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Histology, dimensions, and fluorescein staining characteristics of nodular and cuticular drusen in age-related macular degeneration

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Abstract

Purpose: To enable *in vivo* analysis of drusen composition and lifecycle, we assessed macular nodular and cuticular drusen using histology.

Methods: Median and interquartile range (IQR) of base widths of single (non-confluent) nodular drusen in 3 sources were determined histologically: 43 eyes of 43 clinically undocumented donors, in an online resource; one eye with punctate hyperfluorescence in fluorescein angiography (FA); and two eyes of one patient with bilateral “starry sky” cuticular drusen. All tissues were processed for high-resolution epoxy-resin histology and for cuticular drusen, transmission electron microscopy.

Results: All drusen localized between the retinal pigment epithelium basal lamina and inner collagenous layer of Bruch’s membrane. They were solid, globular, homogeneously stained with toluidine blue, and uncovered by basal laminar deposit and basal mounds. Median base widths

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were 13.0 μm (Source 1, N=128 drusen, IQR 7.7, 20.0 μm), 15.3 μm (Source 2, N=87, IQR 10.6, 20.5 μm), and 7.3 μm (Source 3, N=78, IQR 3.9, 14.1 μm).

Conclusions: In three samples, >90% of solitary nodular drusen were <30 μm, the visibility threshold in color fundus photography; these drusen are hyperfluorescent in FA. Whether these progress to soft drusen, known as high-risk from epidemiology studies and hypofluorescent, may be determinable from multimodal imaging datasets that include FA.

TOC description

Base widths were determined for isolated nodular drusen in donor eyes and two eyes with fluorescein angiography during life, including “starry sky” cuticular drusen. Over 90% of measured drusen were <30 μm wide, the lower visibility limit for color photography. Drusen lifecycles may be accessible in clinical angiographic data sets.

Keywords

age-related macular degeneration; drusen; fluorescein angiography; retinal pigment epithelium; histology; electron microscopy; optical coherence tomography

Introduction

Targeted measures that slow or even prevent neovascular and atrophic end-stages of age-related macular degeneration (AMD) remain a priority. Drusen are the largest known intraocular risk factors for progression, in populations of European descent.[1] The knowledge base available for drusen biology, long-term epidemiologic studies, and cellular-level eye-tracked retinal imaging is substantial. By asking what drusen types are discernible by dye-based angiography, this histopathologic report aims to leverage existing clinical imaging data for insight into drusen composition and lifecycle.

Over 20 years,[2] the Beaver Dam Eye Study of a community population aged 43–86 at baseline showed a continuum from numerous (>20) small hard drusen to advanced AMD. Others recently demonstrated isolated small drusen even in pre-teens.[3] Sarks et al[4] used serial fluorescein angiography (FA) to show that hard drusen may aggregate and form high-risk soft drusen. It has been proposed that noninvasive *in vivo* multimodal imaging, anchored on optical coherence tomography (OCT), is the best way to learn if high-risk drusen form as a direct transformation of small hard drusen.[5] We suggest that dye angiography provides important information in this quest.

In circulation, 70% of fluorescein, a hydrophilic dye, is bound to plasma proteins, and the remainder is unbound within the aqueous phase. Both bound and unbound fluorescein are expected to preferentially diffuse into hydrophilic tissue compartments and avoid hydrophobic compartments (Figure 1). Pauleikhoff et al were the first to suggest that drusen phenotypes of varying lipid composition might differentially stain on FA.[6] They tested this idea by exposing AMD tissue sections to fluorescein in solution. Our approach is detailed histologic analysis of human eyes that underwent FA during life, to identify which lesions correspond to specific angiographic signatures.[4]

Figure 1 shows a model of differential fluorescein uptake by drusen. A large lipid-rich component of soft drusen ('membranous debris') was identified by Sarks et al using electron microscopy of clinically followed eyes,[4] and later attributed to lipoprotein particles of intraocular origin by current author CAC.[7] Microdissection, extraction, and *in situ* electron microscopy indicated electron-dense circular profiles consistent with lipoproteins in nodular drusen, the histologic correlate of hard drusen seen in the fundus. Soft drusen contain a high proportion of electron dense lipid-derived particulate and membrane-like material dispersed in an electron lucent matrix.[8, 9] Nodular drusen could thus bind more fluorescein than soft drusen due to the greater proportion occupied by non-lipid components. Nodular drusen could also appear hyperfluorescent due to RPE absence at the druse dome (Figure 1).[9]

Eyes with "starry sky" appearance in FA have numerous small cuticular drusen (originally called basal laminar drusen).[10, 11] On OCT these correspond to low-lying RPE elevations, a saw-toothed pattern, and broad mound-shaped elevations resembling soft drusen, the latter two with internal hyporeflectivity. The same proteins are immunodetected in cuticular drusen as in extramacular nodular drusen;[12] lipids have not yet been assessed. Morphologic similarities of cuticular and nodular drusen[11] suggest that they might be the same, differing only in abundance. However, cuticular drusen can also exhibit a multilobed structure suggesting dynamic processes beyond confluence, like budding, or collision and uplifting.[11]

To enable analysis of drusen composition and lifecycle using archives of *in vivo* FA, we assessed with histology small drusen from three sources: an online resource of donor eyes with AMD and two separate clinically documented cases with FA during life, including one case with cuticular drusen. We measured base width and height to address visibility in ophthalmoscopy and OCT, respectively.

Methods

Regulatory approval

This laboratory study was approved by the Institutional Review Board of the University of Alabama at Birmingham and the St Vincent's Hospital Human Research Ethics Committee (Sydney Australia). Access to materials in the Sarks Archives complied with a Data Use Agreement (2021STE02369).

Histopathology study

Overview and definitions—To definitively separate tissue layers in the RPE-Bruch's membrane (BrM)-choriocapillaris complex, we prepared tissues for epoxy resin histology and used oil-immersion light microscopy and transmission electron microscopy. Basal laminar deposit (BLamD) is thickened extracellular matrix internal to the native RPE basal lamina (RPE-BL) now visible in OCT as a hyporeflective band.[13] Soft drusen and basal linear deposit (BLinD) are two physical forms (lump vs thin layer) of the same lipid-rich material in the sub-RPE-BL space. This material also aggregates within BLamD as basal mounds (BMounds).[13, 14]

Source 1: Donor eyes—Methods are published for the Project MACULA online resource for AMD histopathology (n=140 eyes, mean death to preservation time = 3.8

hours).[13] Tissue samples were post-fixed by osmium – tannic acid – paraphenylenediamine for extracellular lipid. Epoxy-resin sections 0.8 μm thick were stained with toluidine blue and scanned in their entirety using a 40X objective and a microscope with a robotic stage (numerical aperture 0.95; Olympus VSI 120, CellSens; Olympus, Center Valley PA). Images (~500 MB each) were scaled to tissue units and centered on the fovea or vertical meridian (divergence of Henle fibers) using a custom plugin for ImageJ (https://imagej.nih.gov/ij/download.html).

To identify small RPE elevations, we surveyed 93 aged and non-neovascular AMD eyes. Images were reviewed (author CDE) for small nodular drusen, small soft drusen, excrescences of BLamD, Bmound, and multi-layer deposits (BLamD, BLinD, drusen) that elevate the RPE and thus might be confused with drusen on OCT. Analyzed selections met criteria of adequate stain density, good tissue integrity, and absence of histologic artifact such as folds or bubbles. Screenshots were assembled into presentation software (PowerPoint, Microsoft, Redmond WA) with standardized annotations for selection and sorting. For figures, areas were re-scanned with a 60X oil-immersion objective (numerical aperture = 1.42) and adjusted to maximize the intensity histogram for contrast and white balance (Photoshop CS6, Adobe Systems, USA).

Source 2: eye with nodular drusen and non-exudative type 1 neovascularization in AMD—A 90-year-old woman with bilateral AMD underwent FA at presentation at age 79, 11 years before death. The left eye had an extensive non-exudative type 1 macular neovascular membrane (MNV); the right eye had a fibrovascular scar. Eyes were preserved 6.25 hours post-mortem. Histologic methods, photo-documentation, and analysis approaches are published.[15] High-resolution histology sections were scanned at 20X and matched to 19 clinical OCT scans, each 240 μm apart. Tissue features in the left eye were correlated to *in vivo* OCT by landmarks (author LC). Clinical scans and corresponding histology sections were depicted on a fundus autofluorescence (FAF) image according to locations and coordinates on centered and scaled low magnification images. Drusen on FA were mapped onto the FAF image and matched to the area of histologic drusen. Sections were re-scanned at high resolution for evaluation of drusen type and morphology.

Source 3: two eyes with “starry sky” cuticular drusen—The Sarks-Killingsworth group reported 132 clinically documented cases, 25 of which were processed for high-resolution histology and transmission electron microscopy.[14] As detailed,[16] post-fixation included 2% osmium tetroxide, and sections were stained with methylene blue and basic fuchsin. Deposits in a 61-year-old man were considered cuticular drusen with “starry sky” FA.[17] Clinical images of the left eye at presentation and light and electron micrographs from post-mortem examination were reviewed.[11]

Morphometric analysis

For solitary drusen from all sources, base widths along BrM and maximum heights were measured (ImageJ). Due to the small sample, differences in available images (whole sections vs single micrographs), and stages of disease, these data are not analyzed statistically.

Results

Survey of donor eyes (tissue source 1)

RPE elevations meeting quality criteria were found on 104 images of 43 eyes of 43 donors: nodular drusen (53 images), small soft drusen (8); BLamD with BMounds (33), and multilayer BLamD with BLinD-drusen (10) (Figure 2). Nodular drusen appear solid, globular, and uniformly stained with toluidine blue (Figure 2ABC). They readily contrast with soft drusen material in all its forms, which is granular and light grayish brown (Figure 2DEF), and they lack overlying BLamD and BMounds. Figure 2DEF shows RPE elevated and thinned by BMounds and BLamD, together with soft drusen and BLinD. Most of the RPE elevation due to BLamD- is due to late BLamD, i.e., scalloped layers of dark blue staining extracellular matrix. We did not see complex lobular structures suggestive of cuticular drusen.

Clinically documented case of type 1 MNV (tissue source 2)

Figure 3 illustrates hypofluorescent drusen in a case of non-exudative type 1 MNV secondary to AMD, revealed by an FA administered at presentation 11 years prior to death. As shown in Figure 3C, three spots of atrophy visible on FAF served to link OCT B-scans to histology sections. The optic nerve head and retinal vessels served to register the FA. Figure 3E shows that in a late-phase angiogram, small, uniformly hyperfluorescent dots appeared as the surrounding background haze faded. In the same FA, a window defect at one of the atrophic spots was much larger than the dots, with more irregular outlines (Figure 3E). Fourteen months after the last clinical examination, histology showed small nodular drusen identical to those in the donor eyes (Figure 3F) in the area of the hyperfluorescent dots (Figure 3G). These fell outside the region with type 1 MNV, mostly on its inferior nasal aspect. The distribution of nodular drusen was thus stable over time, like the slow growth of a subfoveal type 1 MNV in this eye.

Clinically documented case of cuticular drusen (tissue source 3)

Bilateral cuticular drusen were imaged with color fundus photography and FA, 6 months before patient death (Figure 4AB). Numerous small yellow dots and corresponding small puncta of hyperfluorescence formed a canonical “starry sky” pattern.[10] By light microscopy, the coloration in Figure 4CD differs from that in Figures 2–3, due to the difference in post-fixation and staining. Like nodular drusen in Figures 2–3, cuticular drusen in this eye (Figure 4C) are globular and have a solid homogeneous appearance and little overlying BLamD. The exteriors of larger confluent deposits (Figure 4D) resemble the smaller deposits in texture and staining density, but the interiors are granular and stained pink. They may also appear multilobed or branched (Figure 4C). By electron microscopy, interiors of individual drusen are solid and homogenous with moderate electron density, or seemingly in transition to multiple scattered electron-dense globules, presumably lipid-containing (Figure 4EFG).

Biometrics of nodular and cuticular drusen

From tissue sources 1–3, 132, 87, and 78 drusen, respectively, were assessed for base width (median, 13.0, 15.3, 7.3 μm) and height (median, 6.2, 9.1, 6.7 μm; maximum, 48.0, 34.8, 19.5 μm) (Figure 5, Supplementary Table 1). Width measurements from sources 1 and 2 were both skewed right due to the similar tissue preparation and microscopy. Source 3 measurements were skewed left, because single-frame electron microscopy images introduced a selection bias against larger deposits. Nevertheless, 91–96% of drusen had base width ≤ 30 μm, the lower visibility limit for color fundus photography,[17] and 95–100% had heights ≤ 40 μm. Heights and widths, within each of the three sources, were highly correlated (R^2 = 0.65–0.72; Figure 6).

Discussion

We find that both isolated nodular drusen and cuticular drusen are hyperfluorescent on FA and have similar morphology and dimensions. For cuticular drusen we additionally observed an apparent transition in internal composition. These data support several concepts elaborated below: nodular and cuticular drusen are the same entity, cuticular drusen are numerous due to predisposing factors in people who have them, and numerous cuticular drusen may confer progression risk using principles learned for other AMD deposits.

Three sources of histologic data from well preserved eyes each offered insights. Source 1, an online histology resource of many short post-mortem donor eyes with minimal disease, was rich in small deposit morphologies. Source 2, with a submacular non-exudative type 1 MNV, exhibited remarkable stability of findings over 11 years, consistent with preservation of visual acuity (20/30). Interpretation was aided by atrophic spots in the FAF image used for registration, one of which appeared as a window defect distinct from drusen-associated fluorescence. This comparison helped solidify the fluorescent dots as druse contents and not incomplete coverage by RPE. Source 3 with bilateral cuticular drusen had excellent transmission electron microscopy. The fact that these drusen were initially called hard drusen[4] suggests that the distinction is not always straightforward. Cuticular drusen were called basal laminar drusen by Gass, who thought that “starry sky” represented outpouchings of a basophilic material at the basal RPE (later called BLamD).[10] Electron micrographs from advanced AMD cases, with high resolution and contrast,[18] revealed a composition and laminar location distinct from soft drusen material. Recent literature using the term cuticular avoids confusion with BLamD. Incomplete RPE coverage atop nodular drusen[9] likely contributes to multimodal imaging characteristics of cuticular drusen (pinstripe hypertransmission defects, dot hypoautofluorescence, and hyperfluorescence).[11]

Our model of drusen biogenesis is outward transport of metabolites and by-products from a relatively functional RPE impeded by transport barriers that move inward over time throughout adulthood. These processes are best appreciated for lipid deposition via lipoprotein particles of RPE origin. Thus, choriocapillaris endothelium dysfunction and BrM changes of cross-linking and glycation lead to lipoprotein binding and accumulation in elastic and inner collagenous layers of BrM, between ICL and RPE-BL first as pre-BLinD, then BLinD, and soft drusen. This is followed by BMounds internal to the native RPE-BL.

Internal remodeling of druse contents as well as cellular invasion of the sub-RPE-BL space, suggestive of an immune response[9, 19] that can range from mild to intense.

By this model, nodular drusen are pan-retinal default deposits that confer a low risk profile when scattered. Nodular drusen were seen to extend root-like processes into BrM,[4] unlike BLinD that detach or accumulate to promote type 1 MNV. Risk alleles of complement genes affect biogenesis of nodular drusen, which are numerous in the equatorial retina.[20, 21] In the macula, variants in CFH, fibulin 5, and fibulin 3 genes manifest clinically as cuticular drusen.[22–24] Cross-sectional classic twin studies have shown that small hard drusen are highly heritable.[25, 26] In 138 twins of 69 pairs, the 20-year incidence of developing ≥20 small hard drusen and drusen ≥63 μm was 5.6% and 13.1%, respectively.[5] This study also reported that unshared environmental effects (smoking, diet, lifestyle, physical activity) explained one-half of incident larger drusen (that will enlarge over time), with aging and smoking equal in impact to genetics.[5] Numerous complement genes are strongly expression in cells of the choroid,[27, 28] and drusen themselves are notably immunoreactive for complement proteins. These genes may regulate outflow in the successive barrier model (above) as well as response by resident and transient immune cells of the choroid.

In contrast to pan-retinal nodular drusen, high-risk soft drusen are dominated by one lipid-rich component and localize to central retina.[7, 29] The high metabolic demand of foveal cone vision, as well as delivery of xanthophyll carotenoid pigments through the RPE, leads to a waste disposal burden on underlying microvasculature that further impedes transfer. This model is supported by the extraordinary concentration of progression risk in the central subfield of the ETDRS grid, appearance of soft drusen material preferentially under the fovea,[13] marked accumulation of esterified cholesterol (a lipoprotein core lipid) in normal aged BrM, fatty acid composition of extracted lipids implicating diet, RPE expression of hallmark genes, and release of apolipoproteins and lipids by highly differentiated RPE in culture. Possibly indocyanine green angiography can reveal this process *in vivo*.[30] Sub-RPE-BL lipid blocks transit of the dye from choroid for uptake by RPE where it is normally visualized. Indeed, soft drusen are hypofluorescent for indocyanine green, and in contrast to nodular drusen, which are hyperfluorescent.

Our data showing an internal transition within cuticular drusen expand this model. The drusen may represent another matrix that can retain lipoproteins. Once trapped, component lipids can become peroxidized, pro-inflammatory, and pro-angiogenic.[31, 32] This process may be accelerated by a layer of mitochondria at the base of the RPE, generating reactive oxygen species at close range. The model in Figure 1 is informed by research on atherosclerotic arterial intima, where lipoproteins also accumulate extravascularly with untoward consequences. This prior work defined multiple physical forms resulting from two chemical forms of cholesterol (unesterified and esterified), solubilized with different proportions of phospholipid.[33] These physical forms differentially distribute in AMD lesions: crystals found in fluid and fibrosis, droplets and liposomes in drusen, BLinD, and BLamD, and lakes (pooled lipid) in drusen and BLinD.[34, 35] Among other factors, reflectivity in OCT involves light scattering from lipid–water interfaces in a size-dependent manner. Thus, it is possible that a transition to a lipid-rich interior, as in cuticular drusen

(Figure 4), may herald not only increased progression risk but also improved *in vivo* visibility, in advanced OCT technologies.

Study strengths include histologic material that validated observations in a large sample with cases of clinically documented FA, high-resolution microscopy, and a well-founded model of drusen formation. Limitations are the small numbers of drusen overall and lack of ultrastructural studies of sources 1–2 to address a compositional transition like that seen in source 3 (Figure 4). Our hypotheses about drusen biogenesis (Figure 1) do not exclude other mechanisms for lipid release (lipoproteins of other sizes, exosomes, extracellular vesicles, shed membranes, organelle fragments) that may occur at specific stages of health and disease. Our intent was to translate cellular and subcellular details from histology, gathered with clinical imaging in mind, into clinical decision-making and trial design. Looking forward, drusen composition and lifecycle can be deciphered in dye angiography data sets in major referral centers and clinical trials that evaluate neovascularization. Multimodal imaging of drusen with widely used non-invasive techniques also remains valuable.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AMD	Age-related macular degeneration
BLamD	basal laminar deposit
BLinD	basal linear deposit
Bmound	basal mound
BrM	Bruch’s membrane
CFP	Color fundus photography

FA	fluorescein angiography
IQR	interquartile range
MNV	macular neovascularization
OCT	optical coherence tomography
RPE	retinal pigment epithelium
RPE+BL-BrM	RPE-basal lamina-Bruch's membrane

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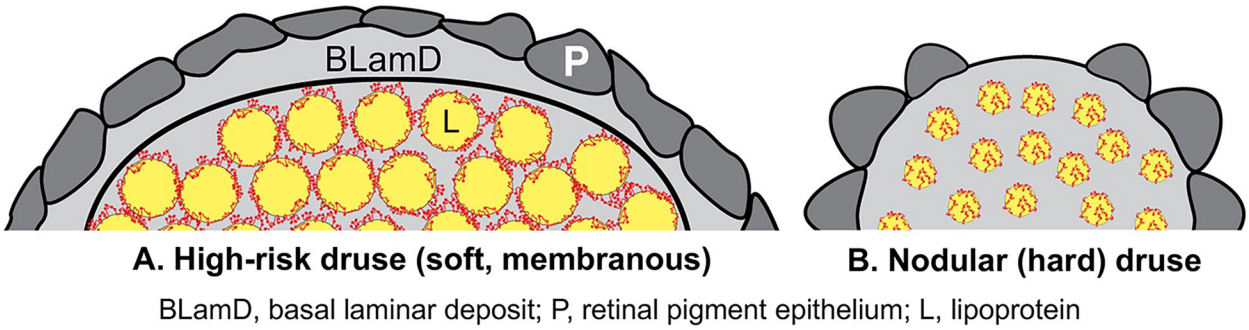

Figure 1. How lipoprotein particles in drusen may impact fluorescein angiography

Drusen terms: Soft and hard drusen are color fundus photography terms whose applicability to OCT is under investigation. Nodular drusen are the histological correlates of hard drusen. The term “membranous” was based the observation of abundant membrane-like structures in soft drusen on electron microscopy. These were later proposed to represent lipoprotein particles at different degrees of aggregation and preservation quality. Herein we retain the term “soft.” *Lipoprotein terms:* Lipoprotein particles with apolipoprotein B (e.g., intestinal chylomicrons and hepatic VLDL) and milk fat globules (lacking apolipoproteins) are the only mechanisms to release non-polar lipids (triacylglycerols, esterified cholesterol) from cells. Atherogenic LDL (enriched in esterified cholesterol) arises from enzymatic remodeling of VLDL in circulation. High levels and specifically localized esterified cholesterol in normal aged Bruch’s membrane and drusen, with RPE expression of hallmark genes, implies intraocular lipoprotein particles of unique composition as a major biogenesis mechanism. *Model for fluorescein angiography:* We propose that the esterified and unesterified cholesterol content of drusen can be largely accounted for by the relative size and packing of apolipoprotein B, E containing lipoprotein particles, based on morphologic and extraction studies of micro-dissected drusen. Soft drusen have large, closely packed particles (panel A). Nodular drusen have small particles interspersed by material rich in proteins and carbohydrates (panel B). For illustration, only apolipoprotein B (of several apolipoproteins) is shown on particle surfaces, and particles are drawn larger than scale. For reference, particles in nodular drusen are ~80 nm in diameter and may be larger in soft drusen. Isolated soft drusen were seen to have higher RPE coverage of their surface than nodular drusen. Loss or retraction of RPE at nodular drusen apices may result from strain imposed the small radius of curvature.

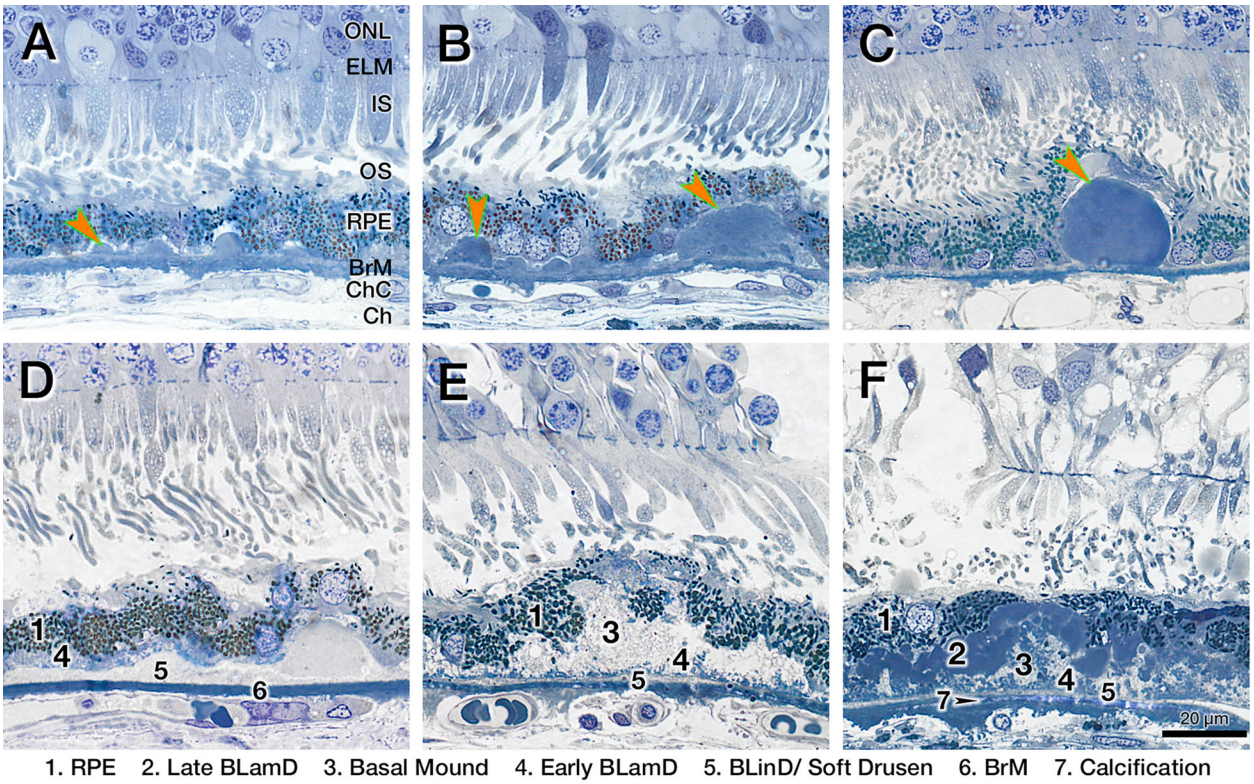

Figure 2. Small deposits elevating retinal pigment epithelium in aging and AMD

A,B,C. Nodular drusen (orange arrowheads) between RPE-BL and inner collagenous layer of BrM are solid, globular, and uniformly stained with toluidine blue. They lack overlying basal laminar deposit (BLamD) and basal mounds. **D,E,F.** Soft drusen material is granular and light grayish brown. BLamD, thickening of extracellular matrix, between RPE and its native BL. **D.** RPE is elevated by small soft drusen and overlying BLamD. **E.** RPE is elevated by a basal mound, i.e., soft drusen material within BLamD. **F.** Most of the RPE elevation is due to late BLamD, i.e., dark blue material that is concave towards BrM. Early BLamD is bright blue palisades close to BrM. Basal mound separates early and late BLamD.

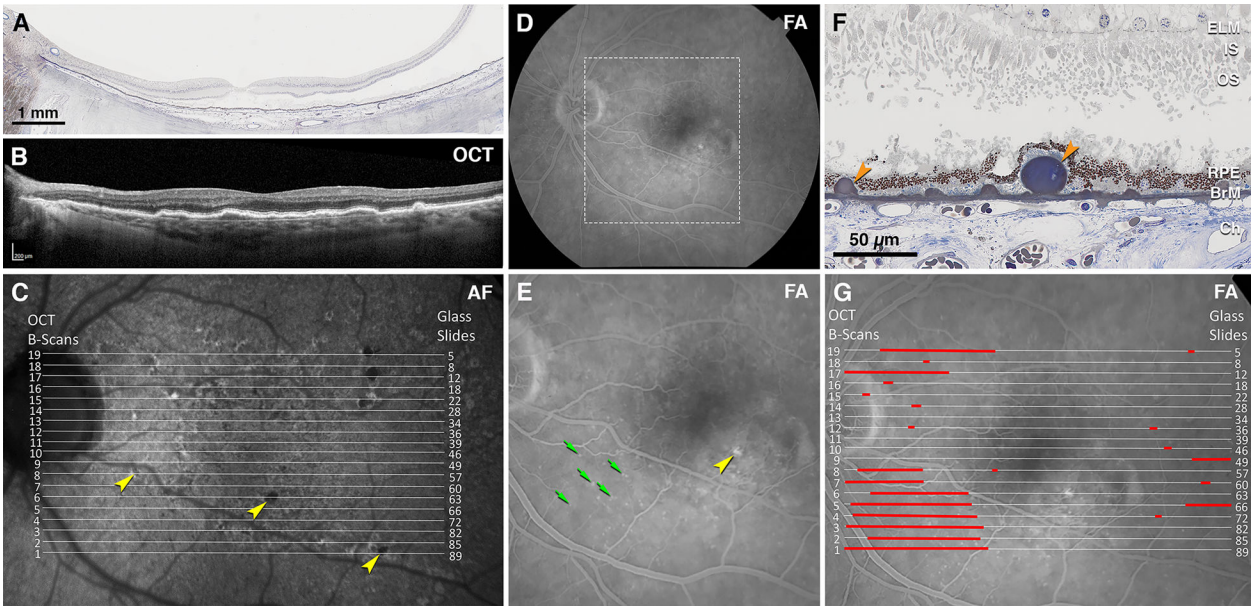

Figure 3. Identifying nodular drusen stained by fluorescein angiography

A 79-year-old woman with bilateral AMD was followed for 11 years before her death. **A, B.** Histologic section and corresponding optical coherence tomography (OCT) B-scan, with shallow irregular RPE elevation harboring non-exudative type 1 macular neovascularization. **C.** Fundus autofluorescence image with an overlay showing OCT B-scans, 43 months before death. Corresponding glass slides for histology indicated. Three atrophic spots (arrowheads) were used for registration. **D, E.** Low and high magnification views of late-phase fluorescein angiography, with drusen indicated (green arrows), at presentation, 11 years before death. Yellow arrowhead, middle of 3 atrophic spots in panel C. **F.** Histologic sections through arrowed areas of E show nodular drusen. **G.** Locations of nodular drusen (red bars) used for quantitative analysis.

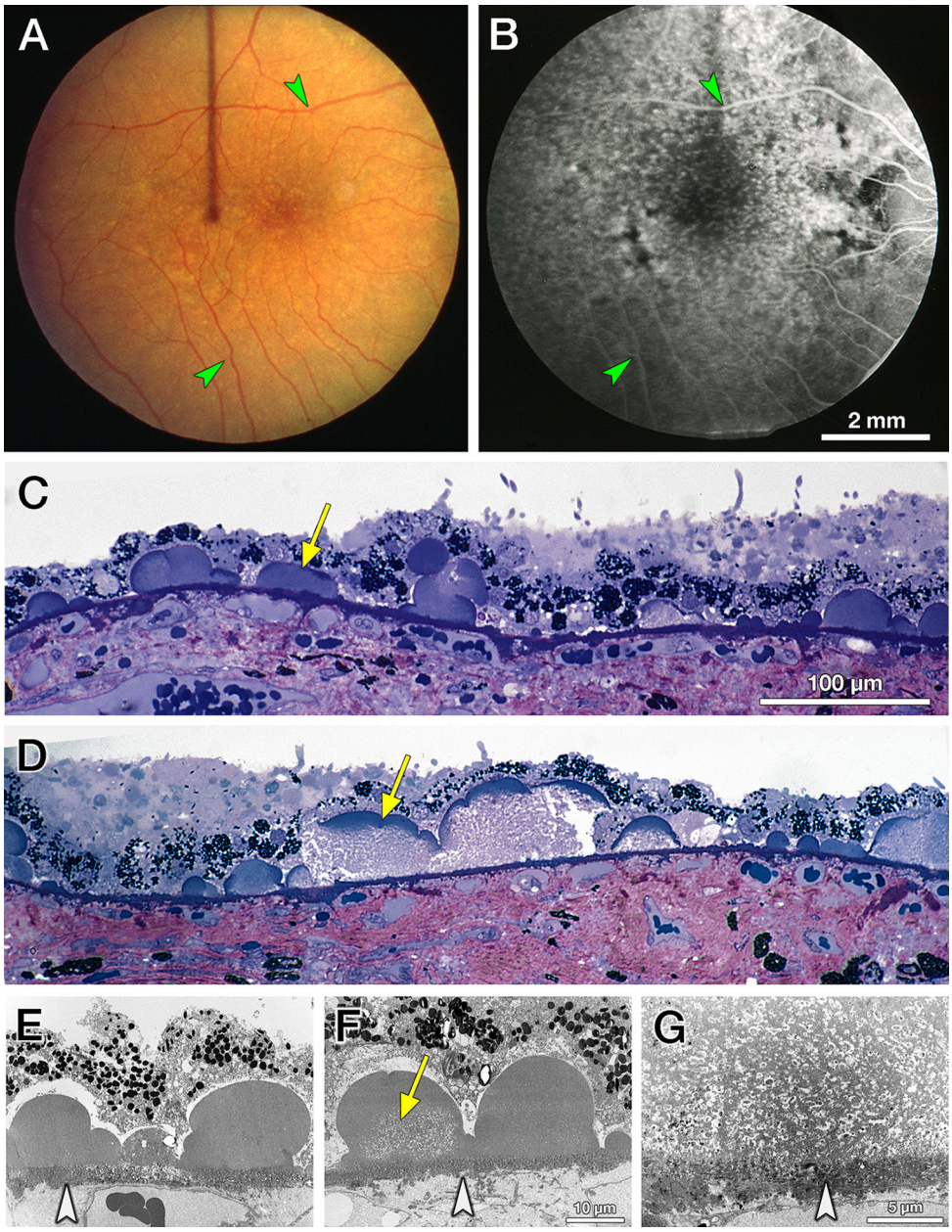

Figure 4. Cuticular drusen in clinical imaging, histology, and electron microscopy

61-year-old man with bilateral cuticular drusen. **A,B.** Green arrowheads indicate the same locations in both fundus images. **A.** Color fundus photograph shows numerous small yellow dots. **B.** Fluorescein angiography shows numerous small puncta of staining. **C, D.** Semi-thin sections stained with methylene blue and basic fuchsin; scale bar in C applies to D. Numerous small drusen (arrow) have a solid homogeneous appearance, little basal laminar deposit, and intact choriocapillaris. **D.** Larger confluent deposits are apparent, in addition to small drusen. Exteriors of the confluent deposits (arrow) resemble the drusen of panel C in texture and coloration. Interiors are granular and stain pink rather than blue. **E, F, G.** Transmission electron microscopy of cuticular drusen. White arrowhead indicates Bruch's membrane. Scale bar in F applies to E and F. **E.** Drusen like those in C are moderately

electron-dense, with homogenous interiors and lacking basal laminar deposits. **F.** One small druse, apparently in transition, has a subregion of less electron-dense puncta (arrow) in the interior. **G.** Interior of druse like those in D has homogeneous background with multiple scattered electron-dense globules, presumed to be lipid-containing.

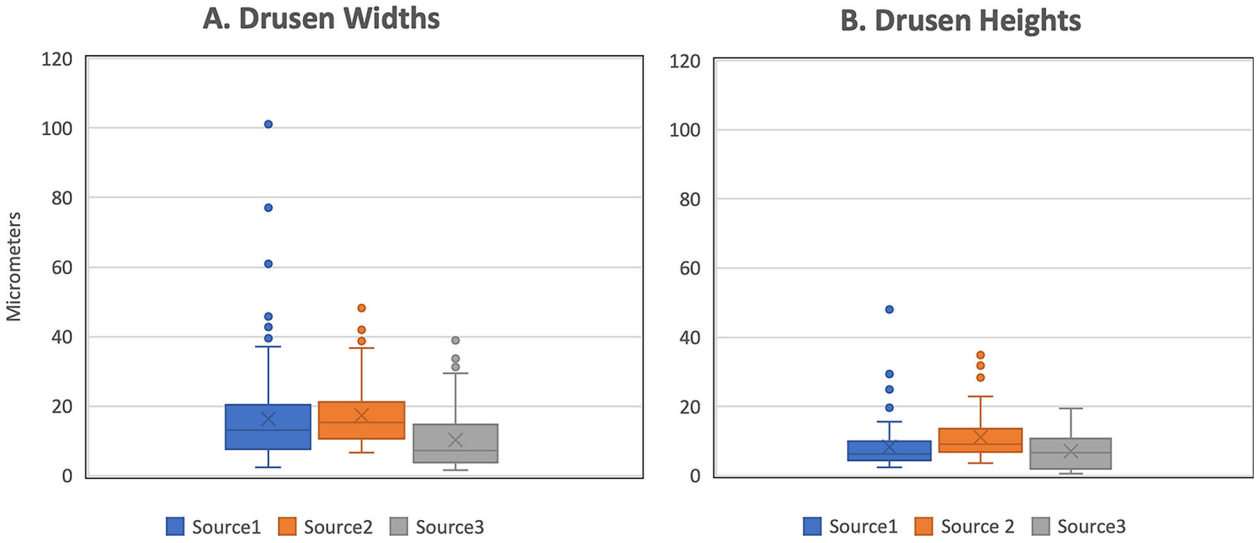

Figure 5. Box plot of nodular and cuticular drusen dimension in three datasets

A. Drusen base widths in tissue sources 1–3. **B.** Maximum drusen heights in tissue sources 1–3, Source 1, 43 clinically undocumented eyes, 132 nodular drusen; Source 2, 1 eye with non-exudative type 1 macular neovascularization secondary to AMD, 87 nodular drusen; Source 3, 1 clinically documented eye, 78 cuticular drusen.

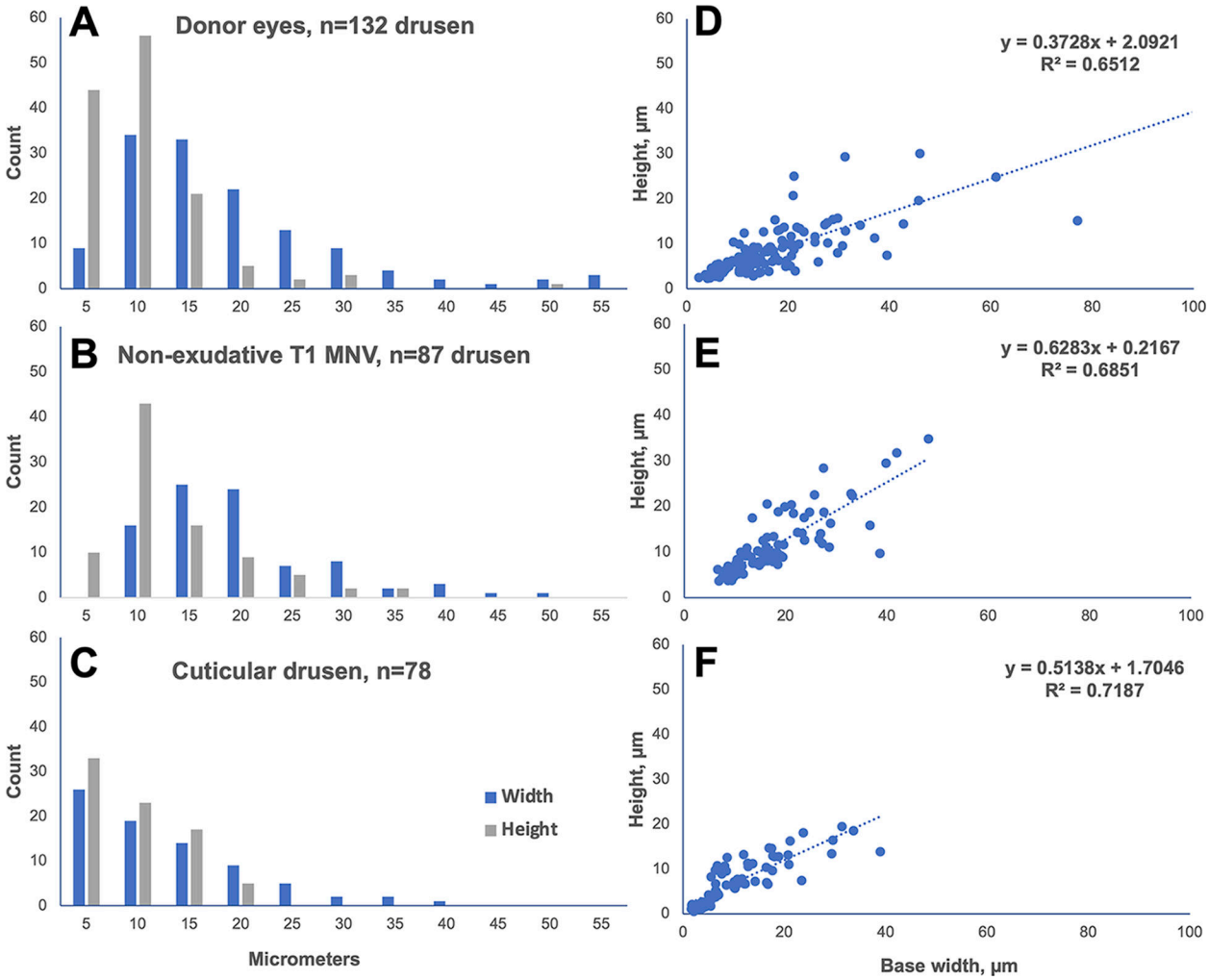

Figure 6. Nodular and cuticular drusen widths and heights are correlated.

A, D. Base widths and maximum heights of nodular drusen (**A**) and correlation between height and width (**D**), in Source 1 (43 clinically undocumented donor eyes). **B, E.** Base widths and maximum heights of nodular drusen (**B**) and correlation between height and width (**E**), in Source 2 (one eye with non-exudative type 1 macular neovascularization secondary to AMD). **C, F.** Base widths and maximum heights of clinically documented cuticular drusen (**C**) and correlation between height and width (**D**), in one eye (Source 3). Measurements in panels A-B were made using light microscopy of sub-micrometer semithin sections. Measurements in panel C were made using non-montaged transmission electron micrographs.