# Rewiring the Retina

WITH TIM GOMEZ, PhD.

Tim Gomez, Professor in UW–Madison's Department of Neuroscience, has studied neural circuit development, and in particular how axons grow and are guided, for over three decades. A major goal of the Gomez lab, together with collaborators in the McPherson ERI, is to understand how neural circuits normally develop and how neural connections may be restored after damage or loss due to injury or disease. Many deficits in neural function, including motor and perceptual deficits, autism, dyslexia, and other learning disabilities, stem from genetic mutations affecting the growth or information-transfer ability of neurons.

Vision—like all human sensory perception, motor activity, and cognitive function—is controlled by an intricate network of neurons that are linked by connecting points called synapses. Signals are sent from neurons through axons, projections which transmit impulses that are received by dendrites extending from other neurons. These signals coordinate activity between billions of neurons and target cells, and form neural circuits throughout the body. The task of assembling these elaborate neural circuits during development and in regeneration requires growth cones, which are specialized structures at the tips of developing axons and dendrites (see image). These growth cones detect many types of cues in the surrounding retinal environment that guide their movement and behavior.

Understanding the axon growth and guidance process is essential to developing regenerative medicine strategies for many retinal diseases that cause vision loss. For example, photoreceptor transplantation holds promise for treating retinitis pigmentosa and age-related

Dendrites

Axon

Synapses

Image by shumpc,
Courtesy Fotosearch
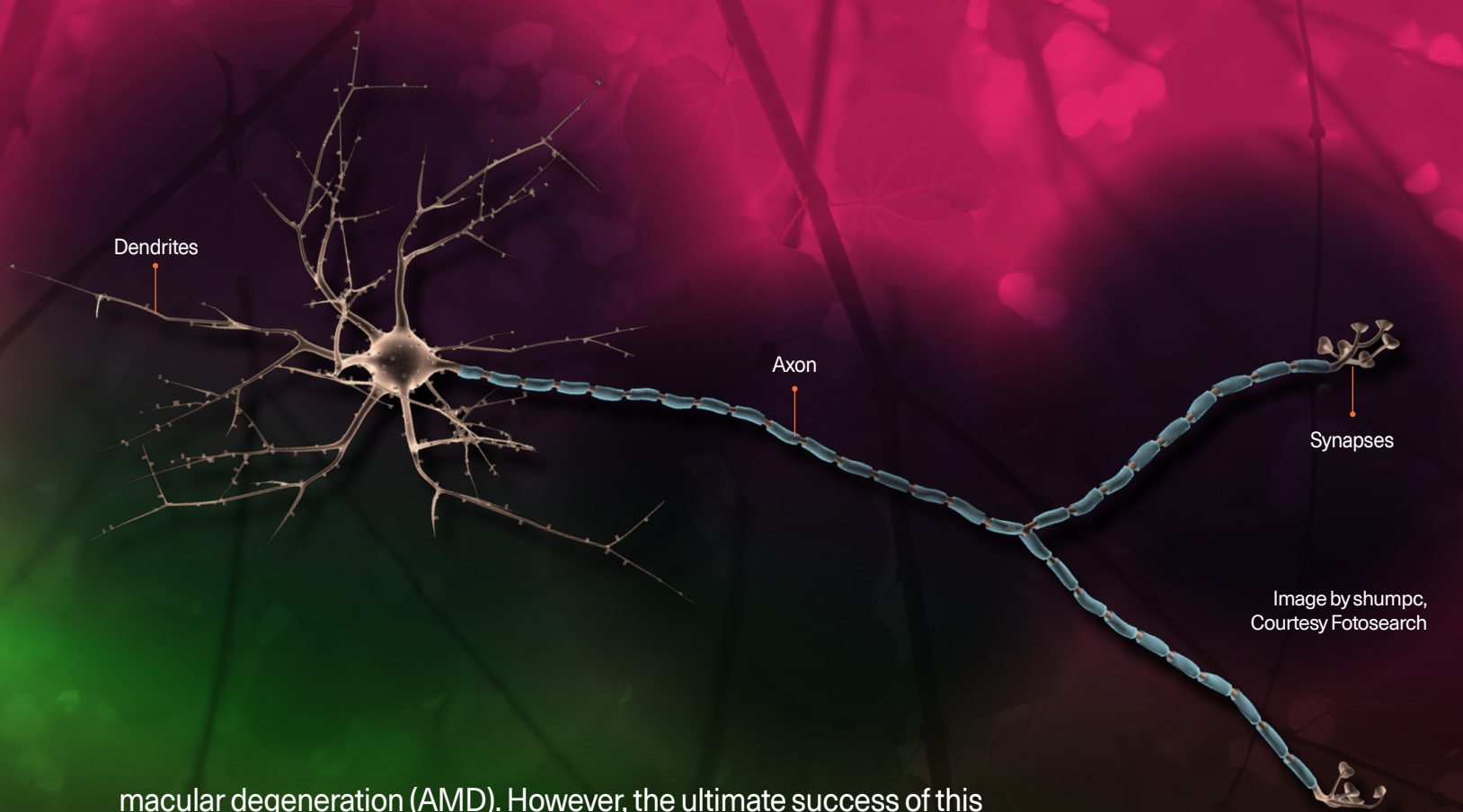
macular degeneration (AMD). However, the ultimate success of this approach requires extensive insight into the basic mechanisms regulating how photoreceptor axons extend, locate targets, and form linking synapses.

The Gomez and Gamm laboratories are currently collaborating to explore photoreceptor axon development and synapse formation. **Sarah Rempel**, a Cellular and Molecular Biology graduate student in the Gomez lab, was part of a team responsible for an important recent discovery. Rempel used protocols and stem cells developed in the Gamm lab to generate human retinal tissues (or "organoids") in a dish. The stem cells were engineered so that the photoreceptors created from them could be easily identified by bright red fluorescence. By studying the stem cell-derived human photoreceptors, the team showed that their axons undergo a developmental switch as they age within organoids. At first, photoreceptor axons extend in a "traditional" way, with their growth cones crawling forward under their own power. Later on, however, the growth cones become "sticky" and immobile. The axons can continue to grow, but only when pulled forward by other cells. Ongoing imaging studies using multi-photon microscopy, which can peer inside retinal organoids, will determine whether these axon behaviors take place in more complex 3D environments. Future studies will identify the molecular basis for this mode switch in axon growth.

Knowledge of the cellular programs that control photoreceptor axon development is an essential step in understanding how to build—and rebuild—human retinal circuits. In turn, these discoveries bring us closer to a future where transplant-based therapies can help individuals with currently untreatable retinal diseases.

From the Director

Dear Friends,

As we carefully continue our research activities in this uncertain time, one thing is certain—we remain committed to moving vision research forward on every front, with a specific focus on the four D's: discovery, diagnosis, development, and delivery. Basic science discovery leads to more and better opportunities to improve disease diagnosis, to develop effective therapies, and to optimize ways to safely deliver those therapeutics to their cellular targets within the eye and visual system. The two projects outlined in this issue of *InSights* are good examples of these core efforts. The cover story on the Gomez Lab's work on neuronal growth cones, as well as the complex multi-lab collaboration on Best disease gene replacement and repair described on these interior pages, together span the discovery to delivery spectrum.

While it is a privilege to highlight the latest work being done by our over 190 McPherson ERI members, it is also rewarding to draw attention to the many honors they receive for their efforts. I am especially pleased to congratulate Dr. Daniel M. Albert, Founding Director of the McPherson ERI, on his 2020 Clinical Sciences Emeritus Faculty Award from the School of Medicine and Public Health's Medical Alumni Association. The award, which recognizes "excellence in the practice of medicine, in academic activities and in research accomplishment," was richly deserved. Dr. Albert's essential role in establishing and guiding the McPherson ERI (after serving ten years as the Chair of the Department of Ophthalmology and Visual Sciences) was one of many factors that led to this recognition. The principles that he helped build into the Institute continue to guide the work we do every day.

Stay well,

David M. Gamm, MD, PhD

Emmett A. Humble Distinguished Director, McPherson Eye Research Institute
Sandra Lemke Trout Chair in Eye Research
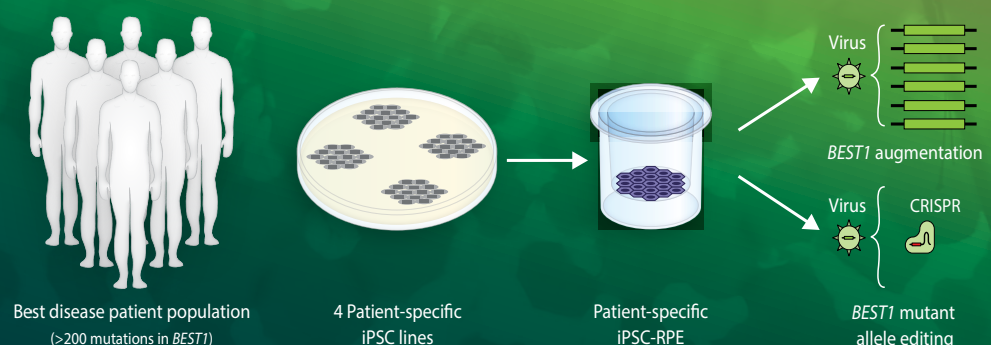
Divide and conquer:
Developing a systematic approach to gene therapy for an inherited form of macular degeneration
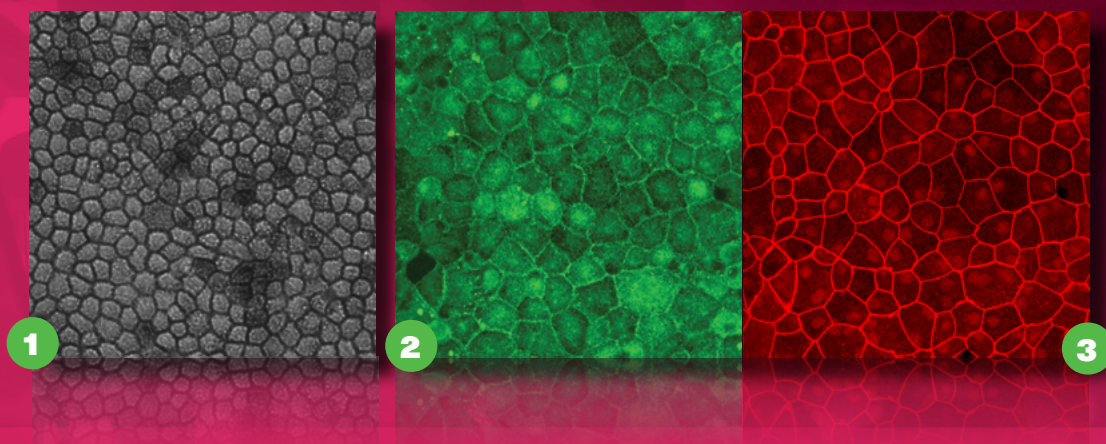
Best disease and gene therapies

Best vitelliform macular degeneration, or Best disease, is a currently untreatable blinding disorder that results in the loss of central vision in thousands of affected individuals. The disease is caused by one of several hundred small mutations in the *BEST1* gene, which codes for a protein that regulates the movement of chloride molecules across a vital layer of the retina called the retinal pigment epithelium (RPE). In a new and groundbreaking study just published in the *American Journal of Human Genetics*, a group of MERI researchers led by Divya Sinha, an Assistant Scientist in David Gamm's lab, and Ben Steyer, an MD-PhD student in Kris Saha's lab, demonstrated that a two-tiered gene therapy strategy may be able to treat all Best disease mutations in a highly effective manner.

Inherited retinal disorders (IRDs) such as Best disease, as well as the many forms of retinitis pigmentosa and Leber's congenital amaurosis (LCA), are good candidates for gene-based therapies as these disorders are caused by mutations in a single gene. Gene therapy is not a one-size-fits-all fix, though, as it must be customized for each gene and, sometimes, for each specific mutation in a particular gene. Thus, to treat all of the Best disease mutations, hundreds of therapies might need to be developed, and some might only be relevant for a handful of people worldwide. To better understand the challenges in developing gene therapies, it is important to understand the concept of dominant and recessive genes, so fasten your seat belts....

McPherson ERI researchers made induced pluripotent stem cells from multiple individuals with Best disease and directed those stem cells to produce RPE, the cell type affected by each of the *BEST1* gene mutations. They then tested the cells to determine what type of gene therapy—gene augmentation or gene editing—was best suited for that particular individual. In all cases, at least one of these two treatment strategies succeeded in eliminating the cellular defects caused by the gene mutation.

Best disease patient population
(>200 mutations in *BEST1*)

4 Patient-specific
iPSC lines

Patient-specific
iPSC-RPE

Virus
BEST1 augmentation

Virus   CRISPR
BEST1 mutant
allele editing

1 A microscopic image of a layer of retinal pigment epithelium (RPE) cells grown in a laboratory dish from induced pluripotent stem cells of an individual with Best disease (left panel). Two panels show fluorescent microscopic images of this person's RPE cells highlighting the distribution of *BEST1* protein (green) **2** and another protein (zonula occludens-1) involved in tightly connecting RPE cells together (red) **3**.

Dominant vs. recessive genetics

To understand this difference, you first need to remember that every gene in your body comes as a pair. Dominant IRDs are caused by mutations in only one of the two genes coding for a specific type of protein, which means that the other gene (and the protein it makes) is normal. Unfortunately, the "bad" protein (think of it as the evil twin) overpowers the good twin, causing disease. To fix a dominant IRD, the bad gene needs to be specifically identified and removed or fixed without inadvertently harming the good twin (and remember, as twins, they are very hard to distinguish). This requires the customized gene editing noted above.

Recessive IRDs, on the other hand, are caused by mutations in both genes. These mutations result in no functional protein being made from either one, so there are no twins at all. As a result, recessive IRDs are less picky in comparison; fixing them requires "only" a new source of the protein. Of course, none of this is truly easy, but the latter scenario involves a much more well-established process known as "gene augmentation." It is also more cost-effective and faster to replace an entire gene than deal with the hundreds of individual mutations that can occur in a gene like *BEST1*.

To use another analogy, dominant "evil" mutations produce workers that actively look to sabotage the efforts of their capable coworkers, whereas recessive mutations produce proteins that never show up for work at all. As it turns out, the latter situation is usually simpler to treat than the former. But the vast majority of cases of Best disease are caused by dominant mutations, so are these people out of luck? The McPherson ERI team aimed for a better answer.

Rethinking the status quo

The McPherson ERI team hypothesized that it may be possible to adequately dilute the influence of the dysfunctional BEST twin by ignoring it entirely and adding many more good *BEST1* twins through gene augmentation. Testing this novel idea required the expertise and resources of four different labs:

- **In the Gamm Lab** (Dept. of Ophthalmology and Visual Sciences), **Divya Sinha** led the stem cell portion of the study. She used stem cell lines from four Best disease patients with four different *BEST1* mutations to generate, study, and treat their RPE cells in the laboratory. Using a specialized viral delivery system, Dr. Sinha performed gene augmentation by introducing a healthy copy of the *BEST1* gene into the RPE cells.

- **Once the Best disease patients' RPE cells** were treated with a boost of "normal" *BEST1* gene, their functional response was evaluated by **Pawan Shahi** in the Pattnaik lab (Dept. of Pediatrics). Shahi used electrophysiology to record the *BEST1* protein activity in the RPE cells and found that three of the four patients showed complete reversal of the cellular dysfunction.

- **What about the remaining patient** whose RPE function hadn't been restored? **Ben Steyer** in Kris Saha's lab (Dept. of Biomedical Engineering) was able to devise a way to rub out the bad twin without touching the good twin. This was done using CRISPR-Cas9 gene editing, a method that has garnered much attention but is just now being tested in humans. Steyer and colleagues found that the patient's RPE cells that failed to improve after gene replacement showed full reversal of the disease after gene editing.

- **One of the potential problems** associated with gene editing is the threat of randomly introducing unintended gene mutations into the patient's genetic code (so-called "off-target effects"). So how does one go about searching for unknown needles in giant genetic haystacks? **Sushmita Roy** (Dept. of Biostatistics and Medical Informatics) uses powerful computational methods to address this problem. Dr. Roy's lab, working with **Katie Mueller** (a PhD student in the Saha lab), found that all other genes besides the bad *BEST1* twin were essentially unaffected by the gene-editing process, a critical step in determining the safety of that therapeutic approach.

Together, these researchers succeeded in showing that a single-gene replacement strategy could be an effective treatment for a large subset of individuals with Best disease, and that gene editing could work for the rest, leaving no one behind. **From a broader perspective, they showed that gene augmentation could be a viable therapy for at least some dominant genetic diseases.** This revolutionary concept could greatly reduce the complexity and cost of developing gene therapies for dominant IRDs and shorten the timeline for clinical trials. And that's cause for optimism. "I'm very excited about these findings," Dr. Sinha noted. "This is what we work for."

Oscar C. Boldt
1924-2020

The McPherson ERI deeply regrets the passing of Oscar C. Boldt, an extraordinary individual who was a founding McPherson ERI Advisory Board member and friend to the Institute for many years. Oscar, who passed away on June 9th of this year, was Chairman of The Boldt Company, one of the nation's largest and most highly respected construction firms. The Boldt Company's sterling reputation is a reflection of Oscar's personal history of service and community support, including his service as a navigator on a 15th Air Force B24 bomber in Italy during World War II. After the war, he returned to Madison to complete his Civil Engineering education. After graduating in 1948, Oscar joined the family's construction company; he guided Boldt Construction for 70 years, crafting many familiar and beautiful Wisconsin buildings including the Wisconsin Institutes for Medical Research (WIMR) towers on UW-Madison's medical campus—a state-of-the-art research complex that holds the McPherson ERI's home offices and labs.

Oscar and his wife Pat, who remains an Honorary Advisory Board member, helped guide and support the McPherson ERI in its early years, and continued in recent years to stay involved with the Institute's progress. Together, they had a second career as benefactors of countless organizations in Appleton and other communities.

Perhaps more than any of these things, what we will miss about Oscar— familiarly known as O. C.—is his great personal warmth, intelligence and common sense. He assisted the Institute with advice both complimentary and constructively honest, and always with a twinkle in his eye. The last line of his obituary sums up the man and his contribution to humanity: "In lieu of flowers... write a note to a friend you haven't spoken with for a long time and tell them how much they mean to you."

Kenzi Valentyn Vision Research Grants 2020

The McPherson Eye Research Institute's Research and Leadership Committees are pleased to announce the 2020 recipients of the Kenzi Valentyn Vision Research Grants, who will each receive a one-year grant of $4000 funded by the Institute's Cycle for Sight event.

Anjani Chakrala is a graduate student in Neuroscience (mentored by Xin Huang). Her project, which focuses on neural representation of overlapping motion surfaces in a part of the brain called the Visual Area MT, will provide new insights into a fundamental function of vision and the close interaction between eye movements and visual perception.

Ralph W. Nelson is a graduate student in Kinesiology – Motor Control & Behavior (mentored by Andrea Mason). His research focuses on assessing visual attention during dual-task walking in children with Autism Spectrum Disorder, with implications for their daily activities.

Abhilash Sawant is a graduate student in Neuroscience (mentored by Raunak Sinha). His award will support work to understand the mechanism and function of postsynaptic inhibition in mouse ON-Alpha retinal ganglion cells, crucial to understanding the retinal substrates underlying dim light vision.

Kenzi Valentyn Vision Research Awards, the McPherson Eye Research Institute's research grant opportunity for trainees, were established in 2017. They are named after Kenzi Valentyn, in honor of her courage and positive attitude throughout her long battle with Kearns–Sayre syndrome, a degenerative disease with symptoms including vision loss, which ended with her passing at age 30 in March 2017. **The McPherson ERI is grateful for the Valentyn family's dedication to vision research.**

You can support the McPherson ERI at vision.wisc.edu/giving. Thank you!