

# Macular degeneration: recent advances and therapeutic opportunities

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**Abstract** | The central retina mediates high acuity vision, and its progressive dysfunction due to macular degeneration is the leading cause of visual disability among adults in industrialized societies. Here, we summarize recent progress in understanding the pathophysiology of macular degeneration and the implications of this new knowledge for treatment and prevention. The past decade has witnessed remarkable advances in this field, including the development of new, non-invasive retinal imaging technologies, the development of animal models for macular disease, and the isolation of many of the genes responsible for both early- and late-onset macular diseases. These advances have set the stage for the development of effective mechanism-based therapies.

As an exercise in functional anatomy, the reader is invited to consider the following question: which are the most valuable several cubic millimetres of tissue in the human body? Or phrased in a slightly different way: which few cubic millimetres of tissue, if lost, would result in the greatest decrement in function or in quality of life? For most organs, the loss of so small an amount of tissue would be inconsequential. Even in brain regions where function is highly localized, such as the visual or motor cortices, the loss of a few cubic millimetres of tissue would generally go unnoticed. However, a few anatomical sites would be substantially affected by such a loss. At or near the top of the list would be the macula, the central region of the retina (and, in particular, the central 2–3 square millimetres of the macula, the fovea), the only retinal region that transmits visual information of high spatial resolution (Figs 1,2a).

This review focuses on a group of diseases, referred to as macular degeneration, that affect the central retina. Symptoms of macular degeneration range from subtle distortions in the perception of fine details to a complete loss of central vision resulting in legal blindness. Macular degeneration principally affects the elderly, with disease incidence rising sharply after the seventh decade (age-related macular degeneration; AMD). In younger individuals, macular degeneration is typically found in the context of any one of several monogenic disorders.

The past several years have witnessed remarkable progress in understanding macular degeneration. New, non-invasive imaging technologies have provided

unprecedented diagnostic capability, animal models have revealed previously unknown pathophysiological mechanisms, and genetic analyses have identified many of the genes relevant to both early- and late-onset macular degeneration. In the text that follows we hope to convey some of the excitement and promise of this research.

## The structure of the retina

The vertebrate retina consists of three layers of neurons and is ~200 µm in thickness (FIG. 1b). The outermost layer (that is, the layer furthest from the centre of the eye) consists exclusively of photoreceptor cells, each of which elaborates a modified cilium, the outer segment, which houses the light-sensing visual pigments and other phototransduction proteins. The distal tips of the outer segments abut the retinal pigment epithelium (RPE), a monolayer of cells that are specialized to service the adjacent photoreceptors. The RPE, in turn, rests atop a specialized basement 'membrane', referred to as Bruch's membrane (the word 'membrane' is used here in the classic histological sense to mean an organized layering of extracellular matrix).

A rich vascular plexus, the choroidal circulation, resides immediately beyond Bruch's membrane, and supplies oxygen and nutrients to the RPE and the photoreceptors. In most mammalian retinas, including the human retina, there is a sharp division of labour between the choroidal and intraretinal vasculatures, with the latter serving only the inner two layers of the

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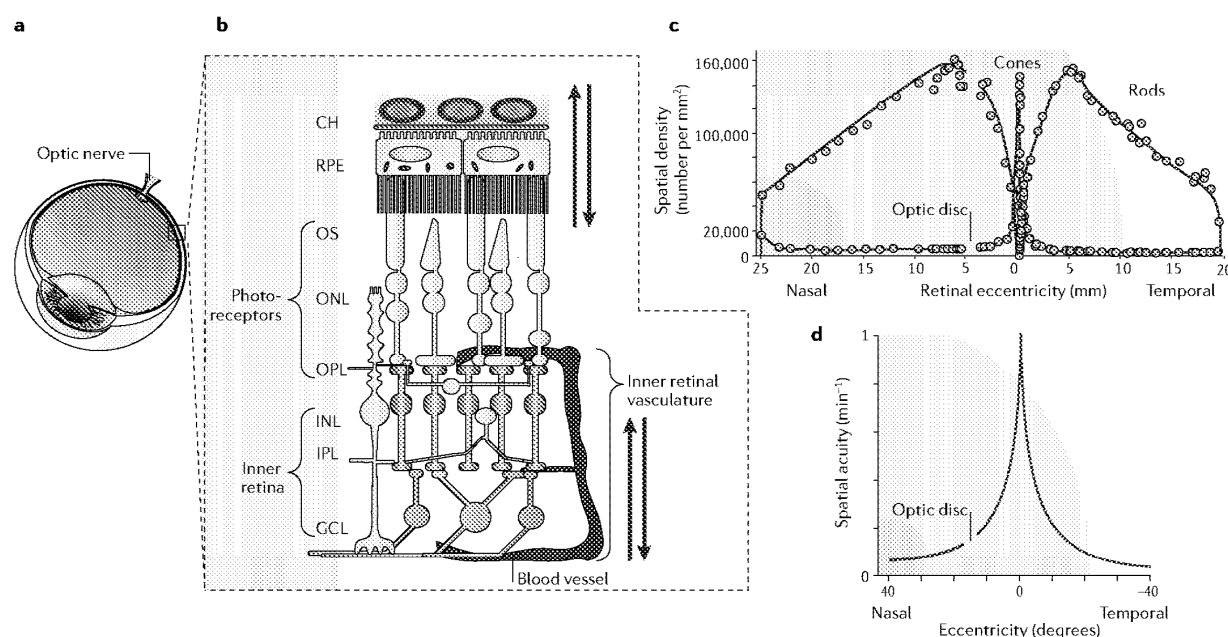


**Figure 1 | The structure of the human eye and retina.** **a** | Cut-away view of the eye, with the cornea at the bottom and the optic nerve at the top. **b** | Schematic diagram of the retina and choroid (CH), showing the major cell types. The choroidal vasculature is at the top and the inner retina is at the bottom. The vasculature of the inner retina is shown on the lower right. Arrows indicate the local diffusion of oxygen, nutrients and waste products between choroidal vasculature and the outer retina

(upper arrows), and the retinal vasculature and the inner retina (lower arrows). **c** | The density of rod and cone photoreceptors changes dramatically as a function of retinal eccentricity<sup>141</sup>. **d** | Visual acuity is highest at the fovea and declines steeply with eccentricity, closely tracking the density of cones. GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer; OPL, outer plexiform layer; OS, outer segments; RPE, retinal pigment epithelium.

retina (FIG. 1b). Why does the outer retina require such a rich blood supply? As Otto Warburg first reported in 1928, when normalized for its weight, the retina is the most oxygen-consuming tissue in the body, with a consumption level ~50% higher than the brain or kidneys<sup>1</sup>. Most of this oxygen consumption occurs in photoreceptors. One drawback of this vascular arrangement, at least on a timescale of decades, is that it constitutively exposes the RPE and the distal outer segments to an oxygen concentration roughly equivalent to that found in arterial blood<sup>2</sup>. This results in the accumulation of oxidative and photo-oxidative damage, the latter reflecting the unique vulnerability of the retina as the only neural tissue that is exposed directly to light and hence to chemically reactive species generated by light absorption.

The RPE serves the photoreceptors in several distinct ways<sup>3</sup>. Because the capillaries of the choroidal vasculature are fenestrated, the RPE constitutes the blood–retina barrier, which is analogous to the blood–brain barrier. The RPE participates in the light-dependent cycling and re-isomerization of retinal (the vitamin A derivative that serves as the chromophore for the visual pigments) from the all-*trans* to the 11-*cis* configuration to regenerate photolysed visual pigments in the outer segments (the retinoid cycle), and it also stores retinyl esters, which can be mobilized to re-enter the retinoid cycle when needed. Of particular relevance to the pathogenesis of macular degeneration, the RPE

daily engulfs and degrades the distal 10% of each outer segment, thus serving as the disposal and recycling arm for the constant renewal of outer segments. As each RPE cell contacts ~50–100 photoreceptor outer segments, and each outer segment has roughly the same biomass as an erythrocyte, each RPE cell daily engulfs and degrades an outer segment mass equivalent to 5–10 erythrocytes. This remarkable level of phagocytic activity continues throughout life with little or no turnover of the RPE cells themselves. One consequence of such prodigious phagocytic activity is a progressive build-up of lipofuscin that comes from the indigestible residue of outer segment metabolism. As discussed below, lipofuscin build-up could be one factor that predisposes the RPE to disease.

#### Monogenic early-onset macular diseases

A small number of monogenic diseases cause early-onset macular degeneration: autosomal recessive Stargardt macular dystrophy, vitelliform macular dystrophy (VMD; Best macular dystrophy), Sorsby's fundus dystrophy (SFD), *malattia leventinese* (identical to Doyme honeycomb retinal dystrophy and dominant radial drusen), and autosomal dominant Stargardt-like macular dystrophy. The genes for each of these disorders have been identified (summarized in TABLE 1), and varying degrees of progress have been made in elucidating the mechanisms by which mutations in these genes cause disease. A brief summary of this progress is presented here.

**Metalloproteinase**

A proteinase that has a metal ion at its active site.

**Druse/drusen**

Discrete sub-retinal pigment epithelium deposits composed of a complex mixture of lipid and protein. From German, meaning 'rock' or 'geode'.

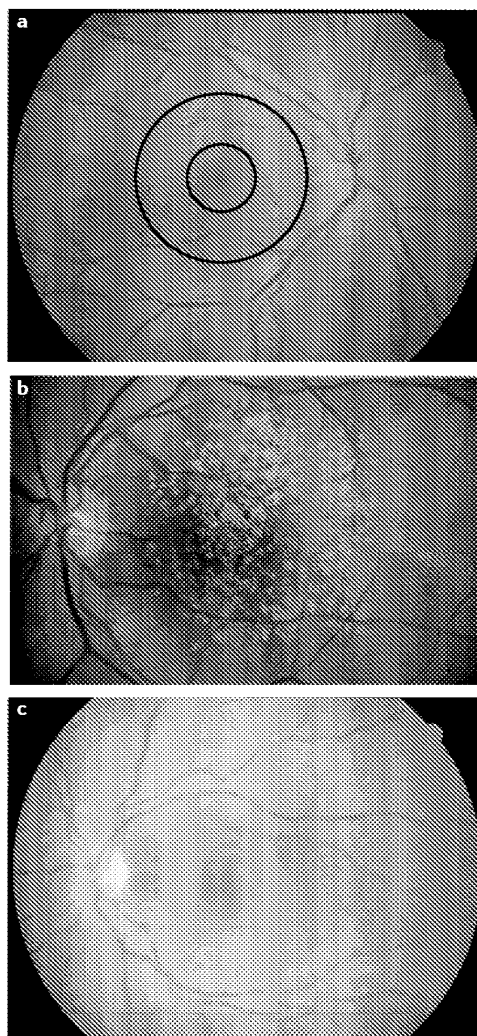


**Figure 2 | The central retina as seen through the ophthalmoscope.** **a** | Typical fundus image. The large and small concentric circles indicate the macula and the fovea, respectively. The retinal vessels enter the globe at the optic disc (right) and form arcades that sweep above and below the fovea (centre). The darker colouration of the central retina derives from the high concentration of the macular pigments zeaxanthin and leutein. **b** | A retina with numerous large drusen. **c** | Autosomal recessive Stargardt disease. Numerous yellow deposits are seen; these arise from the accumulation of retinal pigment epithelium lipofuscin, an important component of which is A2E.

**Vitelliform macular dystrophy.** VMD is an autosomal dominant disorder in which large quantities of yellow material accumulate in or beneath the RPE in a single contiguous region centred on the macula, with an appearance that is reminiscent of an egg yolk. The *VMD* gene codes for an integral membrane protein, bestrophin, which is the founding member of a novel family of anion channels<sup>4–6</sup>. The dominant nature of

VMD has been confirmed in transfection experiments in which VMD-associated and wild-type bestrophins were observed to co-assemble efficiently into heteromeric complexes that reduced channel activity. In the eye, bestrophin is present on the basal plasma membrane of the RPE cell<sup>7</sup>; it might also be present at lower levels in internal membranes. At present, the connection between defective channel activity and macular disease is unclear.

**Sorsby's dystrophy and malattia leventinese.** The genes responsible for malattia leventinese and SFD code for proteins that are part of or regulate the extracellular matrix, and they presumably exert their pathological effect by altering Bruch's membrane. SFD is an autosomal dominant disorder caused by various point mutations that in every case introduce a new cysteine into the carboxy (C)-terminal domain of the tissue inhibitor of metalloproteinase 3 (REF.8) (TIMP3). In SFD there is a massive thickening of Bruch's membrane, possibly due to local inhibition of matrix turnover by metalloproteinases, which impairs the diffusion of small molecules between the choroid and retina<sup>9</sup>. All cases of malattia leventinese investigated so far arise from a single Arg345Trp founder mutation in fibulin-like extracellular matrix protein 1 (EFEMP1, also called fibulin 3), a protein that consists largely of epidermal growth factor (EGF) repeats<sup>10</sup>. At present, it is unclear how this defect leads to the formation of drusen, the sub-RPE deposits that are the hallmark of malattia leventinese and are strongly associated with AMD. It is intriguing that two recent studies indicate that the C-terminus of TIMP3 can bind directly to EFEMP1, suggesting a potential role for EFEMP1 in regulating the localization of TIMP3 and the metabolism of the extracellular matrix<sup>11</sup>, and that TIMP3 binds to vascular endothelial growth factor (VEGF) receptor 2, thereby inhibiting VEGF-stimulated angiogenesis. The latter finding indicates that a failure to appropriately inhibit VEGF might account for the pathological growth of choroidal blood vessels into the retina (choroidal neovascularization; CNV) associated with SFD<sup>12</sup>.

These results are reminiscent of observations with a related extracellular matrix protein, fibrillin 1, which is mutated in Marfan syndrome, a connective tissue disorder with symptoms that include joint laxity and a predisposition to aortic aneurysm<sup>13</sup>. Surprisingly, fibrillin 1 mutations seem to cause disease not through a defect in the structural integrity of the extracellular matrix, but because they disrupt the function of fibrillin 1 as a binding partner and reservoir for transforming growth factor- $\beta$ , which locally orchestrates tissue growth<sup>13</sup>. Taken together, the TIMP3/EFEMP1 and fibrillin 1 data suggest that it would be of interest to systematically analyse Bruch's membrane for bound signalling molecules that might have a role in macular degeneration.

**Recessive Stargardt macular dystrophy.** Autosomal recessive Stargardt's disease is the most common of the early-onset types of macular degeneration, affecting approximately one person in 10,000. Numerous small,

Table 1 | Genes responsible for macular degeneration

| Gene       | Function                                     | Disease                                                                         | Refs      |
|------------|----------------------------------------------|---------------------------------------------------------------------------------|-----------|
| ABCR/ABCA4 | Retinoid transporter                         | Recessive Stargardt disease                                                     | 14–17     |
| VMD2       | Anion channel                                | Vitelliform macular dystrophy; Best macular dystrophy                           | 4–6       |
| TIMP3      | Metalloproteinase inhibitor                  | Sorsby's fundus dystrophy                                                       | 8,11,12   |
| EFEMP1     | Extracellular matrix structural protein      | Malattia leventinese; dominant radial drusen; Doyme honeycomb retinal dystrophy | 10,11     |
| ELOVL4     | Enzyme for long-chain fatty acid synthesis   | Dominant Stargardt-like disease                                                 | 28,29     |
| CFH        | Negative regulator of the complement cascade | Age-related macular degeneration                                                | 73–76, 79 |
| BF         | Positive regulator of the complement cascade | Age-related macular degeneration                                                | 83        |
| LOC387715  | Unknown                                      | Age-related macular degeneration                                                | 80–82     |

yellow deposits in or beneath the RPE give the fundus a distinctive appearance and inspired the alternative name fundus flavimaculatus (flavi = yellow) for this disease (FIG. 2c). Stargardt disease arises from mutations in a photoreceptor-specific ABC transporter, variously referred to as ABCR or ABCA4, which resides in the internal (disc) membranes of photoreceptor outer segments<sup>14–17</sup>. Current evidence indicates that ABCR functions in the retinoid cycle to flip all-*trans* retinal-phosphatidylethanolamine adducts from the luminal to the cytosolic leaflet of the disc membrane, thereby facilitating reduction of all-*trans* retinal to all-*trans* retinol, an essential step in recycling the isomerized chromophore<sup>18,19</sup> (FIG. 3a). As demonstrated in studies of patients with Stargardt disease and *Abcr*<sup>+/-</sup> and *Abcr*<sup>-/-</sup> mice, and consistent with biochemical studies of purified ABCR, a decrease or absence of ABCR leads to a slowing of the retinoid cycle with an acute light-dependent build-up of all-*trans* retinal, delayed dark adaptation and a slow and progressive light-dependent accumulation of a distinctive bis-retinal/ethanolamine adduct, referred to as A2E, in the RPE<sup>19–23</sup> (FIG. 3a).

A2E forms by non-enzymatic reactions in photoreceptor outer segments and RPE phagolysosomes, apparently as a minor and unavoidable by-product of the retinoid cycle<sup>22,24,25</sup> (FIG. 3a). In the presence of oxygen and light, A2E generates free radicals and kills those cells in which it resides<sup>26,27</sup> (FIG. 3b). When housed in complete darkness, *Abcr*<sup>-/-</sup> mice show virtually no change in A2E levels, implying that A2E is quite stable<sup>22</sup>. These data strongly suggest that it should be possible to slow the progressive loss of central vision in patients with Stargardt disease by any strategy that decreases the production of all-*trans* retinal.

**Dominant Stargardt-like macular dystrophy.** Dominant Stargardt-like macular dystrophy is characterized by numerous yellow deposits (of unknown chemical composition) and macular atrophy. The gene responsible codes for ELOVL4, a member of an ancient family of

fatty acid-elongating enzymes, which is expressed in photoreceptors<sup>28,29</sup>. The mechanistic link between defective photoreceptor lipid synthesis and macular disease is at present unclear.

A long-standing and still open question regarding all types of macular degeneration is why there is a predilection for vision loss in the central retina. Each gene identified so far that has a role in monogenic macular degeneration is expressed in both the central and the peripheral retina and/or RPE. As described below, the complement proteins implicated in AMD are present in serum, and some might also be synthesized by the RPE. It is possible that the special susceptibility of the macula is related to the relatively high density of photoreceptors in this region (FIG. 1c).

### Age-related macular degeneration

**Epidemiology of AMD.** In the industrialized world, AMD is the leading cause of severe vision loss in individuals over 60 years of age<sup>30</sup>. The number of people affected depends on how AMD is defined. Most large epidemiological studies make a distinction between early AMD and late AMD (epidemiologists generally use the less weighted term 'age-related maculopathy' (ARM) to refer to both stages). Early AMD is characterized by the presence of large and poorly demarcated drusen ('soft' drusen in clinical parlance) and regions of RPE hypo- and hyperpigmentation, indicative of underlying RPE abnormalities<sup>31</sup> (FIGS 2b,4). By contrast, small and well-demarcated drusen ('hard' drusen) are common and not associated with AMD. Although early AMD is generally not associated with appreciable loss of visual acuity, there is a positive correlation between the number of drusen and a slowing of dark adaptation<sup>32,33</sup>. More importantly, early AMD is associated with a substantial risk of progression to late AMD. Late AMD is characterized by local regions of RPE cell loss (geographic atrophy) and by the growth of new blood vessels from the choroid that breach Bruch's membrane and enter the retina (CNV) where they are often a source of local bleeding<sup>31</sup> (BOX 1; FIG. 4). There is a substantial loss of visual function in the regions affected. Three large surveys (the Beaver Dam Eye Study (Wisconsin, USA), the Rotterdam Study (Netherlands) and the Blue Mountains Eye Study (Australia)), as well as many smaller ones, are in rough agreement that the prevalence of late AMD is ~1.5% in individuals of European descent over the age of 50, and rises progressively with age, reaching ~10% in individuals aged 75 years or older<sup>30,34–36</sup>.

Clinical studies of AMD have been difficult owing to the slow rate of disease progression and the general difficulties of working with an older population in which other health issues present confounding variables. Despite these technical challenges, a number of general principles have emerged. Epidemiological studies show that the strongest risk factors for AMD are family history, age and cigarette smoking<sup>30,37,38</sup>. The first of these almost certainly reflects genetics, as concordance rates for AMD among monozygotic twins are reported in several small studies to be 100%, and family studies indicate a ~twofold elevated risk if there is an affected first-degree

#### Fundus

The posterior region of the eye, including the retina, retinal pigment epithelium and choroid.

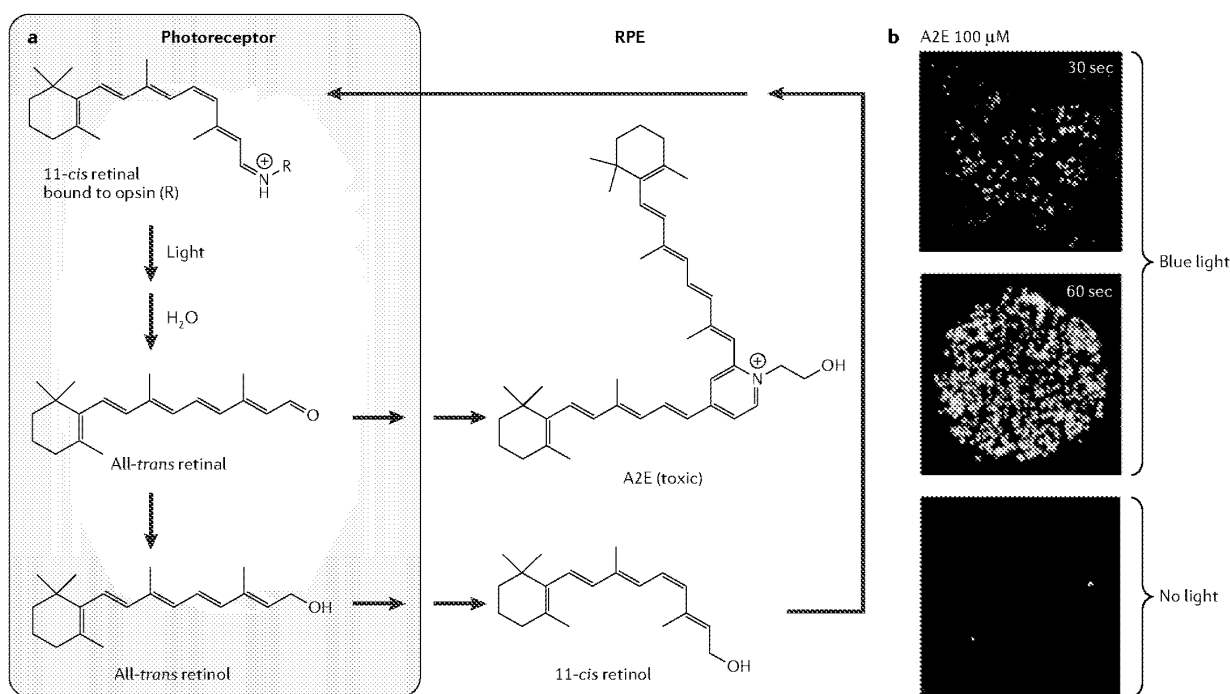


**Figure 3 | The retinoid cycle and A2E.** **a** | The principal chemical species in the retinoid cycle. For the steps shown with more than one arrow, one or more intermediate compounds have been omitted from the diagram. The steps enclosed in the box occur in the photoreceptor outer segment; the remaining steps occur in the retinal pigment epithelium (RPE). A2E, an aberrant side product, is formed by the condensation of two molecules of all-trans retinal with the ethanolamine

of phosphatidylethanolamine, a reaction sequence that begins in the photoreceptor outer segment and is completed in the phagolysosomes of the RPE. **b** | A2E is phototoxic. RPE cells in culture were exposed to 100  $\mu$ M of A2E and either maintained in darkness or exposed to blue light. Cell death, as measured by DNA fragmentation, is produced by exposure to both A2E and light. Panel **b** reproduced, with permission, from REF. 142 © (2000) Association for Research in Vision and Ophthalmology.

relative<sup>37,39,40</sup>. The risk associated with cigarette smoking suggests a role for oxidative damage and/or vascular insufficiency. Consistent with a role for oxidative damage, the Age-Related Eye Disease Study (AREDS) found that daily supplements of zinc and the antioxidants  $\beta$ -carotene and vitamins C and E led to a 25% reduction in the rate of progression to late AMD compared with a placebo group<sup>41</sup>.

**Extracellular deposits and AMD.** AMD is characterized both by relatively discrete deposits of protein and lipid between Bruch's membrane and the RPE (drusen) and by nearly continuous deposits in Bruch's membrane (basal laminar or basal linear deposits; FIGS 4, 5)<sup>42–44</sup>. Whether either or both of these deposits have a causal role in RPE and photoreceptor loss or merely reflect some other underlying disease process is unclear. In favour of a causal role is the likelihood that both types of deposit diminish diffusion from the choroidal circulation to the retina, with adverse effects on both the RPE and photoreceptors. Consistent with this idea is the finding that RPE cells overlying drusen appear vacuolated and swollen<sup>45</sup>. Also suggestive of a causal role are recent immunochemical and proteomic studies of drusen, which show an intriguing and complex protein composition that includes immunoglobulins, activated complement components (C3a and C5a), complement regulators (vitronectin, clusterin and

complement factor H (CFH)), acute phase proteins and the Alzheimer's disease amyloid- $\beta$  (A $\beta$ ) peptide<sup>46–51</sup> (FIG. 5). Most of these are abundant serum proteins. Other serum proteins involved in coagulation and fibrinolysis are also present in and/or immediately adjacent to drusen<sup>46–48,50</sup>. The choroidal circulation is the probable source of these serum proteins.

Other drusen components — lipids, including oxidized lipids, and intracellular proteins, including ubiquitin and cytosolic stress response proteins such as crystallins — are presumed to arise from the overlying RPE cells<sup>50</sup>. With respect to oxidation products, tandem mass spectrometry and immunoblotting has identified carboxyethyl pyrole protein adducts, which arise from docosahexanoate oxidation, in greater abundance in Bruch's membrane and drusen from post-mortem AMD samples relative to controls<sup>50</sup>. Docosahexanoate is a major lipid constituent of outer segments, and therefore the presence of carboxyethyl pyrole adducts in sub-RPE deposits suggests that phagocytosed and oxidized outer segment membranes are extruded by the RPE into Bruch's membrane.

Current evidence suggests that at least some sub-RPE deposits have a dynamic natural history in which accumulation reflects a balance between deposition and clearance. For example, fundus photographs taken several years apart often show both the appearance of new

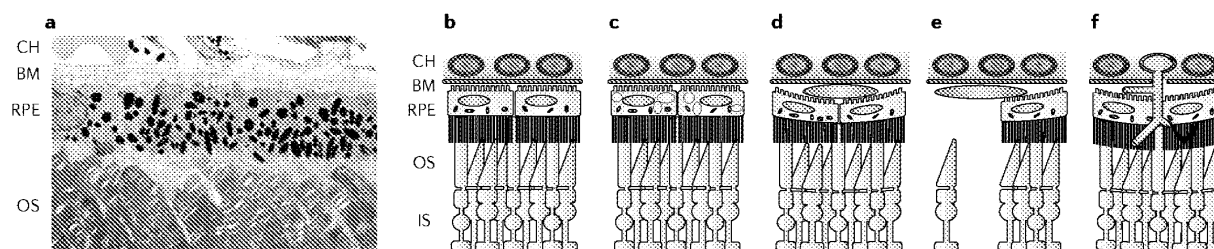


**Figure 4 | The retinal pigment epithelium, Bruch's membrane and the choroid.** **a** | Electron micrograph of the mouse retinal pigment epithelium (RPE) and choroid (CH). The choroidal vasculature is at the top and photoreceptor outer segments (OS) are at the bottom. The apical face of the RPE cell is covered with microvilli that contact the outer segments. On the basal face of the RPE cell a highly folded plasma membrane faces the choroid. Numerous melanin granules (black) are

seen in the RPE cell; the nucleus is at the far left. **b–f**, Schematic illustration of the RPE and Bruch's membrane (BM) histopathology associated with various macular degenerative processes. **b** | Normal retina and RPE. **c** | The RPE with lipofuscin accumulation. **d** | A druse sandwiched between the RPE and Bruch's membrane. **e** | A druse with adjacent geographic RPE atrophy and loss of overlying photoreceptors. **f** | Choroidal neovascularization. IS, inner segments.

drusen and a regression of older ones<sup>52,53</sup>. In the Beaver Dam Eye Study, 12% of eyes with 'soft' drusen at the time of enrolment showed no drusen 5 years later<sup>53</sup>.

The identification of immunoglobulins and complement proteins in drusen fits well with earlier observations of chronic inflammatory cells associated with atrophic RPE and with neovascular lesions<sup>54,55</sup>. Taken together, these data strongly suggest that AMD pathogenesis has a chronic inflammatory component<sup>45,47,48</sup>. A second, albeit rare, connection with chronic inflammation comes from the observation that patients with membranoproliferative glomerulonephritis type II (MPGN-II) develop drusen early in life, and these closely resemble the drusen associated with AMD in both appearance and composition<sup>56–60</sup>. MPGN-II is one of a group of renal diseases in which deposition of complement proteins, immunoglobulins and/or other serum proteins in the glomerular basement membrane is associated with impaired renal filtration, mesangial cell proliferation and thickening of the glomerular basement membrane. Although the initiating event(s) in MPGN-II are obscure, the disease is associated with abnormally low levels of circulating complement factor 3 (C3)<sup>61</sup>, indicating excessive activation of the complement cascade (FIG. 6). A small number of patients with MPGN-II have been reported to harbour partial loss-of-function mutations in *CFH*, the principal negative regulator of the alternative pathway of complement activation<sup>62,63</sup> (FIG. 6), and in some patients with the related disorder MPGN-I, *CFH* is absent due to homozygosity or compound heterozygosity for two complete loss-of-function alleles<sup>64</sup>.

Finally, we note that the pathogenic sequence outlined above for autosomal recessive Stargardt disease might also be relevant to macular disease in the general population, because A2E is a principal component of RPE lipofuscin in the normal human eye and accumulates steadily with age<sup>65,66</sup>. Therefore, individuals with Stargardt disease might simply manifest an accelerated version of the 'normal' accumulation of A2E in much the same way that individuals with familial hypercholesterolemia due to low-density lipoprotein receptor mutations manifest an accelerated rate of atherosclerosis. This line of thinking rests on the plausible hypothesis that, with

age, an increasing burden of A2E-dependent toxicity contributes to the physiological stress experienced by RPE cells in the general population. We note, however, that population studies of lifetime light exposure (which should correlate positively with A2E levels and with photo-oxidative damage) and direct measurements of A2E accumulation by fundus autofluorescence using confocal scanning laser ophthalmoscopy (cSLO) have been equivocal regarding a correlation with AMD risk<sup>30,67</sup>.

**Genetics of AMD.** As described above, twin and family studies have pointed to a substantial genetic component for AMD. The first attempts to identify individual genes relevant to AMD focused on the genes responsible for early-onset monogenic macular diseases, with the general idea that some cases of AMD might be caused by alleles that confer a mild or delayed phenotype. Unfortunately for this hypothesis, genotyping of many thousands of DNA samples from patients with AMD in multiple laboratories failed to identify disease-associated sequence variants in these genes in the vast majority of these patients<sup>68</sup>. However, current evidence suggests that the genes coding for ABCR and fibulin 5, a close relative of fibulin 3/EFEMP1 (the protein that is altered in malattia leventinese), might have a causal role in a few percent of patients with AMD. Two lines of evidence implicate ABCR: ABCR variants Gly1961Glu or Asp2177Asn were found in ~3.5% of ~1,200 AMD patients versus ~1% of ~1,200 controls<sup>69</sup>, and in Stargardt disease pedigrees an elevated frequency of AMD was found in older relatives who are heterozygous for disease-associated ABCR alleles<sup>70</sup>. When different members of the fibulin gene family were screened for sequence variants in a cohort of 402 AMD patients, fibulin 5 missense variants were identified in seven of the AMD patients (~1.5%) but no variants were seen in 429 controls<sup>71</sup>.

Classic linkage analyses of AMD have been technically challenging because the late onset of disease results in only a small number of affected individuals per pedigree. Even in families with a substantial genetic load, younger individuals generally do not show the phenotype, some of the older individuals who might have shown the phenotype die before it is manifest, and

**Hypercholesterolemia**  
Elevated serum cholesterol levels.

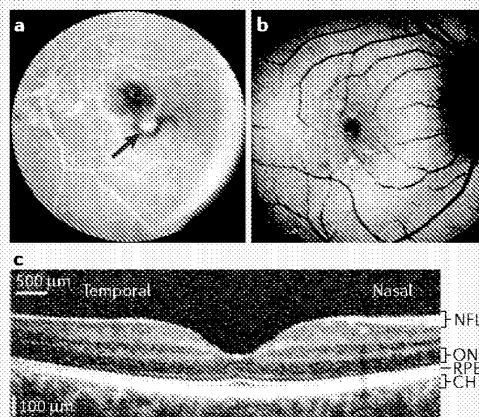
**Confocal scanning laser ophthalmoscopy (cSLO).** A non-invasive technique for fundus examination that produces *en face* images of the retina at high spatial resolution.

### Box 1 | The retina is uniquely accessible to clinical study

With Hermann von Helmholtz's invention of the ophthalmoscope in 1851, the retina, the intraretinal vasculature and the retinal pigment epithelium (RPE) became immediately accessible to direct examination (FIG. 2a). More than 150 years later, the retina remains the only part of the CNS that can be observed directly and in a non-invasive manner, and, in consequence, the assessment of retinal disease routinely includes both functional testing and micro-anatomical examination. In the clinic, routine visual field and visual acuity testing provides an assessment of retinal function with a resolution in the central retina of ~100  $\mu\text{m}$ . In the 1960s, the power of ophthalmoscopy was greatly enhanced by the use of fluorescein angiography to visualize the normal intra-retinal vasculature and any pathological neovascularization, subretinal or intraretinal haemorrhage, and regions of RPE atrophy<sup>126,127</sup>. Panel a shows leakage of fluorescent dye (arrow) secondary to choroidal neovascularization (CNV). RPE atrophy is of special significance in macular degeneration, and can be identified as a zone of hyperfluorescence in which the fluorescein in the choroidal circulation is no longer hidden by RPE melanin.

During the past decade, two new technologies for fundus imaging — confocal scanning laser ophthalmoscopy (cSLO) and optical coherence tomography (OCT) — have shown substantial utility in the investigation and diagnosis of macular degeneration<sup>127–129</sup>. First developed in the 1980s<sup>130</sup>, cSLO offers rapid data acquisition (thereby minimizing image degradation due to eye movements), low levels of illumination, relatively high spatial resolution in the plane of the retina (~20  $\mu\text{m}$ ) and the use of wavelengths with substantial tissue penetration (for example, infrared). cSLO has been used to analyse the location and density of the intrinsically fluorescent lipofuscin in the RPE<sup>66</sup>, and has permitted more precise measurements of blood flow dynamics with fluorescein and indocyanine green angiography. Panel b shows fundus autofluorescence visualized with cSLO<sup>131</sup>; the fluorescence derives from RPE lipofuscin, principally A2E. Three areas currently under development are: the use of cSLO in conjunction with adaptive optics (a method borrowed from astronomy that corrects optical aberrations in real time with a flexible mirror array), a combination that can improve in-plane resolution to ~2  $\mu\text{m}$ <sup>132</sup>; cSLO imaging of individual leukocytes within the retinal vasculature, which could potentially offer a real-time assessment of intra-ocular inflammation<sup>133,134</sup>; and coupling cSLO to a laser beam that monitors eye movements by locking onto features in the retinal image, thereby improving both imaging and therapeutic laser illumination<sup>135</sup>.

Like ultrasound imaging, OCT is based on the principle of interferometry<sup>136</sup>. Low coherence laser light is split into reference and measurement beams, with the latter directed into the eye. Light reflected from intra-ocular structures emerges from the eye and is recombined with the reference beam and allowed to interfere destructively or constructively with it. The path length of the reference beam is systematically varied, so that the resulting pattern of destructive or constructive interference maps out the axial distances of reflective structures within the eye. Currently, OCT generates images with an axial resolution of ~10  $\mu\text{m}$ , which is sufficient to resolve the principal layers of the retina and to be of clinical utility in the diagnosis of retinal oedema, microscopic retinal holes, and retinal and RPE detachments<sup>129</sup>. Still under development are ultra-high-resolution refinements of OCT, with a reported axial resolution of ~3  $\mu\text{m}$ <sup>137,138</sup>, and the integration of adaptive optics and OCT<sup>139</sup>. Panel c shows an *in vivo* cross-section through the human fovea obtained by ultra-high-resolution OCT<sup>138</sup>. The foveal pit is seen at the centre and the nerve fibre layer (NFL), outer nuclear layer (ONL) and choroid (CH) are indicated. This technology provides a spatial resolution approaching that of a tissue biopsy. Finally, cSLO and OCT have recently been integrated into a single instrument to take advantage of the complementary strengths of these two techniques<sup>140</sup>. Panel b reproduced, with permission, from REF. 131 © (2005) Association for Research in Vision and Ophthalmology. Panel c reproduced, with permission, from REF. 138 © (2006) Optical Society of America.



#### Logarithm of the odds (LOD) score

The standard statistical test for genetic linkage, whereby the likelihood of bone fide linkage is compared to the probability that the data reflect a chance association.

#### Single nucleotide polymorphism (SNP)

One of the most common genetic variations in humans. It occurs when a single nucleotide (for example, thymine) replaces one of the other three nucleotides (adenine, guanine or cytosine).

#### Haplotype

Clustered DNA sequence variants that were present together on a common ancestral chromosome. Because of their close proximity they are generally inherited together and serve to define the ancestral chromosomal region.

other potentially relevant members of the pedigree (for example, parents and siblings of affected individuals) will also have died prior to ascertainment. As a result, AMD linkage analyses have generally pooled data from large numbers of small pedigrees, which individually have little statistical power. An additional challenge has been to standardize the grading of the retinal phenotype based on fundus photographs and visual acuity measurements. At present, grading of fundus photographs is not automated, and numerical scores from multiple graders are used to assess the presence, absence and severity of disease. Despite these challenges, linkage analyses have consistently implicated several chromosomal regions

with logarithm of the odds (LOD) scores above 2 as relevant to AMD risk in the general population<sup>72</sup>.

Two of the chromosomal regions implicated by linkage analysis have been emphatically confirmed and refined by association studies using single nucleotide polymorphisms (SNPs). One locus, at chromosome 1q32, shows tight association with the *CFH* gene. One *CFH* haplotype that codes for the protein variant His402 confers a several-fold increase in disease risk in the homozygous state relative to the risk associated with homozygosity for Tyr402, the most common haplotype; His402/Tyr402 heterozygotes have an intermediate disease risk<sup>73–76</sup>. The importance of *CFH* sequence variation

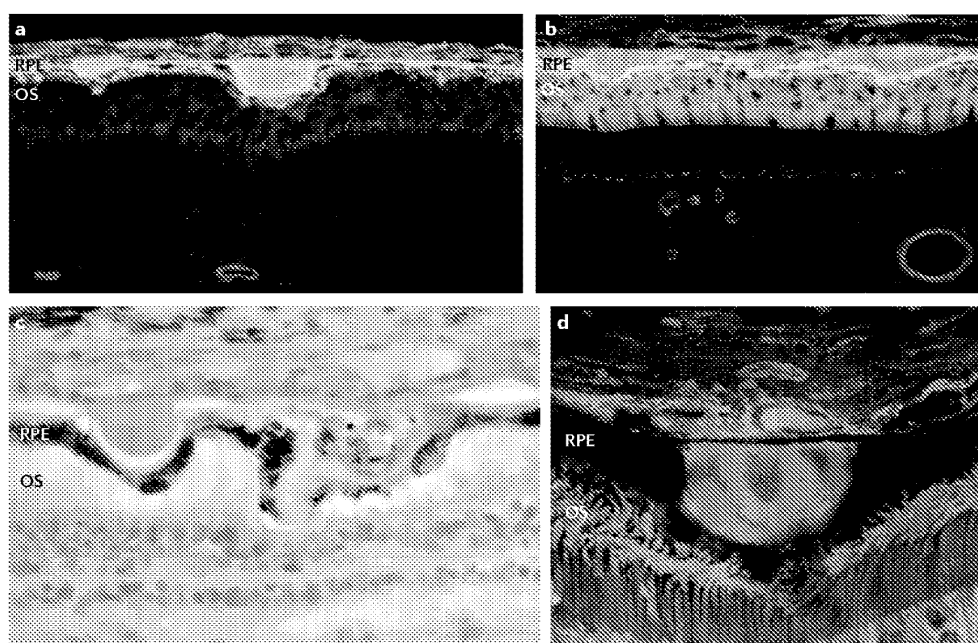


**Figure 5 | Location and composition of drusen.** **a** | Anti-complement factor 5 (C5) immunostaining (green) shows accumulation of C5 or C5 cleavage products beneath the retinal pigment epithelium (RPE). **b** | Wheat germ agglutinin staining (green) highlights rod outer segments (OS). The granular line of bright yellow fluorescence in panels **a** and **b** is RPE lipofuscin, principally A2E. **c** | Hematoxylin and eosin staining showing drusen with disruption of the RPE monolayer and some photoreceptor loss at the right. **d** | Anti-C5b-9 membrane attack complex immunostaining (red) localizes to a druse (centre) and to deposits surrounding choroidal capillaries.

in the general population is underscored by the observation of Hageman *et al.*<sup>74</sup> that in a cohort of 900 AMD patients and 400 controls, homozygosity for the His402 allele is found in 24% of AMD patients but in only 8% of controls. CFH binds to activated C3 (C3b) and promotes its proteolytic inactivation by complement factor I<sup>62,63</sup> (FIG. 6). Position 402 resides in the seventh of twenty homologous repeats (variously referred to as SCRs (short consensus repeats) or CCPs (complement control protein modules)) that comprise the bulk of the CFH protein. Repeats 7, 9, 13 and 20 contain anion-binding sites that are presumed to influence the localization of CFH<sup>77,78</sup>.

Interestingly, a recent analysis of 84 *CFH* gene polymorphisms in 726 individuals with AMD reveals a more complex relationship between sequence variation and AMD risk<sup>79</sup>. Twenty of the 84 polymorphisms showed a stronger association with AMD than did the His402 variant, and many of these sequence changes are located outside the *CFH* coding region. These data suggest that variation in *CFH* expression could be a strong determinant of AMD risk.

A second susceptibility locus, at 10q26, shows tight association with a sequence, *LOC387715*, that by computer-based analyses is predicted to be a gene (a 'hypothetical gene')<sup>80–82</sup>. One haplotype, which codes for Ala69 in the predicted *LOC387715* open reading frame, confers a relatively higher risk for AMD, whereas a second haplotype that codes for Ser69 confers a relatively reduced risk.

Both alleles are common. In ~2,000 German individuals in one study, the frequencies of Ala69/Ala69, Ala69/Ser69 and Ser69/Ser69 haplotypes were 47%, 39% and 13%<sup>81</sup>. In the same study, Ser69/Ser69 homozygotes were at ~eightfold greater risk for AMD relative to Ala69/Ala69 homozygotes, with Ala69/Ser69 heterozygotes showing an intermediate risk. The AMD risks associated with *LOC387715* and *CFH* seem to be independent and multiplicative, as individuals homozygous for high-risk haplotypes at both loci are ~55 times more likely to have AMD than individuals who are homozygous for both low-risk haplotypes. At present, the function of the hypothetical *LOC387715* gene is obscure. The predicted sequence of 107 amino acids includes no known motifs and the only close homologue in the GenBank database is a nearly identical chimpanzee sequence<sup>81</sup>.

Motivated by the connection between CFH and AMD, Gold *et al.*<sup>83</sup> identified common sequence variants in two additional complement regulatory proteins, factor B (BF) and component 2 (C2), that show a two- to threefold difference in frequency in AMD versus control cohorts. BF and C2 are encoded by neighbouring and highly homologous genes located in the major histocompatibility complex. BF is a positive activator of the alternative complement pathway and competes with CFH for binding to C3b<sup>62,63</sup> (FIG. 6). On binding to C3b, BF is cleaved by complement factor D, forming the active C3bBb protease. C3bBb cleaves additional molecules of C3 to C3b (hence its name, 'C3 convertase'), generating

**Major histocompatibility complex (MHC).** There are two classes of MHC molecules. MHC class I molecules are found on the surface of most cells and present proteins that are generated in the cytosol to T lymphocytes. MHC class II molecules are expressed only on the surface of activated antigen-presenting cells, and they present peptides that have been degraded in cellular vesicles to T cells.

Hyperlipidemia  
Elevated serum lipid levels.

a positive feedback loop of activated complement and releasing the pro-inflammatory peptide C3a. C3bBb also proteolytically activates complement factor 5 (C5), which leads ultimately to the assembly of the membrane attack complex (MAC) and to the production of additional proinflammatory peptides. C2 has a somewhat analogous role to BF in the context of the classical pathway of complement activation<sup>62,63</sup>. Proteolytically activated C2 binds to activated C4 to form the complex C4bC2a, which then proteolytically activates C3 and C5.

Taken together, the sequence variants described so far in the *CFH*, *LOC387715*, *BF* and *C2* loci account for more than 50% of the genetic loading for AMD risk. The identification of these main AMD susceptibility loci represents one of the most spectacular triumphs of modern complex trait genetics.

### Animal models

Another important advance in AMD research is the recent development of animal models that substantially reproduce the natural history of AMD. Although rodents lack a fovea and have a lifespan considerably shorter than the time course of most human macular diseases, it seems that the essential features of both early-onset macular disease and AMD can be reproduced in the mouse.

The earliest AMD models focused on CNV following focal laser injury to Bruch's membrane and the RPE<sup>84</sup>. These models have proven valuable for testing anti-angiogenic treatments and they established the concept that Bruch's membrane and the RPE act as a physical barrier to the growth of choroidal vessels. This concept has been further developed with the realization that the RPE secretes both anti-angiogenic signals, such as pigment epithelium derived factor, and the pro-angiogenic factor

VEGF<sup>85,86</sup>. It is likely that CNV occurs in the context of a breakdown in both a physical barrier and the trophic control of angiogenesis. Consistent with this view, high-level transgenic expression of VEGF in photoreceptors or RPE induces neovascular growth from the intraretinal but not from the choroidal vasculature; CNV is seen in these models only if Bruch's membrane and the overlying RPE are disrupted<sup>87</sup>.

In an intriguing link to work on sub-RPE deposits and inflammation, *C3<sup>-/-</sup>* mice or mice in which complement has been depleted with snake venom show an almost complete inhibition of the CNV response following laser injury, indicating a requirement for a functional complement system in this model of CNV<sup>88</sup>. Similarly, genetic ablation of the receptors for C3a or C5a — two proinflammatory cleavage products of C3 and C5 that are found in drusen — also inhibits the CNV response<sup>51</sup>. In both studies, inhibition of CNV seems to reflect a decrease in complement-mediated induction of VEGF.

Several mouse models of AMD implicate a combination of oxidative or photo-oxidative damage and hyperlipidemia — presumably leading to increased lipid oxidation — in the production of basal laminar deposits and drusen. Espinosa-Heidman *et al.*<sup>89</sup> reported that mice maintained on a high-fat diet and exposed to blue-green light developed basal laminar deposits and a thickened Bruch's membrane, both of which were abrogated by treatment with high doses of the antioxidant vitamin E. In an extension of this work, cigarette smoke and hydroquinone, a major oxidant found in cigarette smoke, were shown to promote sub-RPE and Bruch's membrane deposits and a thickening of Bruch's membrane<sup>90</sup>. Oxidative damage is also presumed to be the source of RPE and photoreceptor demise in mice suffering from iron overload due to a lack of the ferroxidases ceruloplasmin and hephaestin, a model that also features sub-RPE deposits and subretinal neovascularization<sup>91</sup>.

Two models show especially good concordance with human AMD pathology, and exhibit age-dependent development of drusen, diffuse sub-RPE deposits and CNV. In the first of these models, mice carrying a targeted knock-in of the human apolipoprotein E  $\epsilon 4$  (*APOE\* $\epsilon 4$* ) allele were maintained on a high-fat and high-cholesterol diet for 1–2 years<sup>92</sup>. In the second model, superoxide dismutase 1 (*Sod1<sup>-/-</sup>*) mice housed and fed under standard conditions were observed to accumulate drusen starting at ~6 months of age, increasing to more than 100 drusen per eye at 18 months<sup>93</sup>. The drusen in the eyes of *Sod1<sup>-/-</sup>* mice contained many of the same immune system and complement components seen in human drusen, including immunoglobulin G (IgG), C5 and vitronectin. Most interestingly, when young *Sod1<sup>-/-</sup>* mice were chronically exposed to white light, with an intensity roughly equal to midday sunlight, multiple drusen developed over several weeks.

Mouse models based on genetic ablation of either the gene for monocyte chemoattractant protein 1 (*MCP1/Ccl2*) or the MCP1 receptor C-C chemokine receptor 2 (*Ccr2*) also exhibit the principal hallmarks of AMD after

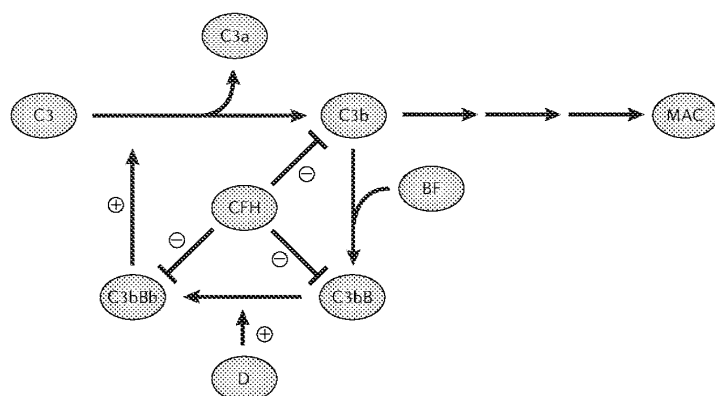


**Figure 6 | The alternative complement pathway and the roles of complement factors B and H.** Proteolytic cleavage of complement factor 3 (C3) generates active fragment C3b which, on association with complement factor B (BF), promotes BF's proteolytic activation to form the C3bBb complex. C3bBb is itself a protease, and it cleaves C3 to form C3b, generating a positive feedback loop for C3 activation. The positive feedback loop is negatively regulated by complement factor H (CFH), which displaces factor Bb from the C3bBb complex and promotes the proteolytic inactivation of C3b by complement factor I (not shown). Sequence variations in the complement factor B and H genes show strong associations with age-related macular degeneration risk. MAC, membrane attack complex.

18 months of age<sup>94</sup>. The phenotype seems to reflect a defect in macrophage recruitment and macrophage-mediated clearance of sub-RPE deposits. With respect to CFH as a risk factor, renal pathology resembling MPGN is observed in *CFH*-deficient pigs and in *CFH*-knockout mice<sup>95,96</sup>, and it will be of great interest to characterize any ocular pathology in these models.

Mouse models exist for several of the early-onset monogenic macular diseases, including SFD, Stargardt disease and autosomal dominant Stargardt-like disease<sup>19,97,98</sup>. The *Abcr*<sup>-/-</sup> mouse has served as a proof-of-principle that a retinoid cycle defect can lead to the accumulation of A2E<sup>22</sup>. More recently, experiments with *Abcr*<sup>-/-</sup> mice have shown that compounds that competitively inhibit RPE65, the isomerase in the RPE that converts retinoids from all-*trans* to 11-*cis*, slow the light-dependent accumulation of A2E<sup>99,100</sup> (FIG. 3).

Finally, surveys of captive primates have identified a colony of cynomolgus monkeys (*Macaca fascicularis*) with early-onset macular degeneration that is ophthalmoscopically and histologically indistinguishable from human AMD<sup>101</sup>. As in the *Sod1*<sup>-/-</sup> mouse, immunostaining of monkey drusen reveals a collection of protein components very much like those identified in human drusen.

### Treatment strategies

Until recently, the only approved treatment for any of the macular degenerative diseases was laser ablation of new choroidal blood vessels. Originally developed in the 1960s when lasers were first used in ophthalmology, the simplest technique relies on the ability of focused laser light to locally heat and kill target cells (photocoagulation)<sup>102,103</sup>. At best, there is scarring at the ablation site but minimal damage to surrounding retinal tissue. An important advance in this area was the development of photosensitizing agents that could be delivered intravenously just prior to laser treatment, a technique referred to as photodynamic therapy<sup>104</sup>. By matching the wavelength of the laser and the absorption peak of the photosensitizing agent, efficient photoexcitation can be confined largely to the irradiated blood vessels. The resulting production of free radicals results in localized vascular occlusion with minimal damage to both the overlying and surrounding retina. Despite their effectiveness, both photocoagulation and photodynamic therapy target only the end-stage of the disease and do not alter its underlying progression.

An alternative approach for the treatment of macular oedema and CNV is the use of anti-VEGF therapies. Initially under development in the context of tumour angiogenesis, anti-VEGF therapies have shown efficacy on a 1–2 year timescale, as measured by stabilization or improvement in visual acuity<sup>105–107</sup>. Currently, three preparations — a polyethylene glycol (PEG)ylated anti-VEGF aptamer (Macugen), a humanized anti-VEGF monoclonal antibody (Avastin) and a high-affinity anti-VEGF Fab (Lucentis) — have been tested by intraocular injection at roughly 1 month intervals. Much of the short-term improvement in visual acuity probably reflects a decrease in macular oedema (measured by

optical coherence tomography (OCT)). This is an expected outcome of blocking VEGF, which is known to promote vascular permeability<sup>108</sup>.

Moving a step closer to the underlying pathophysiology of AMD and to the possibility of a truly prophylactic strategy, it would be desirable to tip the balance between deposition and removal of sub-RPE deposits in favour of removal. One strategy, first explored by Gass<sup>102,109</sup>, is predicated on the observation that a single exposure of individual large drusen to relatively low-intensity laser illumination often causes their regression over the ensuing 1–3 years<sup>110–112</sup>, an effect that seems to be mediated by recruitment of macrophages to the site of laser treatment<sup>113</sup>. The status of this approach as a viable therapy is currently uncertain as some patients can progress more rapidly to late AMD following prophylactic laser treatment<sup>114</sup>. The plausibility of a systemic therapy to enhance removal of sub-RPE deposits is bolstered by work along these lines in the context of Alzheimer's disease and atherosclerosis, both of which are characterized by complex extracellular deposits that involve an immune system component. In the case of Alzheimer's disease, active or passive immunization to Aβ leads to a progressive regression of amyloid plaques<sup>115,116</sup>. Similarly, an extremely low-fat diet and/or aggressive treatment with statins leads to substantial reductions in the rate of accumulation, and in some cases regression, of atherosclerotic plaques<sup>117,118</sup>.

The importance of the complement regulatory proteins CFH and BF in the genetic predisposition to AMD, and the abundance of complement and other immune system proteins in drusen suggest that therapies aimed at decreasing complement activation might also be efficacious. By analogy with anticoagulation therapy, any anti-complement drug would need to be carefully titrated to find an appropriate therapeutic window that does not compromise host defences against infection. From a drug-development point of view, this represents an attractive strategy for two reasons. First, complement activation is mediated by proteolysis, and developing high-throughput cell-free assays to identify and optimize protease inhibitors is straightforward. Second, proteases generally possess an active site that is amenable to binding by high-affinity ligands. Protease inhibitors currently in use include blockers of angiotensin 2 converting enzyme and HIV protease<sup>119,120</sup>.

Another prophylactic strategy is focused on decreasing the number of molecules of all *trans*-retinal produced in the photoreceptor outer segment, with the goal of decreasing the production of A2E. For patients with Stargardt disease, the preclinical evidence (outlined above) that this strategy will slow disease progression is now overwhelming<sup>18,19,22,23,25–27</sup>, although it has yet to be tested in a randomized clinical trial. Isotretinoin (Accutane), an orally bioavailable retinoid used to treat severe acne, is also, adventitiously, a competitive inhibitor of the retinoid isomerase RPE65. Chronic administration of isotretinoin or other RPE65 inhibitors slows both dark adaptation and the production of A2E in *Abcr*<sup>-/-</sup> mice<sup>99,100</sup>. Interestingly, a reduction in the vitamin A level in serum can also slow A2E production<sup>121</sup>.

Optical coherence tomography (OCT). A non-invasive technique for fundus examination that produces cross-sectional images of the retina, retinal pigment epithelium and choroid.

A complementary and non-pharmacological approach to reducing A2E accumulation and photo-oxidative damage would simply be to reduce lifetime light exposure by wearing sunglasses when outside. Sunlight is ~10–100 times brighter than indoor lighting, and therefore a 10-fold decrease in the retina's exposure to sunlight would have little effect on quality of life, and, unlike pharmacotherapy aimed at inhibiting the retinoid cycle, it would not slow dark adaptation.

If, as current evidence suggests, the immune system has a role in the pathogenesis of AMD then it is possible that immune modulators might be able to delay disease. The newly developed mouse models of AMD provide the first real opportunity to explore this idea. The proposed role for the immune system has also led to the suggestion that antecedent infection might trigger or accelerate AMD pathogenesis<sup>74</sup>. This idea has been the subject of intense interest for many years in the context of atherosclerosis. Seroepidemiological evidence shows an association between exposure to *Chlamydia pneumoniae*, an obligate intracellular bacterium, and atherosclerosis, and animal studies demonstrate a causal relationship between *C. pneumoniae* infection and atherosclerotic lesions<sup>122,123</sup>. In the context of AMD, it is intriguing that one small study reported a modestly higher titre of anti-chlamydial antibodies in 25 patients with AMD compared with 18 controls<sup>124</sup>. In a second study, *C. pneumoniae* was detected by immunocytochemistry in CNV tissues from 4 of 9 patients with AMD compared with 0 of 5 patients without AMD<sup>125</sup>.

In any programme aimed at developing a therapy for macular degeneration, it will be important to analyse genomic DNA from all clinical trial participants to determine whether stratification by genetic risk factors

predicts response to therapy. Genetic stratification could allow a subset of responders to be identified in a clinical trial in which the aggregate response is not significant. It will also be of interest to systematically explore exposure to a wide array of viruses and microbes in cohorts of AMD and control patients whose haplotypes at the major AMD-risk loci are known.

## Conclusions

Macular degeneration falls into a class of diseases that are generally considered difficult therapeutic targets: those that are characterized by the slow destruction of a complex non-renewing tissue, as seen, for example, in Parkinson's disease and Alzheimer's disease. At the other end of the therapeutic spectrum, the diseases that have been historically the most preventable and/or treatable are those in which a foreign infectious agent, such as a bacterium, presents highly distinctive targets for a drug or vaccine, or in which a vitamin deficiency can be readily corrected by dietary modification. Inhabiting the middle ground are those diseases of human physiology — such as hypercholesterolemia, hypertension and depression — in which treatment consists of pharmacological manipulation of a complex homeostatic network. The trend of current research suggests that, as we learn more about the pathophysiology of the macular degenerations, we might be able to view them increasingly as disorders of homeostasis. If this is the case, then there are good reasons to be optimistic. If retinal, RPE or immune system physiology can be modified by a treatment that slows the rate of disease progression by as little as 10–20%, and thereby delays the onset of vision loss by 5–10 years, the impact on human health will be substantial.

1. Warburg, O. Über die klassifizierung tierischer gewebe nach ihrem stoffwechsel. *Biochem. Z.* **184**, 484–488 (1928) (in German).
2. Linsenmeier, R. A. Effects of light and darkness on oxygen distribution and consumption in the cat retina. *J. Gen. Physiol.* **88**, 521–542 (1986).
3. Marmor, M. F. & Wolfensberger, T. J. *The Retinal Pigment Epithelium* (Oxford Univ. Press, Oxford, 1998). The classic reference work for RPE biology.
4. Marquardt, A. *et al.* Mutations in a novel gene, *VMD2*, encoding a protein of unknown properties cause juvenile-onset vitelliform macular dystrophy (Best's disease). *Hum. Mol. Genet.* **7**, 1517–1525 (1998).
5. Petukhin, K. *et al.* Identification of the gene responsible for Best macular dystrophy. *Nature Genet.* **19**, 241–247 (1998).
6. Sun, H., Tsunenari, T., Yau, K. W. & Nathans, J. The vitelliform macular dystrophy protein defines a new family of chloride channels. *Proc. Natl Acad. Sci. USA* **99**, 4008–4013 (2002).
7. Marmorstein, A. D. *et al.* Bestrophin, the product of the Best vitelliform macular dystrophy gene (*VMD2*), localizes to the basolateral plasma membrane of the retinal pigment epithelium. *Proc. Natl Acad. Sci. USA* **97**, 12758–12763 (2000).
8. Weber, B. H., Vogt, G., Pruett, R. C., Stohr, H. & Felbor, U. Mutations in the tissue inhibitor of metalloproteinases-3 (TIMP3) in patients with Sorsby's fundus dystrophy. *Nature Genet.* **8**, 352–356 (1994).
9. Jacobson, S. G. *et al.* Night blindness in Sorsby's fundus dystrophy reversed by vitamin A. *Nature Genet.* **11**, 27–32 (1995).
10. Stone, E. M. *et al.* A single *EFEMP1* mutation associated with both Malattia Leventinese and Doyme honeycomb retinal dystrophy. *Nature Genet.* **22**, 199–202 (1999).
11. Klenotic, P. A., Munier, F. L., Marmorstein, L. Y. & Anand-Apte, B. Tissue inhibitor of metalloproteinases-3 (TIMP-3) is a binding partner of epithelial growth factor-containing fibulin-like extracellular matrix protein 1 (EFEMP1). Implications for macular degenerations. *J. Biol. Chem.* **279**, 30469–30473 (2004).
12. Qi, J. H. *et al.* A novel function for tissue inhibitor of metalloproteinases-3 (TIMP3): inhibition of angiogenesis by blockage of VEGF binding to VEGF receptor-2. *Nature Med.* **9**, 407–415 (2003).
13. Judge, D. P. & Dietz, H. C. Marfan's syndrome. *Lancet* **366**, 1965–1976 (2005).
14. Allikmets, R. A photoreceptor cell-specific ATP-binding transporter gene (*ABCR*) is mutated in recessive Stargardt macular dystrophy. *Nature Genet.* **17**, 236–246 (1997).
15. Illing, M., Molday, L. L. & Molday, R. S. The 220-kDa rim protein of retinal rod outer segments is a member of the ABC transporter superfamily. *J. Biol. Chem.* **272**, 10303–10310 (1997).
16. Sun, H. & Nathans, J. Stargardt's ABCR is localized to the disc membrane of retinal rod outer segments. *Nature Genet.* **17**, 15–16 (1997).
17. Molday, L. L., Rabin, A. R. & Molday, R. S. ABCR expression in foveal cone photoreceptors and its role in Stargardt macular dystrophy. *Nature Genet.* **25**, 257–258 (2000).
18. Sun, H., Molday, R. S. & Nathans, J. Retinal stimulates ATP hydrolysis by purified and reconstituted ABCR, the photoreceptor-specific ATP-binding cassette transporter responsible for Stargardt disease. *J. Biol. Chem.* **274**, 8269–8281 (1999). One of the initial descriptions of ABCR's role in the retinoid cycle and in the pathophysiology of Stargardt disease, based on experiments with purified and reconstituted ABCR *in vitro*.
19. Weng, J. *et al.* Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in *abcr* knockout mice. *Cell* **98**, 13–23 (1999). One of the initial descriptions of ABCR's role in the retinoid cycle and in the pathophysiology of Stargardt disease, based on experiments with *Abcr*<sup>-/-</sup> mice.
20. Fishman, G. A., Farberman, J. S. & Alexander, K. R. Delayed rod dark adaptation in patients with Stargardt's disease. *Ophthalmology* **98**, 957–962 (1991).
21. Delori, F. C. *et al.* *In vivo* measurement of lipofuscin in Stargardt's disease — Fundus flavimaculatus. *Invest. Ophthalmol. Vis. Sci.* **36**, 2327–2331 (1995).
22. Mata, N. L., Weng, J. & Travis, G. H. Biosynthesis of a major lipofuscin fluorophore in mice and humans with ABCR-mediated retinal and macular degeneration. *Proc. Natl Acad. Sci. USA* **97**, 7154–7159 (2000). Demonstrates the accumulation of A2E in both a human Stargardt disease patient's eyes and in a mouse model of Stargardt disease.
23. Mata, N. L. *et al.* Delayed dark-adaptation and lipofuscin accumulation in *abcr*<sup>-/-</sup> mice: implications for involvement of ABCR in age-related macular degeneration. *Invest. Ophthalmol. Vis. Sci.* **42**, 1685–1690 (2001).
24. Parish, C. A., Hashimoto, M., Nakanishi, K., Dillon, J. & Sparrow, J. Isolation and one-step preparation of A2E and iso-A2E, fluorophores from human retinal pigment epithelium. *Proc. Natl Acad. Sci. USA* **95**, 14609–14613 (1998).
25. Sparrow, J. R. *et al.* A2E, a byproduct of the visual cycle. *Vision. Res.* **43**, 2983–2990 (2003).

26. Sparrow, J. R. *et al.* Involvement of oxidative mechanisms in blue-light-induced damage to A2E-laden RPE. *Invest. Ophthalmol. Vis. Sci.* **43**, 1222–1227 (2002).
27. Radu, R. A., Mata, N. L., Bagla, A. & Travis, G. H. Light exposure stimulates formation of A2E oxiranes in a mouse model of Stargardt's macular degeneration. *Proc. Natl Acad. Sci. USA* **101**, 5928–5933 (2004).
28. Edwards, A. O., Donoso, L. A. & Ritter, R. A novel gene for autosomal dominant Stargardt-like macular dystrophy with homology to the SUR4 protein family. *Invest. Ophthalmol. Vis. Sci.* **42**, 2652–2663 (2001).
29. Zhang, K. *et al.* A 5-bp deletion in ELOVL4 is associated with two related forms of autosomal dominant macular dystrophy. *Nature Genet.* **27**, 89–93 (2001).
30. Klein, R. in *Age-Related Macular Degeneration* (eds Berger, J. W., Fine, S. L. & Maguire, M. G.) 31–56 (Mosby, St. Louis, 1999).
31. Maguire, M. G. in *Age-Related Macular Degeneration* (eds Berger, J. W., Fine, S. L. & Maguire, M. G.) 17–30 (Mosby, St. Louis, 1999).
32. Eisner, A., Fleming, S. A., Klein, M. L. & Mauldin, W. M. Sensitivities in older eyes with good acuity: eyes whose fellow eye has exudative AMD. *Invest. Ophthalmol. Vis. Sci.* **28**, 1832–1837 (1987).
33. Eisner, A., Stoumbos, V. D., Klein, M. L. & Fleming, S. A. Relations between fundus appearance and function. Eyes whose fellow eye has exudative age-related macular degeneration. *Invest. Ophthalmol. Vis. Sci.* **32**, 8–20 (1991).
34. Klein, R., Klein, B. E. & Linton, K. L. Prevalence of age-related maculopathy. The Beaver Dam Eye Study. *Ophthalmology* **99**, 933–943 (1992).
35. Mitchell, P., Smith, W., Attebo, K. & Wang, J. J. Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. *Ophthalmology* **102**, 1450–1460 (1995).
36. Vingerling, J. R. *et al.* The prevalence of age-related maculopathy in the Rotterdam Study. *Ophthalmology* **102**, 205–210 (1995).
37. Seddon, J. M., Ajani, U. A. & Mitchell, B. D. Familial aggregation of age-related maculopathy. *Am. J. Ophthalmol.* **123**, 199–206 (1997).
38. Thornton, J. *et al.* Smoking and age-related macular degeneration: a review of association. *Eye* **19**, 935–944 (2005).
39. Heiba, I. M., Elston, R. C., Klein, B. E. & Klein, R. Sibling correlations and segregation analysis of age-related maculopathy: the Beaver Dam Eye Study. *Genet. Epidemiol.* **11**, 51–67 (1994).
40. Meyers, S. M., Greene, T. & Gutman, F. A. A twin study of age-related macular degeneration. *Am. J. Ophthalmol.* **120**, 757–766 (1995).
41. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E,  $\beta$ -carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch. Ophthalmol.* **119**, 1417–1436 (2001).
42. Guymer, R. & Bird, A. C. in *The Retinal Pigment Epithelium* (eds Marmor, M. F. & Wolfensberger, T. J.) 693–705 (Oxford Univ. Press, Oxford, 1998).
43. Marshall, J., Hussain, A. A., Starita, C., Moore, D. J. & Patmore, A. L. in *The Retinal Pigment Epithelium* (eds Marmor, M. F. & Wolfensberger, T. J.) 669–692 (Oxford Univ. Press, Oxford, 1998).
44. Zarbin, M. A. Current concepts in the pathogenesis of age-related macular degeneration. *Arch. Ophthalmol.* **122**, 598–614 (2004).
45. Anderson, D. H., Mullins, R. F., Hageman, G. S. & Johnson, L. V. A role for local inflammation in the formation of drusen in the aging eye. *Am. J. Ophthalmol.* **134**, 411–431 (2002).
46. Mullins, R. F., Russell, S. R., Anderson, D. H. & Hageman, G. S. 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.* **14**, 835–846 (2000).
47. Hageman, G. S. *et al.* 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.* **20**, 705–732 (2001).
48. Johnson, L. V., Leitner, W. P., Staples, M. K. & Anderson, D. H. Complement activation and inflammatory processes in Drusen formation and age related macular degeneration. *Exp. Eye Res.* **73**, 887–896 (2001).
49. Johnson, L. V. *et al.* The Alzheimer's A $\beta$  peptide is deposited at sites of complement activation in pathologic deposits associated with aging and age-related macular degeneration. *Proc. Natl Acad. Sci. USA* **99**, 11830–11835 (2002).
50. Crabb, J. W. *et al.* Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. *Proc. Natl Acad. Sci. USA* **99**, 14682–14687 (2002).
51. Nozaki, M. *et al.* Drusen complement components C3a and C5a promote choroidal neovascularization. *Proc. Natl Acad. Sci. USA* **103**, 2328–2333 (2006).
52. Bressler, N. M., Bressler, S. B., West, S. K., Fine, S. L. & Taylor, H. R. The grading and prevalence of macular degeneration in Chesapeake Bay watermen. *Arch. Ophthalmol.* **107**, 847–852 (1989).
53. Klein, R., Klein, B. E., Jensen, S. C. & Meuer, S. M. The five-year incidence and progression of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology* **104**, 7–21 (1997).
54. Penfold, P. L., Killingsworth, M. C. & Sarks, S. H. Senile macular degeneration: the involvement of immunocompetent cells. *Graefes Arch. Clin. Exp. Ophthalmol.* **223**, 69–76 (1985).
55. Penfold, P. L., Madigan, M. C., Gillies, M. C. & Provis, J. M. Immunological and aetiological aspects of macular degeneration. *Prog. Retin. Eye Res.* **20**, 385–414 (2001).
56. Duvall-Young, J., MacDonald, M. K. & McKechnie, N. M. Fundus changes in (type II) mesangiocapillary glomerulonephritis simulating drusen: a histopathological report. *Br. J. Ophthalmol.* **73**, 297–302 (1989).
57. Duvall-Young, J., Short, C. D., Raines, M. F., Gokal, R. & Lawler, W. Fundus changes in mesangiocapillary glomerulonephritis type II: clinical and fluorescein angiographic findings. *Br. J. Ophthalmol.* **73**, 900–906 (1989).
58. Leys, A. *et al.* Subretinal neovascular membranes associated with chronic membranoproliferative glomerulonephritis type II. *Graefes Arch. Clin. Exp. Ophthalmol.* **228**, 499–504 (1990).
59. Leys, A. *et al.* Fundus changes in membranoproliferative glomerulonephritis type II. A fluorescein angiographic study of 23 patients. *Graefes Arch. Clin. Exp. Ophthalmol.* **229**, 406–410 (1991).
60. Mullins, R. F., Aptsiauri, N. & Hageman, G. S. Structure and composition of drusen associated with glomerulonephritis: implications for the role of complement activation in drusen biogenesis. *Eye* **15**, 390–395 (2001).
61. Zipfel, P. F., Heinen, S., Jozsi, M. & Skerka, C. Complement and diseases: defective alternative pathway control results in kidney and eye diseases. *Mol. Immunol.* **43**, 97–106 (2006).
62. Roitt, I. M. *Essential Immunology* (Blackwell Scientific, Oxford, 1994).
63. Holsers, V. M. in *Clinical Immunology: Principles and Practice* (eds Rich, R. R., *et al.*) 363–391 (Mosby, St. Louis, 1996).
64. Dragon-Durey, M. A. *et al.* Heterozygous and homozygous factor H deficiencies associated with hemolytic uremic syndrome or membranoproliferative glomerulonephritis: report and genetic analysis of 16 cases. *J. Am. Soc. Nephrol.* **15**, 787–795 (2004).
65. Delori, F. C., Goger, D. G. & Dorey, C. K. Age-related accumulation and spatial distribution of lipofuscin in RPE of normal subjects. *Invest. Ophthalmol. Vis. Sci.* **42**, 1855–1866 (2001).
66. von Ruckmann, A., Fitzke, F. W. & Bird, A. C. Fundus autofluorescence in age-related macular disease imaged with a laser scanning ophthalmoscope. *Invest. Ophthalmol. Vis. Sci.* **38**, 478–486 (1997).
67. Hopkins, J., Walsh, A. & Chakravarthy, U. Fundus autofluorescence in age-related macular degeneration: an epiphenomenon? *Invest. Ophthalmol. Vis. Sci.* **47**, 2269–2271 (2006).
68. Allikmets, R. Molecular genetics of age-related macular degeneration: current status. *Eur. J. Ophthalmol.* **9**, 255–265 (1999).
69. Allikmets, R. Further evidence for an association of ABCR alleles with age-related macular degeneration. The International ABCR Screening Consortium. *Am. J. Hum. Genet.* **67**, 487–491 (2000).
70. Shroyer, N. F., Lewis, R. A., Vatsenko, A. N., Wensel, T. G. & Lupski, J. R. Cosegregation and functional analysis of mutant ABCR (ABCA4) alleles in families that manifest both Stargardt disease and age-related macular degeneration. *Hum. Mol. Genet.* **10**, 2671–2678 (2001).
71. Stone, E. M. *et al.* Missense variations in the fibulin 5 gene and age-related macular degeneration. *N. Engl. J. Med.* **351**, 346–353 (2004).
72. Fisher, S. A. *et al.* Meta-analysis of genome scans of age-related macular degeneration. *Hum. Mol. Genet.* **14**, 2257–2264 (2005).
73. Edwards, A. O. *et al.* Complement factor H polymorphism and age-related macular degeneration. *Science* **308**, 421–424 (2005).
74. Hageman, G. S. *et al.* A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc. Natl Acad. Sci. USA* **102**, 7227–7232 (2005).
75. Haines, J. L. *et al.* Complement factor H variant increases the risk of age-related macular degeneration. *Science* **308**, 419–421 (2005).
76. Klein, R. J. *et al.* Complement factor H polymorphism in age-related macular degeneration. *Science* **308**, 385–389 (2005).
77. Herbert, A. P., Uhrin, D., Lyon, M., Pangburn, M. K. & Barlow, P. N. Disease-associated sequence variations segregate in a polygenic recognition patch on human factor H revealed in three-dimensional structure. *J. Biol. Chem.* **281**, 16512–16520 (2006).
78. Saunders, R. E., Goodship, T. H., Zipfel, P. F. & Perkins, S. J. An interactive web database of factor H-associated hemolytic uremic syndrome mutations: insights into the structural consequences of disease-associated mutations. *Hum. Mutat.* **27**, 21–30 (2006).
79. Li, M. *et al.* CFH haplotypes without the Y402H coding variant show strong association with susceptibility to age-related macular degeneration. *Nature Genet.* **38**, 1049–1054 (2006).
80. Jakobsdottir, J. *et al.* Susceptibility genes for age-related maculopathy on chromosome 10q26. *Am. J. Hum. Genet.* **77**, 389–407 (2005).
81. Rivera, A. *et al.* Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. *Hum. Mol. Genet.* **14**, 3227–3236 (2005).
82. Schmidt, S. *et al.* Cigarette smoking strongly modifies the association of LOC387715 and age-related macular degeneration. *Am. J. Hum. Genet.* **78**, 852–864 (2006).
83. Gold, B. *et al.* Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. *Nature Genet.* **38**, 458–462 (2006).
84. Ishibashi, T., Miller, H., Orr, G., Sorgente, N. & Ryan, S. J. Morphologic observations on experimental subretinal neovascularization in the monkey. *Invest. Ophthalmol. Vis. Sci.* **28**, 1116–1130 (1987).
85. Blaauwgeers, H. G. *et al.* Polarized vascular endothelial growth factor secretion by human retinal pigment epithelium and localization of vascular endothelial growth factor receptors on the inner choriocapillaris. Evidence for a trophic paracrine relation. *Am. J. Pathol.* **155**, 421–428 (1999).
86. Dawson, D. W. *et al.* Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. *Science* **285**, 245–248 (1999).
87. Oshima, Y. *et al.* Increased expression of VEGF in retinal pigmented epithelial cells is not sufficient to cause choroidal neovascularization. *J. Cell Physiol.* **201**, 393–400 (2004).
88. Bora, P. S. *et al.* Role of complement and complement membrane attack complex in laser-induced choroidal neovascularization. *J. Immunol.* **174**, 491–497 (2005).

- A convincing demonstration that there is an immunological component to experimental choroidal neovascularization, even if it is induced by a laser burn.**
89. Espinosa-Heidmann, D. G., Sall, J., Hernandez, E. P. & Cousins, S. W. Basal laminar deposit formation in APO B100 transgenic mice: complex interactions between dietary fat, blue light, and vitamin E. *Invest. Ophthalmol. Vis. Sci.* **45**, 260–266 (2004).
  90. Espinosa-Heidmann, D. G. *et al.* Cigarette smoke-related oxidants and the development of sub-RPE deposits in an experimental animal model of dry AMD. *Invest. Ophthalmol. Vis. Sci.* **47**, 729–737 (2006).
  91. Hahn, P. *et al.* Disruption of ceruloplasmin and hephaestin in mice causes retinal iron overload and retinal degeneration with features of age-related macular degeneration. *Proc. Natl Acad. Sci. USA* **101**, 13850–13855 (2004).
  92. Malek, G. *et al.* Apolipoprotein E allele-dependent pathogenesis: a model for age-related retinal degeneration. *Proc. Natl Acad. Sci. USA* **102**, 11900–11905 (2005).
  93. Imamura, Y. *et al.* Drusen, choroidal neovascularization, and retinal pigment epithelium dysfunction in SOD1-deficient mice: a model of age-related macular degeneration. *Proc. Natl Acad. Sci. USA* **103**, 11282–11287 (2006).
- Describes genetic evidence that oxidative and photo-oxidative damage induce drusen formation in a mouse model of AMD.**
94. Ambati, J. *et al.* An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. *Nature Med.* **9**, 1390–1397 (2003).
  95. Jansen, J. H., Hogasen, K. & Grondahl, A. M. Porcine membranoproliferative glomerulonephritis type II: an autosomal recessive deficiency of factor H. *Vet. Rec.* **137**, 240–244 (1995).
  96. Pickering, M. C. *et al.* Uncontrolled C3 activation causes membranoproliferative glomerulonephritis in mice deficient in complement factor H. *Nature Genet.* **31**, 424–428 (2002).
  97. Weber, B. H. *et al.* A mouse model for Sorsby fundus dystrophy. *Invest. Ophthalmol. Vis. Sci.* **43**, 2732–2740 (2002).
  98. Karan, G. *et al.* Lipofuscin accumulation, abnormal electrophysiology, and photoreceptor degeneration in mutant *ELOVL4* transgenic mice: a model for macular degeneration. *Proc. Natl Acad. Sci. USA* **102**, 4164–4169 (2005).
  99. Radu, R. A. *et al.* Treatment with isotretinoin inhibits lipofuscin accumulation in a mouse model of recessive Stargardt's macular degeneration. *Proc. Natl Acad. Sci. USA* **100**, 4742–4747 (2003).
- Presents a mouse model of Stargardt disease in which pharmacological inhibition of RPE65 blocks A2E accumulation.**
100. Maiti, P. *et al.* Small molecule RPE65 antagonists limit the visual cycle and prevent lipofuscin formation. *Biochemistry* **45**, 852–860 (2006).
  101. Umeda, S. *et al.* Molecular composition of drusen and possible involvement of anti-retinal autoimmunity in two different forms of macular degeneration in cynomolgus monkey (*Macaca fascicularis*). *FASEB J.* **19**, 1683–1685 (2005).
  102. Gass, J. D. Photocoagulation of macular lesions. *Trans. Am. Acad. Ophthalmol. Otolaryngol.* **75**, 580–608 (1971).
  103. Berger, J. W. & Fine, S. L. in *Age-Related Macular Degeneration* (eds Berger, J. W., Fine, S. L. & Maguire, M. G.) 31–56 (Mosby, St. Louis, 1999).
  104. Michels, S. *et al.* Comparison of early retreatment with the standard regimen in verteporfin therapy of neovascular age-related macular degeneration. *Ophthalmology* **112**, 2070–2075 (2005).
  105. EyeTech Study Group. Anti-vascular endothelial growth factor therapy for subfoveal choroidal neovascularization secondary to age-related macular degeneration: phase II study results. *Ophthalmology* **110**, 979–986 (2003).
  106. Avery, R. L. *et al.* Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. *Ophthalmology* **113**, 363–372 (2006).
  107. Rosenfeld, P. J., Heier, J. S., Hantsbarger, G. & Shams, N. Tolerability and efficacy of multiple escalating doses of ranibizumab (Lucentis) for neovascular age-related macular degeneration. *Ophthalmology* **113**, 632 (2006).
  108. Ferrara, N. Vascular endothelial growth factor: basic science and clinical progress. *Endocr. Rev.* **25**, 581–611 (2004).
  109. Gass, J. D. Drusen and disciform macular detachment and degeneration. *Arch. Ophthalmol.* **90**, 206–217 (1973).
  110. Cleasby, G. W., Nakanishi, A. S. & Norris, J. L. Prophylactic photocoagulation of the fellow eye in exudative senile maculopathy. A preliminary report. *Mod. Probl. Ophthalmol.* **20**, 141–147 (1979).
  111. Wetzig, P. C. Treatment of drusen-related aging macular degeneration by photocoagulation. *Trans. Am. Ophthalmol. Soc.* **86**, 276–290 (1988).
  112. Wetzig, P. C. Photocoagulation of drusen-related macular degeneration: a long-term outcome. *Trans. Am. Ophthalmol. Soc.* **92**, 299–303 (1994).
  113. Duvall, J. & Tso, M. O. Cellular mechanisms of resolution of drusen after laser coagulation. An experimental study. *Arch. Ophthalmol.* **103**, 694–703 (1985).
  114. Hsu, J., Maguire, M. G. & Fine, S. L. Laser prophylaxis for age-related macular degeneration. *Can. J. Ophthalmol.* **40**, 320–331 (2005).
  115. Monsonego, A. & Weiner, H. L. Immunotherapeutic approaches to Alzheimer's disease. *Science* **302**, 834–838 (2003).
  116. Wilcock, D. M. *et al.* Passive amyloid immunotherapy clears amyloid and transiently activates microglia in a transgenic mouse model of amyloid deposition. *J. Neurosci.* **24**, 6144–6151 (2004).
  117. Ornish, D. *et al.* Intensive lifestyle changes for reversal of coronary heart disease. *J. Am. Med. Assoc.* **280**, 2001–2007 (1998).
  118. Nissen, S. E. *et al.* Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. *N. Engl. J. Med.* **352**, 29–38 (2005).
  119. Wong, J., Patel, R. A. & Kowey, P. R. The clinical use of angiotensin-converting enzyme inhibitors. *Prog. Cardiovasc. Dis.* **47**, 116–130 (2004).
  120. Spaltenstein, A., Kazmierski, W. M., Miller, J. F. & Samano, V. Discovery of next generation inhibitors of HIV protease. *Curr. Top. Med. Chem.* **5**, 1589–1607 (2005).
  121. Radu, R. A. *et al.* Reductions in serum vitamin A arrest accumulation of toxic retinal fluorophores: a potential therapy for treatment of lipofuscin-based retinal diseases. *Invest. Ophthalmol. Vis. Sci.* **46**, 4393–4401 (2005).
  122. de Kruijff, M. D., van Gorp, E. C., Keller, T. T., Ossewaarde, J. M. & ten Cate, H. *Chlamydia pneumoniae* infections in mouse models: relevance for atherosclerosis research. *Cardiovasc. Res.* **65**, 317–327 (2005).
  123. Campbell, L. A. & Kuo, C. C. *Chlamydia pneumoniae* — an infectious risk factor for atherosclerosis? *Nature Rev. Microbiol.* **2**, 23–32 (2004).
  124. Kalayoglu, M. V., Galvan, C., Mahdi, O. S., Byrne, G. I. & Mansour, S. Serological association between *Chlamydia pneumoniae* infection and age-related macular degeneration. *Arch. Ophthalmol.* **121**, 478–482 (2003).
  125. Kalayoglu, M. V. *et al.* Identification of *Chlamydia pneumoniae* within human choroidal neovascular membranes secondary to age-related macular degeneration. *Graefes. Arch. Clin. Exp. Ophthalmol.* **243**, 1080–1090 (2005).
  126. Pieramici, D. J. & Bressler, S. B. in *Age-Related Macular Degeneration* (eds Berger, J. W., Fine, S. L. & Maguire, M. G.) 219–236 (Mosby, St. Louis, 1999).
  127. Yannuzzi, L. A. *et al.* Ophthalmic fundus imaging: today and beyond. *Am. J. Ophthalmol.* **137**, 511–524 (2004).
  128. Sharp, P. F., Manivannan, A., Xu, H. & Forrester, J. V. The scanning laser ophthalmoscope — a review of its role in bioscience and medicine. *Phys. Med. Biol.* **49**, 1085–1096 (2004).
  129. Thomas, D. & Duguid, G. Optical coherence tomography — a review of the principles and contemporary uses in retinal investigation. *Eye* **18**, 561–570 (2004).
  130. Webb, R. H., Hughes, G. W. & Delori, F. C. Confocal scanning laser ophthalmoscopy. *Appl. Opt.* **26**, 1492–1499 (1987).
- One of the original descriptions of the cSLO technology.**
131. Bindewald, A. *et al.* Classification of fundus autofluorescence patterns in early age-related macular disease. *Invest. Ophthalmol. Vis. Sci.* **46**, 3309–3314 (2005).
  132. Zhang, Y. & Roorda, A. Evaluating the lateral resolution of the adaptive optics scanning laser ophthalmoscope. *J. Biomed. Opt.* **11**, 14002 (2006).
  133. Hossain, P. *et al.* In vivo cell tracking by scanning laser ophthalmoscopy: quantification of leukocyte kinetics. *Invest. Ophthalmol. Vis. Sci.* **39**, 1879–1887 (1998).
  134. Martin, J. A. & Roorda, A. Direct and noninvasive assessment of parafoveal capillary leukocyte velocity. *Ophthalmology* **112**, 2219–2224 (2005).
  135. Hammer, D. X. *et al.* Compact scanning laser ophthalmoscope with high-speed retinal tracker. *Appl. Opt.* **42**, 4621–4632 (2003).
  136. Huang, D. *et al.* Optical coherence tomography. *Science* **254**, 1178–1181 (1991).
- The invention of OCT.**
137. Schmidt-Erfurth, U. *et al.* Three-dimensional ultrahigh-resolution optical coherence tomography of macular diseases. *Invest. Ophthalmol. Vis. Sci.* **46**, 3393–3402 (2005).
  138. Cucu, R. G., Podoleanu, A. G., Rogers, J. A., Pedro, J. & Rosen, R. B. Combined confocal/en face T-scanned ultrahigh-resolution optical coherence tomography in vivo retinal imaging. *Opt. Lett.* **31**, 1684–1686 (2006).
  139. Fernandez, E. J. *et al.* Three-dimensional adaptive optics ultrahigh-resolution optical coherence tomography using a liquid crystal spatial light modulator. *Vision. Res.* **45**, 3432–3444 (2005).
- Along with reference 138, this paper provides a recent description of state-of-the-art ultra-high-resolution OCT.**
140. Podoleanu, A. G. *et al.* Combined multipolar optical coherence tomography and confocal scanning ophthalmoscopy. *J. Biomed. Opt.* **9**, 86–93 (2004).
  141. Osterberg, G. Topography of the layer of rods and cones in the human retina. *Acta Ophthalmol. Suppl.* **6**, 1–101 (1935).
  142. Sparrow, J. R., Nakanishi, K. & Parish, C. A. The lipofuscin fluorophore A2E mediates blue light-induced damage to retinal pigmented epithelial cells. *Invest. Ophthalmol. Vis. Sci.* **41**, 1981–1989 (2000).

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#### Competing interests statement

The authors declare competing financial interests: see web version for details.

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