

**REVIEW***Clinical advice you can trust*

**Kimberly A. Howes, PhD, Wolfgang Baehr, PhD Salt Lake City, and Krzysztof Palczewski, PhD Cleveland**

PUBLISHED 14 SEPTEMBER 2006

## **Dry AMD: From Theory to Treatment**

**Effective treatment options for dry AMD are not currently available, but genetic studies are showing promise.**

Late stage age-related macular degeneration is classified as dry or wet according to characteristic features of these two pathogenic modes. The dry form, which in its end stage is termed geographic atrophy, is characterized by localized regions of atrophy of the retinal pigment epithelium with associated overlying regions of photoreceptor cell death. The wet form, also called neovascular AMD, involves the growth of new blood vessels from the choroid capillary network across the Bruch's membrane/RPE interface into the neural retina, which results in retinal detachment, subretinal and intraretinal edema, and scarring.

While these late stages show different pathologies, early stages of AMD show similarities in pathogenesis. RPE cells form the outer blood-retinal barrier and provide essential photoreceptor support functions. Dysfunction in the RPE layer is clinically manifested as altered pigmentation and lipofuscin accumulation within the macular region. Drusen accumulation between the RPE and Bruch's membrane and alterations in structural and biochemical properties of Bruch's membrane are similarly associated with aging and early-stage AMD. However, events responsible for the initial insult to the retina and progression to late-stage AMD are not currently understood.<sup>1,2</sup>

Within the last few years, there have been some exciting advancements in treating the wet form of AMD, including verteporfin photodynamic therapy and development of anti-vascular endothelial growth factor (VEGF) compounds, such as pegaptanib sodium (Macugen, OSI/Eyetech) and ranibizumab (Lucentis, Genentech).<sup>3,4</sup> Effective treatment options for dry AMD are not currently available, however.

AMD has been classified as a complex disease with multiple genetic and environmental risk factors. Risk factors for AMD include increased sunlight exposure, Caucasian race, female gender, increased dietary fat and smoking. Mutations in ApoE, ABCA4, FBLN5 and HEMICENTIN-1 genes have been reported to contribute, in part, to AMD progression.<sup>5-12</sup> Early evidence of a genetic component for AMD was reported in a classical study showing hard and soft drusen formation correlated with 57 percent increased AMD risk in monozygotic (identical) twins and 81 percent increased risk in dizygotic (maternal) twins. However, until recently, genetic factors predisposing a large population of patients to AMD and pointing to a central mechanistic pathway in the pathophysiology of this disease have remained obscure. More recent genetic studies are closing this gap.

### **AMD Pathology**

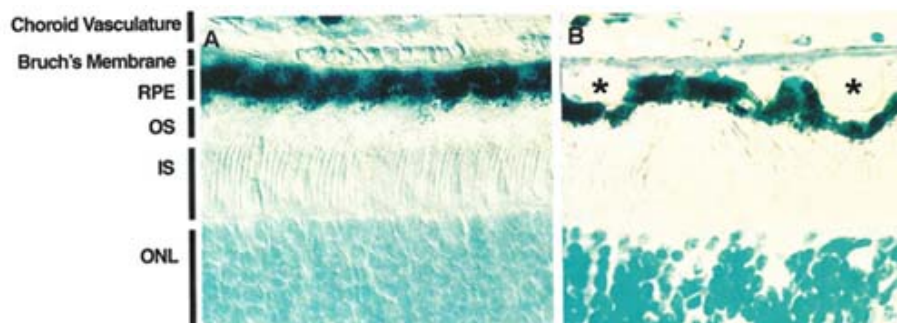


**Figure.1.** Histology from a 58-yr-old donor (A) showing normal Bruch's membrane, retinal pigment epithelium pigmentation, and photoreceptor layers (OS = outer segments, IS = inner segments, ONL = outer nuclear layer(photoreceptor nuclei). (B) An 89-year-old donor with drusen (asterisks), thickening of Bruch's membrane (circled), and RPE pigmentation abnormalities (arrow). Methyl green nuclear stain.

Molecular properties of the choroid/ Bruch's membrane/RPE axis and subsequent perturbations occurring during aging have been extensively studied in order to identify the mechanistic pathways responsible for AMD pathogenesis. Alterations in pigmentation, accumulation of toxic lipofuscin pigments, and formation of drusen, which are extracellular deposits that accumulate between the RPE and Bruch's membrane, are all hallmark features of AMD (See Figure 1).

The RPE plays a critical role in the support of photoreceptor cells. In addition to providing a selective cellular filtration component to the outer blood-retinal barrier, the RPE directly aids the photoreceptor by enzymatic re-isomerization of the visual chromophore and ensuring the daily turnover of photoreceptor shed disc membranes.<sup>13</sup> These roles have been verified by identification of mutations in RPE genes affecting these functions, which result in photoreceptor degeneration. Lipofuscin in the RPE is a byproduct of incomplete outer segment digestion. These highly autofluorescent retinoids accumulate (See Figure 2) when the aging RPE is unable to completely digest the disc membranes.

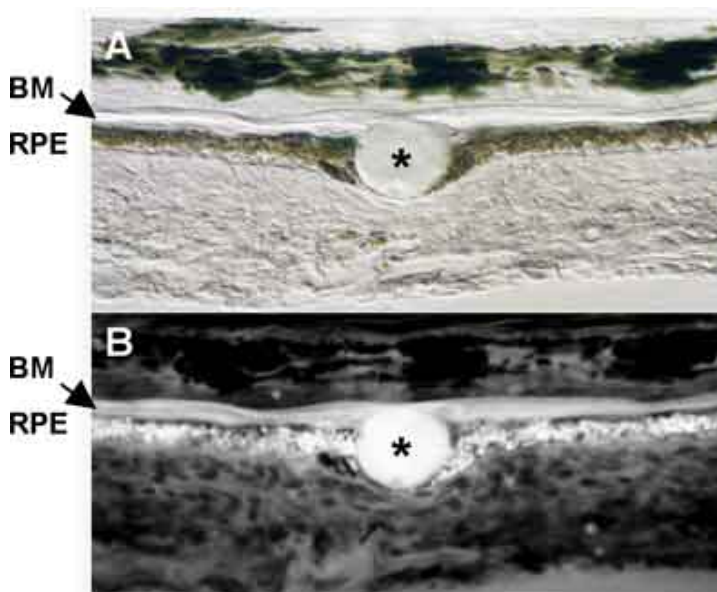


**Figure 2.** A. Brightfield image of a human retina from an elderly donor. B. Autofluorescent image from the same section using a FITC filter set. Note that RPE cells, drusen (\*) and Bruch's membrane (BM) are autofluorescent.

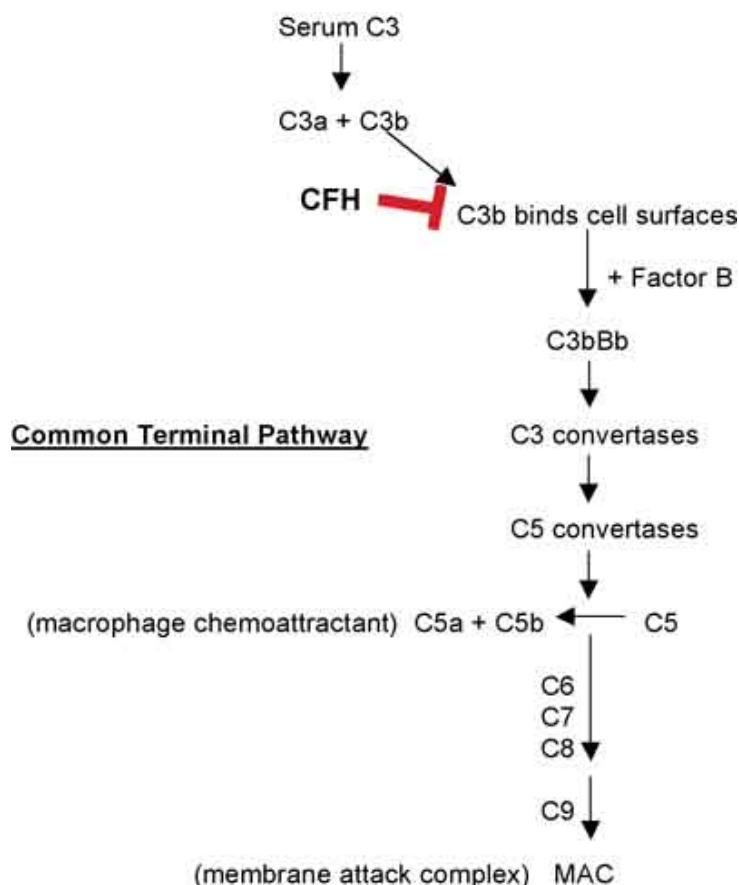
The production of one such lipofuscin fluorophore, A2E, is a marker for RPE dysfunction and is significantly associated with AMD pathogenesis.<sup>14</sup> A2E is a downstream condensation product of all-trans-retinol with phosphatidylethanolamine (PE). A fraction of all-trans-retinol is conjugated to PE within the disc membranes for transfer across the disc membrane by the photoreceptor-specific retinal receptor/transporter encoded by the ABCA4 gene

and subsequent conversion to all-trans-retinol by all-trans-retinol dehydrogenase.<sup>13</sup> Conditions that favor increased levels of all-trans-retinol and decreased conversion to all-trans-retinol lead to accumulation of A2E. A2E accumulation in RPE cells destabilizes membranes, induces mitochondrial release of proapoptotic factors, inhibits proper degradation of ingested photoreceptor outer segments, and sensitizes cells to blue-light damage, all of which result in RPE damage and cell death.<sup>15</sup> Mutation in gene ABCA4 is associated with Stargardt's macular degeneration. Mice with genetic disruption of ABCA4 develop Stargardt-like retinal degeneration, including RPE accumulation of lipofuscin and A2E, and RPE and photoreceptor atrophy.

Drusen, composed of cellular debris and modified proteins and lipids from the RPE and Bruch's membrane, are associated with aging and believed to predispose the retina to disease progression. Drusen are subcategorized into both hard and soft forms, with the soft form conferring a significantly higher risk for AMD progression. Studies show compositional similarities between drusen and amyloid deposits, atherosclerotic plaques and dense deposits in atherosclerosis, amyloidosis, elastosis, and dense deposit disease associated with membranoproliferative glomerulonephritis type II.<sup>16-18</sup> Studies show that molecular and cellular components of drusen are consistent with local cellular (RPE) dysfunction and atrophy.

## Targeting Lipofuscin, A2E Formation

Accelerated lipofuscin and A2E accumulation occurs in patients with AMD and Stargardt macular dystrophy, a juvenile form of macular degeneration sharing many pathological features of AMD, and in mouse models of these retinal disorders. Accumulation of A2E has been associated with RPE dysfunction and atrophy and photoreceptor loss. As such, recent efforts have centered on targeting A2E formation in RPE cells as a therapeutic strategy for AMD and AMD-like retinal disorders. In early studies, it was shown that reducing dietary levels of vitamin A (all-trans-retinol) inhibits lipofuscin and autofluorescence accumulation in the RPE.<sup>19</sup> While impractical as a therapy, these results led to developing an alternative approach for inhibiting vitamin A delivery specifically to the retina.

**Alternative Pathway for Complement Activation****Figure.3. The alternative complement activation pathway.**

Treatment of ABCA4<sup>-/-</sup> mice with Accutane (isotretinoin), which inhibits 11-cis-retinol dehydrogenase (the final step in regeneration of the visual chromophore) and perhaps other steps in the visual cycle, has been shown to reduce all-trans-retinal and subsequent formation of light-sensitive oxidation A2E products, A2E oxiranes.<sup>14,20</sup> While doses necessary to achieve these results in humans would be toxic, these results nonetheless showed that reducing all-trans-retinol can inhibit retinal A2E products *in vivo*.

An alternative strategy for reducing retinol levels specifically in the retina was examined.<sup>20</sup> N-(4-hydroxyphenyl) retinamide (HPR) (fenretinide) is a synthetic amide of all-trans-retinoic acid that has been used for decades, most commonly in cancer treatments, to remove serum retinol bound to carrier protein, retinol-binding protein (RBP).<sup>20</sup> While this treatment has relatively few side effects, treated patients reported effects on night vision. It was subsequently shown that while additional retinol-binding proteins can compensate for retinol delivery to other tissues, the eye relies primarily on RBP for its retinol supply.<sup>21</sup> Therefore, the selective effect of HPR treatments on retinol delivery to the eye was the basis for testing the efficacy of this drug on A2E formation in the ABCA4<sup>-/-</sup> Stargardt mouse model. HPR treatments inhibited the formation of RPE lipofuscin and A2E in these mice. However, prolonged treatment studies are needed, and efficacy in inhibiting the degeneration of RPE and photoreceptors has not yet been ascertained. More potent inhibitors of the retinoid (visual) cycle with prolonged drug activities have been identified.<sup>22</sup> These compounds will need to be further developed before human trials can begin.

Alternatively, death of photoreceptor and RPE cells could result from the accumulation of visual pigments uncoupled from the chromophore.<sup>23</sup> Thus, supplementation of the chromophore for visual pigment regeneration might be required.<sup>24</sup> These two individual strategies, or the combination of both, will require direct testing in humans to determine optimal results.

## A New Therapeutic Target

When RPE cells die, debris serves as a "nucleation" site for drusen formation and subsequent tissue inflammatory responses.<sup>25</sup> Proteomic analysis of drusen from normal vs. AMD patients shows evidence of oxidative protein modifications that may contribute to drusen formation and AMD pathogenesis.<sup>26</sup> Drusen formation in the aging eye has been compared to other aging disorders, such as Alzheimer's disease and atherosclerosis, where plaque formation and deposits induce chronic inflammatory stimuli and contribute to disease progression. Complement C3-, C5- and C5-9 activation components have also been identified in drusen.<sup>27,28</sup>

The alternative complement pathway is initiated by the spontaneous hydrolysis of plasma C3 to C3a and C3b (See *Figure 3*).<sup>29</sup> Unregulated C3b binds to cell surfaces, thereby providing a mechanism for complement activation that will proceed unless downregulated by complement regulating proteins. This mechanism is the basis for the alternative pathway of complement activation. Complement factor H (CFH), an essential member of this regulatory pathway, is a plasma glycoprotein that binds to cell surface sialic acids or neutral or anionic polysaccharides, as well as to C3b. This interaction accelerates C3b degradation and inhibits C3 convertase activation. C3b not neutralized by CFH is available for binding to factor B, resulting in activation of the alternative pathway. In brief, C3b stabilization results in C3-convertase activation, converting C3 to C3a and C3b, with subsequent convertase activation to produce C5a (a macrophage chemoattractant) and C5b. C5b, combines with C6, C7, C8, and C9 to form a membrane attack complex (MAC), with C5b-9 in the terminal complement pathway. Therefore, production of C5a and C5b-9 activates, promotes and amplifies inflammatory responses.

## Complement System and AMD

Considerable advances have been made in the last year linking the complement system to AMD. Several laboratories reported a significant association between AMD risk and mutations in the gene for CFH, the important regulator of the alternative complement pathway (See *Figure 3*).<sup>30-33</sup> A tyrosine residue at position 402 replaced by a histidine residue (a T to C substitution at nucleotide 1277 in exon 9) shows allele frequency between 0.61 to 0.94 in AMD cases and 0.34 to 0.46 in age-matched controls (collectively) among these studies. Further, this variant confers a strong risk for soft drusen formation and both dry and wet forms of AMD.<sup>34</sup>

In a recent study, haplotype analysis of patients carrying the Y402H variant who do not develop AMD revealed factor B and complement component 2 gene variants that confer a protective effect, seemingly counterbalancing effects from the CFH variant.<sup>35</sup> These results strongly support a central role for immune system regulation in the pathogenesis of AMD.

In support of these results, Ccl-2 (monocyte chemoattractant protein, MCP-1) and Ccr-2 (MCP-1 receptor) knockout mice develop AMD-like pathology including drusen formation, thickening of Bruch's membrane, lipofuscin and A2E accumulation in RPE cells, and photoreceptor degeneration. Normally, choroidal macrophages are attracted to C5a and IgG deposits in drusen for subsequent degradation of these immune complexes. Therefore, loss of MCP-1 and its receptor by genetic ablation in this mouse model was proposed to impair the recruitment of macrophages, resulting in chronic complement activation and tissue pathogenesis in these mice.<sup>36</sup>

The recent genetic evidence correlating complement factors with AMD risk strongly supports a major inflammatory component for AMD pathogenesis. While previous identification of environmental and genetic risk factors have contributed to our knowledge of retinal aging disorders, recent identification of immune system involvement fills an

important gap in our understanding of the pathogenesis of AMD. In addition, the current research to control the formation of photosensitizing fluorophores such as A2E during aging represents an equally exciting approach for future therapies. Future research will focus on these pathogenic mechanisms in the aging eye. In addition, new strategies can now be developed that specifically address these injurious pathways, and may finally provide a therapeutic option for dry AMD.

*Dr. Howes and Dr. Baehr are at the Moran Eye Center at the University of Utah, and Dr. Palczewski is in the Department of Pharmacology, Case School of Medicine, Case Western Reserve University, Cleveland. Contact Dr. Howes at the Department of Ophthalmology and Visual Sciences, University of Utah Health Science Center, Salt Lake City, Utah 84112. Phone (801) 585-0499; E-mail: kim.howes@hsc.utah.edu.*

1. Klein R, Peto T, Bird A, Vannewkirk MR. The epidemiology of age-related macular degeneration. *Am J Ophthalmol* 2004;137:486-95.
2. Johnson PT, Lewis GP, Talaga KC, et al. Drusen-associated degeneration in the retina. *Invest Ophthalmol Vis Sci* 2003;44:4481-8.
3. D'Amico DJ. Pegaptanib Sodium for Neovascular Age-Related Macular Degeneration: Two-Year Safety Results of the Two Prospective, Multicenter, Controlled Clinical Trials. *Ophthalmology* 2006; 113:1001.e1-6. Epub 2006 Apr 27.
4. Spaide RF, Sorenson J, Maranan L. Combined photodynamic therapy with verteporfin and intravitreal triamcinolone acetonide for choroidal neovascularization. *Ophthalmology* 2003;110:1517-25.
5. Klaver CC, Kliffen M, van Duijn CM, et al. Genetic association of apolipoprotein E with age-related macular degeneration. *Am J Hum Genet* 1998;63: 200-6.
6. Souied EH, Benlian P, Amouyel P, et al. The epsilon4 allele of the apolipoprotein E gene as a potential protective factor for exudative age-related macular degeneration. *Am J Ophthalmol* 1998;125:353-9.
7. Allikmets R, Shroyer NF, Singh N, et al. Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. *Science* 1997;277:1805-7.
8. Allikmets R, Singh N, Sun H, et al. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nat Genet* 1997;15:236-46.
9. Stone EM, Braun TA, Russell SR, et al. Missense variations in the fibulin 5 gene and age-related macular degeneration. *N Engl J Med* 2004;351:346-53.
10. Schultz DW, Klein ML, Humpert AJ, et al. Analysis of the ARMD1 locus: Evidence that a mutation in HEMICENTIN-1 is associated with age-related macular degeneration in a large family. *Hum Mol Genet* 2003;12:3315-23.
11. 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;102:11900-5.
12. Baird PN, Richardson AJ, Robman LD, et al. Apolipoprotein (APOE) gene is associated with progression of age-related macular degeneration (AMD). *Hum Mutat* 2006;27:337-42.
13. McBee JK, Palczewski K, Baehr W, Pepperberg DR. Confronting complexity: The interlink of phototransduction and retinoid metabolism in the vertebrate retina. *Prog Retin Eye Res* 2001;20:469-529.
14. Radu RA, Mata NL, Bagla A, Travis GH. Light exposure stimulates formation of A2E oxiranes in a mouse model of Stargardt's macular degeneration. *PNAS* 2004;101:5928-33.
15. De S, Sakmar TP. Interaction of A2E with Model Membranes. Implications to the Pathogenesis of Age-related Macular Degeneration. *J. Gen. Physiol.* 2002;120:147-57.
16. Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. *Faseb J* 2000;14:835-46.
17. Luibl V, Isas JM, Kaye R, et al. Drusen deposits associated with aging and age-related macular degeneration contain nonfibrillar amyloid oligomers. *J Clin Invest* 2006;116:378-85.
18. Yoshida T, Ohno-Matsui K, Ichinose S, et al. The potential role of amyloid beta in the pathogenesis of age-related macular degeneration. *J Clin Invest* 2005;115:2793-800.

19. Katz ML, Norberg M. Influence of dietary vitamin A on autofluorescence of leupeptin-induced inclusions in the retinal pigment epithelium. *Exp Eye Res* 1992;54:239-46.
20. Radu RA, Han Y, Bui TV, et al. Reductions in serum vitamin A arrest accumulation of toxic retinal fluorophores: A potential therapy for treatment of lipofuscin-based retinal diseases. *Invest. Ophthalmol. Vis. Sci.* 2005;46:4393-401.
21. Vogel S, Piantedosi R, O'Byrne SM, et al. Retinol-binding protein-deficient mice: Biochemical basis for impaired vision. *Biochemistry* 2002;41:15360-8.
22. Golczak M, Kuksa V, Maeda T, et al. Positively charged retinoids are potent and selective inhibitors of the trans-cis isomerization in the retinoid (visual) cycle. *PNAS* 2005;102:8162-7.
23. Fan J, Woodruff ML, Cilluffo MC, et al. Opsin activation of transduction in the rods of dark-reared Rpe65 knockout mice. *J Physiol (Lond)* 2005;568:83-95.
24. Batten ML, Imanishi Y, Tu DC, et al. Pharmacological and rAAV gene therapy rescue of visual functions in a blind mouse model of Leber congenital amaurosis. *PLoS Med* 2005;2:e333.
25. Donoso LA, Kim D, Frost A, et al. The role of inflammation in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol* 2006;51:137-52.
26. Crabb JW, Miyagi M, Gu X, et al. From the Cover: Drusen proteome analysis: An approach to the etiology of age-related macular degeneration. *PNAS* 2002;99:14682-7.
27. Johnson LV, Leitner WP, Staples MK, Anderson DH. Complement activation and inflammatory processes in Drusen formation and age related macular degeneration. *Exp Eye Res* 2001;73:887-96.
28. Mullins RF, Aptsiauri N, Hageman GS. Structure and composition of drusen associated with glomerulonephritis: Implications for the role of complement activation in drusen biogenesis. *Eye* 2001;15(Pt 3):390-5.
29. Thurman JM, Holers VM. The central role of the alternative complement pathway in human disease. *J Immunol* 2006;176:1305-10.
30. Edwards AO, Ritter R, III, Abel KJ, et al. Complement Factor H Polymorphism and Age-Related Macular Degeneration. *Science* 2005;308:421-4.
31. Haines JL, Hauser MA, Schmidt S, et al. Complement Factor H Variant Increases the Risk of Age-Related Macular Degeneration. *Science* 2005;308:419-21.
32. Klein RJ, Zeiss C, Chew EY, et al. Complement Factor H Polymorphism in Age-Related Macular Degeneration. *Science* 2005;308:385-9.
33. Hageman GS, Anderson DH, Johnson LV, et al. From The Cover: A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *PNAS* 2005;102(12):7227-32.
34. Magnusson KP, Duan S, Sigurdsson H, et al. CFH Y402H confers similar risk of soft drusen and both forms of advanced AMD. *PLoS Med* 2006;3:e5.
35. Gold B, Merriam JE, Zernant J, et al. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. *Nat Genet* 2006;38:458.
36. Ambati J AA, Fernandez S, Sakurai E, Lynn BC, Kuziel WA, Rollins BJ, Ambati BK. An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. *Nature Medicine* 2003;9:1390-7.

Copyright © 2024 Jobson Medical Information LLC unless otherwise noted.

All rights reserved. Reproduction in whole or in part without permission is prohibited.