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Review

Age-Related Macular Degeneration (AMD): Alzheimer's Disease in the Eye?

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Abstract. Age-related macular degeneration (AMD) is a late-onset, neurodegenerative retinal disease that shares several clinical and pathological features with Alzheimer's disease (AD), including stress stimuli such as oxidative stress and inflammation. In both diseases, the detrimental intra- and extracellular deposits have many similarities. Aging, hypercholesterolaemia, hypertension, obesity, arteriosclerosis, and smoking are risk factors to develop AMD and AD. Cellular aging processes have similar organelle and signaling association in the retina and brain tissues. However, it seems that these diseases have a different genetic background. In this review, differences and similarities of AMD and AD are thoroughly discussed.

Keywords: Age-related macular degeneration (AMD), aggregation, aging, Alzheimer's disease, autophagy, lysosome, oxidative stress, proteasome

INTRODUCTION

Age-related macular degeneration (AMD) is the major cause of central blindness in the elderly in Western countries. It is characterized by a progressive loss of color and fine vision attributable to degenerative and neovascular changes in the macula that is a highly specialized region of the central retina unique to humans and other primates (Fig. 1). The global eye disease survey conservatively indicates that 50 million persons worldwide suffer from AMD symptoms and one third of them are blind or severely visually impaired because of AMD [1, 2]. The disease has a tremendous impact on the physical and mental health of the geriatric population and their families and it is becoming a major public health and financial burden. In the absence of an effective treatment for AMD, the number of patients severely disabled by it is expected to duplicate in the next 20 years. The prevalence of early AMD in the age-category of 65–74 years is 15%, in the age-category of 75–84 years 25%, and in persons 85 years and older 30%. A reasonable overall estimate of the prevalence of late AMD in persons aged 65–74 years is 1%, increasing to 5% in persons aged 75–84 years, and up to 13% in persons 85 years and older [3].

The AMD etiology is known to be multifactorial, i.e., in addition to a strong genetic component, environmental risk factors such as smoking, obesity, arteriosclerosis, hypertension, and hypercholesterolemia may predispose to AMD (Fig. 2) [3–5]. At present, chronic oxidative stress and inflammation are strongly linked to AMD pathogenesis [6–8]. AMD is

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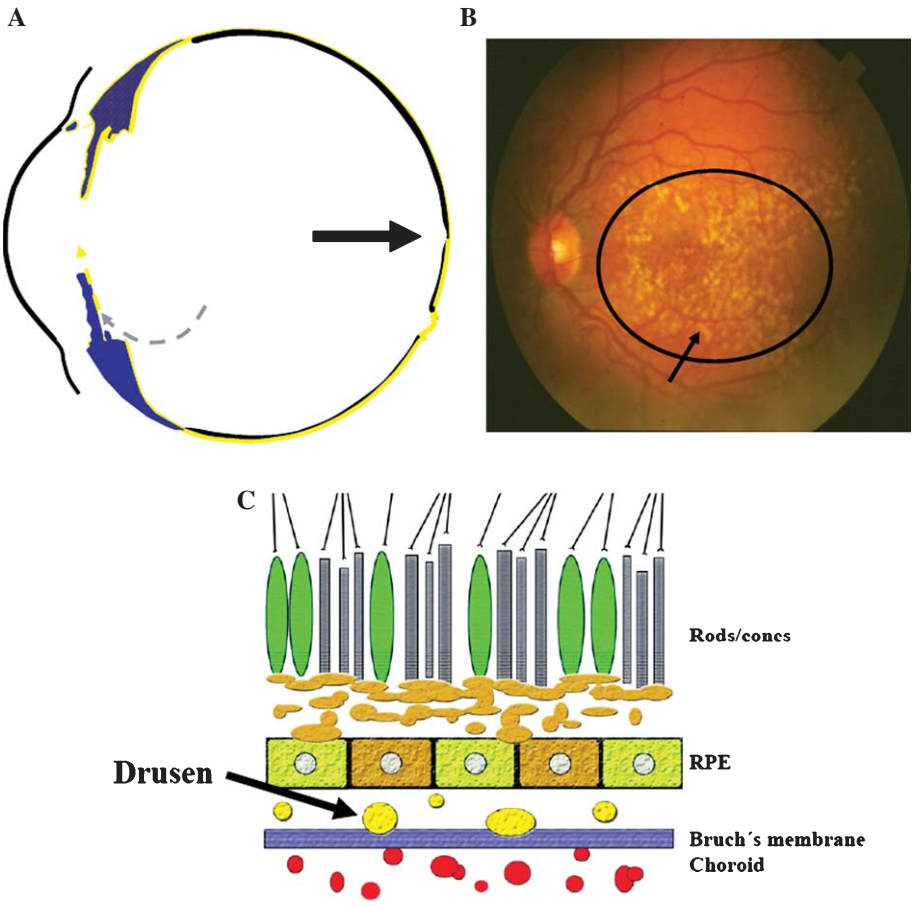

Fig. 1. A) Cross-sectional schematic of the eye from the area of macula, which takes care of fine vision (arrow). B) Fundus color photograph from degenerated macula (black ring) in an AMD patient with abundant yellow/white drusens (arrow). C) RPE (retinal pigment epithelium) is located between neural retina including rods and cones and choroid in the posterior pole of the eye. Drusens are formed between RPE and choroid (arrow).

mainly classified into atrophic and exudative form. A hallmark of AMD is the detrimental accumulation of lysosomal lipofuscin in the postmitotic neuroepithelial cells called retinal pigment epithelial (RPE) cells. Moreover, the presence of extracellular drusen deposits between the basal lamina of the RPE and the inner collagenous layer of the Bruch's membrane are common in AMD (Fig. 1) [9, 10]. High amounts of lipofuscin and drusen predict AMD advancing and severity [11–14]. One of the most essential functions of RPE cells is to take care of neural cells, rods, and cones. In aged RPE cells, this ability is weakened causing secondary adverse effects on neural retina and ultimately leading to the loss of vision. Prior to the onset of AMD, visual defects such as reduced contrast sensitivity, central visual field loss and spatiotemporal sensitivity are experienced by many patients evoking difficulties in coping with routine daily tasks.

In response to chronic oxidative stress and inflammation, choroidal neovascularization may be developed in certain AMD cases (15–20% of all). Chroidal neovascularization is used as diagnosis criteria for exudative AMD. If choroidal neovascularization is not observed then AMD is classified as atrophic form. In the neovascularization process, new vessels sprout from the choroidal capillaries through the Bruch's membrane into the sub-RPE space or into the retinal layers. A thickening, calcification, and fragmentation of Bruch's membrane may predispose to the development of choroidal neovascular membranes [9, 15, 16]. The harmful new blood vessels that are diagnostic for exudative AMD grow through Bruch's membrane, where they can disrupt the membrane and leak blood or fluid into the subretinal pigment epithelial space and evoke fibrogliosis resulting in a disciform scar and severe visual loss if not treated properly (Fig. 1). Anti-

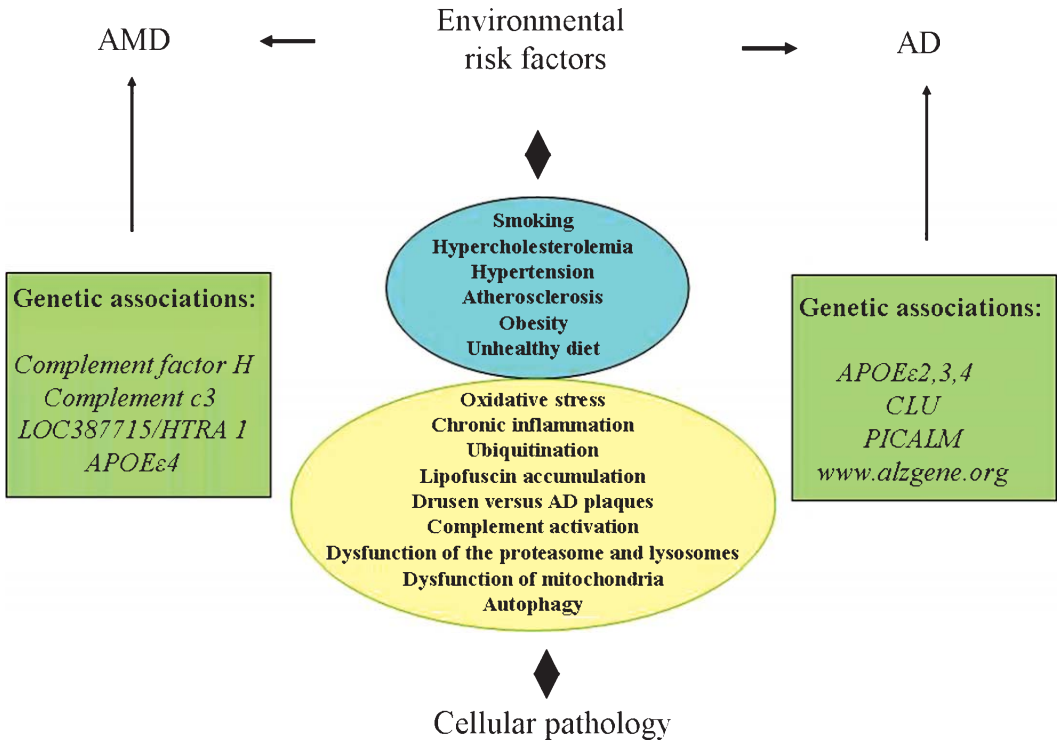

Fig. 2. Schematic presentation of the similarities and differences in the environmental risk factors, cellular pathology, and genetic polymorphisms associated with AMD and AD.

VEGF intravitreal injections for AMD are only useful in patients with neovascular AMD form [17, 18].

Similarly with AMD, the principal risk factor for Alzheimer's disease (AD) is aging. In Western countries, AD affects 1–3% of people aged 60–64 years, and 3–12% of people aged 70–80 years. This proportion increases to 25–35% for people older than 85 years [19]. Cardiovascular dysfunction, such as hypercholesterolaemia, hypertension, obesity and diabetes in mid-life contribute to the development of dementia (Fig. 2) [20–24]. As the aging population increases, the AD cases are expected to quadruple by the year 2050, unless prevention efforts or disease modifying therapies are developed [25]. AD is clinically characterized by the development of early amnesia and executive dysfunction, which eventually spreads across cognitive domains, leading to the complete incapacity and development of end-stage dementia [26]. The major pathological hallmarks of AD are extracellular amyloid-β (Aβ) plaque deposition and intraneuronal neurofibrillary tangle (NFT) formation [25]. Emerging evidence suggests that progressive inflammation and increased oxidative stress play a key role in the early development of AD pathological features. Such mechanisms have also been clearly posted to play a central role in synaptic dysfunction and the loss of neuronal integrity. This may precede the overt appearance of amyloid plaques and NFTs in the brains of affected individuals. Interestingly, insoluble amyloid plaque cores from AD cortex did not impair synaptic plasticity and memory unless they were first solubilized to release Aβ dimers [27]. This reveals that plaque cores are largely inactive but Aβ dimers are synaptotoxic.

DIFFERENCES AND SIMILARITIES IN THE PATHOGENESIS OF AMD AND AD

AMD and AD largely share several clinical and pathological features, including oxidative stress and inflammation. These are well-known inducers of protein aggregation especially in aged post-mitotic cells, such as RPE and neuronal cells. It is interesting that the accumulated deposits have many molecular similarities in AMD and AD. A decreased capacity to remove damaged cellular proteins in postmitotic cells during aging has been strongly implicated in both diseases. Many cellular clearance mechanisms seem to work in

collaboration and are regulated by specific proteins. There are increasing challenges to understand aging processes in different tissues and subsequently to find novel therapy targets to prevent protein aggregation and to maintain effective clearance systems. Therefore, AMD and AD are discussed in parallel in this review.

Genetics in AMD and AD

The genetic risk factors related to the complement system have been highlighted in several studies for AMD. The first associations were observed between the Y402H polymorphism (rs1061170) of the complement factor H gene and AMD in several populations (Fig. 2) [28–34]. Later on, an association between the locus LOC387715/age-related maculopathy susceptibility 2 (*ARMS2*) and high-temperature requirement factor A1 (*HTRA1*) and AMD in populations of different origin has been documented as well (Fig. 2) [35–43]. The *HTRA* polymorphisms are not associated to AD [44]. One common polymorphism (rs2230199) in the complement component 3 has also been associated with AMD [45, 46]. Before the finding of the polymorphisms in the complement system genes, apolipoprotein E (*APOE*) allelic polymorphism was implicated in AMD. Individuals carrying the *APOE-ε4* allele seem to have a lower risk for the disease, whereas the *APOE-ε2* allele is linked to an elevated risk (Fig. 2; [47–50]).

ApoE is a polymorphic protein, which has three common isoforms (E2, E3, and E4) encoded by three alleles (*ε2*, *ε3*, and *ε4*) of the *APOE* gene on chromosome 19q13.2 locus [51]. Based on the role of *APOE* in the recycling of cholesterol and lipids for cell-membrane biosynthesis, these polymorphisms have been speculated to impact retinal membrane renewal and affect macular integrity. Two different hypotheses have been offered to explain the protective mechanism of *APOE-ε4* in the development of AMD. *APOE-ε4*, in contrast to *APOE-ε2* and *ε3* alleles, does not contain disulfide bridges; therefore, being smaller, it may be more effectively transported through Bruch's membrane. The other potential protective mechanism may be explained as follows: *APOE-ε4* has a positive charge, which diminishes hydrophobicity of the Bruch's membrane and thus contributes to better clearance of the debris [52]. In contrast to AMD, the *APOE-ε4* polymorphism represents the strongest thus far identified risk factor for AD, whereas the *APOE-ε2* allele carriers have a reduced risk for developing AD (http://www.alzgene.org) [53–57]. These findings suggest an opposite role for *APOE-ε4* and *APOE-ε2* alleles in AMD and AD. A hypothesis for immune dysfunction via the complement system gene polymorphism in the pathogenesis of both AMD and AD has been suggested. One study reveals that the *CFH T1277C* polymorphism seems to influence the risk of AD and there appears to be an interaction between *CFH T1277C* and *APOE-ε4* alleles. The *CFH T1277C* allele may predispose patients for co-morbidity in AD and AMD [58]. Examination of the common *CFH* Y402H polymorphism in a large case-control cohort to investigate association with late-onset AD susceptibility did not show any evidence that this SNP is associated with AD risk. However, it remains possible that another variant of this gene may modify susceptibility for late-onset AD [59, 60].

The underlying cause of AD is unknown in most cases. More than 40 different genetic alterations have been identified as being associated with AD (see details, http://www.alzgene.org). Amongst them, polymorphisms in *APOE,* clusterin (*CLU*) and phosphatidylinositol binding clathrin assembly protein (*PICALM*) genes currently show the strongest risk effects among AD patients (Fig. 2; http://www.alzgene.org) [53–55, 57, 61, 62]. However, as non-genetic risk factors have been shown to play significant roles in the development of AD, it is likely that interplay between genetic and environmental factors triggers the onset of pathophysiological events eventually leading to AD. In a prospective population-based Rotterdam Study, the presence of AMD and AD were screened and follow-up examinations were conducted from mid-1993 to the end of 1994. Subjects with advanced AMD at baseline showed an increased risk for AD (relative risk = 2.1, 95% confidence interval: 1.1, 4.3; adjusted for age and gender), but this risk decreased after additional adjustment for smoking and atherosclerosis (relative risk = 1.5, 95% confidence interval: 0.6, 3.5). These findings suggested that the neuronal degeneration occurring in AMD and AD may, to some extent, have a common mechanism [63]. This study showed a possible epidemiological connection between AMD and AD. However, none of the AD-related genes are candidates for AMD pathology today. Taken together, it seems that genetic risk factors in AMD and AD have a different origin.

Oxidative stress and mitochondrial and lysosomal dysfunction in AMD and AD

Free radicals and other oxidants are produced as metabolic by-products in an oxygen-containing environment by a large number of physiological and

pathophysiological and aging processes. Derivation of radicals include electron leakage from the mitochondrial electron transport chain, the generation of hydroxyl radicals by Fenton-type reactions and the production of superoxide, hydrogen peroxide and hypochlorite as a consequence of many enzymatic reactions. An imbalance between the generation and suppression of reactive oxygen species (ROS) can lead to undesirable effects due to protein unfolding and damage that especially occur in age-related conditions [64, 65].

Central retina is exposed to the exceptionally high oxidative stress load that is even increased during the aging process [6, 7]. Epidemiologic, genetic, and molecular pathological studies support a role for oxidative stress in AMD pathogenesis (Fig. 2; [5, 6, 66]). The retina and RPE cells serve as an ideal environment for the generation of ROS due to (1) high oxygen consumption, (2) exposure to continuous light, (3) high amounts of polyunsaturated fatty acids (PUFAs) in photoreceptor outer segments, (4) presence of photosensitizers in the RPE and neurosensory retina [6], and (5) daily phagocytosis of the retinal outer segment originated from rods and cones [67]. Oxidative stress is mainly caused by retinal irradiation, lipid peroxidation, photochemical damage of retinal chromophores and respiratory burst [6]. These processes are associated with RPE aging. The postmitotic RPE cells constitute a polygonal monolayer between the neurosensory retina and the fenestrated capillaries of the choroid (Fig. 1C). Degeneration of the RPE cells is one of the most important hallmarks of AMD [66]. Clinically, it can be observed by pigment dispersion, accumulation of intracellular lysosomal lipofuscin, and extracellular drusen deposits (Fig. 1B, C). In senescent cells, especially in postmitotic cells such as the RPE cells, increased oxidative damage evokes protein misfolding and aggregation (see later). One characteristic indication of the cellular aggregation is the progressive lipofuscin accumulation in the lysosomes of RPE cells as a terminal pathway of phagocytosis of shed photoreceptor outer segment disks [68].

Lipofuscin is a highly crosslinked aggregate consisting of oxidized proteins and lipids. It is believed that, once formed, lipofuscin is not degraded by proteasomal or lysosomal enzymes or transported into the extracellular space via exocytosis [66, 69]. This is because the lipofuscin accumulation itself may secondarily disturb proteasomal and lysosomal protein degradation processes and lead to increased extracellular protein accumulation in aged RPE cells (see later; [65, 70, 71]).

Lipofuscin formation is closely linked to mitochondrial function. A small portion of oxidants derived from mitochondria manages diffuse into lysosomes, a compartment rich in cysteine and redox-active iron. Lysosomal iron originates from the degradation of a variety of iron-containing proteins present in the photoreceptor outer segment discs [69, 72]. Mitochondrial hydrogen peroxide and lysosomal iron react in the Fenton reaction producing hydroxyl radicals. Some oxidation products polymerize inside of lysosomes and become undegradable lipofuscin and accumulate in lysosomes of long-lived postmitotic cells, such as RPE cells, which do not dilute the pigment by division [69, 74, 75]. It is speculated that lipofuscin itself is cytotoxic because of its ability to incorporate transition metals [75]. This characteristic of lipofuscin contributes to an increased level of radical formation and oxidatively modified cellular components, such as proteins, lipids, and RNA/DNA, which have been shown to be extensive in aging cells. For example, many proteins isolated from RPE cell lipofuscin are modified with oxidative stress markers, including malondialdehyde, 4-hydroxynonenal, and AGE and RAGE modifications [76, 77]. Recently, it was demonstrated that cytotoxic oxidant production is lipofuscin derived, independent of mitochondria [78]. The ability of lipofuscin to produce oxidants is indeed dependent on the amount of transition iron metals incorporated. However, increased accumulation of lipofuscin, continuous light exposure and phagocytosis in RPE cells induce defects in the mitochondrial functions [75, 79, 80]. As the RPE is an active site of metabolism, impaired mitochondrial function may result in the accelerated degeneration of RPE and the photoreceptor cells due to insufficient nutrient supply [81]. Structural alterations in mitochondria increase during aging process and in AMD pathology [82, 83]. A morphologic analysis of human donor eyes affected by AMD revealed a decrease in the number of mitochondria per cross-sectional area relative to normal age-related changes [82]. Mitochondrial (mt)DNA is more susceptible than nuclear DNA to damage from oxidation and blue light [84–86]. The mtDNA damage in the retina and RPE accumulates during aging [87, 88]. It is suggested that an impaired function of mtDNA-encoded subunits of the electron transport chain may evoke increased superoxide anion production that leads to further mtDNA damage and superoxide anion production [89, 90]. Aging and cigarette smoking are the main risk factors for AMD, both of which are associated with mitochondrial dysfunction [91, 92]. Recent findings in mitochondrial proteomics support the idea that there

is an increased mitochondrial stress and dysfunction in the RPE cells in AMD patients [93]. In summary, reduction of lysosomal and mitochondrial function and increased lipofuscin are supposed to be key points to induce RPE aging and development of AMD (Fig. 2).

It has been speculated that oxidative stress is perhaps the earliest feature of an AD brain. Oxidative damage marked by lipid peroxidation, nitration, reactive carbonyls, and nucleic acid oxidation is increased in vulnerable neurons in AD [94]. Oxidative-stress markers appear decades prior to Aβ deposition in these patients [95–101]. Increased levels of isoprostanes, products of polyunsaturated fatty acid oxidation, are found in patients diagnosed with the prodromal stage of AD [102–104]. In addition, increased levels of lipid peroxidation and nucleic acid oxidation are found in brain tissues, cerebrospinal fluid, plasma, urine, and peripheral leukocytes of mild cognitive impair (MCI) or AD patients [105–107]. A sensitive marker of oxidative stress, heme oxygenase-1, is elevated in postmortem brain tissue of individuals with both AD and MCI [108, 109]. Increased iron has been found at the highest levels both in the cortex and cerebellum from the pre-clinical AD/MCI cases. Interestingly, glial accumulation of redox-active iron in the cerebellum is also evident in preclinical AD patients and it tends to increase as the patients become progressively cognitively impaired [37, 110]. As in AMD, an imbalance in iron homeostasis seems to precede the neurodegenerative processes that lead to AD as well. Extensive literature exists supporting a role for mitochondrial dysfunction and iron accumulation in the pathogenesis of AD [111]. Iron deposition at the preclinical stage of AD may be useful as a diagnostic tool, when using iron imaging methods. It also may represent a potential therapeutic target, by utilizing metal ion chelators in both AD and AMD [37, 75, 112]. AD patients have a striking and significant increase in the mtDNA level in the neuronal cytoplasm and in vacuoles associated with lipofuscin [113], the proposed site of mitochondrial degradation by autophagy (see later). One further indication of increased oxidative stress and mitochondrial dysfunction is the cellular distribution of malondialdehyde (MDA) in brain specimens. Electron microscopy indicated that neuronal MDA formed cap-like linear deposits and associated with lipofuscin [114]. Taken together, increased oxidative stress, accumulation of intracellular iron and lipofuscin deposits and mitochondrial and lysosomal dysfunctions seem to be common pathophysiological events in both AMD and AD pathogenesis (Fig. 2).

Protein aggregation process in AMD and AD

Oxidation ultimately leads to unfolding or conformational changes in proteins, thereby exposing more hydrophobic residues to an aqueous environment. This may lead to a loss of structural or functional activity, including aggregation and accumulation of oxidized proteins as cytoplasmic inclusions [72, 115]. Accumulation of proteins is a recurring event in many age-related diseases, including AMD and AD.

In addition to stress-damaged proteins, the inherited mutations and polymorphisms that alter the sequence of a polypeptide can affect protein folding and stability, triggering disease at birth and during aging. A central cellular mechanism for generating and maintaining normal protein folding is the protein homeostasis or proteostasis network. These processes sustain functional proteins as well as direct their removal from the cell during protein turnover or in response to misfolding. This "yin-yang" balance is critical for normal cellular, tissue, and organismal physiology [116].

The ubiquitin-proteasome system is an error-checking system that directs improperly folded proteins for destruction. There is evidence that molecular chaperones interact directly or indirectly with the proteasome, assuring quite selectively the proteasomal degradation of certain proteins under stress conditions [117]. However, cell type-specific expression levels of the chaperones are demonstrated in neuronal cells [118]. If the chaperone response is unsuccessful, misfolded proteins are tagged with a small polypeptide ubiquitin (Ub) and the complex is directed to the Ub/proteasomal protein degradation pathway (UPP) [119, 120]. A ubiquitin monomer is activated in an ATP-dependent reaction by the Ub-activating enzyme E, ubiquitin conjugating enzyme E2 and in the final step, Ub is transferred to the target protein via a ubiquitin ligase E3 [121]. The UPP is one of the most important cellular systems to remove damaged proteins in response to oxidative stress. Ub was initially documented to be a regulator of protein degradation, but subsequent observations have confirmed its participation also in the regulation of other cellular processes, such as endocytosis, cell cycle, signal transduction, gene regulation, DNA repair and autophagy clearance [120, 122–124].

Proteasomal protein degradation in AMD and AD

The RPE cells live under a chronic oxidative stress due to vitamin A-derived visual cycle metabolism and constant light exposure. During aging process,

the capacity to repair oxidative stress-induced cellular damages is decreased. This may trigger an increased mass of misfolded proteins that have a tendency to gather into detrimental aggregates [72]. However, there is a hypothesis that the accumulation of oxidized and ubiquitinated proteins is due to the decrease of proteasome activity with age [125]. In concordance with this hypothesis, a decreased capacity of UPP to degrade harmful proteins in aged RPE cells has been documented [126]. Moreover, under certain circumstances, oxidative stress itself may inactivate the function of the proteasome and up-regulate the release of proinflammatory cytokines [127], which may account for the chronic inflammation in retina and the accumulation of drusens in the space between Bruch's membrane and RPE that evoke AMD development [66]. In support of these hypotheses, Ethen and colleagues (2007) have documented functional changes of the proteasome in AMD [128].

A common age-related feature observed in many tissues is the accumulation of the Ub-conjugated proteins. These proteins, which have been tagged with Ub for degradation but not efficiently removed, might be detrimental to cell viability. Excessive accumulation of the Ub-protein conjugates is observed in drusens in AMD and in both plaques and tangles in AD [66, 129–133]. It has been shown that a Ub-B mutant protein (UbB + 1), which is a mutant Ub carrying a 19-amino acid C-terminal extension generated by a transcriptional dinucleotide deletion, exists in the AD deposits [133]. Notably, UbB + 1 has been shown to block Ub-dependent proteolysis in neuronal cells [134]. In addition, it causes neuritic beading of mitochondria and is estimated to be a potential mediator of Aβ-induced neurotoxicity [135, 136]. The AD brain is also characterized by the accumulation of oxidized proteins [137, 138], which may further exacerbate the decrease in proteasome activity [139]. Particularly intriguing is the finding that the Ub carboxy-terminal hydrolase L1 (UCH-L1), an enzyme that hydrolyses Ub from poly-ubiquitinated proteins to release Ub monomers, is oxidized in AD and is down-regulated in the specific brain regions of early AD cases [140, 141].

A Ub-like protein called ubiquilin-1 functions as a cytoplasmic "gatekeeper" by controlling AβPP trafficking from the intracellular compartments to the cell surface and thereby influencing the generation of Aβ [142–144]. Interestingly, ubiquilin-1 (*UBQLN1*) gene variants have been shown to associate with AD [142]. However, subsequent replication studies with *UBQLN1* have been controversial in terms of genetic association with AD (see details, http://www.alzgene.org). Increased levels of beta-site AβPP-cleaving enzyme (BACE), whose activity and accumulation are regulated via Ub and the adaptor protein GGA3 (Golgi-localized γ-ear-containing ARF binding proteins), are also linked to the accumulation of the Aβ deposits [145–147]. In addition to the novel signaling mechanism of Aβ accumulation, direct evidence of altered proteasome activity in AD brains has been reported previously [148, 149]. A selective decrease in the proteasome activity in specific brain regions of AD cases has been observed [150]. Very intriguing was the finding that proteasome activity was decreased in the brain regions, such as the hippocampus, that are more susceptible to the AD pathology, whereas other less susceptible brain regions, such as the cerebellum, exhibited no changes in the proteasome activity between AD patients and controls.

In both AMD and AD, detrimentally altered structure or functional activity of proteins and the proteasomal clearance system are observed that summit to increased ubiquitinated protein conjugates. This indicates increased damage of proteins and imbalance of protein clearance via proteasomes in AMD and AD (Fig. 2).

Autophagic clearance in AMD and AD

Recent observations indicate that disturbed autophagy is involved in the protein aggregation in RPE cells and in the AMD pathogenesis [66, 123, 124, 151]. Autophagocytosis is a specific clearance system involved in protein and organelle degradation via the lysosomal pathway. It is categorized into macroautophagy, microautophagy, and chaperone-mediated autophagy [152, 153]. Autophagosomes fuse with lysosomes to form autolysosomes, and endo-autophagosomal components are degraded by lysosomal hydrolases.

AMD-associated stress conditions such as hypoxia, oxidative stress, unfolded protein response and inflammation are typical inducers of autophagy [7, 72, 154]. Autophagy contributes to intracellular quality control and cellular housekeeping, especially by controlling the turnover of damaged and aged proteins. The autophagic process is extremely important in post-mitotic cells, such as RPE and neuronal cells [66, 155]. Enhanced autophagy reduces the toxicity of the protein aggregates that accumulate in many age-associated diseases, but unfortunately the autophagic activity declines during the aging process [154]. If the lysosomal function becomes suppressed, as occurs

during excess lipofuscin loading, then autophagic clearance is no longer functional in RPE cells in response to impaired cellular proteolysis [124]. The preservation of the autophagic activity together with functional lysosomal enzymes is a pre-requisite if one wishes to prevent detrimental intracellular accumulation of damaged proteins [123]. Lysosomes critically regulate the pH-dependent catabolism of extracellular and intracellular macromolecules delivered from the endocytic/heterophagy and autophagy pathways, respectively. The importance of lysosomes in cell survival is underscored not only by their unique ability to effectively degrade metalloproteins and oxidatively damaged macromolecules, but also by their distinct potential to induce both caspase-dependent and -independent cell death with a compromise in the integrity of lysosome function [156]. Oxidative stress and free radical damage play a principal role in cell death induced by lysosome dysfunction and may be linked to several upstream and downstream stimuli, including alterations in the autophagy degradation pathway, inhibition of lysosome enzyme function, and lysosome membrane damage [71]. Neurons are sensitive to lysosome dysfunction and the contribution of oxidative stress and free radical damage to lysosome dysfunction may contribute to the etiology of neurodegenerative disease [156–158]. Genome-wide screening reveals that ROS serve as common mediators upstream of the activation of the type III PI3 kinase (PIK3C3), which is critical for the initiation of autophagy in normal and AD brain [159]. Furthermore, ROS play an essential function in the induction of PIK3C3 and autophagy in response to Aβ peptide, the main pathogenic mediator of AD. In contrast to normal aging, a transcriptional up-regulation of autophagy in the brains of AD patients has been observed, suggesting that there might be a compensatory regulation of autophagy [159]. Similar findings have been documented for AMD [151]. Jaeger and colleagues (2010) showed that BECN1 deficiency disrupts cellular autophagy and autophagosomal-lysosomal degradation and alters AβPP metabolism. Those findings suggest that autophagy and the BECN1-PIK3C3 complex regulate AβPP processing and thus may play an important role in AD pathology [160]. Presenilin-1 is required for lysosomal turnover of autophagic and endocytic protein substrates. Its deletion causes a virtually complete loss of macroautophagy while having a minimal influence on nonlysosomal types of proteolysis [161]. Presenilin-dependent gamma-secretase plays an important role in the development AD by releasing Aβ from AβPP. Gamma-secretase activity is enriched in autophagic vacuoles and enhanced in basal autophagy-disturbed cells through the alpha subunit of eukaryotic translation initiation factor 2 (eIF2alpha) kinase [162]. Furthermore, it is well understood that mitochondrial abnormalities are prominent in AD. Moreira et al. (2007) suggest that mitochondria are the key targets of increased autophagic degradation in AD. However, whether the increased autophagocytosis is a consequence of an increased turnover of mitochondria or whether the mitochondria in AD are more susceptible to autophagy remains to be resolved [163].

As previously discussed, lipofuscin formation may derive primarily from the incomplete lysosomal degradation of mitochondria [69]. Evidence for this includes observations that (1) oxidation of mitochondria is sufficient to generate lipofuscin-like material, (2) mitochondrial proteins and mitochondrial lipid can be found in lipofuscin, (3) lysosomal proteases are present on the surface of lipofuscin, (4) lipofuscin is contained within lysosomal membranes, and (5) inhibition of lysosomal proteolysis contributes to the accumulation of lipofuscin within cultured cells. Recent findings reveal cross-talk between the lysosomal and proteasomal system that may regulate formation of lipofuscin [123]. Lipofuscin can directly or indirectly affect the proteolytic performance within cells [164–166].

p62 is a key molecule to regulate lysosomal (including autophagy) and proteasomal clearance in cells [66]. p62 was first characterized in polyubiquitinated protein aggregates in response to proteasomal depletion [167]. It is highly expressed in the pathophysiological structures of many neurodegenerative diseases, like Lewy bodies in Parkinson's disease, NFTs in AD and huntingtin aggregates [168–171]. To date, it is realized that p62 is one of the main molecules controlling the shuttling of ubiquitinated substrates towards proteasomal degradation [172, 173]. In normal situations, damaged, misfolded or otherwise potentially toxic soluble proteins are degraded by the proteasome machinery. Inhibition of p62 transcription blocks proteasomal sequestration of ubiquitinated proteins and enlargement of inclusions. Therefore p62 plays a vital role in the defense of cells from the toxicity of misfolded proteins by augmenting aggregate formation [174, 175]. In addition to the regulation of proteasomal protein clearance, p62 directly interacts with LC3 via the LRS (LC3 recognition sequence) domain and by this connection it controls autophagic activity [176]. Our recent publication reveals that increased accumulation of perinuclear aggregates and p62 are concentration- and time-dependent, when the proteasome function is

disturbed. The p62 is strongly associated to neurodegenerative aggregation diseases, but it also seems to regulate macroautophagy by a threshold triggering way in RPE cells [175].

Pathogenesis of AMD and AD seems to involve an impairment of both proteasomal and lysosomal protein clearance systems that certainly lead to drusen and amyloid plaque accumulation (Fig. 2).

Similarities of drusens and amyloid plaques

AD is histopathologically characterized by the presence of extracellular senile plaques (SP), predominantly consisting of fibrillar Aβ, intracellular NFTs, composed of hyperphosphorylated tau protein, and vitronectin and the loss of synapses in the selected regions of the brain [177]. AMD is characterized by the presence of drusens, which are extracellular deposits that accumulate between the RPE and Bruch's membrane (Fig. 1). Many protein and lipid constituents of drusens are similar to those found in deposits characteristic of other age-related degenerative disorders, such as AD and other amyloid diseases [132, 178–180]. These include Aβ, clusterin, vitronectin, amyloid P, apolipoprotein E, and inflammatory mediators, such as acute phase reactants and complement components. The finding that C5, C5b9, and C3 fragments, which are components of the complement cascade, are present in drusens as well supports a role for local inflammation in drusen biogenesis [181–183]. Interestingly, Ub is also present in AMD tissue samples, revealing proteasomal-lysosomal axis in the pathogenesis [123, 132, 151]. Polymorphism in complement factor H, a regulator of the alternative complement pathway, significantly increases the risk for AMD [28–31]. It is notable that the association of the complement factor H polymorphism with AD seems to be minimal or non-existing [59–60]. Interestingly, variants of the complement component (3b/4b) receptor 1 gene *CR1* have recently been shown to be associated with AD [62]. The commonalities between AMD and AD can also be seen in a transgenic mouse model that expresses human *APOE4* [184], the *APOE* allelic variant that is a major risk factor for AD [185]. Aged mice of this strain exhibit a retinal phenotype that replicates many features of AMD when the animals are fed a high-fat diet. Interestingly, the pathologic features of this retinal model are attenuated by anti-Aβ antibody, supporting a role for Aβ toxicity in the retina [186]. It is likely that Aβ-induced toxicity in the retina and in the brain is due to the formation of toxic Aβ oligomers [187–188]. When comparing AMD and AD pathology in the animal models, it should be noticed that in rodents there are no functional macula or sharp vision area in the retina. ApoE has been illustrated in drusen [132]. It has also been demonstrated that RPE cells secrete ApoE in response to a variety of hormones, and that the secreted ApoE is associated with HDL. These findings suggest a possible role for ApoE in the AMD pathology via retinal lipid trafficking [189]. Aging or disease-related disruption of normal ApoE function may result in the accumulation of lipoproteins between RPE and Bruch's membrane, which is consistent with lipid deposits in drusen [132, 178]. Indeed, the lipid deposits of drusen are often composed of cholesteryl esters and unsaturated fatty acids. Accumulation of drusen-associated lipids due to the impaired ApoE function could potentially affect the functional integrity of Bruch's membrane and development of AMD [190].

In contrast to AMD, the inheritance of the *APO- ε4* allele is associated with elevated cholesterol levels and an increased risk of developing AD compared to individuals with only the *APOE-ε2* or *APOE-ε3* alleles [47–50, 53, 57]. The hyperphosphorylated tau protein is a crucial tissue hallmark in AD [177]. While tau immunoreactivity does not change with age, it is mildly increased in the RPE of eyes with AMD [191]. Moreover, immunoreactivity for vitronectin, which is a central molecule in drusens [132, 178], is also observed in senile plaques of AD brain. It has been suggested that vitronectin is deposited at the sites of amyloid formation. Therefore, accumulation of misfolded vitronectin may contribute to aggregate formation seen in age-related amyloid diseases [177], such as AMD and AD.

Complement system in AMD and AD

Chronic oxidative stress and inflammation are proposed to be the major causes for the deposition of extracellular drusens between the basal surface of the RPE and a basement membrane complex called Bruch's membrane [8, 9, 66]. Similarly with the drusens, senile plaques in AD contain inflammatory mediators, acute-phase reactants and activated components of the complement system. The complement system, that is an ancient component of the host immune defence, is divided into the classical, alternative and lectin binding pathways. There is evidence that inflammation in the pathogenesis of AMD and AD have similar signaling cascades, even though the cell types in the tissues are different [8, 192]. Formation of senile plaques containing Aβ and the presence of

NFTs, consisting of hyperphosphorylated tau, are characteristic features of AD. Alteration in Aβ processing is one suggested pathogenetic culprit to AD [193–195]. However, it is not known how Aβ leads to neuronal cell death. One proposed mechanism is through the activation of microglial cells, which are phagocytes of the CNS [196], that functionally have similarities with RPE cells, macrophages or dendritic cells in AMD pathology [66]. Activated RPE cells and macrophages in AMD and microglia in AD are known to secret similar inflammatory mediators. Many of these acute-phase proteins can be found in the drusens and senile plaques [9, 197]. The activation of the complement system in AMD and AD is shown in many immunohistochemical analyses and it represents the strong similarities between these two age-related degenerative diseases (Fig. 2).

CONCLUSION

AMD and AD are both age-related neurodegenerative diseases that share similar environmental risk factors comprising of smoking, hypertension, hypercholesterolemia, atherosclerosis, obesity, and unhealthy diet. Cellular pathology associates with increased oxidative stress, inflammation, and impaired proteasomal and lysosomal function that evoke formation of intra- and extracellular deposits. The detrimental deposits consist of largely similar aggregated proteins in both diseases. However, the genetic background seems to be very different between AMD and AD. This retains future challenges to study gene polymorphism effects on the functional levels of proteins and their role in the neurodegenerative diseases. The bright ocular tissues and advanced imaging technology to study the posterior pole of the eye provide interesting opportunities to understand the early signs of AMD that might be associated with AD pathology as well.

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