human tissues and patients. Our views of AMD have advanced substantially through clinical imaging in the last decade, culminating in the Classification of Atrophy Meetings Working Group, which is redefining atrophy to incorporate pathology newly revealed by optical coherence tomography.[1] AMD pathobiology was irreversibly changed by the recent discovery and characterization of a layer of extracellular deposits in the subretinal space, between the photoreceptors and RPE, called subretinal drusenoid deposits (SDDs).[2–5] One striking feature of SDDs is that they mirror the topography of rod photoreceptors in human macula,[6,7] leading to the question of whether there is an equivalent process resulting in a deposition related to foveal cones.

A central thesis of this review is that the biogenesis of soft drusen, the pathognomonic and specific extracellular deposit of AMD[8] and basal linear deposit (BLinD), a diffusely distributed form of the same material, is the leading candidate for such a process. Further, because the fatty acids in cholesterol-rich Bruch's membrane (BrM) lipoproteins, the dominant ultrastructural component of soft drusen, are rich in the fatty acid linoleate (from diet) rather than in docosahexaenoate (from photoreceptors),[9] we seek within the neurosensory retina the cellular relationships and dietary factors that can explain the topography of soft drusen and BLinD in older adults. The delivery of xanthophyll pigments (XPs) to the highly evolved Müller cells supporting foveal cones is one strong candidate. These speculative but testable hypotheses potentially unify many disparate data sets.

Essentials From the Biology of Macula

A neurovascular unit, conceptualized originally for brain and then for inner retina,[10,11] comprises microvessels, neurons, glia, pericytes, and extracellular matrix that link blood flow to the metabolic demands of neurons. The cells and tissues most prominently affected by AMD pathology are those of the outer retinal neurovascular unit,[12] that is, photoreceptors, retinal pigment epithelium (RPE), Müller cells (in neurosensory retina), and the choriocapillaris (ChC) endothelium (in the choroidal vasculature) (Fig. 1, top). Together these cells are served by the choroid, which has the highest blood flow in the body and is regulated in part by the autonomic nervous system and intrinsic choroidal neurons,[13] and contains clinically visible extracellular lipid depots.[14] Between RPE and ChC is a laminated subendothelial extracellular matrix called Bruch's membrane, which functions as a vessel wall laid out flat, in parallel to vascular lumens,[15] rather than circumferentially around them.

The RPE is a monolayer of cuboidal polygonal cells embedded between photoreceptors and BrM. Strong apical to basolateral polarization makes the RPE a key player in

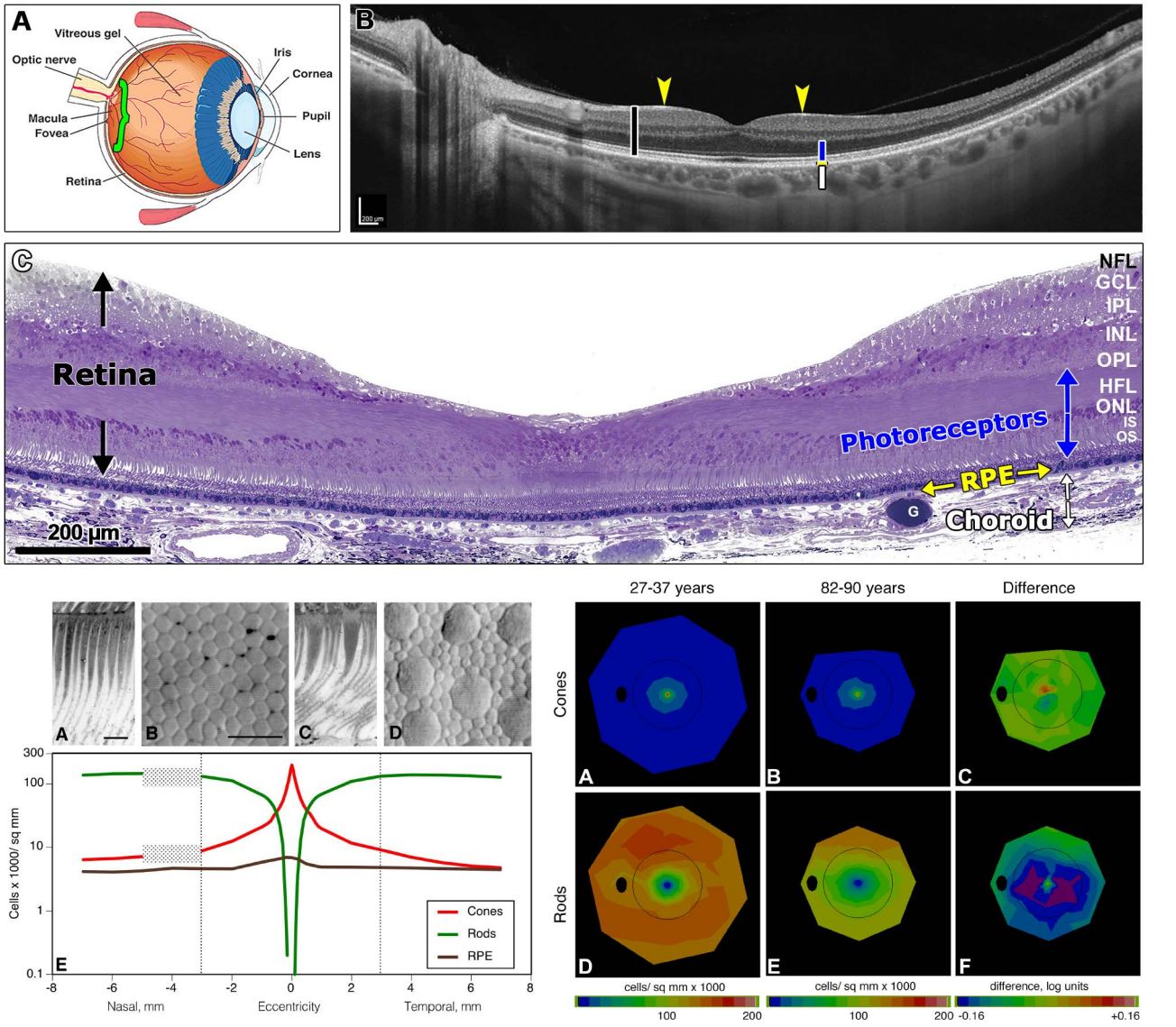

Figure 1. Neurobiology and aging of macula. *Top*: (**A**) Cross-section of a human eye. *Green bracket* shows area in (**B**), including optic nerve head and macula. (**B**) Swept-source optical coherence tomography of a living human neurosensory retina (*black bar*) and choroid (*white bar*), showing the 6-mm-diameter macula and adjacent optic nerve. The retina contains multiple bands of alternating high and low reflectivity that coincide in part with the anatomic layers. *Blue bar* delimits layers occupied by photoreceptors and interleaved Müller glia (M). *Arrows* indicate the fovea. The choroidal vasculature contains lumens of large vessels and is bounded externally by the sclera. (**C**) High-resolution histology of the fovea, in the center of macula and responsible for high acuity vision. The retina, photoreceptor layers, and choroid are indicated by *black*, *blue*, and *white arrows*, respectively. A foveal pit is created by inner retinal neurons, Müller cells, and accompanying retinal vasculature being swept to the side of the visual axis. The RPE is a simple cuboidal epithelium that sits on Bruch's membrane, the inner wall of the choroidal vasculature. Osmium postfixation, epoxy embedding, 1-µm-thick section, toluidine blue stain. *Lower left*: Photoreceptor mosaic and topography of outer retinal cells. (**A**) Foveal cone inner and outer segments, longitudinal section. (**B**) Foveal cone inner segments in a flat-mounted retina of a 34-year-old donor, Nomarski differential interference contrast optics and video. (**C**) Nonfoveal cone and rod inner segments, longitudinal section. (**D**) Cone inner segments (*large*) and rod inner segments (*small*) in the same eye. (**E**) Number of cones (C), rods (R), and RPE per square millimeter of retinal surface in nasal and temporal retina, in young adults, as a function of eccentricity from the foveal center in mm. Peak densities of cones, rods, and RPE in young adults are 200,000/mm^2, 150,000/mm^2, and 10,000/mm^2, respectively. With increasing eccentricity, cone density decreases, and rod density increases, becoming equal at ~0.55 mm. The RPE exhibits central peak with a shallow gradient. *Scale bars*: 10 µm. *Hatched rectangle*, optic nerve head. *Dashed lines*, limits of macula. *Lower right*: Topography of cones and rods in aging human retina,[64] shown as a fundus of a left eye. *Black oval*, optic nerve; *ring*, limits of macula. In (**C**) and (**F**), *warm colors* mean that older group has higher mean density than younger group and *cool colors* mean that older group has lower mean density than younger group. A *yellow-green* map means that differences between groups are small. (**A**) Cones, 27- to 36-year-old donors. (**B**) Cones, 82- to 90-year-old donors. (**C**) Log mean difference in cone density between younger adults and older adults is small and inconsistent. (**D**) Rods, 27- to 36-year-old donors. (**E**) Rods, 82- to 90-year-old donors. (**F**) Log mean difference in rod density between younger adults and older adults is greatest at 0.5 mm to 3 mm from fovea. *Purple* signifies that the log mean difference (aged-young) was < -0.16 log units, that is, that aged eyes had 31% fewer cells than young eyes. G, globule; GCL, ganglion cell layer; HFL, Henle fiber layer; INL, inner nuclear layer; IPL, inner plexiform layer; IS, inner segments; NFL, nerve fiber layer; ONL, outer nuclear layer; OPL, outer plexiform layer; OS, outer segments. *Top*: reprinted with permission from Tian L, Kazmierkiewicz KL, Bowman AS, Li M, Curcio CA, Stambolian DE. Transcriptome of the human retina, retinal pigmented epithelium and choroid. *Genomics*. 2015;105:253–264. © 2015 Elsevier Inc. *Bottom*: reprinted with permission from Jackson GR, Curcio CA, Sloan KR, Owsley C. Photoreceptor degeneration in aging and age-related maculopathy. In: Penfold PL, Provis JM, eds. *Macular Degeneration*. Berlin: Springer-Verlag; 2005:45–62.

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maintaining homeostasis of photoreceptors apically and ChC basally and in the pathology of SDDs apically and drusen basally. The outer blood-retina barrier is maintained by junctional complexes on the RPE, the basolateral surface of which faces the systemic circulation. The inner part of the blood-retina barrier is maintained by endothelial cells and Müller glia around retinal capillaries.

The human macula, 6 mm in diameter, is defined in neurobiology as an area with a continuous layer of ganglion cells[16] and in epidemiology as the area included in the Early Treatment of Diabetic Retinopathy Study grid for grading color fundus photographs.[17] Because the macula constitutes ~3% of the total retina area (of ~1000 mm^2), human eye studies that pool macula and periphery in assays based on whole eyecups overlook potentially important macula-specific signals. Older literature sometimes equates macula and fovea; the fovea is one subregion of the macula. It takes a whole macula to support a fovea.

In 1990, my colleagues and I presented a comprehensive two-dimensional map of cone and rod density in seven short postmortem retinas prepared as unstained whole mounts (Fig. 1, bottom left).[18] This technique enables visualization, topography, and morphologic detail of inner segments, while largely eliminating histologic processing artifacts and counting errors. With accurately localized foveal centers and computerized microscopy, it is possible to determine photoreceptor density over the entire retina. In the human macula, rods outnumber cones: a rod-dominant perifovea surrounds a cone-only foveola that is 0.8 mm in diameter. The foveola has a high peak density (>150,000 mm^2) and a sharp decline with eccentricity. Rods are also numerous (peak >150,000 mm^2) in an elliptical crest at 2- to 5-mm eccentricity that encircle the optic nerve head nasally.[18] Macular cone topography in this study has been replicated repeatedly in vivo by adaptive optics scanning laser ophthalmoscopy imaging.[19,20] Similar laboratory approaches have been recently applied to cell density and autofluorescence of human macular RPE.[21] A demonstrated peak cell density of ~7200/mm^2 RPE cells and a shallow eccentricity-dependent decline is consistent with previous literature.[22,23]

Neurons of the inner nuclear and ganglion cell layers are numerous and are displaced from the foveal center to create the foveal pit.[24] Approximately 95% of macular ganglion cells are midget cells, that is, compact neurons responsible for transmitting signal for high acuity and color vision to the brain.[25] There are at least two ganglion cells for each foveal cone within the central several degrees of vision.[18,26–28] Each foveal cone contacts two midget bipolar cells, splitting the output signal along "private lines" signifying light ON and light OFF to corresponding midget ganglion cells.[29] This unique circuitry means that 40% of human retinal ganglion cells are offset from photoreceptor inner segments defining their receptive fields in visual space.[18] The Henle fiber layer (Fig. 1, top) thus comprises 14% of retinal thickness and contains inner fibers of rod and cone photoreceptors (interleaved with Müller glia and up to 600 μm in length) extending centrifugally to contact bipolar neurons.[16,27,30–33]

Müller glia span the retina between external and internal limiting membranes. In the macula Müller cells equal or exceed the number of foveal cones[34,35] and are Z-shaped owing to the Henle fiber layer. Extramacular Müller cells are vertical and are outnumbered by photoreceptors. Among their many functions,[36] Müller glia deliver vitamin A derivatives required by phototransduction selectively to cones[37,38] (although this function has been explored in only mice to date). Recent evidence supports macular Müller cells as the principal reservoir of the yellow xanthophyll pigments (XP) lutein and zeaxanthin,[39–43] which are replenished by diet (see Aging of Macula below). Owing to their hydrophobicity, XPs localize to the interior of cell membranes. XP is detectable in vivo with behavioral tests involving color matching (heterochromatic flicker photometry)[44] and noninvasive imaging (e.g., two-wavelength autofluorescence, fluorescence lifetimes).[45,46]

Defining the Spaces Between the Layers

Between the photoreceptors and the apical RPE is the subretinal space, a closed physiologic compartment.[47] It is bounded inwardly by the external limiting membrane, a plane of heterotypic junctional complexes between photoreceptors and Müller cells and outwardly by junctional complexes among the RPE cells.[48] Within this compartment is the delicate interphotoreceptor matrix, which includes specialized domains ensheathing every cone and rod photoreceptor[49,50] to mediate intercellular transfers and adhesion.[51]

As defined in a companion article,[9] a three-layer definition BrM allows a tissue compartment and potential space between the RPE-basal lamina and the inner collagenous layer, called the sub–RPE-basal lamina space.[9] Drusen are thus focal deposits located between the RPE-basal lamina and the inner collagenous layer of BrM, in the sub–RPE-basal lamina space. Basal linear deposit is a thin layer of soft druse material, in the same compartment as drusen. Together BLinD and soft drusen comprise the Oil Spill on aging BrM (by this definition)[52] and constitute AMD's specific deposits. SDDs are also extracellular, located between the photoreceptors and RPE. While common in AMD, SDDs also occur in monogenic inherited disorders involving BrM and inherited and acquired disorders of retinoid processing.[53]

Aging of Macula

As shown by population-based epidemiology studies based on color fundus photography, aging is the greatest risk factor for AMD, followed by family history and smoking.[54,55] Intraocular factors most relevant to AMD initiation and progression are the disposition of drusen and hyperpigmentation,[56,57] suggesting that focusing on cell and tissue pathology and pathways underlying these signs will yield insights into molecular pathogenesis.

The relative rate of rod and cone loss characterizes each degeneration affecting photoreceptors,[58–60] and within the neurovascular unit, photoreceptor health is a readout of the support system.[61] In normal eyes from human donors aged 27 to 92 years in which inner segments have been counted in whole mounts for accuracy, rods decline 30% at 0.5- to 3-mm eccentricity, and cones remain stable (Fig. 1, bottom).[62] The rod-vulnerable region is closer to the fovea than the ring of high rod density and autofluorescence attributable to RPE lipofuscin (i.e., lysosome-related, long-lasting inclusion bodies rich in vitamin A derivatives)[21,63] and in fact hugs the rod-free area. Other studies enumerating cells in sectioned tissues agree on relative sparing of foveal cones in aging,[22] which continues well into advanced AMD.[64,65] Rod-mediated dark adaptation, which is limited by retinoid delivery,[66] is slowest within the central 6° of macula (of 12° tested in a small sample).[67]

Major age-related macular changes relevant to AMD besides rod loss and cone resilience are the stability of RPE cell numbers despite lipofuscin accumulation and mitochondrial degeneration.[21,22,68–70] Sprouting of rod terminals in aging are surprisingly more prominent in peripheral retina than in the macula.[71] BrM undergoes cross-linking, thickening, calcification, and lipidization.[72–78] Macular soft and hard drusen accumulate in the sub-RPE-BL space.[79,80] Choriocapillaris apposition to BrM declines,[77] along with increased immuno-

reactivity for membrane attack complex (terminal component of the complement cascade),[81] loss of autonomic nerve fibers,[82] and thinning of the choroid.[77]

Among aging-related factors that could impact rod survival in central macula (Fig. 1, bottom right), we focused on BrM and the sub–RPE-BL space, where AMD pathology is prominent. We investigated BrM lipidization, because a straightforward connection from there to druse lipids, arguably the first druse component described,[83–87] seemed possible. Lipid accumulation in vessel walls can be informed by the pathophysiology of atherosclerotic cardiovascular disease[88] as well as the clinical success in reducing its public health burden.[89]

Soft Drusen Composition Resembles Both Plasma Lipoproteins and Outer Segments

A companion review article[9] details histochemical, ultrastructural, direct assay, gene expression, and cell culture evidence that membranous debris, the principal component of soft drusen as defined by Sarks et al.,[90,91] derives from large lipoproteins containing apolipoproteins B and E, secreted basolaterally by the RPE into BrM. Lipoproteins are multimolecular complexes that resemble oil droplets solubilized for transport through aqueous media with a surface of proteins (apolipoproteins and others), phospholipid, and unesterified cholesterol. Very low-density lipoprotein (of hepatic origin, parent to "bad cholesterol" low-density lipoprotein [LDL]) and dietary chylomicrons (of intestinal origin) are two well studied examples; the brain has high-density lipoprotein (HDL) rich in apolipoprotein E (apoE). As elaborated separately,[9] the predominant ultrastructural component in soft drusen was initially called "membranous debris," which in turn is partly preserved "lipoprotein-derived debris." By using lipid-preserving ultrastructural techniques originally used for elucidating the deposition of plasma LDL in arterial intima,[92,93] it has been possible to see the core-and-surface morphology of lipoproteins that accumulate with age in human BrM, with highest concentration in the macula. When assayed with high-performance liquid chromatography, isolated lipoproteins and BrM extracts are rich in esterified and unesterified cholesterol. Importantly, fatty acids are dominated by linoleate (implicating dietary sources) and not docosahexaenoate (not implicating photoreceptor outer segments). Recently, a culture system of primary porcine RPE was shown to lay down a continuous layer of deposits containing lipid and hydroxyapatite, depending on the substrate the cells were grown on, without supplementation of outer segments and taking up only components of culture medium, which include plasma lipoproteins. These findings give credence to the notion of a diet-driven system.[94] Further, clinical imaging has documented that drusen grow, and RPE migrates anteriorly into the retina, thus no longer maintaining the druse,[95,96] indicating that presence of drusen implies a certain level of RPE functionality. The current model for BrM lipoproteins is that they have two principal sources, plasma lipoproteins delivering lipophilic essentials and phagocytized outer segments, and they provide a mechanism for the RPE to offload unneeded lipids to the systemic circulation and avoid lipotoxicity. Details are available in our companion article.[9]

Using Topography to Dissect Mechanisms

Adhering to the philosophy that improved care for AMD patients is best served by following the biology,[97] we next consider biologic antecedents for these pathways in neurosensory retina. According to the terrain theory of vision,[98] the sampling of visual space by neurons in each species' retina is influenced by the species' normal habitat. Mammalian retinas have an area of high neuronal density specialized for visual acuity, onto which images are registered by coordinated head and eye movements.[99] Conversely, neuronal density and spatial resolution is low in peripheral retina, because space for neurons receiving visual input in the brain is limited and peripheral retina has evolved for functions like motion detection. Retinal neurobiology has taught us that cell populations have emergent properties not appreciated by study of individual cells.[99] Topography is thus a powerful independent variable for dissecting pathogenic mechanisms, while also completing a trajectory from clinical manifestations back to the evolutionary biology that brought humans a macula in the first place.

Topographic considerations can prioritize mechanisms for follow-up. For example, light exposure is often mentioned in the AMD context, yet retinal illuminance is homogeneous to 50° eccentricity[100,101] and not obviously related to the distribution of AMD's characteristic pathology. We have previously considered whether a strong macula-to-periphery gradient (7:1) of esterified cholesterol in aged BrM is related to gradients in photoreceptor density and concluded that these are too small (2.7 for rods, 1.9 for cones).[75] Drawing from hemodynamic considerations in the regional vulnerability of large vessels for atherosclerosis, we also have considered ChC blood flow impacting BrM capacity to retain lipoproteins.[102] However, existing laboratory studies of blood flow have technical limitations,[103,104] and new in vivo methods are under active development.[105,106] Here we ask whether dietary delivery of lipophilic essentials required by macular cells could influence drusen biogenesis.

Do Soft Drusen Have a Predilection for the Macula?

Drusen and hyperpigmentation are the two largest intraocular risk factors for progression to neovascularization and atrophy. Epidemiology, restricted to the 30° view of color fundus cameras,[17,107] has shown that drusen conferring the greatest progression risk reside in the central 1-mm-diameter subfield of the Early Treatment of Diabetic Retinopathy Study grid.[57,108–111] Risk is markedly heightened when normalized for this subfield's small area. Similarly, longitudinal clinical studies using optical coherence tomography have determined that high progression risk associated with drusen volume within the central 3 mm diameter is not increased by using the central 5 mm diameter.[112,113] Via clinical ophthalmoscopy, color fundus photography, and ultrastructural clinicopathologic correlation, Sarks et al.[114] have stated that soft drusen arise within the "inner macula" and are preferentially depleted, relative to hard drusen, by prophylactic laser therapy.

Pathology data supporting a predilection of soft drusen for macula exist but are sparse, because few studies have been designed to test this possibility. In a large series of autopsy eyes analyzed by ex vivo imaging and histology, low-profile "serogranular drusen" in macula[86] have been contrasted to globular drusen in periphery.[115] More peripheral drusen than macular drusen contain apolipoprotein immunoreactivity, whereas all drusen in all regions contain cholesterol forms, suggesting higher lipid concentration in macular drusen.[116] In drusen microdissected from nine eyes of seven AMD donors, soft drusen are found only in the macula,[117] and are less likely to be harvested intact, and more likely to have overlying basal laminar deposit, high coverage by RPE, and interiors dominat-

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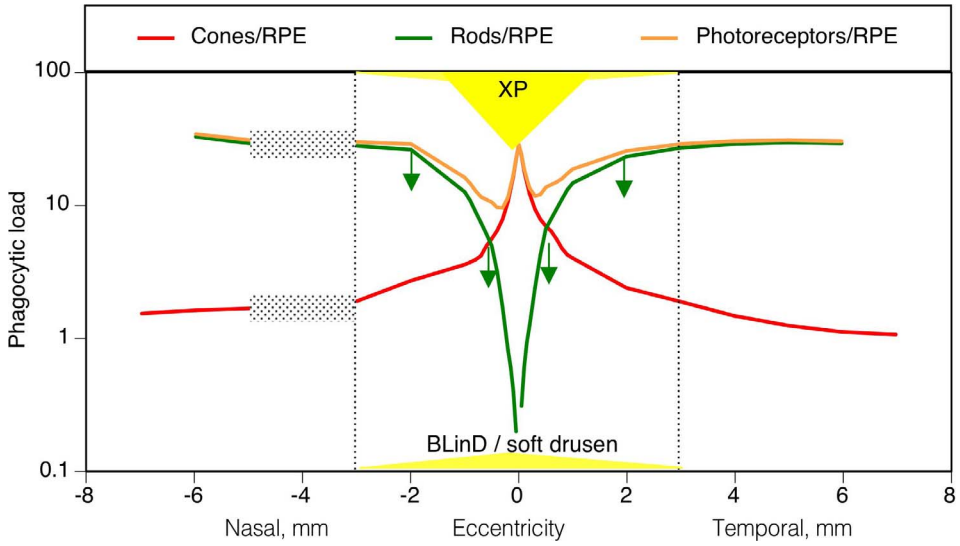

Figure 2. Topography of human macular photoreceptors, retinal pigment epithelium, XP, and BLinD/soft drusen. *Dotted lines* indicate the limits of the 6-mm-diameter macula. *Stippled rectangles* indicate the optic nerve head. *Green downward arrows* indicate the annulus of deepest rod loss in aging (Fig. 1, *bottom right*, *deep blue*). The lateral extents of XP and BLinD/soft drusen are drawn to scale on the eccentricity axis and not-to-scale on the *y*-axis. Data come from different eyes of younger adults: photoreceptors/mm^2 (Ref. 18); RPE/mm^2 (Ref. 18); XP[129]; thickness of BLinD/soft drusen in AMD eyes.[4]

ed by a homogeneous membranous material consistent with the Sarks' descriptions.[117] In a high-resolution histology survey of nonneovascular AMD eyes, BLinD is thickest under the fovea.[4] These suggestive results should be fortified with new high-resolution histology and clinical imaging covering the entire retina, which is now possible. In particular, more data supporting scarcity or absence of soft drusen in the periphery could definitively establish specificity for foveal biology.

Considering Dietary Delivery of Xanthophyll Pigment

Our model of soft drusen biogenesis suggests that fatty acids from diet are combined with unesterified cholesterol both from diet and from outer segments to create large lipoproteins secreted by RPE in BrM.[9] Fatty acids in RPE-secreted BrM lipoproteins are enriched in linoleate (implicating diet as a source) and not docosahexaenoate (from photoreceptor outer segments),[118,119] raising the question of what major dietary pathway(s) supply fatty acids and for what purpose. One possibility is the delivery of vitamin A derivatives for phototransduction. The paucity of well-studied bisretinoid A2E in macula relative to periphery[120–125] (unlike mouse[126]) hints at a unique division of labor between RPE and Müller cells in vitamin A homeostasis, which remains to be explored further. Here we suggest that delivery of XP to Müller cells is a plausible biologic purpose for a large influx of dietary lipids through RPE that is specific to central macula.

The XPs lutein, zeaxanthin, and meso-zeaxanthin[127] are highly concentrated in the fovea, decline by an order of magnitude within 2° of fixation, reach very low levels at the edge of the macula, and remain low throughout the retina.[128–130] The distribution of XPs can be envisioned as three concentric zones centered on the fovea: zeaxanthin is central-most (foveola, 350 μm diameter), overlapped by meso-zeaxanthin (foveal avascular zone, 500 μm diameter); these two are surrounded by lutein (foveal-parafoveal annulus of outer diameter, 2.0 mm).[131] The role of XP in vision is actively investigated under several mechanistic hypotheses. The protection hypothesis suggests that XP protects the retina from cumulative damage of ambient blue light via antioxidant properties.[132] The optical hypothesis suggests that XP improves visual performance and visual comfort by attenuating chromatic aberration and light scatter, via short-wavelength light filtering and dichroism.[133,134] The neural efficiency hypothesis suggests that XP improves vision by a direct interaction with neurons,[135] perhaps conferring an evolutionary advantage.[133] XP biology has expanded beyond the macula to the brain, where these compounds are detected and studied in reference to cognition and aging.[136,137] XP is of clinical significance because dietary supplements containing lutein and zeaxanthin are recommended for some patients with intermediate AMD.[138]

Recent evidence supports macular Müller cells as the principal cellular reservoir of XP. High XP concentration in the Henle fiber layer, foveal center,[128,139–141] and inner plexiform layer is well explained by the morphology of individual Müller glia.[32,142,143] The persistent finding of rings and shoulders in addition to a strong central peak is consistent with Müller cell side branches in the synaptic layers.[144–146] In macular telangiectasia type 2, XP absence is associated with histologically confirmed degeneration of foveal Müller cells.[39,40] Patients with Sjögren-Larsson syndrome exhibit loss of clinically detectable XP and presence of inner retinal cysts suggestive of Müller cell degeneration.[41,147,148] Surgically excised lamellar hole epiretinal membranes are enriched both in Müller cell markers and in XP.[42,43] Autofluorescence imaging suggests that XP persists in central geographic atrophy,[149–151] even after photoreceptor death, because Müller cells remain.[33,64] Attributing strong XP signal to only cone axons in the Henle fiber layer[152] overlooks the numerous rod and Müller processes also in this layer. In 1984 Snodderly et al.[128] rejected Müller cells as a reservoir, in part because little evidence at the time suggested so many foveal glia. Subsequent studies in monkeys[34] and humans[153] indicate equal numbers of Müller glia and macular photoreceptors, at the limited sites examined. This hypothesis does not exclude XPs or their binding proteins also localizing to other cells,[154,155] including cone axons, or the possibility of transfers between Müller cells and photoreceptors.

Figure 2 plots on the same eccentricity axes several mechanisms that may contribute to the apparent vulnerability of parafoveal rods and resilience of foveal cones in aging. The

phagocytic load on RPE is represented by a ratio of photoreceptors/mm^2 to RPE/mm^2 and is computed for cones and rods, separately and summed. This ratio is high at the cone peak, lower in the parafovea, and higher again at the edge of the macula. Yet foveal cones survive into late AMD, and in aging the rods die in an annulus of vulnerability where phagocytic load is lower than elsewhere. These considerations suggest that phagocytic load is less of a factor in AMD initiation than is commonly believed, as previously concluded for monkeys.[23] Figure 2 also shows that BLinD/soft drusen in nonneovascular AMD are found across the macula, being slightly thicker at the foveal center than elsewhere. The annulus of age-related rod loss localizes to the shoulder of this lipid-rich area in BrM. Foveal cones directly overlie lipid deposits but in contrast to rods are sustained by Müller cells harboring XPs, which are present throughout the foveal center and across the annulus of vulnerability.

Factors underlying XP absorption, distribution, metabolism, and excretion are actively investigated and include intestinal absorption and intracellular transport in enterocytes, receptors and transporters in retina and brain target tissues, and participation by the microbiome.[156] Available evidence suggests that retinal XPs are turned over, that is, delivered and removed, on a time scale of months. A detailed time course of XP repletion in two volunteers has shown a steady increase for 140 days after initiation of supplementation, followed by a plateau or slight decrease to 1 year or more.[157] Many studies report that dietary supplementation results in higher MPOD (measured behaviorally or via several imaging technologies) at the earliest time points tested, typically 2 to 4 months and depending on the dose and baseline XP levels in test subjects.[131,158–169] XP removal is less well documented, with limited data suggesting stable, slightly decreasing, or baseline MPOD.[157,169] Mature macaque monkeys consuming a semipurified diet lacking XP lose yellow pigment; these animals were examined after years on this regimen and therefore the minimum time for XP depletion is unknown.[170] Interestingly, XP-deficient animals have hypopigmented spots in the fovea that could represent either drusen or lipoidal degeneration of individual RPE cells, both of which are found in monkeys.[171–174]

We thus hypothesize that XP is a marker for Müller cell protection and enhancement of foveal cone function for acute vision. We also hypothesize that an influx of HDL-mediated XP delivery through RPE provides a major source of fatty acids in BrM lipoproteins, which are a source of peroxidizable, cytotoxic lipids and a barrier to transport between the ChC and outer retinal cells.

HDL (Plasma and Genes) and Xanthophylls

Accumulation of XP in the macula begins with consumed foods, digestion, absorption, and transport in plasma, and ultimately capture, transcellular transfer, and stabilization within retinal cell membranes.[127,132] On a sufficient diet, HDL is the major lipoprotein transporter of lutein (52%) and zeaxanthin (44%); LDL is the major transporter of α- and β-carotene and lycopene.[175] The best studied cellular receptor for plasma HDL is scavenger receptor B-I (SRB-I).[176] Evidence for a facilitated, SRB-I–mediated transport mechanism for lutein absorption in human intestinal enterocytes has been summarized.[132] Animal models lacking apoA-I (principal protein of HDL) lack lutein in retina and retain it in brain, suggesting specific targeting and uptake mechanisms.[177] Evidence accruing for ocular cells includes selective reduction of XP uptake (compared to LDL) by depletion of SRB-I activity in RPE-derived cell lines[178,179] and capacity for XP binding by interphotoreceptor retinoid-binding protein (IRBP) within the subretinal space.[180] In contrast to lutein and zeaxanthin, meso-zeaxanthin is rare in common dietary sources and appears to be converted within the RPE from dietary lutein by a newly recognized function of RPE65, the well-known retinol isomerase of the visual cycle.[181]

Our overall hypothesis adds a new perspective to recurring reports that elevated levels of plasma HDL modestly increase risk (1.15–2.3 fold) for incident early AMD, in population[182–186] and case-control studies,[187] and not in a large cohort of advanced AMD.[188] This effect of AMD is opposite to the well-documented association of elevated plasma HDL with reduced risk for cardiovascular disease. It is possible that elevated HDL level leads to increased XP uptake by the RPE, in turn increasing both the amount available for delivery to photoreceptors and excess lipids to be offloaded to ChC, in RPE-secreted lipoproteins. Yet, positive association between plasma concentrations of lutein and HDL does not always imply an association between detectable retinal XP and plasma HDL concentration.[189]

XP delivery also adds a new perspective to the association of genes of plasma HDL metabolism with AMD, including *APOE*, *CETP*, *ABCA1*, *LIPC*, and *SCARB1*.[190–196] DNA sequence variants in *LIPC* and *ABCA1* associate with intermediate and large drusen.[197] The International Age-related Macular Degeneration Genomics Consortium cohort of 16,144 cases, with 17,832 controls,[195] has been used in Mendelian randomization analyses of genes associated with plasma lipid classes on AMD risk. In one study,[198] three of five variants reached genome-level significance (*LIPC* × 2; *CETP*), placing AMD intermediate between cardiovascular disease (5/17 variants) and Alzheimer (1/4 variants) in lipid gene effects. Another study using this data set has shown that HDL-increasing alleles *CETP* and *LIPC* have opposite effects on AMD risk.[199] A meta-analysis of 21 studies suggests that elevated HDL is a risk factor for any and early AMD.[200] A sequence variant in *SCARB1* (gene encoding SRB-I) is positively associated with plasma lutein but not with behaviorally measured XP.[196]

Not all variants in HDL genes result in higher plasma HDL. Our AMD pathobiology model suggests that genetic variations either modulate concentrations of plasma HDL, intraocularly modulating retinal uptake, intercellular transfer, and membrane stabilization, or both. Many of these gene products are expressed in outer retina (Fig. 3), consistent with an intraretinal HDL system based on apoE, like brain.[4] Immunolocalization of HDL-related genes, using validated antibodies in polarized RPE (in vivo or high-fidelity culture), includes apoE (outer segments, RPE, Müller cells,[94,201–204] drusen,[116,190,201] SDD)[117]; ABCA1 (ATP-binding cassette transporter), RPE cell bodies[205–208]; CETP (cholesteryl ester transfer protein), outer segments, and outer plexiform layer[205]; LIPC (hepatic lipase), all retinal neurons plus RPE, and not in Müller cells[192]; SRB-I, RPE expression,[209,210] activity,[178,211] and localization.[202,206] Lutein has been detected in subretinal fluid removed from rhegmatogenous retinal detachments,[212] consistent with transfer between RPE and retina. Both systemic and ocular mechanisms of HDL-mediated XP delivery could be simultaneously operative. More data are certainly needed.

Conclusions

In this scenario, soft drusen and the Oil Spill on BrM in AMD develop over a lifetime of recycling unneeded lipids, many taken up with plasma HDL delivering XP to the choroid and impaired in egress by aged BrM-ChC. XP is a biomarker of Müller cell health and capacity for sustaining high-acuity, foveal cone-mediated vision, conferring a selective advantage for eyes

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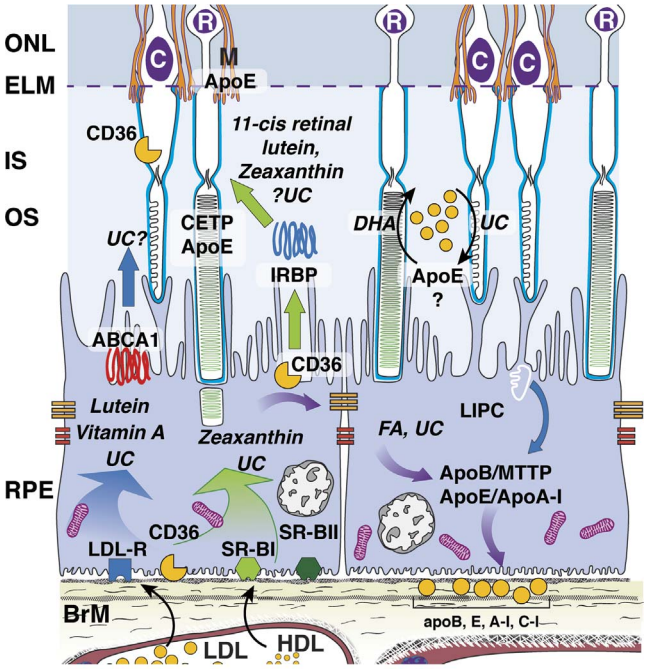

Figure 3. Outer retinal lipid recycling pathways potentially involved in XP transport. Lutein and zeaxanthin, vitamin A, and some unesterified cholesterol (UC) enter RPE via receptor-mediated uptake of plasma lipoproteins, especially at the LDL and SRB-I receptors. These are transferred to photoreceptors by poorly understood mechanisms that may involve interphotoreceptor retinol-binding protein (IRBP, *center*), an apoE-based small HDL particle (*right*), or diffusion (not shown). Because lipids in Bruch membrane lipoproteins are rich in the fatty acid (FA) linoleate (and not docosahexaenoate [DHA]), soft drusen are proposed as a downstream consequence to constitutive dietary delivery of lipophilic essentials to macular cells, and the RPE-mediated recycling of unneeded lipids from these sources and from outer segments, to the circulation via large lipoprotein particles containing apoB and apoE. Lipoproteins accumulate in the sub–RPE-basal lamina largely because of impaired egress through the Bruch's membrane-choriocapillary complex. This is part of a larger system thought to include physiologic release of molecules contributing to subretinal drusenoid deposit (not shown).[4,102] Reproduced with permission from Spaide RF, Ooto S, Curcio CA. Subretinal drusenoid deposits AKA pseudodrusen [published online ahead of print May 30, 2018]. *Surv Ophthalmol*. doi:10.1016/j.survophthal.2018.05.005. © 2018 Published by Elsevier Inc.

with XP in mammalian evolution. XP may be just one representative of the nutritional support system of this superb human asset. We suggest that evolution of the neuroglial relations underlying exquisite foveal vision in humans came with a price, that is, soft drusen and their sequela, long after our reproductive years.

Limitations to this analysis are sparse experimental confirmation of hypotheses largely generated from human tissues and patients. We are fortunate that XP can now be measured noninvasively and objectively in vivo through imaging.[45,146] Further, short-lived mouse models exhibiting relevant phenotypes are now available (XP accumulation,[213] retentive matrix for RPE-secreted lipoproteins[214–216]). Our hypotheses, while speculative, bring together many lines of evidence and do not exclude other major extant hypotheses for AMD biology and may in fact occur in parallel. It is hoped that this conceptual framework can guide the exploration of clinical imaging data sets[46,141,217] and high-throughput analytic assays of tissues.[218,219]

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