The Edward N. & Della L. Thome Memorial Foundation
Awards Program in Age-Related Macular Degeneration Research

2014 Grant Cycle

Radha Ayyagari, Ph.D.
Associate Professor, Ophthalmology
University of California San Diego

"Insights into AMD Derived from the Genetic Mechanisms in Late Onset Retinal Macular Degeneration (L-ORMD)"

Scientific Abstract

The focus of this application is to understand the molecular underpinnings of age-related macular degeneration, with the goal of identifying therapeutic targets to delay the onset or treat the disease. The phenotype of a rare monogenic exudative macular degeneration, also known as late-onset retinal macular degeneration (L-ORMD) is characterized by sub-RPE and sub-retinal drusen accumulation in the fifth decade that result in basal laminar deposits (BLamD), retinal pigment epithelial atrophy, central vision loss and neovascularization in the sixth decade and often misdiagnosed as AMD. We propose to understand the molecular pathology underlying AMD using L-ORMD as a model.

We observed a mutation in the gene C1q tumor necrosis factor--related protein-5 (CTRP5/C1QTNF5) in patients with L-ORMD and generated mutation knock-in (KI) and the gene knockout (KO) mouse models. Both these mouse models showed RPE abnormalities; lipid-rich sub-RPE, sub-retinal, and basal laminar deposits (BLamD); and predominant cone cell loss while most of the rods are preserved. Additional studies indicated that C1QTNF5 might play a role in activation of adenosine monophosphate protein kinase (AMPK) in the RPE. We have ocular tissue that is suitable for microscopy and immunohistochemistry and frozen eye tissue for RNA and protein studies from two human donors with the C1QTNF5 gene mutation. Furthermore, we generated RPE derived from iPSCs of two patients with L-ORMD and two unaffected siblings.

Using the above resources, we propose to characterize the late-onset drusen phenotype in L-ORMD mouse models, study the mechanism underlying the formation of lipid-rich deposits and evaluate pathways associated with late-onset retinal degeneration pathology. The information generated through these studies will be utilized to identify drug targets to prevent the formation of lipid-rich subretinal deposits or remove the existing deposits to delay the onset of age-related degeneration of retinal tissue.

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2014 Grant Cycle

Joseph Carroll, Ph.D.
Associate Professor of Ophthalmology, Biophysics, and Cell Biology, Neurobiology, & Anatomy, Department of Ophthalmology
Medical College of Wisconsin

"Improving Therapeutic Strategies in Age-Related Macular Degeneration Using Adaptive Optics Retinal Imaging"

Scientific Abstract
AMD is the leading cause of irreversible blindness among adults 65 years or older in the western world. One of the irrefutable facts about age-related diseases is that they are present long before they manifest clinically detectable symptoms. The challenge in treating neurodegenerative diseases like AMD is to detect them in their earliest stage, before significant cellular damage has occurred. In addition, by the time patients develop clinically recognizable risk factors, existing interventions have a reduced ability to alter the ultimate course of the disease. An additional challenge is that while there are existing therapies for AMD, they are not equally effective in different patients and treatment practices vary dramatically -- due in part to the lack of an objective, sensitive, means to assess whether a specific intervention is working. Through the Advanced Ocular Imaging Program we have assembled a group of collaborators with expertise in genetics, clinical retina, neurobiology, and optical engineering. Together, we seek to overcome these diagnostic challenges in AMD by employing novel adaptive optics (AO) imaging modalities to study patients with AMD. In aim 1, we propose to use AO and SD-OCT to identify the earliest anatomical changes associated with AMD. Here, we seek to distinguish retinal changes due to normal aging from those that occur as part of the AMD disease process. In aim 2, we seek to develop an imaging-based approach to monitor anti-VEGF treatment response in patients with AMD. Here, we will correlate changes in vascular morphology with conventional clinical assessments of treatment response (e.g. OCT thickness, visual acuity) over time, to test the hypothesis that AO-based measures will have increased sensitivity in detecting changes in response to treatment. We believe that accomplishing these aims will result in improved effectiveness of current and future therapeutic approaches for AMD.

The Edward N. & Della L. Thome Memorial Foundation
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2014 Grant Cycle

Richard Kramer, Ph.D.
Professor of Molecular and Cell Biology, Department of Molecular and Cell Biology
University of California, Berkeley

"Mechanism of Re-animation of the Blind Retina by Synthetic Photoswitch Compounds"

Scientific Abstract
Our ultimate goal is to develop drugs for restoring visual function to patients with end-stage photoreceptor degenerative diseases, including retinitis pigmentosa (RP) and age-related macular degeneration (AMD). A drug treatment for vision restoration would be less invasive than an implanted electronic prosthetic device and more reversible than gene therapy, making this approach advantageous for rapid translation into a medical treatment for humans. Our strategy is to develop synthetic photoswitch compounds that act on endogenous ion channels to bestow light-sensitivity onto retinal ganglion cells (RGCs). Our leading candidate, DENAQ, restores both electrophysiological and behavioral responses to ordinary daylight in mouse and rat models of RP. Remarkably, DENAQ is only effective on RGCs if the rod and cone photoreceptors have already degenerated----it has no apparent effect on RGCs from wild-type mice with healthy retinas. Apparently, the process of photoreceptor degeneration triggers changes in the biochemistry and physiology of RGCs that fortuitously allow DENAQ to act. The selective action of DENAQ suggests that within an individual retina, DENAQ may photosensitize RGCs in regions exhibiting degenerative disease while sparing RGCs in regions that are still healthy. This has profound implications for AMD, where therapeutic intervention is needed only in the macula (less than 5% of the retina) but not in more peripheral areas (more than 95% of the retina). Our goals are 1) to understand mechanistically the changes in RGCs that enable DENAQ to work in a degeneration-specific manner, and 2) test whether DENAQ will act selectively on a small experimentally blinded region of the retina while having no significant effects on the healthy remaining periphery, and 3) test whether photoswitch compounds can slow photoreceptor degeneration. In addition to revealing fundamental information about retinal degeneration, these studies will provide a proof-of-principle for a drug treatment strategy for restoring visual function in AMD.

The Edward N. & Della L. Thome Memorial Foundation
Awards Program in Age-Related Macular Degeneration Research

2014 Grant Cycle

Goldis Malek, Ph.D.
Assistant Professor of Ophthalmology; Assistant Professor of Pathology, Department of Ophthalmology
Duke University

"Targeting the Signaling Pathway of a Lipid Activated Nuclear Receptor for the Treatment of Early Dry Age-related Macular Degeneration (AMD)"

Scientific Abstract
Age-related macular degeneration (AMD) is the leading cause of vision loss in the elderly in the Western world. The early dry subtype is characterized by the accumulation of lipid-rich deposits below the retinal pigment epithelium (RPE). Given that the majority of AMD patients have the early dry subtype and there are no treatments currently available, studies aimed at understanding mechanisms underlying deposit formation, along with animal models that recapitulate disease pathology, are crucial in the identification of appropriate therapies. We have identified a lipid activated nuclear receptor that influences multiple facets of inflammation and is important in lipid metabolism, two pathogenic pathways fundamental to development of AMD. We have confirmed regulation of these pathways in cells vulnerable in AMD including RPE and choroidal endothelial cells. Furthermore, we have examined eyes of mice carrying the null allele for the receptor and have found a significant accumulation of lipid-rich deposits below the RPE, akin to what is seen in human AMD. In this grant, we aim to fully characterize the temporal development of pathology in these knockout mice in order to produce a reliable animal model of early AMD, to be used in further understanding of the biology of the disease as well as testing of novel therapies. We will also determine the therapeutic potential of modulating this receptor in in vitro and in vivo models of sub-RPE deposits.

The Edward N. & Della L. Thome Memorial Foundation
Awards Program in Age-Related Macular Degeneration Research

2014 Grant Cycle

Richard Semba, M.D., M.P.H.
W. Richard Green Professor of Ophthalmology, Department of Ophthalmology
Johns Hopkins University

"Identifying the Roles of ARMS2 and HTRA1 in the Pathogenesis of Age-Related Macular Degeneration Using Functional Proteomics"

Scientific Abstract

Age-related maculopathy susceptibility protein 2 (ARMS2) and serine protease HTRA1 (HTRA1) are strongly associated with a high risk of age-related macular degeneration (AMD), but their roles in the pathogenesis of AMD are not well understood. Protein interactions are critical for establishing protein function and regulating downstream cellular pathways. However, the knowledge regarding ARMS2 and HTRA1 protein interactions remains limited, and whether their interactions and protein expression are altered in human RPE with the ARMS2 A69S and HTRA1 rs11200638 risk variants are unknown. Using a mass spectrometry-based proteomics approach, we identified the two isoforms of ARMS2 and the full-length and two autolytic forms of HTRA1 in human pluripotent stem cell-derived RPE cells. Furthermore, in small-scale pilot studies, we successfully isolated endogenous ARMS2 and HTRA1 from retinal epithelial ARPE-19 cells and identified candidate interacting proteins. The specific aims are to: (1) identify protein interactions of ARMS2 and HTRA1 in human RPE cells and determine how protein levels and interactions are altered with (a) the respective ARMS2 A69S and HTRA1 rs11200638 risk variants, and (b) exposure to cigarette smoke; (2) perform a series of functional studies to determine the impact of ARMS2 or HTRA1 common and risk variants on (a) cell viability, superoxide production, protein carbonylation, expression of drusen-associated proteins, and ultrastructure of human RPE and matrix, and (b) how outcomes are altered by exposure to cigarette smoke; (3) characterize the presence of ARMS2 and HTRA1, and the target proteins identified as interacting with ARMS2 and HTRA1 in human eyes with and without AMD. To meet these aims, we will use a multidisciplinary investigation that combines proteomics, mass spectrometry, cell biology, immunohistochemistry, and bioinformatics. Altogether, our studies will provide important insights into the functions of ARMS2 and HTRA1 in the pathogenesis of AMD, and help identify potential targets for therapeutic intervention.

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Awards Program in Age-Related Macular Degeneration Research

2014 Grant Cycle

Donald Zack, M.D., Ph.D.
Professor of Ophthalmology, Department of Ophthalmology
Johns Hopkins University Wilmer Eye Institute

"Development of a Small Molecule Only Protocol for the Directed Differentiation of RPE from hPSC for the Treatment of AMD"

Scientific Abstract
Age-related macular degeneration (AMD) is the most common cause of irreversible vision loss and legal blindness among the elderly in the US and the developed world. In the most common form of AMD (atrophic or "dry" AMD), damage to the retinal pigment epithelium (RPE) leads to photoreceptor cell dysfunction and cell death, resulting in progressive vision loss. Although dry AMD is currently largely incurable, due to recent advances in stem cell technology RPE transplantation-based treatment is quickly becoming a potential treatment option: pilot clinical trials injecting RPE differentiated from human pluripotent stem cells into patients' subretinal space are currently ongoing. However, obtaining high quality cells that can survive transplantation, retain function, and not be rejected, in quantities sufficient for clinical use, remains a major challenge. In addition, current procedures for differentiation of hES and hiPS cells into RPE require a complex, stepwise regimen that uses a variety of growth factors. To contribute to the development of a streamlined, clinically safe RPE differentiation protocol and one that exclusively relies on small molecules (since growth factors made from bacterial or cultured cells can pose safety concerns as well as contribute to lot to lot variability), we have designed and performed a pilot high-throughput quantitative PCR (HT qPCR) screen that has successfully identified 2 compounds that significantly increase RPE differentiation from human pluripotent stem cells (hPSC). In this Thome application, we propose to use these compounds to optimize RPE production for a wide range of hPSC lines, and also to explore the mechanisms by which these molecules act using next generation sequencing-based RNA-seq. In addition, we propose to extend the success of our HT qPCR approach by screening a larger library of small molecules in order to have a wider range of compounds available for improved and faster RPE differentiation.

Thome Memorial Foundation, Bank of America, N.A. Trustee, Awards Program in Age-Related Macular Degeneration Research

2011 Award Recipients

John Atkinson, M.D.
Three-Year Award Recipient, 2011
Professor of Medicine, Department of Medicine/Division of Rheumatology
Washington University School of Medicine in St. Louis

"A novel mouse model of dry type age-related macular degeneration"

Scientific Abstract
Age-related macular degeneration (AMD) is the leading, worldwide cause of blindness among individuals over the age of 50. The fundamental processes driving this disease are poorly understood. The lack of comprehensive and tractable animal models, especially for dry AMD, is a major impediment. Our proposal takes aim at this limitation. We will evaluate our newly developed animal model of AMD. A tantalizing aspect of our proposal is our recent identification of the expression of CD46, a complement regulatory protein, in the mouse retina. In contrast to its nearly universal expression in humans, mouse CD46 expression was thought to be strictly limited to spermatozoa. Our prior studies involved creating and analyzing a Cd46-/- mice for reproductive studies. However, to our surprise, as the Cd46-/- mice aged, they developed a phenotype closely resembling the dry form of human AMD. Moreover, its deficiency translates into accelerated and more severe disease in a laser model of choroidal neovascularization.

We are excited to characterize this new model and propose: 1) to assess the Cd46-/- mouse as a model for AMD and also determine if there is progression to wet type AMD and 2) to define the expression, structure and function of CD46 in the mouse retina. We will profile the expression pattern of CD46 in the eye, express recombinant murine CD46 and elucidate its complement regulatory functional profile. We have nearly 30 yrs of experience analyzing human CD46 that will be invaluable in this undertaking.

This proposal represents a collaboration between two seasoned research laboratories: the Atkinson lab, which has led the field of complement regulation for decades, discovering CD46 and characterizing much of its biology, and the Bora lab, which has used animal models to elucidate the pathophysiology of inflammatory eye disease, focusing especially on the role of the complement system in the process.

Catherine Bowes Rickman, Ph.D.
Three-Year Award Recipient, 2011
Associate Professor, Departments of Ophthalmology and Cell Biology
Duke University Medical Center

"Contribution of Complement Dysregulation to AMD Pathogenesis"

Scientific Abstract
Age-related macular degeneration (AMD) is the leading cause of irreversible blindness among the elderly in Western industrialized countries. AMD is characterized by the accumulation of extracellular lipid- and protein-containing deposits between the retinal pigment epithelium (RPE) and Bruch's membrane (BrM). Many proteins of the complement system, including an inhibitor of the system, complement factor H (CFH), are found in these deposits and CFH polymorphisms have been implicated as the strongest genetic factor associated with AMD risk, accounting for up to 50% of the population attributable risk percentage. The repercussions of the CFH polymorphism on the entire complement system, as it pertains to the maintenance of the health of the eye, are not yet well understood and it seems likely that other triggers, modulators and/or mechanisms act in concert with CFH in disrupting the delicate equilibrium of the complement system. Among these is another constituent of the sub-RPE deposits, amyloid beta (A beta), which is a known activator of the complement system. We hypothesize that A beta, in the RPE/BrM/choroid region, activates the complement pathway, and this contributes to RPE damage and sub-RPE deposit formation. In support of this hypothesis, we have established A beta as a viable therapeutic target for the treatment of the dry form of AMD. To test this hypothesis in vivo, we have developed a double transgenic mouse by crossing the CFH knock-out (cfh-/-) mouse with our AMD mouse model, the APOE4 mouse (APOE4,APOE4). In Aim 1, we will test the impact of excess complement activation on the onset and progression of AMD-like ocular defects in APOE4/APOE4,cfh-/-and APOE4/APOE4,cfh+/- transgenic mice. In these animals CFH is knocked out or knocked down resulting in decreased inhibition of the complement pathway. In Aim 2, we will determine if RPE/BrM/choroid damage can be attenuated in these mice with A beta-targeted therapies.

Mina Chung, M.D.
Three-Year Award Recipient, 2011
Assistant Professor, Department of Ophthalmology
University of Rochester

"Adaptive Optics Imaging of Geographic Atrophy Progression in Macular Degeneration"

Scientific Abstract
Geographic atrophy (GA) is a severe form of age-related macular degeneration (AMD) and a major cause of vision loss among individuals older than 55 years. No effective treatment yet exists for GA, and its cause remains poorly understood. This gap in understanding represents a

critical barrier in the development of new treatments. This project will address this barrier by focusing on identification of the primary site of cell damage in GA.

The goal of this project is to capitalize on recent innovations in high resolution retinal imaging to test our hypothesis that the sequence of cellular loss in GA begins with loss of rod photoreceptors, followed by cones, and finally retinal pigment epithelial (RPE) cells. Fluorescence adaptive optics scanning laser ophthalmoscopy (FAOSLO) has for the first time enabled the identification of individual cones, rods, and RPE cells simultaneously in living human patients. The impact of this capability is the potential to determine the temporal sequence of retinal disease at a microscopic scale.

Aim1. Test the hypothesis that photoreceptor loss precedes RPE loss in GA. We will microdissect the temporal sequence of cellular damage in GA using AO imaging of the junctional zone surrounding GA lesions to monitor the wave of progression in the photoreceptor and RPE layers simultaneously.

Aim 2. Test the hypothesis that hyperautofluorescence in the junctional zone surrounding GA lesions is preceded by loss of photoreceptors.

Aim 3. Test the hypothesis that loss of rod photoreceptors in GA is preceded by visual cycle disruption in the rod outer segments.

Expected outcomes of the proposed study include determination of the primary cell type affected in GA. Validation of the overall hypothesis could redirect efforts at developing new treatments to the preservation of rod photoreceptor cell health and provide a more efficient outcome measure for future clinical trials.

Anne Eichmann, Ph.D.
Three-Year Award Recipient, 2011
Professor of Medicine (Cardiology), Department of Internal Medicine
Yale University School of Medicine

"Inducing Vessel Quiescence in Age-Related Macular Degeneration"

Scientific Abstract

Excessive angiogenesis is currently treated by inhibition of vascular endothelial growth factor (VEGF), with therapeutic success in wet-type AMD patients. However, treatments involve frequent intraocular injections, and a proportion of patients do not achieve vision improvement. Therefore, developing treatments that improve response to anti-VEGF agents is likely to improve the lives of patients with AMD. VEGF signaling activates negative feedback mechanisms that inhibit VEGF response and promote formation of mature, quiescent vessels via activation of Notch. Therefore, VEGF inhibition in AMD patients reduces Notch signaling, rendering vessels less quiescent. Characterization of additional signaling pathways that

cooperate with Notch to promote quiescence may reveal new targets for AMD therapies to be used in conjunction with anti-VEGF treatments.

Our data strongly support the hypothesis that endothelial-specific BMP9-Alk1 signaling cooperates with the Notch signaling pathway to induce vessel quiescence, and that activation of Alk1 prevents ocular neovascularization in models for AMD. In this proposal, we are addressing the mechanistic basis for this effect at three levels: i) at the cellular level, using in vitro models of sprouting angiogenesis; ii) at the molecular level, by dissecting signaling pathways downstream of Alk1 and Notch, and the molecular mechanisms required to cooperatively induce vascular quiescence; and iii) at the in vivo level, using rodent models of ocular neovascularization. We will define the signaling necessary to induce vascular quiescence downstream of BMP9-Alk1 and Notch, identify the mechanisms that effect BMP9-Alk1 cooperation with Notch, and determine the physiological role of BMP9-Alk1 and Notch signaling in rodent models of AMD. The proposed project will identify signaling pathways necessary for inducing vascular quiescence that have the potential to serve as therapeutic targets in conjunction with anti-VEGF treatment of AMD.

Lindsay Farrer, Ph.D.
Three-Year Award Recipient, 2011
Professor and Chief of Biomedical Genetics, Department of Medicine
Boston University School of Medicine

"Genetic Mechanisms Shared by Eye and Brain Diseases as Novel Therapeutic Targets for Age-Related Macular Degeneration"

Scientific Abstract

There is substantial epidemiological and biological evidence supporting the idea that age-related macular degeneration (AMD) and Alzheimer disease (AD) have common pathogenic mechanisms. We hypothesize that diseases of the retina, including AMD, share etiological mechanisms with neuro-degenerative disease. Our long-range goal is to identify the genetic basis of these mechanisms as a first step toward development of novel therapies. The primary goal of this project is to identify genes and pathways linked to AD that may also contribute to pathogenesis of AMD. We propose to identify genes involved in AD-related pathways that are associated with risk of neovascular and dry forms of AMD by evaluating the association of AMD with single nucleotide polymorphisms (SNPs) in candidate genes using data from several large AMD genome wide association study (GWAS) datasets containing nearly 10,000 AMD cases and 50,000 controls. These genes will be tested for interaction with established AMD genes and environmental risk factors including smoking and hypertension for their synergistic contributions to AMD risk. We will confirm biologically and extend the top-ranked gene findings in several ways including (1) obtaining the complete sequence of genes showing evidence of association from 75 AMD cases and analyzing the sequence data for these genes using bioinformatic approaches in order to identify variants which may directly influence AMD pathogenesis, (2) evaluating association of the top ranked genes and most promising variants

with AMD risk in several large and ethnically diverse cohorts, and (3) investigating expression of the AMD-associated genes in ocular tissues derived from donors with and without clinical histories of AMD. Positive findings from these studies will support the idea that both AMD and AD are systemic diseases. Moreover, we anticipate that the proposed studies will identify additional pathways that can be assessed as pharmaceutic targets to prevent or delay the development of AMD.

Douglas Vollrath, M.D., Ph.D.
Three-Year Award Recipient, 2011
Associate Professor, Department of Genetics
Stanford University School of Medicine

"Modulating metabolism in mice to understand and treat AMD"

Scientific Abstract

Retinal pigment epithelial (RPE) cell dysfunction plays a central role in age-related macular degeneration (AMD). RPE mitochondrial dysfunction is among the mechanisms that contribute to AMD pathogenesis. To gain insight into the RPE response to diminished mitochondrial energy production and the consequences for adjacent photoreceptors, we created a genetic mouse model with RPE-specific postnatal loss of mitochondrial oxidative phosphorylation (OXPHOS). RPE cells lacking OXPHOS survive by transitioning to robust aerobic glycolysis, and initially dedifferentiate and become hypertrophic. They then gradually undergo a series of morphological changes, many of which are reminiscent of those documented in dry AMD, including hypopigmentation, hyperpigmentation, lipid accumulation, increased autofluorescence, loss of cell-cell adhesion, cell migration into the subretinal space, and atrophy. These changes cause diminished photoreceptor function and subsequent degeneration. RPE dedifferentiation is mediated by the mammalian target of rapamycin (mTOR) signaling pathway. Acute oxidative damage to wild-type mice through administration of sodium iodate also causes mTOR activation and RPE dedifferentiation. Thus, two AMD relevant stresses, mitochondrial insufficiency and oxidative damage, cause a similar response in the RPE. Pharmacological inhibition of mTORC1 with rapamycin blunts dedifferentiation and results in preservation of RPE and photoreceptor morphology and function for both models. In light of the similarity between our morphological findings and those seen in AMD, we hypothesize that diminished OXPHOS capacity and RPE mTOR activation are significant contributors to pathology of the disease. Compounds that have been used for decades to treat metabolic diseases in humans are available to inhibit mTOR or stimulate OXPHOS in vivo, but little is known about their effects on the outer retina. We will use these compounds to modulate metabolism in mice with RPE dysfunction to elucidate and counteract AMD pathophysiology. We will also determine the consequences of genetically stimulating aerobic glycolysis the murine RPE.

Howard Weiner, M.D.
Three-Year Award Recipient, 2011
Robert L. Kroc Professor of Neurology
Brigham and Women's Hospital

"Indigneous Microglia and Recruited Monocytes in AMD: Role in Pathogenesis and Immunotherapy by Specific Immune Targeting"

Scientific Abstract
Neuroinflammation is important in the pathoetiology of AMD. However, whether inflammation is neurotoxic and/or neuroprotective during the development of the wet and dry forms of AMD is unresolved. At the center of this debate is the role of retinal microglia versus infiltrating macrophages. Since these two cell subpopulations express the same surface markers, researchers have been unable to differentiate between resident retinal microglia and infiltrating monocytes, and there are no known microglia specific genes that encode cell surface proteins. This is particularly important since the evidence suggests that resident microglia and macrophages may have separate and distinct immune functions. As a result, it has not been possible to specifically target and immunomodulate CNS microglia or recruited monocytes, which is essential for therapeutic intervention to amplify neuroprotective effects, and/or inhibit neurotoxic effects. Our recent discovery of specific biomarkers for resident microglia and recruited inflammatory monocytes provides a unique opportunity to investigate their role in pathoetiology of AMD.

Based on our preliminary studies in the mouse models of Alzheimer's disease and laser-induced CNV, we hypothesize that: 1) Microglia in dry AMD are neuroprotective and prevent geographic atrophy through the phagocytosis and digestion of subretinal b-amyloid deposits and 2) Microglia in wet AMD prevent CNV and vascular leakage. 3) Infiltrating pro-inflammatory monocytes are neurotoxic and eliminate the resident protective microglia and further exacerbate to disease severity in both the Wet and Dry forms of AMD. Target and immunomodulation of these two distinct populations will have a beneficial effect on the course of AMD. To address this hypothesis, we will carry out the following aims: 1) Investigate microglia and macrophages in wet and dry AMD models. 2) Target microglia and recruited macrophages to induce neuroprotection in wet and dry AMD models. 3) Investigate peripheral macrophages and microglia cells in patients with AMD.

Donald Zack, M.D., Ph.D.
Three-Year Award Recipient, 2011
Professor of Ophthalmology
The Wilmer Eye Institute at Johns Hopkins

"Development of a Neuroprotective Molecule for the Treatment of Atophic Age-Related Macular Degeneration"

Scientific Abstract
We have identified the receptor tyrosine kinase inhibitor sunitinib as a potent neuroprotective agent that promotes the survival of photoreceptor cells in vitro and in vivo. In order to more fully characterize sunitinib's unexpected neuroprotective activity on photoreceptors and begin the process to develop analogs appropriate for ocular use, we propose the following specific aims:

SA1. Although sunitinib, in our preliminary studies, is highly neuroprotective of photoreceptors, it does have some toxicity, presumably related to its broad spectrum of activity - it inhibits nearly 100 protein kinases with an IC50 of less than 100 nM, and presumably only one or a few of these kinases are predominant in mediating its neuroprotective activity. Medicinal chemistry approaches will be used to generate sunitinib analogs that have improved photoreceptor neuroprotective efficacy, decreased cellular toxicity, and physiochemical properties more suitable for an ocular formulation.

SA2. Sunitinib's neuroprotective activity is unexpected since among the kinases that it blocks are the receptor tyrosine kinases, which promote cell survival. In this aim we will explore the mechanism by which sunitinib promotes photoreceptor survival.

SA3. We will test the sunitinib analogs, and analogs of other neuroprotective scaffolds, in rodent models of photoreceptor degeneration. In subaim 3A we will continue development of a flow cytometry-based approach that is more rapid and less labor intensive than more established morphometric approaches for assessing and quantifying the degree of photoreceptor damage. This more rapid method should allow us to assess the in vivo activity of more compounds, and to test them at more concentrations. In subaim 3B, the more promising compounds identified in 3A will be tested more definitively using morphometric and functional ERGs methods.

2009 Award Recipients

Rajendra Apte, M.D., Ph.D.
Three-Year Award Recipient, 2009
Assistant Professor, Ophthalmology & Visual Sciences
Washington University School of Medicine in St. Louis

"The Role of Cholesterol in Regulating the Pro-Angiogenic Properties of Senescent Macrophages in Age-Related Macular Degeneration"

Scientific Abstract
The macrophage is a key component of the innate arm of immunity and is critical in regulating initial immune response to tumors, infections and in inflammation. The macrophage is also a central player in sustaining immune privilege in the eye. Immunosenescence is characterized by

age-related changes in both the innate and adaptive compartments of the immune system. Innate immunity, specifically macrophage function, has received particular attention in the eye as it can modulate developmental and post-developmental angiogenesis. Choroidal neovascularization plays a central role in visual impairment and blindness in age-related macular degeneration (AMD), the leading cause of blindness in people over 50 years of age in North America.

The work described in this proposal will help elucidate the mechanisms by which senescence induces a functional drift in macrophages towards a deleterious pro-angiogenic phenotype. These questions are especially relevant to the importance of macrophages in neovascular AMD. These goals will be accomplished by demonstrating that abnormal processing of cholesterol, a dominant component of drusen, causes old macrophages to become pro-angiogenic. The experiments outlined in this proposal are specifically designed to identify critical pathways in the regulation of cholesterol that are potentially important in how macropahges regulate choroidal neovascularization. Our results will directly identify specific targets for translational research for which there are currently available therapeutic agents. Such an approach would facilitate rapid development of clinical protocols in order to develop new agents that prevent blindness from this devastating disease.

Peter Campochiaro, M.D.
Three-Year Award Recipient, 2009
Professor of Ophthalmology, Wilmer Ophthalmalogical Institute
Johns Hopkins University School of Medicine

"Sustained Delivery of Antiangiogenic Peptides for Neovascular Age-Related Macular Degeneration"

Scientific Abstract

Ranibizumab, an Fab that binds vascular endothelial growth factor (VEGF), has provided benefit to patients with neovascular age-related macular degeneration (NVAMD), but there is room for improvement. With monthly injections, 34-40% of patients achieve substantial improvement in vision, but 60% do not. In follow-up portions of phase III studies when patients were switched from monthly dosing to prn treatment, most of the visual gains were lost. VEGF antagonists reduce leakage from choroidal neovascularization (CNV) and suppress growth, but do not cause regression of existing CNV; therefore the minority of patients with NVAMD who achieve substantial improvement with ranibizumab may require frequent injections for the rest of their lives to maintain those gains. Thus, new treatments are needed for NVAMD.

Several endogenous proteins have antiangiogenic activity and also cause regression of established CNV, which could provide benefit in patients not helped by ranibizumab and may "reset the clock" and reduce the need for frequent injections in those that respond to ranibizumab. However, these proteins are large and not ideally suited for use as therapeutic agents. Using bioinformatics, short candidate sequences sufficient for antiangiogenic activity

have been identified. These sequences are around 12-24 amino acids in length and can be readily synthesized using solid-phase methodology; eventually, this methodology could be used to effectively produce the peptides for clinical applications. Synthetic peptides derived from several of the candidate sequences have been demonstrated to suppress endothelial cell proliferation and migration in vitro and neovascularization in vivo in angiogenesis assays, including corneal and laser-induced choroidal neovascularization. Using the most promising peptides identified in the initial screens and nanoparticle technology, we propose to develop a new treatment for NVAMD. Two models of subretinal NV (with testing for suppression and regression of NV in each) that have been demonstrated to have predictive value for benefit in patients with NVAMD will be used to test individual naked peptides and peptides packaged in nanoparticles to identify an optimal therapeutic agent for testing in clinical trials. These studies could lead to a complementary treatment that will further gains already achieved with VEGF antagonists and improve the lives of patients with NVAMD.

Constance Cepko, Ph.D.
Three-Year Award Recipient, 2009
Professor of Genetics, Department of Genetics
Harvard Medical School

"Promotion of Photoreceptor Survival Using HDACs and Gluconeogenic Genes"

Scientific Abstract

Two types of photoreceptors initiate vision in vertebrates: rods, for low light vision, and cones, for bright light and color vision. In humans, cones comprise only 5% of the photoreceptors. However, a specialized central area of the retina, the fovea (within the macula), comprises only cones, and it is this area that is used for all of our high acuity vision. Unfortunately, this is the area which is quite susceptible to degeneration in age related macular degeneration (AMD). There are also many diseases that affect the retina more generally, with 197 genes mapped which lead to loss of vision, most affecting photoreceptors. Gene replacement, using viral vectors, for those diseases where a genetic lesion is known to be causal, is being attempted. However, given the number of genes, and the fact that many of the mutations are dominant, it will be difficult to approach each type of genetic lesion with a specific gene replacement or knock-down. Gene therapy can be envisioned, however, if it is aimed at a common cause for rod and/or cone death, no matter what the underlying cause. This is the basis for this application. We found that the addition of the histone deacetylase 4 (HDAC4) gene to rod photoreceptors promoted their survival in a murine model of retinitis pigmentosa (RP). We more recently found that HDAC4 could promote cone survival when transduced directly to cones in vivo.

We have also investigated the mechanism of cone death in four different murine models for human RP. Following rod death, the cones appear to be nutritionally deprived and undergo self-digestion, or autophagy. We have been able to promote survival and function of cones in one of these animal models by delivering a combination of 3 genes that endowed cones with the

ability to make their own glucose. We wish to optimize this approach by using different viral vectors. If successful, HDAC4 and/or gluconeogenic genes may promote the survival of photoreceptors, and perhaps other neurons, in AMD as well as in other blinding illnesses.

Christine Curcio, Ph.D.
One-Year Pilot Study Recipient, 2009
Professor of Ophthalmology, Department of Ophthalmology
University of Alabama at Birmingham

"Improved Anatomical Endpoints for Treatments of Age-related Macular Degeneration"

Scientific Abstract

Non-invasive imaging of retinal cross-sections at near-histological detail is revolutionizing the clinical management of patients with age-related macular degeneration (ARMD). The newest clinical instruments use spectral domain optical coherence tomography (SD-OCT), based on physical principles similar to ultrasound. Points of in vivo identification essential for maculopathy management and research, such as accurate identification of chorioretinal laminar boundaries and pathologic features, have not been systematically validated. Using a unique resource of human donor eyes, this translational research project will obtain ex vivo SD-OCT images of human macula and optic nerve, followed by high-resolution, wide-field histological cross-sections. Project MACULA (Maculopathy Unveiled by Laminar Analysis) will use 10 donor eyes in each of 5 groups (young adult, normal aged adult, early ARMD, geographic atrophy, and neovascular ARMD) to achieve the following objectives:

1. Compare laminar boundaries visible in ex vivo SD-OCT to those seen in histological cross-sections of the same eyes.
2. Compare specific pathologies seen in ex vivo SD-OCT to those seen in histological cross-sections of the same eyes.
3. Measure for each specimen the thickness of each retinal layer, Bruch's membrane (BrM), and choroid across the macula.
4. Assemble a library of ARMD pathology viewed en face to facilitate interpretation of SD-OCT volume reconstructions and other technologies that reveal that plane.
5. Using whole slide scanning, establish an online atlas of ARMD pathology that can inform next-generation tomographic imaging.

Improved knowledge about macular anatomy will help inform assessment of risk for choroidal neovascularization and assessment of treatments. We anticipate that data will be used by opinion leaders in clinical imaging, instrumentation engineers, ophthalmic educators and illustrators, and developers of new animal models of ARMD.

Patricia D'Amore, Ph.D.
One-Year Pilot Study Recipient, 2009
Director of Research, Departments of Ophthalmology and Pathology
Schepens Eye Research Institute

"An In Vitro Model of Disrupted Retinal Pigment Epithelial Cell-Matrix Interactions"

Scientific Abstract
The hallmark phenotype of AMD is the presence of acellular deposits, drusen, located between the retinal pigment epithelium (RPE) and Bruchâ€™s membrane (BrM). This proposal aims to test the hypothesize that disruption of RPE -extracellular matrix interactions by drusen deposits initiates a cascade of events that leads to an altered differentiation of RPE cells and to the progression of AMD. The studies aim to develop and validate an in vitro model in which RPE interactions with the extracellular matrix are disrupted by drusen-like microparticles and to use the model to investigate the effects of disrupted RPE cell-extracellular matrix interactions on the RPE phenotype and function. To develop a reproducible in vitro model of disrupted RPE cell-extracellular matrix interactions, drusen-like microparticles with surface features and composition similar to human drusen will be fabricated. ARPE-19 cells and primary RPE will be cultured on transwells for four wk to generate an extracellular matrix, removed non-enzymatically, the drusen-like particles distributed across the matrix, and ARPE-19 cells re-plated. Cell-cell and cell-matrix interactions will be examined by SEM. The effect of disrupted RPE cell-extracellular matrix interactions on RPE phenotype and functions will be investigated including, attachment, survival, phagocytosis, junctional complexes, actin cytoskeleton, ultrastructure, and transepithelial resistance as well on the expression level of RPE-secreted molecules including, growth factors, metalloproteinases, and integrins. Results of these studies will provide insight into the pathogenesis of AMD.

Margaret DeAngelis, Ph.D.
Three-Year Award Recipient, 2009
Associate Professor, Ophthalmology and Visual Sciences
University of Utah School of Medicine Moran Eye Center

"Identifying Underlying Mechanisms of Age-Related Macular Degeneration for the Development of Appropriate Preventive and Therapeutic Interventions"

Scientific Abstract
The overall goal of our research is to elucidate key regulatory components in pathways which are implicated in the development of neovascular age-related macular degeneration (AMD) so that appropriate preventive and therapeutic targets can be developed. We propose to accomplish this goal by analyzing key regulatory components in a defined novel network/pathway of genes that show significant altered expression from our recent studies of patients with neovascular AMD compared to their normal siblings using high throughput ChIP-seq based assays (chromatin immunoprecipitation followed by direct sequencing).

Specifically we will determine which genes and their regulatory regions are directly regulated by retinoic acid receptor-related orphan receptor alpha (RORA), an anti-angiogenic transcription factor, that we have identified as a key protective agent against neovascular AMD, in 3 different cohorts (family based; unrelated case-control and prospective nested unrelated case-control), using high throughput ChIP-seq based assays on lymphoblastoid cell lines from our patient cohorts as well as human autopsied eyes with and without neovascular AMD. Examining both patient cell lines and patient ocular retinal tissue will not only help us to determine tissue specificity of RORA binding, but help to elucidate whether or not AMD is a systemic disease or a localized disease, ultimately determining avenues for drug delivery. These studies should help us to 1) determine where in the human genome RORA binds; 2) how the specificity of binding is achieved; 3) how RORA affects gene expression; 4) how this expression is related to genes that function in RORA's pathway(s). We will also evaluate regulatory components in HTRA1/ARMS2, gene(s) from our recent studies that showed epistatic interactions between this gene(s) and RORA. Concurrently with our human in vitro studies, we will characterize the functional consequences of Rora gene expression using a tissue specific conditional knock-out mouse model that lacks Rora (Rora-/-) exclusively in the retina and determining whether Rora or a combination of genes in the Rora pathway are able to rescue retinal phenotypes similar to those found in patients with AMD. We anticipate that this study will lead to the identification of druggable targets for the treatment and prevention of neovascular AMD.

Albert Edwards, M.D., Ph.D.
Three-Year Award Recipient, 2009
Associate Professor, Institute for Molecular Biology
Mayo Clinic

"Pathophysiology of AMD"

Scientific Abstract

This proposal addresses two important questions for advancing our understanding of the pathophysiology of age-related macular degeneration (AMD). The first question is what are the biological pathways that mediate the genetic risks conferred by the age-related maculopathy susceptibility locus 2 (ARMS2) on chromosome 10q26. Genetic variants on haplotypes (segments of DNA inherited as a block) in this region alter the risk of developing AMD. These haplotypes span a hypothetical gene called LOC387715 (or ARMS2 by some) and the promoter region of a protease involved in transforming growth factor signaling and blood vessel homeostatis called high temperature requirement A1 (HTRA1). Which exact DNA sequence changes on these haplotypes alter AMD risk is the subject of intense investigation by us and others. Here we propose to determine the differences in RNA expression (transcriptome) within donor retina and retinal pigment epithelium (RPE) between subjects homozygous for either the protective or risk haplotypes.

The second aim to test the hypothesis that increased activation of the alternative complement pathway in blood could contribute to AMD in humans. This hypothesis arises from the observation by us and others that complement activation is increased in the blood of patients with AMD compared to controls. Further, we have shown that many of the genetic risks that contribute to AMD underlie the increased activation of complement in blood. The simplest explanation is that some of the genetic variation in complement pathways leads to increased activation of complement in the fluid phase (e.g., blood, tissue fluid) and this contributes to formation of AMD. We propose to test this hypothesis by creating animal models of increased activation of complement. We will look for AMD-like endpoints in these animals and determine the impact, if any, of systemic activation of complement on the retina, RPE and Bruchs membrane.

The answers to both questions should have immediate implications for future research in AMD and management of patients. The first question will provide insight into the biological pathways altered by variation at 10q26, while the second question will determine if chronic reduction in complement activation should be considered to reduce the incidence of AMD.

Scott Fraser, Ph.D.
Three-Year Award Recipient, 2009
Anna L. Rosen Professor, Division of Biology
California Institute of Technology

"Novel Diagnostic for AMD"

Scientific Abstract

Age-related macular degeneration (AMD) is the leading cause of severe visual loss in the elderly in the United States. The principal therapy for "wet" (neovascular) AMD is the intraocular injection of anti-angiogenic agents such as ranibizumab (Lucentis®, Genentech). This stabilizes vision in over 90% of patients and significantly improves vision in approximately 1/3 of them. Because most patients with wet AMD have lost reading and driving vision when therapy is initiated, it is important to screen high-risk patients periodically; however, current imaging modalities are not able to detect reliably the early choroidal neovascularization (CNV) of pre-symptomatic wet AMD.

We have modified optical coherence tomography (OCT) to obtain phase contrast (PC-OCT), which enables small blood vessels to be detected. With this contrast, the motion of blood cells in the smallest capillaries and the Brownian motion of blood cells in leakages can be readily detected. We hypothesize that PC-OCT can visualize CNV at pre-clinical stages of wet AMD, visualize regions of leakage in active CNV, and detect the deposition of lipid (fat) in Bruch's membrane, an important risk factor for development of wet AMD.

Our experimental goals are to refine and adapt PC-OCT to optimally visualize vascular structure in the human eye. PC-OCT will follow the status of the eye longitudinally, and tools

will be refined that permit the accurate comparison of data to detect eye changes. Feasibility of PC-OCT for detection of pre-clinical wet AMD will be tested in asymptomatic patients to determine if they have small regions of sub-RPE CNV and subretinal hemorrhage. Feasibility of detecting leakage from CNV will be determined by comparisons between PC-OCT and conventional angiographic imaging. The PC-OCT instrument will also be refined to image lipid accumulation of Bruch's membrane, and high-risk patients will be screened to identify detectable changes occuring before CNV ingrowth takes place.

James Handa, M.D.
Three-Year Award Recipient, 2009
Robert Bond Welch Professor, Wilmer Eye Institute
Johns Hopkins University

"Cigarette Smoking Induces Oxidative Damage and an Enhanced Innate Immune Response during Early Age-related Macular Degeneration"

Scientific Abstract

The long-term objectives are to identify causative pathophysiologic sites of AMD on which to develop target-specific treatments. We will study whether cigarette smoking (CS) causes oxidative damage and activates excessive innate immune mediated inflammation, and if Nrf2, the most powerful redoxsensitive transcription factor, activates a comprehensive antioxidant protective response. Nrf2 signaling decreases with smoking, and its decreased activity can be reversed by the triterpenoids. Herein, we will test the hypothesis using the following aims, that chronic cigarette smoking induces persistent oxidative stress in the fundus such that Nrf2 signaling becomes inadequate, and results in oxidative stress and an uncontrolled innate immune response with the development of AMD.

Specific Aim 1: To test the hypothesis that Nrf2 signaling protects against cigarette smoke-induced oxidative stress and excessive innate immune activation in the fundus. We will use a genetic loss- and gain-of-function strategy by placing mice in a smoking chamber or air for up to 6 months. We will test whether loss of Nrf2 in Nrf2 deficient mice results in an insufficient antioxidant response and oxidative damage. Since CS excessively activates complement and Toll-like receptors (TLR), we will determine whether these innate immune response components participate in the development of AMD. The response will be compared to increased Nrf2 signaling in Keap1 deficient mice. Specific Aim 2: To test the hypothesis that Nrf2 signaling has a differential protective effect among critical fundus cell types after cigarette smoke exposure. Protection by Nrf2 in specific fundus cell types will be delineated using the outcomes defined in aim 1 using Cre-loxP mediated, RPE-specific, photoreceptor-specific, and vascular endothelial cell-specific Nrf2 and Keap1 deficient mice. Specific Aim 3: To determine if pharmacological activation of Nrf2 protects the fundus from developing features of AMD. Instead of a genetic approach, we will use pharmacologic activation of Nrf2 with triterpenoids, which have been proven safe in a Phase I trial. We will determine whether triterpenoids given to wild-type mice exposed to cigarette smoke will activate Nrf2 signaling, and protect against

oxidative and excessive innate immune mediated damage, and prevent changes seen in early AMD.

Bryan Jones, Ph.D.
Three-Year Award Recipient, 2009
Research Assistant Professor, Department of Ophthalmology
University of Utah School of Medicine Moran Eye Center

"Comprehensive Characterization of the Retina-Choroid Interface in Normal Aging and Late Stage AMD Phenotypes Using an Integrated Approach that Includes Computational Molecular Phenotyping"

Scientific Abstract

Age-related macular degeneration (AMD) affect an estimated 18% of Americans from 65 to 74 and 30% older than 74. While AMD represents one of the best characterized diseases from a genetic perspective, we currently know far less about the mechanisms mediating disease progression, particularly in geographic atrophy (GA) and choroidal neovascularization (CNV).

Therefore, this project will: 1) Define normal human retinal, RPE and choroidal histology, and tissue metabolic identity through the aging process, over three decades (60-90 years) typically associated with onset of AMD; and 2) Document and compare disease progression in AMD tissues to those of normal aging, creating indices of disease-related differences and timelines for early, to late stage [geographic atrophy (GA) and choroidal neovascularization (CNV)] disease.

The pathways and mechanisms through which genetic and non-genetic risk factors modulate development of AMD pathogenesis remain largely unexplored. Moreover, current treatment for AMD is palliative and limited to late-stages or exudative forms of the disease. The long-range goal is to provide a rigorous set of AMD-related pathway and biomarker data that can be employed to develop therapies for various AMD phenotypes. We have designed studies to test the central hypothesis that specific, emergent metabolic pathways index and participate in the etiology and pathogenesis of AMD through two aims. The first aim provides a comprehensive cellular metabolic profiling at the choroid-RPE-retina interface from an unprecedented repository of human donor eyes, characterizing the normal aging eye. The second aim compares, in multivariate space, profiles from AMD eyes, including GA and CNV. These aims directly address that (i) retinas are complex heterocellular tissues composed of over 70 classes of cells and (ii) most tissues are altered in inherited retinal degenerative diseases. Defining disease and stage-specific cytoarchitectural and metabolomic responses in AMD is critical for highlighting cellular targets for intervention. We will use unique resources available to us at the John Moran Eye Center (JMEC), including a repository of nearly 4,000 extensively characterized human eyes from donors with and without clinically documented AMD, and a newly developed, high-throughput technology: computational molecular phenotyping (CMP).

Patsy Nishina, Ph.D.
Three-Year Award Recipient, 2009
Professor, Research
The Jackson Laboratory

"Molecular Analysis of Mouse Models That Develop AMD-like Phenotypic Characteristics"

Scientific Abstract
While notable exceptions exist, for most inherited retinal disorders there are few effective treatments or cures and, in most cases, only a rudimentary understanding of the pathogenic pathway(s) involved. A major goal of the current research, therefore, is to identify gene defects and to understand the cellular pathways and molecular mechanisms that lead to disease. Once disease etiology is understood at the molecular level, pharmaceutical or genetic therapies are more easily designed to delay onset of the disease or provide a cure. The first logical step in understanding the molecular mechanisms underlying a disease process in any genetic disease is to identify the mutated gene(s) underlying the condition, a primary goal of this application in which two newly discovered mouse eye models that develop retinal pigmented epithelial atrophy will be studied. At the successful conclusion of this research proposal, we will identify the two genes that underlie the rpea1 and rpea2 mutations that lead to RPE atrophy and subsequent photoreceptor cell death. Our biochemical and histological studies in the rpea1 and rpea2 mutants will provide critical baseline characterization of pre-clinical alterations (i.e. primary lesions) and of the pathology these mutations cause. Finally, these two well-defined mouse models will be made available to investigators in this field to generate further hypotheses about how RPE atrophy might impact AMD.

In this application we will:
Aim 1. Identify the basis for rpea1 & rpea2 and the pathways in which they function.

Aim 2. Identify abnormalities caused by the rpea1 and rpea2 mutations to determine the nature of the pathological changes observed and the affected biological pathways.

Victor Perez, M.D.
Three-Year Award Recipient, 2009
Associate Professor, Department of Ophthalmology, Immunology and Microbiology
University of Miami School of Medicine

"Adaptive Immunity as the Missing Link between Oxidative Stress and Complement Activation in the Pathophysiology of Age-Related Macular Degeneration"

Scientific Abstract
Age-related macular degeneration (AMD) is the leading cause of legal blindness in elderly individuals in the United States and industrialized countries. One hallmark of the disease is the accumulation of debris (termed drusen) below the retinal pigment epithelium (RPE). AMD

occurs in dry and wet forms: dry AMD relates to damage of the macula caused by atrophy whereas wet AMD involves neo-vascularization. Recently, several studies have implicated the immune system in the AMD disease process. Activated complement factor proteins have been found in drusen from AMD patients. Likewise, genetic markers (polymorphisms) within complement factor genes have been associated with development of AMD, suggesting that inflammation is a component of this disease. Oxidative stress is a second major pathway found to play a role in AMD. However, there is limited experimental data linking oxidative damage and complement activation. Our lab has developed a mouse model of dry AMD in which an oxidative stress-induced modification (carboxyethylpyrrole, or CEP) of self antigens leads to AMD-like lesions and complement deposition in the RPE. The research proposed in this application will test the hypothesis that adaptive immune responses generated against retinal products adducted with CEP are the initiating step in the development of AMD. We propose the novel concept that antigen specific T and B cell responses are crucial in the targeting of complement-mediated retinal damage in AMD and their regulation can be modulated to prevent or reverse disease. In addition, new AMD-associated genetic markers within adaptive immunity-related genes will be identified in humans and non-human primates. The immunological characterization of our AMD model will generate new insights into the biology and possible treatment of this serious blinding disease by providing a mechanistic link between oxidative stress and complement activation.

Our specific aims are:
Specific Aim 1: To test the hypothesis that development of T and B cell responses against CEP are important early events in the onset of AMD.

Specific Aim 2: To develop a non-human primate model of dry AMD for translational research.

Specific Aim 3: Identification of novel AMD-associated polymorphisms within adaptive immunity-related (AIR) genes in humans as possible targets for treatment.

Janet Sparrow, Ph.D.
Three-Year Award Recipient, 2009
Anthony Donn Professor of Ophthalmic Science, Department of Ophthalmology
Columbia University

"Limiting RPE Lipofuscin Accumulation by Harnessing Enzyme-Mediated Degradation"

Scientific Abstract
Autofluorescent bisretinoid pigments accumulate with age as the lipofuscin of retinal pigment epithelial (RPE) cells in the eye. These pigments originate in photoreceptor outer segments from reactions of visual cycle retinoid and are deposited in the RPE secondarily. There has long been speculation of a link between RPE lipofuscin accumulation and the pathogenesis of age-related macular degeneration (AMD). Our recent work indicates that products of the photooxidation of RPE bisretinoids serve as activators of the complement system. This finding

is significant given genetic association studies indicating that complement dysregulation underlies the pathogenesis of AMD in a significant proportion of cases.

Efforts in my laboratory are directed toward understanding the composition of RPE lipofuscin, the biosynthetic pathways by which the bisretinoids forms and the mechanisms by which RPE cells are adversely affected by lipofuscin accumulation. The long-term goal of this work is to prevent vision loss in age-related and monogenic forms of macular dystrophy by developing therapies to retard the accumulation of RPE lipofuscin.

Since bisretinoids of RPE cells accumulate over time, it is apparent that these compounds are not degraded by the lysosomal enzymes of the cell. The goal of the proposed research is to develop a therapy to reverse the accumulation of the bisretinoids of RPE lipofuscin. We will test the hypothesis that exogenous enzymes can be delivered to RPE cells for the purpose of safely degrading the bisretinoid constituents of RPE lipofuscin. This approach is particularly relevant for AMD patients who are usually diagnosed later in life and perhaps long after other lipofuscin-targeted therapies could be effective. The specific aims are to i) use non-cellular systems to test the efficacy with which selected groups of enzymes can cleave the known RPE bisretinoids including A2E, all-trans-retinal dimer and A2-DHP-PE; ii) develop approaches for introducing exogenous enzymes to the lysosomal compartment of RPE cells and test for degradation of intracellular bisretinoids; iii) ascertain whether enzyme-mediated cleavage of bisretinoids such as A2E has adverse effects on the cell. These studies involve techniques of cellular and molecular biology along with chromatographic and mass spectrometry analysis.

Douglas Vollrath, M.D., Ph.D.
One-Year Pilot Study Recipient, 2009
Associate Professor, Department of Genetics
Stanford University School of Medicine

"Retinal Degeneration in Mice Due to Loss of RPE Mitochondrial Energy Production: a Model of AMD"

Scientific Abstract

Retinal pigment epithelial (RPE) cell dysfunction and death are widely believed to play central roles in age-related macular degeneration (AMD). Evidence indicates that reduction of RPE mitochondrial function is among the mechanisms that contribute to AMD pathogenesis, but the epithelial changes that result from mitochondrial deficiency are largely unknown and no suitable animal model of a postnatal primary RPE degeneration has been described. To gain insight into the RPE response to diminished mitochondrial function and the consequences for the adjacent choroid and photoreceptors, we created mice with postnatal loss of RPE mitochondrial respiratory chain function through an RPE-specific knockout of a nuclear gene essential for mitochondrial DNA stability. Our model has several features that make it suitable and practical for investigating AMD pathogenesis. The onset of degenerative changes is gradual, but becomes severe before one year of age. While most RPE cells lose mitochondrial

function, a minority do not, modeling the heterogeneity of cell damage seen in AMD. The retinas of affected animals eventually display a number of features seen in the geographic atrophy form of AMD including: 1) abnormal RPE morphology 2) attenuated RPE cells 3) RPE cytoplasmic inclusions 4) RPE autofluorescence 5) loss of RPE epithelial integrity 6) RPE cell migration and 7) progressive photoreceptor degeneration that starts in the posterior retina. We propose to characterize the retinal response to loss of RPE oxidative phosphorylation through deep sequencing of RPE and neural retina mRNA populations and targeted biochemical and cell biological studies. We further propose to determine the effect of dietary restriction on RPE and photoreceptor survival in our model. When thoroughly characterized, our model could serve as a recipient for RPE cell transplantation studies and provide a testing ground for small molecules and growth factors that preserve RPE and/or photoreceptor function in the context of decreased RPE energy production. Dietary restriction has been found to be beneficial not only for extending life span, but also in slowing degenerative changes in many mammalian organs, including the central nervous system. Success of this pilot project could therefore lead to new approaches to the treatment of AMD.