Review

Cell death, clearance and inflammation: molecular crossroads and gene polymorphisms in the pathogenesis of age-related macular degeneration

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Abstract: Age-related macular degeneration (AMD) is the leading cause of irreversible loss of vision and decrease in quality of life in the elderly. A large number of molecular crossroads between cell death, phagocytosis and inflammation have been described in AMD. The different forms of cell death - apoptosis, anoikis, autophagy, necrosis, necroptosis and pyroptosis, have all been studied under different circumstances in the retina and in AMD pathology. Much less is known about the clearance of these dying cells by non-professional (living retinal pigment epithelial (RPE) cells) and professional (macrophages and dendritic cells) phagocytes. The molecular synapse between the dying cells and the phagocytes is far from complete in relevance to AMD. Gene polymorphism of the phagocytic bridging molecules and those involved in inflammation and angiogenesis further complicate the lock-and-key theory in the clearance of dying cells in AMD. The present review gives an overview of the different risk factors, cell death types and clearance mechanisms in the retina, and their implications to inflammation and angiogenesis in AMD. A correlation of the genetic factors affecting AMD to those of other neurodegenerative diseases (Alzheimer's, Huntington and Parkinson's) is also attempted here.

Keywords: cell death, apoptosis, autophagy, phagocytosis, inflammation, gene polymorphism, neurodegenerative diseases, age-related macular degeneration

Background of AMD

Age-related macular degeneration (AMD) is a major cause of visual loss in people over 50 worldwide. It causes irreversible loss of central vision and significant decrease in quality of life of the elderly [1, 2]. Two major forms of AMD are being recognized in a simplified way: dry or early form (also known as geographic atrophy (GA)) and wet or late form (hallmarked by appearance of choroidal neovascularizations (CNVs)) based upon the absence or presence of drusen (yellow deposits) and/or fluid or neovascularizations, respectively, at the macula. The dry form of AMD is the most common and proceeds with thinning or atrophy of the retinal pigment epithelial (RPE) cells of the macula and deposition of drusen. Patients with the dry form of the disease in general do not lose fully their

reading vision [3, 4]. The wet form of AMD is characterized by a growth of abnormal blood vessels from the choroid into the retina via penetration of the Bruch's membrane. Only about 10% of the people with macular degeneration develop the wet form of the disease, which is likely to cause severe visual loss over quite a short time - sometimes even months. AMD represents a major and growing public health problem in developed countries due to the ever increasing ageing population [5, 6].

Risk factors for AMD

It is now clear, AMD turns manifest in the presence of external or specific environmental- as well as internal risk factors such as smoking [7], high blood pressure, high cholesterol level, obesity, or being light skinned, light eye-colored, female subject. The disorder is more common in urban communities. The phenotype of early AMD reflects the influence of all these factors, while late AMD may be influenced by specific genes involved or suspected to be involved in the disease etiology, although full cause-and-effect relationship to AMD has not been proven yet [5, 8-10].

Cell death type and inflammation in AMD pathogenesis

Until recently, apoptosis and necrosis were the only two major types of cell death described in the retina of AMD patients [11, 12]. Pyroptosis and autophagy have not been observed in oxidative stress-induced cell death in the retina [12]. More recently, however, our group and collaborators have detected autophagy in human eyes suffering from AMD [13]. The autophagy activation was hereby found important in the clearance of ELAVL1/HuR-mediated accumulation of SQSTM1/p62 during proteasomal inhibition of human RPE (hRPE) cells. In addition, autophagy and heterophagy dysregulation could be blamed for the RPE dysfunction and the development of AMD [2]. Hypoxia-inducible factor (HIF) is known as the master regulator of hypoxia-induced cellular adaptation that is involved in the nuclear factor kappa beta (NFκB) signaling and the autophagic protein clearance system [14]. NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome activation in the RPE cells during pathogenesis of advanced AMD [15-18] suggests involvement of the inflammasome complex and caspase-1 activation, which together means presence of pyroptosis as a cell death modality in retinal cells. Indeed, different stimuli, like endoplasmatic reticulum (ER) stress inducers, tumor necrosis factor alpha (TNFα), ATP and lipopolysaccharide (LPS) can all activate caspase-1 and -5 in primary cultured hRPE cells and in telomerase immortalized hRPE cells [19]. The zinc chelator (TPEN) can cause cell death in monkey retinal cells coupled with activation of cytosolic calpains and mitochondrial caspase pathways, but without ER stress [20]. Caspase inhibiton is not enough in preventing photoreceptor cell death, supporting the findings that not only apoptosis occurs in retinal or RPE detachment. In fact, the overlap between the different cell death pathways is very much prominent in the retina, the cell morphology appearance being similar to necrosis during RPE detachment, although autophagy also gets activated under such conditions [21]. When the caspase pathway is blocked, receptor interacting protein (RIP) kinases promote necrosis and overcome apoptosis inhibition [22]. Programmed necrosis or necroptosis is a necrosis-like cell death in retinal cells which is rather RIP-3-dependent [12] (supported by unpublished data from our *in vitro* studies on ARPE-19 and primary hRPE cells). RIP-3 is also involved in the ischemic stress response in the retina [23] and plays a central role in rescuing photoreceptor cell death in blind zebrafish [24]. Necroptosis can be efficiently blocked by necrostatins in ARPE-19 cells [25-28], in particular, Nec-1, 3 and 5 [12]. RIP-1-dependent necroptosis is less apparent in ARPE-19 cells, since Nec-1 has no effect on the RIP-1 kinase activity, but pro-inflammatory stimuli as those by TNFα can activate it (unpublished data). Oxidative stress-induced necrotic cell death in the retinal cells by H_2O_2 treatment is rather controversial and can be prevented by Nec-1, but not by caspase inhibitor Z-VAD-FMK, without affecting apoptotic cell death [12]. An interesting association of RIP-7 (also known as leucine rich repeat kinase 2 (LRRK2)) to neurodegenerative disease pathogenesis, in particular Parkinson's disease, is due to a major genetic defect or a dominant missense mutation in this gene [29, 30].

Phagocytic clearance of dying cells and the role of macrophages and dendritic cells in AMD pathology

Photoreceptors and RPE cells found at the edge of AMD atrophy die by apoptosis [31]. The atrophy propagates as the area of apoptosis expands, contributing to a development of visual loss as more and more retinal cells die. The dead cells' debris in the eye is potentially dangerous material which needs removal. The process of clearance can, however, obstruct the physiological engulfment of the photoreceptor outer segments (POS) which occurs on a daily basis and is essential for keeping the retinal homeostasis in a balanced state. The importance of the phagocytosis carried out by RPE cells has been described some 40 years ago [32]. An insufficient clearance of dying retinal cells can lead to degeneration and inflammation [32, 33]. Not much is known, however, about the clearance of other types of dying cells in the retina such as autophagic, pyroptotic and necroptotic RPE cells.

In dry AMD, dead cells' debris from anoikic (cells detached from their extracellular matrix (ECM)) and apoptotic dying RPE cells gets engulfed by non-professional phagocytes (neighboring live RPE cells) in a highly efficient way as shown by our recent findings with ARPE-19 [34] and primary hRPE cells *in vitro* (unpublished data). Probably, other alternative routes of cell death lead to non-professional phagocytosis in the dry form of AMD, since intact, healthy

eyes, have impenetrable tissue barriers to cellular components, while professional phagocytes (macrophages and dendritic cells (DCs)) cannot reach the dead cells under such conditions.

In wet AMD, however, new vessels arise through the blood-retinal barrier, which bring close interaction between the RPE and retinal vascular cells. These new vessels are highly penetrable and leaky, thus professional phagocytes arrive at the scene and induce low-grade inflammation [35]. In this form of AMD, the macrophages and DCs bind to apoptotic cells, thus suppressing inflammation, while the inflammatory response [36]. The uptake of autophagic dying cells may happen in two ways: either by involvement of phosphatidylserine (PS) on the surface of the dying cells and engulfment by non-professional phagocytes (RPE) or in PS-independent way during engulfment by macrophages (**Figure 1**) [37]. *In vitro* data suggest that macrophages activated in this way secrete pro-inflammatory cytokines such as IL-6, TNFα, IL-8 and IL-10 (**Figure 1**)[37]. Autophagic dying cells engulfed by macrophages after LPS induction, abolish the TNFα, IL-6 and IL-8 secretion, but not the IL-1β secretion (through caspase-1 activation).

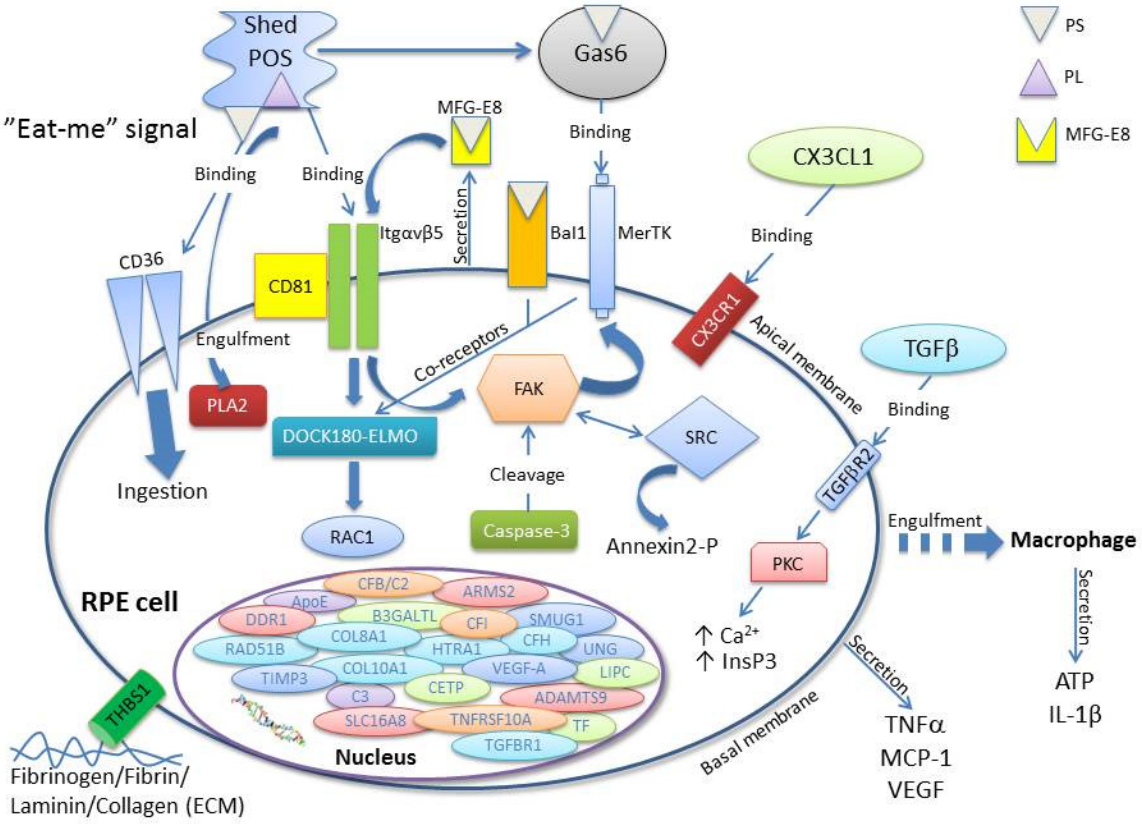

Figure 1. Molecules involved at the phagocytic synapse in the retina. Blue font represents genes in which SNPs have been associated with AMD. Abbreviations: ADAMTS9, a-disintegrin and metalloproteinase with thrombospondin motifs-9; ApoE, Apolipoprotein E; B3GALTL, beta 1,3-galactosyltransferase-like; Bal1, B-agressive lymphoma-1 protein; C3, complement component 3; CFB and CFH, complement factor B and H; CX3CL1, C-X3-C motif ligand 1; CX3CR1, C-X3-C motif receptor 1; DOCK180-ELMO, dedicator of cytokinesis-engulfment and cell motility protein; ECM, extracellular matrix; FAK, focal adhesion kinase; Gas6, Growth arrest-specific 6; HTRA1, high temperature requirement factor A1; InsP3, inositol-1,4,5-triphosphate; MCP-1, monocyte chemotactic protein-1; MFG-8, milk fat globule-8; PKC, Protein kinase C; PL, phospholipid; PLA2, phospholipase A2; POS, photoreceptor outer segment; PS, phosphatydil serine; RAC1, Ras-related C3 botulinum substrate; RPE, retinal pigment epithelium; SMUG1, single-strand selective monofunctional uracil-DNA glycosylate; TAM kinase, TYRO3, AXL, Mer kinase; TF, transferring; THBS1,thrombospondin 1;TGFβ, tumor growth factor beta; VEGF-A, vascular endothelial growth factor-A; UNG: Uracil-DNA glycosylate.

clearance of autophagic dying cells might cause an Moreover, macrophages secrete ATP after engulfing

autophagic dying cells; blockage of potassium (K^+) efflux abolishes the IL-1β secretion from the phagocytic cells. Altogether, a possible downstream pro-inflammatory activation by the clearance of autophagic dying cells seems to be present during engulfment by macrophages [36].

Non-circulatory or resident macrophages (microglia) as well as DCs can also be present in the uveal tract of the eye. Homing or migration of these cells to the choroidal-retinal border is independent of the CXC3 chemokine receptor 1 (CX3CR1) in normal young mice [38]. Forrester *et al*. identified two types of DCs in the choroid based upon their motility/migratory profile: relatively motile, large MHC class II^{mid} DCs, and small MHC class II^{hi} cells capable of forming clusters [38]. Based on their different capacity for translocation to secondary lymphoid tissue, a different role for each is suggested. When choroidal DCs are co-cultured with macrophages and choroidal cell preparations, their antigen presenting function increases as opposed to freshly isolated DCs. The response to stress, such as infection or ageing process (i.e. AMD) is, therefore, dependent upon other resident myeloid cells as well [39, 40]. The phagocytic capacity of macrophages towards dying RPE cells can be enhanced by triamcinolone (TA) ([34]; unpublished data), while substances causing elevation in cyclic AMP (cAMP) [41, 42], phosphodiesterase inhibitors (through cytoskeletal dysruptions) and lysosomotropic agents [43] as well as prostaglandins can decrease the rate of POS debris clearance (**Figure 1**). Exposure of cultured RPE cells originating from RCS rats to carbachol increases the intracellular concentration of inositol triphosphate and enhances the phagocytosis of bound POS [44], although this finding could not be confirmed by other studies [45].

The phagocytosis carried out by RPE cells requires specific binding of the integrin receptor αvβ5 (Itgαvβ5) to POS and internalization of the complex (**Figure 1**). In fact, no other phagocytic cells than RPEs use this receptor for binding and internalization of the engulfed material [46]. Phospholipase A2 has been found to contribute in the recycling of the POS by ARPE-19 cell as well [47]. Moreover, growth-arrest-specific protein 6 (Gas6), a Vitamin K-dependent serum protein, similar to Protein S existing in the hemostatic system, can induce POS phagocytosis and turnover in a Mer-dependent manner [48-50]. The redundancy of these molecules proves the importance of this circadian process [51]. The large plethora of molecules involved in the phagocytic synapse between RPE cells and/or macrophages as phagocytes, on one hand, and POS or dying RPE cells, on the other, are summarized in **Figure 1**.

Lack or failure of phagocytosis may lead to drusen accumulation and later development of AMD [35] due to ECM detachment or anoikis of the RPE cells. Similar to retinal detachment, RPE detachment and cell death may induce production of cytokines and chemokines such as TNFα and monocyte chemoattractant protein-1 (MCP-1), which can mediate activation of macrophages and microglial cells. Chronically activated inflammatory cells can then infiltrate into the outer nuclear layer of the retina and stimulate photoreceptor cell death similar to how it happens in retinal detachment [52, 53].

Genes involved at the retinal phagocytic interface

The apopto-phagocytic interface which is also known as “third synapse” consists of very complex “eat-me” and “don’t-eat-me” molecules found on the surface of engulfed/dying cells, factors released by these cells, bridging apopto-phagocytic molecules and effectors on the side of the phagocytes (**Figure 1**). RPE cells can secrete the glycoprotein milk fat globule-EGF8 (MFG-8) which can further activate the Itgαvβ5 [54-56] and therefore activate the intracellular signaling cascade involving focal adhesion kinases (FAK) [57]. Besides the aforementioned binding of Itgαvβ5 found on the surface of RPE cells to POS, efficient clearance of POS/dying cells in the retina needs involvement of so called TAM (Tyro 3, Axl and Mer-receptor Tyrosine Kinases (MerTK)) receptors expressed on the surface of RPE cells [58]. The latter molecules are related to some retinal dystrophies including retinitis pigmentosa [51]. Selective upregulation of the MerTK receptor via the Itgαvβ5-FAK signalling cascade, and decreased expression of the AXL receptor tyrosine kinase as well as thrombospondin-1 [59] on the surface of retinal phagocytes has been documented previously. Activation of MerTK results in intracellular free calcium release via the inositol-1,4,5-triphosphate (InsP3) pathway [57]. The process of internalization of POS/dying cells in the retina is also dependent upon the scavenger receptor CD36 found on the surface of phagocytic cells (RPE and macrophages) [60-62]. Transforming growth factor-beta (TGFβ) can regulate human RPE phagocytosis by influencing the protein kinase C (PKC)-dependent pathway [63].

Correlations of genetic factors found in AMD and other neurodegenerative diseases

AMD is characterized by abnormal accumulation of cell material at the base of the RPE cell layer. Extensive deposits can be a result of RPE cell dysfunction, disintegration of photoreceptor cells or failure of phagocytic activity that put the individual to a higher risk of developing AMD. Drusen formation is accompanied by appearance of immunomodulatory proteins, thus local pro-inflammatory events. Presence of chronic localized inflammation together with progressive neurodegeneration is observed in patients suffering from Alzheimer’s disease (AD). The two diseases share risk factors, such as ageing, hypercholesterolemia, hypertension, smoking [7] and obesity [5]. Amyloid beta (Aβ) peptide - a major component of the plaques in AD has been demonstrated in retinal drusen as a substructural vesicular component [64]. AD-related apolipoprotein ApoE is also present in deposits concerning both conditions as well

[65]. Imbalance in the Aβ angiogenesis-related factors in RPE cells and lack of neprilysin (Aβ degrading enzyme) in mice causes drusen formation similar to that observed in human AMD [66]. Aβ is implied to co-localize with proteins of the complement system in the amyloid structures, thus contributing to local pro-inflammatory changes [64] in both AD and AMD. Aβ is a target for immunotherapy of AD and the approach has been extended to AMD as well. Systemically administered anti-Aβ increases its elimination from the retina and the brain, and electroretinography abnormalities cease to exist in a mouse model [67]. Although SNP associations in complement factor H (CFH), age-related maculopathy susceptibility protein 2 (ARMS2) and complement component 3 (C3) genes exist in AD, a different genetic model seems to be present in AMD, suggesting a different contribution of the complement activation in the two diseases - complement pathway induction is less pronounced in AD [68]. Logue *et al.* reports no significant overlapping of genetic association between AD and AMD in the form of SNPs [69].

The ageing processes of cells in the retina and the brain goes through similar signaling associations [5]. Molecular genetic findings reveal four micro RNAs (miRNA-9, miRNA-125b, miRNA-146a, miRNA155) as being upregulated in AMD and AD. These miRNAs silence brain and retinal cell-relevant family mRNAs, as well as the CFH gene, a negative regulator of the innate immunity and inflammatory response [70].

Oxidative stress, abnormal cellular homeostasis and chronic inflammation are common risk factors of age-related neurodegenerative diseases, therefore a similar pathogenesis could be associated in different conditions, such as Parkinson's disease. In a retrospective cohort study conducted on Chinese patients, people diagnosed with late AMD had a 2.57 hazard ratio of developing Parkinson's disease [71]. No study has been conducted to date to explore a connection between AMD and Huntington's disease.

Gene polymorphisms found in AMD

The development of AMD is dependent on genetic and environmental factors. Polymorphism SNP studies reveal different loci associated with genetic risk to develop the condition as shown in **Table 1**. The strongest connection is with complement factor H (CFH) on 1q32 [72-78]. Tyr402His appears to be indicative of AMD pathogenesis. Diabetes, age, and gender in the presence of the homozygous C/C genotype in CFH carry an increased risk of AMD [73]. There are also several studies regarding a second major susceptibility gene PLEKHA1/LOC387715/HTRA1 [79] and Factor B polymorphism which play important roles in AMD [80]. Variations in genes of the complement system, such as complement 2 (C2/CFB) [81], C3 [82, 83] and CFI [84] are also linked to AMD [78-81].

APOE gene variants indicate risk for AMD: *APOE*-ε2 allele elevates, while *APOE*-ε4 allele reduces the risk for developing AMD [73, 76, 85, 86]. Studies on a Chinese [85] and Hungarian population [76] show no evidence for association of APOE polymorphism with AMD. Moreover, a separate Hungarian study reports no link between the exudative and dry form of AMD and the polymorphisms in the APOE, CFI, FXIII and MERTK genes [76], but Gas6 c.834+7G.A polymorphism appears to be protective, reducing the odds of wet type AMD to half [76].

Modest effect on AMD of loci variations in CETP, LIPC, TIMP3, VEGFA, TNFRSF10A, COL10A1, COL8A1, COL8A1/FILIP1L, SLC16A8, IER3/DDR1, TGFBR1, RAD51B, ADAMTS9/MIR548A2 and B3GALTL are also reported [87-90] (**Table 1**).

Polymorphism in iron homeostasis genes is addressed in relation to risk for AMD development. GC SNP at rs4481157 and rs8177178 [91] decreases the risk of dry AMD, while GA genotype increases the risk of dry AMD and decreases it for wet AMD [91]. Chowers *et al.* demonstrates that transferrin expression is elevated in AMD patients with respect to a control group [92]. Serum transferrin level is also increased in the AMD group, but the total serum iron level is not changed [93].

Altered iron homeostasis in the retina can produce reactive oxygen species (ROS) contributing to oxidative stress. Higher concentration of iron is found in post-mortem AMD retinas (as compared to unaffected ones) in the photoreceptors, RPE and drusen [94]. Oxidative stress can induce damage in DNA and may affect DNA repair as well. This may lead to misincorporation of uracil to DNA controlled by DNA glycosylases. Polymorphism of the DNA repair genes SMUG1 and UNG is also shown to be related to AMD pathogenesis (**Figure 1** and **Table 1**) [95]. The C/C genotype polymorphism of UNG is associated with an almost three-fold risk of atrophic AMD, while the T/T genotype is a protective polymorphism. Presence of the T allele of g.4235T>C together with A allele of c.-31A>G of SMUG1, however, poses higher risk for developing severe AMD. The C/C genotype of g.4235T>C SNP and G/G genotype of c.-31A>G polymorphism have a protective effect [95]. Progressive maculopathy, similar to AMD develops in retinas of patients suffering from hereditary aceruloplasminemia [31].

Animal models with knocked-out ferroxidase ceruloplasmin (Cp) and homologous hephestin (Heph) have age-dependent iron accumulation followed by subretinal neovascularization and degeneration [96]. Revealing the mechanisms of iron homeostasis and prevention of iron overload in the retina may therefore be advantageous in the treatment of AMD.

The HFE gene is expressed in RPE cells, so patients with hereditary haemochromatosis –a condition caused by a mutation in this gene - accumulate iron locally, predisposing the patient to AMD development. Under such conditions, iron-binding drugs can be useful in the prevention of iron overload [31].

Variations in the fractalkine receptor CX3CR1 show association with progressive age-related conditions, such as atherosclerosis. There are two SNPs; V249I and T280M of this gene, with a higher allele frequency of the M280 being detected in AMD affected population versus controls. The M280 and I249 alleles can produce an abnormal CX3CR1-CX3CL1 interaction and a decreased number of receptor sites [97, 98].

No effect of the TLR4 polymorphism in association with AMD is found in Indian patients [73]. SNPs in the SERPING-1 gene encoding complement factor 1 (C1) inhibitor, such as rs1005510 and rs2511989 show association to wet AMD, exerting a modest risk and a protective effect, respectively, in a study conducted on an American (Caucasian) population [99]. In contrast, a study conducted on Age-Related Eye Disease Study (AREDS) subjects shows no association between the SNPs in SERPING-1 and AMD, nor it finds any other risk variants of AMD-related genes [100].

Vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (PEDF) are the major regulators of angiogenesis, and polymorphisms in these genes can affect angiogenesis–related diseases in the eye [101]. There are known SNPs in the VEGF gene, such as +405C/G (rs2010963), -460 T/C (rs833061), +674 C/T (rs1413711), +936C/T (rs3025039, and -2578C/A (rs699947) in association with AMD. Polymorphisms -460C/T (rs833061) and -634G>C (rs2010963) are associated with diabetic retinopathy. The C/T genotype of -460C>T increases the risk and occurrence of dry AMD, while the T/T genotype lowers it. Dry AMD also associates with the C/C genotype of -634G>C and together with the C/T genotype of -460C>T, the SNPs correlate with the appearance of AMD. The T/T-G/G and T/T-G/C genotypes are all protective for AMD, while C/T-G/G (C/C) genotype increases the risk of atrophic and exudative AMD as detected by a study performed on a Polish population [102, 103]. The VEGF +936 C/T [104] and CFH Y402H SNPs show strong correlation with wet AMD in a Chinese population [77]. SNPs in the genes VEGFA rs699947 and rs833061 and VEGFR2 rs2071559 do not affect the risk of acquiring AMD as detected by a study performed on a Spanish population. VEGFR2 rs2071559 polymorphism shows that carriers of a G/G genotype are more frequent in the subjects with AMD, but is not statistically significant after Bonferroni correction [105]. Fang *et al.* supports these findings and shows no evidence of genetic risk as a cause of the SNPs, except for a rare haplotype in VEGFR2 for neovascular AMD [106].

Prospects for future and cell therapy in AMD

To date, no effective therapy is available for treating the dry form of AMD, although antioxidants and omega-fatty acids show positive effect on prolonging the onset of the disease (AREDS study and modified AREDS). Oral supplements determined from these studies (antioxidant vitamins C and E, lutein, zeaxanthin, and zinc) can reduce the risk of progression to advanced AMD. The use of antioxidants holds a future promise provided it goes through an evidence-based trial process; such attempts are also undertaken with the use of sulphur antioxidants, sulbutiamine and acetylcysteine (NAC) [107], canolol [108], paeoniflorin [109], clusterin [110], curcumin [111] and H_2S [112, 113].

In the late stages of the dry form of AMD, preventing the RPE cell death through limiting oxidative stress-induced necrosis in these cells may hold promise through the use of necrostatins [12].

In the wet form of AMD, inhibitors of the neovascularization process (ranibizumab, bevacizumab and VEGF Trap) [114-116] are all considered impressive drugs, although the frequency of injections is a drawback or a major cause of harmful side-effects. Alternatively, injection of external PEDF [117] or stimulation of its internal production is considered a promising way of blocking neovascularization. Alternatively, Ras pathway inhibitors are considered for treating wet AMD [118]. A secreted extracellular domain of the VEGF receptor-1, sFlt-1, which is a naturally occurring protein antagonist of the VEGF formed by alternative splicing of the pre-mRNA of the full-length receptor is used successfully to achieve strong suppression of retinal or subretinal neovascularization in mice [119]. In addition, resolvins and protectins, which mediate a beneficial effect through preventing NF-κB signaling, are proposed as new targets for regulating the inflammatory responses in AMD [120].

An alternative way of preventing AMD is to enhance the phagocytic capacity by different glucocorticoid-steroid analogues (triamcinolone, dexamethasone) [34]. Potential use of specific agonists of the tyrosine kinase receptors, as well as complement- and anti-amyloid based therapies can also hold a future promise in AMD therapy.

MicroRNAs serving as therapeutic targets focus on silencing miR-23, miR-24 and miR-27 [121, 122] to achieve repression of neovascularization.

Cell therapy for AMD is based upon the use of a wide range of cells including both pluripotent stem cells and multipotent stem cells of fetal and adult origin. Restoring the damaged RPE layer *in vivo* [123], however, faces a cellular polarization problem remaining to be solved before transplantation.

Finally, few experimental drugs for treating AMD undergo testing or clinical trials: α-5-β-1 integrin antibody fragment (Protein Design Labs, Fremont, CA), integrin antagonists (Jerini, Berlin, Germany), bFGF-2 vaccine (Entremed, Rockville, MD), isotretinoin (UCLA, Los Angeles, CA) and phosphodiesterase-5 inhibitor (Pfizer, Groton, CT).

Table 1. List of genetic loci affecting risk for developing AMD and short description about their role. SNPs that have been related to AMD are shown with minor allele frequencies (MAF).

Chromosome location	Gene/locus	Most important SNPs related to AMD	MAF	Pathway relevant to AMD	Function
3p14.1	*ADAMTS9*	N.a.	N.a.	ECM	The protein encoded by the gene is responsible for cleaving proteoglycans of the extracellular matrix, such as aggrecan, versican.
19q13.32	*APOE*	rs7412 rs429358	ε2: 0.14 ε4: 0.08	Lipid metabolism	Protein-encoding gene; functions include binding, internalization and degredation of lipoproteins. ε2 allele brings forward the onset, while ε4 delays development of AMD.
10q26.13	*ARMS2/HTRA1*	rs10490924/ rs11200638	0.3	Other	Protein localized in the cytoplasma, encoded by ARMS2 gene is associated with age-related diseases. HTRA1 regulates IGF and TGFβ family signaling.
13q12.3	*B3GALTL*	N.a.	N.a.	Other	Type 2 membrane protein, an enzyme responsible for the extension of O-fucosylglycan.
6p21.33	*C2/CFB*	rs9332739 rs547154 rs4151667 rs641153	0.03 0.11 0.03 0.1	Complement	Serum glycoprotein, C2a is serine protease of the complement system; deficiencies have been associated with autoimmune conditions. Protective SNPs.
19p13.3	*C3*	rs2230199 rs1047286	0.14 0.16	Complement	Activated protein (C3a) mediates local inflammatory reponses; degranulation of mast cells, enhanced vessel permeability.
16q13	*CETP*	N.a.	N.a.	Lipid metabolism	Transfers molecules relevant to lipoprotein metabolism, may affect susceptibility to atherosclerosis.
1q31.3	*CFH*	rs1061147 rs1061170 rs800292 rs3753394	0.37 0.36 <0.01 <0.01	Complement	Regulation of complement activation via inactivation of C3b as a cofactor.
4q25	*CFI*	rs10033900 rs13117504 rs11726949	0.49 0.41 0.28	Complement	Acts together with CFH, functions as a regulatory molecule in the complement system.
6q22.1	*COL10A1*	rs1999930	0.14	ECM	Encodes type x collagen whic is synthetized in cartilage, also associated to FAK signaling.
3q12.1	*COL8A1/FILIP1L*	rs13081855 rs13095226	0.06 0.07	ECM (COL8A1) angiogenesis (FILIP1L)	Component of corneal (Descemet's membrane) and vessel endothelium, maintenance of vessel wall integrity.
3p22.2	*CX3CR1*	rs3732378 rs3732379.	0.14 <0.01	Other	Functions as a receptor for leukocytes for migration and adhesion.
13q34	*GAS6/AXLLG/AXSF*	rs8191974	0.27	Phagocytosis	Interacts with AXL receptor tyrosine kinase, MerTK amd TYRO3. Involved in cell survival and proliferation. Protective SNP in wet AMD.
6p21.33	*IER3/DDR1*	N.a.	N.a.	Apoptosis (IER3)/ ECM (DDR1)	Encoded protein has a role in cell survival; DDR1 is involved in tyrosine kinase signaling pathways.
15q21.3	*LIPC*	rs10468017	0.2	Lipid metabolism	Enzyme of HDL metabolism and lipoprotein uptake.
14q24.1	*RAD51B*	N.a.	N.a.	DNA-repair	Involved in repair mechanisms during homologous recombination.
22q13.1	*SLC16A8*	N.a.	N.a.	Other	Membrane transport protein, carries monocarboxylates.
12q13.13	*SMUG1*	rs3087404	0.38	DNA-repair	Functions include elimination of uracil from DNA
3q22.1	*TF*	rs4481157	0.47	Other	Tranports iron to proliferating cells.
9q22.33	*TGFBR1*	N.a.	N.a.	Other	Signal transduction of TGFβ from cell surface to the cytoplasm.
22q12.3	*TIMP/SYN3*	rs9621532	N.a.	ECM	Has a role in ECM remodeling through inhibition of metalloproteinases.
8p21.3	*TNFRSF10A*	rs13278062	0.35	Apoptosis	Receptor; transducer of death signaling.
12q24.11	*UNG*	rs2337395	0.39	DNA-repair	Removes uracil from ssDNA.
6p21.1	*VEGFA*	rs833061 rs2010963 rs3025039	0.36 0.33 0.14	Angiogenesis	Main functions include mediation of angio- and vasculogenesis; vessel permeabilization and endothelial cell growth

Conclusion

AMD is a complex, multifactorial disease which involves many pathways ranging from cell death, phagocytosis, inflammation and angiogenesis. Favoring or stimulating one pathway may have countercoup effect on another related pathway, therefore, much caution should be used in deciding which short or long term therapy is the best for treating the different stages of the disease. Population differences in gene polymorphism pose a great challenge and deserve higher attention in making personalized medicine work in the future.

Conflict of interests

The authors declare no conflict of interest.

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