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Choroidal and Sub-Retinal Pigment Epithelium Caverns

Multimodal Imaging and Correspondence with Friedman Lipid Globules

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Purpose: To survey Friedman lipid globules by high-resolution histologic examination and to compare with multimodal imaging of hyporeflective caverns in eyes with geographic atrophy (GA) secondary to age-related macular (AMD) and other retinal diseases.

Design: Histologic survey of donor eyes with and without AMD. Clinical case series with multimodal imaging analysis.

Participants: Donor eyes (n = 139; 26 with early AMD, 13 with GA, 40 with nAMD, 52 with a healthy macula, and 8 with other or unknown characteristics) and 41 eyes of 28 participants with GA (n = 16), nAMD (n = 8), Stargardt disease (n = 4), cone dystrophy (n = 2), pachychoroid spectrum (n = 6), choroidal hemangioma (n = 1), and healthy eyes (n = 4).

Methods: Donor eyes were prepared for macula-wide epoxy resin sections through the foveal and perifoveal area. In patients, caverns were identified as nonreflective spaces on OCT images. Multimodal imaging included color and red-free fundus photography; fundus autofluorescence; fluorescein and, indocyanine green angiography; OCT angiography; near-infrared reflectance; and confocal multispectral (MultiColor [Spectralis, Heidelberg Engineering, Germany]) imaging.

Main Outcome Measures: Presence and morphologic features of globules, and presence and appearance of caverns on multimodal imaging.

Results: Globules were found primarily in the inner choroidal stroma (91.0%), but also localized to the sclera (4.9%) and neovascular membranes (2.1%). Mean diameters of solitary and multilobular globules were 58.9±37.8 μm and 65.4±27.9 μm, respectively. Globules showed morphologic signs of dynamism including pitting, dispersion, disintegration, and crystal formation. Evidence for inflammation in the surrounding tissue was absent. En face OCT rendered sharply delimited hyporeflective areas as large as choroidal vessels, frequently grouped around choroid vessels or in the neovascular tissue. Cross-sectional OCT revealed a characteristic posterior hypertransmission. OCT angiography showed absence of flow signal within caverns.

Conclusions: Based on prior literature documenting OCT signatures of tissue lipid in atheroma and nAMD, we speculate that caverns are lipid rich. Globules, with similar sizes and tissue locations in AMD and healthy persons, are candidates for histologic correlates of caverns. The role of globules in chorioretinal physiologic features, perhaps as a lipid depot for photoreceptor metabolism, is approachable through clinical imaging. *Ophthalmology 2018;■:1–15 © 2018 Published by Elsevier Inc. on behalf of the American Academy of Ophthalmology*

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Our understanding of the choroid's role in health and disease has expanded greatly thanks to clinical imaging technologies based on OCT, especially with techniques such as enhanced depth imaging, en face rendering of chorioretinal structure, swept-source OCT, and OCT angiography (OCTA). Information from spectral-domain (SD) OCT and from angiography (dye and OCT based) together generated many new concepts, including the topography[1] and diurnal variation of choroidal thickness,[2] to name just a few. Furthermore, information from a multimodal imaging approach now can be merged to bring the advantages of each individual technique to bear on a single question, resulting in a more comprehensive understanding. Such capability is important for exploring the multiple cell types and functions represented in the choroidal ecosystem.

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Histopathologic examination has revealed aspects of choroidal biology features that remain to be explored in imaging. In 1934, Jaensch[3] demonstrated that fatty deposits were present in choroidal stroma of eyes of various ages and disease states. In 1966, Friedman and Smith[4] described and illustrated extracellular globules of sudanophilic and osmophilic lipid in a large series of postmortem eyes, many of which were healthy. In all eyes, choroidal stroma surrounding the globules lacked signs of inflammation.

Clinically, in the choroid of eyes with geographic atrophy (GA) secondary to age-related macular degeneration (AMD), Querques et al[5] described caverns identified by SD OCT, OCTA, and indocyanine green angiography (ICGA). Caverns were defined as hyporeflective, angular-to-round, empty features resembling cavities with punctate or linear internal hyperreflectivity, variably localized in the Sattler and Haller layers, with relative preservation of the choriocapillaris. Because of the absence of a hyperreflective boundary, hyperfluorescence on ICGA, and flow signal on OCTA, caverns were suggested to be "non-perfused ghost vessels and stromal pillars."[5] This hypothesis was based on histologic studies[6,7] demonstrating that choriocapillaries can lose functional endothelium and become ghost vessels, that is, spaces between the intercapillary pillars of Bruch's membrane. These spaces in turn may fill with macrophages removing dead endothelium. To explain the appearance of caverns, the authors suggested that similar changes occur in the larger vessels of the Sattler and Haller layers, with the internal punctate hyperreflectivity corresponding to cells. These investigators subsequently described choroidal round hyporeflectivities,[8] which were similar in size to choroidal caverns, yet distinct because of hyperreflective borders and a consistent round shape, and suggested these as nonperfused or hypoperfused vessels, single large cells, or cavern precursors. Among the explanations for choroidal round hyporeflectivities on SD OCT, the authors also included Friedman lipid globules, citing a preliminarily presented survey of these features.[9]

Independently, we observed SD OCT signatures similar to caverns[5] not only in the choroid of several retinal conditions, but also in the sub-retinal pigment epithelium (RPE) space in neovascular AMD (nAMD). We thus investigated multimodal imaging features of both choroidal and sub-RPE nonreflective spaces together, hypothesizing that they were a single entity: caverns. From donor eyes, we determined disease associations, prevalence, tissue localization, and morphologic features of globules in the maculas of AMD and age-matched control eyes. We found that the imaging characteristics and tissue localization of caverns could be explained well by histologic globules, which in turn seem to be part of normal chorioretinal physiologic characteristics.

Methods

Compliance

This observational, consecutive case series was approved by the Western Institutional Review Board in New York and the institutional review board at the University of Milan. The laboratory histopathologic study was approved by the institutional review board at the University of Alabama at Birmingham. The research adhered to the tenets of the Declaration of Helsinki and complied with the Health Insurance Portability and Accountability Act.

Histologic Examination

We used Project MACULA (available at project-macula.cs.uab.edu), a National Eye Institute- and foundation-funded online resource of human AMD histologic results.[10] To create Project MACULA, high-resolution histologic results of 139 maculae were surveyed systematically and documented photographically. Eyes were accessioned for research purposes from nondiabetic white donors to the Alabama Eye Bank from 1996 through 2012. Median death-to-preservation time was 3 hours and 49 minutes (range, 40 minutes−11 hours and 40 minutes). Ophthalmic health records were not available for most donors. Eyes were preserved by immersion in 1% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer after anterior segment excision. Although ex vivo color photography and SD OCT were performed, caverns were not visible, likely because of incomplete light penetrance through edematous retina.

Tissue punches 8 mm in diameter and containing the fovea and the temporal portion of the optic nerve head were postfixed by osmium tannic acid paraphenylenediamine to accentuate extracellular lipid. They were embedded in epoxy resin (PolyBed 812; Polysciences, Warrington, PA) for 0.8-μm thick sections that were stained with 1% toluidine blue for polychromaticity.[11] At 2 levels (fovea and perifovea 2 mm superior to the foveal center), sections were scanned with a ×40 objective (numerical aperture, 0.95) for systematic review, annotation, and layer thickness measurements. One tissue block was resectioned horizontally to provide a view comparable with en face SD OCT. Several eyes were sectioned at silver-gold thickness and viewed with a transmission electron microscope (1200 EXII [JEOL USA, Peabody, MA]; AMTXR-40 camera [Advanced Microscopy Techniques, Danvers, MA]).

As described,[12] AMD cases were defined via histopathologic results as eyes with the presence of 1 large druse (>125 μm in diameter) in the macula or severe RPE changes in the setting of at least 1 druse or continuous basal linear deposit, with or without the presence of neovascularization and its sequelae. Eyes with GA showed at least 1 region 250 μm in diameter lacking a continuous RPE layer (but possibly containing so-called dissociated RPE). Unremarkable eyes were those lacking characteristics of AMD or other chorioretinal disease as discernible in either histologic or ex vivo imaging; these served as comparison eyes.

At each location in a standard grid overlaid on the foveal and perifoveal cross sections, thicknesses were measured and morphologic features were indicated from a layer-specific drop-down menu with a field for free comments (M&A, a custom ImageJ plug-in; available at https://fiji.sc/). Thus, the globule encounter rate is an unbiased estimate of tissue prevalence in these eyes. At the same time, sections were imaged with a light microscope (Nikon Eclipse; Nikon, Melville, NY), a ×60 oil-immersion objective (numerical aperture, 1.4), and digital camera (XC10; Olympus, Tokyo, Japan). From these images, some of which were obtained outside the systematic sampling locations, 117 globules were chosen for morphologic description and diameter measurements (ImageJ; available at https://fiji.sc/). In creating figures, digital light and electron microscopic images were adjusted for exposure, contrast, sharpness, and white balance (Photoshop CS6; Adobe, San Jose, CA).

Clinical Imaging

The clinical study was conducted between June 2016 and December 2016 at 2 different settings: Vitreous Retina Macula