The Pathogenesis of Dry Age-related Macular Degeneration

Lessons learned from animal models.

BY FERNANDO CRUZ-GUILLOTY, PHD; JJ ECHEGARAY; AND VICTOR L. PEREZ, MD

Age-related macular degeneration (AMD) is the leading cause of blindness in developed countries.[1] It is a continual challenge for ophthalmologists and vision researchers to develop new, effective therapeutic regimens that can target the disease in its early stages and prevent progression to severe irreversible blindness.

AMD is commonly divided into two main subtypes: dry AMD, a form of slowly progressive geographic atrophy of the macula, and wet AMD, which rapidly progresses to blindness and involves abnormal formation of blood vessels in the macula, a process known as choroidal neovascularization (CNV). It is believed that the pathology of AMD starts with the chronic, slowly progressing dry form and later develops into the more severe wet form rendering the patient blind.

In the past decade, several chemotherapy agents have been identified as effective drugs to treat wet AMD, reverse vessel formation, and improve vision. However, adequate treatment for dry AMD is lacking. Laser-induced CNV is a widely used animal model of wet AMD in a variety of species, but such an acute injury may not share mechanisms with long-term disease progression.[2] The complexity of pathologic changes associated with dry AMD makes it more difficult to study. A number of both genetic and induced animal models representative of dry AMD have been developed with the prospect of mapping the pathologic mechanisms that trigger the onset of disease. Uncovering these mechanisms would enable the development of both innovative diagnostic assays to detect initial stages of the disease and targeted therapies focused on prevention and early treatment.

Clinical funduscopic examination showing yellow spots called drusen in the areas proximal to the fovea or macula is a diagnostic feature of dry AMD. Histopathologic features of dry AMD include atrophy and loss of retinal photoreceptors and retinal pigment epithelium (RPE), deposition of drusen between the RPE and Bruch membrane along the chorioretinal interphase, and accumulation of lipofuscin (A2E).

Most animal models of dry AMD are based on findings from human studies and therefore attempt to mimic the human histopathology of retinal degeneration.

Most animal models of dry AMD are based on findings from human studies and therefore attempt to mimic the human histopathology of retinal degeneration. These animal models, which are most commonly murine, present either one of the aforementioned histologic features or a combination of them. Although the mouse retina lacks a macula, the vast amount of genetic and biologic manipulations available in the mouse provides researchers with multiple cost-efficient opportunities to dissect the various histologic and clinical manifestations of dry AMD and achieve interdisciplinary study of such a complex disease. For instance, many studies have shown that inflammatory processes are involved in the progression of disease, with the presence of acute-phase proteins, complement deposition, macrophage and microglial infiltration, and cytokine expression in affected tissue.

The aim of this article is to review and delineate the

TABLE 1. KNOWN DRY AMD MOUSE MODELS

Mouse Model	Model Classification	Age of Onset	Most Common Lesions
abcr-/-	Genetic	week 44	Melanosome-phagosome fusion particles, thickening apical RPE and Bruch membrane, lipofuscin granules
ELOVL4	Genetic	2-7 months	RPE vacuolization, undigested outer segments in the subretinal space, pigment granule deposits
Efemp1-R345W	Genetic	4 months	RPE vacuolization, loss of RPE basal infolding organization, activated levels of C3 in the RPE
Timp3S156C/S156C	Genetic	8 months	RPE cell microvilli disruption
Cfh-/-	Inflammatory Gene	12-24 months	Thinning of Bruch membrane, reduced drusen deposition
Ccl2-/- and Ccr2-/-	Inflammatory Gene	9 months	Subretinal drusen deposits, Bruch membrane thickening, ECM disruption, photoreceptor pyknosis, RPE vacuolization, CNV
Cx3cr-/-	Inflammatory Gene	12 months	Subretinal microglial infiltration, retinal thinning
Ccl2-/-/Cx3cr1-/-	Inflammatory Gene	4-6 weeks	Drusenoid deposits, retinal atrophy, RPE vacuolization, lipofuscin depostis, C3d deposition, macrophage infiltration
CEP	Immunology	40-60 days post-immunization	RPE cell hypertrophy and vacuolization, inflammatory cell infiltration, RPE cell lysis, C3d deposition, Bruch membrane thickening
Sod1-/-	Oxidative Stress Gene	7 months	Drusenoid deposits, RPE vacuolization
Sod2 knockdown	Oxidative Stress Gene	4 months	RPE vacuolization and atrophy, thickening of Bruch membrane
ApoE-/-	Metabolic	2 months	RPE vacuolization

known dry AMD mouse models (Table 1). In these models, either a genetic mutation is associated with the development of disease (genetic models) or the disease is induced by exogenous stimulation (induced models).

GENETIC MODELS

Inflammatory gene models. The discovery of activated complement factor proteins in drusen from AMD patients[3] opened the door to the study of immunologic factors involved in AMD. Likewise, genetic polymorphisms within the complement factor H (CFH) gene were found to be associated with development of AMD.[4-7] Several murine models have been developed to dissect the possible mechanisms underlying disease progression in AMD. One of them is the Cfh-/- mouse model.[8] Homozygosity of Cfh genes in humans leads to complement pathway deregulation, while homozygous mice show uncontrolled C3 activation and accumulation in the retina and disorganization of the photoreceptor outer segment. However, contrary to AMD pathological findings, Cfh-/- mice show thinning of Bruch membrane and reduced drusen deposition instead of membrane thickening and increased drusen. However, it has been reported that transgenic mice carrying human CFH mutations can develop AMD-like phenotypes.[9]

There are also models that study the role of inflammatory macrophages in the development of AMD. By genetically altering macrophage recruitment, cytokine signaling, and expression, the Ccl2-/- and Ccr2-/- murine models have both shown histopathologic findings that mimic AMD as early as 9 months of age.[10] The basis behind these models is that Ccl2 (the macrophage chemoattractant protein-1, or MCP-1) binds to its receptor (Ccr2) and serves as the basis for inflammatory signaling involving these cells. Aberrant signaling in this model correlates with subretinal drusen deposits, Bruch membrane thickening, and extracellular matrix (ECM) disruption at

In addition to the immunologic AMD models, there are several genetic models that represent other similar retinal degenerative diseases.

9 months of age. With senescence, these mice develop geographic atrophy of the RPE, photoreceptor pyknosis, RPE vacuolization, and CNV. The latter features are found in mice 16 to 24 months of age.[10]

Retinal microglial cells also play a role in AMD.[11] These cells express the CX3C chemokine receptor 1 (CX3CR1), and homozygosity of the CX3CR1 M280 and V249I alleles is associated with AMD development.[12,13] Twelve-month-old Cx3cr-/- mice show subretinal infiltration of microglia that contain outer segment lipids, and these cells show signs of intracellular drusen-like material with age. Interestingly, combined Ccl2-/-/Cx3cr1-/- mice show accelerated AMD-like pathology that progresses to spontaneous CNV in approximately 15% of compound mutants.[14] Together, these data point to a role for both microglia and macrophages in AMD pathogenesis.

Models testing oxidative stress-associated and lipid metabolism genes. The in vivo model of immune-mediated retinal degeneration described by our group[15] is based on the link between oxidative damage and lipid metabolism. In an attempt to resist a highly oxidative environment, the retina counters with several antioxidant mechanisms, among which superoxide dismutase (SOD) is the most prevalent.[16] Therefore, Sod1-/- mice and Sod2 knockdown murine models were established to test retinal tissue susceptibility to oxidative damage by eliminating these protective pathways. Sod1-/- mice develop drusenoid deposits at around 7 months of age and develop a thicker Bruch membrane with RPE vacuolization by 12 months.[17] The Sod2 knockdown mice start to show earlier AMD-like changes earlier than the Sod1-/- mice, presenting with RPE vacuolization, RPE atrophy, and thickening of Bruch membrane by 4 months of age.[18] These mice later develop basal laminar deposits in the RPE. These findings shed some light on the role of oxidative damage in the development of AMD and stress the importance of retinal susceptibility to oxidative processes as an important precipitating factor in the onset of disease.

Dysregulation of lipid metabolism serves as a starting point for many common diseases such as hypercholesterolemia and atherosclerosis. Lipid deposition is a common risk factor of AMD, and drusen studies have confirmed the presence of apolipoprotein E (ApoE) in these deposits. Because ApoE aids in the metabolism of neural lipids and their repair, the already existing ApoE-/- murine model was used to explore the effects of ApoE deficiency on the retina. These mice showed early changes of mild RPE vesiculation at 2 months, while ApoE-deficient mice who were fed high fat diets showed more AMD-like pathologic features with age, such as Bruch membrane thickening, basal infoldings, and sub-RPE deposits.[19] Taken together, these models provide crucial connections among lipid metabolism, oxidative stress, and the immune system in a complex molecular network associated with AMD development.

Genetic models of retinal disorders similar to dry AMD. In addition to the immunologic AMD models described above, there are several genetic models that represent other similar retinal degenerative diseases. Data obtained from these models may yield valuable information regarding the various mechanisms of disease as well as associations between AMD and other conditions. One of these models features mice with mutations in the abcr gene, which codes for the Rim glycoprotein in outer segments; this model serves as a model for Stargardt disease (STGD), a recessive form of macular degeneration that presents in early childhood with development of macular RPE atrophy and progressive loss of central vision.[20] Abcr-/- mice show pathologic findings at 44 weeks of age that feature accumulation of melanosome-phagosome fusion particles, thickening in apical RPE and Bruch membrane, and an increase in baseline lipofuscin granules and A2E levels in both abcr-/- and abcr+/- mice when compared with wild type (WT) mice.[21,22] This model showed no evidence of drusen development at the time points examined.

The elongation of very long chain fatty acids-4 (ELOVL4) mutant model is also used to study AMD-like pathology because it mimics Stargardt-like macular degeneration (STGD3), an autosomal dominant disease that usually arises in the second decade of life with loss of both central vision and color vision.[23] While homozygous Elovl4-deficient mice are embryonic lethal, transgenic expression of a human mutant form of ELOVL4 (TG E_mut+/-) yields RPE vacuolization, undigested outer segments in the subretinal space, and pigment granule deposits as early as 2 months of age.[24] Significant increases in A2E levels and lipofuscin granules are also seen at 4 months and 7 months, respectively. A 5-base-pair deletion within Elovl4 leads to progressive photoreceptor degeneration at a range of 6 to 18 months of age.[25]

Another autosomal dominant maculopathy, Doyne honeycomb retinal dystrophy, presents with drusen in the macula in young adults and progresses to atrophy, neovascularization, and loss of vision by the fifth and sixth decades of life.[26] This maculopathy has been shown to be caused by an R345W mutation in the EGF-contain-

ing fibrillin-like ECM protein 1 (EFEMP1) gene, which encodes fibulin-3 of the ECM proteins expressed in epithelial basement membranes[27] and has been implicated in AMD pathogenesis.[26] Although Efemp1-/- mice do not show any AMD-like features, the Efemp1-R345W point mutation knock-in mouse has been shown to have RPE vacuolization, loss of RPE basal infolding organization, activated levels of C3 in the RPE, and deposition of collagenous debris at as early as 4 months of age.[28,29]

The Timp3S156C/S156C murine model is used to study Sorsby fundus dystrophy (SFD), a hereditary, late-onset manifestation of retinal degeneration.[30] Mutations in tissue inhibitor of metalloproteinases-3 (TIMP-3) have been shown to have a role in ECM composition.[31] Although SFD patients share many histopathologic findings with AMD patients, such as RPE deposition of ECM debris, Bruch membrane thickening, RPE atrophy, and neovascularization, the Timp3S156C/S156C model has shown only RPE cell microvilli disruption; it lacks other cardinal features of dry AMD.

INDUCED MODELS

Immunology models. Recent data suggest that, in addition to the innate immune compartment (including macrophages and complement), adaptive immune responses (such as autoantibody production) may also be involved in the onset and progression of AMD.[2] A purely immune-mediated model of AMD has presented a promising opportunity to study retinal degeneration from this perspective.[15] In studies of humans with AMD, carboxyethylpyrrole (CEP) adducts were found in greater numbers in patient with drusen.[32] In addition, higher titers of anti-CEP autoantibodies in patient plasma were detected in AMD patients that in age-matched control subjects.[32,33] CEP is an oxidative byproduct of the fatty acid docosahexaenoic acid (DHA). Because DHA is found mostly in the retina, a tissue that is itself highly susceptible to oxidative damage and light exposure, this model directly tests the effect of immunization of WT mice with CEP-modified self-antigens compared with immunization controls.[15] Immunized mice feature AMD-like pathology such as RPE cell hypertrophy and vacuolization, inflammatory cell recruitment in the subretinal space and outer segments, and RPE cell lysis in young mice. Because these features have been shown to persist through time in the immunized mice, this model may serve as an effective way to decrease the threshold of inflammatory processes that put retinal tissue at risk of developing RPE cell dysfunction and retinal degeneration at early time points after immunization, providing a short-term model to study this age-related disease. Currently, a nonhuman primate model using this approach, which would more realistically mirror the human disease, is being developed by our lab and collaborators. Research in this area may lead to the identification of molecular mechanisms underlying the immunologic processes of early-onset AMD.

Light-induced AMD models. Currently, in the standard animal model of CNV for most treatment evaluation experiments, the laser-induced AMD mouse/rat model,[34-39] a krypton laser (blue green or green wavelength) is used to create breaks in Bruch membrane and facilitate development of CNV. Use of a krypton laser with modified settings, including a smaller spot size, higher intensity, and shorter duration (blue green or green wavelength, 50 mm, 50 to 350 to 400 mW, 0.05 s) in C57BL6 mice resulted in a higher frequency (100%) of histologic evidence of CNV.[34]

A recent model studying cyclic light-induced oxidative damage[40] involves exposure of albino rats to 12 hours of 3000-lux cyclic light, resulting in microscopic sub-RPE neovascularization at 1 month, extension of the neovascularization into the outer retina at 3 months, and progression to extensive neovascularization that involves retinal vessels at 6 months. This model also shows some of the hallmarks of dry AMD such as retinal atrophy and drusen-like deposits. This model provides the opportunity to study progression of CNV without mechanical stimulation of the retinal tissue.

Smoking-induced AMD model. In addition to age, smoking is widely known to be a risk factor for AMD.[41,42] To test the effects of cigarette smoke in the development of AMD pathology, a mouse model in which animals are exposed to cigarette smoke was established.[43,44] Affected mice show pathologic traits of dry AMD, such as RPE loss, thickening of Bruch membrane, sub-RPE debris deposition, and accumulation of debris within Bruch membrane at 4 to 5 months of age.

CONCLUSION

The etiology of age-related macular degeneration is a hot topic in vision research, and the challenges of this disease are being addressed from multiple perspectives. It is increasingly clear that both innate and adaptive immunity are involved in the development of AMD. The recent success of inflammatory gene models and immunology-based studies, as well as genetic studies in humans, linking AMD pathogenesis with various components of the immune system, has shed light on the possibility that AMD is a multifactorial disease that is initiated with an abnormal inflammatory process or an error in the regulatory pathways that keep these inflammatory events in check.

The animal models reviewed in this article can enable researchers to dissect the mechanisms of disease at early stages of AMD to help develop innovative therapies that can allow clinicians to detect the disease early, prevent loss of vision, and restore vision in patients who are already in later stages of AMD. We believe that immunoregulatory strategies will make an efficient transition to the clinic in the not-so-distant future. ■

Fernando Cruz-Guilloty, PhD, and J.J. Echegaray are with the Bascom Palmer Eye Institute of the University of Miami Miller School of Medicine.

Victor L. Perez, MD, is an Associate Professor Ophthalmology, Microbiology and Immunology, at the Bascom Palmer Eye Institute of the University of Miami Miller School of Medicine. Dr. Perez reports no financial relationships as they pertain to the content of this article. He can be reached via e-mail at vperez4@med.miami.edu.

1. Javitt JC, Zhou Z, Maguire MG, Fine SL. Willke RJ. Incidence of exudative age-related macular degeneration among elderly Americans. *Ophthalmology*. 2003;110:1534-1539.
2. Morohoshi K, Goodwin AM, Ohbayashi M, Ono SJ. Autoimmunity in retinal degeneration: autoimmune retinopathy and age-related macular degeneration. *J Autoimmun*. 2009;33: 247-254. doi:S0896-8411(09)00117-6 [pii] 10.1016/j.jaut.2009.09.003.
3. Crabb JW, Miyagi M, Gu X, et al. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2002;99,14682-14687.
4. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science*. 2005;308:385-389.
5. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science*. 2005;308:419-421.
6. Hageman GS, Anderson DH, Johnson LV, et al. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2005;102:7227-7232.
7. Edwards AO, Ritter R 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. *Science*. 2005;308:421-424.
8. Coffey PJ, Gias C, McDermott CJ, et al. Complement factor H deficiency in aged mice causes retinal abnormalities and visual dysfunction. *Proc Natl Acad Sci U S A*. 2007;104:16651-16656. doi:0705079104 [pii] 10.1073/pnas.0705079104.
9. Ufret-Vincenty RL, Aredo B, Liu X, et al. Transgenic mice expressing variants of complement factor H develop AMD-like retinal findings. *Invest Ophthalmol Vis Sci*. 2010;51(11):5878-5887. doi:iovs.09-4457 [pii] 10.1167/iovs.09-4457.
10. Ambati J, Anand A, Fernandez S, et al. An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. *Nat Med*. 2003;9: 1390-1397. doi:10.1038/nm950nm950 [pii].
11. Ma W, Zhao L, Fontainhas AM, Fariss RN, Wong WT. Microglia in the mouse retina alter the structure and function of retinal pigmented epithelial cells: a potential cellular interaction relevant to AMD. *PLoS One*. 2009;4:e7945. doi:10.1371/journal.pone.0007945.
12. Combadière C, Feumi C, Raoul W, et al. CX3CR1-dependent subretinal microglia cell accumulation is associated with cardinal features of age-related macular degeneration. *J Clin Invest*. 2007;117:2920-2928. doi:10.1172/JCI31692.
13. Tuo J, Smith BC, Bojanowski CM, et al. The involvement of sequence variation and expression of CX3CR1 in the pathogenesis of age-related macular degeneration. *FASEB J*. 2004:18:1297-1299. doi:10.1096/fj.04-1862fje04-1862fje [pii].
14. Tuo J, Bojanowski CM, Zhou M, et al. Murine ccl2/cx3cr1 deficiency results in retinal lesions mimicking human age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2007;48:3827-3836. doi:48/8/3827 [pii]10.1167/iovs.07-0051.
15. Hollyfield JG, Bonilha VL, Rayborn ME, et al. Oxidative damage-induced inflammation initiates age-related macular degeneration. *Nat Med*. 2008;14:194-198. doi:nm1709 [pii]10.1038/nm1709.
16. Valentine JS, Doucette PA, Zittin Potter S. Copper-zinc superoxide dismutase and amyotrophic lateral sclerosis. *Annu Rev Biochem*. 2005;74:563-593. doi:10.1146/annurev.biochem.72.121801.161647.
17. Imamura Y, Noda S, Hashizume K, et al. Drusen, choroidal neovascularization, and retinal pigment epithelium dysfunction in SOD1-deficient mice: a model of age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2006;103:11282-11287. doi:0602131103 [pii]10.1073/pnas.0602131103.
18. Justilien V, Pang JJ, Renganathan K, et al. SOD2 knockdown mouse model of early AMD. *Invest Ophthalmol Vis Sci*. 2007;48:4407-4420. doi:48/10/4407 [pii]10.1167/iovs.07-0432.
19. Malek G, Johnson LV, Mace BE, et al. Apolipoprotein E allele-dependent pathogenesis: a model for age-related retinal degeneration. *Proc Natl Acad Sci U S A*. 2005;102:11900-11905. doi:0503015102 [pii]10.1073/pnas.0503015102.
20. Walia S, Fishman GA. Natural history of phenotypic changes in Stargardt macular dystrophy. *Ophthalmic Genet*. 2009;30:63-68. doi:910511475 [pii]10.1080/13816810802695550.
21. Mata NL, Tzekov RT, Liu X, Weng J, Birch DG, Travis GH. Delayed dark-adaptation and lipofuscin accumulation in abcr+/- mice: implications for involvement of ABCR in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2001;42:1685-1690.
22. Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH. Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice. *Cell*. 1999;98:13-23. doi:S0092-8674(00)80602-9 [pii]10.1016/S0092-8674(00)80602-9.
23. Stone EM, Nichols BE, Kimura AE, Weingeist TA, Drack A, Sheffield VC.. Clinical features of a Stargardt-like dominant progressive macular dystrophy with genetic linkage to chromosome 6q. *Arch Ophthalmol*. 1994;112:765-772.
24. Karan G, Lillo C, Yang Z, et al. Lipofuscin accumulation, abnormal electrophysiology, and photoreceptor degeneration in mutant ELOVL4 transgenic mice: a model for macular degeneration. *Proc Natl Acad Sci U S A*. 2005;102:4164-4169. doi:0407698102 [pii]10.1073/pnas.0407698102.
25. Vasireddy V, Jablonski MM, Mandal MN, et al. Elovl4 5-bp-deletion knock-in mice develop progressive photoreceptor degeneration. *Invest Ophthalmol Vis Sci*. 2006;47:4558-4568. doi:47/10/4558 [pii]10.1167/iovs.06-0353.
26. Stone EM, Lotery AJ, Munier FL, et al. A single EFEMP1 mutation associated with both Malattia Leventinese and Doyne honeycomb retinal dystrophy. *Nat Genet*. 1999;22:199-202. doi:10.1038/9722.
27. Timpl R, Sasaki T, Kostka G, Chu ML. Fibulins: a versatile family of extracellular matrix proteins. *Nat Rev Mol Cell Biol*. 2003;4:479-489. doi:10.1038/nrm1130nrm1130 [pii].
28. Marmorstein LY, McLaughlin PJ, Peachey NS, Sasaki T, Marmorstein AD. Formation and progression of sub-retinal pigment epithelium deposits in Efemp1 mutation knock-in mice: a model for the early pathogenic course of macular degeneration. *Hum Mol Genet*. 2007;16:2423-2432. doi:ddm199 [pii]10.1093/hmg/ddm199.
29. Fu L, Garland D, Yang Z, et al. The R345W mutation in EFEMP1 is pathogenic and causes AMD-like deposits in mice. *Hum Mol Genet*. 2007;16:2411-2422. doi:ddm198 [pii]10.1093/hmg/ddm198.
30. Weber BH, Lin B, White K, et al. A mouse model for Sorsby fundus dystrophy. *Invest Ophthalmol Vis Sci*. 2002;43:2732-2740.
31. Weber BH, Vogt G, Pruett RC, Stohr H, Felbor U. Mutations in the tissue inhibitor of metalloproteinases-3 (TIMP3) in patients with Sorsby's fundus dystrophy. *Nat Genet*. 1994;8:352-356. doi:10.1038/ng1294-352.
32. Gu X, Meer SG, Miyagi M, et al. Carboxyethylpyrrole protein adducts and autoantibodies, biomarkers for age-related macular degeneration. *J Biol Chem*. 2003;278:42027-42035.
33. Gu J, Pauer GJ, Yue X, et al. Assessing susceptibility to age-related macular degeneration with proteomic and genomic biomarkers. *Mol Cell Proteomics*. 2009;8:1338-1349.
34. Tobe T, Okamoto N, Vinores MA, et al. Evolution of neovascularization in mice with overexpression of vascular endothelial growth factor in photoreceptors. *Invest Ophthalmol Vis Sci*. 1998;39:180-188.
35. Seo MS, Kwak N, Ozaki H, et al. Dramatic inhibition of retinal and choroidal neovascularization by oral administration of a kinase inhibitor. *Am J Pathol*. 1999;154:1743-1753.
36. Frank RN, Das A, Weber ML. A model of subretinal neovascularization in the pigmented rat. *Curr Eye Res*. 1989;8:239-247.
37. Espinosa-Heidmann DG, Suner I, Hernandez EP, Frazier WD, Csaky KG, Cousins SW. Age as an independent risk factor for severity of experimental choroidal neovascularization. *Invest Ophthalmol Vis Sci*. 2002;43:1567-1573.
38. Espinosa-Heidmann DG, Suner IJ, Hernandez EP, Monroy D, Csaky KG, Cousins SW. Macrophage depletion diminishes lesion size and severity in experimental choroidal neovascularization. *Invest Ophthalmol Vis Sci*. 2003;44:3586-3592.
39. Bora PS, Sohn JH, Cruz JM, et al. Role of complement and complement membrane attack complex in laser-induced choroidal neovascularization. *J Immunol*. 2005;174:491-497. doi:174/1/491 [pii].
40. Albert DM, Neekhra A, Wang S, et al. Development of choroidal neovascularization in rats with advanced intense cyclic light-induced retinal degeneration. *Arch Ophthalmol*. 2010;128:212-222. doi:128/2/212 [pii]10.1001/archophthalmol.2009.395.
41. Jager RD, Mieler WF, Miller JW. Age-related macular degeneration. *N Engl J Med*. 2008;358:2606-2617. doi:358/24/2606 [pii]10.1056/NEJMra0801537.
42. Klein R. Overview of progress in the epidemiology of age-related macular degeneration. *Ophthalmic Epidemiol*. 2007;14:184-187. doi:782472683 [pii]10.1080/09286580701344381.
43. Espinosa-Heidmann DG, Suner IJ, Catanuto P, Hernandez EP, Marin-Castano ME, Cousins SW. Cigarette smoke-related oxidants and the development of sub-RPE deposits in an experimental animal model of dry AMD. *Invest Ophthalmol Vis Sci*. 2006;47:729-737. doi:47/2/729 [pii]10.1167/iovs.05-0719.
44. Fujihara M, Nagai N, Sussan TE, Biswal S, Handa JT. Chronic cigarette smoke causes oxidative damage and apoptosis to retinal pigmented epithelial cells in mice. *PLoS One*. 2008;3:e3119. doi:10.1371/journal.pone.0003119.