Prevalence and Morphology of Druse Types in the Macula and Periphery of Eyes with Age-Related Maculopathy

Martin Rudolf,[1] *Mark E. Clark,*[1] *Melissa F. Chimento,*[1] *Chuan-Ming Li,*[1] *Nancy E. Medeiros,*[2] *and Christine A. Curcio*[1]

Purpose. Macular drusen are hallmarks of age-related maculopathy (ARM), but these focal extracellular lesions also appear with age in the peripheral retina. The present study was conducted to determine regional differences in morphology that contribute to the higher vulnerability of the macula to advanced disease.

Methods. Drusen from the macula (n = 133) and periphery (n = 282) were isolated and concentrated from nine ARM-affected eyes. A semiquantitative light microscopic evaluation of 1-μm-thick sections included 12 parameters.

Results. Significant differences were found between the macula and periphery in ease of isolation, distribution of druse type, composition qualities, and substructures. On harvesting, macular drusen were friable, with liquefied or crystallized contents. Peripheral drusen were resilient and never crystallized. On examination, soft drusen appeared in the macula only, had homogeneous content without significant substructures, and had abundant basal laminar deposits (BlamD). Several substructures, previously postulated as signatures of druse biogenesis, were found primarily in hard drusen. Specific to hard drusen, which appeared everywhere, were central subregions and reduced RPE coverage. Macular hard drusen with a rich substructure profile differed from primarily homogeneous peripheral hard drusen. Compound drusen, found in the periphery only, exhibited a composition profile that was not intermediate between hard and soft.

Conclusions. The data confirm regional differences in druse morphology, composition, and physical properties, most likely based on different formative mechanisms that may contribute to macular susceptibility for ARM progression. Two other reasons that only the macula is at high risk despite having relatively few drusen are the exclusive presence of soft drusen and the abundant BlamD in this region. (*Invest Ophthalmol Vis Sci.* 2008;49:1200–1209) DOI:10.1167/iovs.07-1466

From the [1]Department of Ophthalmology, University of Alabama at Birmingham, Birmingham, Alabama; and the [2]Retina Specialists of North Alabama, Huntsville, Alabama.

Supported by National Eye Institute Grant EY06109, Deutsche Forschungsgemeinschaft Grant DFG 13942, Research to Prevent Blindness, the EyeSight Foundation of Alabama, the International Retinal Research Foundation, the Macular Vision Research Foundation, and the Roger H. Johnson Prize (2002).

Submitted for publication November 14, 2007; revised December 1, 2007; accepted January 23, 2008.

Disclosure: **M. Rudolf**, None; **M.E. Clark**, None; **M.F. Chimento**, None; **C.-M. Li**, None; **N.E. Medeiros**, None; **C.A. Curcio**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Martin Rudolf, Department of Ophthalmology, University of Alabama at Birmingham, H020 Callahan Eye Foundation Hospital, 700 South 18th Street, Birmingham, AL 35294-0009; mirudolf@aol.com.

Drusen are focal deposits of extracellular debris located between the basal lamina of the retinal pigment epithelium (RPE) and the inner collagenous layer of Bruch's membrane.[1,2] Found with advanced age in normal human eyes,[3–5] they are regarded as hallmarks of the underlying degeneration of age-related maculopathy (ARM).[6] Drusen identified clinically by retinal fundus examination are yellow-white deposits typically divided into two main morphologic phenotypes "hard" and "soft," distinguishable by the characteristics of their edges and the level of risk conferred for advanced disease.[7–9] Numerous hard drusen in the macula increase significantly the incidence of soft drusen and RPE abnormalities,[10,11] which are, in turn, more likely to progress to the advanced stages of choroidal neovascularization and/or RPE atrophy (age-related macular degeneration, ARMD).[4,10–12]

Recent studies of drusen composition and substructure have refined our understanding of how these lesions form.[13–18] Drusen consist of neutral lipids, with esterified and nonesterified cholesterol constituting ~3.2% of the dry weight.[15,19,20] Drusen also contain carbohydrates,[21,22] zinc,[23] and at least 129 different proteins, including apolipoproteins (e.g., apoE, apoB) and excluding structural extracellular matrix.[18] Druse proteins involved with inflammation (e.g., amyloid-β, immunoglobulin light chains, factor X, C5, and C5b complex) have received heightened attention because complement factors H and B gene sequence variants are associated with increased ARMD risk.[22,24–26] Approximately 30% of drusen exhibit at their bases 15-μm diameter cores that are enriched in nonesterified cholesterol, nonfibrillar amyloid, and peanut agglutinin-binding carbohydrates. These cores may represent nucleation sites for subsequent deposition.[15,21]

A conundrum of ARM pathobiology is that more histologically defined drusen are found in nonmacular regions of the retina, but the macula is more vulnerable to ARMD.[5,12,27–31] This conundrum might be resolved if macular and peripheral drusen could be shown to have different molecular and cellular properties. Limited existing data support this hypothesis. We have noted that macular drusen are more fragile on cryosectioning and have a lower prevalence of apolipoprotein immunoreactivity than do nonmacular drusen in the same eyes.[16] Others have observed that macular drusen lack ex vivo autofluorescence.[5] Furthermore, membranous debris, the principal component of soft drusen, is reportedly present in the central macula only.[32] Regional differences in drusen have been addressed minimally, because few studies have been undertaken to analyze drusen in detail in both the macula and extramacula of the same ARM-affected eyes.[5,16,13,28,33]

We report the results of semiquantitative light microscopic examination of 415 macular and extramacular drusen in nine donor eyes with ARM. Our research questions were: (1) How do macular and peripheral drusen differ morphologically? (2) Can all drusen be categorized as hard or soft? (3) What is the prevalence of these types in different retinal regions? We used our previous methods for identifying ARM in the postmortem fundus[34] and for manually isolating drusen.[15,35] Evaluating

Investigative Ophthalmology & Visual Science, March 2008, Vol. 49, No. 3

TABLE 1. Eye Donors

Donor	Eye	Age	Gender	Preserv.	Drusen (*n*)		Eye Exam before DOD	VA_{CC} and Ocular History
					Macula	Periphery		
1	L	64	F	PF	41	28	9.6 months	20/25–20/40*
2	L	66	F	PF	8	18	36.7 months	20/25–20/40†
3	L	76	F	PF	10	41		
4	R	80	M	PF/GA	12	40	13.9 months	20/300–20/1600‡
5	R	86	F	PF/GA	14	16		
6	L	91	F	PF	17	20		
	R	91	F	PF/GA	7	29		
7	L	102	M	PF	18	36		
	R	102	M	PF/GA	6	54		
Total					133	282		

L, left eye; R, right eye; Preserv., preservative; PF, 4% paraformaldehyde; PF/GA, 1% paraformaldehyde, 2.5% glutaraldehyde; DOD, date of death; VA_{CC}, corrected visual acuity; known ocular history: * dry age-related maculopathy (ARM), cerebral aneurysm, visual field defect; † dry ARM; ‡ normal macula for age, partial optic atrophy.

drusen and their overlying RPE on 12 parameters, we defined a third druse type (compound) and clarified structural differences among types with different associated risk levels for ARMD.

METHODS

Institutional Review at the University of Alabama at Birmingham approved our use of human tissues. Nine eyes with ARM from seven eye bank donors (ages 64–102 years) were obtained within 6 hours of death and were managed in accordance with the guidelines of the Declaration of Helsinki. The eyes were preserved by immersion in either 4% paraformaldehyde or 1% paraformaldehyde/2.5% glutaraldehyde, both in 0.1 M phosphate buffer (Table 1), for 24 hours after corneal removal and were stored in 1% paraformaldehyde at 4°C until they were used. Median time between death and preservation was 2.7 hours.

Before the drusen were harvested, images of the macula and periphery of each eye were taken under a dissecting microscope (SMZ-U; Nikon Instruments, Inc., Melville, NY) using either a digital camera (Coolpix 5000; Nikon, Inc.) or a 35-mm color film (EPJ320T; Eastman Kodak, Rochester, NY). The film was scanned at 1200 dpi (model 4870; Epson America Inc., Long Beach, CA). All eyes were classified as ARM according to the Alabama Age-Related Macular Degeneration Grading System for donor eyes by criteria, such as drusen size, type (soft), and RPE clumping (Fig. 1).[34] We found no evidence of advanced disease (geographic atrophy of the RPE or fibrovascular scar secondary to choroidal neovascularization).

Harvesting of Drusen

Macular drusen were harvested from within a 3-mm radius around the foveal center, the region of the epidemiologic and anatomic macula.[3,36] Extramacular drusen were collected from the remainder of the retina, with a clearance of 3 mm from the macula's outer circumference. As previously described,[35] RPE-capped drusen were mobilized from Bruch's membrane with a borosilicate pipette under stereomicroscopic guidance, herded into small groups, drawn into the pipette, and placed into a capsule (BEEM; Electron Microscopy Sciences, Hatfield, PA). Small drusen, including those considered preclinical (<30 μm),[37] were not isolated individually, but were included in groups of larger drusen and therefore were not systematically excluded.

Embedding

To facilitate handling, washed drusen were covered with a 0.75%-agarose/5%-sucrose solution, then refrigerated to form solid pellets. The pellets were trimmed, postfixed in 1% osmium in 0.1 M sodium cacodylate buffer, 1% tannic acid (gallotannin, $C_{14}H_{10}O_9$), and 1% paraphenylenediamine (OTAP method[20,38]), dehydrated through ethanol and propylene oxide, and embedded in epoxy resin (PolyBed 812; Polysciences, Warrington, PA). One-micrometer-thick sections were cut (Ultracut UCT; Leica Mikrosysteme AG, Vienna, Austria), stained with 1% toluidine-O-blue, and coverslipped for semiquantitative evaluation by light microscopy.

Microscopy and Semiquantitative Evaluation

Sections were examined by bright field microscopy (Eclipse 80i; Nikon Instruments, Inc.). The images were captured with a digital camera (Retiga 4000R Fast) and the accompanying software (Qcapture v2.8.1; QImaging, Burnaby, BC, Canada). For druse identification and orientation, 40× images of sections were merged into a map (Photoshop CS ver. 8.0; Adobe Systems, San Jose, CA). All drusen were numbered and entered in a spreadsheet (Excel 2003; Microsoft, Redmond, WA). Then, drusen were individually examined and photographed with a 60×, plan apochromat oil objective (1.4 numerical aperture) and immersion oil (catalog no. 1261; Cargille Laboratories Inc., Cedar Grove, NJ) and evaluated according to the morphologic parameters described in the next section.

Druse Type Identification

We divided all drusen into three types—hard, soft, and compound—on the basis of morphologic criteria and for hard and soft drusen, reference to widely accepted previous descriptions.[26,39–41] Soft drusen are composed of a loose, amorphous, granular material. Borders are poorly defined, and the druse mound tapers off basally into what is likely a basal linear deposit (BlinD) in the adjacent areas (Figs. 2A, 3C). Hard drusen are round or dome-shaped with well-defined borders. Druse contents are solid and hyalinized (Figs. 2B, 2C). We also found drusen with mixed characteristics (i.e., dome-shaped or sloped borders with contents consisting of both hyalinized and loose material). We classified these drusen as compound (Fig. 2D) and considered whether they might be an intermediate form between soft and hard drusen or an independent type.

Evaluation Criteria and Limits

Each druse was evaluated according to 12 parameters. For each parameter, Table 2 lists the definition, pathobiologic significance, and evaluation scale, with reference to examples illustrated in Figure 3. We first defined for each druse its section plane, because the orientations of drusen in the pellet varied. We discriminated vertical sections that displayed the druse top to base (Fig. 3J) from nonvertical sections (Fig. 3B), which excluded the druse base. Because some features (central subregions and remodeling, Table 2) are thought to be primarily located at the druse base,[15,21] nonvertical sections were censored for

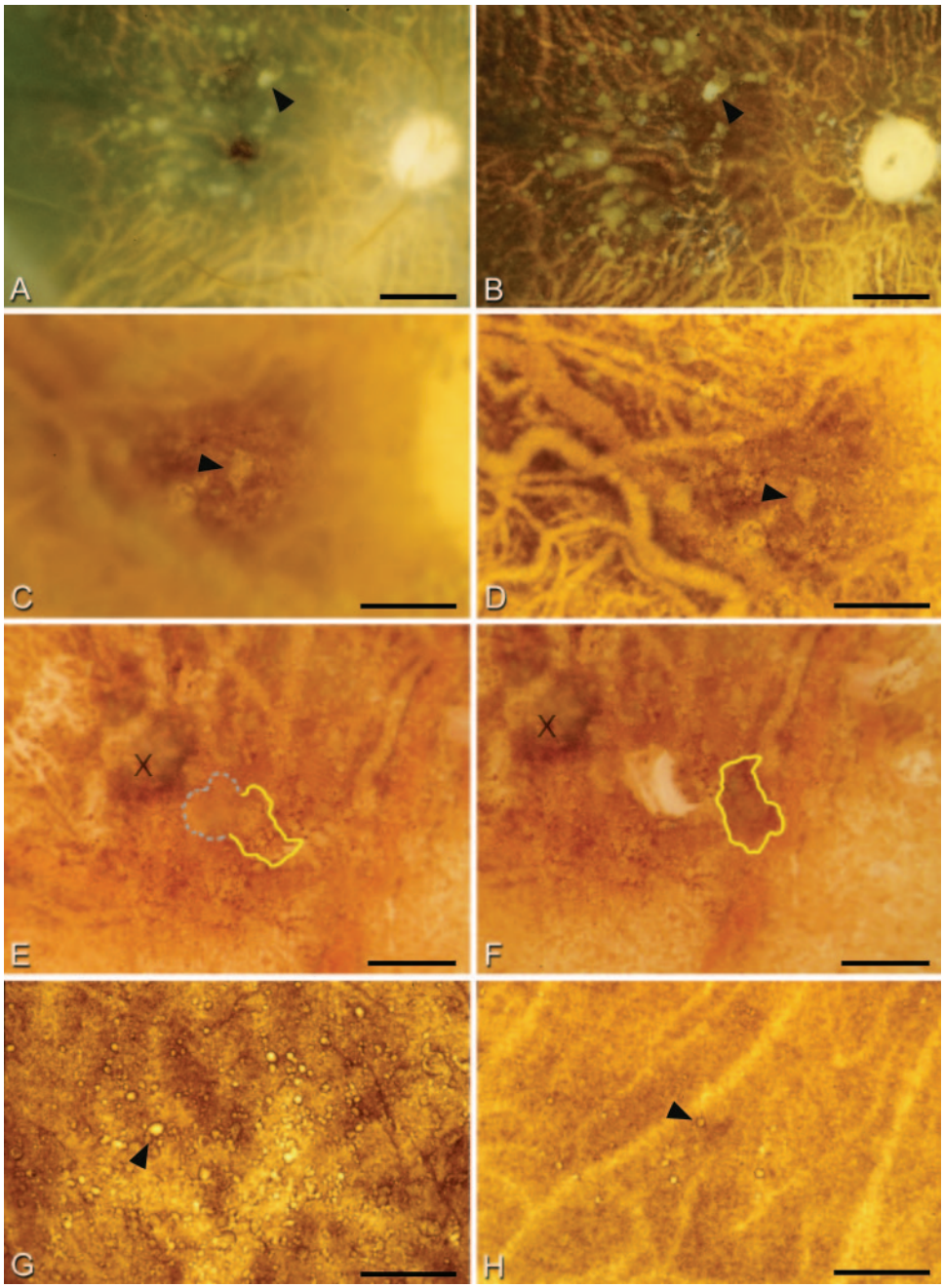

FIGURE 1. Gross appearance of drusen in the macula and periphery of donor eyes. In the specimens (**A**) the retinal vasculature was visible. Prominent in all panels are the major choroidal vessels, which empty at death and appear as lighter stripes in contrast to the heavily pigmented intervening choroidal stroma. The neurosensory retina is in place in the specimens in (**A**) and (**C**) and is removed in all other panels. (**A**) Right macula of case 6R with abundant large drusen. *Arrowhead*: crystalline druse. (**B**) The same macula (retina off) as in (**A**). *Arrowhead*: the same crystalline druse, which is whiter and more glistening relative to the other drusen in the field. (**C**) Right macula of case 7R with large drusen. Drusen are less white than in (**A**) and have a stippled surface indicating overlying RPE. *Arrowhead*: one druse. Semicircular area of depigmentation at *right*, blurred due to the overlying retina, indicates atrophy of the RPE and choroid around the optic nerve head (not visible). (**D**) Same macula (retina off) as (**C**), with the same druse indicated. (**E**, **F**) Pair of images of the macula of case 7L from which a soft druse has been removed. X, the fovea. A druse and surrounding RPE (bordered in *yellow*) has been punctured, releasing oily contents diffusely (cloudy area delimited by *gray dashed line* in **E**) into the surrounding buffer. (**F**) The druse has been cleaved from Bruch's membrane, leaving a window in the RPE through which the underlying choroid is visible. The druse, still bordered by *yellow*, has been inverted so that its basal aspect is now apparent. (**G**) Periphery of case 3L showing abundant drusen, some large (*arrowhead*). (**H**) Periphery of case 1L showing less abundant drusen. The largest drusen (*arrowhead*) are smaller than those in (**G**). Bars, 1 mm.

these parameters. Then, each druse was evaluated for composition properties (integrity, homogeneity), structural features (RPE coverage, BlamD, shells, central subregions, and remodeling), and minority components (cells, pigment, amyloid assemblies, inclusions, and calcification). For integrity, a druse was considered empty if not enough content was preserved to judge the druse interior according to our parameters. The shape of the RPE layer over drusen remained unchanged because the examined eyes were fixed with druse contents in place. To find evidence of former druse content, we examined a fringe of druse remnants clinging to the bowed-up RPE (Fig. 3E). Empty drusen were censored for the parameters' homogeneity, central subregion, remodeling, and minority components. In our study, we evaluated only the most severe RPE change—namely, RPE atrophy/loss—by measuring the completeness of RPE coverage over each druse. RPE coverage was judged as a percentage of the maximum possible RPE coverage (100%), which in vertical sections was the druse top and sloping sides and in nonvertical sections was the whole druse circumference. RPE coverage actually represents the minimum of present RPE for evaluation because additional cells may have broken off in processing. To estimate druse size, we followed two procedures. For vertically sectioned drusen, we measured the length of the base using a digitizing tablet and image analysis software (IP Lab, ver. 3.9.5.r4; BD Biosciences, Exton, PA). For nonvertically sectioned drusen, cross sections were traced, and maximum diameters were determined by the software. Drusen with compromised contents (described later) were traced as though they were intact. We pooled both sets of diameters for Figure 4.

Statistical Evaluation

Our null hypothesis was that druse characteristics are not associated with location (macular and extramacular). Frequency distributions and descriptive statistics were calculated for variables listed in Table 2. We used χ^2 tests to determine whether any observed difference according

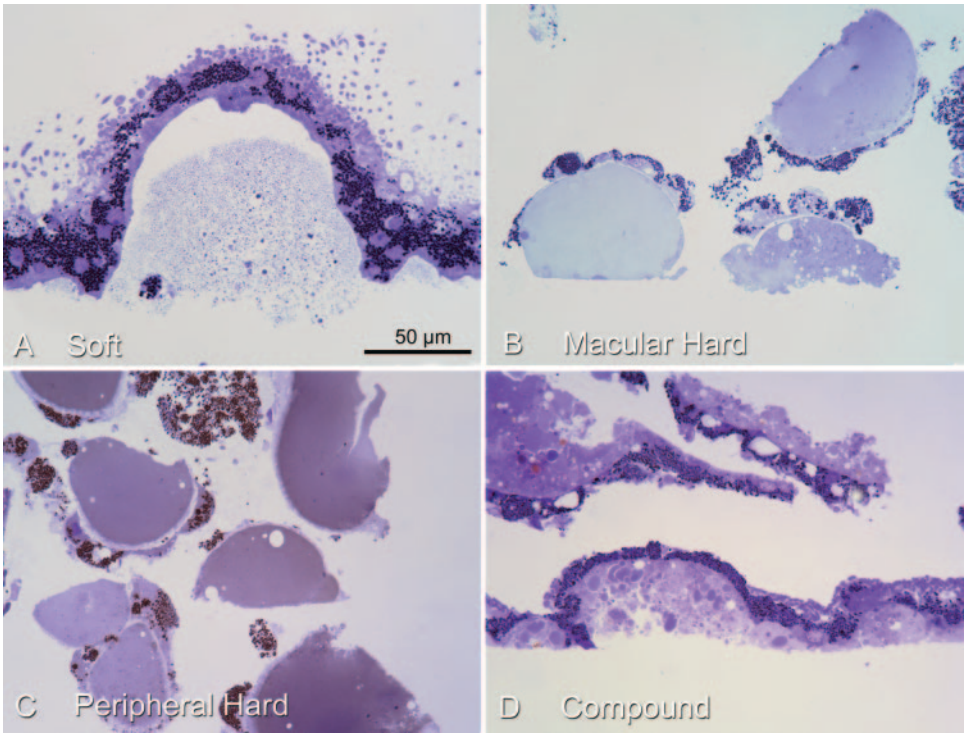

FIGURE 2. Druse types. One-micrometer-thick sections stained with toluidine blue and imaged with a 60× oil immersion objective. All 415 drusen were assigned to one druse type. (**A**) Soft druse, typically loose, amorphous material; sloped shoulders; (**B**, **C**) hard drusen, (**B**, macular, **C**, peripheral), round or dome-shaped, well-defined borders, hyalinized content; (**D**) compound drusen, hyalinized material mixed with loose amorphous contents, not assigned to the two previous types.

to location or druse type was significant. ANOVA was used to test the differences of RPE coverage between peripheral and macular drusen or among different types of drusen. Because data of druse diameters were not normally distributed, the Wilcoxon test was performed to test differences among druse types. All probabilities reported were two-tailed. $P \leq 0.05$ was considered significant. Analyses were performed with commercial software (JMP Statistical Discovery Software ver. 5.1; SAS Institute Inc, Cary, NC).

RESULTS

Donor Eyes and Druse Collection

Characteristics of patients, donor eyes, and known ocular medical history are listed in Table 1. All donors were Caucasians (five women; two men). Their age averaged 80.7 ± 13.6 years. Gross examination of the donor eyes showed easily detectable large drusen in the maculas, an appearance consistent with ARM (Figs. 1A–F).

Macular drusen appeared as singles or in clusters and resembled what clinical grading systems consider hard and soft. During preparation, distinct differences emerged in the handling of different druse types. Because soft drusen were generally difficult to capture singly, a larger area was isolated. Soft drusen were often liquefied or oily, and contents were not always salvageable. Sometimes merely touching a macular soft druse evoked release of a cloudy ejecta (Figs. 1E, 1F). Hard drusen of the macula were often partially or completely crystallized (Fig. 1B). Their isolation was easier than that of soft drusen; but, overall, drusen isolation in the macula was challenging.

Peripheral drusen were readily visible and resembled clinicopathologically described hard drusen (Figs. 1G, 1H). They appeared as cloudy or transparent domes.[35] Their resilience made isolation straightforward, a property reflected by the large number of histologically intact drusen in the final sample. Peripheral drusen never appeared grossly crystallized.

Prevalence of Types

The final total of 415 drusen (Table 3) could all be categorized as one of the three types: soft, hard, or compound (Fig. 2). One hundred thirty-three drusen derived from the macula and 282 from the periphery. Ninety-eight drusen considered soft were found only in the macula (Table 3). Hard drusen were the most abundant druse type ($n = 243$), found in both the macula ($n = 35$) and the periphery ($n = 208$). Seventy-four drusen categorized as compound were found only in the periphery.

Prevalence of Compositional Qualities

Compositional qualities were assessed by the parameters integrity and homogeneity (Table 3). Significant differences between macular and peripheral drusen ($P < 0.001$) matched observations made during preparation.

Integrity, the degree to which druse contents are compromised by preparation, indicates the consistency of the contents. The soft drusen did not usually appear individually but rather as part of a confluent group. These lesions exhibited reduced integrity due to loss of contents, consonant with the findings of oily or liquid interiors lost during isolation. We saved content completely or partially in 66.0% of the soft drusen, compared with 98.5% of the hard drusen ($P < 0.001$). The compound drusen were more compromised than were the hard drusen ($P < 0.001$) but were better conserved than were the soft drusen ($P = 0.037$).

Homogeneity, which we defined as >75% of the cross-sectional area containing a single material, indicates the range of physical and chemical constituents. The soft drusen tended toward homogeneity by this criterion (59.7%), filled with one amorphous material resembling membranous debris (Table 3; Figs. 2A, 3C). The peripheral hard drusen were typically homogenous and hyalinized (80.5%; Table 3, Fig. 2C), unlike the macular hard drusen, of which only 43.3% had homogeneous contents ($P < 0.001$). Composed of loose as well as hyaline material, almost all the compound drusen were considered inhomogeneous in contrast to the soft and hard drusen ($P < 0.001$ for both; Fig. 2D, Table 3).

TABLE 2. Drusen Evaluation Parameters

Parameter	Characteristics	Significance	Evaluation Scale/ Examples in Figure 3†
Section plane	Vertical sections include druse base, top, and center. Non-vertical sections include horizontal and tangential planes	Vertical section required for judging central subregion* and remodeling*	(1) Vertical: A (2) Non-vertical: B
Integrity	Degree in which drusen content can be compromised by preparation technique	Maintenance of druse content provides information about content solidness and consistency	(3) Intact: B (4) Partial: D (5) Empty: E
Homogeneity	Judged on the overall content appearance; homogeneous if ≥75% of coherent druse area consists of one material	Range of physical and chemical constituents	(6) Homogeneous: C,F (7) In homogeneous: G
RPE Coverage	Percentage of maximal possible RPE coverage	RPE health	(8) ~80%: A (9) ~100%: G
Basal laminar deposit‡	Extracellular material between RPE and its basal lamina, overlying druse	Additional accumulation of extracellular debris	(10) A, C, E
Shell‡	Solid, homogenous outline of druse	Specific substructure	(11) B, F, I
Central subregion*‡	Recognizable dense structure at the druse base	Specific substructure	(12) F, L
Remodeling*‡	Focal disaggregation at the druse base	Sign of possible druse turn-over	(13) J
Cells‡	Cells within or underneath druse	Suspected role in druse genesis	(14) L
Pigment granules‡	Melanin granules, as in the RPE	Suspected role in druse genesis, as parts of degenerated RPE cells	(15) H
Amyloid assemblies‡	Concentric, ring-like structures	Suspected role in drusen genesis	(16) K
Calcification‡	Crystalline (birefringent) bodies	End-stage of drusen development	(17) A
Inclusions‡	Sharply defined, lucent round structures	Unknown	(18) H

* These parameters are dependent on the section plane. Central subregion and remodeling are located at the druse base, which is preserved in vertical sections only.

† Examples in Figure 3 are indicated by letters (for panels) and numbers (for the features).

‡ These parameters were judged as Present or Absent. References to Figure 3 show present features. Absent is not illustrated.

Prevalence of Structural Features

RPE coverage and the prevalence of BlamD, shells, central subregions, and remodeling recesses by druse type and location are described in Table 4.

RPE coverage was significantly lower, at ~80%, over the hard drusen ($P < 0.001$), of both the macula and periphery ($P = 0.665$). In contrast, RPE coverage, a measure of RPE health, was not compromised over the soft or compound drusen (100% and 97%, respectively). We observed that pigmentation and regularity of RPE cells over these druse types were also altered to some degree—changes not captured by the parameter RPE coverage.

BlamD, a diffuse lesion between the RPE and druse body (Fig. 3C) that confers risk for ARMD when abundant, was a typical macular feature. Almost all the soft (89.7%) and hard (78.8%) macular drusen exhibited BlamD, with no significant difference between the two ($P = 0.109$). In contrast, BlamD was found in just two of three peripheral hard drusen and in one of three compound drusen ($P < 0.001$). BlamD thickness appeared qualitatively thicker in the macula than in the periphery (Figs. 3A, 3C, 3E for the macula versus 3F, 3I for the periphery).

Shells are contrasted sharp outlines of a druse (Fig. 3D), elsewhere demonstrated as rich in apolipoproteins and cholesterol.[15,16,35] They were more frequent in the periphery (53.8%), compared with <28.4% in the macula ($P < 0.001$, Table 4). We detected no differences among druse types in each location ($P_{macular} = 0.242$, $P_{peripheral} = 0.104$).

At the druse base, we evaluated the prevalence of central subregions (Fig. 3L), which include the lectin-binding, nonesterified cholesterol-rich cores. Central subregions were exclusively found in the hard drusen, predominantly in the periphery (21.1%) and less so in the macula (3.7%).

At the druse base, we also evaluated remodeling (Fig. 3J), characterized by a delicate recess, possibly indicating druse turnover or cellular invasion. Such remodeling was not reliably detected in the soft drusen, mostly because of the inherent liquidity and resultant loss of soft druse contents. The highest prevalence (16.0%) was found in the compound drusen, compared with 7% to 8% of the hard drusen, regardless of location ($P = 0.155$).

Prevalence of Minority Components

Prevalences of cells, pigment granules, amyloid assemblies, inclusions, and calcification are listed in Table 5.

Cells (Fig. 3L) of any identity were undetectable in the macular soft or hard drusen, but they occurred rarely (3%) in the peripheral hard drusen and regularly (12.5%) in the compound drusen ($P = 0.003$). Without specific staining, we could not definitively determine cell types (e.g., leukocytes, dendritic cells), but many cells resembled altered RPE.

Pigment granules (Fig. 3H), perhaps signifying a deposition from dying RPE cells, were found in the soft, macular hard, and peripheral hard drusen equally ($P = 0.389$), in an average of 6% to 9% of these lesions. In contrast, pigment granules were undetectable in the compound drusen.

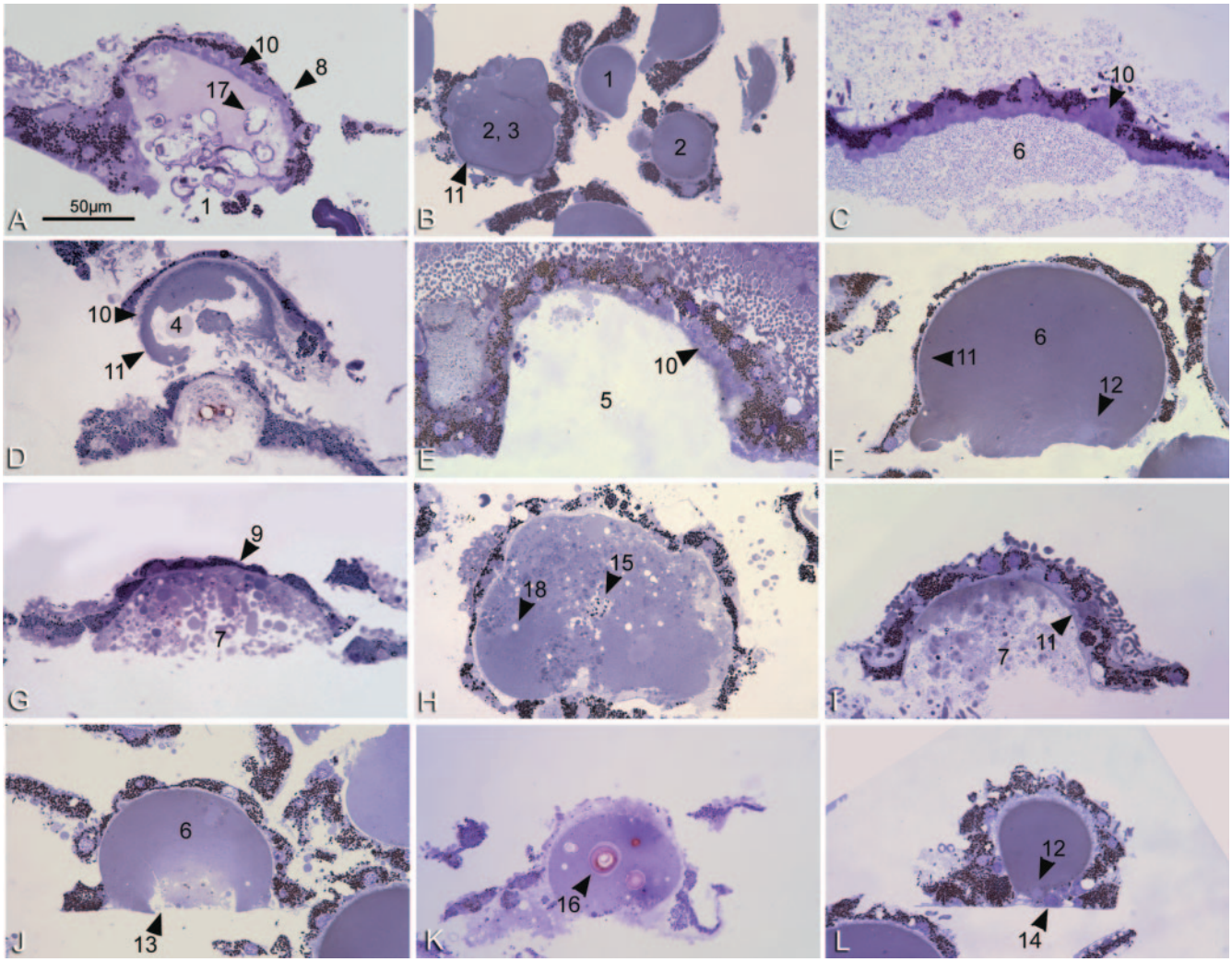

FIGURE 3. Examples of evaluated druse parameters. One-micrometer-thick sections stained with toluidine blue and imaged with a 60× oil immersion objective. The images depict the evaluated parameters (Table 2). Examples are marked with an arrowhead and a number: 1, vertical section plane; 2, nonvertical section plane; 3, full integrity; 4, reduced integrity; 5, empty druse; 6, homogeneous content; 7, inhomogeneous content; 8, reduced RPE coverage; 9, full RPE coverage; 10, BlamD; 11, shell; 12, central subregion; 13, remodeling recess; 14, internal cells; 15, pigment granules; 16, amyloid assemblies; 17, calcification; and 18, inclusions. Soft drusen (**C**, **E**, **H**); macular hard drusen (**A**, **B**); peripheral hard drusen (**D**, *top*; **F**, **I**, **J**, **K**, **L**); compound drusen (**D**, *bottom*; **G**).

Amyloid assemblies, concentric ring–like structures (Fig. 3K), were found most often in the compound drusen (28.1%, $P < 0.001$). More macular hard drusen (13.5%) exhibited amyloid than did soft drusen (3.1%) and peripheral hard drusen (2.0%), which did not differ from each other ($P = 0.059$).

Inclusions, sharply delimited, round, lucent areas of undetermined function (Fig. 3H), were seen in a few soft drusen (3.1%) and frequently in all other drusen. The prevalence in the peripheral hard drusen (53.7%) was significantly higher ($P < 0.001$) than in the macular hard drusen (25.8%), and it was highest (67.7%) in the compound drusen.

Calcification is crystalline and represents an end-stage druse (Fig. 3A). It was a typical macular hard druse feature (43.3%), rarely found in the other druse types ($P < 0.001$).

Druse Size

The median diameter of peripheral hard drusen (47.3 μm) was smaller than all others (Fig. 4, $P = 0.001$). The median diameter of soft drusen was not significantly larger than the macular hard ($P = 0.540$) or compound ($P = 0.076$) drusen. However, diameter measurement also included many small drusen, so it

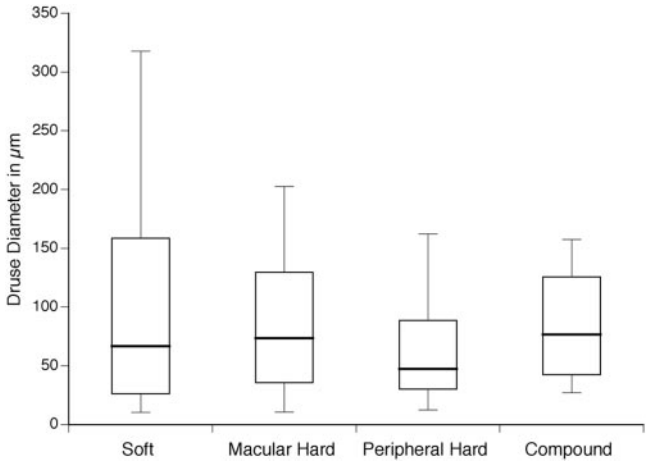

FIGURE 4. Druse diameters, by druse type. Diameters were determined for drusen sectioned in the vertical and nonvertical planes separately and then pooled. *Box plot*: the median and quartiles; *bars*, minimum and maximum diameter for each type.

TABLE 3. Prevalence of Composition Characteristics

			Prevalence (%)				
			Integrity			Homogeneity	
Region	Druse Type	*n*	Intact	Partial	Empty	Yes	No
Macula	Soft	98	20.6	45.4	34.0	59.7	40.3
	Hard	35	57.6	33.3	9.1	43.3	56.7
	Compound	0	—	—	—	—	—
Periphery	Soft	0	—	—	—	—	—
	Hard	208	81.5	16.6	1.9	80.5	19.5
	Compound	74	30.1	53.4	16.4	20.0	80.0

does not properly reflect the extensive range of soft druse sizes (Fig. 4). On average, 15% of the drusen of all types were <30 μm in diameter (considered small, preclinical by Sarks et al.[37]). Because the isolation of solitary soft drusen was particularly challenging, larger areas around prominent drusen were isolated. In the periphery of these drusen, numerous small ones were found, resulting in a higher percentage (23.5%) of small drusen in this group. Therefore, for the soft drusen, maximum diameter was more informative. The largest soft druse, at 318 μm, was 50% larger than the macular hard drusen and twice as large as the peripheral hard drusen.

DISCUSSION

This report provides improved descriptions of macular and extramacular drusen, with implications for models of how these extracellular lesions develop and participate in ARM progression. An enriched druse preparation, first developed for biochemical studies,[18] allowed us to obtain single sections through numerous drusen, rather than sectioning large areas of retina. Other strengths include the strict separation of macular and extramacular regions and the fact that all eyes had a fundus appearance consistent with ARM, in contrast with prior literature, which focused only on macula or periphery, pooled drusen from different regions, did not specify location, or did not document maculopathy status.[13,14,18,41]

Limitations are the incompatibility of the fixation method with immunohistochemical localization of specific proteins, the loss of soft druse contents in processing, and the random sectioning plane. Nevertheless, these data, generated from drusen identically handled from two regions of the same eyes, suggest macular drusen are collectively more fragile and show less evidence of dynamic processes inferred from detailed morphologic study of peripheral drusen. We summarize our findings by druse type (Table 6), with reference primarily to Sarks' clinicopathologic classification, which includes clinical, light microscopic, and electron microscopic levels.[41–44] Of note, we accounted for all druse substructures with existing descriptors, suggesting that the morphologic catalog of druse contents is nearly complete.

Hard Drusen

Hard drusen are the most abundant druse type throughout the retina. Therefore, these lesions will dominate any analysis in which drusen are pooled across regions.[18] In general, hard drusen are solid and more resistant to processing-related damage than other drusen. Notably, we found a significant decline of RPE coverage only in hard drusen, consistent with aberrant expression of vitronectin and amyloid A over small drusen.[25,26] However, several hard druse characteristics differed critically depending on their location. Relative to the extramacular drusen, the macular drusen were bigger, exhibited more and thicker BlamD, tended toward inhomogeneity, and were richer in substructure. Calcifications, for instance, were found in almost half of the macular hard drusen. These lesions also contained more amyloid assemblies and cells. In contrast, the peripheral hard drusen were almost exclusively homogenous and displayed more shells, inclusions, and central subregions.

Of note, several substructures such as central subregions, previously postulated as signatures of druse biogenesis, were found primarily in the hard drusen. We refrained from calling these structures cores (proposed nucleation sites) without information about lectin binding, cholesterol content, and nonfibrillar amyloid content (not to be confused with amyloid assemblies, presented earlier).[15,21] Nevertheless, the 21% of extramacular hard drusen containing central subregions resemble the 32% of these drusen with nonesterified cholesterol-rich cores[15] and the 27% of hard and soft drusen with histochemically identified cores.[21] We also found more shells, shown

TABLE 4. Prevalence of Structural Features

			Prevalence of Structures (%)			
Region	Druse Type	Average RPE Coverage (%)	BlamD	Shells	Central Subregion	Remodeling
Macula	Soft	100.0 ± 1.9	89.7	28.4	0.0	*
	Hard	78.9 ± 2.3	78.8	17.2	3.7	8.3
	Compound	—	—	—	—	—
Periphery	Soft	—	—	—	—	—
	Hard	80.9 ± 1.5	62.6	53.8	21.1	7.1
	Compound	96.5 ± 2.3	31.9	63.2	0.0	16.0

* Remodeling was not reliably detected in soft drusen, mostly due to loss of druse contents but also due to the liquid nature of these contents, even if intact.

TABLE 5. Prevalence of Drusen Minority Components

Region	Druse Type	Prevalence of Structures (%)				
		Cells	Pigment Granules	Amyloid Assemblies	Inclusions	Calcification
Macula	Soft	0	6.4	3.1	3.1	1.6
	Hard	0	6.7	13.5	25.8	43.3
	Compound	—	—	—	—	—
Periphery	Soft	—	—	—	—	—
	Hard	3.0	8.9	2.0	53.7	2.0
	Compound	12.5	0	28.1	67.7	1.6

elsewhere to be rich in apoE, apoC-I, and esterified cholesterol.[15,16,35] Likely to be hydrophobic, the presence of shells may imply a focal uplifting of the lipid wall, a layer of lipoprotein-like particles particularly abundant in the aged macula.[15,35,45–47] Other hard druse substructures also may signify modulation of these lesions. The remodeling profiles we found equally in macula and periphery resemble the moth-eaten or washed out appearance of regressing hard drusen.[37] Another sign of druse progression is calcification, found in our study almost exclusively in the macular hard drusen. Calcification can easily fill an entire druse, and in this form could be easily remarkable in a fundus examination. Calcification of Bruch's membrane is specifically associated with ARM-associated choroidal neovascularization in the macula.[48]

Macular hard drusen appear in fundi of up to 94% of adults.[3,4] Eyes with sparse drusen <63 μm in diameter are not considered to have, or to be at risk for, ARM.[4,5,10,12,37] In contrast, abundant hard macular drusen increases risk for pigmentary abnormalities and incident soft drusen.[10,49] Our hard drusen matched best the small hard, hyalinized drusen of Sarks' clinicopathologic classification as well as pseudosoft cluster-derived drusen (i.e., fusing hard drusen). The hard drusen exhibited compromised RPE coverage, as seen by others, with RPE loss starting on top and leading to patchy RPE atrophy funduscopically.[43,50] Reduced coverage may contribute to the findings of geographic atrophy in eyes with abundant hard drusen.

TABLE 6. Salient Druse Type Features

Druse Type	Salient Features
Soft	• Found in macula • Mostly homogenous, loosely knit content • Basal laminar deposits • Full RPE coverage • No significant substructures
Hard	• Predominant drusen type throughout retina • RPE coverage is significantly reduced • Shells are common • Central subregions are a specific feature
Location-dependent features of hard drusen:	
• Macula	• More often basal laminar deposits • Majority inhomogeneous
• Periphery	• More shells • Most central subregions • Majority is solid and homogeneous
Compound	• Found in periphery • Inhomogeneous, semi-loosely knit content • Majority with shells • Highest rate of remodeling

Compound Drusen

A druse type formally introduced herein is the compound druse, found only in the periphery. Compound druse contents resemble Sarks' description of drusen developing a mixed and more coarsely and granular content as they regress.[37,44] However, previous observations of regressing drusen are incompatible with our findings. First, the RPE usually atrophies over regressing drusen, but RPE coverage was uncompromised over the compound drusen. Second, we found this druse type exclusively in the periphery, unlike the previous descriptions. Third, regressing drusen should be smaller, but our compound drusen are larger than peripheral hard drusen. Fourth, regressing drusen should share at least some characteristics of younger drusen (e.g., central subregions), but they did not.

Compound drusen do not seem to be an intermediate form between soft and hard drusen. Their integrity was appreciably compromised, but as much as soft drusen. In contrast to the soft and peripheral hard drusen the majority of the compound drusen was inhomogeneous and rich in substructure. The prevalence of shells, cells, inclusions, amyloid assemblies, and remodeling was highest in this druse type, suggesting that some aspects of druse formation postulated for hard drusen apply to these lesions as well. Although the identity of druse-resident cells was not determined, our data permit some inferences. In one hypothesis, RPE cells are perceived as the origin of druse material,[51] suggesting that identifiable RPE content should be found frequently in drusen. However, pigment granules were completely absent from compound drusen. If instead these cells are macrophages,[52] their activity would support the notion of remodeling recesses as part of druse regression.

Soft Drusen

Soft drusen are universally considered the most fateful of ARM lesions visible in the fundus. Our soft drusen match previous descriptions in size (up to 350 μm[43]) and morphology.[32,41–44] Of particular note, we found them only in the macula. Usually, the area surrounding individual large soft drusen contained several small merging drusen and thick BlamD. We found the oily liquid content very fragile on isolation, consistent with previous observations.[16] Soft drusen typically contain microscopically homogenous material without significant internal substructure, suggesting that models of biogenesis based on cores, shells, or amyloid assemblies may not apply to these lesions.[32] Unexpectedly, RPE coverage was not compromised

in soft drusen. Clinically, RPE atrophy ensues only when confluent large drusen cause a serous RPE detachment.[43]

Our observations on soft drusen are likely to be applicable to BlinD, a specific, insidious, but experimentally elusive ARM lesion that accumulates between the RPE basement membrane and the inner collagenous layer of Bruch's membrane.[1,32,53] Soft drusen and BlinD can be understood as a focal or diffuse accumulation of the same drusenoid material,[32] explaining why we found areas adjacent to individual soft drusen also altered. Like soft drusen, the principal component of BlinD is membranous debris, so called because it contains coiled membranes and vesicular outlines of putative RPE origin.[20,32] We have shown that this material is actually solid neutral lipid-rich particles requiring specialized postfixation for optimal visualization.[20] Nevertheless, the current findings of loose, oily druse content would be consistent with a preponderance of such material. Membranous debris is reportedly confined to the macula,[32] matching our finding that soft drusen are likewise regionally restricted. Thick BlamD, another diffuse extracellular deposit, was associated with almost every soft druse, concordant with indications that no membranous debris is found without BlamD.[20,32,48]

Clinically, intermediate and large drusen reach confluence with comparable rapidity,[41] possibly due to the liquefied loose content of soft drusen. Eyes with large soft drusen are at higher risk for ARMD.[10–12] Neovascularization rising from the choriocapillaris can spread easily between the RPE and Bruch's membrane in a cleavage plane lined by what we have demonstrate in the present study is easily disrupted drusenoid material.[20,32]

Summary

With regard to why only the macula is at high risk for ARMD despite having relatively few drusen, our data show that two important reasons are the exclusive presence of soft drusen and the abundant BlamD in this region. Soft drusen, like BlinD, are structurally unstable, and BlamD is usually associated with funduscopically visible RPE alterations (hyperpigmentation, irregularity, hypertrophy, or attenuation) that signify declining RPE health. Our analysis of drusen substructure indicates multiple pathways for drusen formation in different parts of the eye. Future laboratory work should recognize the importance of specifying the location of study drusen and specifying the limitations under which nonmacular drusen serve as more readily obtainable surrogates for macular drusen. The location-specific information presented herein are useful, not only for assessing emerging animal models, but also for facilitating exploitation of resources such as eye pathology laboratories, in which rapidly preserved surgical specimens may include drusen of uncertain retinal location.

Acknowledgments

The authors thank the Alabama Eye Bank for timely retrieval of donor eyes and J. Brett Presley and Hardik Kapadia for technical assistance in tissue processing.

References

1. Green WR. Histopathology of age-related macular degeneration. *Mol Vis*. 1999;5:27.
2. Abdelsalam A, Del Priore L, Zarbin MA. Drusen in age-related macular degeneration: pathogenesis, natural course, and laser photocoagulation-induced regression. *Surv Ophthalmol*. 1999;44:1–29.
3. Klein R, Klein BE, Linton KL. Prevalence of age-related maculopathy. The Beaver Dam Eye Study. *Ophthalmology*. 1992;99:933–943.
4. Bressler NM, Bressler SB, West SK, Fine SL, Taylor HR. The grading and prevalence of macular degeneration in Chesapeake Bay watermen. *Arch Ophthalmol*. 1989;107:847–852.
5. Lengyel I, Tufail A, Hosaini HA, Luthert P, Bird AC, Jeffery G. Association of drusen deposition with choroidal intercapillary pillars in the aging human eye. *Invest Ophthalmol Vis Sci*. 2004;45:2886–2892.
6. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Surv Ophthalmol*. 1995;39:367–374.
7. Davis MD, Gangnon RE, Lee LY, et al. The Age-Related Eye Disease Study severity scale for age-related macular degeneration: AREDS Report No. 17. *Arch Ophthalmol*. 2005;123:1484–1498.
8. Klein R, Davis MD, Magli YL, Segal P, Klein BE, Hubbard L. The Wisconsin age-related maculopathy grading system. *Ophthalmology*. 1991;98:1128–1134.
9. Ferris FL, Davis MD, Clemons TE, et al. A simplified severity scale for age-related macular degeneration: AREDS Report No. 18. *Arch Ophthalmol*. 2005;123:1570–1574.
10. Klein R, Klein BE, Knudtson MD, Meuer SM, Swift M, Gangnon RE. Fifteen-year cumulative incidence of age-related macular degeneration: the Beaver Dam Eye Study. *Ophthalmology*. 2007;114:253–262.
11. Klein R, Klein BE, Tomany SC, Meuer SM, Huang GH. Ten-year incidence and progression of age-related maculopathy: The Beaver Dam eye study. *Ophthalmology*. 2002;109:1767–1779.
12. Wang JJ, Rochtchina E, Lee AJ, et al. Ten-year incidence and progression of age-related maculopathy: the blue Mountains Eye Study. *Ophthalmology*. 2007;114:92–98.
13. 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.
14. Hageman GS, Mullins RF. Molecular composition of drusen as related to substructural phenotype. *Mol Vis*. 1999;5:28.
15. Li CM, Clark ME, Rudolf M, Curcio CA. Distribution and composition of esterified and unesterified cholesterol in extra-macular drusen. *Exp Eye Res.* 2007;85:192–201.
16. Malek G, Li CM, Guidry C, Medeiros NE, Curcio CA. Apolipoprotein B in cholesterol-containing drusen and basal deposits of human eyes with age-related maculopathy. *Am J Pathol*. 2003;162:413–425.
17. Mullins RF, Johnson LV, Anderson DH, Hageman GS. Characterization of drusen-associated glycoconjugates. *Ophthalmology*. 1997;104:288–294.
18. 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 USA*. 2002;99:14682–14687.
19. 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. *Ophthalmology*. 1992;99:1548–1553.
20. Curcio CA, Presley JB, Malek G, Medeiros NE, Avery DV, Kruth HS. Esterified and unesterified cholesterol in drusen and basal deposits of eyes with age-related maculopathy. *Exp Eye Res*. 2005;81:731–741.
21. Mullins RF, Hageman GS. Human ocular drusen possess novel core domains with a distinct carbohydrate composition. *J Histochem Cytochem*. 1999;47:1533–1540.
22. 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.
23. 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.
24. 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.

25. Johnson LV, Leitner WP, Staples MK, Anderson DH. Complement activation and inflammatory processes in Drusen formation and age related macular degeneration. *Exp Eye Res*. 2001;73:887–896.
26. Hageman GS, Luthert PJ, Victor Chong NH, 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. *Prog Retin Eye Res*. 2001;20:705–732.
27. Friedman E, Smith TR, Kuwabara T. Senile choroidal vascular patterns and drusen. *Arch Ophthalmol*. 1962;69:114–124.
28. Lewis H, Straatsma BR, Foos RY. The prevalence of macular drusen in postmortem eyes. *Am J Ophthalmol*. 1986;102:801–803.
29. Lewis H, Straatsma BR, Foos RY. Chorioretinal juncture: multiple extramacular drusen. *Ophthalmology*. 1986;93:1098–1112.
30. Lewis H, Straatsma BR, Foos RY, Lightfoot DO. Reticular degeneration of the pigment epithelium. *Ophthalmology*. 1985;92:1485–1495.
31. Wang JJ, Foran S, Smith W, Mitchell P. Risk of age-related macular degeneration in eyes with macular drusen or hyperpigmentation: the Blue Mountains Eye Study cohort. *Arch Ophthalmol*. 2003;121:658–663.
32. Sarks S, Cherepanoff S, Killingsworth M, Sarks J. Relationship of basal laminar deposit and membranous debris to the clinical presentation of early age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2007;48:968–977.
33. 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.
34. Curcio CA, Medeiros NE, Millican CL. The Alabama Age-Related Macular Degeneration Grading System for donor eyes. *Invest Ophthalmol Vis Sci*. 1998;39:1085–1096.
35. Li CM, Clark ME, Chimento MF, Curcio CA. Apolipoprotein localization in isolated drusen and retinal apolipoprotein gene expression. *Invest Ophthalmol Vis Sci*. 2006;47:3119–3128.
36. Polyak SL. *The Retina*. Chicago: University of Chicago; 1941.
37. Sarks SH, Arnold JJ, Killingsworth MC, Sarks JP. Early drusen formation in the normal and aging eye and their relation to age related maculopathy: a clinicopathological study. *Br J Ophthalmol*. 1999;83:358–368.
38. Guyton JR, Klemp KF. Ultrastructural discrimination of lipid droplets and vesicles in atherosclerosis: value of osmium-thiocarbohydrazide-osmium and tannic acid-paraphenylenediamine techniques. *J Histochem Cytochem*. 1988;36:1319–1328.
39. Green WR, Enger C. Age-related macular degeneration histopathologic studies. The 1992 Lorenz E. Zimmerman Lecture. *Ophthalmology*. 1993;100:1519–1535.
40. Green WR, McDonnell PJ, Yeo JH. Pathologic features of senile macular degeneration. *Ophthalmology*. 1985;92:615–627.
41. Sarks JP, Sarks SH, Killingsworth MC. Evolution of soft drusen in age-related macular degeneration. *Eye (Lond)*. 1994;8:269–283.
42. Sarks SH. Ageing and degeneration in the macular region: a clinicopathological study. *Br J Ophthalmol*. 1976;60:324–341.
43. Sarks JP, Sarks SH, Killingsworth MC. Evolution of geographic atrophy of the retinal pigment epithelium. *Eye (Lond)*. 1988;2:552–577.
44. Bressler SB, Bressler NM, Sarks S, Sarks J. Age-related macular degeneration: nonneovascular early AMD, intermediate AMD, and geographic atrophy. In: Ryan SJ, Schachat AP, eds. *Retina.* Philadelphia: Elsevier Mosby; 2006:1041–1074.
45. Ruberti JW, Curcio CA, Millican CL, Menco BP, Huang JD, Johnson M. Quick-freeze/deep-etch visualization of age-related lipid accumulation in Bruch's membrane. *Invest Ophthalmol Vis Sci*. 2003;44:1753–1759.
46. Huang JD, Presley JB, Chimento MF, Curcio CA, Johnson M. Age-related changes in human macular Bruch's membrane as seen by quick-freeze/deep-etch. *Exp Eye Res*. 2007;85:202–218.
47. Johnson M, Dabholkar A, Huang JD, Presley JB, Chimento MF, Curcio CA. Comparison of morphology of human macular and peripheral Bruch's membrane in older eyes. *Curr Eye Res*. 2007;32:791–799.
48. Spraul CW, Grossniklaus HE. Characteristics of Drusen and Bruch's membrane in postmortem eyes with age-related macular degeneration. *Arch Ophthalmol*. 1997;115:267–273.
49. Bressler NM, Silva JC, Bressler SB, Fine SL, Green WR. Clinicopathologic correlation of drusen and retinal pigment epithelial abnormalities in age-related macular degeneration. *Retina.* 1994;14:130–142.
50. Sarks SH. Drusen patterns predisposing to geographic atrophy of the retinal pigment epithelium. *Aust J Ophthalmol*. 1982;10:91–97.
51. el Baba F, Green WR, Fleischmann J, Finkelstein D, de la Cruz ZC. Clinicopathologic correlation of lipidization and detachment of the retinal pigment epithelium. *Am J Ophthalmol*. 1986;101:576–583.
52. Penfold PL, Madigan MC, Gillies MC, Provis JM. Immunological and aetiological aspects of macular degeneration. *Prog Retin Eye Res*. 2001;20:385–414.
53. Curcio CA, Millican CL. Basal linear deposit and large drusen are specific for early age-related maculopathy. *Arch Ophthalmol*. 1999;117:329–339.