

Pathophysiology of Age-Related Macular Degeneration: Implications for Treatment

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Keywords

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Abstract

Age-related macular degeneration (AMD) is a complex, multifactorial, progressive retinal disease that affects millions of people worldwide and has become the leading cause of visual impairment in developed countries. The disease etio-pathogenesis is not understood fully, although many triggers and processes that lead to dysfunction and degeneration of the retinal pigment epithelium (RPE) have already been identified. Thus, the lack of cellular control of oxidative stress, altered proteostasis, dysfunction of lipid homeostasis, and mitochondrial dysfunction form an internal feedback loop that causes the RPE to fail and allows accumulation of abnormal misfolded proteins and abnormal lipids that will form drusen. An inadequate antioxidant response, deficits in autophagy mechanisms, and dysregulation of the extracellular matrix (ECM) help to increase the deposition of abnormal drusen material over time. The drusen then act as inflammatory centers that trigger chronic inflammation of the sub-retinal space in which microglia and recruited macrophages

are also involved, and where the complement system is a key component. Choriocapillaris degeneration and nutritional influences are also classic elements recognized in the AMD pathophysiology. The genetic component of the disease is embodied in the recognition of the described risk or protective polymorphisms of some complement and ECM related genes (mainly *CFH* and *ARMS2/HTRA1*). Thus, carriers of the risk haplotype at *ARMS2/HTRA1* have a higher risk of developing late AMD at a younger age. Finally, gut microbiota and epigenetics may play a role in modulating the progression to advanced AMD with the presence of local inflammatory conditions. Because of multiple implicated processes, different complex combinations of treatments will probably be the best option to obtain the best visual results; they in turn will differ depending on the type and spectrum of disease affecting individual patients or the disease stage in each patient at a specific moment. This will undoubtedly lead to personalized medicine for control and hopefully find a future cure. This necessitates the continued unraveling of all the processes involved in the pathogenesis of AMD that must be understood to devise the combinations of treatments for different concurrent or subsequent problems.

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Introduction

Age-related macular degeneration (AMD), a progressive degenerative retinal disease affecting the central macular region that involves the complex of photoreceptors, retinal pigment epithelium (RPE), Bruch's membrane (BrM), and the choroid [1], is a major cause of visual loss in elderly individuals in developed countries [2]. Outer retinal changes in normal aging include photoreceptor loss, BrM thickening, choroidal thinning, and one type of small subretinal deposits called hard drusen. But in AMD, there is a slow shift toward exacerbation of these changes [3]. Earlier non-sight-affecting stages of AMD classically feature deposition of large extracellular subretinal debris called soft drusen which locate between the BrM and the basal membrane of the RPE, and/or the so-called subretinal drusenoid deposits (SDDs) or reticular pseudodrusen (RPD) located over the apical side of the RPE, suggesting that potential pathophysiologic mechanisms on both sides of the RPE need to be taken into account [4]. A number of different systems exist for classifying these deposits, including categorization based on size, location, shape, appearance with multimodal imaging, microscopic appearance, and molecular composition [5]. SDD and soft drusen are similar in protein content but differ in lipid composition. In the natural disease course, these deposits grow in size and number and then regress. This drusen life cycle is accompanied by pathological alterations in the RPE, including detachment of individual RPE cells from their basement membrane and migration into the neurosensory retina [1]. In fact, AMD is a spectrum of diseases with different stages. The initial intermediate, usually asymptomatic, slowly progressive stage of AMD (iAMD) is characterized by the presence of the previously mentioned large soft drusen/SDDs and RPE changes. From this point, the disease progresses toward advanced stages that lead to visual loss with two possible clinical forms that in some cases present together. First, the appearance of incomplete or complete Retinal Pigment Epithelial and Outer Retinal Atrophy (iRORA/cRORA) occurs in what roughly correlates with the so-called geographic atrophy (GA), the characteristic lesion of the dry, nonexudative, or atrophic AMD (aAMD) [6–8]. Second, the wet, neovascular, or exudative AMD (nAMD) causes more rapid visual acuity (VA) loss [1, 3, 9], due to the appearance of macular neovascularization (MNV) lesions classified by optical coherence tomography (OCT) into type 1 (located under the RPE), type 2 (subretinal), and type 3 (retinal angiomatous proliferation). This classification of MNV allows to establish a

long-term visual prognosis, an estimate of the number of treatments that a certain case may require, and a stratification of the risk for secondary GA that may appear thereafter [7, 8, 10]. Nevertheless, nonexudative MNV can be present for months or years without developing any exudation [11].

The disease etiology and pathophysiology are not fully understood but include a long series of elements and processes that interact with each other and follow complex pathways. AMD is a multifactorial disease that involves aging, genetic susceptibility, and environmental risk factors. The etiopathology of AMD can be explained based on certain genetic variants together with alterations in the regulation of gene expression modulated by epigenetics, with the participation of environmental factors such as aging, smoking, hypertension, diet low in antioxidants, and obesity, which are also recognized as the main risk factors [12, 13]. The pathogenetic mechanisms involve defects in autophagy, proteolysis, and lipid homeostasis; an altered response to oxidative stress with mitochondrial dysfunction; alterations in the extracellular matrix (ECM) metalloproteinase system; an inflammatory response in the context of chronic inflammation with important participation of the complement system; and aging of the choroidal microvascularization [1, 14–16].

A wide spectrum of retinopathies presents also with drusen or drusen-like phenotypes, some of which are inherited monogenic conditions appearing in middle-aged patients that entail dysregulation of the complement cascade and/or disruption in the visual cycle that can lead to BrM thickening, such as dominant drusen due to mutations in the endothelial growth factor (EGF) containing the fibulin extracellular matrix protein 1 (*EFEMP1*) gene. Thus, drusen and/or SDDs are likely to represent phenotypic convergence of a number of diverse biologic processes that, despite being different diseases, lead to a similar phenotype. In fact, some genetic variants on these genes that cause monogenic diseases in middle-aged patients also are genetic risk factors for AMD, a distinct multifactorial disease that develops in older patients [5, 17]. This report reviews the published literature on AMD pathophysiology and is intended to update the currently available knowledge.

Methods

We conducted an extensive search of research studies that explain the mechanisms involved in disease onset; the studies have different hypotheses regarding the onset and often complement each other. The literature search was performed using the follow-

ing reference databases: PubMed, Medline, Web of Science, Scopus, and Cochrane Library Plus. The search strategies included the following medical subject heading terms: age-related macular degeneration, AMD, ARMD, physiopathology, pathogenesis, genetics, epigenetics, autophagy, oxidative stress, inflammation, mitochondria, matrix, and complement. Articles published in English and Spanish between 2015 and 2021 were included. We selected all experimental and clinical studies that fit our search. All articles underwent critical reading and qualitative analysis along with a cited reference search. The most important factors in the pathophysiology of AMD and its implications to fix treatment strategies are discussed below.

Oxidative Stress

The neuroretina, like the brain, is a highly metabolic tissue that is sensitive to noxious microenvironmental stimulations. However, unlike the brain, the macula is exposed continuously to high light stimulation, mainly ultraviolet A (UVA), which can generate large amounts of oxidized materials. Blue light exposure has been proven to be deleterious to RPE cells due to increased oxidative stress and cell death [18]. Furthermore, amyloid beta-peptides proteolytically derived from the amyloid precursor protein can accumulate in drusen deposits in AMD and there are clear links between amyloid precursor protein expression/proteolysis and UVA light exposure resistance of *in vitro* cultures of ARPE-19 cells. Then amyloid beta can activate the alternative complement pathway. It has been claimed that these effects can be mitigated by using blue light-filtering devices [19]. Nevertheless, there are conflicting results in the scientific literature regarding its use to prevent the onset of AMD in humans, and more studies are needed to demonstrate with certainty the benefit beyond what was observed *in vitro* [20].

Oxidative stress increases with age in retinal tissue and induces a para-inflammatory response [21]. It is highly associated with elevated intracellular reactive oxygen species (ROS), which are generated mainly by the electron transport chain in the inner membrane of mitochondria and consist of hydrogen peroxide, superoxide (O_2^-), and hydroxyl radicals. ROS can oxidize organelles, nucleic acids, proteins, or lipids [22], as well as a para-inflammatory response [21] that in turn can cause cellular damage. Also, reactive nitrogen species such as nitric oxide and its byproducts, nitrate, nitrite, peroxynitrite, and the peroxyl radical have a direct role in cellular signaling, vasodilation, and inflammation. Reactive nitrogen species can trigger nitrosative damage in proteins, lipids, deoxyribonucleic acid (DNA), and other molecules and, in the ret-

ina, can induce the photoreceptor cell death observed in AMD [21, 23].

Besides, the RPE of AMD patients has increased susceptibility to oxidative stress because its capacity to neutralize ROS declines with aging due to decreased antioxidant production (Fig. 1) [24]. Under normal conditions, nuclear factor erythroid-derived factor 2/antioxidant response element (NRF-2/ARE) and peroxisome proliferator-activated receptor coactivator protein 1 (PGC-1) are two central elements that control ROS levels in the RPE cells that may well be involved in the pathogenesis of the disease, since the NRF2/ARE pathway is a master regulator of endogenous antioxidant protection in the RPE [25]. NRF-2 translocates from the cytosol to the nucleus in response to oxidative stress, where it initiates the expression of several antioxidant defense systems and activates gene transcription involved in autophagy and proteolysis pathways. However, the PGC-1 family regulates a cascade of processes that govern the transcriptional control of mitochondrial biogenesis, cellular respiration function, and the mitochondrial antioxidant defense system [26]. Hence, NRF-2 and PGC-1 α are key players in the process of RPE damage associated with increased oxidative stress, protein aggregation, lipofuscin accumulation, mitochondrial damage, and mononuclear phagocyte infiltration into the retina, which also occurs in processes associated with AMD. Both the ubiquitin-proteasome system (UPS) and lysosome/autophagy clearance rates may be insufficient because NRF-2/PGC-1 α diminishes in aged RPE cells, leading to photoreceptor function loss [27].

Nutritional antioxidant supplements have been proposed to attenuate progression from iAMD to advanced disease. The Age-Related Eye Disease Study 2 (AREDS2) is a controlled randomized clinical trial using omega-3 fatty acids and/or lutein/zeaxanthin supplements in which that goal was achieved. In fact, prescribing these type of supplements is the only available therapeutic strategy to reduce late AMD incidence at present, but its effectiveness appears limited [28, 29].

Mitochondrial "Vicious Cycle" of Aging

Nordgaard et al. [30] reported that cellular respiration decreased in the RPE of AMD patients and that adenosine triphosphate (ATP) production was lower compared to controls. Besides the reduced expression of the α -, β -, and δ -ATP synthase subunits, subunit VIb of the cytochrome C oxidase complex, mitofilin, mtHsp70, and the mitochondrial translation factor Tu indicate mitochon-

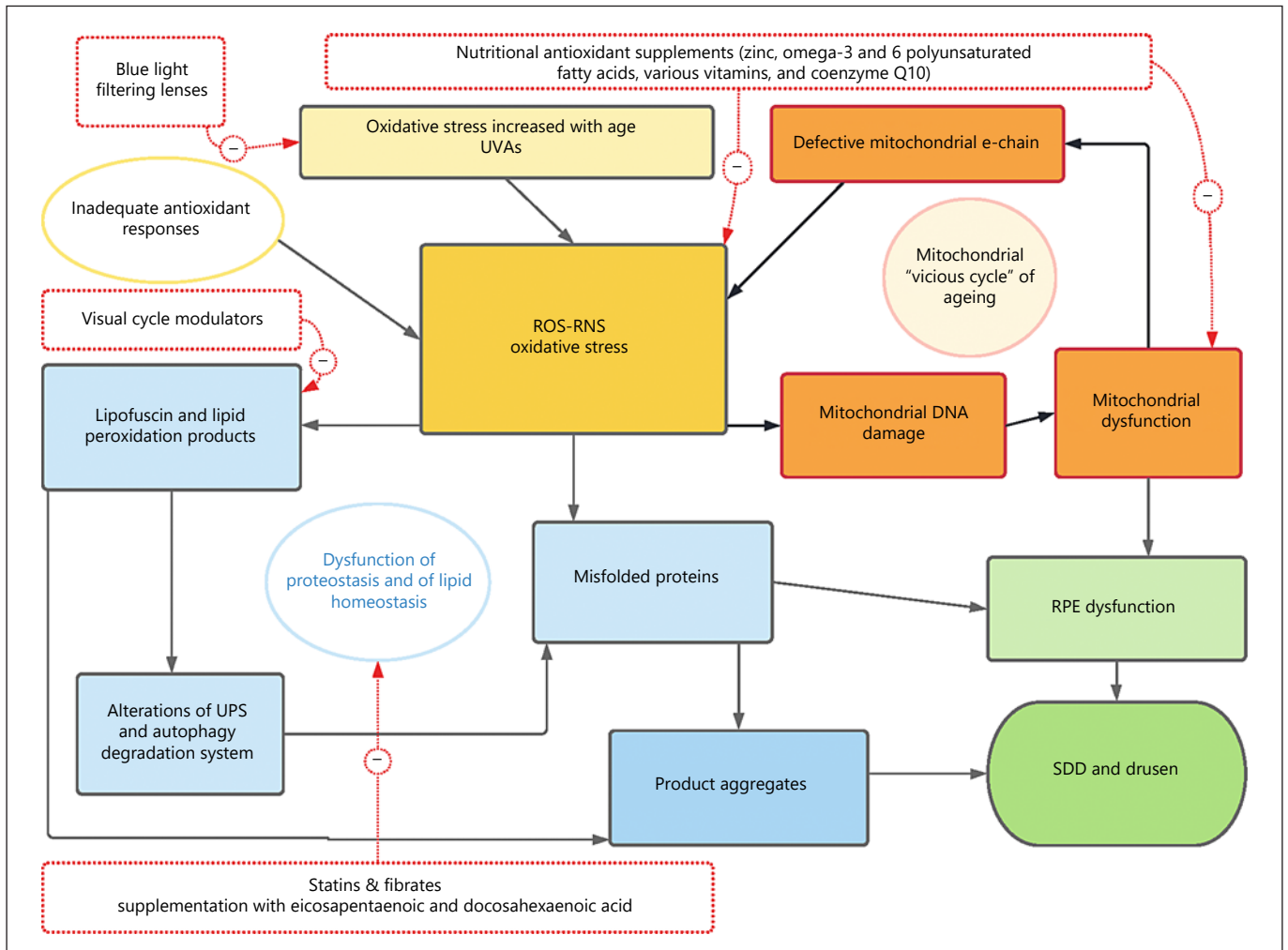


Fig. 1. Elevated ROS levels, mitochondrial failure, and accumulation of waste lipidic and protein aggregates are the primary inducers of RPE dysfunction leading to the appearance of abnormal deposits in early-intermediate AMD. The treatments directed to the

different therapeutic targets are framed in red boxes with dashed lines. UVAs, ultraviolet A; DNA, deoxyribonucleic acid; ROS, reactive oxygen species; RNS, reactive nitrogen species; RPE, retinal pigment epithelium; UPS, ubiquitin-proteasome system.

drial dysfunction. Converging evidence suggests an association between AMD and deficient mitochondrial importation of nuclear-encoded proteins, since mtHsp70 is required for the ATP-dependent importation of nuclear-encoded proteins into the mitochondrial matrix, and this may result in many matrix-localized functions including both the tricarboxylic acid cycle and β -oxidation [30].

Ferrington et al. [16] proposed that mitochondrial dysfunction initiates a cascade of events resulting in increased ROS production that exacerbates mitochondrial dysfunction if not properly removed from the cell. Functional analysis of the two major energy-producing path-

ways, oxidative phosphorylation and glycolysis, revealed changes in protein content and gene expression due to changes in the epigenetic landscape, leading to decreased bioenergetics and increased resistance to oxidative stress. These adjustments allow the cell to survive, but this system fails under conditions of chronic assault in the diseased retina [16]. In addition, poly(ADP-ribosylation) compartmentalized to the mitochondria can be converted from a homeostatic process under normal conditions to a mechanism of cell death when oxidative stress is accompanied by energy depletion [31]. All of this would be explained within the so-called mitochondrial “vicious cycle” theory of aging that proposes that ROS cause accu-

mulation of mutations in the mitochondrial DNA and e-chain coding genes, which subsequently increases ROS production. ROS overproduction would in turn generate further damage to mitochondrial DNA and increase the magnitude of the process in that cycle that ultimately leads to progressive tissue deterioration and dysfunction [32].

Dysfunction of Proteostasis and Lipid Homeostasis

Dysfunction of proteostasis due to uncontrolled oxidative stress in the RPE may explain the drusen formation in the early stages of AMD (Fig. 1). Normally, RPE cells participate in the renewal of the photoreceptor outer segments in a process of heterophagy from which numerous waste products are derived that must be properly managed to achieve cellular homeostasis. When this fails, waste products accumulate in those extracellular deposits referred to as drusen and SDDs. These disruptions in waste clearing lead to the accumulation of harmful lipid and protein aggregates that can act as a physical barrier to intracellular transport and disturb the proper functioning of RPE cells. Oxidized unsaturated fatty acids and misfolded or damaged proteins have been found among drusen components, and both appear to be involved in the pathogenesis of AMD [33].

All proteins require correct folding for stability and functionality, but ROS can interfere with folding, causing their destruction and making them prone to aggregate each other. Therefore, a high amount of ROS combined with a deficient antioxidant system leads to the detrimental aggregation of drusen-forming proteins, which will be accompanied by insufficient protein clearance [16].

Genetic, epidemiologic, and histopathologic studies indicate a causal role for abnormal lipid metabolism in the AMD pathogenesis. Serum high-density lipoprotein levels are correlated positively with AMD susceptibility, and cholesterol-rich sub-RPE and subretinal deposits are hallmarks of AMD progression. Excess cholesterol in turn entraps the anionic lipid bis(monoacyl)glycerophosphate (BMP) in the RPE lysosomes. BMP is a cofactor for acid sphingomyelinase, the lysosomal enzyme that hydrolyzes sphingomyelin to ceramide. In the RPE, ceramide affects microtubule stability and membrane dynamics, with consequences for metabolic and mitochondrial health. In humans, increased ceramide levels in the serum and RPE have been reported in patients with AMD but not in unaffected controls. Recent studies have suggested that bis-retinoids lead to a secondary accumula-

tion of cholesterol and ceramide due to a biophysical interaction between these lipids [34].

Also, the impaired ability of the aging RPE to participate in processing lipids in the subretinal space seems to be especially implicated in the formation of drusen [35]. The large volumetric component of drusen is lipids (>40%), principally esterified cholesterol, unesterified cholesterol, and phosphatidylcholine, which are thought to be derived from large lipoproteins of intraocular origin. SDDs contain unesterified cholesterol and undetectable esterified cholesterol, whereas sub-RPE basal laminar drusen contain both. Unesterified cholesterol is also a major structural element in cell membranes and is part of complex lipoprotein particles, especially those present in BrM. Nevertheless, the distinct lipid and mineral content of SDDs versus soft drusen indicates that SDDs are not just drusen in the wrong place [35].

Another important detail that must be considered is that AMD is associated with progressive accumulation of lipofuscin, melanolipofuscin, and melanosome granules in RPE cells. Low lipofuscin and high melanolipofuscin content within foveal RPE cell bodies and abundant lipofuscin at the perifovea suggest a different genesis, plausibly related to the population of overlying photoreceptors [36]. Also, redistribution and loss of autofluorescent granules are among the earliest AMD changes and could impact the fundus autofluorescence (AF) signal attributable to RPE at these locations [37]. Excessive accumulation of lipofuscin in the RPE also reflects that the cellular product degradation system is inefficient.

Super-resolution structured illumination microscopy has demonstrated that there are hundreds of AF organelles in individual human RPE cells viewed in a projection image [38]. Thus, digital maps of quantitative AF, cell density, and packing geometry of those can provide metrics for cellular-resolution clinical imaging [39]. Using these techniques, there is evidence that quantitative AF levels are not increased in eyes with large drusen compared with healthy eyes and that they significantly decline over time in the AMD eyes, highlighting that the relationship between quantitative AF levels and RPE health does not seem to be straightforward [40]. Quantifying RPE organelle number and its packing characteristics may be also important for validating the signal source for precise new diagnostic imaging technologies such as quantitative (blue), near-infrared autofluorescence for melanin, and OCT-based technologies, especially polarization sensitive OCT [36].

Lipofuscin is an autofluorescent pigment consisting of peroxidized lipids and phospholipids. It comprised vita-

min A metabolites called bis-retinoids that formed via nonenzymatic condensation reactions of retinaldehyde and phosphatidylethanolamine in the photoreceptor outer segment discs. Bis-retinoid oxidation products have both hydrophilic and amphiphilic properties, allowing their diffusion through the lipofuscin granule membrane into the RPE cell cytoplasm. These products contain cytotoxic carbonyls, which can modify cellular proteins and lipids. Protein glycation caused by these substances can activate inflammatory processes [16, 41]. In addition, lipofuscin may be cytotoxic by binding to the proteasome and lysosomal proteases that would inhibit degradation of oxidized proteins [42]. In fact, the inhibition of the proteasomal system is a major aspect of the cytotoxic effects of lipofuscin. Likewise, the main component of lipofuscin, N-retinylidene-N-retinylethanolamine (A2E), is a product of the interaction between all-*trans*-retinal and ethanolamine and may induce increased expression of VEGF, interleukins, and other inflammatory molecules in the retina, which stimulate new blood vessel formation in nAMD [43]; photosensitization then causes telomeric shortening and DNA damage of RPE cells, which may trigger their senescence and death, a process that would contribute to outer retinal atrophy in aAMD [44]. In brief, bis-retinoid fluorophores are photosensitizers and phototoxic to RPE and neuroretina cells, compromising RPE health and function by promoting oxidative stress and interfering with multiple homeostatic mechanisms, and they are likely an aggravating factor in the pathogenesis of AMD.

Besides, cytochrome P450 (CYP450) is an enzyme that plays a crucial role in the metabolism of long-chain polyunsaturated fatty acids (LCPUFAs) into epoxydocosapentaenoic acids and epoxyeicosatetraenoic acids that ultimately regulate vascular function, and it has been suggested that CYP450 monooxygenase plays a vital role in inhibiting nAMD via LCPUFA metabolites [45]. Polymorphisms in several cholesterol pathway genes including apolipoprotein E (*APOE*), ATP Binding Cassette Subfamily A Member 1 (*ABCA1*), *lipase C* (*LIPC*), Advanced Glycosylation End-Product Specific Receptor (*AGER*), and cholesterol ester transferase (*CETP*) genes have been found to be associated with the disease [46–48]. *LIPC* and *ABCA1* associate with intermediate and large drusen [49]. Regarding *APOE*, low-profile “serogranular drusen” in the macula have been contrasted to globular drusen in the periphery in a large series of autopsy eyes analyzed by ex vivo imaging and histology, finding that more peripheral than macular drusen contain apolipoprotein immunoreactivity, whereas all drusen in all regions con-

tain cholesterol forms [49]. And although it is known that *APOE* binds to complement factor H (CFH), thereby regulating complement activation, its concentration in AMD particles did not differ from those found in controls either [50, 51].

Regarding possible treatments that address the altered lipid homeostasis and given the possible associations between local and/or systemic dyslipidemia and AMD, some have proposed the use of statins, but large randomized controlled trials and prospective cohort studies are still necessary to definitively answer the question of what the role of statins and other lipid-lowering medications such as fibrates is [52]. Besides, omega 3 and 6 fatty acids when deficient can lead to photoreceptor degradation and accumulation of lipid-filled drusen in the RPE and sub-RPE space. Thus, the supplementation with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has been promoted to slow the progression from iAMD to nAMD, mainly supported by observational studies [29].

Visual cycle modulators have also been tested for the treatment of aAMD. The objective of this kind of treatment is reduction of RPE lipofuscin and therefore enhancement of RPE survival. The rationale derives from clinical imaging showing hyper-AF surrounding GA, attributed to a high intracellular concentration of lipofuscin and cell culture studies suggesting that exposure to bis-retinoid components of lipofuscin is deleterious, as previously said. Longitudinal clinical imaging and human eye pathology studies have provided a subcellular basis for fundus AF imaging while simultaneously questioning lipofuscin and its components as causative agents in AMD pathogenesis. Of relevance is that visual cycle modulators have persistent side effects, including dyschromatopsia and, importantly, slowed dark adaptation, which may mask AMD natural history or exacerbate symptoms in some patients, influencing how the results of trials will be interpreted and applied in clinical practice [53].

ECM Dysregulation

Alterations in the regulation of the ECM at the level of BrM by modification of the balance between the matrix metalloproteinases (MMPs) and the tissue inhibitors of the MMPs (TIMPs) seem to play a role in the formation and growth of drusen and, therefore, in the pathogenesis of AMD [54]. The MMP/TIMP complex is essential in the regulation of ECM turnover under normal conditions.

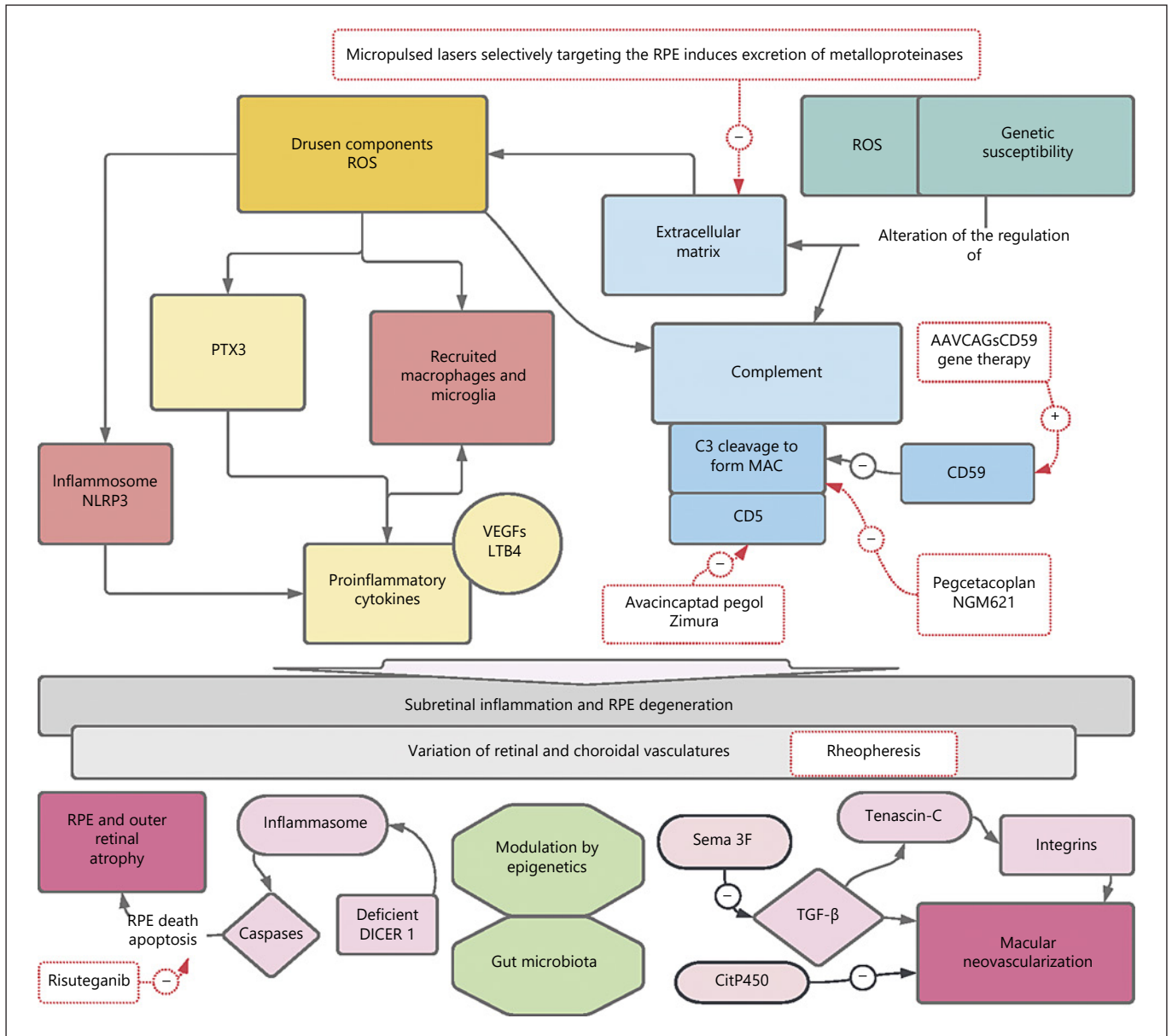


Fig. 2. Inflammation is the central element that causes AMD to progress to its advanced stages. Many factors are involved in the process, and the appearance of new blood vessels and/or extensive cell death depend at least partly on epigenetic modulation. The treatments directed to the different therapeutic targets are framed in red boxes with dashed lines. PTX3, pentatrix 3; NLRP3, NOD-

like receptor pyrin domain containing; MAC, membrane attack complex; VEGF, vascular endothelial growth factor; LTB4, leukotriene B4; ROS, reactive oxygen species; RPE, retinal pigment epithelium; Sema 3F, semaphorin 3F; CitP450, cytochrome P450; TGF- β , tumor growth factor- β .

However, its expression can be modified during aging and other pathological states, which will change the relationship between its components and cause major changes in the ECM, such as thickening and the formation of subretinal deposits [55]. Defective remodeling and degradation of the ECM can also cause accumulation of cel-

lular waste material, thereby favoring the formation of drusen and subsequent inflammation in the subretinal space (Fig. 2).

The agents that produce this imbalance between MMPs and TIMPs are not fully understood because the complex interactions between all their components and

the intricate and numerous pathways involved make it difficult to find a first effector responsible for the loss of balance of the ECM regulatory complex. Nevertheless, oxidative stress, C3 activation of the complement system, activation of the renin-angiotensin-aldosterone axis, and a polymorphism in the high-temperature requirement protein A1 (*HTRA1*) gene have been related to modifications in the expression or activity of some MMPs and TIMPs and in the degradation of the elements of BrM, which may explain the appearance of subretinal deposits in AMD [55]. In addition to *HTRA1*, another gene that largely explains the heritability in AMD is *age-related maculopathy susceptibility 2* (*ARMS2*), and although its precise role is unknown, genome-wide association study (GWAS) data reveal that patients homozygous for both the *CFH* gene and the *ARMS2/HTRA1* risk alleles had over 50 times the risk of developing AMD [56]. Carriers of the risk haplotype at *ARMS2/HTRA1* have a higher risk of developing late AMD at a younger age [57]. *HTRA1* is a serine protease secreted by the RPE involved in the tight regulation of the ECM, the levels of which are raised in patients with AMD. EFEMP1 is a substrate of *HTRA1*, which is an essential protein in the maintenance of the BrM structure and regulates tumor growth factor- β (TGF- β) involved in the stimulation of angiogenesis. Thrombospondin, a potent inhibitor of angiogenesis, is another target of *HTRA1* [58]. All this suggests the role of *HTRA1* in regulating the ECM, which leads to increasing the size and number of drusen and potentially playing a role in neovascularization appearance. Regarding the same hypothesis, 10 variants in single nucleotide polymorphisms in seven ECM genes (*COL15A1*, *COL8A1*, *MMP9*, *PCOLCE*, *MMP19*, *CTRB1-CTRB2*, and *ITGA7*) are associated only with advanced stages of AMD, suggesting that the extracellular remodeling pathways of the RPE matrix play a central role in the development of advanced AMD [59].

Another important player is tenascin-C, which belongs to a family of ECM proteins and is expressed in the choroidal neovascular membranes in eyes with AMD. Tenascin-C is secreted by transdifferentiated RPE cells and promotes the development of new vessels via integrin α V in a paracrine manner [60]. Integrins are transmembrane proteins on the surface of the RPE cells that bind to ECM proteins such as laminin, fibronectin, and collagen and mediate the phagocytosis of the outer segment particles of the photoreceptors by the RPE. Some integrins are expressed during neovascularization processes linked to endothelial cell migration and proliferation. Hence, these two molecules are considered to be involved in the patho-

genesis of nAMD and possible therapeutic targets [45]. In fact, intravitreal risuteganib is an integrin inhibitor, with antiapoptotic and antiangiogenic effects that may also improve mitochondrial activity, which has shown significant vision improvement in patients with nonexudative AMD without drug-related adverse events [61]. In contrast, semaphorin 3F might protect against subretinal neovascularization as seen in mice, thus enhancing its action was proposed as a potential treatment [62].

Argon laser therapy was shown to resolve the drusen and it has been suggested that this could have a role in slowing progression of the disease. The reason for macular improvement after an injury to the RPE with such a kind of laser is still vague, but it is proposed that there is an improvement in the Bruch membrane conductive response that could be due to excretion of metalloproteinases and RPE relocation, providing its prophylactic use for iAMD. But thermal lasers failed to stop AMD progression, probably due to direct retinal injury. Instead, techniques using micropulsed lasers selectively targeting the RPE have been used as they do not cause neuroretina damage [63]. The treatment seems to be effective in patients without coexistent RPD, but it does not fully prevent AMD progression and may be inappropriate in those with RPD [64].

Alterations of Autophagosome/Lysosomal and UPS

Aggregates of misfolded and oxidized proteins and lipids formed after exposure of RPE cells to ROS must be degraded by cellular clearance systems to avoid their progressive accumulation. When these protective mechanisms fail, the increased cellular waste products themselves may damage the systems responsible for their clearance and contribute to their accumulation and the secondary formation of more ROS, as in the case of lipofuscin mentioned previously. The accumulation of modified proteins can also have a proinflammatory effect by activating the inflammasome nucleotide-binding oligomerization domain (NOD)-like receptor pyrin domain containing 3 (NLRP3) and promoting angiogenesis. Under normal conditions, the UPS and the autophagosome/lysosomal degradation system (autophagy) share the task of maintaining cellular proteostasis to prevent the formation of subretinal deposits [33, 65]. Both, in coordination with each other, actively recognize and select the material to be degraded and recycled. When the proteolytic activity of the UPS decreases, the autophagic flux increases to preserve proteostasis. However, in aging RPE, the

expression of UPS genes and subsequently the proteolytic activity of the UPS are reduced, deviating the flow of products to the autophagy system. In addition, the decreased activity of the proteasome system can lead to the accumulation of damaged respiratory chain proteins, which causes mitochondrial dysfunction and increased cellular levels of ROS [66]. For its part, the protein sequestosome 1 (p62/SQSTM1) has multiple roles since it acts as a bridge transporting protein between the UPS and the cellular autophagocytic system, as well as regulating the antioxidant response in the RPE to oxidative stress [67]. p62/SQSTM1 is a fundamental piece in the activation and organization of the autophagy process and the protection of the RPE against ROS by inducing dehiscence of the NFR-2-Keep 1 complex in the NRF-2/ARE pathway, which allows the nuclear translocation of NRF-2 and its biologic action with the expression of antioxidant defense systems. Ambient factors such as cigarette smoke can induce inactivation of p62/SQSTM1 of the RPE, which increases accumulation of protein aggregates, and a decreased autophagy and NRF2-mediated antioxidant response, which partially explains the role of smoking in the disease [68].

In the final step of the autophagy process, a lysosome fuses with the autophagosome, giving rise to the autolysosome, where ubiquitin, p62/SQSTM1, and microtubule-associated protein 1A/1B-light chain 3 are degraded. These are considered biomarkers of protein aggregation and autophagic activity [69]. Based on this, Golestaneh et al. [70] reported that the levels of light chain 3 and p62/SQSTM1 in RPE cells of patients with AMD did not decrease when subjected to starvation/fasting compared with normal subjects, inferring that autophagic flux is reduced in AMD. Another observation is that p62/SQSTM1 accumulates in the macular area of patients with AMD and in large drusen, supporting the hypothesis that the autophagy defect is involved in early stages of the disease [69]. However, the p62 protein decides the fate of the cell since it may be involved in the cell-survival pathway by activating the TNF receptor-associated factor 6-NF-K β pathway, activating the caspase-8 through its polyubiquitination, and inducing the cell death signaling complex, ultimately causing cell death [71].

Therefore, a dysfunctional autophagy process in patients with AMD may be a key element in understanding why RPE cell dysfunction and drusen formation occur, leading to the onset of the early stages of AMD (Fig. 1). In fact, cell survival and programmed cell death are intimately linked to the autophagy process and, although the specific mechanism of cell death in the RPE in AMD (py-

roptosis, necroptosis, or apoptosis) is unknown, the disease could be explained by failed regulation of this cellular process [71]. Many natural products possibly constraining AMD severity have been used and investigated based on their mode of action as antiapoptosis inducers or as cell cycle arrest modulators, but the level of evidence supporting their use is low [72].

Chronic Inflammation

Many plasma proteins involved in inflammation are found in drusen, which could point to systemic inflammation as the possible origin of AMD [14]. Besides, the elevated plasma levels of complement components and some inflammatory cytokines such as interleukin 6 (IL-6) and tumor necrosis factor (TNF- α) observed in patients with AMD support the possible systemic origin of the disease too [73, 74]. A good example of this is pentraxin 3 (PTX3), which is part of the innate immune system and has a role in controlling inflammation in response to stimuli such as IL-1 β , TNF- α , or high ROS levels, and whose plasma levels are elevated in patients presenting with nAMD [75]. PTX3 is also considered an inflammatory marker in many inflammatory diseases and is related to premature death of RPE cells. Hwang et al. [76] reported that oxidative stress-induced PTX3 enhances expression of antioxidant enzymes and is involved in the expression of some AMD marker genes such as complement factor I (*CFI*), *CFH*, *APOE*, and toll-like receptor 4. Other factors inducing chronic inflammation, such as periodontal disease, have been linked to AMD development. Periodontal disease induces chronic inflammation and bacteremia in susceptible individuals and has also been associated with elevated systemic biomarkers such as TNF- α , C-reactive protein (CRP), IL-1 β , and IL-6 [77]. But most importantly, the products of oxidative stress trigger chronic low-grade inflammation (pathophysiological parainflammation) processes in AMD patients [78].

Role of Drusen as Inflammatory Centers

Inflammation is a biologic repair process controlled by the inflammasomes. These are members of the NOD innate immune system family of receptors including the NLRPs that are triggered by diverse danger signals and ultimately activating caspase-1, which leads to the production of inflammatory cytokines IL-1 β and IL-18 and cell death by pyroptosis [79].

Inflammation is clearly present in the pathophysiology of AMD. It involves numerous factors and explains a large

part of the events that occur in the disease, as drusen would be the inflammatory center necessary for progression to advanced stages of AMD (Fig. 2). The formation of extracellular deposits, inflammasome activation, inefficient ECM turnover, and crowding of inflammatory cells in the subretinal space are processes that might explain the extensive cell death in GA and also the appearance of new blood vessels in advanced nAMD. Degenerative changes in the RPE, i.e., altered proteostasis systems, increased oxidative stress, and attenuation of the oxidative stress protection mechanisms, mentioned previously, favor accumulation of molecules in drusen that can induce inflammation in the subretinal space [14]. Lipids, lipoproteins, organelles, lipofuscin, amyloid- β , APOE, albumin, TIMPs, matrix MMPs, melanin granules, complement components (C3, C5, factor H), immunoglobulins, CRP, vitronectin, fibrinogen, and pentraxins are some components of these extracellular deposits [14, 55]. As previously said, drusen then can act as true inflammatory centers because they are comprised of substances that attract inflammatory cells and activate the inflammasomes. More specifically, the presence of lipofuscin, amyloid β , A2E, 4-hydroxynonenal, end products of lipid peroxidation, and intracellular protein aggregates already discussed, are signals that activate the NLRP3 inflammasome pathway in the RPE [80]. Activation of the NLRP3 inflammasome in addition to the production of cytokines such as IL-1 β consolidates the inflammatory process in the subretinal space and induces caspase-mediated programmed cell death or pyroptosis, a process that when extensive can be responsible for RPE and outer retina atrophy.

Recruited Macrophages and Microglia

The retina has traditionally been considered an immunologically privileged organ but now is known to have an endogenous immune system that is coordinated by immunocompetent cells including the microglia, RPE, and macrophage/dendritic cells that play important roles in maintaining and restoring retinal homeostasis. Evidence suggests that a number of innate immunity pathways are involved in para-inflammation in the retina in aging, which is characterized by microglial, complement, and vascular endothelial activation; breakdown of the blood-retinal barrier; increased cytokine/chemokine production; leukocyte infiltration; and appearance of fibrosis [21].

Macrophages can be proangiogenic or antiangiogenic depending on several microenvironment factors affecting their activation. Proinflammatory M1 macrophages inhibit angiogenesis, while M2 macrophages promote neo-

vascularization. This is regulated by FasL, which is affected highly by MMP activity, which cleaves FasL from the cell surface and diminishes its proapoptotic function during the angiogenic responses. Because of that, blood levels of soluble FasL might be a biomarker of AMD [81]. In turn, soluble FasL recruits more macrophages that accumulate in the subretinal space and perpetuate the inflammation. However, inflammatory macrophages are also an important source of proinflammatory cytokines such as IL-1 β implicated in cone and rod degeneration [82] and choroidal neovascularization [83], or TNF- α , which inhibits visual cycle genes in the RPE [84]. Another possible mediator in the inflamed retina is leukotriene B4 (LTB4), which can be produced by macrophages, RPE, and glial cells, since LTB4 receptor 1 (BLT1) is highly expressed on the surface of recruited macrophages in aging mice, and its activation stimulates secretion of proangiogenic and profibrotic markers, favoring the appearance of new blood vessels [85].

The Role of Complement

Since histologic evidence shows the presence of complement factors in drusen, the involvement of the complement system in AMD pathogenesis has been widely accepted and studied, although not completely understood [86]. In fact, analysis of associations between distinct AMD phenotypes and local complement activation represents an important and innovative approach in AMD research. A scheme summarizing its participation is shown in Figure 2.

Complement is a defense mechanism of the organism against pathogens that, with its activation by any of its pathways (classical, lectin, alternative, or intrinsic), causes an inflammatory tissue response. All pathways converge in the cleavage of complement factor C3 by the C3 convertase to form C3a and C3b. Further pathways include cleaving of complement factor C5 to C5a and C5b, where C5b in association with factors C6, C7, C8, and C9 forms the terminal complex C5b-9, also referred to as the membrane attack complex (MAC). MAC will lead to a pore in the target cell membrane that ultimately causes cell lysis and death. This cascade is controlled by a series of regulatory factors that prevent persistent inflammation. MAC can be downregulated by complement inhibitors like CD59 [87, 88]. CFH is an important inhibitor of the system by competing with complement factor B (CFB) to bind C3b, accelerating the resultant dissociation of the alternative system C3 convertase, and it also acts as a cofactor to facilitate the CFI-mediated C3b inactivation [88].

The initiation of the complement cascade in the retinal environment of drusen triggers inflammation and high levels of oxidative stress, leading to activation of the MAC on the RPE, which accounts for the extensive cell death in cRORA/iRORA characteristic of aAMD. The effect of the inflammasome must be added since some complement components can promote its activation on their own [89]. Besides, photoreceptors are much more susceptible to MAC because they have fewer regulators in their membrane, and, therefore, they can be more easily damaged, considering that they do not have the immunologic barrier provided by the already damaged RPE. Moreover, neoepitopes generated by oxidative stress and found in the neovascular lesions may participate in amplifying damage, presumably through uncontrolled activation of the alternative complement pathway [89]. The initial activation of the alternative pathway could join with the classical pathway via release of neoantigens that follows destruction of the RPE, its recognition by the immune system, and the production of antibodies [90]. The action of anaphylatoxins such as C3a, C4a, and C5a, which act as chemotactic factors, must be added and may be responsible for the mononuclear infiltrate of the retina. Other components such as opsonins C3b, iC3b, and C4b when present in the outer retinal layers could target otherwise healthy RPE cells or photoreceptor outer segments for phagocytosis [89]. Finally, the expression of the complement regulatory proteins CD46 and CD59, inhibitors of the MAC, may be reduced by oxidized lipoproteins and other waste products present on the membrane of RPE cells of patients with AMD [91].

Large GWASs have implicated the role of the complement system in the initiation and progression of AMD [92]. The first gene to be associated with AMD was *CFH*, the main function of which is inhibition of the alternative complement pathway. Its p.Y402H variant is the best known genetic risk factor for AMD, especially in Caucasians [89], whereas the p.I62V variant is more prominent in Asian populations [14]. *CFH* binds to malonaldehyde, a product of lipid peroxidation that induces inflammation and damage in RPE cells, but the Y402H polymorphism markedly reduces its ability to bind malonaldehyde, suggesting it disturbs the normal blocking of inflammation processes [93, 94]. *CFH* Y402H results in weaker binding of *CFH* to CRP, making RPE cells more resistant to this inflammation mediator and also affects *CFH* binding to heparan sulfate proteoglycans leading to accelerated lipoprotein accumulation in BrM and drusen progression [14, 95]. Cluster variants in the *CFH regulator 1* and 3 genes also may be related to AMD risk [96, 97].

Another interesting variant in the *CFH* gene is the C-terminal variant R1210C, which induces a partial deficit of *CFH* that leads to a monogenic retinal disease with a drusen-like phenotype of a middle-aged appearance along with renal disease [98].

The *CFI* gene rs10033900 polymorphism is potentially associated with the risk of advanced AMD. Individuals who carry the CC genotype or C-allele possibly have a lower incidence of AMD, whereas individuals carrying the T-allele or TT genotype may have a higher susceptibility to AMD [99]. Further genetic variants have also been associated with AMD in other complement genes such as *C2*, *C3*, *CFB*, *FI*, *FB*, *FD*, *C9*, *CFHR1*, and *CFHR3* that involve the alternative and the classical complement pathways. The presence of variants in genes leading to increased expression of C3a and *CFB* proteins has been associated with VEGF secretion in the RPE, which, in addition to promoting the formation of MNV, can attract leukocytes and activate the complement cascade, causing loss of these protective factors against development of AMD [10, 15, 88, 100]. The fact that complement activation can stimulate secretion of angiogenic factors has led to the hypothesis that some phospholipid compounds that comprise drusen may act as neoepitopes and trigger the formation of autoantibodies that activate the classical complement pathway, resulting in increased VEGF secretion and formation of MNV in nAMD [90].

How these specific genetic variations influence lesion composition and treatment is being explored, although, in most genetic studies, nAMD and cRORA have been lumped into the single category “advanced AMD.” A specific single-nucleotide polymorphism (SNP) in the 10q26 region was associated with RPE hyperpigmentation, large drusen, and larger neovascular lesions. Also, the risk allele of the SNP rs11200638 in *HTRA1* appears to be linked to bilaterality, classic CNV, and increased CNV size. The association of the high-risk allele of the SNP rs1061170 in *CFH* Y402H with predominantly classic CNV is controversial. Type 3 neovascularization has been shown to share certain risk and protective alleles in *CFH*, *ARMS2/HTRA1*, *CFB*, and *C2* with AMD, both in Asian and white populations. Finally, treatment response of neovascular lesions appears to be influenced by genetics of complement since the low-risk *CFH* Y402H genotype associates higher rates of visual improvement after treatment with intravitreal bevacizumab and vice versa [51, 59, 89, 96, 101].

Targeting at C3, two therapeutic drugs have been devised for aAMD so far. First, intravitreal Pegcetacoplan is an investigational targeted C3 therapy designed to con-

trol excessive complement activation. It has been tested in late-stage clinical trials, where intravitreal injections monthly or every other month of pegcetacoplan slowed the rate of GA growth by 29% and 20%, respectively, compared with the sham treatment group. Nevertheless, they were associated with an unexpected increased incidence of new-onset neovascularization, identified to be dose-dependent. The conversion from a nonexudative MNV to exudative MNV in these eyes has been suggested to be the main cause, and it may have an immunologic basis, but further study of these hypotheses is being pursued in an ongoing global phase 3 study [102–104]. Second, NGM621 is a humanized IgG1 monoclonal antibody engineered to potently inhibit the activity of C3, which was safe and well-tolerated in phase I trials [88].

Avacincaptad pegol is a pegylated RNA aptamer targeting at C5 that can reduce 27.4–27.8% mean rate of GA growth over 1 year compared to controls, which is of statistical significance in the phase II/III trials [88]. Zimura is another complement inhibitor targeting C5 that in combination therapy with ranibizumab for nAMD was safe and well-tolerated, and approximately 60% achieved VA improvement greater than or equal to three lines, which was better than the results of anti-VEGF monotherapy [88].

Gene therapy has the potential of providing long duration of action with a single procedure. AAVCAGsCD59, also named as HMR59, uses an AAV2 gene therapy intravitreally delivered and is designed to induce the generation of a soluble CD59 protein, which binds the incomplete MAC and blocks the binding of the C9 protein required to complete the complex. In phase I clinical trials, it was safe and well-tolerated, and although the trial was not designed to judge efficacy, it is encouraging that 9 of 11 cases demonstrated a slower rate of GA growth [88].

Variations of Retinal and Choroidal Vasculatures

The degeneration of the choriocapillaris and the RPE are parallel processes and present in all AMD stages. The retina has a high energy demand and high oxygen consumption, and meeting this need demands a sufficient blood supply through the choroid. The choriocapillaries, photoreceptors, and RPE cells must act as symbionts since the RPE provides glucose from the choroidal circulation to the photoreceptors, the rods assist the cones by secreting rod-derived cone viability factor, and both produce lactate to nourish the RPE [105]. Any failure in this sophisticated metabolic ecosystem leads to serious function-

al and structural consequences in the photoreceptor outer segments. Therefore, choriocapillaris involution, unresolved chronic low-grade inflammation that alters retinal metabolism, and/or PRE dysfunction cause inefficient photoreceptor outer segment renewal, all key points implicated in the pathogenesis of AMD [105]. These three elements are closely interconnected and interdependent, since degeneration of one leads to involution of the other two, and although we do not know the exact sequence of involvement of these elements [106], we know that in all types of AMD there is involution of the choriocapillaris associated with outer retinal degeneration. In AMD, local hemodynamic changes occurring in the choriocapillaris compromise the flow of oxygen and nutrients to the retina and alter the transport of waste products by the RPE [106]. Some vascular changes observed in early stages of the disease are decreased vascular density of the choriocapillaris, increased endothelial cell loss, altered cell polarity in the endothelium with abnormal fenestrations and altered diaphragms, and prolonged choroidal vascular filling [107]. Because of the perfusion deficit and inefficient transport secondary to the regression of choroidal vascularization, the clearance of extracellular products is slowed, which may favor the appearance and growth of drusen. Considering this, the vascular density of the choriocapillaris is related inversely to the density of the drusen, suggesting that adequate choroidal microcirculation might prevent drusen formation [108]. In addition, a recent study using OCT-angiography showed that the deficit of flow in the choriocapillaris predicts the formation and growth of drusen, which was identified as a risk factor for developing advanced forms of AMD together with the presence of large and intermediate drusen [108].

In nAMD, the most important milestone that occurs is the formation of MNV that curiously grows in regions where perfusion is especially precarious, stimulated by VEGF produced by the RPE in response to insufficient blood supply and subsequent hypoxia [108, 109]. The VEGF family gene encodes a family of glycoproteins including VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and VEGF-F and placental growth factor, with a role in vascular proliferation and migration of endothelial cells for both physiological and pathological angiogenesis. Hypoxia-inducible factor-1 (HIF-1) is a heterodimer made up of HIF-1 and HIF-1. Among others, genes that are transcriptionally regulated by HIF-1 include VEGF and its receptor VEGFR1; platelet-derived growth factor-B (PDGF-B) and its receptor PDGFR; stromal-derived factor-1 and its receptor CXCR4; angiopoietin-2 (Ang2); and vascular endothelial-protein tyrosine phosphatase

(VE-PTP). Ang2, a key molecule in the angiogenesis pathway, is produced almost exclusively by endothelial cells and functions as a vessel-destabilizing molecule through its competition with Ang1 and inhibition of Tie2. Like VEGF, Ang2 is upregulated by hypoxia, and ocular levels of both Ang2 and VEGF are elevated in eyes with nAMD. VEGF and Ang2 may work together to induce pathological neovascularization and increased vascular permeability [110, 111]. Finally, tyrosine kinase is an enzyme that in the eye, binds both VEGF and PDGF-G to cell surface receptors to propagate signal transduction into the cell [112].

Most anti-VEGF drugs used in clinical practice inhibit the VEGF-A such as bevacizumab, ranibizumab, and brolucizumab, whereas aflibercept inhibits the VEGF-A & B and placental growth factor. Also, a robust pipeline of drugs for nAMD has merged over the last years, including anti-VEGF-A (abicipar pegol); anti-VEGF-C & D (OPT-302); tyrosine kinase inhibitor that blocks VEGFR1 and PDGF-B (sunitinib); and a bispecific Ang2 and VEGF-A antibodies (faricimab). Results of clinical trials indicate that faricimab has a greater effect than inhibition of VEGF-A or Ang2 individually showing sustained efficacy, thereby reducing treatment burden in nAMD [110, 112, 113].

In aAMD the regression of the choriocapillaris is also very important, along with outer retinal atrophy that increases to affect the entire macular area as GA. Choroidal vascularity index impairment in the subfoveal area measured with OCT-angiography is also considered a predictor of GA progression in the clinical setting, and it could be considered as a new potential biomarker in the efficacy evaluation of new GA interventions [114]. Among the triggers of degeneration of the choroidal vasculature, choroidal aging per se is the primary culprit, followed by cardiovascular risk factors that are frequently associated with AMD. The mechanisms that cause choroidal vascular aging include the same ones mentioned previously for RPE (oxidative stress, inflammation, deficient proteostasis, mitochondrial dysfunction/DNA damage, and cellular senescence), and these should be implicated as the first ones involved in AMD onset [115]. Finally, it has been observed that, in association with smoking, diabetes, hypertension, and obesity, changes in the choroid appear similar to those observed in AMD, suggesting that these risk factors affect the aging mechanisms mentioned previously. Finally, several treatments such as eye drops of M-1101 (MacuClear) or rheopheresis have been used to improve blood flow, hoping to stop progression to advanced disease with little success [116].

Gut Microbiota and Nutritional Factors

The development of AMD is affected by environmental factors including disruptions in lipid, carotenoid, and inflammatory pathways induced mainly by diet and smoking [117]. Many observational studies have reported the potential role of micronutrient supplementation in lowering the risk of progression from the early to advanced stages of AMD [28]. Recent studies have shown that their efficacy might be mediated by intestinal microbiota; however, although different lines of evidence indicate the impact of dietary habits (high-fat and high-glucose or high-fructose diets), micronutrients (vitamins C, E, and D, zinc, beta-carotene, lutein, and zeaxanthin), and omega-3 fatty acids on the modulation of the gut microbiota and their relationship with AMD risk and progression, it remains unclear whether a causal relationship truly exists [118]. Moreover, an animal model highlighted the role of high-fat and high simple-sugar diets on the development of AMD through derangement of the gut microbiota that leads to systemic low-grade inflammation. Recent evidence indicates strict interaction between the gut microbiota and retina, referred to as the “gut-retina axis” [119]. A better understanding of the mechanisms that underlie this connection may help clinicians promote healthy nutritional habits and adequate micronutrient intake to elucidate if modulation of the gut microbiota through dietary intervention delays the course of the disease. AREDS supplementation reversed some adverse intestinal microbial changes in AMD patients. Besides, microbiota-induced metabolite production and gene expression occur in pathways important in AMD pathogenesis, thus providing a novel potential therapeutic target [28, 120].

Epigenetics Modulation

Epigenetics in association with environmental factors has aided a partial understanding of the heritability of AMD, its progression through different stages, and why the disease has different clinical forms [121]. First, patients who are carriers of some genetic risk variants develop AMD only when they are exposed to certain environmental factors. This observation would mean that for these genes to be activated, a previous alteration in the epigenetic regulation mediated by some of these external agents is necessary [121]. In addition, environmental (smoking, hypertension, obesity, diet low in antioxidants) and genetic (*CFH*, *ARMS2/HTRA1*) risk factors

and factors associated with race and age might condition individual susceptibility and lead to oxidative damage and inflammation, initiating passage from the initial to intermediate stages of AMD to the advanced neovascular and/or atrophic forms of the disease [122]. Epigenetic mechanisms involved in AMD include DNA methylation, histone modification, chromatin remodeling, and gene inhibition by noncoding ribonucleic acids [13].

Small but significant DNA methylation differences are seen in the blood of patients with nAMD near the *ARMS2* locus. The methylation level of one of the 5'-C-phosphate-G-3' (CpG) sites is correlated significantly with the genotype of the risk SNP rs10490924, suggesting a possible epigenetic mechanism of risk. Another consistent, replicable change in DNA methylation in the promoter region of protease serine 50 (*PRSS50*) has also been found. These methylation changes may identify sites in novel genes that are susceptible to nongenetic factors known to contribute to AMD development and progression. However, many questions remain. Did the disease-associated differential methylation observed in the blood and retina arise before AMD onset and contribute to the disease phenotype, or was it a secondary effect of the disease process or treatment? And what is the functional significance of the methylation changes in the *ARMS2* promoter? And are similar methylation changes present in the macular RPE of patients with AMD? [123]. Similarly, Porter et al. [124] reported a large genome-wide methylation study using human RPE cells that showed a relationship between differentially methylated loci and regions in *SKI*, *GTF2H4*, and *TNXB* genes and early/intermediate AMD. The involvement of the *SKI* and *GTF2H4* genes in the pathogenesis of AMD remains to be determined. However, the *TNXB* gene encodes isoform 1 of tenascin XB, a glycoprotein of the ECM predominantly located in the outer reticular lamina of the basement membrane of cells and related to Ehlers-Danlos syndrome [124]. Patients with AMD also have significantly reduced mRNA levels of the mu1 and mu5 isoforms of glutathione S-transferase (*GSTM1* and *GSTM5*) in the RPE. A comparison of choroid and neurosensory retina with age-matched controls suggested that epigenetic repression by hypermethylation of the *GSTMU1* and *GSTMU5* genes would have occurred, which may increase susceptibility to oxidative stress in retinas with AMD [125]. In addition, decreased methylation levels were found in the *IL17* receptor gene in patients with AMD, with consequent increased expression of this receptor that mediates transduction of IL-17 inflammatory activity [126].

In addition to methylation, another important epigenetic mechanism in AMD is regulation in the heterochromatin-euchromatin relationship that mediates histone acetylation-deacetylation. Wang et al. [127] observed a global decrease in chromatin accessibility of the RPE cells in the early stages of AMD that was similar to that that originated in pluripotent stem cells exposed to cigarette smoke and associated with the overexpression of histone deacetylase 11 with a global decrease in chromatin access; this triggered senescence and cell death in nonexudative AMD.

Another recent study of AMD cybrids reported increased RNA/protein levels for five methylation-related and four acetylation-related genes, along with lower levels of two methylation and three acetylation genes compared to normal cybrids. This activity inhibits the MAC and has an antiangiogenic capacity, so that its deficiency may favor expansion of the retinal inflammatory process and ultimately cause progression to nAMD [128].

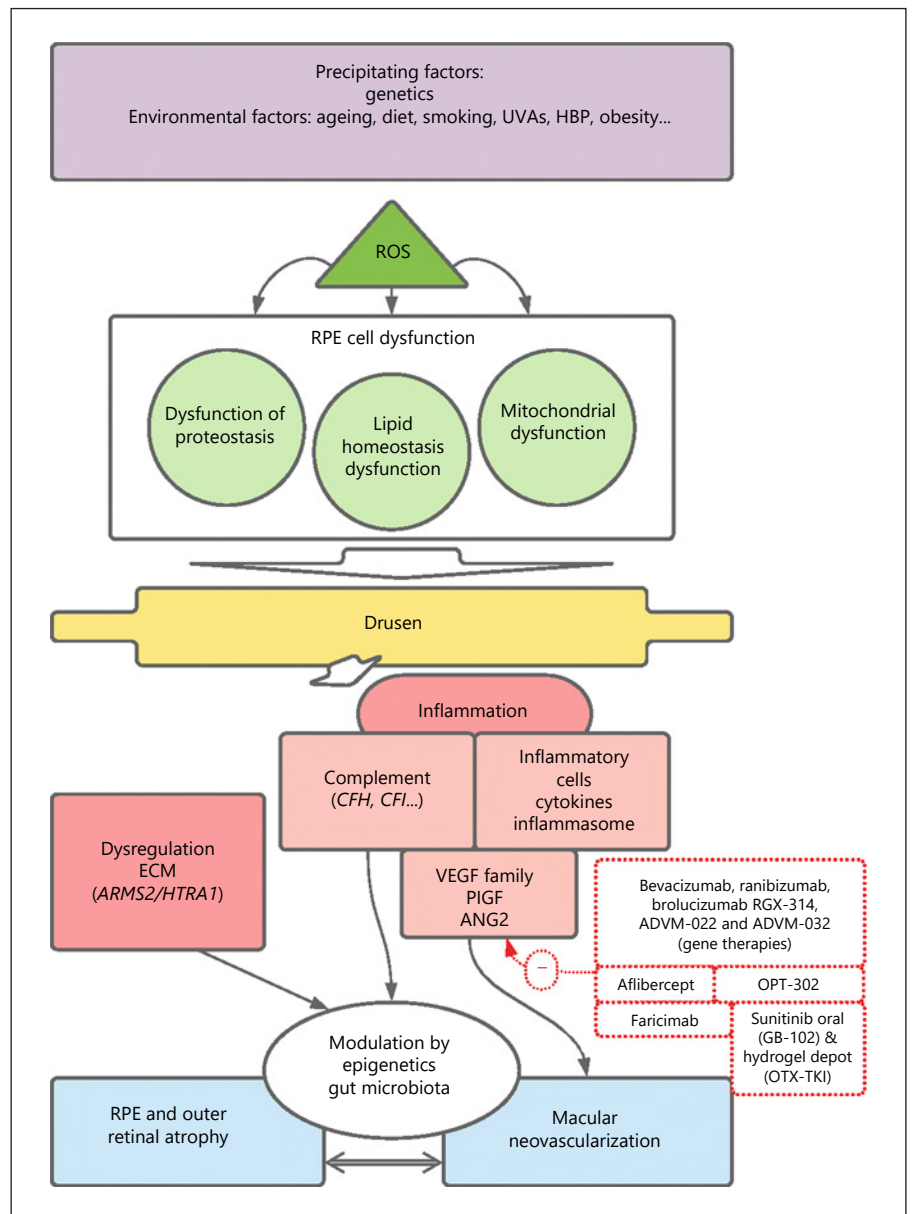
Finally, the expression of three micro (mi-)RNAs has been observed to be decreased in ischemic retinas. Of these, mi-RNA-150, whose presence reduces VEGF levels and thus neovascularization, may be implicated directly in nAMD [121]. In addition, overexpression of miRNAs normally present in abundance in the brain and retina, such as miRNAs-9, miRNA-125b, miRNA146a, and miRNA-155, causes CFH deficiency, which might explain the inflammatory degeneration that occurs in AMD and Alzheimer's disease [129]. Also, the mi-RNA processing enzyme 1 (*DICER1*) is related to transposable elements, which can be inhibited by Alu transcripts. Both deficiency of the former and excess of the latter in the RPE seen in AMD can activate the NLRP3 inflammasome and initiate the cascade of events that lead to extensive cell death and subsequently to the atrophy characteristic of cRORA/iRORA.

Therefore, the characterization and understanding of epigenetic events in AMD should be studied to elucidate their most relevant contribution to the pathogenic mechanisms of AMD since they might provide new targets and a rational basis for the design of much-needed therapeutic strategies. Reprogramming epigenetic information may be a future therapeutic option [130].

Conclusion and Consequent Therapeutic Options

The main players in the AMD pathogenic processes are summarized in Figure 3. Elevated ROS levels, mitochondrial failure, ECM dysregulation, and accumulation

Fig. 3. Summary of the pathophysiology of AMD. An initial genetic and local susceptibility is necessary for the disease to occur and develop, together with the participation of the different factors involved. The treatments directed to the different therapeutic targets are framed in red boxes with dashed lines. ROS, reactive oxygen species; UVAs, ultraviolet A; HBP, high blood pressure; RPE, retinal pigment epithelium; *CFH*, complement factor H gene; ECM, extracellular matrix; VEGF, vascular endothelial growth factor; PIGF, placental growth factor; Ang2, angiopoietin 2; *ARMS2/HTRA1*, high-temperature requirement protein A1/age-related maculopathy susceptibility 2 gene.



of waste lipidic and protein aggregates are the primary inducers of RPE dysfunction, leading to the appearance of abnormal deposits in early to intermediate AMD. The unsolved question that remains here is: what causes a cell that has been fighting oxidative stress for a lifetime to be overcome by it? The accumulation of ROS and malfunctioning of NRF-2/ARE and PGC-1 systems due to mere senescence and global cellular dysfunction might be the triggers. In addition, the lifetime exposure to different stressors (UVA, diets poor in antioxidants, etc.) might gradually be involved. Third, the changes conditioning a

diminished response to oxidative stress may be centered first on the gene regulatory systems involved in the antioxidant response, which perhaps through epigenetics, then leads to ROS accumulation, as exemplified by the case of hypermethylation of the *GSTM1* and other genes mentioned previously.

Inflammation is another important player. Drusen are true centers of chronic retinal inflammation that can activate a potentially damaging immune response and cause progression of AMD to its advanced stages if this response is not well regulated. The complement system and

Table 1. Treatments with its targets and summary of results

Target/effect	Intervention	Results
Blue light exposure	Blue-light filtering lenses	More clinical studies needed to demonstrate the benefit beyond what was observed in vitro
Oxidative stress, mitochondrial dysfunction & gut microbiota	Nutritional antioxidant supplements (zinc, omega-3 and 6 polyunsaturated fatty acids, various vitamins, and coenzyme Q10)	Only available treatment to reduce late AMD, but its effectiveness appears limited
Lipid homeostasis	Lipid-lowering medications such as statins & fibrates	Controlled trials and prospective cohort studies still needed
	Supplementation with eicosapentaenoic and docosahexaenoic acid	Observational studies showed certain efficacy
Decrease the toxic lipofuscin	Visual cycle modulators	Persistent side effects: slowed dark adaptation
Regulation of the extracellular matrix	Micropulsed lasers selectively targeting the RPE induces excretion of metalloproteinases	Prevents AMD progress in patients without coexistent reticular pseudodrusen
Antiapoptotic effect	Many natural products	The level of evidence supporting its use is low
C3 inhibitors	Pegcetacoplan	Slowed the rate of GA growth by 20–29%. Side effect: conversion from a nonexudative to exudative MNV
	NGM621	Safe and well-tolerated in phase I trials
C5 inhibitors	Avacincaptad pegol	Reduced 27.4–27.8% GA growth over 1 year compared to controls
	Zimura	In clinical trials 60% achieved VA improvement ≥ 3 lines. Safe and well-tolerated
Increase CD59 activity to block the MAC formation	AAVCAGsCD59 gene therapy (=HMR59)	Safe and well-tolerated in clinical trials. In conjunction with anti-VEGF injections has demonstrated a reduced treatment burden
VEGF-A inhibitors	Bevacizumab	Widely used. Able of maintaining VA on a long-term basis of at least 4–5 years although undertreatment may worsen final outcomes
	Ranibizumab	
	Brolucizumab	
	Abicipar pegol	Side effects: high rate of intraocular inflammation
	RGX-314 (gene therapy expressing a protein similar to ranibizumab)	50% of patients remaining free of anti-VEGF injections at 18 months in best cohort results
	ADVM-022 and ADVM-032 (gene therapy expressing a protein similar to ranibizumab)	Phase I trial with many patients not requiring any rescue anti-VEGF injections
VEGF-A & B + PIGF inhibitor	Aflibercept	Widely used in clinical practice with similar results to VEGF-A inhibitors
VEGF-C & D	OPT-302	Significant improvement in monotherapy or in combination with aflibercept or ranibizumab
VEGF-A + Ang2 inhibitors	Faricimab	>effect than inhibition of VEGF-A or Ang2 individually
Tyrosine kinase inhibitor, anti-VEGFR1 and PDGF-B	Sunitinib oral (GB-102) & hydrogel depot (OTX-TKI)	A clinical trial in combination with aflibercept is underway currently
Reduced apoptosis, angiogenesis (VEGF-A) and integrin function + improved mitochondrial activity	Risuteganib	Significant visual acuity improvement in patients with nonexudative AMD. No drug-related adverse events
Reduction in blood viscosity that would improve microcirculation	Rheopheresis	There appears to be a rationale for its use in iAMD but further results of clinical trial are awaited
Epigenetics	Reprogramming epigenetic information	Not tested for AMD

PIGF, placental growth factor.

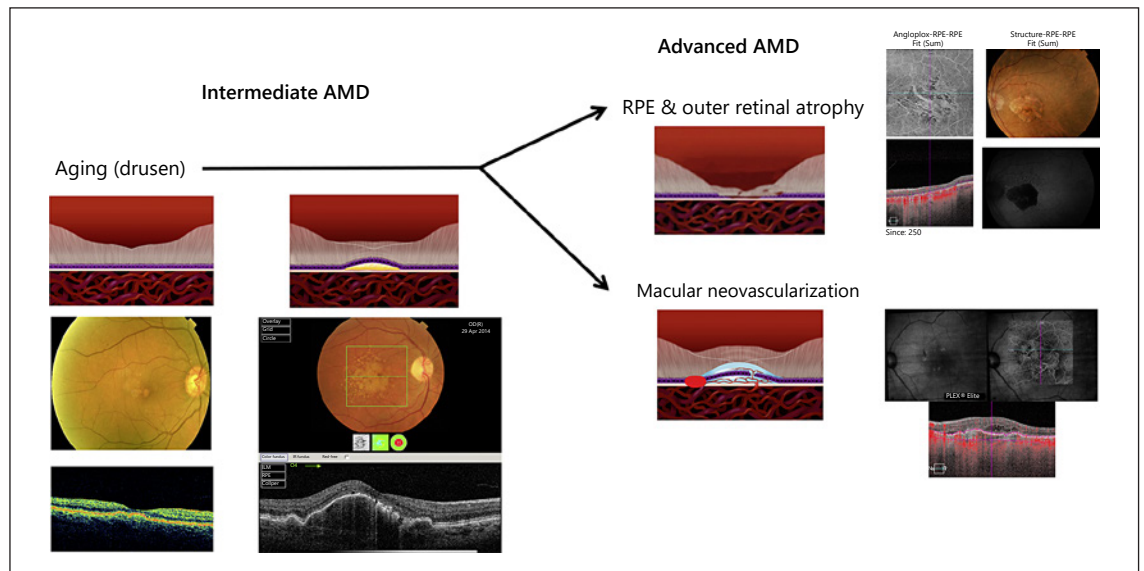


Fig. 4. Different stages of AMD starting at normal aging, going through intermediate AMD and from this point to advanced disease, including both RPE and outer retinal atrophy and macular neovascularization.

recruited macrophages and microglia involvement are widely accepted. Nevertheless, drusen, a necessary but not sufficient element for the development of advanced forms of AMD, explains the association of the disease with *CFH* and *ARMS2/HTRA1* polymorphisms, which lead to a defect in the regulation of complement and the ECM. Thus, all the processes that trigger the loss of the RPE and the appearance of new blood vessels are linked closely to the individual genetic susceptibility governed by these genes, which gives the ECM and complement central roles in inflammation.

Other questions that arise are why do some patients develop nAMD and others aAMD, and why do some patients develop nAMD in one eye and aAMD in the other? A possible answer lies in the epigenetic modulation of inflammation that takes place locally in each case. Thus, a series of specific conditions must be present in the surrounding inflammatory environment for the secretion of VEGF and LTB₄ that stimulate new vessel formation in nAMD, just as other specific circumstances are required for activation of the processes that lead to extensive RPE and photoreceptor cell death in cRORA/iRORA.

From the previously mentioned factors, it can be inferred that a large part of the susceptibility to both developing AMD and its progression to advanced stages is probably unmodifiable. Thus, the initial susceptibility of the individual may be determined by his/her risk factors and mainly by age. Nevertheless, intervention into expo-

sure to some modifiable environmental factors such as smoking, diet, or UVAs should be implemented. Also, the ability to respond to oxidative stress genetically determined and regulated by epigenetic mechanisms may be modifiable.

Management of nAMD has advanced dramatically due to the introduction of effective intravitreal treatments with anti-vascular EGF (anti-VEGF) capable of maintaining VA on a long-term basis of at least 4–5 years, although undertreatment may worsen final outcomes [131]. Unfortunately, no such advances have been made in the treatment of aAMD so far, and no effective therapy can fully prevent progression from the intermediate to the advanced stage of the disease [72, 132]. The development of atrophy in patients in whom the neovascular component is controlled also must be addressed. Therefore, a better understanding of the biologic basis of this complex heterogeneous disease will allow development of new personalized therapies to prevent visual loss or restore lost vision.

Regarding the etiopathogenic basis of possible treatments (Table 1), patients can take drugs or dietary supplements that either potentiate the antioxidant response or are themselves antioxidants and allow control of intracellular ROS levels. In fact, the prescription of antioxidant supplements such as zinc, omega-3, and 6 polyunsaturated fatty acids, various vitamins [133], and coenzyme Q10 for mitochondrial dysfunction [134] is one of the few

actions undertaken today to prevent progression from early to advanced disease.

Considering the progression to advanced AMD and that the genetic load plays a dominant role in this progression, therapies can be imagined in which the loss of function of complement and matrix regulators might be counteracted by replacing them or by inhibiting the damage caused by these systems. In fact, some complement inhibitors are currently being tested in clinical trials to halt GA progression [135, 136], but some of these therapeutic options have shown unfavorable benefit-risk ratios and further studies are warranted [137]. Another interesting point would be that once nAMD is established, treatment with anti-VEGFs might be combined with another treatment focused on modulating subretinal inflammation as a secondary prevention of fibrosis or atrophy that can occur further along in the disease evolution and lead to additional visual loss [72, 138, 139]. Reprogramming epigenetic information may also be a future therapeutic option [130]. Finally, in patients with outer retina atrophy, photoreceptor loss can be reduced by limiting the damage and degeneration using neuroprotective agents [140].

In summary, although we do not know the precise primary trigger in AMD, we do know many of the central players in numerous events that mark the disease course: proteostasis dysfunction due to uncontrolled oxidative stress and increased ROS, mitochondrial dysfunction, inadequate antioxidant response, deficient autophagy, ECM dysregulation, choroidal involution, chronic inflammation in which complement and recruited microglia and macrophages play a pivotal role, and finally epigenetics that are key players in the onset and natural disease course. Because of these multifactorial determinants, different complex combinations of treatments may be the

best option to enhance visual results in patients with AMD; the results will differ depending on the stage or spectrum of disease in each patient (Fig. 4). This will lead to the application of personalized medicine to control and hopefully cure the disease in the future. It is necessary to both continue unraveling all the processes involved in the pathogenesis of AMD and understand them in their entirety.

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Conflict of Interest Statement

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Julian Garcia-García was involved in reviewing the bibliography and drafting on the manuscript. Rosa M. Coco-Martin was involved in the conceptual design and drafting of the manuscript. All the authors were involved in the development of the manuscript and agreed to publish it.

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