Age Related Macular Degeneration: a Complex Pathology

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Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness worldwide. At present, 8-7% of the worldwide population has AMD and by 2020 around 196 million expected to have AMD which is further increasing to 288 million in 2040 [1]. There are two types of AMD, the “dry” and “wet” forms. Wet AMD includes classic and occult choroidal neovascularization (CNV) and polypoidal choroidal vasculopathy (PCV). The chronic form “Dry AMD” typically develops first and is characterized by the deposition of acellular, polymorphous debris between the retinal pigment epithelium (RPE) and Bruch’s membrane (BM) called “drusen”. The excessive “drusen” deposition may lead to damage of the RPE, degeneration of collagen or elastin in BM, the outer retina and the choroid vasculature. This may lead to wet form of AMD where abnormal vessels grow within the sub-RPE space or grow out into the retina by rupturing the RPE, this form occurs at the late stage. Therefore, dry AMD is considered a precursor for the wet AMD. Caucasian AMD patients predominantly exhibit late stage geographic atrophy of dry “AMD” while Asian AMD patients frequently have CNV or PCV forms of “wet AMD” with few or no drusen. Wet AMD represents only 10 to 15% of the overall prevalence of AMD but is responsible for more than 80% of cases of legal blindness [2]. Overall, AMD is a progressive, polygenic and multi factorial disease with a genetic contribution. The etiology and pathogenesis of AMD are not well understood and remain a major challenge to understand. This review discusses recent advancement in genetics and genomics, and the molecular pathways involved in AMD pathogenesis.

AMD Genetics

Over the years, the involvement of genetics in the development of AMD has been very well studied and established. Genome-Wide Association Studies (GWAS) have revealed more than 30 risk loci (e.g. 1q25-31, 9p13, 9p24, 10q26, 15q21, and 17q25) and have implicated several candidate genes—CFH, C3, C2-CFB, CFI, HTRA1/ ARMS2, CETP, TIMP3, LIPC, VEGFA, COL10A1, TNFRSF10A, and APOE with AMD [5-7]. Among them, chromosome loci 1q32 and 10q26 are major disease regions associated with the susceptibility of AMD [5,8-17] including PCV [18-20]. The genetic variants in complement factor H at chromosome loci 1q32 and additional complement-related genes firmly established a link between the complement cascade and AMD biology, which have been implicated in mediating drusen formation [21]. CFH Y402H is a major AMD susceptibility variant in Caucasians and has been shown that heterozygote alleles conferred a 4.6-fold where-as homozygote alleles have a 7.4-fold increased risk, as compared with the homozygote non-risk genotype [14]. On the other hand, chromosome loci 10q26 is more complex due to the strong linkage disequilibrium (LD) across this region comprising of three genes: pleckstrin homology domain containing family A member 1 (PLEKHA1), age-related maculopathy susceptibility 2 (ARMS2) and high-temperature requirement A serine peptidase 1 (HTRA1) [8,14-17]. Because of strong LD, statistical genetic analysis alone is incapable of distinguishing the effect of an individual gene in this locus and has yielded widely conflicting results [15,17,22-28]. As a result, the functional involvement of HTRA1, ARMS2 or PLEKHA1 in AMD remains uncertain, despite strong genetic evidence. So far, rs10490924, indel polymorphisms of ARMS2, and rs11200638 of HTRA1 promoter region are most significantly AMD associated haplotypes at this locus [29]. The HTRA1 gene encodes an evolutionarily conserved multifunctional serine protease that is ubiquitously expressed in mammalian tissues but ARMS2 is only expressed in certain primates with unknown function. The subcellular localization of ARMS2 is controversial and studies suggesting that it present in mitochondria, extracellular matrix, or as a non coding RNA [16,17,27]. An increased level of HTRA1 is suggested to play a potential role in the pathogenesis of AMD [15,23-25]. Therefore, we studied the functional involvement of HTRA1 by transgenically expressing human HTRA1 in mouse RPE and showed that increased HTRA1 induced characteristic features of PCV, including branching networks of choroidal vessels (BVM) and polypoidal lesions (polyps). Ultrastructural study revealed degeneration of both the elastic lamina and tunica media of choroidal vessels, as well as the degradation of the elastic lamina of Bruch’s membrane in hHTRA1- mice. Another group also reported the degradation of EL in BM when over expressing mouse HTRA1 in RPE [30]. The phenotypes of hHTRA1- mouse we generated share remarkable similarities to the
well established clinical features of human PCV (e.g. BVN, polyps, late geographic hyper fluorescence, pigment epithelium detachment, and hyper fluorescent plaque) [31-33]. The hHTRA1” mouse is the first PCV model and no other animal models exist with these features. The strengths and limitations of available AMD animal models are comprehensively reviewed by Pennesi ME [34]. HTRA1 is clearly important in maintaining the vasculature by inhibiting the signaling of TGFβ family members [35,36]. Loss-of-function mutations in HTRA1 were linked to familial ischemic cerebral small-vessel disease [37,38]. In the eye, knockout of HTRA1 leads to reduced blood vessels in mouse retina [39]. However, several studies demonstrated that AMD associated variants at 10q26 locus are not correlated with the expression level of HTRA1 in AMD-affected eyes [26, 27,40-42]. Recently, it is shown that AMD linked synonymous SNPs within exon 1 of HTRA1 makes it conformationally defective. This conformationally defective HTRA1 is more susceptible to proteolysis and has a reduced binding capacity to IGF-1, which supports cellular division and growth therefore may compromise photoreceptors and choriocapillaris survival [43]. Currently, all three possibilities (up-regulation, down-regulation or no change) in HTRA1 levels with AMD-associated variants are being investigated. HTRA1 is the leading candidate for the 10q26 genetic risk. However, more studies are necessary to establish a firm link.

**Inflammation and AMD**

In recent years, numerous clinical-genetic studies documented the crucial role of inflammation and immune-mediated processes (e.g. complement activation) in the pathogenesis of AMD. The ectopic levels of complement components C3a and C5a, C5 and C5b-9 terminal complement complex [44-46], complement factor H (CFH) [13, 47], membrane cofactor protein (MCP) [48], and C-reactive protein (CRP) [49] are observed in AMD patients and clearly indicating that complement activation is crucial in AMD pathogenesis. In fact, the hallmark of AMD, “drusen”, contains large amount of components involved in the complement pathway [44,50-57]. In addition, it’s been shown that Membrane Attacking Complex (MAC) formation is increased in the photoreceptors that may trigger the apoptotic processes inducing retinal degeneration [50-53,57-58]. The deposition of esterified/unesterified cholesterol (7kCh) and glycation/lipoxidation end products (AGEs/ALEs) has been identified 58]. The deposition of esterified/unesterified cholesterol (7kCh) and the apoptotic processes inducing retinal degeneration [50-53,57-58]. In addition, it’s been shown that Membrane Attacking Complex (MAC) formation is increased in the photoreceptors that may trigger the apoptotic processes inducing retinal degeneration [50-53,57-58]. The deposition of esterified/unesterified cholesterol (7kCh) and glycation/lipoxidation end products (AGEs/ALEs) has been identified 58]. The deposition of esterified/unesterified cholesterol (7kCh) and the apoptotic processes inducing retinal degeneration [50-53,57-58]. In addition, it’s been shown that Membrane Attacking Complex (MAC) formation is increased in the photoreceptors that may trigger the apoptotic processes inducing retinal degeneration [50-53,57-58]. The deposition of esterified/unesterified cholesterol (7kCh) and glycation/lipoxidation end products (AGEs/ALEs) has been identified 58]. The deposition of esterified/unesterified cholesterol (7kCh) and the apoptotic processes inducing retinal degeneration [50-53,57-58]. In addition, it’s been shown that Membrane Attacking Complex (MAC) formation is increased in the photoreceptors that may trigger the apoptotic processes inducing retinal degeneration [50-53,57-58]. The deposition of esterified/unesterified cholesterol (7kCh) and glycation/lipoxidation end products (AGEs/ALEs) has been identified 58]. The deposition of esterified/unesterified cholesterol (7kCh) and the apoptotic processes inducing retinal degeneration [50-53,57-58]. In addition, it’s been shown that Membrane Attacking Complex (MAC) formation is increased in the photoreceptors that may trigger the apoptotic processes inducing retinal degeneration [50-53,57-58]. The deposition of esterified/unesterified cholesterol (7kCh) and glycation/lipoxidation end products (AGEs/ALEs) has been identified 58].

**Autophagy and AMD**

Recently, autophagy has caught the attention of AMD researchers. Autophagy plays a critical role in removing misfolded or aggregated proteins, clearing damaged organelles, such as mitochondria, endoplasmic reticulum and peroxisomes [72]. It also eliminates intracellular pathogens to keep post-mitotic cells healthy and functional [73]. The autophagy processes are highly active in the RPE layer because RPE cells are subject to oxidative stress, high oxygen tension, lifelong light illumination, and are involved in daily phagocytosis of photoreceptor outer segments. As we read in the previous section, the physiological balance between various interlinked pathways (e.g vascular growth factor pathways, lipid pathways and oxidative stress pathways) has been perturbed in AMD which may impair the autophagy process. Some exosome and autophagy markers have been detected in drusen [74]. Inflammation and local hypoxia are the hallmarks of autophagy and are present in the aging choriocapillaris, RPE cells, and neural retina [75]. It is well known that oxidative stress leads to mitochondrial DNA damage, increases ROS generation and reduces the metabolic capacity. The increased mitochondrial stress and dysfunctional autophagy in the RPE cells of AMD patients also support the involvement of autophagy in the pathology of AMD [76,77]. The association between the variant of CST3, (encoding cystatin C), an inhibitor of lysosomal cysteine proteases, and AMD has been established. Also, increased serum levels of cystatin C found in AMD patients are correlated with the risk of development of advanced AMD [78,79]. In addition, in-vitro studies on lysosome function on RPE cells also provided insights on the disruption of lysosomal functions and possible role of lysosomes in the development of AMD [79-82]. Vascular dysfunctions also result in oxidative stress, that is, overproduction of ROS, which induces further changes in the retinal and choroidal vasculature. Such changes can also be evoked by hypoxia, since it stimulates synthesis and release of hypoxia-inducible factor-1 (HIF-1) and vascular endothelial growth factor (VEGF) that contribute to neoangiogenesis (NV). Recent reports suggest that dysfunctional autophagy activates inflammasomes probably through the dysregulation of mitochondrial homeostasis [83,84]. To date, there is no consensus as to whether autophagy inhibitors or activators would be beneficial in AMD therapy.

**Treatment**

There is no cure for AMD. Nevertheless, AMD treatment may prevent severe vision loss or slow the progression of the disease considerably, for example, anti-angiogenic drugs (anti-VEGF) and photodynamic therapy with verteporfin (PDT-V) are very...
effective for wet AMD. However, the anti-VEGF therapy is not very effective in treating PCV compared with classic CNV (or type 2 neovascularization). Monoclonal antibodies Ranibizumab (Lucentis) and Bevacizumab (Avastin) are used to treat "wet form" of AMD by targeting all isoforms of VEGF-A. Currently, bevacizumab is the most widely used anti-VEGF agent throughout the world due to its significantly lower cost and similar efficacy compare to Lucentis. Another new promising drug is Aflibercept (known as VEGF-Trap) is a human recombinant fusion protein which consists of extracellular domains of VEGF receptor 1 and 2 (VEGFR-1 and -2) fused with the Fc portion of IgG1. It binds to VEGF-A, VEGF-B, and placental growth factor (Pigf). It has a higher affinity for VEGF compared to other anti-VEGFs, including bevacizumab and ranibizumab. For more detail we recommend reading the recent review from Hanout et al. 2013 [85]. Indeed, currently very little is available to prevent the progression to more serious stages for “dry” AMD’s patients. Colloquially, quitting smoking and a healthy diet of dark green leafy vegetables and fruits supplemented by zinc and anti-oxidant vitamins (Vitamins E, C, and beta carotene) are recommended.

**Conclusion**

AMD is a genetically well-characterized disease with a high complexity. Despite several important findings in the last decade, we still do not have a clear picture of biological pathways that are actual culprits for AMD. Based on recent findings, the dysfunction and/or degenerative damages photoreceptors, RPE and BM of the macula, are initiated by “attacks” from drusen, aging, genetic and environmental risk factors. These primary factors create a para-inflammatory environment which may provoke the infiltration of macrophages, lymphocytes, neutrophils and various cytokines to the degenerated tissue sites in AMD patients and cause further damage and lead to “wet AMD” (Figure1). We have witnessed remarkable progress in identifying genetic risk factors for AMD. However, investigations of the underlying disease mechanisms by causal alleles are needed. It is also important to elucidate factors and/or signaling pathways that regulate inflammation, oxidative stress, and autophagy of this disease in order to develop effective preventive and treatment therapies.

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**References**


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