# Therapeutic targets in age-related macular disease

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**Age-related macular disease (AMD) accounts for more than 50% of blind registration in Western society. Patients with AMD are classified as having early disease, in which visual function is well preserved, or late disease, in which central vision is lost. Until recently, there was no therapy available by which the course of the disorder could be modified. Now, the most common form of late-stage AMD — choroidal neovascularization — responds to treatment with anti-VEGF therapies; although visual loss is modified in a portion of these cases, no therapeutic approach exists that alters the evolution from early to late disease. However, as discussed in this Review, research over the last few years has demonstrated several features of AMD that are likely to be amenable to treatment. Potential targets for treatment are described, and possible therapeutic approaches are discussed.**

Age-related macular disease (AMD) is a common ocular condition characterized by loss of vision in the center of the visual field as a result of damage to the retina. It usually affects adults over the age of 50 years. Importantly, AMD is recognized as causing more than 50% of blind registration in Western society and is now designated as one of the major blinding diseases in the world (1–4). In contrast to the current high prevalence of AMD, in the 19th century, it was considered a rare disorder and described as "chorioretinal disease in senile persons" (5). There is evidence that the current increased prevalence of AMD cannot be fully explained by increased life expectancy (6). Furthermore, the disease is now recognized as a burden in societies in which it was considered rare 30 years ago. Low prevalence of AMD in some populations not of European descent has been well documented in the past (7, 8). However, the disorder has become common in the last 2 decades in urban communities in Japan (9–12), and in the last 3 years, the rate of hospital referrals has doubled (M. Uyama, personal communication). Publications imply that a similar change in prevalence may be occurring elsewhere in east Asia (13–15). There is also a strikingly high prevalence of macular disease in elderly Inuit in Greenland (16, 17).

For many years, familial involvement in AMD had been acknowledged, and numerous genes have been identified recently as conferring risk for developing the disease, including complement factor H (*CFH*), *CFB*, complement component 2 (*C2*), *C3*, and the age-related maculopathy susceptibility 2/HtrA serine peptidase 1 (*ARMS2/HTRA1*) gene locus at 10q26 (18–25). As most of these genes encode proteins involved in the complement cascade, which contributes to pathogen clearance by the innate immune system, it seems probable that dysregulation of the innate immune system plays an important role in the pathogenesis of AMD. Of particular interest is CFH. One polymorphism in CFH that generates the Y402H CFH variant is found in 50% of individuals with AMD and is predicted to cause deregulation of the complement cascade (18–25). Protective *CFH* haplotypes also exist (21, 26). Moreover, mutations in *CFH* cause type 2 mesangiocapillary nephropathy, a condition characterized by thickening of the basement membrane of the renal glomeruli and changes to the fundus (the interior surface of the eye, opposite the lens) that simulate AMD (27). In addi-

tion to the genetic component of AMD risk, environmental factors play a role in disease development (28). There is overwhelming evidence that both smoking and obesity confer risk and that they are additive to the genetic influences (29).

The ocular structures involved in the disease process are photoreceptor cells, specialized neurons in the outer retina responsible for converting photons of light into an action potential; retinal pigment epithelium (RPE), the monolayer of polar cells that gives metabolic support to the photoreceptor cells; Bruch membrane, a complex of collagen and elastic layers interposed between the RPE and choroid; and choriocapillaris, the capillary bed in the inner choroid that provides the metabolic needs of the outer retina (Figure 1). These structures are metabolically interdependent, and there is considerable metabolic exchange across the Bruch membrane. The outer segment of the photoreceptor cell is composed of a stack of membranes formed as discs, and these contain the pigment that absorbs photons of light and initiates the visual process. Photoreceptor cells consist of 2 types: cones mediate vision in bright light (i.e., photopic vision), and rods mediate vision in dim light (i.e., scotopic vision). Each day, the distal part of the outer segment of each photoreceptor cell is shed and phagocytosed by the RPE. The phagosome merges with lysosomes to form a phagolysosome. Lysosomal enzymes degrade the material in the phagosome, and some of the material is recycled back to the photoreceptor cells to form new outer segment discs. The remaining degraded material is believed to be discharged outward into the Bruch membrane to be cleared by the choriocapillaris. Undegraded material in the RPE forms electron-dense residual bodies, which accumulate in the RPE with age. An important component of the residual body is composed of combinations of all-trans retinal and ethanolamine (A2-E), is autofluorescent, and is highly resistant to degradation. The formation of this fluorophore is initiated in the outer segment. It is thought that all-trans retinal is released from photopigments into the intradiscal space after bleaching and transferred to the cytoplasmic space after binding to the membrane lipid phosphatidyl ethanolamine. If 2 molecules of all-trans retinal bind to the membrane lipid, the complex is not flipped. As the discs become older, there are increasing amounts of retinal/ethanolamine combinations. The compound is taken into the RPE cell during phagocytosis, whereupon it is converted into lipofuscin in the acidic environment of the phagolysosome. These processes become dysregulated in AMD,
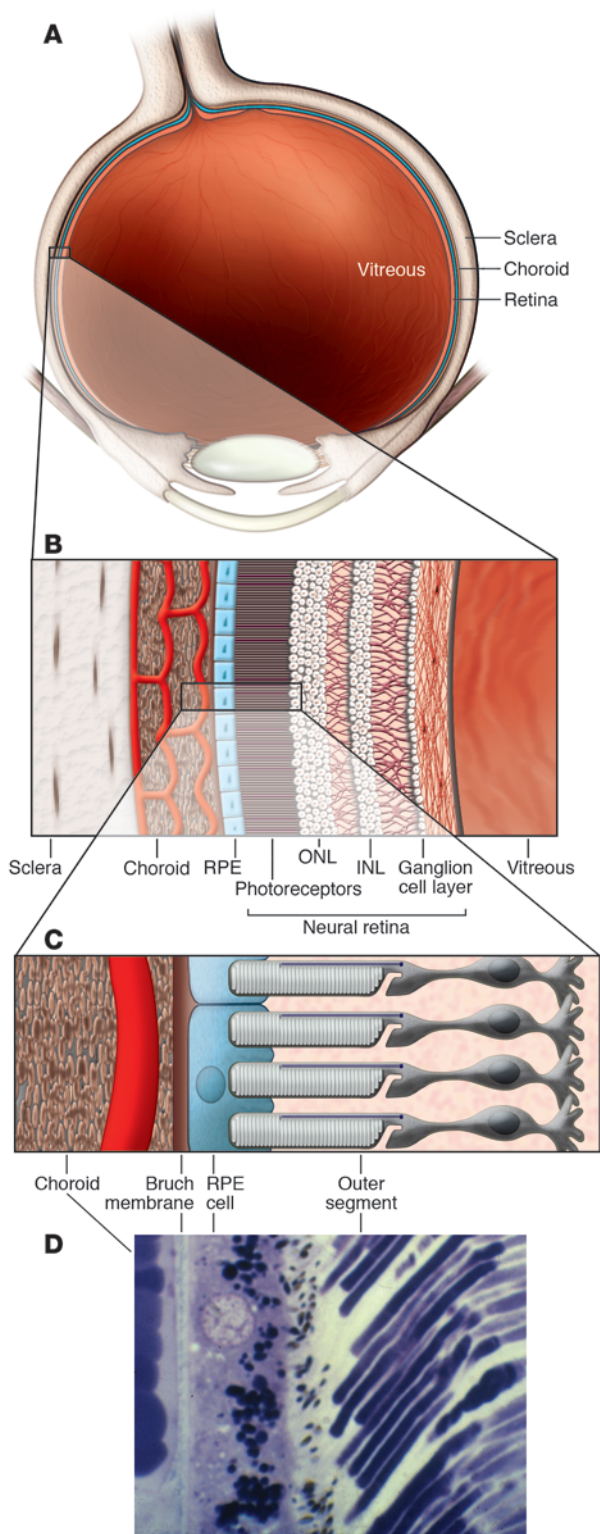
**Figure 1**
Ocular anatomy relevant to AMD. (**A**) Cross-section of an eye showing the retina lining the inside of the eye. (**B**) Cross-section of the retina showing the neural retina and, external to it, the RPE and choroid. INL, inner nuclear layer; ONL, outer nuclear layer. (**C** and **D**) Diagram (**C**) and light microscopic view (**D**) of retinal tissues involved in AMD. The apical microvilli of the RPE interdigitate with the distal portions of the photoreceptor outer segments. External to the RPE is the choroid, with Bruch membrane interposed between them.

However, in the fundus, focal deposits called drusen are seen in the Bruch membrane. Their distribution and size varies from one patient to another, although these attributes are highly concordant between the eyes of an individual. There may also be irregular pigmentation of the RPE.

The 3 forms of late-stage AMD cause loss of central vision. The most common form is choroidal neovascularization (CNV), which occurs when blood vessels of the choriocapillaris grow inward into or through the Bruch membrane (Figure 2, A and B). Retinal pigment epithelial detachment (PED), in which fluid accumulates between the RPE and Bruch membrane (Figure 2C), is relatively uncommon. In geographic atrophy (GA), there is well-defined loss of RPE and photoreceptor cells. GA is generally considered to be the default pathway of the disease process, whereas PED and CNV occur as reactive events during its evolution. Successful treatment exists for CNV, but no proven therapy is available for PED or GA, and there is no way to manipulate the events that occur in early-stage disease. In this Review, I attempt to identify therapeutic targets upon which new treatment strategies might be based. Because AMD is a multifactorial disorder in which risk factors are common in the community, it is difficult to distinguish qualitatively between age-related changes and AMD, and it is likely that the difference is only the severity of the changes. As a consequence, many studies have concentrated on age-related changes alone, on the assumption that the findings will further the understanding of AMD.

## Structural changes to the choroid in AMD

There is relatively little literature on histopathological changes in the choriocapillaris in individuals with AMD because of the paucity of specimens examined. In young healthy individuals, the choriocapillaris is formed of a sinusoidal complex, which is fenestrated and lacks tight junctions. It is believed that the development of the choriocapillaris is determined in part by outward constitutive VEGF expression by the RPE (30–34).

One morphometric study found that the density of the choriocapillaris decreases with age in eyes without AMD (35). In another study, neoprene casts were used to show the change with age from a sinusoidal system to a tubular vascular system (Figure 3 and ref. 36). In advanced AMD, loss and narrowing of the choriocapillaris occurs (37–40).

Sorsby fundus dystrophy is a monogenic macular degeneration disorder that causes loss of central vision in the third or fourth decade of life; it shares many pathological features with AMD (41). It is characterized by major thickening of the Bruch membrane and a prolonged choroidal filling phase upon fluorescein angiography (42). Normally, when fluorescein sodium is injected intravenously, the dye leaks freely from the choriocapillaris and becomes bound to polar compounds in the Bruch membrane. When photographed, this gives a homogeneous background fluorescence within 1 second of dye entry into the ocular circulation. In Sorsby

and changes occur in these tissues throughout the eye, although they are most marked at the macula, which subserves central vision and contains a high density of cones.

AMD can be divided clinically into early and late stages. In early-stage disease, there are few symptoms, and visual acuity is normal.
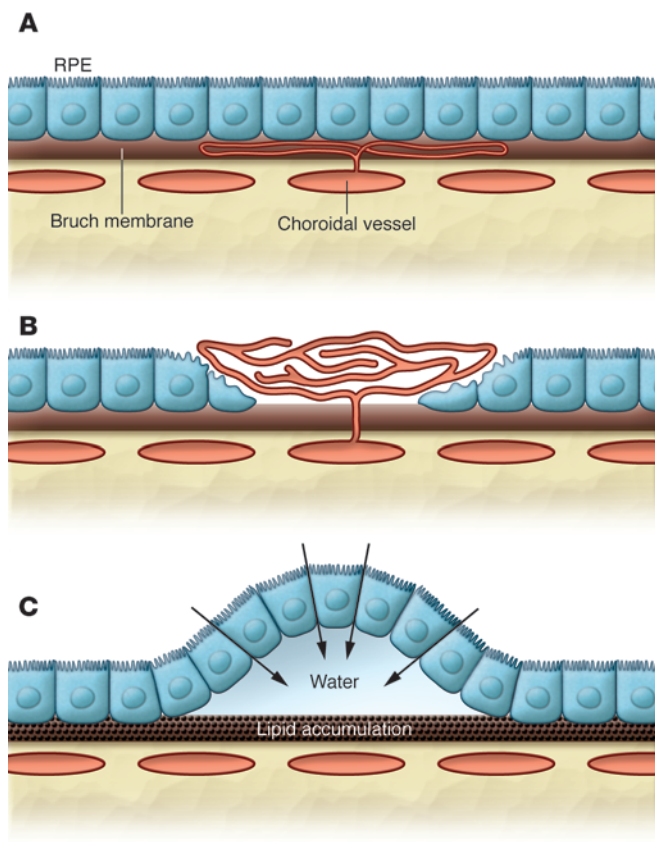
**Figure 2**
Exudative lesions in AMD. (**A** and **B**) In CNV, blood vessels grow inward toward the choroid and may proliferate within Bruch membrane (**A**) or transgress the RPE to grow in the subretinal space (**B**). (**C**) In RPE detachment, lipid accumulates in Bruch membrane, rendering it hydrophobic. The RPE moves water outward toward choroid (arrows); as it fails to pass freely outward, the water induces detachment of the RPE from Bruch membrane.

sequent reduction of metabolic supply to the outer retina may play an important contributory role to the generation of disease. The choroid is not currently seen as a good target for therapy.

## Structural changes to the Bruch membrane in AMD

A direct relationship between aging and thickness of Bruch membrane has been established by both electron and light microscopy (49, 50). However, in one study, comparing Bruch membrane thickness with age showed great variation in the elderly on both electron microscopy and light microscopy (51). Thus, about half of the change in thickness must be explained by factors other than age, such as genetic or environmental influences. The thickening is thought to be caused, at least in part, by incomplete clearance of waste material discharged outward by the RPE, causing deposit buildup.

Several studies on the nature of the deposits have been undertaken. Clues concerning content and its potential influence on the function of thickened Bruch membrane were consequent upon discussion of the pathogenesis of PED. There is constant outward movement of ions, and therefore water, from the outer retina toward choroid, and it was hypothesized that reduced hydraulic conductivity of the Bruch membrane would hamper movement of water toward the choroid, causing it to accumulate in the sub-RPE space (Figure 2C and ref. 52). This concept demands that the Bruch membrane contain high lipid content that would increase the resistance of fluid flow. A series of investigations was undertaken to test these hypotheses, and support was derived from histopathological, biochemical, biophysical, and clinical observations (53–59). A study of frozen tissue using histochemical staining on human donor eyes from individuals ranging in age from 1 to 95 years showed accumulation of lipids with age that varied greatly in both quantity and form of lipids in the elderly (Figure 4 and ref. 53). Some eyes stained for neutral lipids alone, some stained predominantly for phospholipids, and others stained equally for both neutral lipids and phospholipids. To confirm these observations, material extracted by lipid solvents from tissue of fresh eye-bank eyes was analyzed by thin-layer and gas chromatography (54, 55). After separation, the chemical species were identified by mass spectroscopy. This study confirmed that the quantity of total lipid in the Bruch membrane increases with age. Little or no lipid was

fundus dystrophy, the background fluorescence may take more than 10 seconds to become continuous. This delay is likely caused by loss of fenestrae and changes in the capillary bed from a sinusoidal to a tubular state. It is thought that the diffusely thickened Bruch membrane represents a barrier to diffusion of VEGF toward the choroid, resulting in changes in the capillary bed (43, 44). This angiographic abnormality has also been identified in patients with early AMD (45). The potential significance of this clinical observation has been established by the demonstration that discrete areas of the retina exhibit threshold elevation for vision under low-light conditions (i.e., scotopic vision) of up to 3.4 log units as well as slow dark adaptation (of which the patients are symptomatic), which correspond closely to regions of abnormal choroidal perfusion (46, 47). Loss of photopic sensitivity was less marked.

Modification of the choroid in AMD is most likely to be a response to alteration of neighboring tissues, although the alternative — that there may be intrinsic change — has been suggested (48). The con-



**Figure 3**
Scanning electron images of the inner surface of choroidal casts. (**A**) In a young donor, the choriocapillaris is a dense sinusoidal network, with no view of the larger vessels of the outer choroid. (**B**) In an 87-year-old subject, the capillaries are tubular, with large gaps between the capillaries. Figure reproduced courtesy of J. Olver.
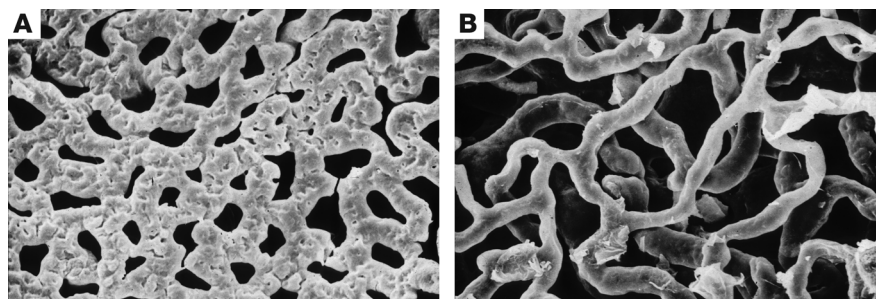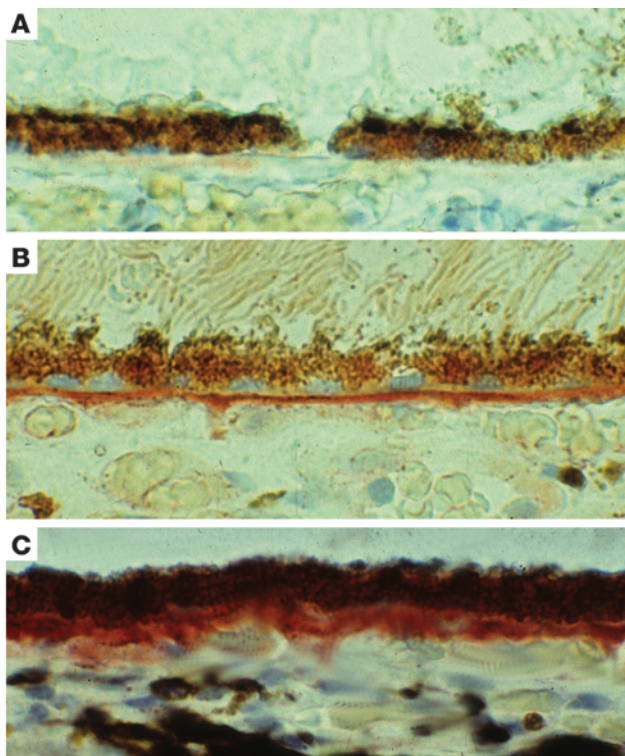
**Figure 4**
Lipid stained in frozen sections of the eye. Light micrographs of cross-sections of frozen retinas from 16-year-old (**A**), 44-year-old (**B**), and 84-year-old (**C**) donors stained with Oil Red O, demonstrating that lipid quantities in Bruch membrane increase with age.

extracted from specimens from donors younger than 50 years; in specimens from donors older than 50 years, the increase was exponential. Eyes from donors over the age of 60 years showed wide variation of total lipid extracted from donors of similar age, and the ratio of phospholipids to neutral fats was different from one specimen to another from donors of similar age. The ratio of neutral lipids to phospholipids did not correlate with the total quantity of lipid. The finding that the major lipid species were phospholipids and fatty acids, rather than cholesterol and cholesterol esters, and that only 50% of the phospholipids was phosphatidyl choline led to the conclusion that the lipids were of cellular (presumably RPE), rather than plasmatic, origin (55). In this respect, the lipid deposits are different from those in atherosclerotic plaques and arcus senilis, which derive from plasma. As a consequence, a relationship between AMD and hyperlipidemia would not necessarily be expected, and lipid-lowering drugs would be likely to be unhelpful (56). Using different extraction methods, Curcio and colleagues found that cholesterol and cholesterol esters were the major lipids in the Bruch membrane, rather than phospholipids (57). However, as in the previous study (55), it was concluded that the lipids were of RPE origin, on the basis of the nature of the cholesterol (57). Finally, measurements of the hydraulic conductivity of the Bruch membrane showed reduction with age (58, 59); after the age of 50 years, there is a close direct linear relationship between resistance of fluid flow and lipid content. Thus, there is good evidence that the biophysical properties of Bruch membrane change upon aging. It is likely that, when extreme, these changes cause PED, but they likely have a profound effect on the aging retina and play a major role in AMD in all its forms.

Clinical observations were sought to support the concept that the biochemical content as well as the thickness of the Bruch membrane influence subsequent clinical behavior. It was hypoth-

esized that fluorescein angiography would give an indication of the lipid content of focal Bruch membrane deposits, and that this may mirror the presence or absence of diffuse lipid deposits. It was suggested that drusen that are hydrophilic would allow free diffusion of water-soluble sodium fluorescein into the abnormal deposit, and that dye would bind to polar molecules, thus making them hyperfluorescent on fluorescein angiography. Conversely, if the drusen were hypofluorescent, it would suggest that they are hydrophobic, as a result of the presence of neutral lipids. This conclusion was supported by histological observations in which it was shown that in vitro binding of sodium fluorescein correlated well with the biochemical contents of drusen (60). Drusen rich in neutral lipids did not bind fluorescein, whereas those with little lipid content bound fluorescein strongly.

The highest resistance to water flow in the Bruch membrane was predicted to be found in eyes destined to suffer PED tearing, in which tangential stress in the detached tissues is sufficient to cause them to rupture. The determination that an RPE tear in one eye conferred high risk for a similar event in the fellow eye (61) provided the opportunity to test the concept in a clinical setting. Drusen fluorescence during angiography was compared in the fellow eye of an eye with a tear with drusen and in the fellow eye of one with subretinal neovascularization; the drusen were larger, more confluent, and less fluorescent on angiography in the former than in the latter (62). Thus, clinical observations support the general concept of lipids having a major influence on the biophysical properties of the Bruch membrane and contributing to AMD.

There is considerable lipid trafficking through the Bruch membrane, and lipids are believed to accumulate as they fail to pass freely through a thickened Bruch membrane. This demands that the Bruch membrane becomes thicker as a requirement for this lipid accumulation. Analysis has been undertaken of proteins in aging Bruch membrane, since this is likely to initiate the thickening. Recent studies have shown that several proteins associated with the immune system, such as C3, C5b–C9, and CFH, are present in high quantity in the Bruch membrane in AMD (63). These observations underline the potential importance of a disordered immune system in AMD. However, unlike inflammation elsewhere in the body, there is no infiltration of the Bruch membrane by inflammatory cells. β-Amyloid has also been identified (64–67). In the inner part of the Bruch membrane, there are high levels of vitronectin that might be protective against immune attack (68, 69). The origin of the proteins is in doubt, as there is RPE expression of some of the constituents, and a major contribution may come from plasma (70). The state of the proteins is unknown, but circumstantial evidence suggests that some proteins, including CFH, are oligomerized, which may be induced by high levels of zinc or other metallic ions (71, 72). In the Bruch membrane, levels of zinc are very high, and levels of bioavailable zinc are many times greater than those necessary to cause oligomerization of CFH in vitro (71). The variant of CFH associated with a high risk of developing AMD, Y402H, is predicted to oligomerize more readily than those associated with a low risk of disease, since the amino acid change provides

an additional zinc binding site. Thus, at least some of the proteins accumulating in the Bruch membrane may not have the biological properties of monomers, and therefore may not affect ocular function via their inflammatory roles (73, 74). Rather, they may act as a barrier to metabolic exchange because of their cumulative bulk.

Further insight into the possible mechanisms of material accumulation in Bruch membrane was derived from observations in the $Cfh^{-/-}$ mouse (75). A knockout does not necessarily generate a phenotype homologous with that generated by a polymorphism, since absence of a protein is different from an amino acid change that modifies the function of a protein. In addition, the immune system in mice is dissimilar from that in humans, and mice do not have a macula. However, if reduction of CFH activity is important, the observations may help in understanding AMD. In the $Cfh^{-/-}$ mouse, there is thickening of the basement membrane of the glomerulus, but, surprisingly, the Bruch membrane is thinner than in age-matched mice. This suggests that dysregulation of the immune system alone may not explain thickening of the Bruch membrane and that the presence of the CFH protein is important to the process.

Thus, there is considerable evidence that thickening of the Bruch membrane causes impedance of metabolic exchange and fluid movement that is likely to be important in the pathogenesis of AMD. Several therapeutic approaches might be considered. Reduction of the availability of the constituent proteins by chronic use of antiinflammatory agents may slow the disease process. Once thickening is established, breaking down the oligomers may be achieved with the use of antibodies or possibly zinc buffers, as has been suggested in Alzheimer disease (76). However, there are potential risks in rapidly generating monomers, such as complement attack on the RPE (73). Alternatively, the lipids might be mobilized. All these approaches would increase hydraulic conductivity and improve metabolic exchange between the RPE and choriocapillaris. In addition, increased hydraulic conductivity would allow diffusion of VEGF expressed by the RPE and induce increased choroidal circulation and fenestral density.

## Structural changes to the RPE in AMD

Accumulation of residual bodies that fluoresce can be used as an index of aging in the RPE. A quadratic relationship exists between age and both autofluorescence and residual body quantity, as measured by autofluorescence imaging by light microscopy and electron microscopy, respectively (51). The slowing of accumulation of each in the elderly is not surprising, since the population of photoreceptors decreases in late life (77). However, the relationship between age and autofluorescence is not close, given the wide variation observed in the elderly (34). It was concluded that 50% of the variation in both autofluorescence and residual bodies is not explained by aging, the suspicion being that genetic or environmental factors play a role in determining the variance. Most surprising was the weak relationship between autofluorescence and residual body volume (51). This was direct, which was expected, since the autofluorescence is derived from the residual bodies. In retrospect, the variation among specimens should not have been surprising, since only a small proportion of the material in residual bodies fluoresces, and this proportion may be influenced by things such as vitamin A content in the diet (56). Consistent with this, if rodents are given a diet low in vitamin A, the residual bodies do not fluoresce (78). In littermates given a diet high in vitamin A, the residual body content of the RPE is similar, but they fluoresce brightly. From this observation, it might be concluded that those

patients with high autofluorescence levels have a diet high in vitamin A. Alternatively, they may have a genetic predisposition, such as would be imposed by variants in the ATP-binding cassette, subfamily A, member 4 (*ABCA4*) gene. Such a variant would increase lipofuscin content in the photoreceptor outer segment and therefore RPE, as is seen in Stargardt disease (which is caused by ABCA4 mutations) and in the *Abca4*-null mouse (79, 80). Further support for the importance of dietary vitamin A comes from the Reykjavik eye study, in which GA was found to account for 80% of AMD cases with visual loss in Iceland, in contrast with approximately 25% in the United Kingdom (81). The dietary vitamin A intake of this community is very high because of the large amounts of fish in the diet and the customary practice of taking vitamin A supplements. As might be expected, the prevalence of GA in Oslo is intermediate between Reykjavik and London (82).

The clinical relevance of these finding is underlined by the ability now to image RPE autofluorescence in vivo (83). This is achieved using a confocal scanning laser ophthalmoscope (SLO) with an excitation wavelength of 488 nm generated by an argon laser. Emission is recorded above 500 nm by inserting a barrier filter. Evidence that the signal originates from lipofuscin in the RPE is derived from the work of Delori and coworkers (84), who found that the spectral characteristics were typical for lipofuscin and that the source of the signal was external to the neural retina and internal to the choroid, implying its derivation from the RPE. It has been shown using SLO that in the early stage of AMD, the distribution of autofluorescence varies from one patient to another. Autofluorescence is homogeneous in about half, but diffusely irregular or focally increased in the remainder (85). Drusen do not appear to explain the differences, since they do not autofluoresce substantially. It has been shown that in bilateral early AMD, the autofluorescence pattern is symmetrical, which suggests that the autofluorescence characteristics reflect the form of disease in an individual that may be determined by genetic or environmental influences. In patients with unilateral visual loss from AMD, focally increased autofluorescence in the good eye is associated with GA in the other eye and predicts later development of GA in the good eye. This conclusion was reinforced by the observation that a high level of autofluorescence can be found around the perimeter of GA, and if so, the area becomes atrophic within 1 year (86).

The underlying molecular mechanisms by which changes in the RPE result in GA development have been subject to debate. It has been argued that the cytoplasmic volume occupied by the residual bodies may interfere with cell metabolism (87). It has been shown that lipofuscin is a free radical generator that may cause cell damage (88). In addition, there is evidence for toxic effects of individual lipofuscin components. A2-E has surfactant-like properties on biomembranes that have been shown in one study to increase intralysosomal pH by modulating the influence of the ATP-dependent lysosomal proton pump. The increased pH would, in turn, inhibit the activity of lysosomal hydrolases (89). Furthermore, A2-E has been shown to cause leakage of lysosomes in vitro (90). Release of lysosomal content may contribute to RPE dysfunction and cell death. Another study failed to confirm a rise in lysosomal pH in the presence of lipofuscin, possibly because lower quantities of lipofuscin were used, but it did show that lipid degradation was reduced (91). Thus, both studies suggest that lipofuscin reduces the activity of phagolysosomal enzymes.

The possible consequences of reduced RPE lysosomal degradation have been investigated in vivo. Interference with degradation of lyso-
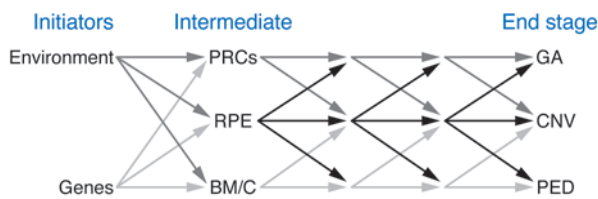
**Figure 5**
Putative disease pathways in AMD, from the initiators of the process through the intermediate disease processes to the final event. The initiating events are considered to be generated by known as well as unknown genetic and environmental factors that may affect the Bruch membrane/choroid (BM/C), RPE, or photoreceptor cells (PRCs). The metabolic interdependence of the tissues is such that changes in any one would inevitably influence the others, giving rise to a complex of intermediate disease mechanisms of which little is known. The final events of GA, CNV, and PED are partially understood. It is likely that effective therapeutic intervention at any stage of the disease evolution would be of benefit and that successful treatment directed at one tissue would have a beneficial effect on other tissues.

somes was achieved in 11-week-old Sprague-Dawley rats by intravitreal injection of the lysosomal protease inhibitor E-64 (92). A single injection caused transient accumulation of phagolysosome-like inclusion bodies in the RPE. Furthermore, 2 or 3 injections on alternate days caused progressive accumulation of these inclusions, and these were associated with changes in intracellular organelles, such as loss of smooth endoplasmic reticulum and RPE cell conformation. This was accompanied by shortening and loss of photoreceptor outer segments without prior dysmorphic changes, photoreceptor loss, reduction of fenestrae in the choroidal capillaries, and invasion of the Bruch membrane by fibroblasts and pericytes. Intravitreal injection of vehicle for comparison induced no structural changes.

It was considered likely that RPE changes induced by inhibition of lysosomal proteases reflected reduced metabolism of lipids, reduced basolateral VEGF expression, and consequent loss of fenestrae in the choriocapillaris. The shortening and loss of the outer segments was thought to be caused by impaired morphogenesis of disk membranes rather than a direct effect of E-64 on photoreceptor cells, because there was no vesiculation of disk membranes (92). The shortening of the outer segment could be explained by a lack of available lipids due to the inability of the RPE to recycle materials rescued from the phagosome. These findings are suggestive of greater dependence upon the availability of products of phagosomal degradation for photoreceptor outer segment renewal than was previously considered, and also indicate that acquisition of plasma-derived material is insufficient to sustain this process fully. The ability of the RPE to recycle lipids has been well illustrated (93, 94). The observed changes in rats treated with a lysosomal protease inhibitor are similar in many respects to age-related changes in the RPE, photoreceptors, and choroid, although there are major differences between such an acute experiment and the consequences of lifelong metabolic activity, as well as species differences between rats and humans. However, both experimental evidence and clinical observations illustrate potential pathogenetic mechanisms of GA and explain its association with focal increased autofluorescence if the latter is witness to the inability to recycle phagosomal contents.

Another potential intriguing consequence of the presence of lipofuscin is the demonstration that photodegradation products of the fluorophore activate the complement cascade, which may be relevant to Bruch membrane thickening (95). Thus, lipofuscin may contribute to age-related ocular changes and AMD through multiple mechanisms.

Measurement of visual function over areas of increased autofluorescence showed loss of scotopic function much greater than that of photopic vision, as great as 3.5 log units (96). Whether the loss is caused by cell loss or cell dysfunction has not been addressed.

Thus, there is good evidence that increased lipofuscin is important to the genesis of GA. Given that vitamin A is essential to the formation of lipofuscin, dietary supplementation with vitamin A may be inadvisable. Therapeutic trials to reduce lipofuscin accumulation by restricting the availability of vitamin A to the retina are currently underway. Initial results have shown the potential benefits of this approach in *Abca4*^{−/−} mice (97). On theoretical grounds, light restriction might also be helpful, since release of retinal is induced by light. Agents that increase lysosomal activity or lower phagolysosomal pH might also be effective, as this might counteract the effects of lipofuscin upon degradation of material in the phagosome.

## Structural changes to the outer retina in AMD

Relative to other retinal structures, there is little information on the physical changes in the neural retina in AMD, of which the photoreceptors are the most important component with respect to AMD. From early histological studies, it was concluded that loss of photoreceptor cells occurs progressively in early-stage AMD, although it was thought this was a consequence of RPE dysfunction (98, 99). Clinical studies have served to support this conclusion: 2 papers describe the results of studies on individuals with GA using a specialized imaging technique known as ocular coherence tomography (OCT; refs. 100, 101). Areas of the fundus beyond the edge of atrophy, in which the retina appeared normal by ophthalmoscopy, apart from drusen, were imaged to determine the thickness of the photoreceptor layer. In some subjects, there was an abrupt change from the lack of photoreceptor cells in the area of atrophy to a normal-thickness outer nuclear layer. However, in the majority, there was evidence of major loss of photoreceptor cells for a considerable distance beyond the edge of atrophy. Consequently, it appears probable that the functional losses of more than 3 log units recorded in areas of high autofluorescence and slow choroidal filling on fluorescein angiography are caused, in part or in full, by cell loss rather than by cell dysfunction. Thus, all the current evidence suggests that loss of photoreceptor cells may be profound even in early AMD, but varies greatly from one individual to another.

Observations in *Cfh*^{−/−} mice may also be relevant to photoreceptor cell loss in AMD (75). The visual function of these mice was reduced compared with age-matched animals, despite a lack of Bruch membrane thickening. In these mice, the photoreceptor outer segments were dysmorphic, and there was increased C3 expression in the outer retina. The relevance of C3 to outer segment morphology, and how this might be related to the belief that alternate complement pathway dysregulation is important to the pathogenesis of AMD, are unknown. As a result of these observations, it was concluded that the consequences of the CFH polymorphism associated with a high risk of developing AMD may not be restricted to its influence upon the Bruch membrane. Of possible relevance is that the distribution of *CFH* in RPE in vitro appears to be toward the apical domain, which suggests possible expression into the outer retina rather than choroid (102).

Preservation of photoreceptor cells might be best achieved by improving RPE function, although reducing the accumulation of

lipofuscin may be achieved through manipulation of A2-E formation in the photoreceptor outer segment. Restriction of vitamin A availability would achieve this end. Neuroprotective agents might be beneficial, as has been hypothesized in the treatment of glaucoma (103). The relevance of the immune system to the health of photoreceptor cells is unknown, but there is indication that it may be important on the basis of the expression of CFH and presence of C3 in the outer retina. This may be a target for treatment when more is known.

## Neovascularization

There is evidence to suggest that the growth of blood vessels inward from the choroid is caused by an imbalance of growth factors (104). VEGF, which stimulates growth, and pigment epithelial-derived factor (PEDF), which suppresses growth, have received the greatest attention. The RPE has been shown to constitutively express VEGF outward toward the choroid and PEDF inward toward the neurosensory retina (105). Humans with CNV show increased VEGF and reduced PEDF levels (106, 107). In experimental CNV induced by photocoagulation, new vessel growth occurs in the first few days after application. Within a few weeks, the RPE covers the inner surface of the lesion, and the new vessels shut down (108). During the growth phase, RPE expression of VEGF and PEDF is increased and reduced, respectively (109, 110); during spontaneous resolution of the new vessel complex, the situation is reversed. That the RPE is responsible for controlling the behavior of the vascular response is illustrated by Yamagishi et al. (111). At various intervals following photocoagulation, sodium iodate, which kills the RPE, was injected intravitreally. When given at the time of laser application, new vessel formation did not occur. When given 4 days after laser application, the vessels continued to grow over a long period, rather than shutting down, as would have been expected otherwise. It was therefore concluded that diffusible factors derived from the RPE were responsible for both the initiation of new vessel growth and its subsequent shutdown.

Thus, the concept has developed that the normal nonvascular nature of the Bruch membrane is caused by RPE suppression of inward growth of choroidal blood vessels, and that this changes in response to age-related changes. The stimulus to change in growth factor production by RPE is unknown, but it may be consequent upon lack of metabolic supply from plasma, possibly as a result of reduced diffusion of material through the thickened Bruch membrane or of reduced metabolic supply secondary to changes in the choriocapillaris.

A similar mechanism could account for retinal angiomatous proliferation (RAP; ref. 112). This appears to occur in the setting of early-stage AMD and involves invasion of the outer retina by retinal blood vessels; it is not known whether it precedes or occurs simultaneously with CNV. Just as growth factors may prevent vascularization of the Bruch membrane, so may they account for the avascular nature of the outer retina. This would involve growth factors secreted through the basolateral plasma membrane domain of the RPE cell in the first instance, and through the apical domain in the second. Thus, RAP may be caused by a change in the balance of growth factors arising from the apical domain, rather than the basal lateral plasma membrane domain; alternatively, both domains may play a role.

Treatment trials have shown that anti-VEGF agents are of major benefit to individuals with CNV (113, 114), although monthly intravitreal injections are ergonomically difficult to achieve. All the evidence suggests that the stimulus for neovascularization is determined by a single diffusible agent, unlike the situation in most cancers. The conclusion that VEGF is the sole or principal growth factor stimulating ocular neovascularization, whatever the clinical setting, is supported by observations in retinopathy of prematurity and experimental retina arterial occlusion (115, 116).

Interestingly, it appears that VEGF exposure to the apical domain of the RPE causes reduction of electrical resistance in confluent culture, suggestive of loss of tight junction integrity and of certain polar attributes (117). Thus, the therapeutic effect of anti-VEGF agents may be mediated through their effects on the RPE as well as on blood vessels.

## Conclusions

In age-related disease, changes occur in the choroid, Bruch membrane, RPE, and outer retina. Both genetic and environmental influences have been identified as conferring risk of disease and are presumably the initiators of disease. However, there is doubt as to what component of this tissue complex initiates the disorder. It is possible that the order of change may be different from one patient to another. If this is the case, it would be important to determine the tissues most affected in any individual, since treatment directed at one tissue may be appropriate to some, but not others. This would require accurate phenotyping, which may be possible with autofluorescence imaging or OCT (118). However, given the metabolic interdependence of these tissues, modulation of change in one tissue would inevitably have secondary effects on neighboring tissues (Figure 5). Currently, there are concepts as to the pathogenesis of the end stages of AMD, although less is known of the intermediate disease mechanisms. From the standpoint of therapy, interference at any stage of development of the disease might be beneficial. In marked contrast to the recent past, there is currently very active research into the various pathogenetic processes. There has been increasing understanding of the disease processes as a consequence of successes in genetics, cell biology, and biochemistry.

This Review is by no means exhaustive, and additional factors have been considered that are not tissue specific, such as free radical damage and mitochondrial dysfunction (119, 120). There is a large body of circumstantial evidence to support each, although neither is proven. There is some urgency for successful treatment of this disorder, which causes a major health burden in communities of western European origin and threatens to become so in other societies. Given the new information described herein and ongoing investigations, novel rational patient management techniques should be established in the foreseeable future.

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