medicine at MICHIGAN

Fall 2007
A PUBLICATION OF THE UNIVERSITY OF MICHIGAN MEDICAL SCHOOL

Body and Soul

By Sally Pobojewski • Photos by Scott Galvin

Secrets of the *Cilia*

{ long-overlooked organelles hold some hefty keys to human health }

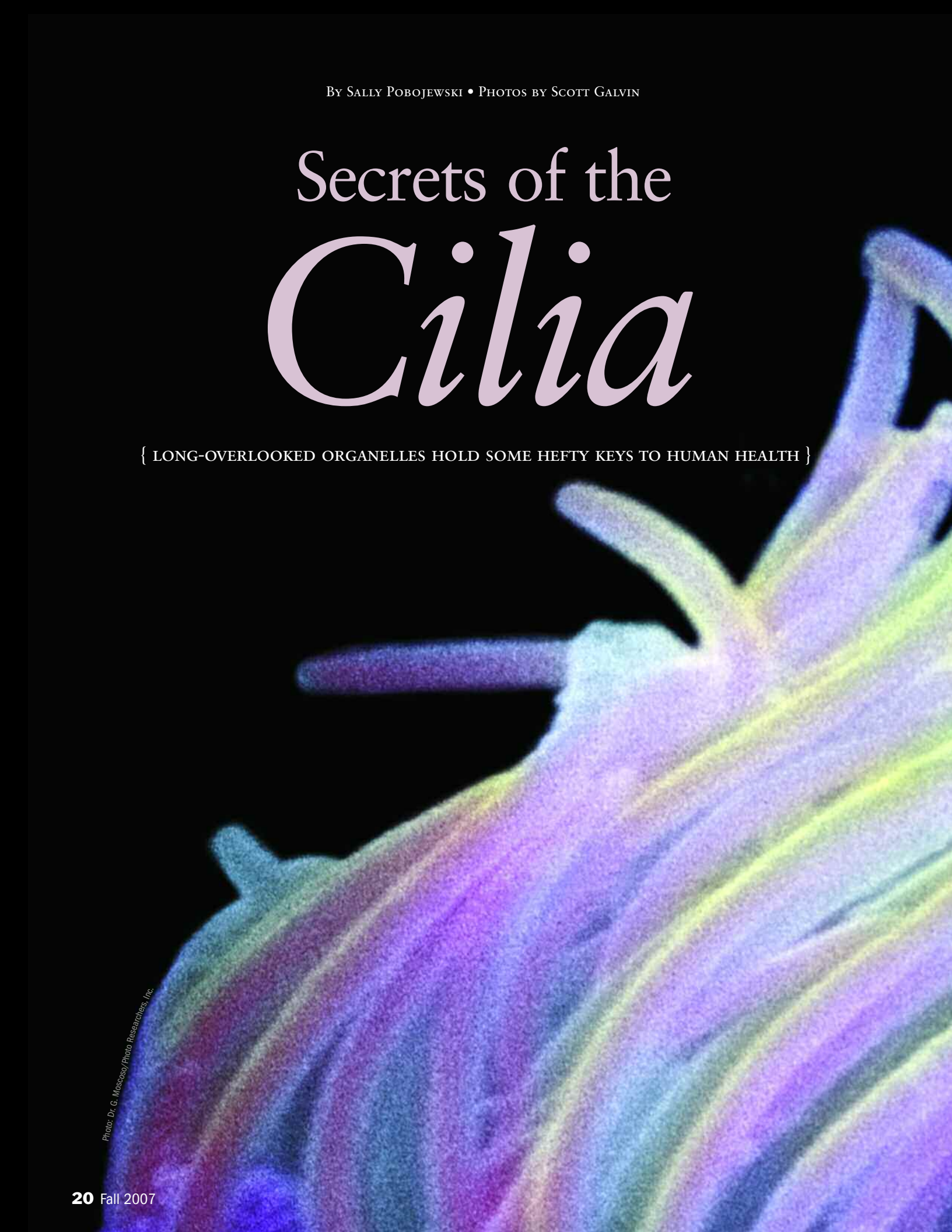
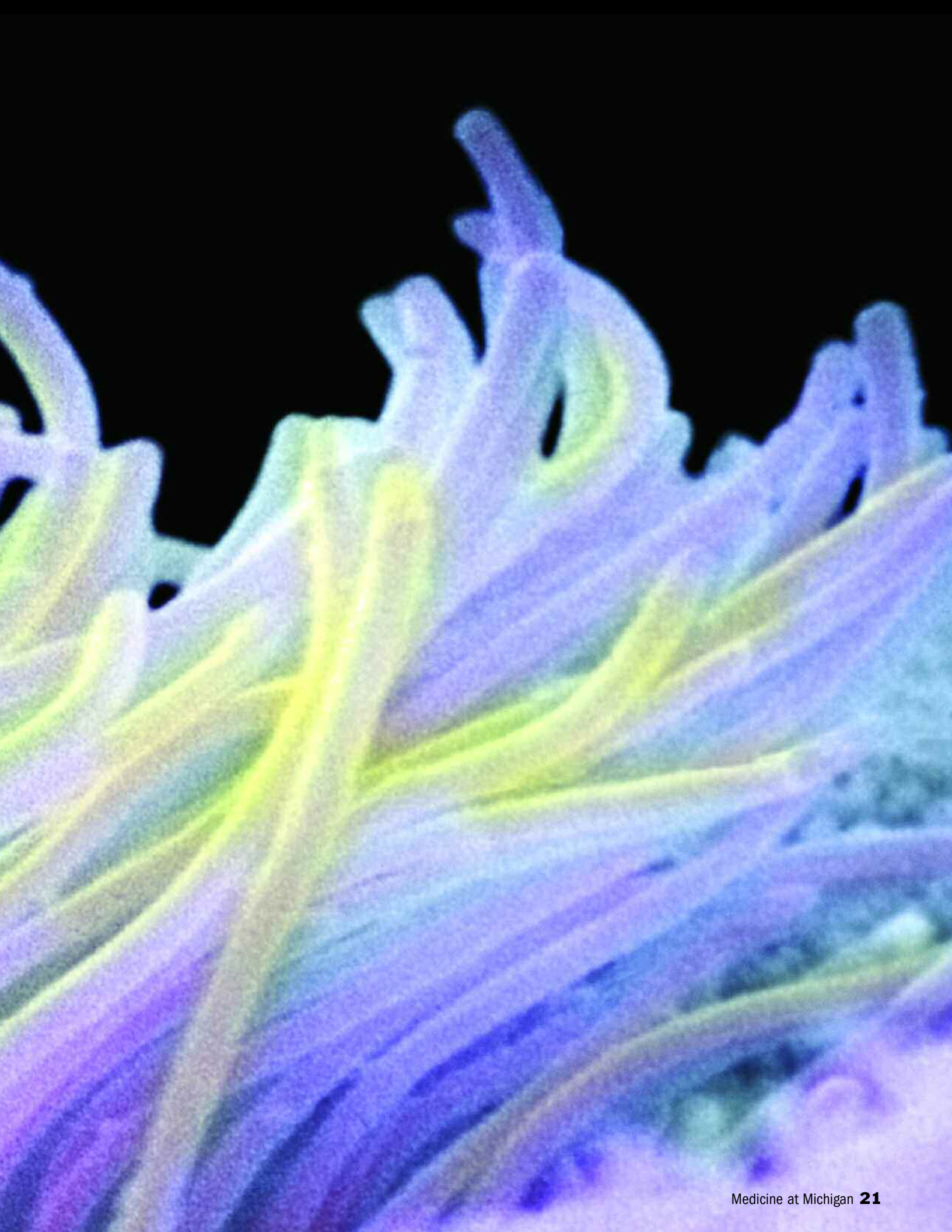


Photo: Dr. G. Moscoso/Photo Researchers, Inc.



Every year, thousands of babies lose the genetic lottery and are born with mutations in genes known as PKD1 or PKD2. Most of the time, the babies are normal at birth. But as they grow to adulthood, their kidney tissue will slowly be destroyed and replaced by large fluid-filled cysts. Eventually, about 50 percent will develop kidney failure and need dialysis or a kidney transplant to survive.

It's called polycystic kidney disease, or PKD, and within the world of genetic disorders, it's a common, life-threatening condition that affects 600,000 Americans. Physicians have no treatment, no cure, and many questions about PKD. Ben Margolis, M.D., a professor of internal medicine and of biological chemistry, believes the answers will be found in cilia.

Scientists like Margolis are just beginning to understand how much life depends on these tiny hair-like sensory antennae — thinner than a strand from a spider's web — that are found on the surface of nearly every cell in virtually every organism on Earth. From roundworms to fruit flies, from algae to zebra fish, from mice to humans, evolution has relied upon cilia to help cells sense changes in their external environment.

Thanks to cilia, you can see the words on this page, smell fresh coffee brewing in the morning and hear birds chirping outside the window. Cilia regulate the growth of kidney cells and control how an embryo develops. They sweep particles and mucus out of the respiratory tract, nudge eggs down fallopian tubes, and help neurons in the brain grow new connections.

Until recently, cilia didn't get much respect from scientists, because they were considered to be nothing more than leftovers from our distant evolutionary past — the cellular equivalent of wisdom teeth. But when researchers discovered that defects in cilia can cause human disease, the scientific community suddenly became very interested.

Highway for the Light

Anand Swaroop, Ph.D., a scientist at the Kellogg Eye Center, studies photoreceptor cells called rods and cones, which are found in the retina lining the back of the eye. These specialized neurons capture photons of light and transform them into electrical signals, which are processed by the brain to allow us to see.

According to Swaroop, the human retina contains about 120 million photoreceptor cells, and they are some of the hardest working cells in the body. Each photoreceptor produces about 6,000 light-grabbing molecules of rhodopsin or cone opsin every minute. That's a total of 6 billion molecules synthesized every second in each human retina. All the molecular components required to make these proteins must travel through one slender cilium, which connects the photoreceptor's inner and outer segments.

"It's a very vulnerable connection, and that's why we have so many blinding diseases that involve ciliary or transport defects," says Swaroop. "Any defect in the synthesis, regulation, transport or transduction of all these molecules can quickly lead to the degeneration of photoreceptors. These are non-dividing cells, so if you lose too many of them, you go blind."

Because the cilia connection in photoreceptors is so delicate, Swaroop says even small losses in protein function can compromise vision, even if cilia on other types of cells are not affected.

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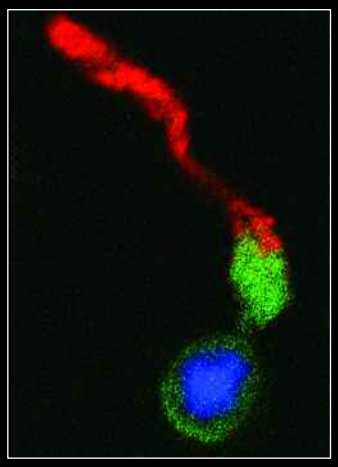
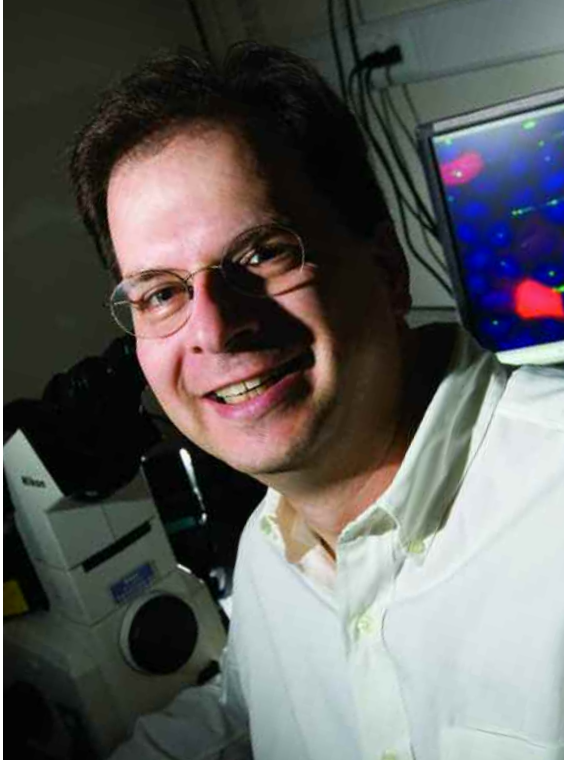


Photo: Sunil Parapuram, Ph.D. (Swaroop Laboratory)

This photoreceptor cell has been stained to show the nucleus (blue), the outer segment (red) and the inner segment (green). Everything the cell needs to make rhodopsin or cone opsin — molecules vital to human sight — passes through one tiny cilium that connects the inner and outer segments of the cell.

SCIENTISTS ARE JUST BEGINNING to understand how much life depends on these tiny hair-like sensory antennae. Long thought to be evolutionary "leftovers," cilia only recently gained scientific respect as researchers have learned that defects in them can cause human disease.



Ben Margolis

So far, scientists have linked about 10 human diseases to cilia-related defects. Researchers at the Medical School are studying these diseases, called "ciliopathies," to understand how cilia work and what happens when they don't.

"There are many diseases involving cilia and many more we don't even know about yet," says Margolis. "This is a new field — only about 10 years old — and we still have more questions than answers."

Someday, research on cilia could lead to new treatments for cystic kidney diseases or a cure for the blinding disorder called retinitis pigmentosa. Learning more about olfactory cilia involved in the sense of smell may even make it possible for future physicians to use scratch-and-sniff tests to diagnose many common diseases.

U-M scientists are enthusiastic about the importance of cilia to human health and medicine, but they caution that many years of research will be required before they can answer even the most basic questions about cilia's structure and function.

After all, it's taken millions of years for evolution to fine-tune the intricate connections between cells and cilia. The cilium will not give up its secrets easily.

GOING WITH THE FLOW

Most people tend to take their kidneys for granted. Filtering blood and making urine may not be the most elegant jobs in the human body, but they are among the most important.

Each about the size of a fist, the kidneys are made up of small filtration chambers connected by a drainage system of tubules. The interior surfaces of these tubules are lined with a layer of epithelial cells. And on the surface of every one of those epithelial cells is a single cilium.

Margolis has spent decades studying the proteins that control how epithelial cells develop in the kidney. Epithelial cells are in a constant state of turnover. As old cells die and are sloughed off the inner surface, the body must grow new cells to replace them.

The body keeps tight controls on the development of new epithelial cells, according to Margolis, because they can't grow just any way they want to. All epithelial cells have polarity, meaning they are oriented in a specific direction in space and develop in a specific order. In kidney epithelial cells, the cilium always forms on the interior, or apical, side, so urine passing through tubules on its way to the bladder can flow over the cilium and bend it in the direction of the flow.

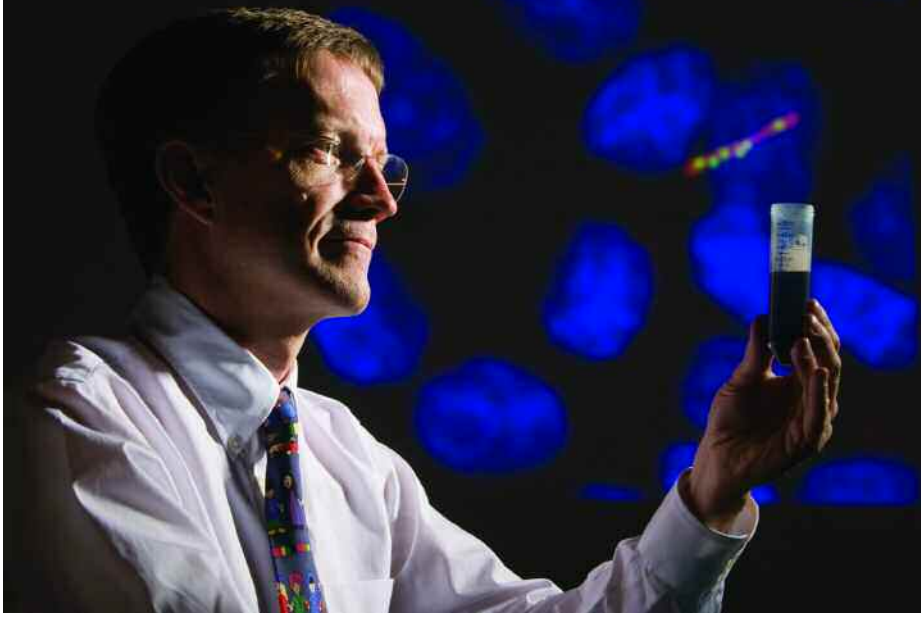
"The leading theory in polycystic kidney disease is that cilia sense urine flow and bend in response," Margolis says. "A calcium channel mechanosensor at the base of the cilium senses bending. When the cilium bends, it sends calcium into the cilium, which sends a signal to the cell telling the kidney everything is cool."

This sensing mechanism could have several important functions, according to Margolis. If tubules are blocked and the kidneys stop functioning, the signal could trigger tubule cells to start dividing in an effort to bypass the blockage. The sensing mechanism also could be important in directing kidney cells to grow in the proper direction.

"In polycystic kidney disease, we think this sensing mechanism goes awry," Margolis says. "There is still normal urine flow, but the kidney's sensing system is broken."

Margolis explains that people with PKD are born with one normal copy of a polycystin gene and one mutated copy. The body uses the genetic code stored in polycystin genes to make many of the proteins found in cilia on kidney epithelial cells.

Kidney epithelial cells can function normally with one mutated polycystin gene, according to Margolis. But if a second mutation knocks out the normal copy of the gene, it prevents the cilium's signal from reaching the cell. No longer able to sense the normal flow of urine, Margolis believes the epithelial cell loses its polarity and starts dividing uncontrollably in all directions to form a cyst. As mutations accumulate, more kidney cells are affected and more cysts develop.➤


Friedhelm Hildebrandt

"If we can understand how defects in cilia block their signals to epithelial cells, we may be able to stop or reverse the progressive kidney damage," Margolis says. "PKD is a slowly progressing disease, so even if we can just slow the growth of kidney cysts, people may be able to outlive it."

MAKING CONNECTIONS

After a kidney epithelial cell divides to make two new cells — a process called mitosis — each cell must build a new cilium. The process begins with the centrosome, a structure that organizes microtubules which pull apart the cell's DNA during mitosis to make two sets of chromosomes. Once cell division is complete, the centrosome moves to the apical side of the new epithelial cell to form a foundation called a basal body. The cell then builds a cilium on the basal body by moving proteins up a scaffold made of microtubules generated by the centrosome. If something goes wrong during this complex process, the cell won't have a cilium.

"Cilia are protruding organelles in the middle of the cell, and they have to be built like a high-rise is built," says Friedhelm Hildebrandt, M.D., the Frederick G.L. Huetwell Professor for the Cure and Prevention of Birth Defects, who is also a professor of pediatrics and of human genetics, and a 2007 Doris Duke Clinical Scientist. "One needs an elevator to bring the tubulin scaffold out there, and motor proteins to transport the cargo up and down the scaffold."

The trafficking of proteins up and down the cilium is called intraflagellar transport, and it is one of the most intriguing and baffling of cilia's many secrets. Somehow the basal body knows which proteins to send up the cilium, depending on the cell's function. But how this decision is made or what happens to proteins as they move up one side of the cilium and down the other is still a mystery.

Some of the molecular cargo moving up and down the cilium includes polycystin-1, polycystin-2 and other proteins involved in cystic kidney disease. Hildebrandt says scientists now believe that proteins from almost all the genes involved in cystic kidney disease are located in cilia, in basal bodies or in the centrosomes.

Hildebrandt and his research team study genes that, when mutated, cause a type of cystic kidney disease called nephronophthisis (pronounced nephrono-THI-sis), a rare degenerative disease that leads to kidney failure in infants, children and young adults. Kidney damage from nephronophthisis is similar to that of polycystic kidney disease, except the kidneys get smaller instead of larger and have more scarring. So far, Hildebrandt has identified 10 genes with mutations that cause different types of the disease.

In the process of searching for nephronophthisis genes, Hildebrandt's research team discovered some interesting things about cilia. For example, children with a mutation in the gene for NPHP5 not only had nephronophthisis, they also had a blinding disease called retinitis pigmentosa. The connection between kidney disease and eye disease, Hildebrandt says, is found in cilia. Just like kidney epithelial cells, photoreceptor cells in the retina of the eye depend on cilia to function normally.

FROM ROUNDWORMS TO FRUIT FLIES, from algae to zebra fish, from mice to humans, evolution has relied upon cilia — found on the surface of nearly every cell in virtually every organism on Earth — to help cells sense changes in their external environment.

Tracking down the NPHP6 gene led to another connection with a rare disorder called Joubert syndrome. Babies with Joubert syndrome are born with nephronophthisis, retinitis pigmentosa and severe mental retardation caused by defective cilia on brain neurons.

One of the most devastating cilial diseases is an inherited disorder called Bardet-Biedl syndrome. Depending on the combination of mutant genes they inherit, children with the syndrome can have retinitis pigmentosa, mental retardation, extra fingers and toes, cystic kidney disease, diabetes, obesity, an impaired sense of smell and/or infertility.

How can mutations in just a few genes lead to defects in so many different parts of the human body? Since

all known Bardet-Biedl genes generate proteins that are present in cilia, basal bodies or centrosomes throughout the body, even one mutation can have multiple — and seemingly unrelated — effects.

"It seems that defective ciliary proteins can lead to disease in virtually all organ systems," says Hildebrandt.

Consider that a protein involved in cargo transport on cilia has been found in plaques and tangles from brains of people with Alzheimer's disease. The abnormal growth of cancer cells may be associated with a defect in centrosomes. Defective cilia have been linked to neural tube defects like spina bifida. Scientists have recently learned that signaling molecules called Hedgehog and Wnt, which regulate every phase of cellular and embryonic development, don't work without cilia.

It's ironic how much the normal functioning of the human body depends on a common cellular structure that was basically ignored by scientists until just 10 years ago. Now that researchers finally realize how important they are to human health and disease, cilia already may have lost their biggest secret.

Friedhelm Hildebrandt's research has been supported by Irv and Carol Smokler, U-M alumni from Boca Raton, Florida.

Picking Up the Scent

Evolution has a lot of resources invested in the human sense of smell, according to Jeffrey R. Martens, Ph.D., an assistant professor of pharmacology who joined the Medical School faculty three years ago. It takes the activity of at least 300 human genes — more than 1 percent of the entire human genome — to smell the difference between a banana and a steak.

Cilia are on the front lines of the body's olfactory system. They grow from the ends of long olfactory neurons — the only neurons in the body with a direct connection between the outside environment and the brain.

Millions of intertwined olfactory cilia fill a mucus layer lining the inside of the nasal passages. When you inhale an odor, odorant molecules bind to matching receptor proteins on the surfaces of cilia from specific olfactory neurons to create a biochemical signal.

The human nose needs so many olfactory cilia to sort out the seemingly infinite number of different combinations in the odorant molecules we inhale every day, according to Martens.

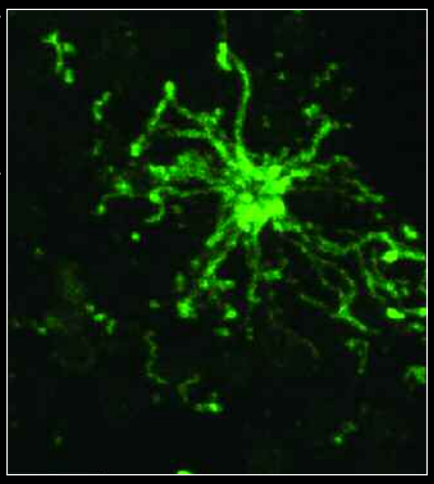


Photo: Courtesy of the Martens Laboratory

Olfactory cilia grow from the ends of long olfactory neurons like these that carry scent signals directly to the brain.

Genetic mutations that affect cilia or their proteins can disable this delicate sensory machinery. The result is anosmia, or the inability to smell — a condition that Martens says often goes undiagnosed by physicians and unnoticed even by people who have it.

"If your sense of smell has been deficient since birth or declines gradually over time, you may not realize that anything is wrong," he says.

Martens adds that many diseases and medical conditions — including obesity, developmental disorders, Leber Congenital Anamosis (a form of childhood blindness), sexual dysfunction, Alzheimer's disease and depression — may be associated with defects in olfactory cilia that affect the sense of smell.

"Olfactory function tests may be a useful, non-invasive screening tool for these and other cilia-related diseases," Martens says.

—SP