



RESEARCH NEWS

Discovering New AMD Drug Treatments

APARNA LAKKARAJU, PhD

Inflammation is the body's response to fighting stressors like pathogens, radiation and chemical exposure. Because uncontrolled inflammation can cause tissue damage and promote disease, this response has to be finely controlled over the entire human lifespan. Defects in proteins that limit inflammation are associated with many diseases including age-related macular degeneration (AMD), which destroys central, high-resolution vision and currently affects over 30 million people globally. Many of these proteins belong to the complement pathway, which is the part of the immune system responsible for clearing pathogens and damaged cells. How abnormal activation of the complement system can damage the retina and lead to vision loss has been an open question. In work recently published in the *Proceedings of the National Academy of Sciences (PNAS)*, Aparna Lakkaraju, Assistant Professor of Ophthalmology and Visual Sciences, along with colleagues and McPherson ERI members Li Xuan Tan and Kimberly Toops, shed light on this enigma and identify novel drug targets that prevent inflammation in models of macular degeneration.

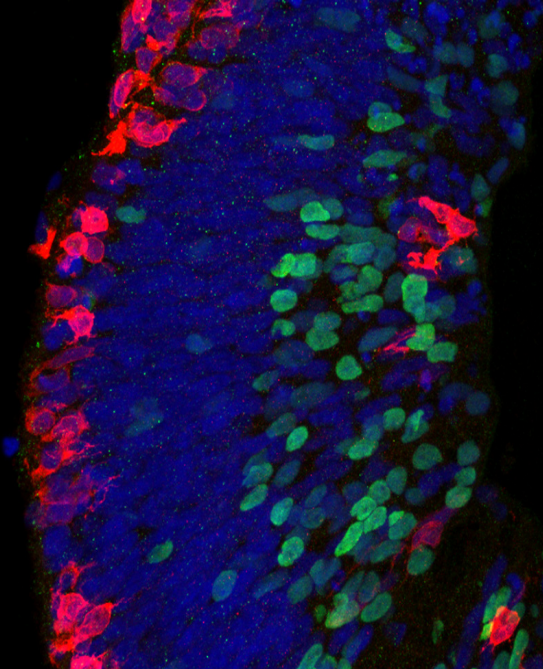
The initial site of insult in AMD is the retinal pigment epithelium (RPE), which sits between the light-sensing photoreceptors and the choroidal blood supply and performs numerous functions essential for healthy vision. Another unique feature of the RPE is its role in maintaining "ocular immune privilege", by forming a barrier that prevents the entry of invading pathogens and limiting immune and inflammatory responses within the retina. Because of this function, it has been hypothesized that the RPE prevents unregulated complement activation in the retina. This is essential because the final step of the complement cascade is the formation of pores or holes in cell membranes that compromise the integrity of the cell and eventually lead to mitochondrial damage and oxidative stress.

Dr. Lakkaraju's team set out to answer two questions: first, how does the RPE protect itself from the harmful consequences of these membrane pores? And second, how are these protective mechanisms derailed in macular degeneration? To address these questions, the Lakkaraju lab used a combination of high-speed, high-resolution live imaging of primary RPE monolayers and a mouse model of Stargardt inherited macular degeneration. Using these approaches, they showed that analogous to the "rapid response teams" in hospitals that provide emergency critical care to patients, the RPE mobilizes two mechanisms

within seconds-to-minutes of complement exposure to stave off mitochondrial injury. The first response is the activation of a protein called CD59, which inhibits the final step required to form pores on the membrane after complement attack. Once these pores are formed on the cell membranes, Dr. Lakkaraju's team discovered that the RPE has a second response: using compartments called lysosomes as duct tape to seal the holes and preserve cell integrity. Previous research from the Lakkaraju lab showed that RPE in models of macular degeneration have excess cholesterol, which causes traffic jams within the cell by activating an enzyme called acid sphingomyelinase (ASMase). In the present study, they found that these traffic jams interfere with both of the rapid response mechanisms in the mouse model of macular degeneration, leading to mitochondrial fragmentation.

The group then used this information to identify two novel drug targets to counteract these cellular traffic jams: removing cholesterol or inhibiting ASMase. Several FDA-approved drugs currently on the market for various indications are known to inhibit ASMase. Dr. Lakkaraju's team showed that one of these FDA-approved drugs used to treat depression, when administered to mice in their drinking water, completely protected the RPE from complement-induced damage. The Wisconsin Alumni Research Foundation has filed a patent application on the use of ASMase inhibitors to treat inherited and age-related macular degenerations. The Lakkaraju lab is now evaluating over 15 FDA-approved drugs known to inhibit ASMase for their ability to protect the RPE and retina from inflammation.

Given the long and arduous road to regulatory approval for new drugs, "drug repurposing" or finding novel uses for existing drugs holds great promise for intractable diseases like AMD. The ASMase inhibitors identified by Dr. Lakkaraju satisfy several criteria for the "ideal" AMD therapeutics because they are low molecular weight drugs with documented safety profiles that can be orally administered. Moreover, epidemiological studies show that use of one class of these inhibitors is associated with a significant decrease in the onset of AMD. Dr. Lakkaraju believes that these strengths constitute a powerful rationale to fully investigate the promise of ASMase inhibitors as potential therapies that could benefit millions of AMD patients around the world.



FROM THE DIRECTOR:

Dear Friends of the McPherson Eye Research Institute,

Researchers who join the McPherson ERI share a common fascination with all aspects of vision science. At our cross-disciplinary events and seminars, it's inspiring to see faculty and trainees from fields as varied as engineering, neuroscience, psychology, and computer science discuss cutting-edge vision research with other members who interact with human (and animal) patients affected by blinding disorders. The collaborations sparked from these encounters continue to generate advances that help turn hope into reality.

In the illustration below we offer a glimpse into how one of these collaborations is seeded and grows. It is an example of many such cooperative endeavors that McPherson ERI members have participated in through the years. As the boundaries between the sciences become increasingly blurred, supporting cross-campus interactions has become an uncomfortable burden for some, but a validation for those who embrace and steward it. The University of Wisconsin and the McPherson ERI have been in the vanguard of collaborative research, and it is now common to see problems of vision disorders addressed through joint efforts of ophthalmological researchers, biomedical engineers, and neuroscientists.

Of course communicating our work to the hopeful patients we serve is very important to me and my McPherson ERI colleagues. One of our most meaningful get-togethers recently took place on campus in late August, when Dr. Bikash Pattnaik and I hosted approximately 40 McPherson ERI friends and supporters (many of whom have been active in our annual Cycle for Sight spinning bike fundraiser) for a progress update on vision research. A substantial number of our visitors, and Cycle for Sight participants in general, are blind or visually impaired. They participate in the ride in order to help shape the progress of vision research on campus, and at the informal presentation and discussion had many excellent questions about the nature and pace of that research. At the McPherson ERI, our goal is to move vision research down the field as thoughtfully and rapidly as possible, keeping our minds and hands working toward achieving major wins for patients. There is great reason to be optimistic about near-term research advances, and as we make progress, we'll continue to update you – and as always, we remain grateful for your interest and support.

David M. Gamm, MD, PhD

RRF Emmett A. Humble Distinguished Director, McPherson ERI
Sandra Lemke Trout Chair in Eye Research

Photoreceptor Replacement

ANATOMY OF A COLLABORATION

Team-based research is a founding principle of the UW McPherson Eye Research Institute, but how does a complex scientific collaboration really work? The inner workings of one such effort (of many) is illustrated by McPherson ERI member-researchers who, along with researchers at Johns Hopkins University, recently received a major grant from the National Institutes of Health. As part of the National Eye Institute's "Audacious Goals" initiative, \$12.4 million in grant support was offered to advance technology to regenerate light-sensing photoreceptors in the retina and – ultimately – reverse blindness. Due in large part to the McPherson ERI's depth of experience and worldwide reputation in the fields of retinal stem cell biology and engineering, combined with the powerful drug screening expertise of our Johns Hopkins colleagues, the UW-Madison/Johns Hopkins team was chosen as one of six research groups nationwide to receive funding.

Photoreceptors are the specialized cells in the retina – the rods and cones – which initiate the process of vision by converting light into electrical signals that are then sent through the optic nerve to the brain. Damaged or lost photoreceptor cells (which the eye cannot repair on its own) cause many well-known blinding diseases, including age-related macular degeneration, diabetic retinopathy, and retinitis pigmentosa. Through the work of McPherson ERI researchers and others around the world, it's become possible to "manufacture" photoreceptors and envision potential cures for these diseases. There are still many hurdles to overcome to achieve this incredible goal; however, the UW-Madison/Johns Hopkins team is thoughtfully designed to engage these roadblocks efficiently and with great determination. Here's a step-by-step look at this collaboration....

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Photoreceptor cells connect with other cell types across a small gap – the synapse – between cells. **Dr. Xinyu Zhao**, a neuroscientist, will develop tests to measure these synaptic connections. This essential step will help assure that the end result of this collaboration achieves our ultimate goal of devising better strategies to promote full, functional photoreceptor replacement.



1

David Gamm leads the UW-Madison team tasked with generating stem-cell derived photoreceptors and testing their capacity to form connections with other cells in the retina. The Gamm Lab at UW-Madison developed technology to manufacture photoreceptor cells from induced pluripotent stem cells (iPSC), which are reprogrammed from donors' blood or skin cells. Remarkably, these photoreceptor cells self-organize into full-fledged "mini-retinas" that have the layered complexity of a developing retina.



2

Joe Phillips is the scientist in charge of photoreceptor production in the Gamm Lab for this project. Stem cell-derived production of photoreceptors is a highly delicate process with a host of variables; deriving a single iPSC line can take months, and producing photoreceptors from the iPSCs and testing their capabilities takes yet more time, expertise, and knowledge.



3

Generating photoreceptor cells is, at this point, relatively far along. Connecting those photoreceptor cells, once manufactured, to other cells in the retina is less well established. **Don Zack** heads the Johns Hopkins laboratory charged with finding conditions and drugs that promote these connections. The Zack Lab (including scientists Bibhu Mishra, Cindy Berlinicke, and Baranda Hansen) will robotically screen many thousands of molecules to discover those that may promote these connections.



4

How will we know if these compounds work? Neuroscientist **Tim Gomez**, along with biomedical engineers **Justin Williams** and **Bill Murphy**, are developing assays (rigorous tests) to measure the extension of axons, the thread-like projections of photoreceptor cells whose growth is critical to forming connections with other retinal cells. And in order to understand how the compounds work (and not just *if* they work), Dr. Gomez will perform studies to better understand the internal machinery that photoreceptors must use to extend and guide those axons to their correct targets.



RESEARCH AND MEMBER NOTES:

Annual Vision Science Poster Session

The McPherson ERI's fall vision science poster session is the Institute's annual opportunity for members, along with their lab associates and trainees, to showcase current research. Approximately one hundred people attended this year's session, held October 4th. Posters underscored the breadth of vision research on the UW-Madison campus, with more than a dozen departments represented. Topics featured advances in understanding and treating diseases that affect vision (generating a stem cell line for enrichment of cone photoreceptors; identifying genes that cause age-related retinal abnormalities and neurodegeneration; finding a protective factor to prevent retinal ganglion cell atrophy and death that occur in glaucoma). Other posters addressed questions of visual function and use (discerning how children with autism spectrum disorder use informative verbs in reading; studying the warping of perceptual space in virtual reality environments). The conversations and ideas generated by placing dozens of researchers in close proximity are always a poster session highlight, accelerating new connections and sparking new avenues of research.

New Recipients: Retina Research Foundation Endowed Chair & Professorship

T. Michael Nork, MD, MS (Ophthalmology and Visual Sciences) has been named the McPherson Eye Research Institute's *Retina Research Foundation Kathryn and Latimer Murfee Chair* for 2016-2019. Dr. Nork is a clinician-scientist trained in ocular pathology and vitreoretinal surgery, and his research focuses on both the cellular and functional aspects of retinal ischemia and its role in retinal diseases. Retinal ischemia—a condition in which oxygen supplied to the retina is greatly reduced or lost—can be caused by a variety of conditions including stroke, accident, diabetes, and retinal vein detachment. Dr. Nork is currently focusing on retinal circulation, using non-invasive imaging methods in living eyes to study blood flow and working to advance animal models that can help move novel therapies forward.

Bikash R. Pattnaik, PhD, MS (Pediatrics) has been named the McPherson Eye Research Institute's *Retina Research Foundation M. D. Matthews Research Professor* for 2016-2019. Assistant Professor Pattnaik and trainees in his lab group are studying the physiology of vision and ion channels in retinal degeneration, focusing on vision loss due to ion-channelopathy—a genetic or acquired disorder in the function of potassium or chloride channels within cell membranes that causes early onset blindness. Dr. Pattnaik is investigating ion channel dysfunction through molecular-genetic and electrophysiological studies. He is also using retinal pigment epithelium cells derived from induced pluripotent stem cells (iPSC-RPEs) to test drug therapies that may lead to functional cure of diseases like Leber congenital amaurosis, a blinding disease estimated to affect one in 80,000 children.

2016 Romnes Faculty Fellowships Awarded to McPherson ERI Members

Two of the twelve UW-Madison faculty honored with Romnes Faculty Fellowships this year are members of the McPherson ERI. Romnes awardees receive an unrestricted \$50,000 for research, supported by the Wisconsin Alumni Research Foundation (WARF).

Kristyn S. Masters (Biomedical Engineering) uses tissue engineering to create in vitro models of diseased tissues and organs—including heart valves, blood vessels, and cells that initiate wound healing—with the goal of identifying therapeutic targets for treating the disease. She is also designing a model of the retina in order to characterize age-related macular degeneration (AMD) and use the engineered tissue to identify or screen new AMD therapies.

William Murphy (Biomedical Engineering; Orthopedics and Rehabilitation) aims to create new biomaterials inspired by the materials found in nature, to understand stem cell behavior and induce tissue regeneration. His ongoing studies include novel biomaterials to design, manufacture, and deliver peptides, proteins, DNA, RNA, cells and tissues—applications that extend to angiogenesis in the eye, retinal degeneration, and ocular cell therapy.

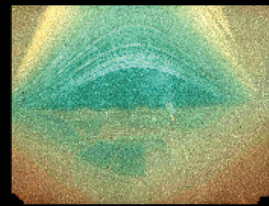
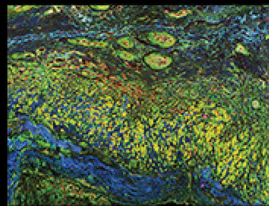
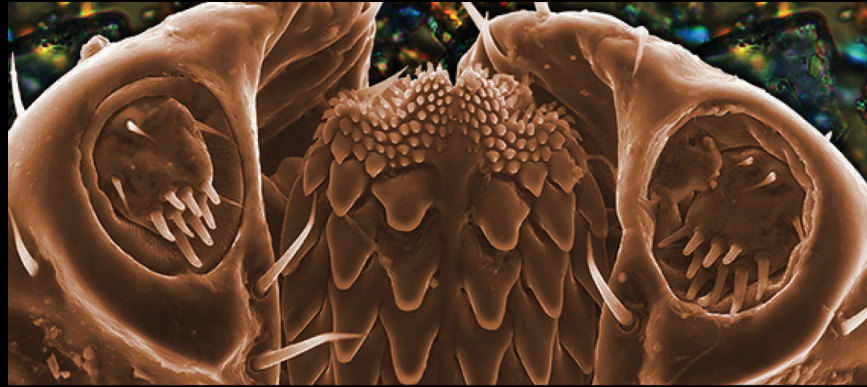


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2017**

JOIN THE RIDE! Please visit
cycleforsight.wisc.edu in
December to register or to
donate to vision research



SEPT 12 - DEC 16, 2016

In the McPherson Eye Research Institute's
MANDELBAUM & ALBERT FAMILY VISION GALLERY

9th floor, Wisconsin Institutes for Medical Research, 1111 Highland Ave
Parking available in UW-Madison lots 82 or 60 and in ramps 63 or 76

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Cool Science Image contest sponsored by Promega

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