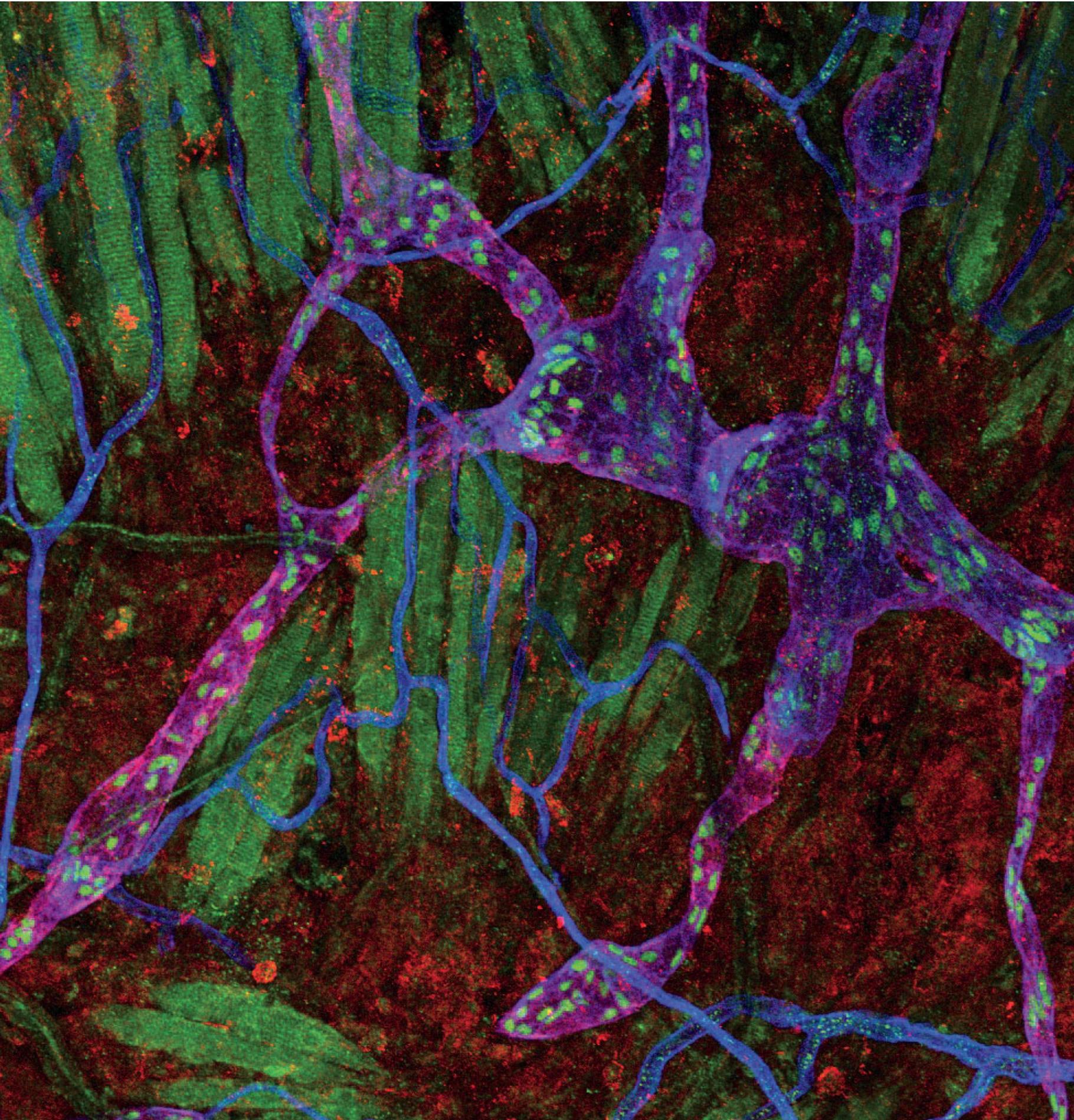


## INVESTIGATIONS

*Explorations and revelations taking place in the medical school*



Lymphatic vessels (purple)—which drain lymphatic fluid from tissue—next to blood vessels (blue)

# THE FAMILY THAT ASSAYS TOGETHER

THE GENETICS OF LYMPHEDEMA REVEALED  
AT A REUNION | BY ALLISON A. CURLEY

**N**early two decades ago, Pitt's David Finegold (BS '68, MD '72, Res '75), professor of pediatrics and medicine, and Robert Ferrell, professor of human genetics in the Graduate School of Public Health, were chatting over a cup of coffee. Finegold mentioned that his wife, physiatrist Judith Esman (Res '87), worked with patients with lymphedema, an often-disabling retention of fluid (usually in the limbs) that results from abnormal drainage of the lymphatic system. Ever the curious geneticist, Ferrell asked his usual question: "Is it ever inherited?" As it happened, Esman was treating a father and his twin daughters for Milroy's disease, a hereditary form of lymphedema. In fact, many members of this family were affected, and they were willing to participate in research. Finegold and Ferrell couldn't pass up the opportunity to study an obscure disorder that was clearly genetic, and so they set out to identify the genes underlying Milroy's disease.

Some days later, the researchers rented out a local fire hall and hosted a family reunion. At one end of the hall were cookies and coffee and at the other, blood draws and clinical exams. The team collected DNA samples from 40 family members; others mailed in samples later. Such was the beginning of the largest-ever genetic study of heritable lymphedema, the Pittsburgh Lymphedema Family Study, which now includes more than 300 families worldwide.

Three years in, Finegold and Ferrell identified the first-known causative gene, which codes for a protein named VEGFR3 and is important in the development of the lymphatic

system. They went on to find three more of the seven known causative genes—and have pinpointed the genetic culprits in about a third of the families they've studied.

The lymphatic system is a network of vessels that collect fluid from tissue throughout the body and deliver it to the bloodstream and lymph nodes. Hereditary (or primary) lymphedema, in which the system develops abnormally, occurs in roughly one in 6,000 people.

Far more common is secondary lymphedema. Secondary lymphedema occurs in about 30 percent of women treated for breast cancer; for many years, doctors assumed it was a result of the trauma of surgery, chemotherapy, or radiation. But not everyone in treatment gets lymphedema, so Ferrell and Finegold reasoned that trauma probably isn't the only cause. They suspected that genes played a role in this form of lymphedema, too, and set out to prove it. "If we're right, and we can identify a subset of women who are going to be at risk,

edema—had mutations in the gene coding for connexin 47 (Cx47). The researchers had previously implicated the gene in primary lymphedema.

Drawing on the expertise of research assistant professor of cell biology Catherine Baty, who joined the team in 2008, the researchers also examined the functional consequences of the Cx47 mutations. Connexins are the primary components of structures called gap junctions; recent evidence suggests they are important in the movement of lymph fluid. The investigators reasoned that the deficits resulting from the mutations may be subtle, because patients don't develop the condition until after the insult of cancer treatment. Using cultured human cells with mutations introduced, they showed that proteins trafficked normally to the cell surface but exhibited other functional impairments.

Ultimately, the team hopes that knowledge of the underlying genes could lead to a

**Secondary lymphedema occurs in about 30 percent of women treated for breast cancer.**

we can potentially start preemptive treatment, such as massage and compression garments," says Finegold.

In April, the team published results of a study that examined several candidate genes in a population of women with secondary lymphedema following breast cancer treatment. They found that these women—but not healthy women or those with breast cancer who hadn't experienced secondary lymph-

cure. In fact, drugs modifying the function of connexins are already available; they were initially developed for cardiovascular disease.

The findings could also have implications for wound healing among other conditions linked to fluid imbalance, says Ferrell. He calls lymphedema a "window on lymphatics" and a way to identify genes that are important in the functioning of the lymphatic system as a whole. ■

**“CAPTAIN” GENE  
A NEW TARGET  
FOR FIBROID THERAPY  
BY DANA YATES**

# JUST FOLLOWING ORDERS

The National Institutes of Health reports that, by age 50, half of all women will have fibroids. Consisting of muscle cells and other tissues found in and around the wall of the uterus, fibroids cause no symptoms for some women. For others, however, fibroids can lead to a range of problems. They include frequent urination, lower back pain, heavy menstrual bleeding, painful sex, a sensation of fullness or pressure in the lower abdomen, miscarriages, and premature labor. In extreme cases, fibroids can weigh up to 100 pounds, causing severe disfigurement. Fibroids are the leading cause of hysterectomies in the United States.

But what causes them? A discovery made by the University of Pittsburgh’s Aleksandar Rajkovic indicates that, for the majority of women, a single gene mutation is to blame.

Rajkovic, an MD/PhD and associate professor in the School of Medicine’s Department of Obstetrics, Gynecology, and Reproductive Sciences, is division chief of genetics at Magee-Womens Research Institute. He is also the senior author of a study that identified the genetic pathway that contributes to the development of uterine fibroids. The team’s findings were published in the online journal *PLoS One* in March.

“Right now, the only treatment for fibroids

involves surgically removing them from the uterus. But that solution isn’t perfect,” he says. “For approximately 50 percent of women, the fibroids come back, and redoing the surgery is associated with even more complications.” Among them, infection, loss of the whole uterus, and death.

As a gynecologist who is familiar with those risks, Rajkovic is focused on finding noninvasive ways to eliminate fibroids. Using genome-sequencing technology, Rajkovic’s group figured out the exact order of the chemical building blocks of fibroid and healthy uterine tissues.

“We wanted to know if there were differences or genetic changes in the tissues that allowed fibroids to grow,” he says.

By examining the fibroid and normal uterine tissues from five women who had undergone hysterectomies, the research team learned that three of the women had fibroids with mutations in a gene called MED12. A regulatory gene, MED12 acts like a captain, telling the genes downstream what to do.

The researchers broadened their exploration and turned to a biobank for additional tissue samples, looking for the MED12 mutation in 143 uterine fibroids. The results: approximately 70 percent of the samples contained the mutation, whereas normal uterine

LEFT: Genome sequencing of normal uterine tissue. RIGHT: Highlighted letters show a genetic change on the X chromosome. A single gene mutation, MED12, causes fibroid tumor growth—it was found in 70 percent of the fibroid samples Rajkovic studied.

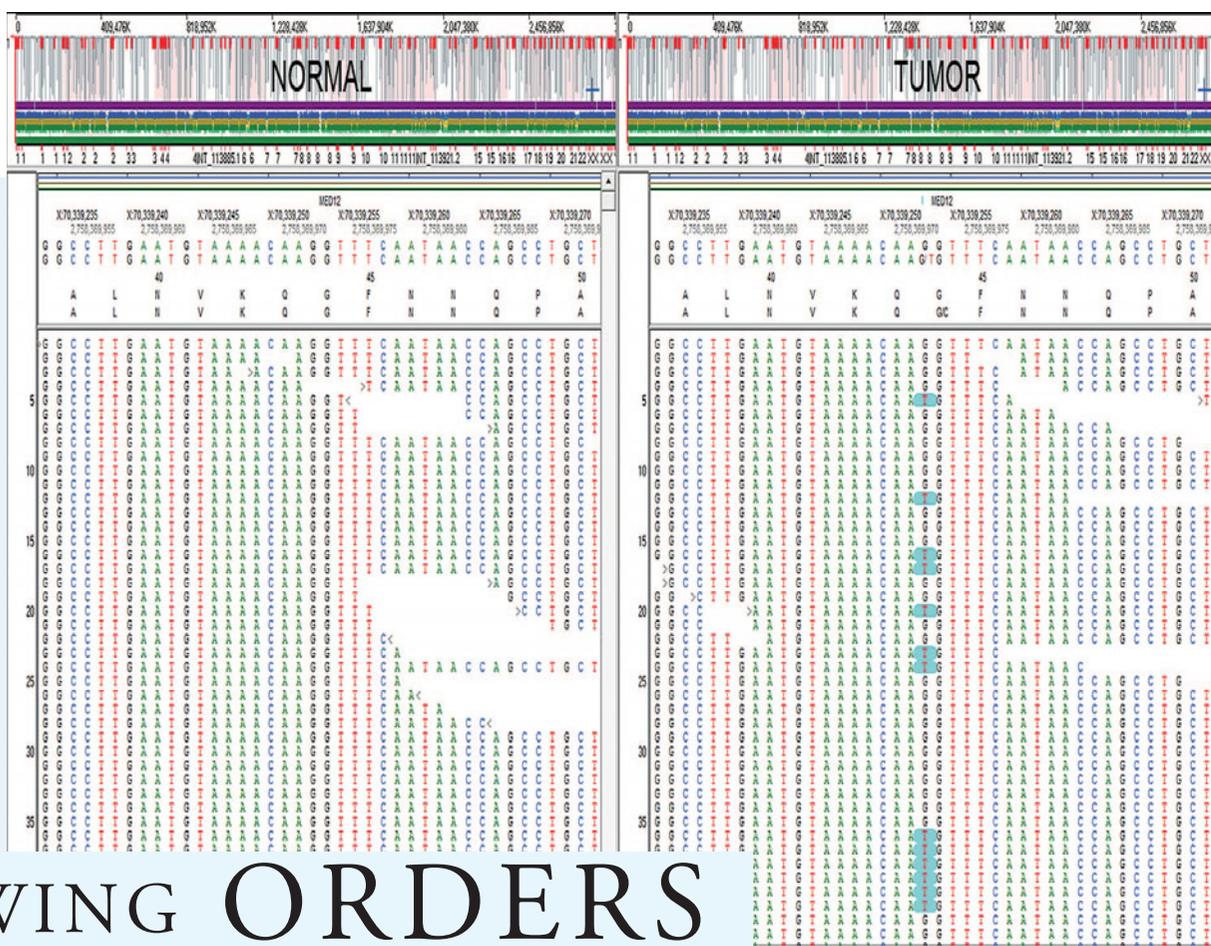
tissue samples did not.

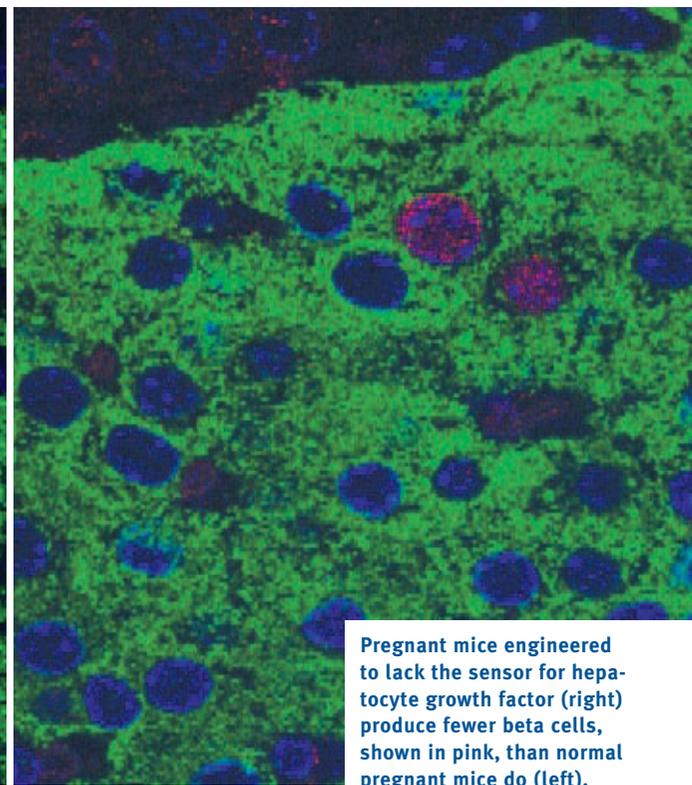
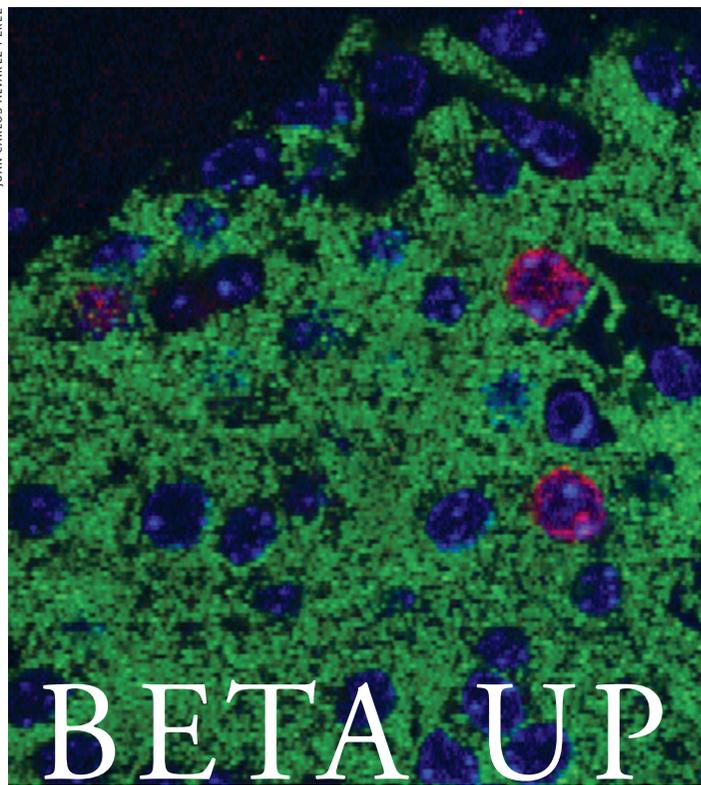
“Ultimately, we hope to target the other players that MED12 regulates,” says Rajkovic.

“They are the major carriers of the information that MED12 is trying to convey, and we could modify their function pharmacologically.”

Rajkovic’s research group is now exploring the mechanism between MED12 and other genes that take orders from it. To that end, the researchers are working with mice with modified MED12 genes. The team also wants to determine whether the MED12 mutation makes fibroid regrowth more or less likely after surgical treatment.

And what about the fibroid samples that lack the MED12 mutation? In those cases, Rajkovic speculates, multiple genes may be involved, interacting with environmental factors to spur fibroid growth. Future research studies will investigate that process, but Rajkovic is still busy unraveling the MED12 mystery. ■





Pregnant mice engineered to lack the sensor for hepatocyte growth factor (right) produce fewer beta cells, shown in pink, than normal pregnant mice do (left).

# BETA UP

## CAN HGF STRIKE OUT GESTATIONAL DIABETES?

BY MELINDA WENNER MOYER

**F**or an expectant mother, a diagnosis of gestational diabetes—which afflicts 135,000 pregnant women in the United States every year—can be terrifying. For the remainder of her pregnancy, she must carefully monitor her blood sugar as well as her baby’s size to minimize the risk of labor difficulties and stillbirth. Further, she and her baby will always be at heightened risk for type 2 diabetes. Historically, researchers have understood very little about the cause of gestational diabetes, but a new study by a team led by Adolfo Garcia-Ocaña, a PhD associate professor of medicine at the University of Pittsburgh, suggests that the difference between a healthy pregnancy and a diabetic one may boil down to problems with a single liver protein and its molecular sensor.

This protein, called hepatocyte growth factor (HGF), has actually been a longtime interest of Garcia-Ocaña’s. It was discovered in 1990 by Pitt’s George Michalopoulos, MD/PhD and chair of Pitt’s Department of Pathology. Soon after arriving at Pitt in 1997, Garcia-Ocaña wondered: Given HGF’s ability to induce cellular growth in tissue, could the protein be used to grow more beta cells (found in the pancreas) in people with type 1 and type 2 diabetes—diseases characterized in part by a paucity of beta cells? In 2000, Garcia-Ocaña

genetically engineered mice so that their beta cells over-produced HGF, and the mice grew more and bigger beta cells than normal mice did. HGF, it seemed, played an important role in beta-cell growth and replication.

It occurred to Garcia-Ocaña that beta-cell growth was also an important aspect of a healthy pregnancy. “A mother adapts to enhanced metabolism during pregnancy in a way that increases the number of insulin-producing cells and increases the amount of insulin that’s being produced by these cells,” he explains. After delivery, her body kills off the extra beta cells. He knew from research published in 1995 that during pregnancy, HGF levels in the body skyrocket. “So we thought, *Hmmm. Maybe HGF plays a role in gestational diabetes.*” Perhaps the condition arises when HGF is unable to do its job, he imagined.

In order for pancreatic cells to respond to HGF, they have to express its sensor, c-MET, on their surface. Garcia-Ocaña wondered what would happen if HGF couldn’t communicate with c-MET during pregnancy. To find out, he genetically engineered female mice to be unable to produce c-MET on their beta cells. Then he watched what happened.

At first, the mice did just fine. They seemed perfectly normal. But then, when they became pregnant, “the expansion of insulin-producing

cells was blunted, and insulin secretion was also diminished,” he explains.

In other words, “These mice developed gestational diabetes.”

When Garcia-Ocaña studied the mice further, he discovered that their bodies prematurely killed off beta cells during late pregnancy, too.

Garcia-Ocaña and his collaborators—who include the School of Medicine’s Cem Demirci, Sara Ernst, Juan Carlos Alvarez-Perez, and Gabriella Casinelli, among others—published the findings online in March in *Diabetes*.

He doesn’t yet know whether women who develop gestational diabetes tend to have problems with HGF or c-MET or both. So his team will analyze blood samples from this population to find out more.

Another unanswered question is whether HGF and c-MET affect beta-cell growth directly, or do so by way of additional proteins or receptors.

Garcia-Ocaña wonders whether doctors might one day treat or prevent gestational diabetes with therapies that regulate abnormal HGF/c-MET signaling.

He’s considering the implications for type 1 or 2 diabetes, as well. Boosting the growth of insulin-producing beta cells might treat or prevent those disorders, too. ■