Biometrics, impact, and significance of basal linear deposit and subretinal drusenoid deposit in age-related macular degeneration

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Supplementary material – Methods and Supplementary Figure 1

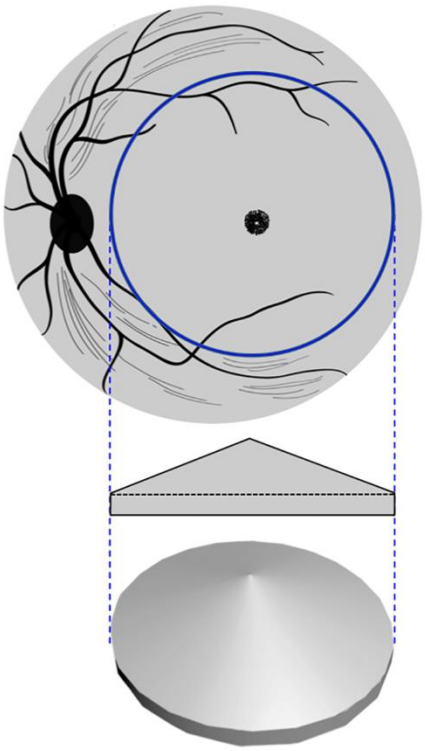
Histologic preparation

Eyes were accessioned from non-diabetic white donors to Advancing Sight Network (formerly the Alabama Eye Bank, Birmingham AL USA). Ophthalmic histories were not available for most donors. Eyes were collected from 1995-2012 and retained in fixative. Eyes with drusen are underrepresented, because some specimens were removed for other studies.[53] All eyes were preserved in 1% paraformaldehyde and 2.5% glutaraldehyde in a 0.1M phosphate buffer with an average death-to-preservation time of 3:49 (range 0:40-11:40).

Macula-wide, full-thickness tissue punches were obtained with an 8.25 mm diameter trephine (#68825-L, Howard Instruments, Tuscaloosa AL). Punches were post-fixed and embedded in epoxy resin (PolyBed 812, Polysciences, Warrington PA), tissues were sectioned at 0.8 µm starting at the superior edge of the punch, as indexed by the microtome counter, and stained with toluidine blue. Sections were saved at ~2 mm from this starting point to capture the rise of rod density in superior perifovea. More sections were saved at ~4 mm from the top to capture the rod-free zone. Sections at each of these two locations (Superior and Central) were scanned in their entirety and digitized using image stitching software (CellSens, Olympus).

Histologic diagnosis

Early-intermediate AMD was defined histologically[18] as the absence of MNV and its sequelae, AND the presence of one large druse (>125 µm in diameter) OR severe RPE dysmorphia in the setting of drusen or continuous BLinD. Control eyes did not meet criteria for AMD and lacked histologic evidence for other chorioretinal pathology.



Supplementary Figure 1. Geometric model of basal linear deposits (BLinD).

Top, in a 6 mm diameter foveal-centered area corresponding to the ETDRS grid, thicknesses of BLinD were measured at pre-determined locations in high-resolution histological sections. Middle and bottom, BLinD is thick at the foveal center and thin (but non-zero) at the macular edge. It was thus modeled as a low conical solid of rotation on top of a circular disk of uniform thickness. The height and slope of the surface of the cone was determined by fitting regression lines to the distribution of thicknesses as a function of eccentricity, as described in the Methods.