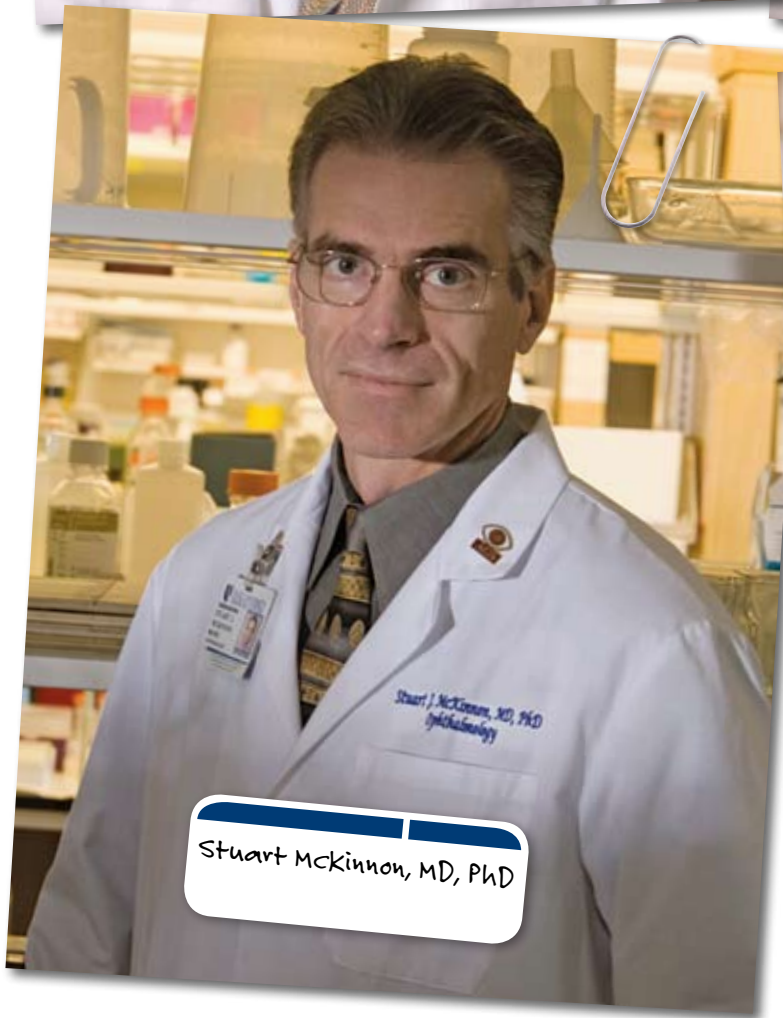




Rand Allingham, MD



Pedro Gonzalez, PhD



Stuart McKinnon, MD, PhD



Vasanth Rao, PhD



The Glaucoma Detectives

Duke Eye Center's researchers are searching through cells and genes, hunting for the real cause of glaucoma

URNS OUT, IT'S NOT SO SIMPLE.

For years the medical community thought elevated pressure inside the eye was the main cause of most glaucoma cases, the leading source of incurable blindness in the United States and the world. Based on that belief, most treatments were aimed at reducing intraocular pressure.

Recently, however, there has been a growing awareness that perhaps as many as a third of the people who develop glaucoma have normal or near-normal intraocular pressure. In fact, in some populations, including Mexican-Americans and Japanese, over 80 percent of those with glaucoma have normal eye pressure.

So if increased intraocular pressure *isn't* the only cause of most glaucoma, *what is?*

That's what researchers at the Duke Eye Center are trying to figure out. In the laboratories of the Albert Eye Research Institute, they are delving into the inner function of the eye from every angle. Once they pinpoint the cause, it's just a matter of time before better treatments and, ultimately, a cure for this devastating disease is found.

Glaucoma is an optic nerve disease (actually a group of more than 60 diseases) characterized by the loss of optic nerve tissue, which ultimately leads to vision loss and blindness. Elevated eye pressure is a major risk factor for most forms of glaucoma because it can cause damage to the optic nerve. However as many as 25 to 30 percent of individuals who develop glaucoma do so with normal or near-normal intraocular pressure, and their glaucoma is indistinguishable from that of people who have elevated pressure. Of all the individuals who have an elevated eye pressure, only about 10 percent will develop glaucoma.



One of the biggest challenges with glaucoma is that, in most cases, it is an asymptomatic disease. It rarely causes pain or symptomatic vision loss until late in the course of the disease. Vision loss from glaucoma is not reversible, so while we strive to find a cure for glaucoma, *diagnosing* it at a treatable stage remains a major goal.

"Your eye pressure can be normal, and you can develop glaucoma, or your pressure could be elevated, and you may not develop glaucoma. So pressure is a terrible way to screen whether you have glaucoma," explains Rand Allingham, MD, chief of Duke's Glaucoma Service.

Worldwide an estimated 70 million people have glaucoma; seven million of these people are blind. That makes glaucoma a major public health issue, one that creates a tremendous burden on the individuals, their families, and society-at-large.

One of the biggest challenges with glaucoma is that, in most cases, it is an asymptomatic disease. It rarely causes pain or symptomatic vision loss until late in the course of the disease. Vision loss from glaucoma is not reversible, so while researchers strive to find a cure for glaucoma, *diagnosing* it at a treatable stage remains a major goal.

Current treatments for glaucoma include medication, laser surgery, and conventional surgery that lower eye pressure to slow or stop the progression of the disease, and, in some cases, surgery to clear a plugged fluid drain. Even in cases where pressure is not elevated, treatment is directed at lowering eye pressure (from the high-normal range to the low-normal range). Now it is still the only proven way to control vision loss from glaucoma.

When elevated eye pressure does occur, it is caused by a blocked fluid drain in the eye. Aqueous humor is the colorless liquid that fills the eyeball. It is pumped through the eye continuously, even before birth. It circulates within the eye, nourishes it, and keeps the eye inflated. The aqueous fluid is constantly produced by the ciliary body, and it must be drained continuously through a fluid flow drain. When the drain doesn't work efficiently, eye pressure increases. The elevated eye pressure damages the optic nerve, more specifically the nerve fibers within the optic nerve that carry all visual information to the brain.

In many glaucoma cases where pressure is elevated, the fluid flow drain isn't working well. Duke basic science researcher Pedro Gonzalez, PhD, associate professor in ophthalmology, is studying how the drain works at the molecular level.

"Glaucoma usually has a very slow progression and, in general, it affects people after the age of 40," says Gonzalez, who worked at the National Eye Institute laboratories before coming to Duke. "We want to know, first, how normal drain tissue works, and then what goes wrong with that mechanism in glaucoma. If we can understand what is responsible for the failure of this tissue in the fluid drain, we can develop treatments to delay the disease's progression."

Gonzalez and his research team use pig eyes for most of their studies. They take tissue samples from the front of the eye (the part responsible for draining the aqueous humor), pump fluid into these samples, and measure the speed at which the liquid drains. They also modify cells in this tissue by genetically introducing, altering, or removing genes related to the drainage process to determine the impact on the rate of fluid drainage. They are studying this process in both healthy eye tissue and tissue from eyes with glaucoma.

Gonzalez's lab is currently focusing on oxidative stress as one of the main factors that could cause glaucoma. Oxygen is used by cells to breathe and to obtain energy. But it can also form a series of molecules that can damage the cells in the drainage system.

"We think it may be possible for us to develop medications that could prevent the damage caused by oxidative stress so the drain tissue will be functional for a longer time. In the laboratory, we're testing molecules that seem to be promising in helping to protect the cells from this oxidative damage, and we're hoping to be able to begin testing in animal models very soon."

Researcher Stuart McKinnon, MD, PhD, associate professor of ophthalmology, is starting there. The optic nerve, which is in the retina in the back of the eye, is made up of about 1.5 million nerve





fibers that arise from ganglion cells that transmit signals between the eye and the brain. In glaucoma these ganglion cells begin to die, which causes vision loss. McKinnon is trying to understand the molecular process by which this cell death occurs.

“Now that we know elevated intraocular pressure is not the cause of glaucoma, it’s important to find therapies to protect the optic nerve itself. We’re trying to determine the important events in the cell death process and ways to delay or prevent those events from happening,” McKinnon explains. “Glaucoma is a long-term, chronic disease, and the longer we can delay the process, the more useful vision people will have. If we can make the optic nerve healthy and strong so it’s not susceptible to damage by elevated eye pressure, then we won’t have to worry as much about controlling that pressure.”

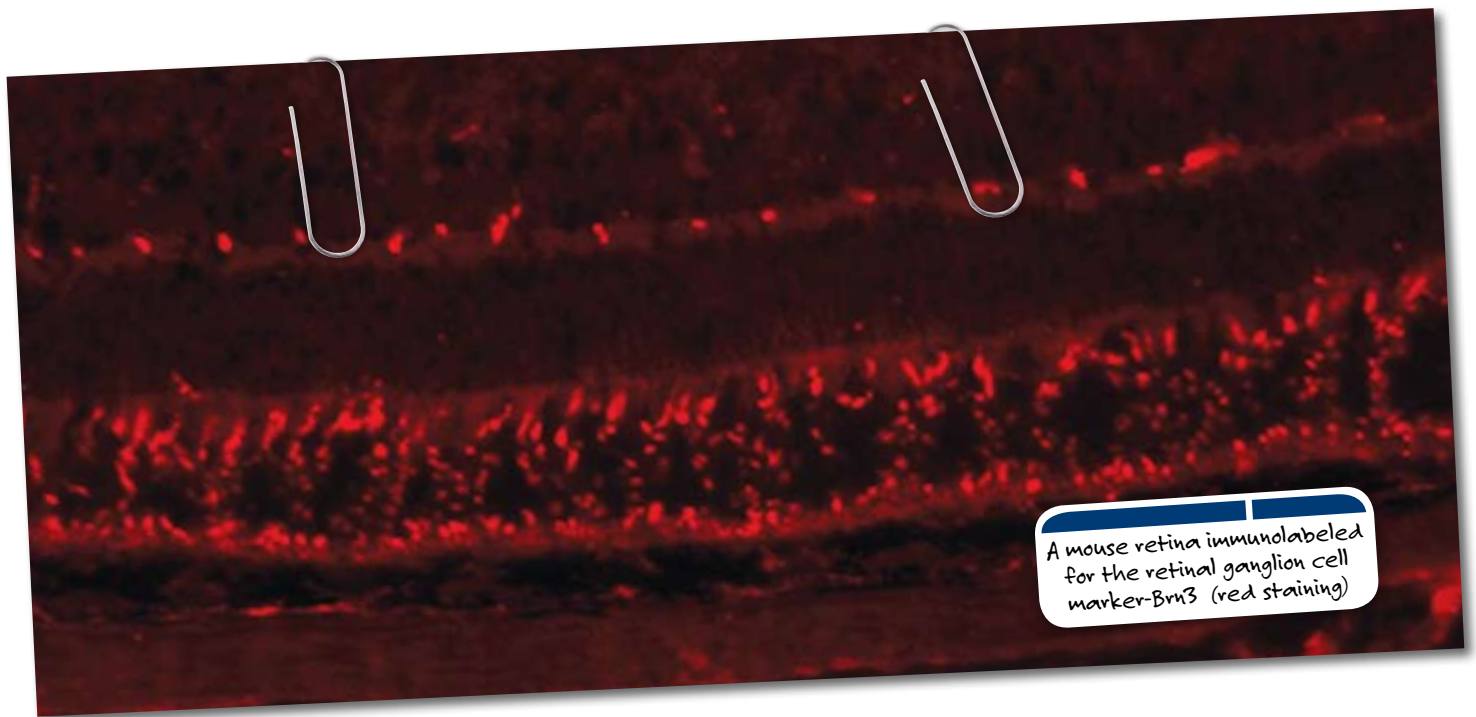
McKinnon’s lab uses rodents to test different therapeutic approaches, such as using viruses to deliver genes that will produce proteins capable of protecting the ganglion cells. The scientists elevate the pressure in one eye of each animal and then deliver either a treatment or a sharp control treatment to these eyes. They then determine whether there is any protection from glaucoma damage in optic nerves in the treated group when compared to the optic nerves from the control group. If successful in animal models, McKinnon will take these treatments to human clinical trials with

the ultimate goal of translating this laboratory research into effective treatments for glaucoma patients.

McKinnon was the first researcher to discover that molecular events that take place in glaucoma are similar to those that occur in Alzheimer’s disease, a finding recently confirmed by British researchers. Proteins that affect the brains of people with Alzheimer’s also appear to cause the death of the optic nerve cells in glaucoma. This means that therapies used to treat Alzheimer’s could be used to

Now that we know elevated intraocular pressure is not the cause of glaucoma, it’s important to find therapies to protect the optic nerve itself. We’re trying to determine the important events in the cell death process and ways to delay or prevent those events from happening.

treat glaucoma, McKinnon says. Pharmaceutical companies are already conducting human clinical trials for several medications that are FDA-approved for treating Alzheimer’s disease to see if they are effective in treating glaucoma. McKinnon says gene therapy and immunological treatments may also be promising, given those findings. He is conducting a pharmaceutical company-sponsored trial to determine if immunotherapy directed against amyloid-beta,



A mouse retina immunolabeled for the retinal ganglion cell marker-Brn3 (red staining)

the neurotoxin molecule found in the senile plaques in Alzheimer's disease, can prevent optic nerve damage in his rodent glaucoma model.

In the research laboratory next door to McKinnon's, Vasanth Rao, PhD, associate professor in ophthalmology with a secondary appointment in the Department of Pharmacology and Cancer Biology, is applying his expertise in cell biology and cytoskeletal signaling to both the aqueous fluid flow drain and the optic nerve. Rao's research efforts are divided between glaucoma and cataract.

"The immediate goal of our lab in the area of glaucoma is to understand the cell biology of the aqueous humor drainage pathway," Rao says. "Every cell has a structural framework, the cytoskeleton, which operates like the human skeleton in that it holds up and gives shape to the cell. Our lab is trying to understand how the cytoskeleton of the cells around the drain works, and how it influences other cellular activities under normal conditions and in eyes with glaucoma, so that we can find specific molecules in these cells that we can target with drugs or molecular therapy to increase fluid flow and lower intraocular pressure."

Using human and pig eyes as samples, Rao's lab has already found several molecular targets that increase aqueous outflow, and pharmaceutical companies are now running clinical trials on some of the promising drugs and drug-delivery systems to target these molecules effectively and safely. The research team is also using animal models to investigate whether issues with the cytoskeletal signaling of the drain cells may be linked to the development of glaucoma.

Rao's research interests complement those of David Epstein, MD, professor of ophthalmology and chairman of the Department of Ophthalmology, a renowned glaucoma researcher in his own right. Rao is also collaborating with McKinnon to explore, at the cellular level, how changes in cytoskeleton and intraocular pressure affect the survival—or death—of the ganglion cells in the back of the eye.

And then there's genetics. Researchers have found that possessing certain genes can cause an individual to be more susceptible to developing some forms of glaucoma. More specifically it appears

that the genes make someone more susceptible to damage of the optic nerve or fluid drains, explains Allingham, who has been leading a large-scale study to find the genes that cause glaucoma in the United States and in Ghana, West Africa, where the prevalence of glaucoma is especially high—even in the younger population.

"Many forms of glaucoma are inherited. If we can understand exactly what is inherited genetically that increases our risk of glaucoma, then we'll be able to do DNA testing to identify those at high risk long before damage occurs. If the risk is very high, we could even treat the disease preventively."

Once the genetic links are understood, as well as the molecular workings of the optic nerve and fluid flow drain, targeted treatments could be developed that are specific to the cause of each form of glaucoma, rather than treating high eye pressure generally, Allingham says. "That's important because elevated eye pressure and/or vision loss is the last thing that develops – a series of other things have gone wrong before that. Understanding the genetics can help us intervene earlier in the disease process, before it gets too far along."

Allingham, Gonzalez, McKinnon, Rao, and other glaucoma laboratory researchers and clinician-scientists at Duke are in close communication, sharing their findings and collaborating on several projects. Allingham's genetic findings can tell Gonzalez which genes or proteins to look at in the drain tissue he's studying, for instance, or guide McKinnon to study those genes in his mice. Or Gonzalez may find proteins that appear to play an important role in fluid flow in the eye, and Allingham can use that information to look at candidate genes.

What is the cause of glaucoma? The answer, when it is found, will clearly be complex. As these Duke Eye Center "detectives" pursue every lead, each new insight raises more questions and more investigative avenues to pursue. But together, they're approaching the eye from every angle—which means it won't be long before their suspect is cornered.

