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CARBOXYETHYLPYRROLE PLASMA BIOMARKERS IN AGE-RELATED MACULAR DEGENERATION

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SUMMARY

Age-related macular degeneration causes irreversible central blindness in people over the age of 50 and is increasing in prevalence among elderly populations. There are currently limited treatment options available for the exudative form of the disease and no formal treatments for the geographic atrophy form, aside from lifestyle change and incorporation of antioxidant supplements in the diet. As such, it is important to be able to assess high-risk AMD patients as early as possible in order to prescribe preventive measures. Carboxyethylpyrrole (CEP) is a promising plasma biomarker suited to this purpose. Both CEP immunoreactivity levels, as well as anti-CEP autoantibody titers, are significantly elevated in AMD patients and thus provide the potential to assess AMD susceptibility with approximately 80% accuracy when evaluated alongside genomic AMD markers. Moreover, strong evidence implicates CEP as functionally related to AMD pathogenesis, a role which must be explored further. This avenue of research will foster improved understanding of the disease itself and perhaps reveal better therapeutic targets and options. Further research into the role of CEP in AMD pathogenesis and the application of CEP as an AMD biomarker is merited.

INTRODUCTION

Age-related macular degeneration (AMD) is the world's leading cause of irreversible central blindness in people over the age of 50 (1-3). Since the prevalence of AMD is expected to increase by 50% to approximately 3 million people in the U.S. alone by the year 2020 (4), the disease constitutes a major public health concern. To date, there is no effective cure for the disease and the only available treatments include either diet and lifestyle changes for early-stage patients and anti-vascular endothelial growth factor (anti-VEGF) antibody injection to prevent angiogenesis in exudative AMD cases (5).

Early AMD is characterized by extracellular deposition of debris (drusen) on Bruch's membrane, which separates the retinal pigment epithelium (RPE) from the choriocapillaris. The disease may result in geographic atrophy, in which case areolar loss of the photoreceptor and RPE cells in the macula occurs, or exudative/neovascular AMD, which is characterized by choroidal neovascularization (CNV), retinal exudates and hemorrhages. CNV is only present in 10-15% of total AMD cases, and yet it accounts for severe vision loss and blindness in more than 80% of all AMD patients seen by ophthalmologists (5). Although the disease pathogenesis is not fully understood, it is widely agreed that AMD is an inflammatory disease resulting from different immuno/inflammatory pathways, such as dysregulation of the complement system (6, 7). In humans, several complement pathway genes have been associated with AMD, including *CFH*, *C2* and *C3* (8). Strong genetic associations have also been made with human *HTRA1* (serine protease) and *ARMS2* (mitochondrial protein of unknown function) (8, 9). Nonetheless, AMD is a multifactorial disease with environmental components as well. Specifically, oxidative damage is a significant risk factor, as it has been shown that smoking increases the chance of developing AMD (10) and incorporating antioxidants and zinc into the diet reduces this risk (11).

In the last 10 years, significant progress has been made in establishing plasma biomarkers for use alongside genomic markers to characterize AMD susceptibility (7, 12-14). One promising biomarker, carboxyethylpyrrole (CEP), belongs to the family of 2-(ω-carboxyalkyl)pyrrole adducts. Protein adducts form with reactive fragments generated from oxidation of cellular polyunsaturated fatty acids, such as docosahexaenoic acid (DHA) (Fig. 1) (12, 15). Studies have shown that the use of anti-CEP immunoreactivity measurements in plasma and titers of anti-CEP autoantibody levels provide a good means to assess AMD susceptibility (14), particularly when used alongside genomic markers (13).

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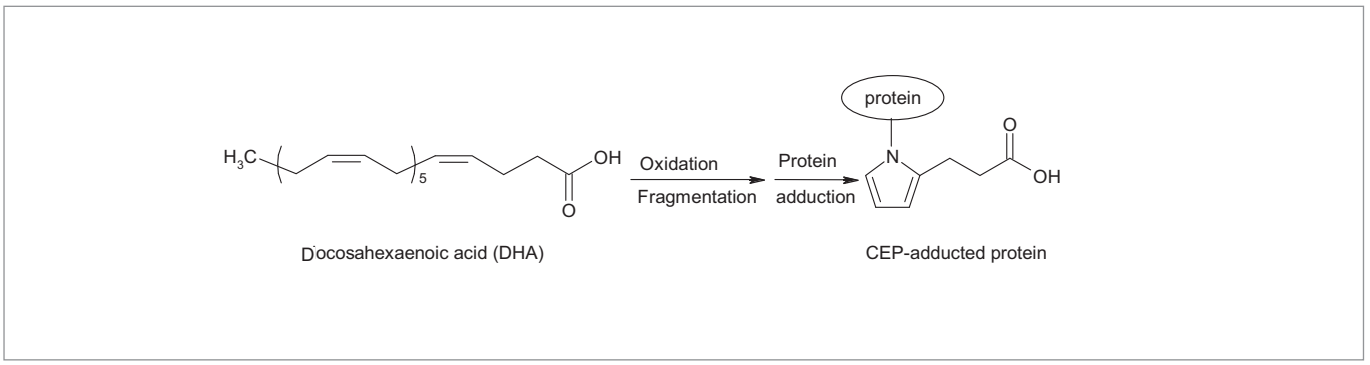

Figure 1. Carboxyethylpyrrole (CEP) structure and formation. Oxidation of DHA creates CEP fragments that react with cellular protein to form CEP adducts.

CEP AS AN AMD BIOMARKER

One of the earliest hallmarks of AMD susceptibility is the accumulation of drusen on Bruch's membrane. Small hard drusen are found in people over the age of 50 and are considered in most cases to be a part of normal aging (5). However, excess and large soft drusen may cause lesions in the RPE and chronic inflammation, and are thus thought to play a role in AMD pathogenesis (6). Proteomic analysis revealed significantly larger quantities of CEP protein adducts in drusen obtained from AMD donors as opposed to those extracted from age-matched normal donors (12). CEP had been previously identified as being uniquely generated from free radical-induced oxidation of DHA, the most oxidizable polyunsaturated fatty acid, comprising ~80 mol % of all lipids in photoreceptor outer segments (16). Considering the great potential for photooxidative stress in ocular tissues, CEP may be an ideal marker for oxidative damage in RPE cells. This stands in direct contrast to other carboxyalkylpyrroles, such as ω-(2-carboxyheptyl)pyrrole (CHP; oxidized from linoleic acid) and ω-(2-carboxypropyl)pyrrole (CPP; oxidized from arachidonic acid), which may be generated due to various other causes (14).

As a consequence of the link between oxidative damage and AMD (10), CEP was tested as a potential marker for AMD susceptibility. Immunocytochemical analysis showed that CEP localizes to photoreceptor rod outer segments and RPE cells in both mouse and human retinas (12, 14). Additionally, anti-CEP immunoreactivity was significantly higher in ocular tissue from human AMD retinas than from healthy donor retinas (12, 14). Gu et al. (14) reported that anti-CEP immunoreactivity in AMD human plasma (n = 19; average age 82 years) was 1.5-fold higher ($P = 0.004$) than in both age-matched controls (n = 19; average age 83 years) and in young controls (n = 9; average age 27 years; $P = 0.05$). Additionally, they found that sera from AMD patients demonstrated mean titers of anti-CEP autoantibody 2.3-fold higher than controls ($P = 0.02$). The autoantibody results were confirmed using a dot blot assay, which revealed 2.5-fold higher anti-CEP autoantibody levels in AMD eyes than in age-matched controls (14). Of the patients in this study who had both elevated anti-CEP immunoreactivity and autoantibody measurements above the non-AMD mean, 92% had AMD. The authors concluded that using both anti-CEP immunoreactivity and autoantibody titer measurements together resulted in the best predictive value for AMD susceptibility. Although statistically significant, the data were nonetheless weak due to a very small sample size.

A later study used a significantly larger sample size (916 AMD and 488 control donors) and determined a 1.6-fold increase in mean plasma anti-CEP immunoreactivity ($P < 0.0001$) and a 1.3-fold increase in mean plasma anti-CEP autoantibody ($P < 0.0001$) titer in AMD donors with respect to control donors (13). This study found similar c-statistic values to those presented by the earlier study (14) when using both anti-CEP plasma immunoreactivity levels and autoantibody titers to predict AMD likelihood (13). Interestingly, proteomic CEP markers alone can distinguish between AMD and normal donors with approximately 76% accuracy and when analyzed together with genomic markers, the discriminatory accuracy increased to about 80%. Donors with *ARMS2*, *HRTA1*, *CFH* and *C3* AMD risk alleles were found to be approximately 5-10 times more likely to have AMD, whereas when coupled with elevated CEP markers the odds ratios increased an additional 2- to-3-fold. Furthermore, donors with AMD risk genotypes at the *ARMS2/HRTA1* loci were about two times more likely to have elevated CEP markers in their plasma, whereas donors with *CFH/C3* risk alleles showed no appreciable increase (13).

Both of the above studies present compelling evidence for the use of CEP plasma biomarkers to predict AMD susceptibility (13, 14), particularly when analyzed alongside genomic markers that have been previously linked to AMD (8, 17-21). However, work remains to be done to define confounding factors surrounding the use of these biomarkers (13). For instance, the authors indicate that smoking seemed to have a relatively low impact on plasma CEP marker levels within both the AMD and control groups (13), which contrasts with the well-established link between smoking and AMD (10, 22, 23). Additionally, the study by Gu et al. determined that both hypertensive and hyperlipidemic patients had slightly increased levels of CEP markers (13), and by and large it seems there is a link between cardiovascular disease and AMD in many population studies (24-28). It is, however, uncertain as to whether or not CEP markers may be affected in this case and further analysis is required in this area. Furthermore, diabetics with AMD exhibited no increase in plasma CEP adducts, whereas those without AMD appeared to have elevated CEP autoantibody levels. Again, further analyses are warranted in these cases, especially due to the small sample size of the study.

Aside from biological confounding factors, importantly, there is as yet no standard method for analyzing patient samples, such as whether or not patients be required to fast, how samples are stored and how they are assayed (13). These varying experimental conditions across studies may have a role in differing and confusing results.

CEP is not the only biomarker available for AMD. Various studies have implicated A2E, carboxymethyllysine (CML) and pentosidine as potential biomarkers for AMD. A2E is found primarily in lipofuscin granules in RPE cells and lipofuscin deposits on Bruch's membrane (29, 30). A2E accumulates progressively with age (31), induces VEGF expression in ARPE-19 cells (29) and has proangiogenic properties in vivo (32), which constitutes it as a possible contributor to wet AMD. However, no studies have yet been performed which attempt to associate A2E levels with humans afflicted with AMD. Plasma protein pentosidine and CML, two advanced glycation end products, have been examined alongside CEP. Both CML and pentosidine were found in significantly higher levels in AMD serum ($P < 0.0001$) in a cohort of 58 AMD and 32 control donors; using CEP adducts in combination with CML and pentosidine increased the discriminatory accuracy of both (7). Being able to use these biomarkers and any others that may be found in the future will prove useful in taking preventive measures for people with a high risk of developing AMD.

A FUNCTIONAL ROLE FOR CEP IN AMD

It is not surprising that CEP biomarkers are useful in assessing AMD susceptibility, because CEP seems to have a functional role in AMD pathogenesis, specifically in the neovascularization process observed in neovascular AMD (Fig. 2) (33). Ebrahem et al. provided convincing evidence that CEP-modified peptides or proteins induce neovascularization in animal models (34). First, in vivo implantation of CEP adduct-containing methylcellulose discs in chick embryos induced angiogenesis similarly to exogenous VEGF implantation. Additionally, rat corneas which received CEP adduct-containing pellets underwent neovascularization in a pattern similar to that induced by VEGF positive controls. Neovascularization was also observed in mouse corneas implanted with CEP pellets, but a differential response was observed in eyes that received pellets containing mixtures of either CEP and anti-CEP or CEP and anti-VEGF antibody. Neovascularization was only attenuated when anti-CEP antibodies were included in the CEP pellet, whereas anti-VEGF antibody addition failed to eliminate new blood vessel growth. Moreover, subretinal injection of CEP-modified proteins into laser-induced CNV mouse models exacerbated the extent of CNV. Based on these data alone, it seems that CEP may independently induce neovascularization, and when considering that ARPE-19 cell cultures treated with CEP do not secrete VEGF, it seems likely that an intercellular signaling cascade involving multiple cell types or pathways could be

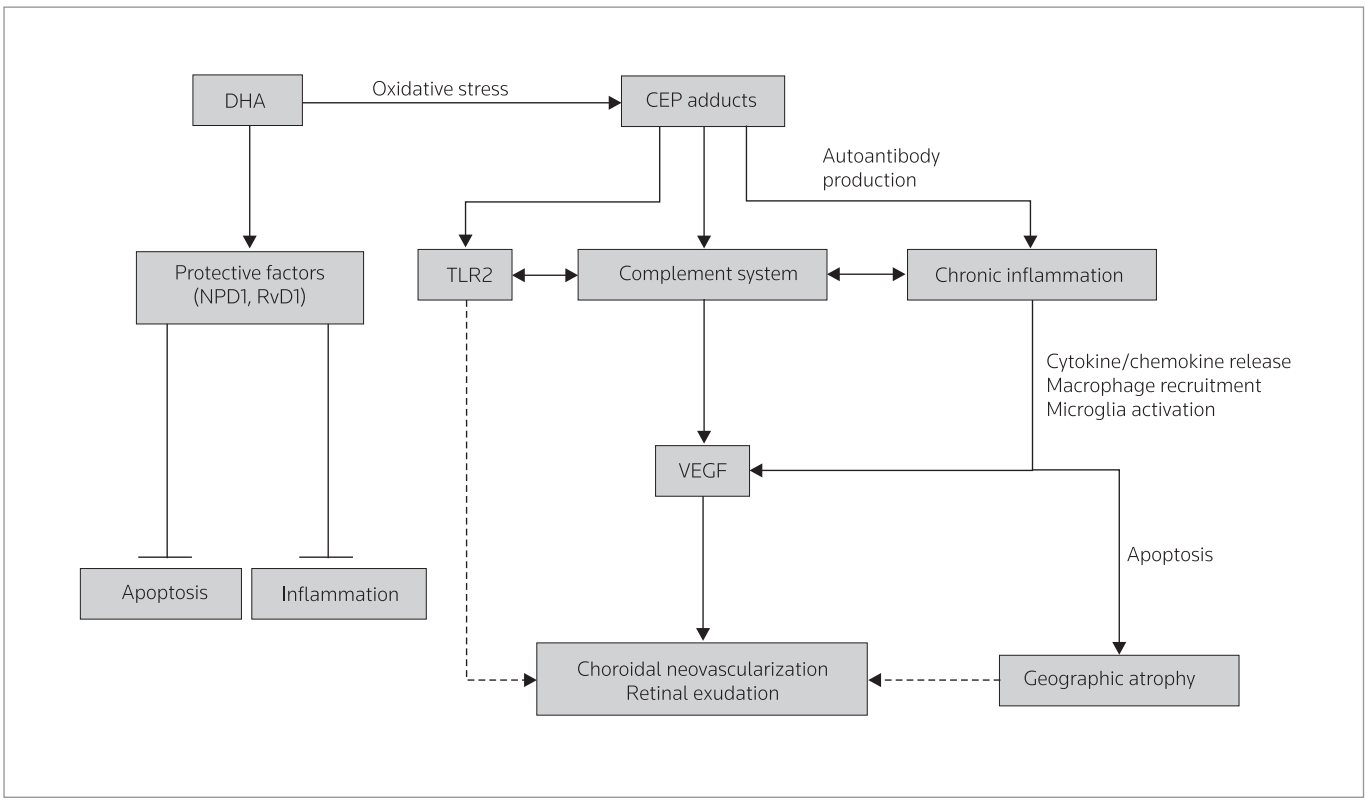

Figure 2. Carboxyethylpyrrole (CEP) functionality in AMD pathogenesis. CEP adducts generated from oxidation of docosahexaenoic acid (DHA) likely activate inflammatory and complement pathways that result in neovascularization and/or geographic atrophy. Neovascularization occurs via vascular endothelial growth factor (VEGF) and another VEGF-independent pathway involving Toll-like receptor 2 (TLR2). Chronic inflammation may also result in geographic atrophy via a macrophage-regulated process, and may even lead to neovascularization over time. DHA normally regulates protective mechanisms in the cell. NPD1, neuroprotectin D1; RvD1, resolvin D1.

involved. Subsequently, CEP was shown to induce angiogenesis via toll-like receptor 2 (TLR2) in a VEGF-independent mechanism in endothelial cells from human umbilical vein, mouse lung and mouse aorta (35). No studies with respect to AMD have been performed.

It must be noted, however, that not all data suggest that CEP plays a role in exudative AMD, as two studies by the same group failed to detect CNV in CEP-immunized mice (36, 37). Mice immunized with CEP-adducted serum albumin develop high autoantibody levels and characteristic dry AMD features, including drusen formation and RPE lesions that mimic geographic atrophy (36). The long-term response to CEP immunization includes thick swelling in retinal tissues, in addition to RPE cell lysis, pyknosis and infiltration of macrophages and lymphocytes. Importantly, the invading cells were reported not to play a role in lesion formation due to the fact that they contained melanin pigment, indicating that they were recruited post-lesion by cytokine secretion in order to clear debris. Additionally, not all lesions were found to have been infiltrated by inflammatory cells (37).

There is no consensus on how CEP works within the AMD disease state. Looking at AMD without considering CEP, one leading hypothesis regarding disease pathogenesis purports that chronic inflammation mediated by dysregulation of complement proteins, M1 and M2 macrophages, and microglia activation leads to geographic atrophy (Fig. 2). CNV development in later AMD stages is explained by observations implicating M2 macrophages to have pro-angiogenic effects (see 38, 39 for a thorough review). Because mice immunized with CEP adducts produce anti-CEP antibody and fix complement component-3 in Bruch's membrane (36), the process appears to be complement-regulated. Evidence exists linking the complement system to a variety of developmental processes, including regulation of angiogenesis (40-42). Complement activation leads to recruitment of the membrane attack complex, which mediates targeted cell lysis. Within the activation cascade, binding of anaphylatoxins to blood basophils and mast cells induces degranulation, smooth muscle contraction and increased vascular permeability. It has been previously shown that the anaphylatoxins C3a and C5a, the downstream effectors of the complement system, are present in AMD drusen and stimulate choroidal neovascularization via VEGF in vivo and in vitro, and that anti-C3a and anti-C5a antibody treatment prevents angiogenesis (43). In contrast, immunocompromised mice (Rag-deficient mice that lack B and T cells) treated with CEP adducts mount no immune response and develop no signs of AMD (36). It is therefore possible that complement dysregulation, together with processes involved in antibody production, causes a prolonged inflammatory/immunological response that contributes to the AMD pathology.

However, complement activation has also been convincingly shown to inhibit neovascularization via macrophage-regulated mechanisms in mouse models of retinopathy of prematurity (ROP) (41). AMD and ROP pathogenesis differ in that different tissues are involved, namely, the RPE is an important factor in AMD but not in ROP models. Thus, the overall message is that the complement system is intricately involved in neovascularization and further study is required to evaluate its role in AMD pathogenesis. It could very well be that CEP triggers an autoimmune response mediated by the complement system, and that immune cell recruitment by proinflammatory cytokines worsens the condition and results in RPE damage. The finding that CEP induces VEGF-independent neovascularization via TLR2 (35) suggests that the link between increased CEP adduct levels in AMD patients and activation of the complement system could be due to TLR2 (Fig. 2), which would subsequently implicate a role for innate immunity in regulating angiogenesis. Consequently, manipulation of the TLR2 signaling pathway in the retina may be a viable therapy for AMD patients. At least one other AMD marker, A2E, has been shown to activate the complement system via the alternative pathway and contribute to the chronic inflammation observed in AMD pathologies (44-45), and is also proangiogenic in vivo (32). Research into the largely unknown interplay between these various factors will undoubtedly be the key to fully understanding AMD pathogenesis.

CEP AND ω_3 FATTY ACIDS

The negative effects caused by CEP accumulation question the treatment value of ω_3 fatty acid supplements recommended for AMD patients (46, 47). DHA, one of the ω_3 fatty acids and the precursor of CEP, has been shown to inhibit apoptosis in RPE cells (48, 49), protect cells from oxidative stress and promote survival via two of its metabolic products, neuroprotectin D1 (NPD1) and resolvin D1 (RvD1) (50, 51), and suppress retinal inflammation in an AMD mouse model (47). One hypothesis is that ω_3 fatty acids repress nuclear factor NF-κB signaling in RPE cells and thus prevent an inflammatory response (52). If CEP accumulation is responsible for at least a subset of all AMD cases, then increasing DHA consumption could potentially worsen the AMD condition in those patients who are more susceptible to oxidizing DHA into CEP. It is also important to consider ω_3 fatty acids along with ω_6 fatty acids. Studies have shown that more important than absolute quantities of the fatty acids are their relative proportions (53). For example, in animal models, a high ω_6/ω_3 ratio results in abnormal electroretinograms (54, 55), and there are suggestions that ω_6 fatty acids have proinflammatory properties (56). Thus, further advances into understanding the biochemistry between DHA and CEP, as well how the effects are mediated based on the ω_6/ω_3 fatty acid ratio, are required to validate the efficacy of this treatment option. In particular, the controversy could be resolved if we knew whether the formation of CEP is substrate (DHA)-dominant or shunted, with conversion to CEP favored in individuals with at-risk genetic or epigenetic profiles, as well as a macular microenvironment.

CONCLUSIONS

AMD poses a tangible threat to public health in the future (1-2, 5). It is of vital importance to be able to identify at-risk individuals for AMD as early as possible so that proper preventive measures can be taken prior to disease progression. Towards early detection, plasma biomarkers such as CEP provide a good measurement for assessing risk (14), particularly when they are analyzed along with genetic predisposition (13) and other potential disease biomarkers, such as CML and pentosidine (7).

Currently, the biggest drawbacks include a limited number of studies and no clear progress towards using preliminary data to create a practical clinical assay. Moreover, strong evidence implicating the aforementioned biomarkers as functionally related to AMD patho-

genesis must be further explored, particularly the finding that CNV may result from at least one other VEGF-independent mechanism (34), which may be induced via TLR2 (35). Exploring these avenues of research will provide a better understanding of the disease itself and perhaps reveal better therapeutic targets and options. It may potentially divulge other biomarkers that may aid in predicting not only AMD susceptibility, but also indicating to what stage the disease has progressed and how the disease has responded to therapy. For instance, Hollyfield et al. reported that CEP is sufficient to induce AMD in a mouse model that causes geographic atrophy of the macula (36, 37), whereas Ebrahem et al. reported that CEP plays a role in exudative AMD (34). According to these studies, it could be the case that CEP accumulation initiates geographic atrophy and may potentially induce CNV in later stages after sufficient time is allowed or some other pathogenic pathway is activated. Further research into the role of CEP in AMD pathogenesis and the application of CEP as an AMD biomarker is merited.

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DISCLOSURES

The authors state no conflicts of interest.

REFERENCES

1. Pascolini, D., Mariotti, S.P., Pokharel, G.P., Pararajasegaram, R., Etya'ale, D., Negrel, A.D., Resnikoff, S. *2002 global update of available data on visual impairment: A compilation of population-based prevalence studies.* Ophthalmic Epidemiol 2004, 11(2): 67-115.
2. Congdon, N., O'Colmain, B., Klaver, C.C. et al. *Causes and prevalence of visual impairment among adults in the United States.* Arch Ophthalmol 2004, 122(4): 477-85.
3. *Visual impairment and blindness*. World Health Organization, April 2011. http://www.who.int/mediacentre/factsheets/fs282/en/index.html. Accessed June 6, 2011.
4. Friedman, D.S., O'Colmain, B.J., Munoz, B. et al. *Prevalence of age-related macular degeneration in the United States.* Arch Ophthalmol 2004, 122(4): 564-72.
5. Jager, R.D., Mieler, W.F., Miller, J.W. *Age-related macular degeneration.* N Engl J Med 2008, 358(24): 2606-17.
6. de Jong, P.T. *Age-related macular degeneration.* N Engl J Med 2006, 355(14): 1474-85.
7. Ni, J., Yuan, X., Gu, J., Yue, X., Gu, X., Nagaraj, R. H., Crabb, J.W. *Plasma protein pentosidine and carboxymethyllysine, biomarkers for age-related macular degeneration.* Mol Cell Proteomics 2009, 8(8): 1921-33.
8. Chen, Y., Bedell, M., Zhang, K. *Age-related macular degeneration: Genetic and environmental factors of disease.* Mol Interv 2010, 10(5): 271-81.
9. Yu, Y., Bhangale, T.R., Fagerness, J. et al. *Common variants near FRK/COL10A1 and VEGFA are associated with advanced age-related macular degeneration.* Hum Mol Genet 2011, Epub ahead of print.
10. Seddon, J.M., Willett, W.C., Speizer, F.E., Hankinson, S.E. *A prospective study of cigarette smoking and age-related macular degeneration in women.* JAMA 1996, 276(14): 1141-6.
11. Age-Related Eye Disease Study Research Group. *A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8.* Arch Ophthalmol 2001, 119(10): 1417-36.
12. Crabb, J.W., 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(23): 14682-7.
13. Gu, J., Pauer, G.J., Yue, X. et al.; and the Clinical Genomic and Proteomic AMD Study Group. *Assessing susceptibility to age-related macular degeneration with proteomic and genomic biomarkers.* Mol Cell Proteomics 2009, 8(6): 1338-49.
14. Gu, X., Meer, S.G., Miyagi, M., Rayborn, M.E., Hollyfield, J.G., Crabb, J.W., Salomon, R.G. *Carboxyethylpyrrole protein adducts and autoantibodies, biomarkers for age-related macular degeneration.* J Biol Chem 2003, 278(43): 42027-35.
15. Gu, X., Sun, M., Gugiu, B., Hazen, S., Crabb, J.W., Salomon, R.G. *Oxidatively truncated docosahexaenoate phospholipids: Total synthesis, generation, and peptide adduction chemistry.* J Org Chem 2003, 68(10): 3749-61.
16. Fliesler, S.J., Anderson, R.E. *Chemistry and metabolism of lipids in the vertebrate retina.* Prog Lipid Res 1983, 22(2): 79-131.
17. Edwards, A.O., Ritter, R., Abel, K.J., Manning, A., Panhuysen, C., Farrer, L.A. *Complement factor H polymorphism and age-related macular degeneration.* Science 2005, 308(5720): 421-4.
18. Katta, S., Kaur, I., Chakrabarti, S. *The molecular genetic basis of age-related macular degeneration: An overview.* J Genet 2009, 88(4): 425-49.
19. Dewan, A., Liu, M.G., Hartman, S. et al. *HTRA1 promoter polymorphism in wet age-related macular degeneration.* Science 2006, 314(5801): 989-92.
20. Sobrin, L., Reynolds, R., Yu, Y. et al. *ARMS2/HTRA1 locus can confer differential susceptibility to the advanced subtypes of age-related macular degeneration.* Am J Ophthalmol 2011, 151(2): 345-52.
21. Ryu, E., Fridley, B.L., Tosakulwong, N., Bailey, K.R., Edwards, A.O. *Genome-wide association analyses of genetic, phenotypic, and environmental risks in the age-related eye disease study.* Mol Vis 2010, 16: 2811-21.
22. Kabasawa, S., Mori, K., Horie-Inoue, K. et al. *Associations of cigarette smoking but not serum fatty acids with age-related macular degeneration in a Japanese population.* Ophthalmology 2011, 118(6): 1082-8.
23. Cackett, P., Yeo, I., Cheung, C.M.G. et al. *Relationship of smoking and cardiovascular risk factors with polypoidal choroidal vasculopathy and age-related macular degeneration in Chinese persons.* Ophthalmology 2011, 118(5): 846-52.
24. Fraser-Bell, S., Wu, J., Klein, R., Azen, S.P., Hooper, C., Foong, A.W.P., Varma, R. *Cardiovascular risk factors and age-related macular degeneration: The Los Angeles Latino Eye Study.* Am J Ophthalmol 2008, 145(2): 308-16.
25. Haas, P., Aggermann, T., Steindl, K. et al. *Genetic cardiovascular risk factors and age-related macular degeneration.* Acta Ophthalmol 2011, 89(4): 335-8.
26. Klein, R., Klein, B.E.K., Tomany, S.C., Cruickshanks, K.J. *The association of cardiovascular disease with the long-term incidence of age-related maculopathy: The Beaver Dam Eye Study.* Ophthalmology 2003, 110(6): 1273-80.
27. van Leeuwen, R., Ikram, M.K., Vingerling, J.R., Witteman, J.C.M., Hofman, A., de Jong, P. *Blood pressure, atherosclerosis, and the incidence of age-related maculopathy: The Rotterdam Study.* Invest Ophthalmol Vis Sci 2003, 44(9): 3771-7.
28. Hogg, R.E., Woodside, J.V., Gilchrist, S. et al. *Cardiovascular disease and hypertension are strong risk factors for choroidal neovascularization.* Ophthalmology 2008, 115(6): 1046-52.

29. Iriyama, A., Fujiki, R., Inoue, Y. et al. *A2E, a pigment of the lipofuscin of retinal pigment epithelial cells, is an endogenous ligand for retinoic acid receptor.* J Biol Chem 2008, 283(18): 11947-53.
30. Ng, K.P., Gugiu, B., Renganathan, K. et al. *Retinal pigment epithelium lipofuscin proteomics.* Mol Cell Proteomics 2008, 7(7): 1397-405.
31. Murdaugh, L.S., Wang, Z., Del Priore, L.V., Dillon, J., Gaillard, E.R. *Age-related accumulation of 3-nitrotyrosine and nitro-A2E in human Bruch's membrane.* Exp Eye Res 2010, 90(5): 564-71.
32. Iriyama, A., Inoue, Y., Takahashi, H., Tamaki, Y., Jang, W.D., Yanagi, Y. *A2E, a component of lipofuscin, is pro-angiogenic in vivo.* J Cell Physiol 2009, 220(2): 469-75.
33. Ramkumar, H.L., Zhang, J., Chan, C.C. *Retinal ultrastructure of murine models of dry age-related macular degeneration (AMD).* Prog Retin Eye Res 2010, 29(3): 169-90.
34. Ebrahem, Q., Renganathan, K., Sears, J. et al. *Carboxyethylpyrrole oxidative protein modifications stimulate neovascularization: Implications for age-related macular degeneration.* Proc Natl Acad Sci U S A 2006, 103(36): 13480-4.
35. West, X.Z., Malinin, N.L., Merkulova, A.A. et al. *Oxidative stress induces angiogenesis by activating TLR2 with novel endogenous ligands.* Nature 2010, 467(7318): 972-6.
36. Hollyfield, J.G., Bonilha, V.L., Rayborn, M.E. et al. *Oxidative damage-induced inflammation initiates age-related macular degeneration.* Nat Med 2008, 14(2): 194-8.
37. Hollyfield, J.G., Perez, V.L., Salomon, R.G. *A hapten generated from an oxidation fragment of docosahexaenoic acid is sufficient to initiate age-related macular degeneration.* Mol Neurobiol 2010, 41(2-3): 290-8.
38. Patel, M., Chan, C.C. *Immunopathological aspects of age-related macular degeneration.* Semin Immunopathol 2008, 30(2): 97-110.
39. Ding, X., Patel, M., Chan, C.C. *Molecular pathology of age-related macular degeneration.* Prog Retin Eye Res 2009, 28(1): 1-18.
40. Rutkowski, M.J., Sughrue, M.E., Kane, A.J., Ahn, B.J., Fang, S., Parsa, A.T. *The complement cascade as a mediator of tissue growth and regeneration.* Inflamm Res 2010, 59(11): 897-905.
41. Langer, H.F., Chung, K.J., Orlova, V.V. et al. *Complement-mediated inhibition of neovascularization reveals a point of convergence between innate immunity and angiogenesis.* Blood 2010, 116(22): 4395-403.
42. Kahr, W.H. *Complement halts angiogenesis gone wild.* Blood 2010, 116(22): 4393-4.
43. Nozaki, M., Raisler, B.J., Sakurai, E. et al. *Drusen complement components C3a and C5a promote choroidal neovascularization.* Proc Natl Acad Sci U S A 2006, 103(7): 2328-33.
44. Zhou, J., Kim, S.R., Westlund, B.S., Sparrow, J.R. *Complement activation by bisretinoid constituents of RPE lipofuscin.* Invest Ophthalmol Vis Sci 2009, 50(3): 1392-9.
45. Sparrow, J.R. *Bisretinoids of RPE lipofuscin: Trigger for complement activation in age-related macular degeneration.* Adv Exp Med Biol 2010, 703: 63-74.
46. Ho, L., van Leeuwen, R., Witteman, J.C. et al. *Reducing the genetic risk of age-related macular degeneration with dietary antioxidants, zinc, and {omega}-3 fatty acids: The Rotterdam Study.* Arch Ophthalmol 2011, 129(6): 758-66.
47. Tuo, J., Ross, R.J., Herzlich, A.A. et al. *A high omega-3 fatty acid diet reduces retinal lesions in a murine model of macular degeneration.* Am J Pathol 2009, 175(2): 799-807.
48. German, O.L., Insua, M.F., Gentili, C., Rotstein, N.P., Politi, L.E. *Docosahexaenoic acid prevents apoptosis of retina photoreceptors by activating the ERK/MAPK pathway.* J Neurochem 2006, 98(5); 1507-20.
49. Rotstein, N.P., Politi, L.E., German, O.L., Girotti, R. *Protective effect of docosahexaenoic acid on oxidative stress-induced apoptosis of retina photoreceptors.* Invest Ophthalmol Vis Sci 2003, 44(5): 2252-9.
50. Bazan, N.G. *Neuroprotectin D1 (NPD1): A DHA-derived mediator that protects brain and retina against cell injury-induced oxidative stress.* Brain Pathol 2005, 15(2): 159-66.
51. Mukherjee, P.K., Marcheselli, V.L., de Rivero Vacari, J.C., Gordon, W.C., Jackson, F.E., Bazan, N.G. *Photoreceptor outer segment phagocytosis attenuates oxidative-stress induced apoptosis with concomitant neuroprotecting D1 synthesis.* Proc Natl Acad Sci U S A 2007, 104(32): 13158-63.
52. Kaarniranta, K., Salminen, A. *NF-kappaB signaling as a putative target for omega-3 metabolites in the prevention of age-related macular degeneration (AMD).* Exp Gerontol 2009, 44(11): 685-8.
53. Schweigert, F.J., Reimann, J. *[Micronutrients and their relevance for the eye - Function of lutein, zeaxanthin and omega-3 fatty acids.].* Klin Monbl Augenheilkd 2011, 228(6): 537-43.
54. Pawlosky, R.J., Denkins, Y., Ward, G., Salem, N. Jr. *Retinal and brain accretion of long-chain polyunsaturated fatty acids in developing felines: The effects of corn oil-based maternal diets.* Am J Clin Nutr 1997, 65(2): 465-72.
55. Neuringer, M., Connor, W.E., Van Petten, C., Barstad, L. *Dietary omega-3 fatty acid deficiency and visual loss in infant rhesus monkeys.* J Clin Invest 1984, 73(1): 272-6.
56. Kishan, A.U., Modjtahedi, B.S., Martins, E.N., Modjtahedi, S.P., Morse, L.S. *Lipids and age-related macular degeneration.* Surv Ophthalmol 2011, 56(3): 195-213.