Curcio, C. A. Soft drusen in age-related macular degeneration: biology and targeting, via the Oil Spill Strategy.
Supplementary Material

Supplementary Table 1: Drusen components, by retinal region		
Component	**Abundance**	**Region examined**
Membranous debris; suggested as lipoprotein-derived debris	Soft drusen, BLinD	Macula [1-6]
EC, UC, phospholipid, unspecified oil red O binding lipid; attributed to lipoprotein particles	All drusen (filipin); most drusen (oil red O); >40% of hard druse volume; EC pools in soft drusen	Unspecified; [7] Macula; [8] Macula vs Periphery; [9, 10] Periphery [11]
Modified lipids (7-ketocholesterol, isolevuglandin)	All drusen	Periphery; [12] Macula [13]
Apolipoproteins (apoB, A-I, C-I, E)	60-100% of hard drusen; higher rates in periphery than macula	Macula; [14] Unspecified;[15] Macula vs Periphery; [10] Periphery [16]
Melanin/ lipofuscin granules	6% of hard and soft drusen	Macula vs Periphery [9]
Cells (dendritic, others)	3-6% of hard drusen only	Macula vs Periphery; [9] Unspecified [17]
Refractile hydroxyapatite spherules (calcium phosphate); also called amyloid vesicles (0.25 -10 μm)	2% of hard drusen, 40% of compound drusen, frequent in eyes with many drusen, some AMD eyes; 43% of macular hard drusen, 1.6% of soft drusen, 2% of peripheral hard drusen by light microscopy; all drusen by specific label	Periphery; [11] Macula+Periphery; [9] Macula; [18] Unspecified [19]
Advanced glycation end-products (AGE; pentosidine, carboxymethyl lysine)	Prominent	Macula; [20] Unspecified; [21]
Non-fibrillar amyloid	Prevalence n.a.	Macula [22]
Clusterin	All drusen	Macula [23] Periphery; [24]
TIMP3	All drusen	Unspecified; [25, 26] Periphery [24]
Vitronectin	All drusen	Periphery; [24] Unspecified [27]
C-reactive protein	Some drusen	Unspecified; [26, 28] Macula [29]
Complement factor H, C3 fragments, C5	Many pathway components seen	Unspecified [27, 28, 30, 31] Macula; [29] Periphery [24]

Membrane attack complex (C5b-9)	Terminal step of complement activation	Macula (hard drusen); [32-35] Unspecified [26, 30, 36]
RGR-d	All drusen	Macula [37]
αA- and αB-crystallin	N.A.; higher in BrM, more in AMD drusen	Macula+Periphery [38] Macula+Periphery [39]
Ubiquitin	Most drusen in most eyes	Unspecified [40, 41]
Carbohydrates	All drusen	Unspecified [42]
Zinc	Many drusen	Macula+Periphery [43, 44]
Iron	Many drusen	Macula+Periphery [44]
Exosome markers CD63, CD81, and LAMP2	N.A.	Unspecified [33]
Bestrophin, membrane-bound	N.A.	Unspecified [45]

Notes:
Retinal regions, Macula – only Macula was studied; Periphery – only Periphery was studied; Macula vs Periphery – both regions were studied and compared; Macula+Periphery – drusen from both regions were both studied and reported together; Unspecified, not indicated and not determinable from illustrations.
Localization methods, immunohistochemistry, histochemistry, immuno-gold transmission electron microscopy; Direct assays, proteomics, western blot, microprobe synchrotron X-ray fluorescence for zinc; N.A. not available; Varying estimates of druse components are due to differences in location of samples and druse types examined. "All drusen" means "all drusen sampled."

1. Sarks JP, Sarks SH, Killingsworth MC. Evolution of geographic atrophy of the retinal pigment epithelium. *Eye* 1988;2:552-577.
2. Sarks JP, Sarks SH, Killingsworth MC. Evolution of soft drusen in age-related macular degeneration. *Eye* 1994;8:269-283.
3. Sarks SH, Arnold JJ, Sarks JP, Gilles MC, Walter CJ. Prophylactic perifoveal laser treatment of soft drusen. *Aust N Z J Ophthalmol* 1996;24:15-26.
4. Curcio CA, Messinger JD, Sloan KR, McGwin G, Jr, Medeiros NE, Spaide RF. Subretinal drusenoid deposits in non-neovascular age-related macular degeneration: morphology, prevalence, topography, and biogenesis model. *Retina* 2013;33:265-276.

5. Pilgrim MG, Lengyel I, Lanzirotti A, et al. Sub-retinal pigment epithelial deposition of drusen components including hydroxyapatite in a primary cell culture model. *Invest Ophthalmol Vis Sci* 2017;58:708–719.
6. Balaratnasingam C, Cherepanoff S, Dolz-Marco R, et al. Cuticular drusen: clinical phenotypes and natural course defined using multimodal imaging. *Ophthalmology* 2018;125:100-118.
7. Farkas TG, Sylvester V, Archer D, Altona M. The histochemistry of drusen. *American Journal of Ophthalmology* 1971;71:1206-1215.
8. Pauleikhoff D, Zuels S, Sheraidah GS, Marshall J, Wessing A, Bird AC. Correlation between biochemical composition and fluorescein binding of deposits in Bruch's membrane. *Ophthalmol* 1992;99:1548-1553.
9. Rudolf M, Clark ME, Chimento M, Li C-M, Medeiros NE, Curcio CA. Prevalence and morphology of druse types in the macula and periphery of eyes with age-related maculopathy. *Invest Ophthalmol Vis Sci* 2008;49:1200-1209.
10. Malek G, Li C-M, Guidry C, Medeiros NE, Curcio CA. Apolipoprotein B in cholesterol-containing drusen and basal deposits in eyes with age-related maculopathy. *Am J Pathol* 2003;162:413-425.
11. Anderson DH, Talaga KC, Rivest AJ, Barron E, Hageman GS, Johnson LV. Characterization of beta amyloid assemblies in drusen: the deposits associated with aging and age-related macular degeneration. *Exp Eye Res* 2004;78:243-256.
12. Rodriguez IR, Clark ME, Lee JW, Curcio CA. 7-ketocholesterol accumulates in ocular tissues as a consequence of aging and is present in high levels in drusen. *Exp Eye Res* 2014;128:151-155.
13. Charvet CD, Saadane A, Wang M, et al. Pretreatment with pyridoxamine mitigates isolevuglandin-associated retinal effects in mice exposed to bright light. *J Biol Chem* 2013;288:29267-29280.
14. Klaver CC, Kliffen M, van Duijn CM, et al. Genetic association of apolipoprotein E with age-related macular degeneration. *Am J Hum Genet* 1998;63:200-206.
15. Anderson DH, Ozaki S, Nealon M, et al. Local cellular sources of apolipoprotein E in the human retina and retinal pigmented epithelium: implications for the process of drusen formation. *Am J Ophthalmol* 2001;131:767-781.
16. Li C-M, Clark ME, Chimento MF, Curcio CA. Apolipoprotein localization in isolated drusen and retinal apolipoprotein gene expression. *Invest Ophthalmol Vis Sci* 2006;47:3119-3128.
17. Hageman GS, Luthert PJ, Chong NHC, Johnson LV, Anderson DH, Mullins RF. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. *Progr Ret Eye Res* 2001;20:705-732.
18. Dentchev T, Milam AH, Lee VM, Trojanowski JQ, Dunaief JL. Amyloid-beta is found in drusen from some age-related macular degeneration retinas, but not in drusen from normal retinas. *Mol Vis* 2003;9:184-190.
19. Thompson RB, Reffatto V, Bundy JG, et al. Identification of hydroxyapatite spherules provides new insight into subretinal pigment epithelial deposit formation in the aging eye. *Proc Natl Acad Sci U S A* 2015;112:1565-1570.

20. Ishibashi T, Murata T, Hangai M, et al. Advanced glycation end products in age-related macular degeneration. *Arch Ophthalmol* 1998;116:1629-1629.
21. Handa JT, Verzijl N, Matsunaga H, et al. Increase in the advanced glycation end product pentosidine in Bruch's membrane with age. *Invest Ophthalmol Vis Sci* 1999;40:775-779.
22. Luibl V, Isas JM, Kayed R, Glabe CG, Langen R, Chen J. Drusen deposits associated with aging and age-related macular degeneration contain nonfibrillar amyloid oligomers. *J Clin Invest* 2006;116:378-385.
23. Sakaguchi H, Miyagi M, Shadrach KG, Rayborn ME, Crabb JW, Hollyfield JG. Clusterin is present in drusen in age-related macular degeneration. *Exp Eye Res* 2002;74:547-549.
24. Wang L, Clark ME, Crossman DK, et al. Abundant lipid and protein components of drusen. *PLoS ONE* 2010;5:e10329.
25. Fariss RN, Apte SS, Olsen BR, Iwata K, Milam AH. Tissue inhibitor of metalloproteinases-3 is a component of Bruch's membrane of the eye. *Am J Pathol* 1997;150:323-328.
26. Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. *FASEB J* 2000;14:835-846.
27. Hageman GS, Mullins RF, Russell SR, Johnson LV, Anderson DH. Vitronectin is a constituent of ocular drusen and the vitronectin gene is expressed in human retinal pigmented epithelial cells. *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 1999;13:477-484.
28. Anderson DH, Mullins RF, Hageman GS, Johnson LV. A role for local inflammation in the formation of drusen in the aging eye. *Am J Ophthalmol* 2002;134:411-431.
29. Bhutto IA, Baba T, Merges C, Juriasinghani V, McLeod DS, Lutty GA. C-reactive protein and complement factor H in aged human eyes and eyes with age-related macular degeneration. *British Journal of Ophthalmology* 2011;95:1323-1330.
30. Johnson LV, Ozaki S, Staples MK, Erickson PA, Anderson DH. A potential role for immune complex pathogenesis in drusen formation. *Experimental Eye Research* 2000;70:441-449.
31. Anderson DH, Radeke MJ, Gallo NB, et al. The pivotal role of the complement system in aging and age-related macular degeneration: hypothesis re-visited. *Prog Retin Eye Res* 2010;29:95-112.
32. Seth A, Cui J, To E, Kwee M, Matsubara J. Complement-associated deposits in the human retina. *Invest Ophthalmol Vis Sci* 2008;49:743-750.
33. Wang AL, Lukas TJ, Yuan M, Du N, Tso MO, Neufeld AH. Autophagy and exosomes in the aged retinal pigment epithelium: possible relevance to drusen formation and age-related macular degeneration. *PLoS ONE* 2009;4:e4160.
34. Mullins RF, Schoo DP, Sohn EH, et al. The membrane attack complex in aging human choriocapillaris: relationship to macular degeneration and choroidal thinning. *Am J Pathol* 2014;184:3142-3153.

35. Chirco KR, Scott Whitmore S, Wang K, et al. Monomeric C-reactive protein and inflammation in Age-related Macular Degeneration. *J Pathol* 2016.
36. Gerl VB, Bohl J, Pitz S, Stoffelns B, Pfeiffer N, Bhakdi S. Extensive deposits of complement C3d and C5b-9 in the choriocapillaris of eyes of patients with diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2002;43:1104-1108.
37. Kochounian H, Johnson LV, Fong HK. Accumulation of extracellular RGR-d in Bruch's membrane and close association with drusen at intercapillary regions. *Exp Eye Res* 2009;88:1129-1136.
38. Crabb JW, Miyagi M, Gu X, et al. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. *Proc Natl Acad Sci U S A* 2002;99:14682-14687.
39. Nakata K, Crabb JW, Hollyfield JG. Crystallin distribution in Bruch's membrane-choroid complex from AMD and age-matched donor eyes. *Exp Eye Res* 2005;80:821-826.
40. Loeffler KU, Mangini NJ. Immunolocalization of ubiquitin and related enzymes in human retina and retinal pigment epithelium. *Graefes Arch Clin Exp Ophthalmol* 1997;235:248-254.
41. Leger F, Fernagut PO, Canron MH, et al. Protein aggregation in the aging retina. *J Neuropathol Exp Neurol* 2011;70:63-68.
42. Mullins RF, Hageman GS. Human ocular drusen possess novel core domains with a distinct carbohydrate composition. *J Histochem Cytochem* 1999;47:1533-1539.
43. Lengyel I, Flinn JM, Peto T, et al. High concentration of zinc in sub-retinal pigment epithelial deposits *Exp Eye Res* 2007;84:772-780.
44. Flinn JM, Kakalec P, Tappero R, Jones B, Lengyel I. Correlations in distribution and concentration of calcium, copper and iron with zinc in isolated extracellular deposits associated with age-related macular degeneration. *Metallomics* 2014;6:1223 - 1228.
45. Gouras P, Braun K, Ivert L, Neuringer M, Mattison JA. Bestrophin detected in the basal membrane of the retinal epithelium and drusen of monkeys with drusenoid maculopathy. *Graefes Arch Clin Exp Ophthalmol* 2009;247:1051-1056.