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Retinal Ultrastructure of Murine Models of Dry Age-related Macular Degeneration (AMD)

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Abstract

Age-related macular degeneration (AMD) is the most prevalent form of irreversible blindness worldwide in the elderly population. The pathology of dry AMD consists of degeneration of photoreceptors and the RPE, lipofuscin (A2E) accumulation, and drusen formation. Mice have been widely used for generating models that simulate human AMD features for investigating the pathogenesis, treatment and prevention of the disease. Although the mouse has no macula, focal atrophy of photorecptors and RPE, lipofuscin accumulation, and increased A2E can develop in aged mouse eyes. However, drusen are rarely seen in mice because of their simpler Bruch's membrane and different process of lipofuscin extrusion compared with humans. Thus, analyzing basal deposits at the ultrastructural level and understanding the ultrastructural pathologic differences between various mouse AMD models are critical to comprehending the significance of research findings and response to possible therapeutic options for dry AMD.

Based on the multifactorial pathogenesis of AMD, murine dry AMD models can be classified into three groups. First, genetically engineered mice that target genes related to juvenile macular dystrophies are the most common models, and they include *abcr*$^{-/-}$ (Stargardt disease), transgenic *ELOVL4* (Stargardt-3 dominant inheritary disease), *Efemp1*$^{R345W/R345W}$ (Doyne honeycomb retinal dystrophy), and *Timp3*$^{S156C/S156C}$ (Sorsby fundus dystrophy) mice. Other murine models target genes relevant to AMD, including inflammatory genes such as *Cfh*$^{-/-}$, *Ccl2*$^{-/-}$, *Ccr2*$^{-/-}$, *Cx3cr1*$^{-/-}$, and *Ccl2*$^{-/-}$/*cx3cr1*$^{-/-}$, oxidative stress associated genes such as *Sod1*$^{-/-}$ and *Sod2* knockdown, metabolic pathway genes such as *neprilysin* $^{-/-}$ (amyloid β), transgenic *mcd/mcd* (cathepsin D), *Cp*$^{-/-}$/*Heph*$^{-/Y}$ (ferroxidase ceruloplasmin/hepaestin, iron metabolism), and transgenic *ApoE4* on high fat and high cholesterol diet (lipid metabolism). Second, mice have also been immunologically manipulated by immunization with carboxyethylpyrrole (CEP), an oxidative fragment of DHA found in drusen, and found to present with dry AMD features. Third, natural mouse strains such as *arrd2/arrd2 (Mdm* gene mutation) and the senescence accelerated mice (SAM) spontaneously develop features of dry AMD like photoreceptor atrophy and thickening of Bruch's membrane.

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All the aforementioned models develop retinal lesions with various features that simulate dry AMD lesions: focal photoreceptor degeneration, abnormal RPE with increased lipofuscin, basal infolding, decreased melanosomes and degeneration. However, Bruch's membrane changes are less common. Most mice develop retinal lesions at an older age (6–24 months, depending on the models), while the *Ccl2*$^{-/-}$*/cx3cr1*$^{-/-}$ mice develop lesions by 4–6 weeks. Although murine models present various degrees of retinal and/or RPE degeneration, classical drusen is extremely rare. Using electron microscopy, small drusenoid deposits are found between RPE and Bruch's membrane in a few models including *Efemp1* $^{R345W/R345W}$, *Ccl2*$^{-/-}$*/cx3cr1*$^{-/-}$, *neprilysin* $^{-/-}$, transgenic *mcd/mcd*, and *ApoE4* transgenic mice on a high fat diet. High A2E levels are measured in the retinas of *abcr*$^{-/-}$, transgenic *ELOVL4*, and *Ccl2*$^{-/-}$*/cx3cr1*$^{-/-}$ mice. In summary, murine models provide useful tools for studying AMD pathogenesis and evaluating novel therapies for this disease. This review compares the major dry AMD murine models and discusses retinal pathology at the ultrastructural level.

Keywords

Age-related macular degeneration; AMD; dry; mouse model; retina; ultrastructure; pathology

1. Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible visual impairment and blindness in the elderly population (World Health Organization 2009; Friedman et al., 2004). The development of AMD is a slow progressive process that occurs with aging and mainly affects people over age 60 (Gehrs et al., 2006). People with AMD experience a loss of high-resolution vision and perceive distortions and loss of images in the center of their visual field, making driving and reading very difficult. Many environmental risk factors, especially smoking, have been associated with an increased risk of developing AMD. The genetics of macular degenerative diseases, or maculopathies, is complicated, and genes associated with AMD are being identified. Mendelian inherited macular degeneration (MD) that often develops in the early ages, can be either autosomal dominant or recessive. Complex MD can result from chloroquine and clofazimine drug toxicities (Wolfensberger, 1998). AMD still has an unknown etiology, though oxidative stress to the retinal pigment epithelium (RPE) cells along with immune dysregulation are leading theories of AMD pathogenesis (Ding et al., 2009).

1.1. Epidemiology of AMD

AMD accounts for more than 50% of blindness cases in Caucasian Americans, the group most affected by early and late AMD in the United States (Congdon et al., 2004). The prevalence of the disease increases with age, and by age 75 early AMD affects 30% of the population. An estimated 1.75 million Americans were affected by AMD in 2000, and the number is expected to rise to 2.95 million in 2020 (Friedman et al., 2004). Worldwide, 30–50 million people are affected by AMD, and limited population studies in Asia demonstrate a prevalence of AMD that is lower than the United States (Wong et al., 2006; Gehrs et al., 2006).

1.2. Pathology of AMD

Aging induces retinal changes in RPE cell size and shape and results in the accumulation of lysosomal residual bodies called lipofuscin granules. With age, photoreceptor cells (rods much more than cones, peripheral more than central retina) and ganglion cells, decrease in number and density; RPE cells also show parallel changes and progressive cell loss (Gao and Hollyfield, 1992; Panda-Jonas et al., 1995). Recently, it has been noted that abnormalities in the distal cone axon occurs with aging and increases susceptibility to AMD (Shelley et al., 2009). There is an age-related increase in thickness in Bruch's membrane and the internal limiting membrane (Sarks, 1976; Ding et al., 2009). Cholesterol and calcium have been found to accumulate in

Bruch's membrane throughout adulthood (Sarks, 1976; Curcio et al., 2001; Pauleikhoff et al., 1990). These age-associated changes set the stage for AMD development in individuals with a genetic predisposition and exposure to environmental risk factors.

AMD was first described as "symmetric central chorioretinal disease occurring in senile persons" (Hutchinson and Tay, 1875). The signs of degenerative retinal change in AMD have been described in histopathologic studies of AMD eyes (Green, 1993; Sarks, 1976). The classic pathology of early AMD is multiple small or intermediate drusen (drusen size of ≥ 63 microns but < 125 microns) in the macular area (Coleman et al., 2008). The classic pathology of advanced AMD is described as having two forms: the 'geographic atrophic (dry)' and 'neovascular/exudative (wet)' forms. The 'dry' form of AMD, non-neovascular AMD, including intermediate AMD with many intermediate or large drusen (drusen size of ≥ 125 microns) and RPE alterations and advanced AMD with geographic atrophy, consists of many perturbations in the RPE, Bruch's membrane, photoreceptors, and choriocapillaris, leading to photoreceptor degeneration and atrophy. New blood vessel formation from the choroid, known as choroidal neovascularization (CNV), is the hallmark of 'wet' AMD. This form, often accompanied by hemorrhages, serous exudates, and edema in the neuroretina, is called exudative or neovascular AMD.

1.2.1. Early stage AMD—In the early stages of AMD, pathologic findings are found mostly in the RPE and Bruch's membrane. The RPE progressively accumulates lipofuscin, leaving partially digested photoreceptors in the cytoplasm. The number and density of RPE cells in the macula also decrease. Early in the disease process, basal deposits, a type of waste between the RPE and Bruch's membrane, can be identified by electron microscopy. There are two types of basal deposits: basal laminar deposits (BlamD) and basal linear deposits (BlinD). BlamD are a granular electron dense material with wide-spaced collagen between the plasma membrane and basal lamina of the RPE (Green, 1993). BlinD are lipid-rich electron dense substances with granules and vesicles external to the RPE basement membrane in the inner collagenous zone. BlinD are a more specific marker of AMD progression to late disease, and BlamD are a more reliable indicator of RPE atrophy and photoreceptor degeneration (van der Schaft et al., 1992; Curcio and Millican, 1999; Sarks, 1976). Thick BlamD closely correlate with the presence of AMD (Spraul and Grossniklaus, 1997).

Other features of early AMD include thickening and loss of the normal architecture of Bruch's membrane and drusen formation. Drusen are subretinal extracellular deposits composed of glycoproteins and lipids in the inner collagenous zone of Bruch's membrane (Sarks, 1976). Drusen can be described as soft or hard. Large soft drusen are thought to arise from BlinD, which are 24-fold more common in eyes with AMD than age-matched controls (Curcio and Millican, 1999). Hard, or nodular, drusen are smooth surfaced, dome-shaped structures between the RPE and Bruch's membrane that are clinically common. At the ultrastructural level, drusen are a fine, granular, amorphous material the same electrodensity of the RPE basement membrane with vesicles, tubular structures, curly membranes and abnormal collagen (Sarks, 1976).

Studies analyzing the composition of drusen have shown that drusen contain acute phase proteins (C-reactive protein, vitronectin, α-antichymotrypsin, amyloid P component, and fibrinogen), complement pathway components (C3, C5 and C5b-9 complex) and inhibitors (clusterin), apolipoproteins B and E, mucopolysaccarides, lipids, mannose, and sialic acid (Farkas et al., 1971; Mullins et al., 2000). Lower levels of the endoplasmic reticulum chaperone protein ERp29 are found in neurodegenerative diseases, in the AMD maculae, and the aging retina. Low ERp29 levels impacts chaperone function and lead to the accumulation of misfolded proteins which may aggregate and interfere with intracellular movement, allowing for the aggregation of incompletely folded compounds such as lipofuscin and drusen to cause

RPE damage (Li et al., 2004; Ethen et al., 2006). Loss of mitochondria is also reported in AMD retina (Ding et. al, 2009). Lipofuscin accumulation in RPE cells, basal deposits, drusen, and thickening of Bruch's membrane are all seen in early AMD and may continue to be present in late stages of the disease. Additionally, patients with drusen and CNV have higher circulating levels of anti-retinal antibodies compared to controls (Patel et al., 2005), and this profile does not significantly change with progression from early AMD to advanced stage AMD (Cherepanoff et al., 2006).

1.2.2. Advanced stage AMD—In the advanced stage of dry AMD called geographic atrophy, the RPE has well-demarcated atrophy and the neurosensory retina is severely affected (Coleman et al., 2008). Areas of geographic atrophy may have a single layer of macrophages between the RPE basement membrane and the inner collagenous layer of Bruch's membrane with residual pigmented material. The retina adjacent to the areas of geographic atrophy (junctional zones) is hyperpigmented and has hypertrophied RPE cells with occasional multinucleated giant cells (Dastgheib and Green, 1994). RPE atrophy is often accompanied by degeneration and loss of the outer layers of the retina (photoreceptors, outer nuclear layer, and external limiting membrane). Cone degeneration and red-green cone hypertrophy are also found. Photoreceptor cell loss is most prominent in the parafovea and can progress to total photoreceptor loss and disciform degeneration (Curcio et al., 1996; Sarks, 1976; Shelley et al., 2009). Additionally, sclerosis of the choriocapillaris is found without breaks in Bruch's membrane (Sarks, 1976).

When Bruch's membrane becomes calcified and focal breaks occur, new blood vessels from the choroid form in the space under the RPE, and this CNV is the hallmark of exudative/neovascular, or 'wet' AMD (Spraul and Grossniklaus, 1997). IgG antibodies against retinal antigens are present in a complex and specific pattern in wet AMD eyes, which differs from dry AMD eyes and healthy controls (Joachim et al., 2007). Neovascular AMD often causes sudden loss of vision and presents with serous or hemorrhagic detachment of either the retinal pigment epithelium or sensory retina. Subretinal fibrous tissue, or minimal subretinal fibrosis, and widespread RPE atrophy are also often noted (Coleman et al., 2008). Hemorrhages, exudates, and neovascular fibrous tissue may replace normal retinal architecture and result in photoreceptor atrophy (Green, 1999; Kim et al., 2002). The presence of an inflammatory cell component at the site of breaks in Bruch's membrane strongly suggests that the immune system is intricately involved in disease progression (van der Schaft et al., 1992; Spraul and Grossniklaus, 1997; Anderson et al., 2002; Chan et al., 2008; Patel and Chan, 2008).

In this review, we focus on murine models for dry AMD, including intermediate stage and geographic atrophy. We will emphasize the histopathology, particularly the retinal ultrastructure, and molecular pathologic findings in the presented mouse models.

2. The use of murine models for AMD

Since 1924 when the first inherited form of retinal degeneration was identified, the mouse has been the main model organism for the study of diseases involving retinal degeneration (Keeler, 1924; Hafezi et al., 2000). Mouse strains with spontaneously arising retinal degeneration (the Rd mice) have been described, and genes causing retinal degeneration have been identified, characterized, and manipulated at the molecular level (Hafezi et al., 2000). Mouse models for retinitis pigmentosa (RP), cone-rod dystrophies (CORD), and other eye diseases have led to a wealth of understanding about the molecular pathogenesis of their responsible diseases and have provided model systems in which therapeutic options have been tested (Chang et al., 2005). AMD, unlike retinal degenerative diseases that affect peripheral vision and the peripheral retina, preferentially affects central vision and the macula. It is difficult to model this disease in the mouse because the mouse lacks a macula. However, certain clinical,

pathological, physiological and biochemical features of AMD, such as focal deep retinal lesions, progressive A2E accumulation, abnormal ERGs, and RPE and photoreceptor degeneration, can be demonstrated in retinal lesions in mice (Rakoczy et al., 2006)

AMD is difficult to study because of its late onset, complex genetics, and the influence of environmental factors. Therefore, it can be very useful to apply models that demonstrate pathologic findings of the disease with an earlier presentation and Mendelian inheritance (Zack et al., 1999). These models help reveal the biochemical and genetic perturbations that occur in the disease state and allow scientists and clinicians to test therapeutic options and get answers to questions that would be more difficult and expensive to ask in clinical trials. A success story is the analysis of a rare juvenile form of glaucoma that led to the discovery of a gene that was found to be responsible for some types of primary open angle glaucoma (Stone et al., 1997). Groups that have studied Stargardt disease and Best disease, early forms of macular degeneration with Mendelian inheritance patterns, have analyzed the role of the perturbed genes and proteins at the molecular level, and these models will be discussed in more detail (Weng et al., 1999). Modeling macular degeneration in mice helps us readily learn aspects of AMD that may be difficult to tease out in animals with a macula. Aged primates may develop AMD, but they only exhibit symptoms in their second decade of life. The slow time course of the disease, exorbitant cost, and the ethical and technical issues in making transgenic primates make this model system less useful (Marmorstein and Marmorstein, 2007). The power to genetically modify and manipulate the structure of the mouse retina will elucidate the biological importance of the different cellular components of the retina and immune system and can bring us closer to understanding the etiology of AMD.

2.1. Differences between the human and mouse retina

The main difference between the human and mouse retina is that the mouse has no maculae. The photoreceptor cell populations also differ. Because mice are derived from a nocturnal species, the mouse retina is composed of mainly rod photoreceptor cells (97%), and cones comprise the remaining 3% of photoreceptors (Carter-Dawson, 1979). The mouse also has only two types of cones: S-cones (blue light) and M-cones (green light), whereas humans have three types of cones: S-cones, M-cones, and L-cones (red light) (Szel et al., 1992). The mouse retina has a distribution of 3.1% horizontal cells, 41% bipolar cells, 16% Müller cells, and 39% amacrine cells. Primates have 9% horizontal cells, 40% bipolar cells, 22% Müller cells, and 28% amacrine cells (Jeon et al., 1998). The average cone density in the mouse retina is the same as the primate retina at 3–4 mm eccentricity. Evolutionarily, cones evolved first, and it is possible that a fixed number of cones set the framework for retinal organization (Wikler and Rakic, 1990). Therefore, the mouse retina is most structurally similar to the human peripheral retina, making it a useful model for retinal degenerative diseases (Figure 1).

In the aged human eye, residual body phagosomes often pass through the cytoplasm toward Bruch's membrane, and lysosomal bodies can be seen in the basal portion of the RPE cytoplasm or near the basement membrane (Hogan and Alvarado, 1967; Mishima and Hasebe, 1978). Drusen formation in Bruch's membrane has been hypothesized to come from RPE phagosomes via the basal pathway of secretion into Bruch's membrane (Mishima and Hasebe, 1978). However, in mice, the accumulation of lysosomal bodies occurs apically in the RPE cytoplasm, causing dilation of RPE microvilli over time, and lysosomal bodies are extruded into the subretinal spaces to preserve RPE function (Mishima and Kondo, 1981). That is, mice do not have a tendency to accumulate lysosomes basally or secrete residual bodies into Bruch's membrane like humans (Mishima and Kondo, 1981). For this reason, drusen and residual bodies in Bruch's membrane are rarely seen in aged mice or transgenic mouse models.

Bruch's membrane has a simple structure in mice, consisting of the RPE basement membrane, intermediate connective tissue collagen fibers, and the choriocapillaris basement membrane.

The total thickness is about 0.4 μm, increasing to 1.1 μm in 2 year-old mice. Age-related changes in mice are less remarkable than in humans and include thickening, hyalinization, and patchy basophilia. The number of collagen fibrils and amorphous material increase little with age (Mishima and Hasebe, 1978). Extended and enlarged basal cytoplasmic infoldings are found in mice over 12 months of age, and large amounts of filamentous fibrous material with amorphous deposits can be seen between basal infoldings in the mouse RPE. However, striations of banded material and electron-dense clumps in the basal infoldings of human RPE is not seen (Mishima and Hasebe, 1978). Aged mice have parallel undulating filaments in the RPE, large accumulations of lysosomal dense bodies (similar to what is seen in humans with only a difference in cytoplasmic location), and enlarged basal infoldings (Mishima and Hasebe, 1978). A great deal can be learned about the pathogenesis of macular degeneration from studying models of retinal degeneration and transgenic mouse models that produce lesions similar to those found in patients with AMD. The power to genetically modify and manipulate the structure of the mouse retina will elucidate the importance of cone versus rod biology and the etiology of AMD.

2.2. Pathophysiologic findings of murine AMD models

Despite the fact that the mouse does not have a macula, AMD mouse models are used to recapitulate the pathology and genetics of AMD. In mouse models, the pathologic hallmarks of AMD remain relatively the same: lipofuscin and basal deposit accumulation, soft drusen development, RPE and photoreceptor atrophy, and CNV. Many models show one or more of the features of AMD, and few show late-stage ‘dry’ AMD progression to ‘wet’ AMD (Rakoczy et al., 2006). Aged mice have been shown to have an accumulation of lysosomal dense bodies in the apical portion of the cytoplasm, vacuolization of the cytoplasm, and slightly extended basal infolding. There is also more lipofuscin and its byproduct A2E in the aged mouse retina (Mishima and Kondo, 1981). The production of lipofuscin and A2E is increased in the *abcr*$^{-/-}$, *ELOVL4*-mutant, *Efemp1*$^{R345W/R345W}$, *Ccr2*$^{-/-}$, *sod1*$^{-/-}$, and *Neprilysin*$^{-/-}$ mouse models (Imamura et al., 2006; Iwata et al., 2001; Vasireddy et al., 2009; Weng et al., 1999; Marmorstein et al., 2007; Ambati et al., 2003). Mouse models are examined for the presence of drusen, which has been shown to confer risk for AMD. The first sign of drusen is the development of BlinD, which has been seen in AMD patients but is rarely encountered in mice (e.g. *Neprilysin*$^{-/-}$ and *mcd/mcd* mice). There are basal deposits in the *Timp3*$^{S156C/S156C}$, *mcd/mcd*, ApoE4 TR, APO B100, APO*E3-Leiden, *Ccl2*$^{-/-}$, *Ccr2*$^{-/-}$, *Ccl2*$^{-/-}$/*Cx3cr1*$^{-/-}$, *Efemp1*$^{R345W/R345W}$, *Sod2* knockdown, *neprilysin*$^{-/-}$, *mcd/mcd*, CEP-immunized, and SAMP8 among other mouse models (Iwata et al., 2001; Kliffen et al., 2000; Heckenlively et al., 1995; Fogarasi et al., 2008; Zhang et al., 2002; Espinosa-Heidmann et al., 2004; Chan et al., 2008; Weber et al., 2002; Rakoczy et al., 2002; Marmorstein et al., 2007; Sandbach et al., 2001; Justilien et al., 2007; Hollyfield et al., 2008; Majji et al., 2000). Photoreceptor loss or derangement is seen in the *abcr*$^{-/-}$, *ELOVL4*-mutant, *cfh*$^{-/-}$, *Ccl2*$^{-/-}$, *Ccr2*$^{-/-}$, *Ccl2*$^{-/-}$/*Cx3cr1*$^{-/-}$, Sod2 knockdown, mcd/mcd, Cp$^{-/-}$/Heph$^{-/Y}$, ApoE4 TR, arrd2/arrd2, Mfrp^{rd6}, *Nr2e3*^{rd7}, and *cpfl3* among other mouse models (Vasireddy et al., 2006; Weng et al., 1999; Ambati et al., 2003; Heckenlively et al., 1995; Coffey et al., 2007; Chan et al., 2008; Justilien et al., 2007; Malek et al., 2005; Chang et al., 2008; Kameya et al., 2002; Akhmedov et al., 2000; Chang et al., 2006). The *Ccr2*$^{-/-}$, *Ccl2*$^{-/-}$/*Cx3cr1*$^{-/-}$, ApoE(−), and APO*E3-Leiden mouse models, demonstrate the presence of dry and wet AMD features with RPE degeneration, photoreceptor loss, and CNV (Chan et al., 2008; Ambati et al., 2003; Dithmar et al., 2000; Kliffen et al., 2000). Animal models of CNV are reviewed by Grossniklaus and colleagues (in this issue). The detailed pathological findings of murine dry AMD models will be discussed in detail in this review.

3 Murine models of dry AMD

3.1. Genetically engineered mice

The majority of dry AMD murine models have been genetically engineered to disrupt genes that are postulated to have a role in AMD pathogenesis (Rakoczy et al., 2006). A summary of the mouse models, their relevant genetic modifications, and human disease associations is included (Table 1). Mouse models that were created by genetic engineering will be described with their relevance to dry AMD.

3.1.1. Genes that lead to macular or retinal degeneration—The most well established murine models of dry AMD target genes previously identified to have a key role in the pathogenesis of AMD with predictable Mendelian inheritance. These diseases include Stargardt disease, Stargardt-3 dominant disease, and Malattia leventinese or Doyne's honeycomb retinal dystrophy. These models are especially popular because the mutated genes are known to cause juvenile macular dystrophy with the phenotype of RPE and photoreceptor atrophy in the macula.

3.1.1.1. abcr$^{-/-}$ (Stargardt disease) murine model: Mutations in the human *ABCR* gene for Rim protein (RmP), a glycoprotein in outer segment disc rims in the ATP-binding cassete (ABC) transporter family, result in the phenotype known as Stargardt's disease (STGD), a recessive form of macular degeneration that presents in childhood with progressive central visual loss and RPE atrophy overlying the macula (Stargardt, 1909; Allikmets et al., 1997). Heterozygous mutations in *ABCR* have been shown to be responsible for AMD, and *abcr*$^{+/-}$ mice have been shown to have delayed dark adaptation, a clinical feature of AMD and STGD (Mata et al., 2001). Ultrastructural examination of the RPE in *abcr*$^{-/-}$ mice at 44 weeks of age reveals an apical accumulation of electron-dense bodies, likely melanosome or melanosome-phagosome fusion particles (Feeney-Burns and Eldred, 1983), and thickening and disorganization of the basal RPE underlying Bruch's membrane that is more prominent in the *abcr*$^{-/-}$ mouse compared to the *abcr*$^{+/-}$ mouse (Mata et al., 2001). The baseline number of lipofuscin granules is increased in the *abcr*$^{-/-}$ mouse compared to WT mice, and accumulation of lipofuscin granules also occurs at a faster rate with age. As expected, A2E levels were elevated in both the *abcr*$^{+/-}$ and *abcr*$^{-/-}$ mouse models compared to WT mice (Mata et al., 2001; Weng et al., 1999). However, no significant RPE degenerative changes were noted. At 44 weeks, the photoreceptor layers were relatively unremarkable (Weng et al., 1999). Additionally, *abcr*$^{-/-}$ mice have thickening of Bruch's membrane but no evidence of drusen (Weng et al., 1999).

3.1.1.2 ELOVL4-mutant (Stargardt-3 dominant inheritary disease) murine model: Stargardt-like macular degeneration (STGD3) is an autosomal dominant disease with progressive central vision and color vision loss that begins in the second decade of life and has been associated with mutations in the elongation of very long chain fatty acids-4 (*ELOVL4*) gene (Stone et al., 1994). Three different mutations in the *ELOVL4* gene have been identified: a 5-bp deletion mutation, two 1-bp mutations, and a nonsense mutation (Zhang et al., 2001). ELOVL4 is normally heavily expressed in the endoplasmic reticulum of rod and cone photoreceptor cells; however, mutant ELOV4 acts as a dominant negative protein (Vasireddy et al., 2005) and loses its ER retention signal, causing deranged intercellular trafficking and accumulation of mutant ELOV4 in the Golgi apparatus (Ambasudhan et al., 2004). Complete loss of the *Elovl4* gene causes embryonic lethality, and haploinsufficiency results in an essentially normal retinal phenotype (Raz-Prag et al., 2006). Transgenic mice expressing a mutant form of human *ELOVL4 (TG E_mut*$^{+/-}$) develop RPE changes, including vacuolization with accumulation of debris and undigested outer segments in the subretinal space and pigment granule deposits as early as two months of age. The number of lipofuscin granules and A2E

levels were significantly higher in *TG E_mut*$^{+/-}$ mice at 7 months and 4 months, respectively, compared to controls (Vasireddy et al., 2009; Karan et al., 2005). The *Elovl4* 5-bp deletion knock-in mouse model demonstrated progressive photoreceptor degeneration, predominately of cones, from 6–18 months of age (Vasireddy et al., 2006).

3.1.1.3 Efemp1$^{R345W/R345W}$ (Doyne honeycomb retinal dystrophy) murine model: Malattia leventinese (ML), or Doyne's honeycomb retinal dystrophy (DHRD), is an autosomal dominant, fully penetrant, inherited maculopathy that presents in early adulthood with drusen in a honeycomb form in the macula and optic nerve head (Collins, 1913). The burden of drusen increases, leading to geographic atrophy, CNV, and central vision loss by age 40–50 (Tree, 1937). Doyne's retinal dystrophy has been shown to be caused by a R345W mutation in the *EGF-containing fibrillin-like ECM protein 1* (*EFEMP1*) gene, which encodes fibulin-3, a protein of unknown function (Stone et al., 1999). EFEMP1 is a member of the fibulin family of extracellular matrix (ECM) proteins that are expressed in epithelial basement membranes and play a role in the assembly of elastin fibers (Timpl et al., 2003). Genetic studies have implicated other fibulins in the pathogenesis of AMD (Stone et al., 2004). *Efemp1*$^{-/-}$ mice were shown to have features of premature aging but not macular degeneration (McLaughlin et al., 2007). The R345W mutation was first identified in individuals with familial DHRD/ML but was not identified in a large cohort of control or AMD subjects (Stone et al., 1999).

An *Efemp1*$^{-R345W}$ point mutation knock-in mouse was made and found to have many pathologic features of dry AMD (Fu et al., 2007). *Efemp1*$^{R345W/R345W}$ mice have vacuoles in RPE cells that appear at 6 months and increase in number; by 12 months of age, the normal organization of the basolateral infolds of the RPE is lost(Marmorstein et al., 2007; Fu et al., 2007). Activated C3 is detected in RPE cells and Bruch's membrane (Fu et al., 2007). The *Efemp1*$^{R345W/R345W}$ mice have Bruch's membrane abnormalities: deposits of wide-spaced collagen are noted at two months, and significant amorphous electron dense debris of membrane coated vesicles are diffusely in the collagenous and elastic layers of Bruch's membrane at 12 months of age (Marmorstein et al., 2007; Fu et al., 2007). Basal deposits appear in *Efemp1*$^{R345W/R345W}$ and *Efemp1*$^{+/R345W}$ mice at 4 months of age; at 8 months of age, they are larger in size, and at 12 months of age, the deposits merge into continuous patches preferentially in the central retina (Marmorstein et al., 2007). Basal deposits contain Timp3, an Efemp1 interacting protein, and mutant fibulin-3. The neural retina in the *Efemp-1* $^{R345W/R345W}$ mutant mouse was normal from 6 to 18 months of age (Marmorstein et al., 2007; Fu et al., 2007).

3.1.1.4 Timp3$^{S156C/S156C}$ (Sorsby fundus dystrophy) murine model: Sorsby fundus dystrophy (SFD) is a rare, late-onset, hereditary degenerative disease of the retina and choroid that causes rapid central vision loss and progressive peripheral vision loss, ultimately leading to blindness (Sorsby et al., 1949). Histopathologic studies of SFD patients have shown that these patients have sub-RPE deposits of extracellular matrix material (collagen, elastin and glycoaminoglycans) and Bruch's membrane thickening, leading to CNV and RPE atrophy, reminiscent of AMD pathology (Chong et al., 2000). SFD patients were found to have point mutations in the *tissue inhibitor of metalloproteinases-3* (*TIMP3)* gene, and TIMPS, the inhibitors of the metalloproteinases (MMPs), have a role in the composition of the ECM (Weber et al., 1994; Matrisian, 1992).

A mouse model was constructed with a mutant *Timp3* gene (Weber et al., 2002). There was no significant difference in the thickness or structure of Bruch's membrane when *Timp3*$^{+/S156C}$, *Timp3*$^{S156C/S156C}$, and wild-type mice were compared at 8 months of age, but Bruch's membrane thickness increased in *Timp3*$^{S156C/S156C}$ mice as granular debris in the inner layers of the ECM accumulated by 30 months of age (Weber et al., 2002). The RPE cells in the *Timp3*$^{+/S156C}$ mice had local disorientation of the apical processes and reduced thickness

and complexity of basal microvilli. The RPE cells in *Timp3*$^{S156C/S156C}$ aged mice had a disrupted basal layer with loss of the palisade-like orientation of microvilli. The RPE processes no longer faced the outer segment of the photoreceptors; rather, the processes were horizontally aligned near the RPE cell bodies and formed a vesiculated barrier between the outer segment and RPE (Weber et al., 2002). The *Timp3*$^{+/S156C}$ and *Timp3*$^{S156C/S156C}$ mice had no derangements of the neural retina from 8 months to 30 months old.

3.1.2. Genes relevant to AMD—While it is known that genetics and environmental factors both contribute to AMD development and progression, the roles of genetic factors in AMD have been described in detail in recent years (Swaroop et al., 2007). Twin studies have demonstrated that genetic factors contribute to 46–71% of the overall variation in severity of macular degeneration. When one or both twins have AMD, concordance is 55% among monozygotic twins and 25% among dizygotic twins for AMD (Seddon et al., 2005). Single nucleotide polymorphisms in several genes, especially the Y402H variant of *complement factor H* (*CFH*) (Haines et al., 2005; Klein and Weleber, 1998; Klein et al., 2005; Edwards et al., 2005; Hageman et al., 2005), have been associated with AMD pathogenesis (Swaroop et al., 2007; Ding et al., 2009). Missense mutations in the gene encoding fibulin-5 have also been associated with AMD (Stone et al., 2004). Several studies have demonstrated an increased risk for AMD in patients with polymorphisms in *C2*, *BF*, *CX3CR1*, *ARMS2/HTRA1*, and *APOE* (Gold et al., 2006; Maller et al., 2006; Klaver et al., 1998; Francis, 2008).

3.1.2.1. Inflammatory genes: Chronic para-inflammation, a tissue adaptive response to noxious stress, contributes to the initiation and progression of obesity, type 2 diabetes mellitus, atherosclerosis, and age-related neurodegenerative diseases including AMD (Xu et al., 2008; Xu et al., 2009). The pathology of AMD lesions demonstrates chronic persistent inflammatory damage, including macrophage infiltration, microglial accumulation, and inflammatory components in drusen, such as complement factors, pro-inflammatory cytokines and chemokines (van der Schaft et al., 1992; Spraul and Grossniklaus, 1997; Anderson et al., 2002; Chan et al., 2008; Patel and Chan, 2008). Mutations in many of the genes that have been attributed to AMD result in a pro-inflammatory state in the eye, which are signified by increased C-reactive protein and decreased inhibitory complement factors (Boekhoorn et al., 2007; Seddon et al., 2004). Because of the central role of inflammation in AMD, mouse models that target specific aspects of the inflammatory process have been created to understand their significance in AMD and will be discussed in detail.

3.1.2.1.1. Cfh*$^{-/-}$ *murine model: CFH negatively regulates the complement system by inhibiting the alternative pathway either by promoting Factor I-mediated inactivation of C3b or by displacing Factor Bb from the C3bBb complex (Alsenz et al., 1985). CFH dysfunction may lead to excessive inflammation and tissue damage and contribute to the pathogenesis of AMD (Johnson et al., 2006). Homozygous deficiency of CFH in humans and mice results in complement factor alternative pathway dysregulation, the inflammatory renal disease membranoproliferative glomerulonephritis type II (MPGN2), and an AMD-like phenotype (Raines et al., 1989; Leys et al., 1991).

To further understand the role of *CFH* in the eye with relation to AMD, a *cfh*$^{-/-}$ mouse model was made and found to have poor visual function at two years of age (Coffey et al., 2007). The *cfh*$^{-/-}$ mice had C3 accumulation in the retina, secondary C3 deficiency, and uncontrolled C3 activation, which may increase phagocytic uptake and cause neural damage to the retina (Mullins et al., 2000; Coffey et al., 2007). Ultrastructurally, *cfh*$^{-/-}$ mice have significant thinning of Bruch's membrane (by 29%) and consistently disorganized, misaligned photoreceptor outer segments. There is a decrease in the amount of sub-RPE electron-dense material when compared to age-matched wild-type mice (Coffey et al., 2007). CFH itself is a main component of drusen, and the loss of this protein may reduce the volume of sub-RPE

desposits. The outer segment photoreceptors in *cfh*$^{-/-}$ mice are disorganized and misaligned with an inappropriate interface with RPE cells. Melanosomes, lipofuscin granules, and melanosome-lipofuscin granules were dispersed throughout the RPE in *cfh*$^{-/-}$ mice, compared to primarily apical localization of organelles observed in controls (Coffey et al., 2007). Though there is a touted association between CFH and AMD, the *cfh*$^{-/-}$ mice do not have many features of AMD; rather, thinning of Bruch's membrane and decreased drusen were found. However, disorganized photoreceptor outer segments and organelle localization in the RPE can be found in AMD eyes.

3.1.2.1.2 Ccl2*$^{-/-}$ *and Ccr2*$^{-/-}$ *murine models: To analyze the role of macrophage dysfunction in AMD, mice genetically altered to affect macrophage recruitment were created (Ambati et al., 2003). The monocyte chemoattractant protein-1 (MCP-1, or Ccl-2) binds to the C-C chemokine receptor-2 (Ccr-2) and mediates adhesion of inflammatory cells to vessels, controlling their extravasation to tissues (Kuziel et al., 1997). MCP-1/CCL2 is essential for monocyte recruitment and influences cytokine expression related to helper T-cell responses (Lu et al., 1998). Both *Ccl2*$^{-/-}$ and *Ccr2*$^{-/-}$ murine models were found to have features of AMD (Ambati et al., 2003).

After 9 months of age, subretinal deposits with features of drusen were observed in all *Ccl2*$^{-/-}$ mice, and the number of deposits increased with age. Bruch's membrane was markedly thickened with internal fragmentation and disruption of the collagen and elastin layers in 10 to 12 month old *Ccl2*$^{-/-}$ mice compared to age-matched wild-type mice (Ambati et al., 2003). At 9 months of age, RPE cells became swollen, and vacuolated and high electron density intracellular dense bodies, likely melanosomes or melanolipofuscin fusion granules, accumulated. Additionally, the number of lipofuscin granules and the A2E signal increased with age in senescent *Ccl2*$^{-/-}$ mice (Ambati et al., 2003).

As *Ccl2*$^{-/-}$ mice aged, they exhibited many of the findings of advanced AMD, including geographic atrophy, progressive outer retinal degeneration, and CNV (Ambati et al., 2003). At 14 months of age, photoreceptors were healthy, but attenuated and pyknotic photoreceptors developed by 16 months of age, while wild type mice still had normal photoreceptors (Ambati et al., 2003). The RPE cells in *Ccl2*$^{-/-}$ mice had marked vacuolization with a degenerative nucleus and few pigment granules at 16 months of age. There were abundant melanocytes filled in the choroid but no choriocapillaries in the 16-month old *Ccl2*$^{-/-}$ mouse. At 20 months of age, *Ccl2*$^{-/-}$ mice began to overexpress VEGF in the RPE, had dilated choriocapillaries, and began to have fragmentation in the outer layer of Bruch's membrane (Ambati et al., 2003). In this model, deposition of IgG, C5, vitronectin, CD46, serum amyloid P, and advanced glycosylation endproducts were present in *Ccl2*$^{-/-}$ mice in a distribution similar to what has been previously reported in humans AMD eyes (Ambati et al., 2003; Mullins et al., 2000).

The *Ccr2*$^{-/-}$ mice developed a phenotype very similar to *Ccl2*$^{-/-}$ mice. After 9 months of age, subretinal deposits similar to drusen were noted in the *Ccr2*$^{-/-}$ mice. Bruch's membrane was noticeably thickened with collagen and elastin layer disruption at 10 months of age. Lipofuscin granules accumulated at 9 months and increased in number with age. Vacuolization of the RPE cells was also noted at 9 months (Ambati et al., 2003). *Ccr2*$^{-/-}$ mice also displayed signs of late AMD with geographic atrophy, outer retinal degeneration, and CNV. Fundoscopic evidence of geographic atrophy was visible in *Ccr2*$^{-/-}$ mice at 18 months of age. Changes in the neural retina, especially cell loss and atrophy of the outer nuclear layer of photoreceptors, became evident around 16 months of age in the *Ccr2*$^{-/-}$ mice and were similar to findings in the *Ccl2*−/− mouse (Ambati et al., 2003). The choriocapillaris became severely attenuated, and the RPE became hypopigmented and devoid of basal infoldings and most organelles by 16 months of age. At 24 months of age, the *Ccr2*$^{-/-}$ mouse had CNV with endothelial cell and fibrocyte invasion of the sub-RPE space through a Bruch's membrane defect. Complement

deposition and activation was in a pattern similar to the *Ccl2*$^{-/-}$ mice (Ambati et al., 2003). The similar phenotype and features of both early 'dry' and late 'wet' AMD in the *Ccl2*$^{-/-}$ and *Ccr2*$^{-/-}$ mouse models highlight the role of macrophage recruitment in the pathogenesis of AMD.

3.1.2.1.3 Cx3cr1*$^{-/-}$ *murine model: Studies of the CCR2 and CCL2 receptors in transgenic mice have been shown to alter macrophage recruitment and influence AMD progression to CNV (Tsutsumi C, 2003). The role of retinal microglial cells in AMD is being investigated. Retinal migroglial cells express the CX3C cheomkine receptor 1 (CX3CR1). Homozygosity of the *CX3CR1* M280 allele and V249I allele, associated with impaired microglial cell migration, has been shown to increase the risk of AMD (Combadiere et al., 2007; Tuo et al., 2004). The perimacular area in AMD eyes has lower expression of *CX3CR1* than the perimacular area of control subjects, possibly implicating decreases in *CX3CR1* in age-related changes in the macula (Tuo et al., 2004; Chan et al., 2005). Activated microglia have been shown to directly injure photoreceptor cells (Roque et al., 1999) and increase autofluorescence due to lipofuscin deposition (Xu et al., 2008).

At 12 months of age, ultrastructural analysis of senescent *Cx3cr1*$^{-/-}$ mice revealed subretinal cells that contained intracellular lipid deposits and outer segment remnants between the photoreceptor outer segment and RPE. At 18 months of age, the engorged subretinal microglial cells appeared drusen-like on fundoscopy (Combadiere et al., 2007). At this age, the *Cx3cr1*$^{-/-}$ mice had significant (40%) thinning of the outer retina, mainly the outer nuclear layer, which progressed to marked degeneration of the photoreceptor layer by 4 months of age when compared to wild-type mice. After laser injury, *Cx3cr1*$^{-/-}$ mice showed signs of significant CNV compared to wild-type mice (Combadiere et al., 2007). These studies suggest a role of microglial cells in aging and AMD pathogenesis (Xu et al., 2009; McGeer et al., 2005).

3.1.2.1.4 Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ *murine model: The *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ mouse model has demonstrated early onset of the AMD phenotype with high penetrance (Tuo et al., 2007). *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ mice began exhibiting drusen-like lesions on fundoscopy as early as 4–6 weeks of age. These lesions progressed to large, confluent areas of yellow deposits in the deep retina and subretinal space by 4–6 months of age and flattened atrophic areas by 6 months of age (Chan et al., 2008). Other AMD features include an abnormal and thickened Bruch's membrane with irregular deposits. Drusen, while present, were smaller (5–15 μm) than classic human drusen seen in AMD patients. Abnormalities of RPE cells included RPE hypopigmentation, depigmentation, vacuolization, loss of melanosomes, an increase in lipofuscin (Figure 2), and elevated A2E, which led to RPE degeneration. More basal infoldings were observed in *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ mice when compared to age-matched wild-type controls (Figure 2). Photoreceptor outer segment disorganization and photoreceptor atrophy were also observed in *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ mice (Figure 2). Compared to wild-type mice, *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ mice have significantly fewer photoreceptor terminals and synapses (Zhang et al., unpublished). Spontaneous CNV was present in about 15% of *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ mice (Tuo et al., 2007).

Characterization of the immunologic mileu in *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ mice revealed several abnormalities. *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ mice had increased C3 and CD46. C3d deposition was increased in Bruch's membrane, drusen-like lesions, RPE, photoreceptors, and choroidal capillaries (Ross et al., 2008), similar to previous reports in humans with AMD (Hageman et al., 2005). Lower levels of the chaperone protein ERp29 are found in *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ mice compared to wild-type controls (Tuo et al., 2007). Macrophage infiltration, microglial accumulation, and anti-retinal antibody levels were increased in *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ mice compared to wild-type controls (Ross et al., 2008). The *Ccl2*$^{-/-}$*/Cx3cr1*$^{-/-}$ mouse model

demonstrates many AMD features: small drusenoid deposits, RPE and Bruch's membrane degenerations, photoreceptor atrophy, CNV, and simulation of the immunologic and pathologic environments found in AMD patients (Herzlich et al., 2008; Ross et al., 2008; Ding et al., 2009). It is one of the few models to have high penetrance and early onset- all of which facilitate experimentation (Chan et al., 2008).

3.1.2.2. Oxidative stress associated genes: The retina has the highest oxygen consumption of any tissue in the body (Sickel, 1972). Oxidative stress, or cellular and molecular damage caused by reactive oxygen species (ROS), has been implicated in aging and age-related eye diseases (Finkel and Holbrook, 2000). There is increasing evidence that cumulative oxidative damage is involved in AMD pathogenesis. Smoking, a common mechanism for generating oxidative stress, is a well-documented AMD risk factor, and a recent meta-analysis showed that current smoking nearly triples AMD incidence (Neuner et al., 2009). ROS include unstable species, such as superoxide anions and hydroxyl radicals, and stable freely diffusible substances like hydrogen peroxide. In the neutrophil, NADPH oxidases as part of a cytosolic enzyme system, contribute to oxidative stress. Most intracellular ROS production, however, is derived from the mitochondria in the electron transport chain.

Endogenous protection against oxidative stress is encountered and regulated through enzymatic and nonenzymatic antioxidant denfense by catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) (Finkel and Holbrook, 2000). One of the main retinal antioxidant systems is the superoxide dismutase (SOD) family of three isoenzymes that catalyze the dismutation of the superoxide radical: Cu, Zn-SOD (SOD1) is cytosolic; Mn-SOD (SOD2) is in the mitochondrial matrix, and extracellular SOD (SOD3) is interstitial and secreted (Valentine et al., 2005). In the retina, SOD1 has the highest activity (Behndig et al., 1998). To investigate the role of oxidative stress in AMD pathogenesis, transgenic mice targeting the SOD oxidative stress-recovery pathways have been made and examined.

3.1.2.2.1. Sod1$^{-/-}$ murine model: SOD1 levels are very high in the retina, and SOD1 is known to protect against oxidative damage, a possible trigger for AMD. Imamura et al. showed that *Sod1*$^{-/-}$ mice have accelerated age-related pathologic changes in the retina reminiscent of AMD, including drusen, thickening of Bruch's membrane, and CNV (Imamura et al., 2006).

Drusen were visible on fundoscopy of *Sod1*$^{-/-}$ mice after 7 months of age, and lesions increased in number with age. At 10 months of age, 86% of mice had drusen, compared to very few drusen in age-matched wild-type mice (Imamura et al., 2006). These sub-RPE dome-shaped deposits have many characteristics of drusen in human AMD eyes, including positive staining for vitronectin, CD46, activated complement components, TIMP3, and Igs (Crabb et al., 2002; Imamura et al., 2006). Based on human studies that show light exposure is a risk factor for AMD (Wenzel et al., 2005; Cruickshanks et al., 1993), *Sod1*$^{-/-}$ mice were exposed to fluorescent light, and more light exposure was found to induce more drusen (Imamura et al., 2006).

At 12 months of age, RPE vacuolization and degenerative changes were noted in *Sod1*$^{-/-}$ mice. Ultrastructural analysis demonstrated disrupted junctional integrity of the RPE in *Sod1*$^{-/-}$ mice, signified by altered expression of junctional adherence proteins N-cadherin and β-catenin and their translocation from the cell membrane to the cytoplasm (Imamura et al., 2006). A biomarker of oxidative DNA damage was present at higher levels in *Sod1*$^{-/-}$ mice when compared to controls. Bruch's membrane was markedly thickened, with a thickness approximately 6-fold greater in *Sod1*$^{-/-}$ mice compared to age-matched wild-type mice. Photoreceptor loss was shown in 17% percent of *Sod1*$^{-/-}$ mice. Approximately 10% of mice older than 10 months had CNV (Imamura et al., 2006). The similarities of the lesions in this

mouse model to the human AMD lesions suggest that the *Sod1*$^{-/-}$ mouse is a suitable murine model for AMD.

3.1.2.2.2. Sod2 knockdown murine model: The antioxidant enzyme manganese superoxide dismutase (MnSOD), encoded by *SOD2*, is in the mitochondrial matrix and converts superoxide anions produced in aerobic respiration to hydrogen peroxide. To study the role of this enzyme, *Sod2*$^{tm1Cje-/-}$ mice were made, but they all died of dilated cardiomyopathy by 10 days of age (Li et al., 1995). The *Sod2*$^{tm1Cje-/-}$ mice were treated with the Sod mimetic mangenese 5,10,15,20-tetrakis (4-benzoic acid) porphyrin (MnTBAP), and the animal survival time nearly doubled. These animals died of spongiform encephalopathy with normal brain mitochondria (Melov et al., 1998). Ocular pathology of the MnTBAP-treated *Sod2*$^{tm1Cje-/-}$ mice revealed that the 10 day-old *Sod2*$^{tm1Cje-/-}$ mice had thinning only of the central photoreceptor layer (Sandbach et al., 2001). By 20 days of age, most retinal layers were all significantly reduced and contained markedly edematous mitochondria in the *Sod2*$^{tm1Cje-/-}$ mice compared to control animals. Photoreceptor damage at three weeks of age is early when compared to the *Sod1* and *Gpx-1* antioxidant knockdown models, which only show retinal damage at 1 year of age (Gosbell et al., 2006; Imamura et al., 2006). There were no differences in the RPE basement membrane or basal infoldings, and lipofuscin granules were rare in *Sod2*$^{tm1Cje-/-}$ mice and controls (Sandbach et al., 2001).

To further study the role of *Sod2* deficiency in older mice, a *Sod2*-deficient mouse was made using a subretinal injection of an AAV-ribozyme-mediated knockdown of *Sod2* mRNA in the RPE of wild-type mice (Justilien et al., 2007). The *Sod2* knockdown mice had RPE and Bruch's membrane changes and accumulated A2E and lipofuscin granules in the RPE (Justilien et al., 2007). One month after injection, the RPE exhibited hypopigmentation with a normal neural retina. Between 2 and 4 months after injection, the RPE cells underwent vacuolization and atrophy with shortening and disorganization of the outer and inner segment of the photoreceptors and thinning of the outer nuclear layer (Justilien et al., 2007). After 4 months, there was a 40% increase in Bruch's membrane thickness, specifically in the inner and outer collagenous zones, in *Sod2* knockdown mice compared to age-matched wild-type mice. Basal laminar deposits (BlamD) are present between the basal laminar infoldings and the plasma and basement membrane. ERG *a*-wave and *b*-wave amplitudes were noted to decrease by 33% and 41%, respectively, after 4 months. A2E levels were greater than twofold higher in *Sod2* knockdown mice 4.5 months after injection, compared to controls (Justilien et al., 2007). The *Sod2* knockdown murine model emphasizes the role of oxidative stress in AMD pathogenesis, as the phenotype includes RPE hypopigmentation and increased lipofuscin, Bruch's membrane thickening, basal laminar deposits, and photoreceptor disorganization.

3.1.2.3. Metabolic pathway associated genes: When lipid and protein-rich sub-RPE and Bruch's membrane deposits accumulate in AMD patients, hydraulic conductivity and the transport of fluids and hydrophilic substances through Bruch's membrane becomes impaired (Moore et al., 1995). Derangements in cholesterol metabolism may affect hydraulic conductivity across Bruch's membrane, cause the accumulation of its toxic metabolites such as 7-ketocholesterol, and contribute to AMD pathogenesis (Javitt and Javitt, 2009). Photoreceptors depend on choriocapillary circulation and the RPE for nutrients and clearance of metabolic waste products; impairment of this process is related to the pathogenesis of late AMD (Moore et al., 1995; Sarks, 1976). Lysosomal degradation of the photoreceptor outer segments is mediated in part by protease Cathepsin D, and the inactivation of this protease has been shown to cause metabolic derangements in RPE cells and induce AMD-like lesions in *mcd/mcd* transgenic mice (Zhang et al., 2002). Increased deposition of Amyloid β, as seen in Alzhimer's disease, has been shown to cause AMD-like retinal pathology in the *neprilysin*$^{-/-}$ murine model (Yoshida et al., 2005). Derangements in iron metabolism leading to iron overload have been shown to cause retinal degeneration and the accumulation of

inclusion bodies in the RPE (He et al., 2007). Mouse models with increased serum cholesterol include *ApoE* and *ApoB-100* transgenic mice, and these mice have been shown to have AMD-like lesions (Cousins et al., 2002; Klaver et al., 1998). The retinal pathology of the aforementioned models with metabolic dysfunction will be discussed in detail.

3.1.2.3.1. Neprilysin$^{-/-}$ murine model: Amyloid β (Aβ) is a physiologic peptide, the steady state of which is maintained by a balance between synthesis and degradation (Saido, 1998). Immediately after production, Aβ is normally degraded by peptidases such as neprilysin (Iwata et al., 2001). Aging is associated with decreased levels of neprilysin and increased Aβ peptide deposition and aggregation (Iwata et al., 2002). Aβ oligomers and aggregates in the brain form senile plaques, which are pathologic, immunogenic, and implicated in Alzheimer's disease (Saido, 1998; Wisniewski et al., 1997). Aβ oligomers result in microglia recruitment and neuronal apoptosis (Uchihara et al., 1997). Recent evidence has shown that drusen contains Aβ, a specific finding in drusen from AMD eyes (Johnson et al., 2002; Anderson et al., 2004; Dentchev et al., 2003).

In vitro data has shown that the oligomeric form of Aβ: OAβ(1–42) reduces mitochondrial redox potential and increases the production of reactive oxygen species without inducing apoptosis in RPE cell cultures, a finding that is partially reversible with antioxidant pre-treatment (Bruban et al., 2009). Subretinal injection of OAβ(1–42) in wild-type mice induces RPE abnormalities and photoreceptor loss; however, the retinal ultrastructure was not depicted in this model (Bruban et al., 2009). The retinal pathology of senescent transgenic mice with disruption of the neprilysin gene was also examined (Yoshida et al., 2005). In 27 month-old *Neprilysin*$^{-/-}$ mice, the RPE developed vacuolization, loss of the tight and adherence junctions, prominent basal infoldings, and subretinal accumulation of amorphous deposits and photoreceptor outer segments, implying RPE dysfunction. *Neprilysin*$^{-/-}$ mice have extensive BlamD and some BlinD. Bruch's membrane appears normal, as does the photoreceptor layer. The *Neprilysin*$^{-/-}$ mice have enhanced VEGF expression and diminished PEDF expression with no evidence of CNV or leakage on fluorescein angiography (Yoshida et al., 2005).

3.1.2.3.2. mcd/mcd murine model: Phagocytosis of photoreceptor outer segments is a fundamental vision-preserving physiologic function of the RPE (Young and Bok, 1969). An early sign of RPE dysfunction in AMD eyes is indigestion of photoreceptor outer segments, with visible remnants in the RPE cytoplasm. Accumulation of this material results from lysosomal abnormalities in the RPE (Hayasaka, 1983) or Bruch's membrane changes inhibiting exocytosis (Starita et al., 1996). Cathepsin D (CatD) is an aspartic protease that is involved in the lysosomal digestion of the outer segments (Rakoczy et al., 1997). An age-related increase in the inert indigestible substrate inactive catD, or procathepsin D (proCatD) in RPE cells, is postulated to cause vision impairment by impairing photoreceptor outer segment proteolysis (Wiederanders and Oelke, 1984; Zhang et al., 2002). To further study the role of catD *in vivo*, a homozygous (*mcd/mcd*) transgenic mouse model was made that expresses a form of catD that lacks the catD cleavage site (Zhang et al., 2002). The retinal phenotypes of these mice were described by Rakoczy and colleagues (Rakoczy et al., 2002).

On fundoscopic exam at 9 months of age, RPE hypopigmentation, hyperpigmentation, and drusenoid lesions were present in most *mcd/mcd* mice, while no lesions were present in wild-type mice. After 10 months of age, RPE cells in *mcd/mcd* mice were found to have focal attenuation, hypertrophy, and hypopigmentation. By 18 months of age, minimal Bruch's membrane thickening, drusen-like sub-RPE deposits, and extensive BlamD and BlinD were observed. An increase in apoptotic photoreceptors in *mcd/mcd* mice was noted when compared to age-matched wild-type controls, and progressive thinning of the ONL layer of photoreceptors was present at 12 months with disorganization of the INL. ERG abnormalities

(reduced *a*-wave and *b*-wave amplitudes) were present in transgenic *mcd/mcd* mice at 12 months of age. No changes in the choriocapillaris were noted (Rakoczy et al., 2002).

To investigate whether the observed phenotype in the *mcd/mcd* mice is a direct result of the amount of proCatD, a transgenic mouse (*mcd2/mcd2*) was made with additional deletions in the catD cleavage side such that *mcd2/mcd2* mice cannot make any active catD (Zhang et al., 2005). The *mcd2/mcd2* mice experienced earlier signs of retinal degeneration than the *mcd2/mcd2* mice. Three month-old *mcd2/mcd2* mice showed focal areas of RPE disorganization, clumping, proliferation, and pleomorphism with attenuation, depigmentation, and atrophy. These early findings progressed with age, and by 12 months old almost all the animals developed RPE proliferation. These RPE findings, collectively referred to as "pigment mottling," are accompanied by progressive photoreceptor outer segment disturbances and photoreceptor atrophy by 12 months. The basal lamina and Bruch's membrane were unaffected. Tight junctions and basal polarity of the RPE nucleus was lost in the *mcd2/mcd2* mice (Zhang et al., 2005).

3.1.2.3.3. $Cp^{-/-}Heph^{-/Y}$ murine model: Iron is an essential cofactor for many enzymes, but ferrous iron (Fe^{2+}) can cause oxidative damage via the Fenton reaction. If Fe^{2+} causes oxidative damage in the macula, it may contribute to AMD pathogenesis (Hahn et al., 2003; Hahn et al., 2004). In iron overload states, retinal degeneration and photoreceptor toxicity have been observed (Doly et al., 1986). Compared to normal eyes, AMD eyes have a statistically significant increase in total iron in the RPE and Bruch's membrane and in AMD lesions (Hahn et al., 2003). Ceruloplasmin (Cp) is a multicopper feroxidase that, when secreted, circulates in the blood without crossing the blood-brain barrier (Patel and David, 1997). *Cp* is expressed in human and mouse retinas in the inner nuclear layer (Klomp and Gitlin, 1996; Klomp et al., 1996), and *Cp* expression increases in Müller glia after photic injury (Chen et al., 2003). Cp is necessary for iron export from cells, and $Cp^{-/-}$ mice have mild iron overload with increased susceptibility to oxidative stress (Patel et al., 2002). Hephaestin (Heph) is a multicopper feroxidase that is 50% identical to Cp and facilitates iron export. *Heph* is naturally mutated in *sex-linked anemia (sla)* mice, which have low Heph ferroxidase levels (Vulpe et al., 1999; Chen et al., 2004). Mice with a combined deficiency in Cp and Heph ($Cp^{-/-}Heph^{-/Y}$ mice) were made and found to have age-related iron accumulation, secondary increases in ferritin, and retinal degeneration with AMD-like features (Hahn et al., 2004).

By 5–6 months of age, the $Cp^{-/-}Heph^{-/Y}$ mice had increased iron levels, and the highest iron levels were in the RPE and photoreceptor OS. Iron-laden electron dense vesicles, likely lysosomes or lysosome-melanosome fusion vesicles, were visible in the RPE cells of 5 month-old $Cp^{-/-}Heph^{-/Y}$ mice only. At 6–9 months old, $Cp^{-/-}Heph^{-/Y}$ mice had severe retinal degeneration with RPE hypopigmentation, hypertrophy, hyperplasia, and necrosis. Hypertrophic RPE cells were full of lipofuscin, phagosomes, and lysosomes with partially digested photoreceptor outer segment membranes. This RPE hypertrophy is greater than what has been observed in AMD retinas (Hahn et al., 2004; Hadziahmetovic et al., 2008). $Cp^{-/-}Heph^{-/Y}$ mice had no clinically observable drusen. Focal subretinal deposits with banded structures and wide-spaced collagen, similar to BlamD, were observed in the 9 month-old $Cp^{-/-}Heph^{-/Y}$ mice. Local photoreceptor degeneration and thinning was found in the ONL along with inner segment vacuolization (Hahn et al., 2004). In 12 month-old $Cp^{-/-}Heph^{-/Y}$ mice, 90% of RPE cells were hypertrophic, and subretinal macrophage infiltration was evident. The complement system is activated in $Cp^{-/-}Heph^{-/Y}$ mice. Evidence of focal CNV was also present in 75% of 12–13 month-old mice and 50% of 7–9 month-old mice (Hahn et al., 2004; Hadziahmetovic et al., 2008).

3.1.2.3.4. Murine models of aberrant cholesterol metabolism: Cholesterol deposition in Bruch's membrane is one of the most common age-related changes in the human retina

(Pauleikhoff et al., 1990). The deposition of lipid- and proteoglycan-rich extracellular material is a common risk factor for atherosclerosis and AMD (Snow and Seddon, 1999). Mice with higher plasma LDL levels via transgenic methods and/or high fat and cholesterol diets have more circulating lipoproteins and are more prone to both atherosclerosis and degeneration of Bruch's membrane, a key factor in AMD pathogenesis (Sallo et al., 2009; Rudolf et al., 2004). Degenerative changes in Bruch's membrane have been recognized by several groups in association with elevated serum lipid levels in both wild-type mice and transgenic mice in advanced age (Cousins et al., 2003; Miceli et al., 2000; Cousins et al., 2002; Dithmar et al., 2000; Rudolf et al., 2004). Laser photodisruption of the RPE in mice fed a high fat diet accumulate BlamD, indicating that compromise to RPE lipid metabolism in a hypercholesterolemic mouse leads to BlamD (Dithmar et al., 2001).

3.1.2.3.4.1. ApoE transgenic murine models: Apolipoprotein E (apoE) is a polymorphic CNS apolipoprotein that is important for plasma lipid metabolism, cholesterol and lipid mobilization, and neuronal cell membrane maintenance and repair (Pitas et al., 1987; Mahley, 1988). ApoE has been found in drusen and BlamD (Klaver et al., 1998). *ApoE*$^{-/-}$ (Apolipoprotein E deficient) mice have elevated total plasma cholesterol and VLDL. Starting at 2 months of age, these mice form vacuolated electrolucent vescicles in Bruch's membrane, similar to BlinD, that increase with age (Dithmar et al., 2000). Several genetic association studies show that *APOE* polymorphisms provide a significant risk for AMD: the *APOE ε4* allele confers a decreased AMD risk; the *APOE ε2* allele is associated with a slightly increased AMD risk compared to APOE ε3 allele homozygotes (Klaver et al., 1998; Baird et al., 2004; Bojanowski et al., 2006). These findings contrast with studies that show a positive association between Alzheimer's disease, atherosclerosis, multiple sclerosis, stroke and the APOE ε4 allele (Kalaria, 1997; Weller and Nicoll, 2003; Enzinger et al., 2004).

To compare the *apoE2*, *apoE3*, and *apoE4* alleles, transgenic (TR) mice models were made expressing one of the three human APOE isoforms (Sullivan et al., 1997). The apoE2, apoE3, and apoE4 TR mice were fed a low or high fat diet, and their retinal pathology was compared and summarized (Malek et al., 2005). The young (12–13 week-old) *apoE* TR mice (irrespective of the fat content in their diet) had a normal ocular phenotype. The aged (65–127 week-old) apoE3 TR mice on a high-fat diet were ultrastructurally normal except for minor RPE vacuolization. Aged apoE2 TR mice on a high-fat diet had a more severe phenotype with RPE vacuolization, mottling, hyperpigmentation and hypopigmentation, disorganized basal infoldings, sub-RPE deposits, and Bruch's membrane thickening (Malek et al., 2005).

Aged *apoE4* TR mice on a high-fat diet had the most severe degenerative phenotype with RPE hyperpigmentation, hypopigmentation, atrophy, disorganization of basal infoldings with electron-dense deposits, many electron-dense sub-RPE basal deposits, lipid-rich "drusenoid" deposits, disorganization with varying thickness of Bruch's membrane, and CNV of varying severity (Malek et al., 2005). In studies of patients with Alzheimer's disease and the APOE4 allele, there is increased deposition of Aβ; in the apoE4 TR mouse, CFH and Aβ form a complex in sub-RPE deposits, which may cause local inflammation leading to the progression of the disesase seen in the apoE4 TR mouse model (Wellington, 2004; Strohmeyer et al., 2002; Malek et al., 2005; Bruban et al., 2009).

Another ApoE transgenic mouse model is the APO*E3-Leiden mouse, a mouse that produces a dysfunctional form of human APOE3 and has hyperlipoproteinemia (van den Maagdenberg et al., 1993). After 9 months on a high fat/cholesterol diet, 100% of APO*E3 Leiden mice eyes have BlamD, while 33% of APO*E3 Leiden mice on a normal diet have BlamD (Kliffen et al., 2000). Interestingly, more BlamD were found in atherosclerotic APO*E3 Leiden mice compared to non-atherosclerotic APO*E3 Leiden mice (Kliffen et al., 2000), supporting existing epidemiologic data linking AMD and atherosclerosis (Vingerling et al., 1996; Klein

et al., 1999). BlamDs are the main pathologic finding in this model, along with an increase and elongation of the RPE basal infoldings (Kliffen et al., 2000). Further electron microscopy is needed to analyze the ultrastructure of the RPE and Bruch's membrane in this model.

3.1.2.3.4.2. Apo B100 transgenic murine model: Insertion of the apoB-100 gene into mice creates a sharp increase in plasma LDL after a high-fat diet, a lipid profile similar to what has been seen in humans (Bjelik et al., 2006; Purcell-Huynh et al., 1995). The apoB100 transgenic mice have higher serum total and LDL cholesterol and a statistically significant cholesterol-dependent increase in Bruch's membrane thickness (Sallo et al., 2009). The apoB-100 transgenic mice fed with a high cholesterol diet develop BlamD (Sallo et al., 2009; Espinosa-Heidmann et al., 2004; Fujihara et al., 2009) and highly specific BlinD (Fujihara et al., 2009; Curcio and Millican, 1999) with outer collagenous zone deposits and loss of basal infoldings (Fujihara et al., 2009). When young apoB-100 mice were placed on a high-fat diet and exposed to acute photo-oxidative stress in the form of blue-green laser light or cigarette smoke, sub-RPE deposits, specifically BlamD, developed at two months of age. Additionally, diffuse Bruch's membrane thickening with granular deposits, choriocapillaris hypertrophy, and reduplication of the basement membrane was observed (Espinosa-Heidmann et al., 2004; Espinosa-Heidmann et al., 2006). The RPE of apoB-100 transgenic mice on high-fat diets have misaligned, disorganized, amorphous material between the RPE plasma and basement membrane, and atrophic basal processes with occasional vacuolization. No signs of photoreceptor damage or atrophy were found in the apoB-100 transgenic mouse (Sallo et al., 2009).

3.2. Immunologically manipulated carboxyethylpyrrole (CEP) immunized mouse

Ample evidence has implicated AMD as an immunologically-mediated disease (Patel and Chan, 2008). One hypothesis is that a signal from the outer retina initiates immune involvement in AMD. One potential signal is carboxyethylpyrrole (CEP), an adduct from the oxidation fragment of deocosahexaenoic acid (DHA), an easily oxidizable long-chain polyunsaturated fatty acid that is abundant in the outer retina (Anderson, 1970; Gu et al., 2003). CEP forms when an oxidation fragment of DHA covalently interacts with an ε-lysyl amino group in a tissue protein (Gu et al., 2003; Crabb et al., 2002). CEP, a hapten, acts as a biomarker of oxidative stress, a well-known factor in AMD pathogenesis (Snow and Seddon, 1999). In an AMD patient's outer retina and plasma, there are more CEP-modified proteins when compared to age-matched controls (Crabb et al., 2002). CEP is also pro-angiogenic via a VEGF-independent pathway (Ebrahem et al., 2006).

Serum albumin is one of the major proteins modified with CEP, and one group used this concept to immunize mice with CEP-modified mouse serum albumin, generating a strong immunologic response against endogenous CEP adducts in the outer retina (Hollyfield et al., 2008; Crabb et al., 2002). The severity of retinal pathology correlated directly with the CEP-antibody titer. The RPE cells in CEP-immunized mice had vesiculation, pyknosis, lysis, and areas of RPE loss with overlying swollen photoreceptors. Fundoscopy of immunized mice revealed patchy, reticular changes that were not present in controls (Hollyfield et al., 2008). Compared to age-matched controls, CEP-immunized mice have significant BlamD accumulation throughout the retina. Bruch's membrane was significantly thicker (about 3 fold) in immunized mice. Immunized mice had C3d, a C3b degradation product in Bruch's membrane, signifying that an intact immune system responds to CEP immunization with sub-RPE complement deposition, a well-known feature of AMD (Hollyfield et al., 2008; Patel and Chan, 2008). CEP-immunization has also been shown to exacerbate angiogenesis in mice with laser-induced breaks in Bruch's membrane, a finding that is reversible with administration of a monoclonal anti-CEP IgM antibody (Ebrahem et al., 2006).

3.3. Natural mice strains with features of AMD

Several naturally occurring mouse strains have shown features of retinal degeneration (Chang et al., 2002). Retinal degeneration is a broad term for conditions that cause irreversible vision loss, and over 140 genes have been identified in human retinal degenerations. The first identified retinal degeneration model was Rds^{Rd2}, an autosomal dominant slow retinal degeneration identified in inbred mice that accumulated an insertion of foreign DNA into an exon of *Rds* (Sanyal and Hawkins, 1986; van Nie et al., 1978). While many of the mouse models have been used to study retinitis pigmentosa, certain mouse models of retinal degeneration help provide insight into the molecular pathology of photoreceptor loss in AMD (Chang et al., 2002; Rakoczy et al., 2006). An accelerated aging model, the Senescence-Accelerated Mouse (SAM) family, is a collection of mouse strains with naturally occurring premature aging and associated pathologies (Takeda, 1997). As AMD is an age-related phenomenon, the retinas of these mice may provide insight into the causes of naturally occurring alterations in the aging human retina.

3.3.1. arrd2/arrd2 (Mdm gene mutation) murine model—A murine model of a naturally occurring, severe, late-onset, age-related, retinal degeneration (*arrd2*) has been identified to have a nonsense mutation in the *Mdm1* gene on mouse chromosome 10 that is inherited in an autosomal recessive manner (Chang et al., 2008). Analysis of a cohort of AMD patients with the human ortholog of *MDM1* on chromosome 12q did not reveal an association between *MDM1* and AMD, though this location has previously been reported to be an AMD susceptibility locus (Chang et al., 2008; Iyengar et al., 2004; Fisher et al., 2005). At 4 months of age, the *arrd2/arrd2* mice had early photoreceptor outer segment degeneration, increased phagosomes and debris in the RPE, normal ONL, swollen synaptic complexes, and large mitochondria. At 6 months, the *arrd2/arrd2* mice had subnormal rod and cone ERG responses and an undetectable ERG response at 22 months. At 9 months of age, the *arrd2/arrd2* mice had fragmented photoreceptor outer segments, abundant phagosomes with loss of the apical processes in the RPE, thinning of the outer plexiform layer (INL and ONL), swollen and necrotic synaptic terminals, and mitochondrial edema. No basal deposits or Bruch's membrane changes were noted in *arrd2/arrd2* mice. In this model, cone degeneration occurs slower than rod degeneration. The *arrd2/arrd2* mice also have retinal vascular attenuation, RPE hypo-pigmentation and atrophy at 14 months. This phenotype has features similar to human progressive rod-cone degeneration and AMD (Chader, 2002; Klein, 2007).

3.3.2. $Mfrp^{rd6}$ retinal degeneration murine model—One of the naturally occurring retinal degeneration and retinitis pigmentosa models, rd6, is inherited in an autosomal recessive manner and maps to mouse chromosome 9 with a human homolog on chromosome 11q23 (Hawes et al., 2000). A splice-donor mutation with the loss of an exon in a gene encoding membrane-type frizzled protein *(Mfrp)* was identified in the rd6 mouse (Kameya et al., 2002). *Mfrp*, expressed at high levels exclusively in the RPE and ciliary epithelium and not the neural retina, has strong homology to the cysteine-rich domain of frizzled, a protein that is an important member of the Wnt family of proteins (Kameya et al., 2002; Xu and Nusse, 1998). Aberrations in the Wnt/frizzled signaling pathway have been implicated in photoreceptor degeneration in retinitis pigmentosa patients, and abnormal Wnt/frizzled signaling in the RPE may be responsible for photoreceptor degeneration (Jones et al., 2000; Kameya et al., 2002). The homozygous $Mfrp^{rd6}$ mice have pan-retinal drusen-like deposits on fundoscopy at 8 weeks of age that progress to advanced retinal degeneration by 8 months of age, and these lesions are associated with macrophage invasion of the sub-retinal space and retinal dysplasia (Hawes et al., 2000). By 3 months of age, $Mfrp^{rd6}$ RPE cells accumulate lipofuscin-like material and pigment. The photoreceptor layer degenerates to 33% of its original thickness by 4.5 months of age, 15% of normal by 7 months of age, and becomes 1 cell layer thick by 24 months of age (Kameya et al., 2002). Homozygous $Mfrp^{rd6}$ mice have

diminished ERG responses beginning at 1 month of age, supportive of a slow progressive retinal rod and cone dysfunction that culminates in extinguished ERG activity by 70 weeks of age. No aberrations in Bruch's membrane have been reported, but further analysis of the retinal ultrastructure is necessary.

3.3.3. Nr2e3^{rd7} retinal degeneration murine model—The retinal degeneration 7 mouse has a mutation in the *Nr2e3* gene responsible for Goldmann Favre disease, or human enhanced S-cone syndrome (ESCS) (Haider et al., 2000; Chang et al., 1998). The inheritance pattern is autosomal recessive, and *Nr2e3* is mapped to mouse chromosome 9 and human chromosome 15q23. The *Nr2e3^{rd7}* mouse has an exon 4,5 deletion in the *Nr2e3* gene, causing a frameshift mutation that results in a premature stop codon (Akhmedov et al., 2000). *Nr2e3* is part of the cellular and nuclear receptor family for steroid hormones and is involved in cone cell proliferation, maintenance of photoreceptor topography and intercellular interactions, and expression of rod photoreceptor cell-specific genes (Fuller, 1991; Haider et al., 2001; Cheng et al., 2004). At one month of age, large evenly-spaced retinal lesions are visible throughout the retina of homozygous *Nr2e3^{rd7}* mice by fundoscopy (Hawes et al., 1999). Histologic analysis of the photoreceptor ONL demonstrates retinal dysplasia with whorls and rosettes, which decrease by 5 months of age and disappear when the photoreceptor layer flattens to 5-cell layers thick. There is additional retinal vascular attenuation and mottled retinal pigmentation at 16 months of age (Chang et al., 1998; Haider et al., 2001; Akhmedov et al., 2000). ERGs of homozygous *Nr2e3^{rd7}* mice show progressive reduction of both rod and cone signals starting at 5 months of age, and wave amplitudes were 50% of normal by 16 months of age (Akhmedov et al., 2000). The retinal ultrastructure demonstrates disorganized photoreceptor outer segments and apical microvilli retardation in the RPE cells (Won et al., 2008).

3.3.4. cpfl3 (Gnat2 gene mutation) cone degeneration murine model—Most AMD cases have regional cone photoreceptor loss (Sarks, 1976). Additionally, cone dysfunction is an important clinical predictor of AMD severity (Hogg and Chakravarthy, 2006). Shelley and colleagues recently showed that, in AMD, cones lose their synaptic connections before dying, and signs of photoreceptor degeneration are found in cones before rods. Rod photoreceptor cell death also expedites cone cell death (Shelley et al., 2009). Therefore, studying models of cone photoreceptor cell dysfunction is important in understanding AMD pathogenesis and testing therapeutic options.

A mouse with a naturally occurring mutation in cone photoreceptor function loss-3 (*cpfl3*) is being used a model for rod monochromism, or achromatopsia, an autosomal recessive disease in which there is a loss of functional retinal cone photoreceptors, resulting in a disease that presents in infancy with horizontal nystagmus, photophobia, color blindness, and poor visual acuity (Aligianis et al., 2002). A mutation in exon 6 of *Gnat2* results in a missense mutation of the α-subunit of transducin, preventing photoreceptor hyperpolarization in the light transduction cascade, resulting loss of function in the structurally normal cones in *cpfl3/cpfl3* mice. Fundoscopy of *cpfl3/cpfl3* mice shows no retinal lesions and only dilated retinal vessels at 8 months of age. *Gnat2^{cpfl3}* mice have normal rod responses on ERG with an abnormal, significantly reduced, photopic response that was extinguished by 9 months of age. Histopathology demonstrates increased vacuolization and photoreceptor outer segment shortening with increased age (Chang et al., 2006). However, the ocular ultrastructure has not yet been described. While this model does not have the Bruch's membrane, RPE, and choriocapillaris changes seen in AMD patients, it demonstrates the cone pathology and dysfunction that can be present in the AMD macula.

3.3.5. SAM (senescence-accelerated mouse) model strains—Aging, or senescence, has been shown to be associated with photoreceptor loss in humans and in murine models

(Gao and Hollyfield, 1992; Katz and Robison, 1986). The Senescence-Accelerated Mouse (SAM) family of 12 murine lines demonstrates accelerated senescence that has been developed by selective inbreeding of the AKR/J strain (Takeda et al., 1994). The SAM mice have many characteristic pathologic phenotypes that develop after a period of normal development, including lordokyphosis, hair loss, skin lesions, amyloidosis, and early death. There are nine senescence-prone inbred strain mice SAMP(1–9) and four senescence-resistant inbred mice SAMR(1–4) (Takeda et al., 1994). The SAMP mice are short-lived and have double the aging features when compared to the SAMR mice at 8 months of age (Takeda, 1997; Takeda et al., 1994).

Each SAM strain has a distinct strain-specific phenotype and genetic identity, though mice in the same family (SAMP or SAMR) have similar biochemical and immunogenic markers (Kitado, 1994). Genetic studies of the SAM mice reveal that several endogenous provirus genes, polytropic murine leukemia virus 25 (*Pmv-25*), modified polytropic murine leukemia virus 17 (*Mpmv-17*), and modified polytropic murine leukemia virus 29 (*Mpmv-29),* are found exclusively in the SAMP strains, and polytropic murine leukemia virus 35 (*Pmv-35*) is found exclusively in the SAMR strains (Kitado, 1994).

The SAMP8 mice have a shortened life-span, learning and memory deficits, features of AMD, and no signs of brain atrophy (Kawamata et al., 1997). As AMD is an age-related phenomenon, another strain of SAM mice with a longer life-span, SAMR1 mice, have age-related retinal pathology reminiscent of AMD (Sarks, 1976; Majji et al., 2000). Neuronal cell loss was investigated in SAMP1 and SAMR1 mice at various time points compared to control mice. By 25 months of age, SAMR1 mice had near complete loss of the photoreceptor layer peripherally and significant loss of the photoreceptor and ganglion cells centrally (Shoji et al., 1998). The SAMP1 strain has been shown to have age-related changes in the RPE and Bruch's membrane (Ogata et al., 1992; Takada et al., 1994; Takada et al., 1993). The RPE cells in SAM mice have swelling of basal infoldings and accumulation of lipofuscin granules that increase with age (Ogata et al., 1992). In SAMP8 mice, early disruption of the RPE basal microvilli begins at 8 months of age and progresses to severe RPE degeneration by 12 months of age (Majji et al., 2000). In Bruch's membrane, fragmentation between the elastic and outer collagenous layers occurs with age (Ogata et al., 1992). As the SAMP1 and SAMP8 mice age, deposition of fine fibrils in Bruch's membrane increases, resulting in a four-fold increase in thickness of Bruch's membrane by 8 months of age (Takada et al., 1993; Majji et al., 2000). BlamD, amorphous deposits in the sub-RPE space on the RPE side of Bruch's membrane, were also found (Majji et al., 2000). There is also an age-related increase in the thickness of the choriocapillaris basement membrane, which was found to have anti-type IV collagen antibodies by gold particle labeling (Takada et al., 1993; Takada et al., 1994). Intra-Bruch's membrane choroidal neovascularization was also seen in 40% of the SAMP8 mice older than 11 months of age (Majji et al., 2000).

4. Summary

The molecular pathology of dry AMD has demonstrated a role of oxidative stress, inflammation, and metabolic dysregulation (Ding et al. 2009). Pathologic hallmarks of dry AMD include drusen accumulation (BlamD and BlinD), Bruch's membrane thickening, RPE lipofuscin accumulation followed by degeneration, and photoreceptor degeneration and atrophy. Although mice do not have a macula and lipofuscin extrusion follows a different process, mice have been the models for retinal disease because the mouse retina closely resembles the human nasal and peripheral retina. The described murine models of dry AMD have drusenoid lesions and RPE atrophy on fundoscopy, ERG abnormalities, A2E accumulation, sub-RPE basal deposits, and Bruch's membrane thickening (Table 2). One of

the key advantages of murine models is the ability to relatively easily manipulate mice genetically, surgically, pharmacologically and via changes in their diet and environment.

The aforementioned dry AMD murine models fall into three categories: genetically engineered mice, immunologically manipulated mice, and naturally occurring mouse strains. Most murine models are genetically engineered to manipulate genes that cause macular degeneration-like disease in humans or are relevant to AMD pathogenesis. Inflammatory genes *Cfh*, *Ccl2*, *Ccr2*, and *Cx3cr1*, oxidative stress associated genes *Sod1* and *Sod2*, and metabolic pathway genes *Neprilysin*, *mcd*, *Cp*, *Heph*, *ApoE* and *ApoB100* have been manipulated to create models with features of dry AMD (Figure 3). The immunologically manipulated mouse model was immunized against CEP, an endogenous biomarker of oxidative stress, and its phenotype consisted of immune dysregulation and complement deposition as seen in AMD. The naturally occurring mouse models include retinal degeneration models with mutations in *Mdm1*, *Mfrp*, *Nr2e3*, and *Gnat2* with significant photoreceptor pathology and the senescence-accelerated mouse (SAM) models with systemic and retinal age-related pathology, including Bruch's membrane thickening, basal deposits, and CNV. These models contrast with the murine models of wet AMD that are mostly induced mechanically.

5. Future directions and challenges

AMD is a challenging disease to study because of its complex genetics, late onset, and confounding environmental risk factors. As with other human diseases, finding a model system for AMD has been a demanding task. Working with primates, the only mammals with a macula, may have scientific advantages. Aged primates have macular degeneration that presents in the late 20s with soft drusen and an immunophenotype of drusen very similar to that of humans (Umeda et al., 2005). Monkey models of both late-onset macular degeneration and early-onset macular degeneration are bred in Japan, and the genetics of affected monkeys is being studied vigorously with the hope of identifying new AMD-related genes (Umeda et al., 2005). Primate research is often difficult, given a limited supply of animals, economic constraints, the slow course of disease, and ethical challenges in making genetically manipulated monkeys.

Mice, while very amenable to genetic manipulation, diverged from humans about 65 million years ago. The study of immune responses in mice has brought great insight to human immunology. Despite the obvious differences in size and lifespan, sequencing of mouse and human genomes reveals great conservation of function and only about 300 unique genes (Waterston et al., 2002). The general structure of the immune system in mice and humans is also very similar, though discrepancies in both the innate and adaptive immune system exist (Haley, 2003; Mestas and Hughes, 2004). While many biologic paradigms translate between the species, the trend toward more specific and sophisticated therapeutics makes it very important to understand the limitations of the extrapolation of mouse research to human disease.

In spite of the great advances made in mouse immunology in the past fifty years, few observations made in the murine system have made a clinical impact. Therapies that have been shown to work in mice have failed in human trials, and therapies that do not work in mice have shown success in humans (Shepherd and Sridhar, 2003; Panitch et al., 1987). Several reasons for these discrepancies exist. Inbred mouse strains are excellent for experimentation; however, results seen in these mice may be different than those seen in genetically diverse outbred strains. An outbred mouse population would be more similar to a human population, but these mice are neither easily available nor amenable to large-scale scientific experimentation. On the other hand, there are successful therapies for human disease that arose from mouse models. Indeed, the usage of cyclosporine, rapamycin, or daclizumab for uveitis is based on its effectiveness

in experimental autoimmune uveitis (Nussenblatt, 2002; Martin et al., 1995; Nussenblatt et al., 1983).

An important role for mouse models in AMD research is to serve as a preclinical test of safety and efficacy of new therapeutic options. Recently, human embryonic stem cell (hESC)-derived RPE cells were subretinally transplanted into the *ELOVL4*-mutant mouse and rats with retinal degeneration (RSC rats). These mice were followed for a prolonged period (>220 days) and shown to recover visual function and photoreceptor integrity without complications (Lu et al., 2009). We have also reported that high omega-3 diet ameliorates retinal AMD-like lesions in $Ccl2^{-/-}/Cx3cr1^{-/-}$ mice (Tuo et al., 2009). Mouse models with pathology that is similar to AMD at the ultrastructural level allow for robust preclinical studies of novel AMD therapeutics.

Mouse models will likely play a critical role in the future of AMD research, as they may answer many specific questions at the molecular level, potentially providing further insight into the pathogenesis of this disease. Data from mouse experiments has generated significant basic scientific progress and has set the stage for several new clinical trials for dry AMD. A detailed understanding of the differences between mouse and human biology and pathology will help guide and improve the efficacy of future research therapeutic trials. Ultimately, meaningful advances in AMD research are made when the distance from the “bedside to the bench” shrinks. With greater access to human samples for basic research, scientists and physicians will be able to further understand the pathophysiology of AMD in humans and make meaningful mouse models and therapies based on clinical and scientific insights.

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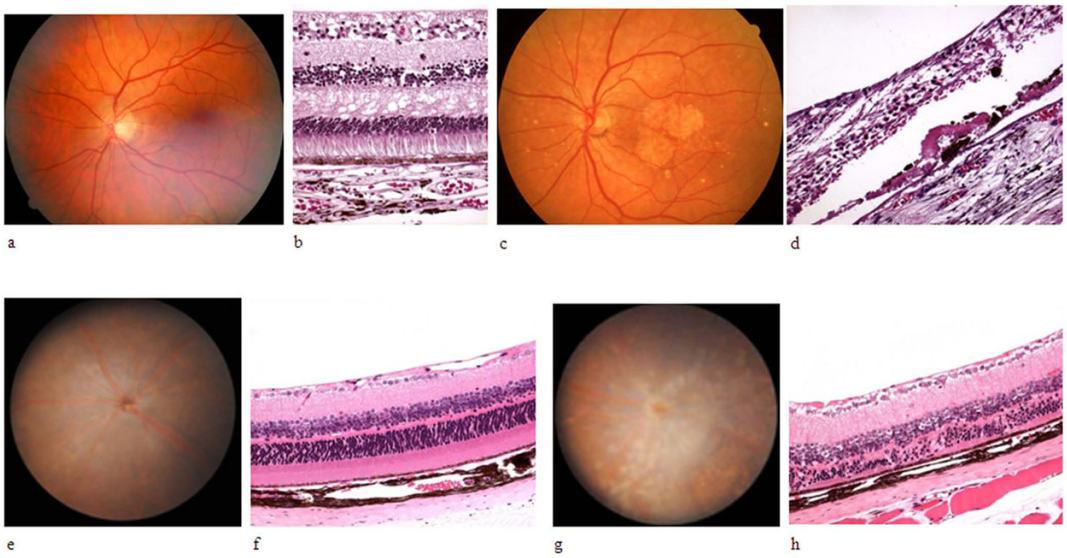

Figure 1. A comparison of the mouse and human retina and AMD-like pathology

a) A normal human fundus, b) light microscope histologic picture of the normal human maculae, c) the fundus of a patient with dry AMD with drusen and scarring in the maculae, d) light microscope histologic picture of a patient with dry AMD, e) A normal mouse fundus, f) light microscope histologic picture of the normal mouse retina, g) fundus picture of a 3 month-old *Ccl2*$^{-/-}$/*Cx3cr1*$^{-/-}$ mouse with drusenoid deposits and a retinal scar, h) light microscope histologic picture of a 3 month-old *Ccl2*$^{-/-}$/*Cx3cr1*$^{-/-}$ mouse with RPE hyperpigmentation and hypertrophy and photoreceptor outer segment disorganization and atrophy

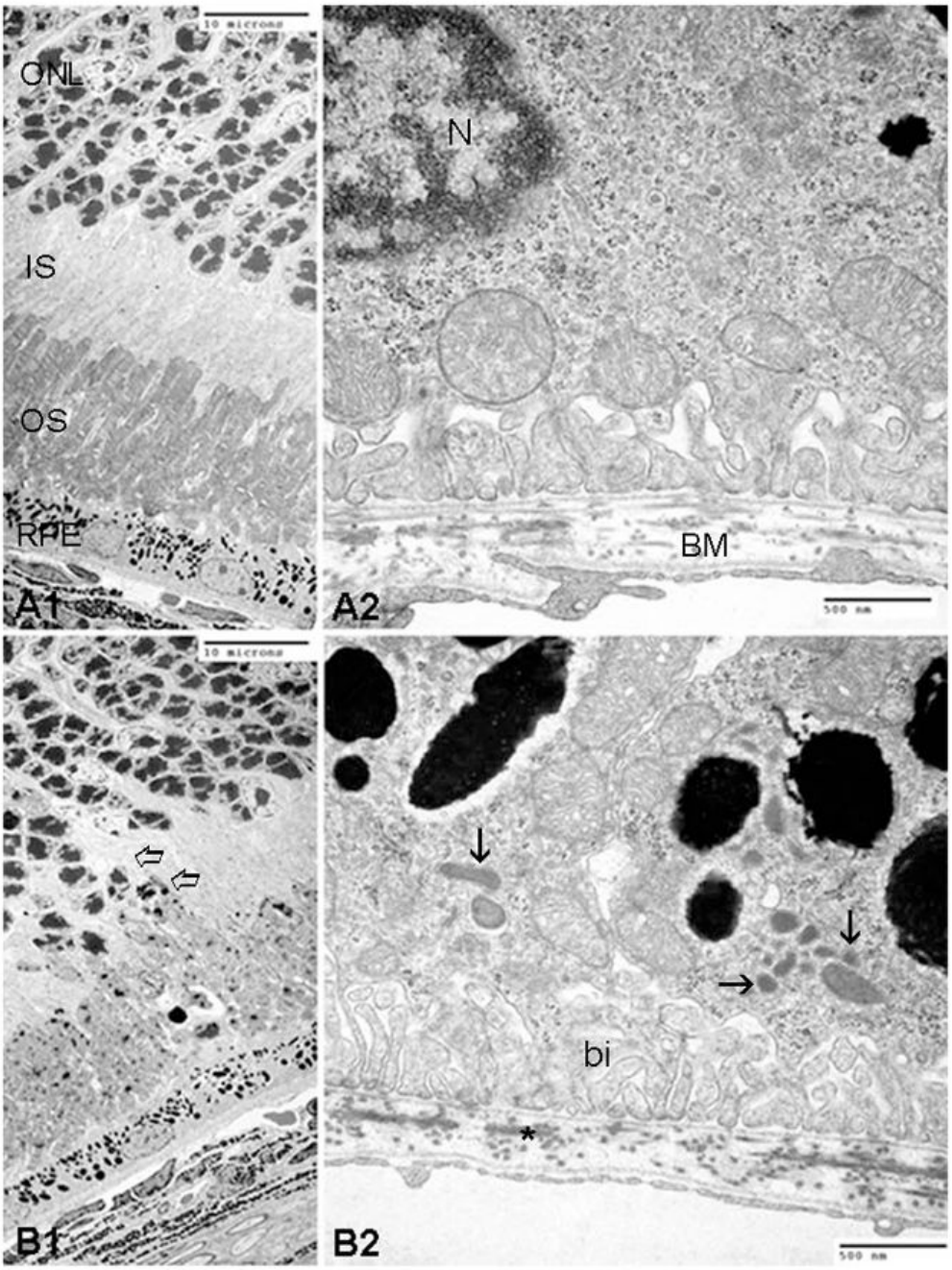

Figure 2.

Transmission electron micrographs illustrated outer retinal degeneration in a 21-days old *Ccl2*$^{-/-}$/*Cx3cr1*$^{-/-}$ mouse. Comparing with WT mice (A1–2), *Ccl2*$^{-/-}$/*Cx3cr1*$^{-/-}$ mice revealed disorganization and migration of photoreceptors (open arrow in B1), rich lipofuscin (arrows in B2) and more basal infoldings (bi in B2) in RPE, as well as higher electron-dense of elastic layer in BM (asterisk in B2). Scale bars, 10 μm (A1, B1); 500 nm (A2, B2). (ONL, outer nuclear layer or photoreceptor nuclei; IS, inner segments of photoreceptors; OS, outer segments of photoreceptors; RPE, retinal pigment epithelial cells; BM, Bruch's membrane)

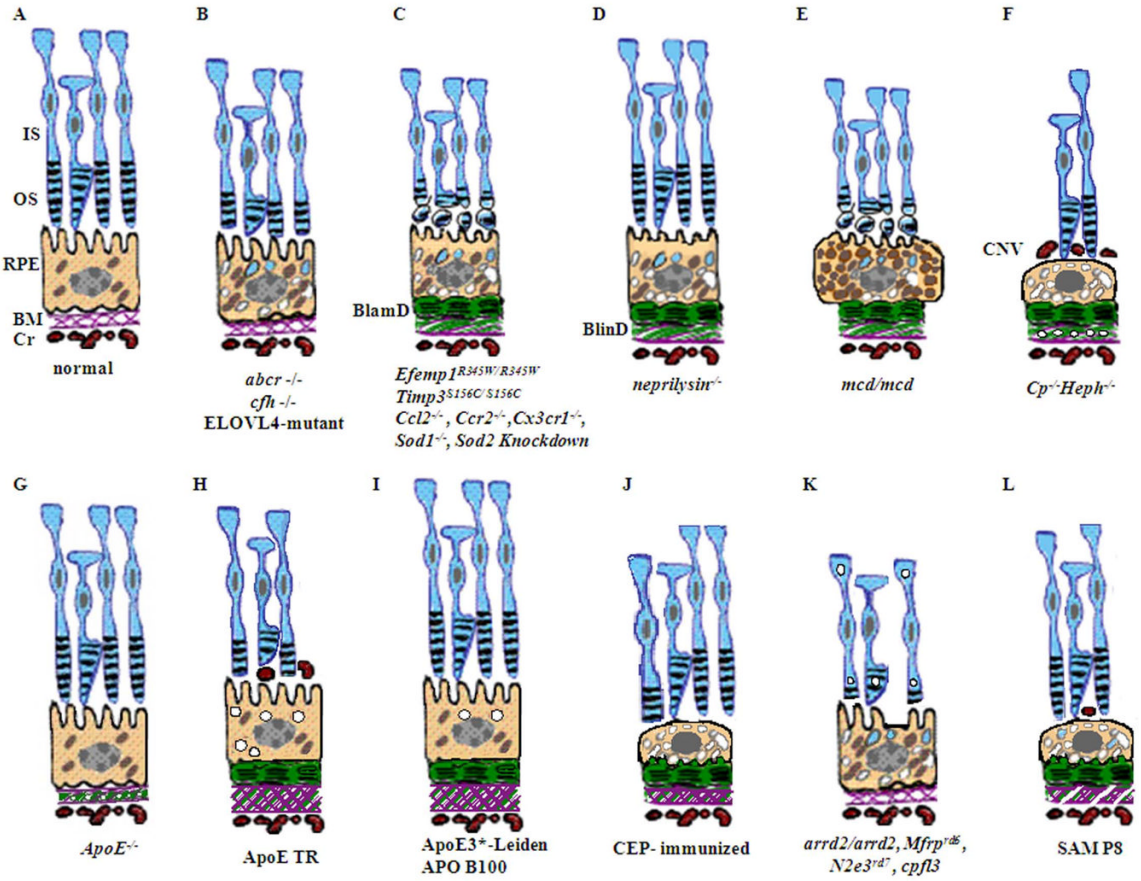

Figure 3.

Diagrams of classic ultrastructural changes in the photoreceptor, RPE, Bruch's membrane and choroid in different mouse AMD models (IS, photoreceptor inner segment; OS, photoreceptor outer segment; RPE, retinal pigment epithelial cells; BM, Bruch's membrane; Cr, Choroid; BlamD, basal laminar deposits; CNV, choroidal neovascularization)

Table 1

Genetics of dry AMD models. This table presents a summary of the mouse and human genetics of the transgenic, immunologically modified, and naturally occurring dry AMD murine models and related diseases.

No.	Mouse model	Human Disease association	Human Gene	Chromosome Location	OMIM #	Genetic Modification	Mouse gene(s)	Encoding protein(s)	Reference(s)
1	*abcr−/−*	Stargardt disease	*ABCA4*	1p21-p13	*601691	knock-out	*Abca4*	RmP	Weng et al., 1999; Mata et al., 2000
2	*ELOVL4*-mutant	Stargardt-3 dominant inheritary disease	*ELOVL4*	6q14	*605512	transgenic	*Elovl4*	Elov4	Karan et al., 2005
3	*Efemp1*$^{R345W/R345W}$	Doyne honeycomb retinal dystrophy	*EFEMP1*	2p16	*601548	transgenic	*Efemp1*	fibulin-3	Marmorstein et al., 2007; Fu et al., 2007
4	*Timp3*$^{S156C/S156C}$	Sorsby fundus dystrophy	*TIMP3*	22q12.1-q13.2	*188826	knock-in	*Timp3*	TIMP3	Weber et al., 2002
5	*cfh*$^{-/-}$	AMD	*CFH*	1q32	+134370	knock-out	*Cfh*	CFH	Coffey et al., 2007
6	*ccl2*$^{-/-}$	coronary artery disease, tuberculosis	*CCL2 (MCP1)*	17q11.2-q12	+158105	knock-out	*Mcp1*	MCP1	Ambati et al., 2003
7	*ccr2*$^{-/-}$	atherosclerosis, rheumatoid arthritis	*CCR2*	3p21	*601267	knock-out	*Ccr2*	MCP1 receptor	Ambati et al., 2003
8	*cx3cr1*$^{-/-}$	AMD, coronary artery disease, HIV	*CX3CR1*	3pter-p21	*601470	knock-out	*Cx3cr1*	Fractalkine receptor CX3CR1	Combadiere et al., 2007
9	*ccl2*$^{-/-}$/*cx3cr1*$^{-/-}$	coronary artery disease	*CCL2, CX3CR1*	17q11.2-q12, 3pter-p21	+158105, *601470	double knock-out	*Mcp1, Cx3cr1*	MCP1, CX3CR1	Tuo et al., 2007
10	*sod1*$^{-/-}$	amyotrophic lateral sclerosis	*SOD1*	21q22.1	*147450	knock-out	*Sod1*	SOD1	Imamura et al., 2006
11	*sod2*$^{-/-}$	Leber's hereditary optic neuropathy, idopathic cardiomyopathy	*SOD2*	6q25.3	*147460	knock-down	*Sod2*	SOD2	Sandbach et al., 2001; Justilien et al., 2007
12	*neprilysin* $^{-/-}$	Alzheimer's disease	*NEPRILYSIN*	3q21-q27	*120520	knock-out	*Neprilysin*	Amyloid β	Iwata et al., 2001
13	*mcd/mcd* mice	neuronal ceroid lipofuscinosis	*CTSD*	11p15.5	*116840	transgenic	*mcd*	Cathepsin D	Rakoczy et al., 2002
14	*Cp*$^{-/-}$/*Heph*$^{-/Y}$	aceruloplasminemia	*CP*	3q23-q24	*117700	knock-out	*Cp, sla*	ferroxidase ceruloplasmin/hepaestin	Hahn et al., 2004
15	*ApoE*$^{-/-}$	hyperlipoproteinemia	*APOE*	19q13.2	+107741	knock-out	*ApoE*	ApoE	Dithmar et al., 2000
16	APO*E3-Leiden	hyperlipoproteinemia	*APOE*	19q13.2	+107741.0015	transgenic	-	apoE3	Kliffen et al 2002
17	ApoE4 TR	Type III/V hyperlipoproteinemia, Alzhimer's disease	*APOE*	19q13.2	+107741.0016	transgenic	-	apoE4	Malek et al., 2005
18	APO B100	hypobetalipoproteinemia	*APOB*	2p24	+107730	transgenic	-	apoB100	Espinosa-Heidmann et al. 2004; Callow et al. 1994
19	*arrd2/arrd2*	progressive rod-cone degeneration, AMD	*MDM1*	12q14.3	*164785	none	*Mdm1*	Mdm1	Chang et al., 2008
20	*Mfrp*^{rd6}	retinitis pigmentosa	*MFRP*	11q23	*606227	none	*Mfrp*	MFRP	Kameya et al., 2002
21	*Nr2e3*^{rd7}	Enhanced S-cone syndrome (ESCS)	*NR2E3*	15q23	*604485	none	*Nr2e3*	NR2E3	Ahkmedov et al., 2000
22	*cpfl3*	achromotopsia	*GNAT2*	1p13	+139340	none	*Gnat2*	GNAT2	Chang et al., 2006
23	SAMP	aging	unknown	unknown	unknown	unknown	*Mpmv, Pmv*	unknown	Takada et al., 1994
24	SAMR	aging	unknown	unknown	unknown	unknown	*Pmv-35*	unknown	Majji et al., 2000; Takada et al., 1994

Table 2

Retinal pathology of dry AMD models. This table presents a summary of the clinical, biochemical, and retinal pathologic findings in dry AMD murine models.

No.	Mouse model	Clinical information			Biochemical data		Pathologic information				
		Age of onset	Clinical exam	Type of lesion	ERG changes	A2E level	RPE changes	Basal deposits	BM changes	Photoreceptor atrophy	CNV
1	*abcr−/−*	44 w	not xamined	-	reduced *a*-wave mplitude	elevated	lipofuscin, melanosomes vacuolization,	none	thickening	shortening of the PR OS	none
2	*ELOVL4*-mutant	7 mo	retinal lesions	RPE atrophy	reduced *b*-wave responses	elevated	lipofuscin, debris, central retinal RPE atrophy	present	none	OS disk disorganization, geographic atrophy	none
3	*Efemp1*$^{R345W/R345W}$	4 mo	no lesions	none	normal *a*-waves and *b*-waves	not measured	vacuolization, loss of basal infoldings	BlamD	with amorphous debris	None	none
4	*Timp3*$^{S156C/S156C}$	8 mo	no lesions	none	normal *b*-waves	not measured	basal layer disruption	present	thickening	None	present
5	*cfh*$^{-/-}$	2 yrs	retinal lesions	hyperfluorescent	reduced *a*-wave and *b*-wave amplitudes	not measured	lipofuscin, melanosomes, disorganized organelles	decreased	thinning	disorganized PR OS	
6	*Ccl2*$^{-/-}$	9 mo	retinal lesions	drusenoid	not performed	elevated	vacuolated, lipofuscin, menalosomes	present	thickening	pyknotic PR, geographic atrophy	present
7	*Ccr2*$^{-/-}$	9 mo	retinal lesions	drusenoid	not performed	elevated	hypopigmentation, loss of basal infolding	present	thickening	ONL atrophy, geographic atrophy	present
8	*Cx3cr1*$^{-/-}$	12 mo	retinal lesions	drusenoid	not performed	not measured	intracellular lipid deposits and phagosomes	present	none	outer retinal thinning, progressive degeneration	only after laser injury
9	*Ccl2*$^{-/-}$/*Cx3cr1*$^{-/-}$	4–6 w	retinal lesions	drusenoid	reduced *a*-wave and *b*-wave amplitudes	elevated	hypopigmentation, vacuolization, lipofuscin, melanosome loss --> degeneration	present	thickening	OS disorganization and atrophy	present
10	*Sod1*$^{-/-}$	7 mo	retinal lesions	drusenoid	not performed	not measured	vacuolization, degeneration	present	thickening	ISand OS loss	present
11	*Sod2* knockdown	4 mo	not examined	-	reduced *a*-wave and *b*-wave amplitudes	elevated	lipofuscin, hypopigmentation, basal lamina deterioration, vacuolization, mitochondrial abnormalities	BlamD	thickening	disorganized PR OS, inner retinal thinning	none
12	*neprilysin* $^{-/-}$	27 mo	not examined	-	not performed	not measured	vacuolization, loss of tight and adherens junctions, distorted basal infolding, degeneration	BlamD and BlinD	none	none	none; VEGF upregulation, PEDF downregulation
13	*mcd/mcd* mice	12 mo	retinal lesions	RPE pigmentary changes and drusenoid deposits	reduced *a*-wave and *b*-wave amplitudes	not measured	hypertrophy, hypopigmentation, and attenuation	BlamD and BlinD	none	ONL thinning, INL disorganization	none
14	*Cp*$^{-/-}$/*Heph*$^{-/Y}$	5–6 mo	no lesions	none	not performed	not measured	lipofuscin, lysosomes, endosomes, and phagosomes, hypopigmentation; RPE hypertrophy, hyperplasia, necrosis	present	vacuolization	focal areas of PR loss, progressing to eventual loss of IS, OS, and ONL thinning	present

No.	Mouse model		Clinical information		Biochemical data			Pathologic information			
		Age of onset	Clinical exam	Type of lesion	ERG changes	A2E level	RPE changes	Basal deposits	BM changes	Photoreceptor atrophy	CNV
15	*ApoE*$^{-/-}$	2 mo	not examined	none	not performed	not measured	normal	BlinD	vacuolization	none	none
16	ApoE4 TR	65 wk-127 wks	not examined	drusenoid	not performed	not measured	vacuolization, hyperpigmentation, hypopigmentation, atrophy, disorganized basal infoldings none detected;	BlamD	thickening	thinning of the ONL	present
17	APO*E3-Leiden	9 mo	not examined	-	not performed	not measured	however, unable to assess	BlamD	thickening	none	none
18	APO B100	3 mo	not examined	-	not performed	not measured	occasional vacuolization	BlamD	thickening	none	none
19	CEP-immunized mouse	3 mo after immunization	retinal lesions	patchy reticular changes	not performed	not measured	vesiculation, pyknosis, lysis, and areas of RPE loss	BlamD	thickening	swollen PR	only after laser injury
20	*arrd2/arrd2*	4 mo	retinal lesions	RPE hyper- and hypopigmentation vessel attenuation	reduced amplitude, ultimately extinguished waves	not measured	phagosomes, loss of apical processes, hypopigmentation, atrophy	none	none	INL and ONL thinning, necrotic synapses, OS fragmentation	none
21	*Mfrp*^{rd6}	3 mo	retinal lesions	drusenoid	reduced amplitude, ultimately extinguished waves	not measured	lipofuscin	none	none	PR degeneration in all layers	none
22	*Nr2e3*^{rd7}	1 mo	retinal lesions	drusenoid	reduced *a*-wave and *b*-wave amplitudes	not measured	apical villi retardation	none	none	ONL dysplasia with whorls and rosettes, progressive thinning	none
23	*cpfl3*	8 mo	no lesions	none	reduced photopic response	not measured	none	none	none	vacuolization, PR OS shortening	none
24	SAMP8	8 mo	not examined	-	not performed	not measured	lipofuscin, swelling of basal infoldings, microvilli disruption, severe degeneration	BlamD	thickening, fragmentation	none	present

Abbreviations: RPE, retinal pigment epithelium; CNV, choroidal neovascularization; ERG, electroretinography; BM, Bruch's membrane; mo, month; A2E, N-retinylidene-N-retinylethanolamine; BlamD, basal laminar deposits; BlinD, basal linear deposits; PR, photoreceptors; OS, outer segment; ONL, outer nuclear layer; IS, inner segment; VEGF, vascular endothelial growth factor; PEDF, pigment epithelium-derived factor