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Perspective

Bruch's membrane and the vascular intima: is there a common basis for age-related changes and disease?

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Abstract

Several clinical and epidemiological studies have concurrently illuminated established cardiovascular risk factors in age-related macular degeneration (AMD), raising the possibility that cardiovascular disease and AMD may share a similar pathogenic process. The vascular intima and the Bruch's membrane share several age-related changes and are the seat of many common molecules. Diseases of these structures may represent parallel responses to the tissue injury induced by multiple intercalated factors such as genetic variations, oxidative stress, inappropriately directed immune response or inflammatory disease complex. However, there are marked differences in the age-related changes in these two structures. The strategic location of the Bruch's membrane between the retinal pigment epithelium and the choriocapillaris can at least partially explain the differential susceptibility of AMD to cardiovascular risk factors. Unlike the vascular wall that is exposed to changes from the endothelium, the Bruch's membrane is subject to changes from both the endothelium (choriocapillaris) and epithelium (retinal pigment epithelium). Moreover, although both the vascular wall and Bruch's membrane become lipid laden with age, the lipid composition is characteristically different. This review examines the morphological and biochemical alterations in the senescent Bruch's membrane and its analogy to the vascular wall to evaluate the concurrence of atherosclerosis and AMD.

Key words: Bruch's membrane, vascular intima, age-related macular degeneration, atherosclerosis.

Introduction

Age-related macular degeneration (AMD) is the leading cause of severe visual loss in patients above the age of 50 in industrialized countries.[1–3] Despite the high prevalence and public health impact, the aetiology of AMD remains largely unknown. Several clinical and epidemiological studies have concurrently illuminated a few cardiovascular risk factors in AMD such as increasing age, smoking, dyslipidaemia, hypertension, obesity, impaired glucose metabolism and a sedentary lifestyle.[4–6] However, advancing age and smoking are the only proven cardiovascular risk factors for AMD. The impact of other risk factors such as hypertension, diabetes and hypercholestrolaemia on AMD remains inconclusive.[5]

The ageing processes in the Bruch's membrane (BM) and the vascular wall seem to be extricably bound to the pathogenesis of AMD and atherosclerosis, respectively. In his vascular model, Friedman proposed that alterations in the BM are a key factor that explains the association of atherosclerosis and AMD.[4] This review examines the morphological and biochemical alterations in the senescent BM and its analogy to the vascular intima to evaluate the concurrence of atherosclerosis and AMD.

A structural comparison of the BM and the vascular wall

The BM is a thin (2–4 μm) connective tissue interposed between the metabolically active retinal pigment epithelium (RPE) and its source of nutrition, the choriocapillaris. This penta-laminar structure is classically described as consisting of the basement membrane of the RPE, an inner collagenous zone (ICZ), a fenestrated elastic layer, an outer collagenous zone (OCZ) and the basement membrane of the endothelium of the choriocapillaris.[7] In contrast, a healthy arterial wall of a major blood vessel is composed of only three histologically specific layers: the innermost layer (intima) consists of a single layer of endothelial cells; the media is composed of smooth muscle cells, collagen and elastic fibrils; and the third distinct layer is a fibrous adventitia.[8]

In order to investigate the comorbidity of atherosclerosis and AMD, a useful analogy of the BM is the arterial wall of a major blood vessel such as the carotids.[9] Both the fenestrated choriocapillaris and the endothelial monolayer of the arterial wall possess a subcellular stratified extracellular

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matrix (ECM): the BM for the choriocapillaris and the media and adventitia for the vascular intima. Although both these structures have similar ECM, the key difference is the virtual absence of smooth muscle cells in the BM. Moreover, unlike the vascular ECM, the BM is strategically located between an endothelium and an epithelium (RPE) and is therefore subject to changes from both sides. In contrast, the vascular wall matrix is affected by changes caused by endothelial dysfunction only.

Although the cause for endothelial dysfunction of the choriocapillaris is akin to that of atherosclerosis, RPE dysfunction may arise secondary to causes that do not primarily affect its nutrition from the choricapillaris, and this includes oxidative insults, local inflammatory processes, senescence and genetic defects. This may further explain the susceptibility of AMD to cardiovascular risk factors.

Macroscopic changes of the BM and the vascular wall

The fundamental age-related change in both structures is typified by increased thickness. The age-associated thickening of the arterial wall consists mainly of intimal thickening.[10] The carotid intima-media thickness increases two- to threefold between 20 and 90 years of age.[8] Similarly, the BM undergoes diffuse thickening, with thickness being reported to increase by 135% in 10 decades.[7,11] The maximum thickness occurs in the substrata of the OCZ.[12] However, there is marked heterogeneity in the intimal and BM thickness among individuals at a given age.[8,13]

Disease risk is associated with age-related thickness of these structures. Carotid intima-media thickness is used as a predictive non-invasive test for atherosclerotic burden.[14] Likewise, a prolonged choroidal filling phase during fluorescein angiography signals the presence of diffuse BM thickening.[13,15] In addition, serial measurement of the thickness of the BM may be a useful prognostic parameter in longitudinal studies of AMD.

Cell biology of the vascular wall and the BM

The three anatomical changes that occur in the BM with age are the progressive accumulation of debris, lipid deposition and alteration of the ECM. A similar build-up is seen in the arterial wall with accumulation of lipids, cellular waste products, and fibrin and calcium deposits resulting in plaque formation.[10]

The deposition of PAS-positive (periodic-acid-schiff) granular, vesicular and filamentary deposits in the ICZ of BM has been identified as early as the first decade of life.[7] There are three morphological forms of sub-RPE deposits.[16–18] The clinically visible deposits known as drusen are discrete extracellular deposits situated between the basal lamina of RPE and ICZ of BM. They are further subclassified according to their size and structure, with the soft and confluent forms being a clinical hallmark for AMD.

Green and Enger defined the second type of deposits as basal linear deposits (BLD) that form a thin layer of membranous profiles below the RPE.[18] Sarks *et al.* termed them as entrapment sites as these membranous bodies are thought to be released from the basal plasma membrane of the RPE, but are unable to enter the ICZ owing to the tight meshwork of collagen fibrils beneath them.[18–20] Curcio and Millican demonstrated that BLD and large drusen with membranous contents are strongly associated with early age-related maculopathy compared with basal laminar deposits.[21]

Basal laminar deposits are the third type of sub-RPE deposit to be found in the BM, consisting of diffuse heterogenous material that lies internal to the RPE basal lamina.[21] Ultrastructural and histochemical analyses suggest long-spacing collagen to be a dominant constituent of these deposits.[16,21]

As age advances, the debris accumulates and contaminates all the collagenous layer of the BM, and is seen on both sides of the elastic lamina, thus forming the bulk of the age-related debris in the collagenous layers, especially in the OCZ.[12] These deposits may occur simultaneously in some eyes, which may indicate that they have different aetiologies, although histological evidence has failed to clarify the exact cause–effect. Depending on their proximity to the RPE, deposits may represent incompletely digested waste material emanating from a dysfunctional RPE,[22–25] or it may be the sequelae of dysfunctional choriocapillary endothelium.[26] Experimental evidence also suggests that these deposits may be due to altered remodelling of the membrane,[27] or an inappropriately directed immune response.[28,29]

Furthermore, the cell biology of the arterial intima suggests abundant granulovesicular debris in the intima, probably originating from disintegration of vascular smooth muscle cells and dead macrophage foam cells.[30] Although the source of the deposits in both diseases may not be directly linked, a common component may be that the cellular debris in both structures acts as a focus for inflammation and local immune response.

Molecular composition of drusen and atherosclerotic deposits

The link between atherosclerosis and AMD is further substantiated by the similarity of molecular composition of drusen and arteriosclerotic deposits.[31] The proteins identified in the drusen/BM include both locally derived (neural retina, RPE and choroid) and extracellular non-ocular components. The oxidative modifications of some of these components may also be the primary catalyst in drusen formation. Most of these components are also present in atherosclerotic lesions.

The complement system plays a key role in the body's immune response. C5b-9 complexes have been isolated in intact cells, disintegrated cells and cell debris enmeshed in the ECM of thickened vascular intima and atherosclerotic plaque.[32] Some of these cells represent activated or dead macrophages. They trigger inflammatory events and pro-

gression of atherosclerotic lesions. Likewise, immunohistochemical analyses of all phenotypes of drusen and the BM have also revealed C3, the terminal complement complex C5 and the membrane attack complex C5b-9 terminal complexes. Although C3 and C5 mRNA are present in the neural retina, RPE and choroids,[33] the source of most of the complement factors may be from plasma. In addition, several complement inhibitors have been identified in drusen such as clusterin, CD46 and CR1.[34] The localization of these complement proteins and their regulators provide evidence of local inflammatory response. The granulovesicular nuclear fragments, oxidized lipoproteins and advanced glycation end-products may be potential activators of the complement cascade in both the vascular intima and BM.[28,35,36] The fact that immunological injury may be an initiating factor in the development of AMD and atherosclerosis is further substantiated by the isolation of human leucocyte antigen markers in drusen and atherosclerotic lesions.[37,38] However, the multifactorial nature of both disease processes makes a simple relationship with a single major histocompatibility complex determinant unlikely.

Lipid accumulation

An important age-related change that forms the base for atherosclerosis is lipid accumulation in the thickened intima. In the arterial intima, plasma lipoproteins are transported across the endothelium and trapped among fibrils of ECM.[39] These initial lesions are characterized by microscopically visible lipid droplets that coalesce to form fatty streaks, and finally stratified pools of lipid are found in the intimal thickening.

A similar age-related exponential accumulation of lipids occurs in the BM. The BM becomes sudanophilic and exhibits increased staining with Oil red O.[40–44] In eyes of patients less than 60 years old, these lipid-containing small round solid particles are scarcely scattered in both collagen layers. In older eyes, the lipid droplets occupy up to one-third of the ICZ and also form a thin lipid layer external to the RPE basement membrane.[45]

There is circumstantial evidence that ultrastuctural changes of the BM, especially the lipid deposition and thickening and reduced basal convolutions of the RPE basal lamina,[45,46] result in an exponential decline in hydraulic conductivity of the BM with age.[47,48] The combination of greater volume of intervening material together with the greater separation of the choriocapillaris from RPE contributes to the decrease in exchange efficiency between these structures and aids deposition of material in the BM.[49]

Although both the BM and the arterial intima become lipid laden with age, the lipid composition is dissimilar. Moreover, there is a wide variation of the nature and proportion of lipids deposited in the BM. Unlike the plasma-derived low-density lipoproteins infiltration in the vascular intima, the predominant lipids in the BM consist of phospholipids and fatty acids.[40–44] Approximately 50% of the phospholipids are phosphatidylcholine, suggesting that the lipids in the BM are more likely of a cellular origin (a potential source being the photoreceptor outer segment membranes) than from plasma. However, recent studies using filipin histochemistry and hot stage polarizing microscopy revealed that lipid contents of BM consist of both esterified and unesterified cholesterol. This suggests a vascular origin akin to atherosclerosis.[8,45,50] Both polyunsaturated and monounsaturated cholesterol esters were found distributed throughout BM and localized to drusen. However, saturated fatty acids were not seen in the BM.[50] This further substantiates the fact that unlike the vascular intima, the BM is subjected to noxious agents from both the epithelial and endothelial sides.

The cholesterol transporters, apolipoprotein E (ApoE) and ApoB, are components of drusen and ApoE has also been identified in atherosclerotic plaque.[51–53] The inheritance of ApoE4 alleles is linked to the age of onset in atherosclerosis with ApoE4 carriers being highly susceptible to atherosclerosis. In contrast, the allele E4 has a protective effect on AMD.[54] In addition to plasma ApoE, the RPE is a source of ApoE mRNA and it could be involved in local lipid homeostasis and a local disruption could result in an lipid accumulation in the BM.[55]

Matrix disregulation

The matrix changes associated with age-related arterial changes include: increased collagen content, increased collagen cross-linking, increased fibronectin, decreased elastin associated with calcification and fragmentation of the elastic lamina, and increased glycosaminoglycans (GAG).[56] Similar changes are observed in the ageing BM.

Collagen synthesis increases with age in both structures.[57,58] Types I and III collagens form the bulk of the total plaque protein in atherosclerosis.[57] Likewise, Newsome *et al.* found an age-related increase in collagen I in the BM with age.[27] Other collagens noted in the BM include types III, IV and V.[58] In addition, atypical banding periodicity has been observed in the collagen produced. The ICZ consists more of 640A type collagen and the OCZ contains more 1000A type collagen with age.[7] The meshes formed by the tightly interwoven collagen fibres in the ICZ also become irregular and coarse with age.[59] The long-spacing collagen is a material with periodicity ranging between 100 and 140 nm found mainly in the OCZ and extends as intercapillary pegs to areas where the choriocapillaris has undergone age-related atrophy.[60] This material has also been isolated in the aortic media.[61] Moreover, increased cross-linking results in a linear decline of solubility of the collagens with age.[57,58] In both structures, the increased insoluble collagen may contribute to debris accumulation and serve as a depot for lipoproteins, growth factors and cytokines.

The amount of non-collagen protein in BM also increases significantly with age as suggested by an increase in deposition of non-collagen amino acids in the macular area.[58] The elastic layer becomes basophilic; the elastic fibres increase in number and become more electron dense. Needle-like crys-

tals are deposited within the fibres.[7] The BM also undergoes calcification and fragmentation, rendering the membrane to loose its elasticity and become more brittle. The degree of calcification and fragmentation of BM correlates with the severity of wet AMD, although it is seen in both types of AMD.[62]

Similar changes occur in the elastic layer of the arterial wall. The exact mechanism of calcification of the elastic fibres is not known, but it may be the result of production of abnormal elastic fibres, degeneration of elastic fibres or other alterations in the ECM.[63] Degradation of the arterial wall elastin is a characteristic feature in atherogenesis. Serum concentration of elastin-derived peptides is elevated in atherosclerotic patients and reflects elastin turnover.[64] Increased serum concentration of elastin-derived peptides is a potential indicator of advanced atherosclerosis such as plaque instability and is also a predictor of rupture in atherosclerotic aortic aneurysms.[65]

Changes in GAG also occur in the ECM of both the vascular intima and BM. Sulphated GAG, which are components of the vascular matrix, increase during the early stages of atherosclerosis.[66] Binding of dermatan sulphates to low-density lipoproteins correlates positively with lipid accumulation in the intima and progression of atherosclerotic lesions, respectively. Chondroitin sulphate normally retards the passage of plasma particles and maintains the viscoelastic property of the vessel wall that is structurally altered in advanced lesions.[67] In contrast, heparan sulphate, a major component of basement membrane, decreases with the severity of atherosclerotic lesions.[68] These changes do not correspond with changes in the BM. An increase in the content and structure of GAG in BM has been identified.[69] The increased volume of GAG may partially be responsible for the altered metabolism of the collagens and the resultant increased negative field may also contribute to the deceased filtration across the BM.[70] An increased proportion of heparan sulphate in the basement membranes of the BM and simultaneous decrease in proteoglycan filaments containing chondroitin sulphate and dermatan sulphate associated with the collagen fibrils have been noted.[70,71] It may be that the main source of dermatan sulphate in the intima is the smooth muscle cells and the absence of these cells in the BM correlates with its decrease. In addition, there may be differences in macrophage recruitment and accumulation in the two structures and proteolytic degradation by the macrophages may account for the significant differences in changes in proteoglycan subtypes. The difference in the proteoglycan subtypes may partially be responsible for the altered lipid content in these structures.

Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are required by both BM and arterial intima for ECM remodelling, protein processing and angiogenesis.[72] The age-related increased collagen content, the release of reactive oxygen radicals by the lipid-laden BM and intima, and the presence of inflammatory cytokines, cellular transformation and growth hormones in both the structures may upregulate these enzymes, especially MMP-2 and MMP-9.[73,74] Both MMP-2 and MMP-9 are gelatinases and readily digest denatured collagen. In addition, MMP-2 digest types I, II and III collagens.[72] Localization of MMP-2 and MMP-9 expression to areas of new vessel formation in the BM and to areas of adventitial vasovasorum suggests their role in the growth of neovascular complexes in both AMD and atherosclerosis.[74,75]

One of the endogenous tissue inhibitors of MMPs (TIMPs) regulates the activation of MMPs and also has other independent actions.[74] Of the four TIMPs characterized to date, TIMP-3 is the only member found exclusively in ECM explaining the presence of TIMP-3 in both the intima and BM.[74] In addition, it binds tightly to sulphated GAG. Western blotting and quantitative reverse zymography have demonstrated an age-related increase in TIMP-3 in BM and its concentration is shown to correlate with the amount of ECM and the quantity of drusen.[76] In BM, TIMP-3 controls ECM turnover, limits neovascularization[77] and may play a role in apoptosis.[78] Mutations of TIMP-3 associated with Sorsby's fundus dystrophy suggest that TIMP-3 may result in aberrant protein interaction and increased cell adhesiveness, which may cause defective turnover of the BM.[79] A similar upregulation of TIMP-3 in atherosclerotic aorta has been noted.[80]

Conclusion

It is clear that the vascular intima and the BM share several age-related changes with the involvement of several common molecules. This may be because they are exposed to similar genetic variations, oxidative stress, or immune or inflammatory disease complexes. On the basis of this review, one may argue that atherosclerosis and AMD may share some aspects in a response to the resultant tissue damage.

References

1. Vingerling JR, Dielemans I, Hofman A *et al.* The prevalence of age-related maculopathy in the Rotterdam Study. *Ophthalmology* 1995; **102**: 205–10.
2. Mitchell P, Smith W, Attebo K, Wang JJ. Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. *Ophthalmology* 1995; **102**: 1450–60.
3. Klein R, Klein BE, Jensen SC, Meuer SM. The five-year incidence and progression of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology* 1997; **104**: 7–21.
4. Friedman E. The role of the atherosclerotic process in the pathogenesis of age-related macular degeneration. *Am J Ophthalmol* 2000; **130**: 658–63.
5. Evans JR. Risk factors for age-related macular degeneration. *Prog Retin Eye Res* 2001; **20**: 227–53.
6. Snow KK, Seddon JM. Do age-related macular degeneration and cardiovascular disease share common antecedents? *Ophthalmic Epidemiol* 1999; **6**: 125–43.

7. Hogan MJ, Alvarado J. Studies on the human macula. IV. Aging changes in Bruch's membrane. *Arch Ophthalmol* 1967; **77**: 410–20.
8. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a 'set up' for vascular disease. *Circulation* 2003; **107**: 139–46.
9. Curcio CA, Millican CL, Bailey T, Kruth HS. Accumulation of cholesterol with age in human Bruch's membrane. *Invest Ophthalmol Vis Sci* 2001; **42**: 265–74.
10. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 2000; **20**: 1262–75.
11. Ramrattan RS, van der Schaft TL, Mooy CM, de Bruijn WC, Mulder PG, de Jong PT. Morphometric analysis of Bruch's membrane, the choriocapillaris, and the choroid in aging. *Invest Ophthalmol Vis Sci* 1994; **35**: 2857–64.
12. Killingsworth MC. Age-related components of Bruch's membrane in the human eye. *Graefes Arch Clin Exp Ophthalmol* 1987; **225**: 406–12.
13. Bird AC. Bruch's membrane change with age. *Br J Ophthalmol* 1992; **76**: 166–8.
14. Meschia JF, Gerber TC. Editorial comment – vascular thickness and calcification as markers of atherosclerotic burden. *Stroke* 2003; **34**: 2372–3.
15. Pauleikhoff D, Chen J, Bird AC, Wessing A. The Bruch membrane and choroid. Angiography and functional characteristics in age-related changes. *Ophthalmology* 1992; **89**: 39–44.
16. Sarks SH. Ageing and degeneration in the macular region: a clinico-pathological study. *Br J Ophthalmol* 1976; **60**: 324–41.
17. Feeney-Burns L, Ellersieck MR. Age-related changes in the ultrastructure of Bruch's membrane. *Am J Ophthalmol* 1985; **100**: 686–97.
18. Green WR, Enger C. Age-related macular degeneration histopathologic studies. The 1992 Lorenz E. Zimmerman Lecture. *Ophthalmology* 1993; **100**: 1519–35.
19. 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**: 358–68.
20. Loeffler KU, Lee WR. Basal linear deposit in the human macula. *Graefes Arch Clin Exp Ophthalmol* 1986; **224**: 493–501.
21. Curcio CA, Millican CL. Basal linear deposit and large drusen are specific for early age-related maculopathy. *Arch Ophthalmol* 1999; **117**: 329–39.
22. Burns RP, Feeney-Burns L. Clinico-morphologic correlations of drusen of Bruch's membrane. *Trans Am Ophthalmol Soc* 1980; **78**: 206–25.
23. Ishibashi T, Sorgente N, Patterson R, Ryan SJ. Aging changes in Bruch's membrane of monkeys: an electron microscopic study. *Ophthalmologica* 1986; **192**: 179–90.
24. Young RW. Pathophysiology of age-related macular degeneration. *Surv Ophthalmol* 1987; **31**: 291–306.
25. 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**: 576–83.
26. Friedman E, Smith TR, Kuwabara T. Senile choroidal vascular patterns and drusen. *Arch Ophthalmol* 1963; **69**: 220–30.
27. Newsome DA, Hewitt AT, Huh W, Robey PG, Hassell JR. Detection of specific extracellular matrix molecules in drusen, Bruch's membrane, and ciliary body. *Am J Ophthalmol* 1987; **104**: 373–81.
28. 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**: 705–32.
29. Penfold PL, Madigan MC, Gillies MC, Provis JM. Immunological and aetiological aspects of macular degeneration. *Prog Retin Eye Res* 2001; **20**: 385–414.
30. Klurfeld DM. Identification of foam cells in human atherosclerotic lesions as macrophages using monoclonal antibodies. *Arch Pathol Lab Med* 1985; **109**: 445–9.
31. 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**: 835–46.
32. Rus HG, Niculescu F, Vlaicu R. The relationship between macrophages and C5b-9 complement complexes in human atherosclerosis. *Clin Immunol Immunopathol* 1988; **48**: 307–16.
33. 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**: 411–31.
34. 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**: 887–96.
35. Hansson GK, Libby P, Schonbeck U, Yan ZQ. Innate and adaptive immunity in the pathogenesis of atherosclerosis. *Circ Res* 2002; **91**: 281–91.
36. Libby P. Inflammation in atherosclerosis. *Nature* 2002; **420**: 868–74.
37. Dahlen GH, Slunga L, Lindblom B. Importance of Lp(a) lipoprotein and HLA genotypes in atherosclerosis and diabetes. *Clin Genet* 1994; **46** (1 Spec No.): 46–51.
38. Goverdhan SWM, Howell H, Bacon IH, Chisholm K, Avery AJ. Lotery association of HLA polymorphisms in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2004; **45**: E-Abstract 1822.
39. Berliner JA, Navab M, Fogelman AM *et al.* Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. *Circulation* 1995; **91**: 2488–96.
40. Holz FG, Sheraidah G, Pauleikhoff D, Bird AC. Analysis of lipid deposits extracted from human macular and peripheral Bruch's membrane. *Arch Ophthalmol* 1994; **112**: 402–6.
41. Spaide RF, Ho-Spaide WC, Browne RW, Armstrong D. Characterization of peroxidized lipids in Bruch's membrane. *Retina* 1999; **19**: 141–7.
42. Farkas TG, Sylvester V, Archer D, Altona M. The histochemistry of drusen. *Am J Ophthalmol* 1971; **71**: 1206–15.
43. Sheraidah G, Steinmetz R, Maguire J, Pauleikhoff D, Marshall J, Bird AC. Correlation between lipids extracted from Bruch's membrane and age. *Ophthalmology* 1993; **100**: 47–51.
44. 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**: 1548–53.
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**: 1753–9.
46. Moore DJ, Clover GM. The effect of age on the macromolecular permeability of human Bruch's membrane. *Invest Ophthalmol Vis Sci* 2001; **42**: 2970–5.
47. Starita C, Hussain AA, Pagliarini S, Marshall J. Hydrodynamics of ageing Bruch's membrane: implications for macular disease. *Exp Eye Res* 1996; **62**: 565–72.
48. Fisher RF. The influence of age on some ocular basement membranes. *Eye* 1987; **1**: 184–9.
49. Marshall J. The ageing retina: physiology or pathology. *Eye* 1987; **1**: 282–95.
50. Haimovici R, Gantz DL, Rumelt S, Freddo TF, Small DM. The lipid composition of drusen, Bruch's membrane, and sclera by hot stage polarizing light microscopy. *Invest Ophthalmol Vis Sci* 2001; **42**: 1592–9.
51. Pahor M, Elam MB, Garrison RJ, Kritchevsky SB, Applegate WB. Emerging noninvasive biochemical measures to predict cardiovascular risk. *Arch Intern Med* 1999; **159**: 237–45.
52. 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**: 1252.
53. 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**: 413–25.
54. Baird PN, Guida E, Chu DT, Vu HT, Guymer RH. The 2 and 4 alleles of the apolipoprotein gene are associated with age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2004; **45**: 1311–15.
55. 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**: 767–81.
56. Goncalves I, Moses J, Dias N *et al.* Changes related to age and cerebrovascular symptoms in the extracellular matrix of human carotid plaques. *Stroke* 2003; **34**: 616–22.
57. Barnes MJ, Farndale RW. Collagens and atherosclerosis. *Exp Gerontol* 1999; **34**: 513–25.
58. Karwatowski WS, Jeffries TE, Duance VC, Albon J, Bailey AJ, Easty DL. Preparation of Bruch's membrane and analysis of the age-related changes in the structural collagens. *Br J Ophthalmol* 1995; **79**: 944–52.
59. Yamamoto T, Yamashita H. Scanning electron microscopic observation of Bruch's membrane with the osmium tetroxide treatment. *Br J Ophthalmol* 1989; **73**: 162–7.
60. Krey H. Morphology of the peripheral choriocapillaris in patients of various ages. *Klin Monatsbl Augenheilkd* 1975; **167**: 295–301.
61. Morris CJ, Bradby GV, Walton KW. Fibrous long-spacing collagen in human atherosclerosis. *Atherosclerosis* 1978; **31**: 345–54.
62. Spraul CW, Lang GE, Grossniklaus HE, Lang GK. Histologic and morphometric analysis of the choroid, Bruch's membrane, and retinal pigment epithelium in postmortem eyes with age-related macular degeneration and histologic examination of surgically excised choroidal neovascular membranes. *Surv Ophthalmol* 1999; **44** (Suppl. 1): S10–32.
63. Walker ER, Frederickson RG, Mayes MD. The mineralization of elastic fibers and alterations of extracellular matrix in pseudoxanthoma elasticum. Ultrastructure, immunocytochemistry, and X-ray analysis. *Arch Dermatol* 1989; **125**: 70–6.
64. Baydanoff S, Nicoloff G, Alexiev C. Age related changes in the level of circulating elastin-derived peptides in serum from normal and atherosclerotic subjects. *Atherosclerosis* 1987; **66**: 163–8.
65. Petersen E, Wagberg F, Angquist KA. Serum concentrations of elastin-derived peptides in patients with specific manifestations of atherosclerotic disease. *Eur J Vasc Endovasc Surg* 2002; **24**: 440–4.
66. Stevens RL, Colombo M, Gonzales JJ, Hollander W, Schmid K. The glycosaminoglycans of the human artery and their changes in atherosclerosis. *J Clin Invest* 1976; **58**: 470–81.
67. Stary HC, Chandler AB, Glagov S *et al.* Special report: a definition of initial, fatty streak and intermediate lesions in atherosclerosis: a report from the committee on vascular lesions of the council on arteriosclerosis, American heart association. *Arterioscler Thromb* 1994; **89**: 2462–78.
68. Williams KJ, Tabas I. The response to retention hypothesis of early atherogenesis. *Arterioscler Thromb Vasc Biol* 1995; **15**: 551–61.
69. Kliffen M, Mooy CM, Luider TM, Huijmans JG, Kerkvliet S, de Jong PT. Identification of glycosaminoglycans in age-related macular deposits. *Arch Ophthalmol* 1996; **114**: 1009–14.
70. Hewitt AT, Nakazawa K, Newsome DA. Analysis of newly synthesized Bruch's membrane proteoglycans. *Invest Ophthalmol Vis Sci* 1989; **30**: 478–86.
71. Takada Y, Ohkuma H, Ogata N, Matsushima M, Sugasawa K, Uyama M. [Proteoglycan in Bruch's membrane of senescence accelerated mouse: localization and age-related changes.] *Nippon Ganka Gakkai Zasshi* 1994; **98**: 469–76 (in Japanese).
72. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res* 2003; **92**: 827–39.
73. Sethi CS, Bailey TA, Luthert PJ, Chong NH. Matrix metalloproteinase biology applied to vitreoretinal disorders. *Br J Ophthalmol* 2000; **84**: 654–66.
74. George SJ. Tissue inhibitors of metalloproteinases and metalloproteinases in atherosclerosis. *Curr Opin Lipidol* 1998; **9**: 413–23.
75. Steen B, Sejersen S, Berglin L, Seregard S, Kvanta A. Matrix metalloproteinases and metalloproteinase inhibitors in choroidal neovascular membranes. *Invest Ophthalmol Vis Sci* 1998; **39**: 2194–200.
76. Kamei M, Hollyfield JG. TIMP-3 in Bruch's membrane: changes during aging and in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 1999; **40**: 2367–75.
77. Moses MA, Langer R. A metalloproteinase inhibitor as an inhibitor of neovascularization. *J Cell Biochem* 1991; **47**: 230–5.
78. Baker AH, Zaltsman AB, George SJ, Newby AC. Divergent effects of tissue inhibitor of metalloproteinase-1, -2, or -3 overexpression on rat vascular smooth muscle cell invasion, proliferation, and death in vitro. TIMP-3 promotes apoptosis. *J Clin Invest* 1998; **101**: 1478–87.
79. Yeow KM, Kishnani NS, Hutton M, Hawkes SP, Murphy G, Edwards DR. Sorsby's fundus dystrophy tissue inhibitor of metalloproteinases-3 (TIMP-3) mutants have unimpaired matrix metalloproteinase inhibitory activities, but affect cell adhesion to the extracellular matrix. *Matrix Biol* 2002; **21**: 75–88.
80. Carrell TW, Burnand KG, Wells GM. Clements JM, Smith A. Stromelysin-1 (matrix metalloproteinase-3) and tissue inhibitor of metalloproteinase-3 are overexpressed in the wall of abdominal aortic aneurysms. *Circulation* 2002; **105**: 477–82.