Photoreceptor degeneration: genetic and mechanistic dissection of a complex trait

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Abstract | The retina provides exquisitely sensitive vision that relies on the integrity of a uniquely vulnerable cell, the photoreceptor (PR). The genetic and mechanistic causes of retinal degeneration due to PR cell death — which occurs in conditions such as retinitis pigmentosa and age-related macular degeneration — are being successfully dissected. Over one hundred loci, some containing common variants but most containing rare variants, are implicated in the genetic architecture of this complex trait. This genetic heterogeneity results in equally diverse disease mechanisms that affect almost every aspect of PR function but converge on a common cell death pathway. Although genetic and mechanistic diversity creates challenges for therapy, some approaches — particularly gene-replacement therapy — are showing considerable promise.

The major cause of adult blindness in industrialized countries is the progressive dysfunction and death of retinal photoreceptors (PRs). PR degeneration is arguably the most genetically heterogeneous disorder in man: mutations in 184 loci — 146 of which have been identified — seem to account for little more than half of all of the monogenic subtypes[1], and the number of loci that influence susceptibility to age-related macular degeneration (AMD) — a common and genetically complex multifactorial cause of PR degeneration — is expanding. PR degeneration can be viewed as a complex trait that is influenced by many genes — with variants of large and small effect — and by environmental factors. Here, we focus on genetically simple and complex forms of progressive PR cell death or degeneration — other forms of retinal degeneration, such as glaucoma, involve different retinal cells and are not considered here.

'Inherited' forms of PR degeneration[2,3] are defined by their predominantly monogenic inheritance and are a common cause of visual impairment, with a prevalence of ~1 in 3,000. The most common subtype is retinitis pigmentosa (RP), which is one of the two main causes of blindness in 20–64 year olds[4]. Its clinical features are summarized in BOX 1. Few phenotypic features reliably distinguish the >44 genetic subtypes of RP, although some subtypes are part of clinical syndromes that include non-ocular features. Other inherited PR degenerations include macular, cone and cone–rod degenerations, which are clinically distinguishable from RP (BOX 1). These disorders can present at any stage of life but predominantly cause severe visual loss in early to middle age. Their overall impact is, however, dwarfed by AMD, a multifactorial cause of PR degeneration that accounts for more than one-half of all blindness and visual impairment in industrialized countries[5,6]. The prevalence of AMD rises exponentially with age; mild to moderate AMD occurs in 30% of individuals over 75 years old[6,7].

The inherited and multifactorial forms of PR degeneration are distinct in several ways, but they share a key feature that accounts for most of the visual disability — the loss or dysfunction of PR cells as a primary or secondary event. Clinical ascertainment of the broad spectrum of PR defects has been efficient, as has their genetic dissection, and a large catalogue of implicated loci has emerged (see Supplementary information S1 (table)). Mechanistic understanding lags behind genetic dissection, but the main cellular functions that are affected are now apparent. Our understanding of the genetic and mechanistic architecture of PR degeneration is arguably more complete than for any other complex human trait.

The genetic and mechanistic diversity of PR degeneration presents challenges for therapy, but there have been impressive recent successes. In this Review, we consider the architecture of genetic variants that influence

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Box 1 | **Clinical features of photoreceptor degeneration**

The most common cause of inherited photoreceptor (PR) degeneration is retinitis pigmentosa (RP), which typically presents with poor night vision (due to rod dysfunction) in early or middle life. It progresses to loss of the mid-peripheral field of vision, which gradually extends and leaves many patients with a small central island of vision due to the preservation of macular cones[1]. The most common form of RP results from a primary defect in rods, but this almost invariably leads to secondary cone loss (hence, it is a rod–cone degeneration). Other types of RP show a primary dysfunction of both rods and cones. Disorders in which cones are more severely affected than rods (cone–rod degeneration) or in which they are the only cell type involved (cone degeneration) are also not uncommon. These cone and cone–rod degenerations are distinct from inherited macular degenerations, which result from anatomically circumscribed primary defects in macular rods, cones or retinal pigment epithelium (RPE). Early loss or distortion of central vision is common in cone and cone–rod degenerations and inherited macular degenerations, but these can usually be distinguished by electrophysiological and other tests owing to the more generalized PR defect in the cone and cone–rod degenerations.

RP is a feature of >30 different syndromes in which non-ocular signs are also present. These syndromes account for 20–30% of all RP. Leber congenital amaurosis (LCA) also results from PR loss but is distinct from RP due to the onset of retinal blindness at or within a few months of birth. There is genetic overlap between LCA and later-onset PR degenerations, as mutations in at least six genes can cause both types of disorder (Supplementary information S1 (table)). Different mutations in centrosomal protein 290 kDa (*CEP290*), which encodes a ciliary protein, can give rise to isolated LCA or syndromal forms of RP with extraocular features, such as renal cystic disease (Senior–Loken syndrome), polydactyly and obesity (Bardet–Biedl syndrome), cerebellar malformation (Joubert syndrome) or developmental anomalies of the renal, biliary or central nervous systems (Meckel syndrome) (Supplementary information S1 (table)).

In contrast to RP and the cone and cone–rod degenerations, in which onset is usually well before middle age, inherited macular degenerations present both in early and post-reproductive life, and some resemble the more genetically complex age-related macular degeneration (AMD). About 20% of typical AMD subjects have other affected family members but monogenic forms are rare. AMD is rare under the age of 60 and its clinical features result from the age-related build-up of focal and diffuse sub-RPE or basal deposits in the macula, which leads to RPE and secondary PR dysfunction and death. The initial symptoms include distortion of central vision, progressing to patchy loss of central vision (geographic atrophy) and, in 10–20% of subjects, a sudden and catastrophic loss of central vision resulting from the invasion, exudation or haemorrhage of abnormal blood vessels into the macula (neovascular AMD).

PR degeneration, some examples of mechanistic insights and, finally, how these could influence treatment in the future.

Photoreceptors and retinal structure

The unique structural and functional organization of the vertebrate retina is finely adapted to the initial capture and processing of visual signals, but this organization also makes it unusually vulnerable to dysfunction[8,9] (FIG. 1). The retina consists of an outer monolayer of cells, which make up the retinal pigment epithelium (RPE), and an inner neural retina, which is a trilaminar network of different neuronal types and their connections[9]. The outer layer of the neural retina contains the light-sensitive PR cells — rods and cones — the apical outer segments of which are densely packed with membranes containing the visual pigment opsin covalently bound to the light-sensitive chromophore 11-*cis* retinal. The inner nuclear and ganglion cell layers process the light signals generated by PRs and transmit them through the optic nerve to the brain. Rod and cone PRs are unevenly distributed across the retina in many species. In primates, the central retina has a 5–6 mm diameter 'macula', which is specialized for high acuity vision. At the centre of the macula is the fovea, which is the region of highest acuity and only contains cones[10].

There is a close interdependence among PRs, the RPE and the choroid that nourishes them, so dysfunction in any of these components can cause secondary dysfunction in the others. The primary source of PR disease can also lie outside the retina — for example, the liver can produce abnormal metabolites or immune regulators that influence PR cell death. However, regardless of the primary disease site, if the outcome is PR dysfunction and death, vision starts to fail.

Genetics of photoreceptor degeneration

Inherited degenerations. Inherited PR degeneration is usually defined as a monogenic form of progressive PR cell death[2], although there is one report of digenic inheritance[11]. Furthermore, an excess of isolated cases in segregation analyses[12] suggests that polygenic inheritance and/or environmental factors (for example, drug toxicity) could account for a minority of affected individuals. In one segregation analysis of RP[12], families were classified as autosomal dominant (24%), autosomal recessive (41%) and X-linked (22%), and the remaining 12% of cases were presumed to result from non-genetic factors, non-Mendelian inheritance (for example, mitochondrial or *de novo* mutations) or complex inheritance. Databases suggest a similar spread of inherited subtypes for cone, cone–rod and inherited macular degenerations (see the Retinal Information Network (RetNet) database).

Most of the genes that cause inherited PR degeneration contribute a small fraction of cases (Supplementary information S1 (table)). The most common single genes that cause RP are retinitis pigmentosa GTPase regulator (*RPGR*; 10–20% of cases[13]), rhodopsin (*RHO*; 8–10% of cases[14]) and usherin (*USH2A*; ~3% of cases[1]). *RPGR* has a carboxy-terminal exon with a highly repetitive structure that is prone to small insertion–deletions (indels); this mutation 'hot spot' accounts for more than half of the 358 known *RPGR* mutations[15,16]. The gene most commonly mutated in inherited macular degeneration is ATP-binding cassette, subfamily A, member 4 (*ABCA4*), which causes autosomal-recessive juvenile macular degeneration (Stargardt disease), cone–rod degeneration or RP, depending on the mutational severity[17].

Almost all causal mutations associated with inherited PR degeneration are rare (minor allele frequency (MAF) <<0.01). For example, most of the >500 *ABCA4* mutations are rare (see The Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff) and consistent with mutation–selection balance. One exception is the mildly deleterious *G863A* allele, which only causes disease in combination with a more severe *ABCA4* allele and has a carrier frequency varying from <0.3% in south-western Europe to 5.5% in north-western Europe[18]. Most of the associated genes have hundreds of disease alleles, but sometimes one predominates. For example, the *2299delG* allele of *USH2A* causes a substantial proportion of cases of Usher syndrome, but it is still rare in the general population (MAF <0.01)[1,19,20].

Retinal pigment epithelium
A monolayer of pigmented neuroepithelial cells located between the vascular choroid and photoreceptor layer of the neural retina. It forms part of the blood–retinal barrier and has a close metabolic relationship with adjacent photoreceptors.

Outer segment
The apical extension of vertebrate photoreceptors that forms part of a modified cilium and contains densely packed membranous discs or folds containing the visual pigment and other components of the phototransduction apparatus. It is connected to the inner segment by a narrow connecting cilium.

Opsins
An evolutionarily conserved family of G-protein-coupled receptors that can function as light-sensitive photopigments when coupled to a light-sensitive chromophore, such as 11-*cis* retinal.

Choroid
The vascular layer of the eye lying between the retina and its fibrous scleral coat. The choroidal vasculature nourishes the outer part of the retina, including photoreceptors.

Few common variants have been found to influence inherited PR degeneration. One example is a common allele in the RPGR-interacting protein 1-like (*RPGRIP1L*) gene, *A229T*, which affects the likelihood of PR degeneration in the context of other ciliopathy mutations (see below)[21]. Another is a common variant of precursor mRNA (pre-mRNA)-processing factor 31 (*PRPF31*), which is suggested to be a modifier of penetrance in a dominant form of RP[22]. Low-penetrance or modifier genes are difficult to identify compared with high-penetrance alleles, but they are almost certain to contribute to phenotypic variability. It is even harder to find rare phenotypic modifiers, but interactome analyses have uncovered rare modifiers in Bardet–Biedl syndrome (BBS) and related ciliopathies[23].

The genetic architecture of inherited PR degeneration therefore shows hundreds of rare alleles, most of large effect, in at least a few hundred genes. Only a few loci have common alleles or significant aggregate allele frequencies[24]. This suggests the analogy of a long chain with many links, any of which can be broken or weakened to cause or predispose to PR degeneration.

Age-related macular degeneration. Genetic contributors to AMD have been more difficult to discover. A landmark discovery came in 2005 when genetic association studies identified the complement factor H (*CFH*)[25–28] and age-related maculopathy susceptibility 2 (*ARMS2*)[29] genes. A common indel polymorphism in the 3′ UTR of *ARMS2* has been proposed to be the causal variant, although this remains unresolved[30]. The *CFH* gene forms part of the regulator of complement activation (RCA) cluster, which contains 15 complement-related genes[31], many of which are in strong linkage disequilibrium with each other, so it is still unclear how many RCA loci are causally associated with AMD.

The locus and allelic spectrum underlying AMD is very different from inherited PR degenerations. Most

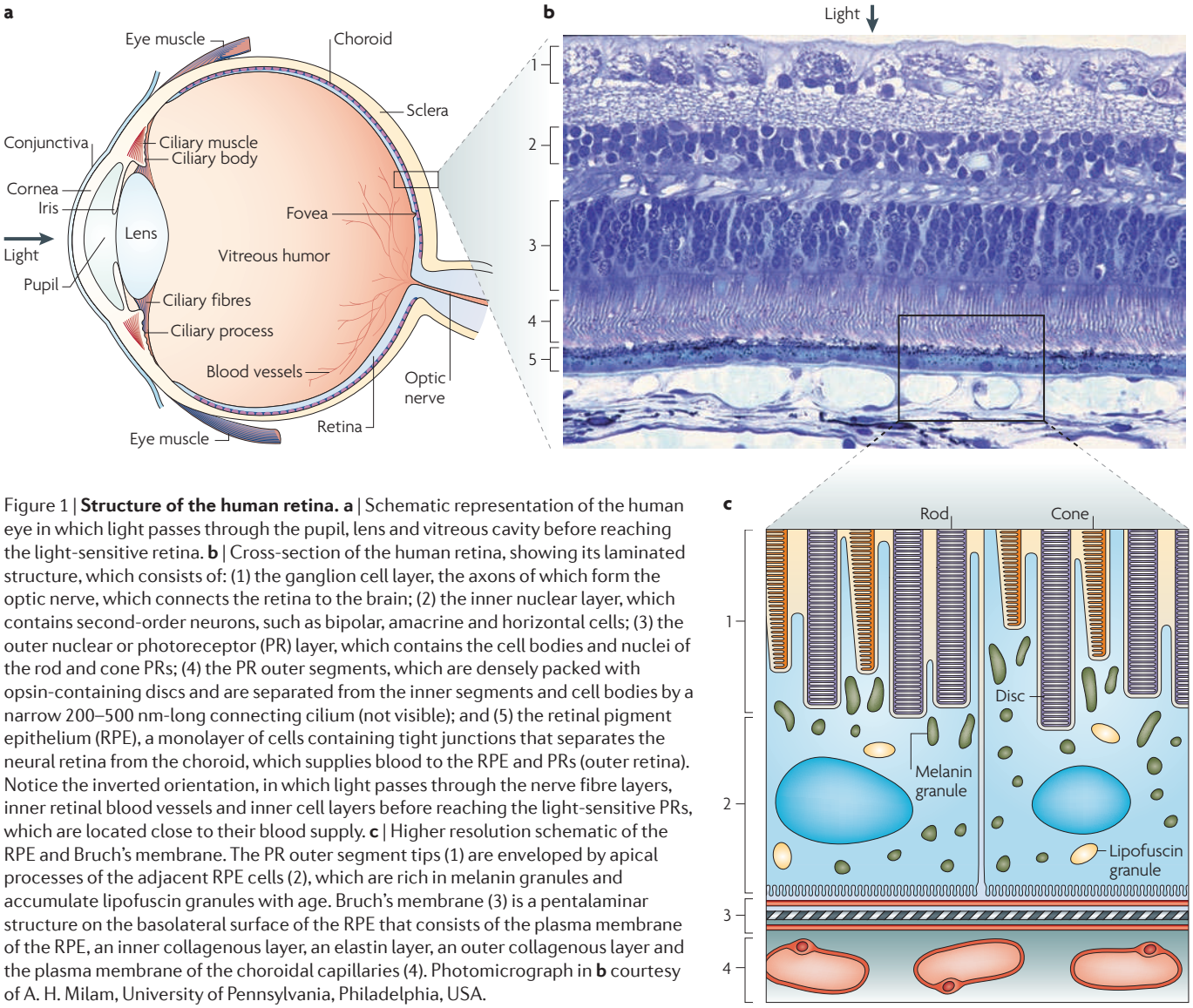


Figure 1 | **Structure of the human retina. a** | Schematic representation of the human eye in which light passes through the pupil, lens and vitreous cavity before reaching the light-sensitive retina. **b** | Cross-section of the human retina, showing its laminated structure, which consists of: (1) the ganglion cell layer, the axons of which form the optic nerve, which connects the retina to the brain; (2) the inner nuclear layer, which contains second-order neurons, such as bipolar, amacrine and horizontal cells; (3) the outer nuclear or photoreceptor (PR) layer, which contains the cell bodies and nuclei of the rod and cone PRs; (4) the PR outer segments, which are densely packed with opsin-containing discs and are separated from the inner segments and cell bodies by a narrow 200–500 nm-long connecting cilium (not visible); and (5) the retinal pigment epithelium (RPE), a monolayer of cells containing tight junctions that separates the neural retina from the choroid, which supplies blood to the RPE and PRs (outer retina). Notice the inverted orientation, in which light passes through the nerve fibre layers, inner retinal blood vessels and inner cell layers before reaching the light-sensitive PRs, which are located close to their blood supply. **c** | Higher resolution schematic of the RPE and Bruch's membrane. The PR outer segment tips (1) are enveloped by apical processes of the adjacent RPE cells (2), which are rich in melanin granules and accumulate lipofuscin granules with age. Bruch's membrane (3) is a pentalaminar structure on the basolateral surface of the RPE that consists of the plasma membrane of the RPE, an inner collagenous layer, an elastin layer, an outer collagenous layer and the plasma membrane of the choroidal capillaries (4). Photomicrograph in **b** courtesy of A. H. Milam, University of Pennsylvania, Philadelphia, USA.

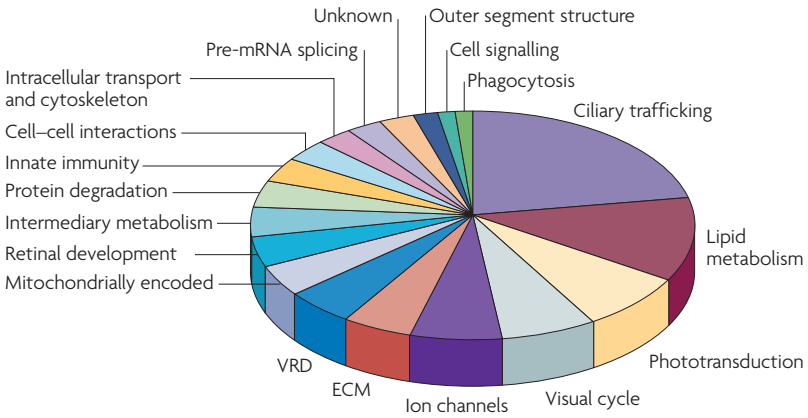


Figure 2 | **Functional categorization of genes that influence photoreceptor degeneration.** Pie chart showing the functional categorization of 146 genes implicated in PR degeneration. The data are from the Retinal Information Network (RetNet) database or are cited in the main text. For further details of the genes, see Supplementary information S1 (table). ECM, extracellular matrix; pre-mRNA, precursor mRNA; unknown, function undetermined; VRD, vitreoretinal degeneration.

alleles that influence AMD risk are common, and there are few well-established examples of rare susceptibility variants[6,5]. Common variants in six genes account for about half of the heritability in AMD[32]. A handful of rare coding variants have been reported in the fibulin 5 (*FBLN5*) and *CFH* genes in AMD[33,34], but there are few other examples. The effect sizes of the *CFH* and *ARMS2* susceptibility alleles are unusually large by the standard of most complex traits, with odds ratios (ORs) in the ranges of 2–11 at the *CFH* locus[35] and 3–8 at the *ARMS2* locus, depending on disease severity and allele dosage[29]. The other AMD-associated genes, complement component 3 (*C3*), *C2*, *CFB*, *CFI* and apolipoprotein E (*APOE*), show small effects (OR 1.2–1.7 per allele) that are more typical of complex diseases. In most cases, the causal variant or variants have not been identified, although there are some strong candidates[36–38], so true effect sizes remain to be established.

Differences in genetic architecture. Why is the genetic architecture so different between monogenic (simple) and polygenic (complex) forms of PR degeneration? The most obvious reason is their differing exposure to purifying selection. Inherited PR degeneration is usually evident within reproductive life, whereas AMD is very rare before the age of 60. Alleles with large effects on visual function within the reproductive period are expected to be at low frequency, much as observed. The selection coefficient need not be high to maintain such alleles at frequencies close to mutation–selection balance[39]. Recent human population expansions and bottlenecks, which have resulted in an excess of young and rare mutations, may skew the allele distribution even further towards low frequencies[40,41]. Even in a late-onset disorder like AMD, common variants with large functional effects, such as the *CFH 402H* allele, are unlikely to have reached such high allele frequencies (MAF ~0.3 in Europeans) in the absence of some adaptive advantage, perhaps maintained by balancing selection. For example, the *402H* allele influences binding of the CFH protein to anionic surfaces[38] and may have provided a selective advantage, as CFH is an important regulator of the innate immune system[42].

There are also a handful of genes — for example, epidermal growth factor-containing fibulin-like extracellular matrix protein 1 (*EFEMP1*) and tissue inhibitor of metalloproteinase 3 (*TIMP3*) — in which rare high-penetrance alleles cause late, post-reproductive onset of PR degenerations that are similar to AMD (Supplementary information S1 (table)). Why are the alleles that cause these disorders also rare? Interestingly, their allelic diversity tends to be low — often a single or a few causal alleles — which suggests that rather than being maintained at low frequency by mutation–selection balance, these alleles have a recent origin.

Pathways to cell death

The genes known to influence PR degeneration affect almost all aspects of cellular structure and function (FIG. 2). Mutations affecting PR-specific functions, such as phototransduction or the visual cycle, are only marginally more numerous than mutations affecting more general functions, such as protein folding, lipid metabolism or the extracellular matrix. Most show widespread rather than PR-specific expression patterns. Surprisingly, the category with most disease-causing genes is PR ciliary function. Why does such a diverse range of cellular functions lead to the common end point of PR cell death? First, we discuss the mode of cell death and then briefly summarize some of the major mechanisms underlying PR degeneration.

Apoptotic mechanisms. Early papers on cell death in PR degeneration concluded, on the basis of morphology and TUNEL staining, that apoptosis was the predominant mode of cell death[43,44]. However, it was subsequently shown that TUNEL staining can detect both apoptotic and necrotic or autolytic cells[45,46], and it was recently shown in mouse models that cell death can be caspase-independent or show features of autophagy[47,48]. It is now accepted that caspase-independent and -dependent mechanisms are both involved, often cooperatively, in apoptotic cell death[49], including PR degeneration. Different cell-death mechanisms may be predominant during different stages of the disease or overlap at any one stage — however, apoptosis remains the predominant mode of cell death.

No simple answer has emerged as to why mutations in so many different genes cause PR degeneration[2,3,50]. We argue that the unique physiology and biochemistry of the PR underpins its vulnerability to cell death; PRs sit on a knife edge separating function and survival from dysfunction and death, and almost any defect seems capable of tipping them towards cell death.

Light damage. The most obvious factor that makes PRs vulnerable to degeneration is light exposure. Visible and ultraviolet light are insufficiently energetic to ionize most biomolecules[51], but oxygen enhances the ionizing effect of light[52] so that cellular damage can occur when reactive oxygen and nitrogen species (RONS) are generated

Rhodopsin
A member of the opsin family of G-protein-coupled receptors that is found in vertebrate rod photoreceptors, where it is covalently coupled to the light-sensitive chromophore 11-*cis* retinal to form the visual pigment.

Ciliopathy
A disorder of cilia — the small hair-like organelles that are attached to the surface of almost all polarized cells. Cilia can subserve motor or sensory functions, the latter being particularly important in photoreceptors, which contain a modified cilium.

Complement
A group of about 30 circulating proteases involved in innate immunity that are normally inactive but that can be activated by foreign or altered self antigens, initiating a cascade leading to cell lysis or phagocytosis.

Odds ratio
The odds of carrying a genetic variant (or other hazard exposure) in cases compared with controls. It can be used as a measure of effect size in case–control association studies. An odds ratio significantly different from one suggests that the genetic variant is associated with the disease or trait.

by light acting on photosensitizing molecules, such as retinoids. The focusing of light onto the central macula of the retina (FIG. 1) is essential for high-acuity vision, but it makes this region vulnerable to light damage.

Wavelengths of ~500 nm (blue light) — similar to the absorption spectrum of rhodopsin[53] — cause most PR cell death, and there is growing evidence that light damage to PRs requires the release of all-*trans* retinal from light-activated rhodopsin[54,55]. Photo-excitation of all-*trans* retinal generates singlet oxygen and can cause photo-oxidative damage. If mutations affecting the visual cycle (FIG. 3) block the recycling of all-*trans* retinal to 11-*cis* retinal, toxic bis-retinoids (such as the all-*trans* retinal dimer) and adducts (such as *N*-retinylidene-*N*-retinyl-ethanolamine (A2E)) build up with advancing age. Toxic bis-retinoids can undergo photo-oxidation to generate RONS[56,57] and form the major constituent of the auto-fluorescent ocular pigment lipofuscin. Lipofuscin is not readily degraded and is increased in tissue samples from a variety of PR degenerations[55,57,58]. These retinoid derivatives are normally kept at low concentrations in PRs by enzymes and transporters but can accumulate in RPE owing to the daily phagocytosis of the distal (oldest) 10% of PR outer segments.

Some types of PR degeneration are accelerated by light[1,59]. Avoidance of light slows or even halts some degenerations but has no effect on others[58]. New *in vivo* imaging techniques show the excessive build-up of lipofuscin in RPE cells in an unexpectedly broad range of PR degenerations[60–62]. Based on increased RPE autofluorescence, defects that are potentially exacerbated by light include: visual cycle defects (for example, *ABCA4* or retinol dehydrogenase 12 (*RDH12*) mutations[63]); RPE phagocytosis defects (for example, mer proto-oncogene tyrosine kinase (*MERTK*) mutations[64]); defects in the stability of outer segment discs (for example, peripherin 2 (*PRPH2*) mutations[65]); and PR cilia defects that seem to slow outer segment turnover (for example, *RPGR* mutations[60]).

Lipid oxidation. The extremely lipid-rich outer segments of PRs, which account for the majority of the surface area of these cells, provide another source of vulnerability. The lipid content of PRs is about 15% of wet weight, compared with 1% of wet weight in most cells[66]. The very-long-chain polyunsaturated fatty acid (PUFA) content of outer segment membranes is also high; for example, PRs have the highest concentration of the PUFA docosahexaenoic acid (DHA) in the body. PUFAs are readily oxidized to highly reactive electrophilic aldehydes and other compounds, including malondialdehde (MDA) and 4-hydroxy-2-nonenal (HNE), that initiate destructive free radical chain reactions[67]. The daily phagocytosis of outer segments by RPE may have evolved as a mechanism to prevent the build-up of these oxidized lipids, as well as to prevent build-up of the bis-retinoids discussed above[68].

Disorders of PUFA synthesis can cause inherited PR degeneration. For example, mutations in the elongation of very-long-chain fatty acids-like 4 (*ELOVL4*) gene cause an autosomal dominant macular degeneration that resembles Stargardt disease[69]. The ELOVL4 protein catalyses the elongation step in the synthesis of C28 and C30 saturated and polyunsaturated fatty acids[70]. C28–C36 fatty acids are found in abundant phosphatidycholine species in rod and cone outer segments[71]. Several mouse models of *ELOVL4* disease show increased retinal accumulation of lipofuscin and A2E, which suggests that RPE and secondary PR cell death in this disorder results from photosensitization and increased RONS formation, similar to *ABCA4* defects[71].

In AMD, lipid peroxidation products, such as HNE and MDA, form covalent adducts with proteins, including lysosomal cysteine proteases, which results in reduced lysosomal proteolysis and exocytosis of undegraded protein adducts from the basolateral RPE[72]. Some adducts — such as carboxyethylpyrrole (CEP) protein adducts, which are derived from oxidized DHA and are more abundant in AMD than in normal human retinas[73] — activate the complement pathway. CEP-adduct immunized mice have AMD-like features,

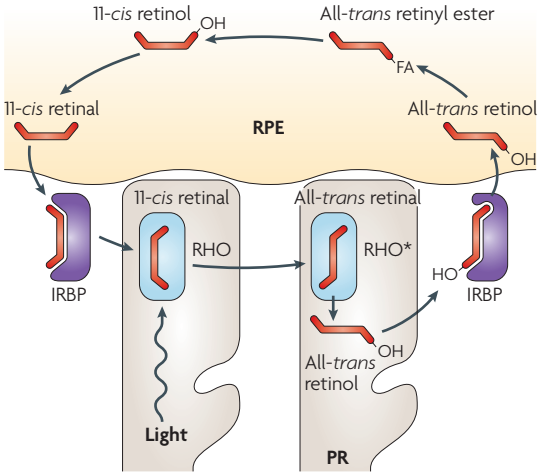


Figure 3 | **The rod photoreceptor visual cycle.** The visual cycle is a pathway of enzymatic reactions that recycle the retinoids that are used during light detection in photoreceptor (PR) cells. The activation of the PR pigment rhodopsin (RHO, RHO* when activated) by light occurs through the isomerization of 11-*cis* retinal, the chromophore that is bound to rhodopsin, to all-*trans* retinal. All-*trans* retinal is released from rhodopsin, conjugated with the membrane lipid phosphatidylethanolamine and transported to the cytoplasm by ATP-binding cassette, subfamily A, member 4 (ABCA4) (not shown). After modification to all-*trans* retinol by a retinol dehydrogenase (hydroxyl group shown as OH), it is transported to the RPE, where it is esterified to a fatty acyl group (FA) by lecithin retinol acyltransferase (LRAT) to form all-*trans* retinyl ester. All-*trans* retinyl ester is subject to *trans*-isomerization to 11-*cis* retinal through the actions of two further enzymes (RPE65 and 11-*cis* retinol dehydrogenase). After transport back to the PR cell, 11-*cis* retinal binds rhodopsin, rendering it sensitive to light. Retinoid-binding proteins, such as interstitial retinol-binding protein (IRBP, also known as RBP3), cellular retinol-binding protein and cellular retinaldehyde-binding protein, are involved in the transport of the hydrophobic retinoids in an aqueous environment.

Balancing selection
Selection that favours the maintenance of more than one polymorphic allele in a population by mechanisms such as frequency-dependent selection or heterozygote advantage.

Phototransduction
The biochemical process by which a light signal is absorbed by visual pigments in photoreceptors, amplified and converted into a neuronal response.

TUNEL staining
A terminal deoxyuridine 5′-triphosphate nick-end-labelling assay. It involves the enzymatic labelling of the 3′ ends of partially degraded DNA in a cell undergoing apoptosis (and some other forms of cell death).

Apoptosis
A type of programmed cell death in which specific cellular machinery causes the cell to self-destruct. Membranous vesicles are formed and are removed by phagocytic cells.

Caspase
A family of intracellular proteases that cleave specific protein targets at cysteine-aspartic acid residues and are responsible for the breakdown of the cell during some types of (caspase-dependent) apoptotic cell death. They are synthesized as inactive procaspases that are activated by proteolytic cleavage (often by other caspases).

Autophagy
A catabolic process involving the degradation of a cell's own components by means of the lysosomal machinery.

Reactive oxygen and nitrogen species
Oxygen- or nitrogen oxide-containing free radicals that contain one or more unpaired electrons (such as superoxide or nitric oxide) together with non-radical oxygen or nitrogen oxide derivatives (such as hydrogen peroxide or peroxynitrite).

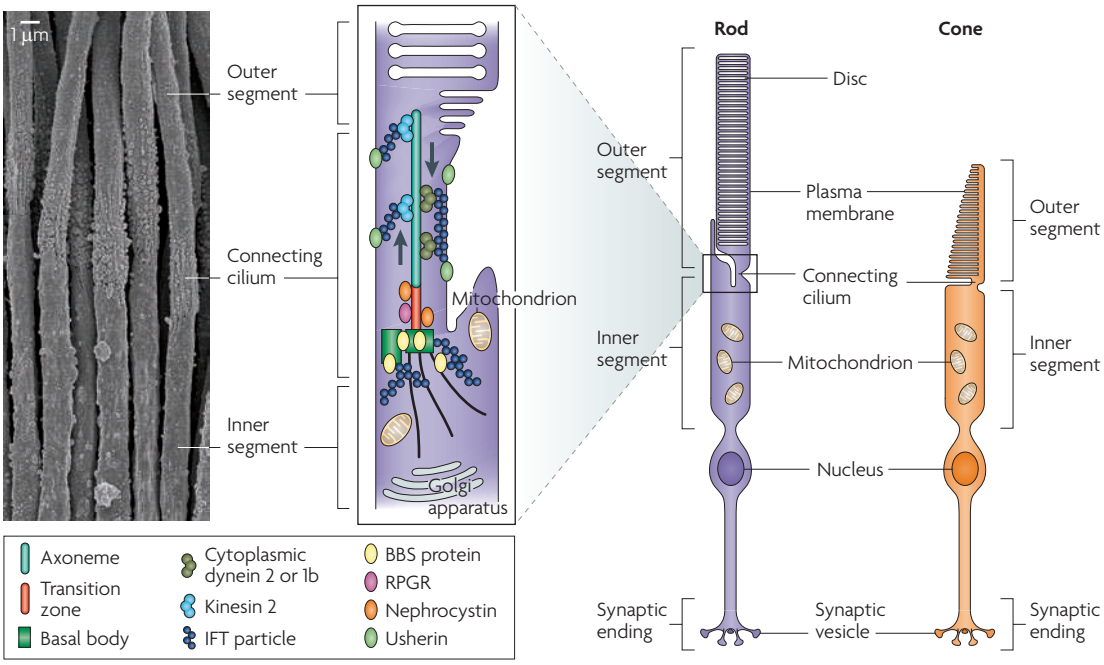


Figure 4 | **The photoreceptor connecting cilium.** The connecting cilium (shown in the electron micrograph on the left) is the counterpart of the transition zone of a primary cilium and (with the basal body and outer segment) forms part of a modified sensory cilium that is characteristic of vertebrate photoreceptors (shown schematically on the right). The connecting cilium consists of a backbone, called the axoneme, that is comprised of nine microtubule doublets arranged in a circle. The cilium is anchored to the basal body, which organizes microtubule assembly (centre panel). The inner segment contains the major metabolic and polarized trafficking machinery. The outer segment contains the phototransduction apparatus. There is a high flow of proteins and membranes from the inner to the outer segments through intraflagellar transport (IFT) mechanisms, driven by kinesin 2 motors (towards the tip of the axoneme) or by dynein motors (towards the basal body), so the cilium is crucial for the function and survival of photoreceptors. BBS, Bardet–Biedl syndrome; RPGR, retinitis pigmentosa GTPase regulator. Electron micrograph courtesy of P. Munro, UCL Institute of Ophthalmology, University of London, UK. The central diagram is modified, with permission, from *Nature Reviews Molecular Cell Biology* REF. 86 © (2007) Macmillan Publishers Ltd. All rights reserved.

Lipofuscin
General term for a variety of coloured lipid-soluble pigments that accumulate in cells, especially during ageing.

Alternative complement pathway
A part of the innate immune system that involves a cascade of steps, starting with the binding of complement component 3b (C3b) to foreign or host cell surfaces and leading to the formation of a terminal membrane attack complex, which lyses target cells.

Microglia
Resident macrophages of haematopoietic origin located in the retina and other parts of the central nervous system. They can become motile and phagocytic after activation by specific changes in their external environment.

Inner segment
The middle part of the photoreceptor, which contains densely packed mitochondria, a basal body, a Golgi complex, endoplasmic reticulum and microtubules. It is separated from the outer segment by the connecting cilium.

which supports a role for these compounds in activation of the complement pathway in AMD[74].

Complement activation and neuroinflammation. In AMD, there is strong evidence of complement-mediated inflammatory damage to RPE and Bruch's membrane (FIG. 1). PR loss occurs later, following the death of RPE cells or the invasion of the PR cell layer by abnormal blood vessels. Variants in genes that regulate the alternative complement pathway are associated with AMD, as discussed above. Major environmental influences, such as smoking, also influence this pathway. The extracellular deposits found between the basolateral RPE and Bruch's membrane, which are characteristic of AMD, include complement-pathway and acute-phase proteins. The most likely explanation for this is that these deposits reflect the activation of the innate immune system by oxidized lipids — including the bis-retinoid components of lipofuscin[75] and CEP adducts[73,76] — and by oxidized proteins that are extruded from the basolateral RPE. Genetic variants that reduce the ability to downregulate the alternative complement pathway may therefore predispose macular RPE cells and their associated PRs to cell death. Complement activation products, such as C3a and C5a[77], CEP adducts[78] and bis-retinoids[79], also stimulate invasion of the macula by abnormal blood vessels, leading to pathological neovascularization.

As in several types of central nervous system neurodegeneration, a neuroinflammatory role for microglia has been proposed in some inherited PR degenerations. Microglia are resident macrophages[80,81] that become activated and migrate to regions undergoing PR cell death, where they can be either protective or neurotoxic. Neurotoxicity results from the release of pro-inflammatory cytokines and RONS, such as nitric oxide and superoxide. The resultant oxidative environment may contribute to non-cell autonomous (bystander) PR damage, as PR degeneration can be slowed by corticosteroids that reduce microglial RONS formation[82].

Ciliary transport defects. The Achilles heel of the PR seems to be the modified cilium that connects the inner segment and the outer segment (FIG. 4). The outer segment cannot synthesize the proteins and membranes that it requires for phototransduction, so these must travel at high flow rates through the connecting cilium[83]. Almost one-quarter of known PR degeneration genes are associated with ciliary structure or function (FIG. 2). This might reflect the proteomic complexity of vertebrate cilia, in which >1,000 proteins have been identified[84]. Many ciliary proteins

are also expressed in other cellular compartments and in non-ocular cells, which accounts for the non-ocular features of many ciliopathies that include RP[85,86]. The precise functions of these cilia-associated proteins are still being elucidated but, to date, most seem to have primary effects on ciliary structure and trafficking rather than polarity or signalling. For example, a complex involving seven BBS proteins (known as the BBSome) seems to regulate the docking and fusion of vesicles from the Golgi complex at the base of the connecting cilium[87]. A complex involving several Usher syndrome proteins is thought to be involved in functions such as vesicular docking or transport in inner-ear hair cells and in PRs[88]. Disruption of ciliary trafficking is expected to compromise all outer segment functions, from ion movements and consequent energy utilization to phototransduction and RPE phagocytosis, and is therefore expected to have far-reaching cellular consequences that increase vulnerability to cell death.

Endoplasmic reticulum stress. Oxidative stress due to protein misfolding is the most common single cause of neurodegeneration in the central nervous system[89]. The situation is similar in PRs, in which rhodopsin mutations often cause misfolding and retention in the endoplasmic reticulum (ER), leading to ER stress[90]. As rhodopsin is abundant (it constitutes 85–90% of outer segment protein mass) and hydrophobic, such misfolding can trigger the unfolded protein response (UPR) in the ER[91]. The UPR seems to result from increased RONS signalling in response to repeated attempts to fold reduced and unfolded protein substrates[92]. UPR signalling pathways aim to enhance the folding capacity and to decrease the amount of misfolded protein[93]. If the amount of misfolded protein exceeds the capacity of the UPR, cellular stress occurs, which can activate pro-apoptotic signalling pathways. For example, the UPR is activated by the Pro23His rhodopsin mutation in transgenic rats, but this is followed by a rise in expression of the transcription factor CCAAT/enhancer-binding protein homologous protein (CHOP), which is a component of the ER stress-mediated pathway that activates pro-apoptotic genes[94]. In addition to rhodopsin, mutations in at least 13 other PR degeneration genes cause misfolding of their protein products and ER stress[95].

Metabolic stress and mRNA processing. Metabolic stress is a common trigger for apoptotic cell death[96]. Mutations that affect several proteins involved in intermediary metabolism or fatty acid metabolism can cause PR degeneration, often as part of more widespread neurodegeneration (FIG. 2; Supplementary information S1 (table)). Isolated PR degeneration can also have a metabolic cause. For example, mutation in the NAD-specific mitochondrial enzyme isocitrate dehydrogenase 3 (IDH3) causes RP. Mutations in the gene encoding another metabolic enzyme, inosine-5′-monophosphate dehydrogenase 1 (IMPDH1), probably do not cause PR degeneration through metabolic dysfunction; the mutations cause loss of the ability to regulate the translation of specific retinal mRNAs — including rhodopsin — at polyribosomes, rather than catalytic defects[97]. Mutations in the ubiquitously expressed pre-mRNA processing factors PRPF31, PRPF8, PRPF3 and PAP1, all of which cause RP, may impose a similar type of cellular stress as a result of the unusual pre-mRNA processing requirements of the retina due to rhodopsin and other PR-specific transcripts[98].

Survival signalling. A key goal in developing therapies for inherited PR degeneration is to prevent the secondary, non-cell-autonomous death of cones, which accounts for the greatest disability in disorders such as RP (BOX 1). Many reasons have been suggested for secondary cone cell death, including lack of rod-derived survival or neurotrophic factors[99,100], nutrient deprivation due to an abnormal cone–RPE interface resulting from rod loss[101], release of toxic metabolites by dying rods[102], oxidative stress due to retinal hyperoxia[103] and 'collateral damage' caused by activated microglia[81,82]. It seems likely that more than one mechanism is involved. For example, insulin infusion and various antioxidants reduce cone cell death in mouse models, which provides evidence for both nutrient deprivation[101] and oxidative stress[104,105] being involved in cone cell death.

Survival factors are also important in rod degeneration, as four different neurotrophic factors (ciliary neurotrophic factor (CNTF), basic fibroblast growth factor, brain-derived neurotrophic factor and nerve growth factor) and one 'viability' factor (rod-derived cone viability factor) delay rod degeneration in some animal models of RP[99,100]. CNTF shows efficacy in 13 different animal models and has progressed to a phase II clinical trial in RP[106]. Histone deacetylase 4 (HDAC4) has recently been found to confer neuroprotective activity, which could provide another therapeutic lead[107].

Altered bioenergetic function. Oxygen can be highly toxic, as it can directly oxidize essential biomolecules or be partially reduced to RONS, which in excessive amounts can cause cell death[51]. Therefore, aerobic organisms maintain tight control of cellular oxygen concentrations. In PR degeneration, bioenergetic function often declines, causing the oxygen tension in the inner and outer segments to rise, often dangerously. The constriction of inner retinal arterioles, one of the hallmarks of RP, has been proposed to result from hyperoxia and vascular damage that extends from the PRs even into the inner retina[108,109], exacerbated by the inability of choroidal vessels to autoregulate in response to falling oxygen demand[103]. Mitochondrial oxidative phosphorylation (OXPHOS) is the major consumer of oxygen in the retina[110] and is therefore the first line of defence against oxygen toxicity, but if cellular demand for ATP falls, so does the antioxidant capacity of OXPHOS to fully reduce oxygen to water. A number of animal models of RP show clear evidence of rising oxygen levels as the disease progresses, particularly in PR inner segments[111]. Inherited defects in phototransduction, the visual cycle and outer segment maintenance are all expected to cause reduced energy utilization and consequent hyperoxia. The biological defences against hyperoxia are not as robust as those against hypoxia, so even a small rise in cellular

Intermediary metabolism
The intermediate steps, catalysed by enzymes, in which foodstuffs are metabolized in cells and converted into cellular components.

Neurotrophic factors
(Also known as neurotrophins.) A broad group of secreted proteins that support the survival, differentiation or function of neurons.

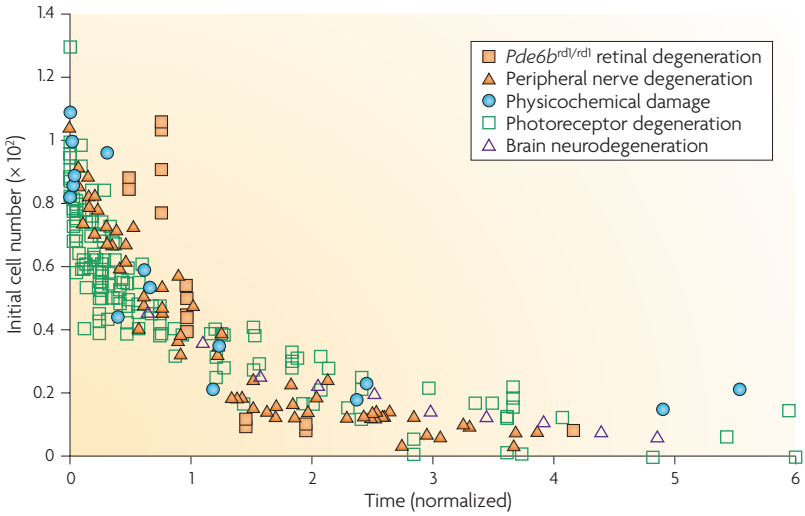


Figure 5 | **Stochastic mechanisms in cell death.** Model showing that a (stretched) exponential kinetic mechanism is almost universally applicable in neurodegenerative diseases of brain and retina. The kinetics of cell death resemble an exponential decay process, although the rate slows slightly as the degeneration advances. The data are from a range of neurodegeneration models normalized to the exponential parameter and follow a single time curve, consistent with a near universal mechanism underlying the cell death process. The only degeneration that deviates from the curve is seen in the phosphodiesterase 6B (*Pde6b*)$^{rd1/rd1}$ mouse, which shows the fastest known retinal degeneration (orange squares) and seems to be atypical. Orange triangles represent peripheral nerve degeneration data; blue circles represent physicochemical damage data; open squares represent data from different inherited photoreceptor degenerations; and open triangles represent brain neurodegeneration data. The graph is modified, with permission, from REF. 115 © (2005) Elsevier, and shows the trend of the data but not the accurate points.

oxygen could disproportionately influence the likelihood of cell death due to changes in RONS signalling.

Integrating diverse cellular stresses

Some themes, such as increased RONS and bioenergetic dysfunction, recur in the PR cell-death mechanisms discussed above, but how do the different mechanisms converge on a final common death pathway? Similar mechanistic themes have been identified for neurodegenerative disorders that affect the brain[112,113], but how the diverse disease pathways are integrated and relate to the kinetics of PR cell death — in which genetically equivalent cells die at different times, sometimes several decades apart, as a result of a stochastic process[114,115] — is more difficult to explain.

All investigated examples of inherited PR cell death are consistent with a kinetic model in which the probability of apoptosis, averaged across all cells, is more or less constant over time, declining slightly in later stages (stretched exponential kinetics)[114,115]. Cell death therefore resembles an exponential decay process in which the rate of PR loss is proportional to the number of surviving PRs, and the rate constant differs according to disease severity[115,116] (FIG. 5). In any one PR degeneration, individual PRs can have different probabilities of cell death, but these are predicted to vary around a mean value for a given genotype and species[116]. In this model, all PRs start with a relatively high probability of cell death compared with other cells, reflecting their intrinsic vulnerability, but this is increased further by almost any mutation. The slight decline in cell death rate with time probably reflects compensatory processes. The consistency of this exponential process across different forms of neurodegeneration suggests a universal mechanism of neuronal cell death[114,115]. It clearly excludes a model in which the probability of cell death increases with time, as might be expected if RONS-mediated damage resulting from factors such as photo-oxidation or protein misfolding accumulates as the degeneration progresses.

How can the kinetic and mechanistic data be reconciled? One proposed model argues that the diverse cellular stresses associated with the >180 different inherited PR degenerations are integrated by mitochondrial signalling pathways, which exert dominant control over the propensity of a cell to undergo apoptosis[116,117]. It is suggested that each PR lineage acquires genomic damage to varying extents, which differentially affects mitochondrial bioenergetic function and sets apoptotic signalling pathways accordingly[117] (BOX 2). Stochastic factors would therefore result in the early death of some PRs, whereas others survive for a lifetime.

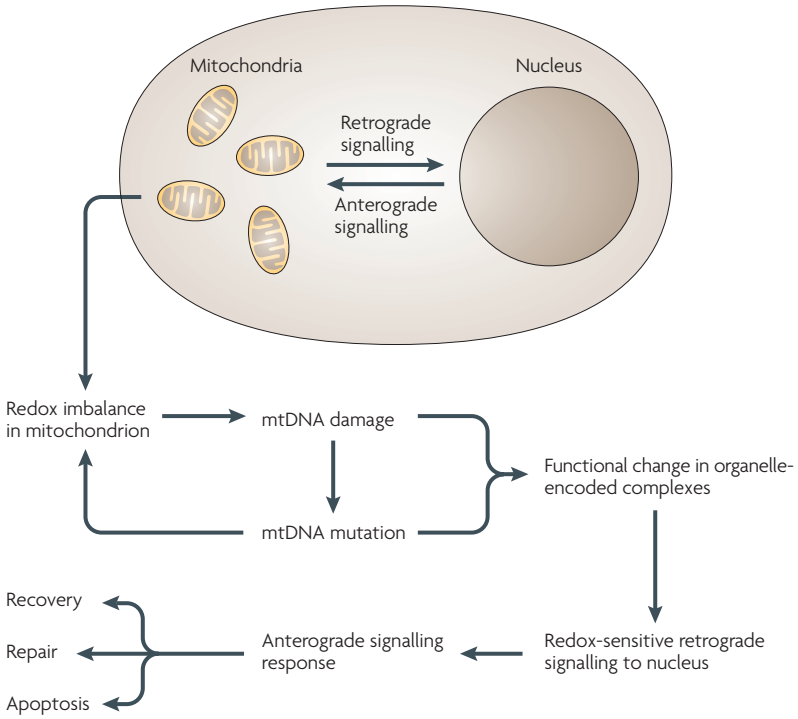
The observed kinetics of cell death can only be reconciled with the numerous cellular stresses discussed above if these all lead to a quasi-steady-state level of pro-apoptotic signalling early in the disease, perhaps reflecting an equilibrium between damage and compensatory responses, such as repair or upregulation of survival factors. The central role of mitochondria in control of pro-apoptotic signalling in response to diverse cellular stresses is consistent with biochemical[118] and evolutionary data[117] as well as the kinetics of PR degeneration[114,115].

Therapy: light at the end of the tunnel?

Incremental advances have been made in either slowing PR degeneration or improving PR function using neuroprotective factors[99–101,106,119], antioxidants[104,105,120], drugs[82,121,122], stem cells or progenitor cells[123,124]. However, in humans and animal models, ocular delivery of a functional gene using a viral vector (gene-replacement therapy) has restored vision to a remarkable degree in a recessive form of Leber congenital amaurosis (LCA) caused by *RPE65* mutations[125–127]. This opens the door to a widely applicable means of rescuing vision across many other PR degenerations[128,129].

However, the large number of disease genes and mechanisms that cause PR degeneration suggests that substantial problems might still lie ahead. Treatment will need to be tailored to each of the different PR degeneration genes, many of which have hundreds of different alleles with potentially different pathogenetic effects. Mutations will need to be classified as loss or gain of function, implying the need for large-scale functional assessment of mutant proteins and large-scale testing of individual vectors for safety and efficacy. The small number of common susceptibility variants that influence AMD might provide a more attractive proposition, but even so it is questionable whether retinal surgery would be worth the risk if it only reduces the chance of blindness from, for example, 10% to 4%.

Box 2 | **Mitochondrial integration model: a final common cell death pathway**



Different neurodegenerative conditions are caused by defects in diverse cellular pathways, but the kinetics of cell death are often similar (FIG. 5). What could be the explanation for this? One way of viewing this question is to compare rates of neuronal cell death caused by equivalent mutations in the same genes across different species[116]. Rates of neurodegeneration in five mammalian species were compared for ten different inherited neurodegenerations caused by functionally equivalent mutations. The results showed an inverse relationship between rate of degeneration and the lifespan of the species. Equivalent mutations in these functionally highly conserved genes differed by up to 100-fold in the resultant rates of degeneration depending only on the species in which they occurred. A plausible explanation was proposed to lie in the strong inverse correlation between constitutive mitochondrial reactive oxygen and nitrogen species (RONS) formation and lifespan[116,148]. Other factors, such as basal metabolic rate, body mass index, phylogenetic group and chance were shown to be unlikely.

Mitochondria are known to integrate a diverse range of cellular life and death responses through changes in neurotrophin or stress signalling, but why should mitochondrial RONS exert such a powerful influence on these responses? One possible explanation comes from the proposal that the extraordinary vulnerability of the mitochondrial genome to oxidative damage is evolutionarily advantageous, as it provides a cumulative sensor for redox damage in cell lineages[117] (see figure). Cellular redox imbalance leading to mutations in the mitochondrial DNA (mtDNA) provides a signal that can traverse cell generations and activate retrograde signalling pathways (mitochondrion to nucleus) in cells, including post-mitotic cells such as photoreceptors. Redox-induced damage to many of the ~1,500 nuclear genes that are expressed in mitochondria and that also influence bioenergetic function could have a similar effect on retrograde signalling pathways, but the nuclear genome is at least an order of magnitude less sensitive to damage than the mitochondrial genome.

These redox-sensitive signalling pathways have been well characterized in many organisms and usually involve RONS-induced post-translational modification of functionally crucial thiols in specific enzymes or transcription factors, which enables these proteins to signal to the nucleus[149–151]. The nucleus generates an anterograde (nucleus to mitochondrion) response, which has been proposed to set the baseline level of pro-apoptotic signalling and the resultant probability of cell death[117]. This provides a way in which the organism can detect and eliminate cells that compromise its fitness as a result of cumulative genomic damage. The figure is modified, with permission, from REF. 117 © (2009) Elsevier.

The 100-fold higher rate of recessive over dominant mutations in mouse mutagenesis screens suggests that most PR degenerations are likely to be functionally recessive due to loss-of-function mutations[130,131]. The potential of gene-replacement therapy is that it offers a realistic means of slowing cell death in this major subgroup, almost regardless of the initiating cellular stress. However, genes that cause autosomal recessive forms of PR degeneration are under-represented in current databases owing to the difficulty in identifying them. Causal genes have been assigned to little more than half of all PR degeneration patients; identifying the remainder, many of which may be very rare or hard to detect, may show diminishing returns. Generic treatment approaches that work across genetic subtypes therefore offer advantages, although progress has been slow to date.

Assessment of therapeutic success using any treatment method will be difficult, as disease progression is slow and 1–2 years of careful clinical assessment in homogeneous patient groups will usually be required (in contrast to the rapid functional read-out for rare visual cycle defects[125–127]). Also, the widespread occurrence of retinal remodelling[132–135], such as loss of normal lamination or synaptic rearrangements, presents further challenges. Remodelling seems to be a neuronal–glial response to PR loss that is seen in many humans and animals with PR degeneration[136,137], even at early stages of the disease, which suggests that it may be hard to reverse.

Dominant (gain-of-function) mutations require alternative therapeutic approaches, such as subretinal delivery of small interfering RNA together with gene replacement, which are currently under development[138,139]. However, these diseases are often quite advanced before they receive clinical attention[140,141], by which time the retina may be metabolically abnormal. The scope of genetic testing for early diagnosis is limited by the fact that about half of affected subjects have no family history of disease. This raises the question of whether the hard-won knowledge of disease mechanisms discussed above will help to overcome some of these potential hurdles.

Capitalizing on mechanistic knowledge

Therapeutic intervention during the early stages of PR degeneration, particularly using gene therapy or combined approaches, has an excellent prospect of delaying or preventing further cell death. In early or slowly progressing disease, vision may even be substantially improved, as with *RPE65* mutations. This is particularly important for congenital disorders, such as LCA, in which late intervention may limit success because the brain has insufficient plasticity to achieve high-acuity vision[142].

If the mitochondrial integration model of cell death is correct, each PR cell in an affected individual will have an increased probability of cell death owing to the early establishment of a raised level of pro-apoptotic signalling specific to the particular genotype. To slow cell death, a lower level of pro-apoptotic signalling will need to be established by therapeutic intervention. However, there are likely to be secondary changes in the remaining PRs and elsewhere in the retina as a result of PR death.

Necrosis
Localized cell death resulting from external agents, such as injury or infection, in which there is rupture of the plasma and organellar membranes with release of the cellular contents, often leading to inflammation.

For example, mutation in *PRPH2* may cause abnormal outer segment disc formation, which leads to shortened outer segments, in turn reducing phototransduction, ion channel conductance and the high level of energy utilization that is required for maintaining ionic gradients. Oxygen utilization will fall and a pro-oxidant state ensues, establishing a higher level of pro-apoptotic signalling. Phagocytosis of outer segment discs may slow, so photosensitizing lipids accumulate. Adaptive changes are likely to accompany each step in the sequence, and some slowing of the cell death rate may follow as these become more effective. In this scenario, stopping disease progression would require the re-growth of outer segments and the resumption of phototransduction and ion channel conductance activity on a sufficiently large scale across the retina to more or less restore the bioenergetic *status quo*. If many rods have been lost, this may never happen. Intervention using drugs that block the final step of the apoptotic process[122] might cause the persistence of PRs that are bioenergetically beyond repair, which would lead to necrosis and inflammation.

What solutions can be offered? Unless treatment can be initiated early, there is a strong rationale for combining gene therapy with local delivery of antioxidants and/or neurotrophins, which may be synergistic. However, the best and safest combinations remain to be established. Mitochondrially targeted antioxidant enzymes[105] or small molecules conjugated to lipophilic cations[143], which can reach substantially higher intra-mitochondrial concentrations than in plasma, are also potentially useful. Combination therapies are not attractive to grant panels, regulatory authorities or pharmaceutical companies, but they already underpin current treatments for many types of cancer and infectious disease. Large pharmaceutical companies are now interested in the substantial market for the prevention of AMD, and recent advances in understanding the genetic basis of the disease and the roles of complement and neuroinflammation are likely to be beneficial. Approaches might include using small-molecule inhibitors of the alternative complement pathway, inhibiting lipofuscin formation and angiogenesis or locally suppressing microglia activation.

After retinal cell loss becomes severe, cell replacement using fetal progenitor, embryonic, neural, haematopoietic or induced pluripotent stem cells are important approaches. Some of these show considerable promise, although most are at a relatively early stage in terms of clinical translation[144]. Human embryonic stem cells can differentiate into RPE and PRs that are potentially suitable for transplantation, although possible tumorigenicity remains a concern[124,145–147]. The whole field is evolving very rapidly, so for the millions of PR-degeneration sufferers worldwide who have residual PRs, the prospect of halting disease progression no longer seems unrealistic.

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Competing interests statement
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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
ABCA4 | *ARMS2* | *CFH* | *FBLN5* | *PRPF31* | *RHO* | *RPE65* | *RPGR* | *USH2A*
The Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff: http://www.hgmd.cf.ac.uk
OMIM: http://www.ncbi.nlm.nih.gov/omim
BBS | LCA
Retinal Information Network (RetNet): http://www.sph.uth.tmc.edu/Retnet
UniProtKB: http://www.uniprot.org
RBP3

FURTHER INFORMATION
Alan F. Wright's homepage:
http://www.hgu.mrc.ac.uk/people/a.wright.html
Shomi S. Bhattacharya's homepage: http://www.ucl.ac.uk/ioo/pdf/PI/Professor%20Shomi%20Bhatt.pdf

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