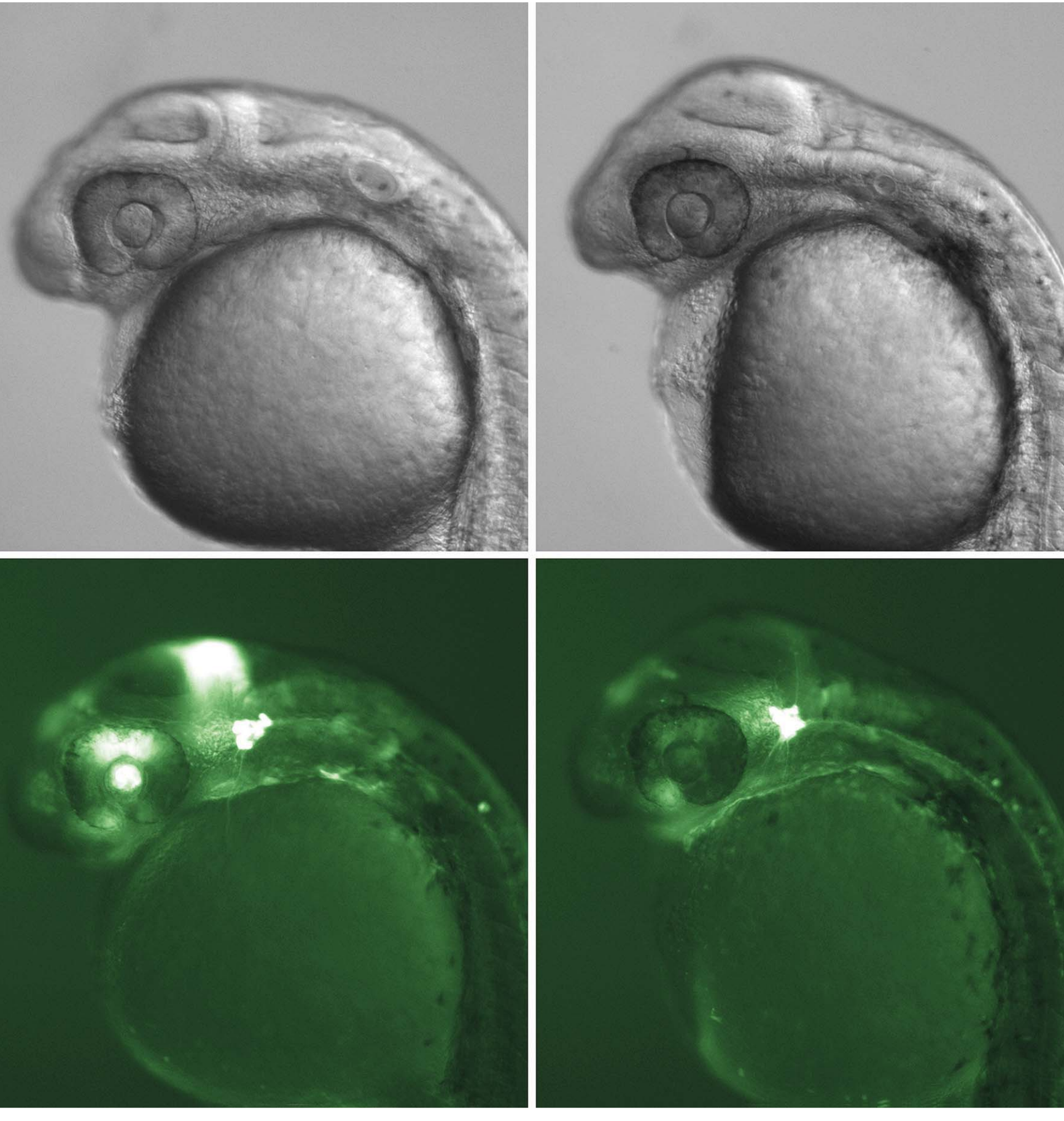
Explorations and revelations taking place in the medical school



ONE BIG HEART
COMING RIGHT UP

TSANG MANIPULATES A GROWTH FACTOR PATHWAY

BY JOE MIKSCH

For many, the utility of fish begins and ends with their estimable service as food. Michael Tsang, University of Pittsburgh assistant professor of microbiology and molecular genetics, uses fish to search for answers, molecule by molecule, regarding how embryos develop.

What he's learned even allowed him to control the size of a developing heart.

Tsang employs the zebra fish, an inch-and-a-half-long striped swimmer, which shares 70 to 80 percent of its genes with humans. Zebra fish reproduce and develop rapidly. The zebra fish embryo—this is key—develops outside the mother, making it easier to examine than, say, a mouse embryo. And the University of Pittsburgh's Biomedical Science Tower 3 hosts one of the largest colonies of the critters in the world.

Tsang, a PhD who previously worked at the National Institutes of Health, developed his novel zebra fish–centric molecular screening model so he could investigate embryonic development via fibroblast growth factors (FGFs). The FGF pathway is vital to brain, heart, and limb formation. FGFs, there are 22 of them, also play critical roles in wound healing and angiogenesis.

Tsang has created transgenic zebra fish embryos that act as biosensors for fibroblast growth factor activity vital to brain, heart, and limb formation. These fish embryos are under a microscope in standard light (top) and blue light (bottom). In the bottom right image, the growth factor isn't active in the eyes or parts of the developing brain.

COURTESY MOLINA ET AL BMC DEVELOPMENTAL BIOLOGY (2007)

When FGF receptors malfunction, diseases such as Crouzon syndrome, which is marked by facial deformities, can develop.

To prevent disease, doctors may sometimes want to block the FGF pathway. Other times, they may find it preferable to ramp it up.

Figuring out which molecules might have an impact on FGF signaling is no easy task.

Tsang's solution was to create a line of transgenic zebra fish. He injected a piece of DNA consisting of two elements, a promoter and a reporter, into the fish.

Promoters, as the name indicates, help facilitate genetic transcription and can switch on gene expression. Tsang's promoter comes from a gene normally regulated by FGF signaling. The reporter, which contains a code for a jellyfish protein called GFP (green fluorescent protein), glows under blue light.

Tsang's lab then bred his genetically unique zebra fish and looked for places where the glow was prevalent.

"This indicates to us where FGF is active in the embryo just by looking at it," Tsang says. "This fish is like a live biosensor for FGF activity."

With his model, Tsang can treat his transgenic zebra fish with molecules, hoping to find ones that increase or suppress FGF activity.

Things go pretty quickly once the molecule being investigated gets into the rapidly growing zebra fish embryo.

"We add our compounds, and we can look for GFP expression in six hours. We add them in the morning, and we'll know what happened by the afternoon," Tsang says.

If GFP doesn't appear, Tsang knows that the molecule blocks FGF signaling. If there's more GFP than normal, FGF signaling is increased. If the embryo dies, Tsang says, "then we know it's toxic, and it's no good to continue on with that compound."

Tsang's collaborators on this project include Andreas Vogt, research assistant professor of pharmacology and chemical biology; John Lazo, Allegheny Foundation Professor of Pharmacology; Ivet Bahar, John K. Vries Chair of Computational Biology; and Billy Day, professor of pharmaceutical sciences and chemistry.

Of the 5,000 molecules Tsang's colleagues have so far screened, they've found one (called BCI) that hyperactivates FGF signaling. It turns out that BCI blocks a particular FGF inhibitor from doing its job. So, Tsang now knows the identity of a molecule that affects FGF function and that molecule's target, as well as the mechanism by which BCI works to augment FGF activity.

He is beginning to use this knowledge to learn how the timing and location of FGF expression modulates embryonic development.

Using BCI and changing the fate of embryonic cells, Tsang's lab has managed to increase the size of a zebra fish's heart.

"By adding BCI, we've seen less blood and blood-vessel progenitors and more cardiac tissue, because we enhanced FGF signaling at a particular time in development.

"If you block FGF signaling, you get the opposite effect," he says. ■

SAYING "HI" TO THE BAD GUY

THE BODY'S SENTINELS COULD PREVENT ORGAN REJECTION

BY REID R. FRAZIER

You might call the task Herculean, or Sisyphean, if you like. How do you get the body to accept a new organ without killing the body in the process? Immune systems attack transplanted organs, but immunosuppression regimens can cause high blood pressure; increase the risk of cancer, heart disease, and stroke; and leave the body vulnerable to infection. In time, the problems they cause take the lives of roughly one quarter of all transplant recipients for many types of organ grafts.

Finding a way for the body to accept a new organ without turning off the entire immune system is the holy grail of transplant immunology. "It's a Nobel Prize, if you can find a way to transplant without drugs," says Mark Hardy, Auchincloss Professor of Surgery and director emeritus of transplantation at Columbia University. Bone marrow transplantation, radiation, and blood transfusions are just a few of the methods scientists have tried to induce tolerance for a new organ.

The University of Pittsburgh's Angus Thomson thinks he's on to a way to make the immune system more tolerant of a transplanted organ. Thomson is known for his work with dendritic cells, sentinel cells that trigger the immune response. He believes doctors can use dendritic cells to make organs feel at home in their new surroundings.

The concept is a little like convincing a snarling Rottweiler guarding a house to suddenly hop up on its hind legs and lick your face.

The Scotland born Thomson, a fellow in the Royal Society of Edinburgh, Scotland's national academy of science and letters, thinks there's a way to train the Rottweiler to recognize your face as friendly.

Thomson, Distinguished Professor of Surgery with appointments in immunology, as well as microbiology and molecular genetics, was among the first to use dendritic cells in transplant immunology in the 1990s. He and his collaborators showed that these cells, particularly immature ones, could be "trained" to regulate the immune response in lymphocyte white blood cells.

Some call this approach a "negative vaccine," because it undoes the immune response.

"If you can program [dendritic cells] the way we want to, they can tell the lymphocytes to switch off rather than on," says Thomson. (Or, as Hardy notes, "You kind of encourage the cell to be stupid.")

This changed the way transplant scientists viewed dendritic cells, Hardy says. Dendritic cells, the thinking went, "were there to be attacked, not to be encouraged," says Hardy, who himself has studied how to "tolerize" dendritic cells by exposing them to foreign tissue.

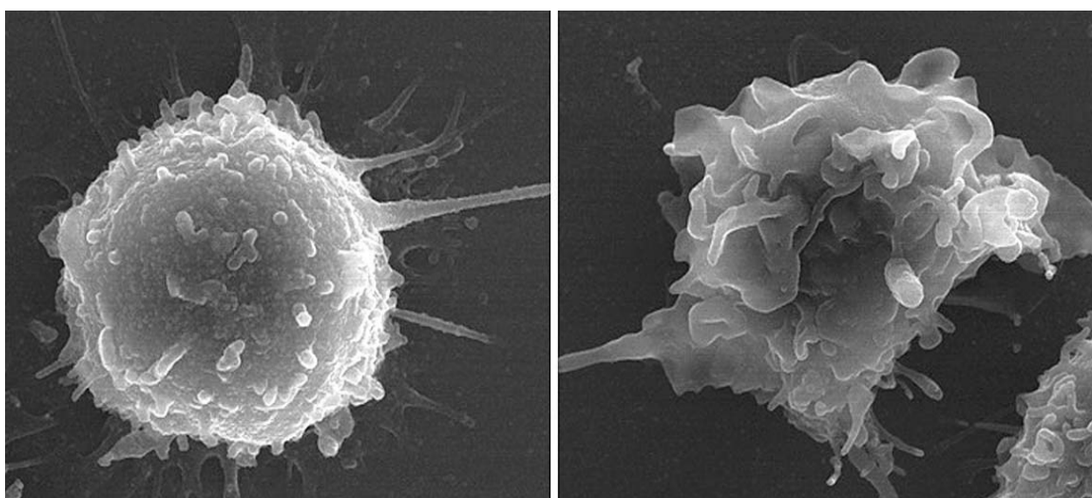
"[Thomson's breakthrough] was very significant and very important and stimulated other people to focus their research in the area," he says.

Transplant doctors had good reason to fear dendritic cells—shape-shifting structures that trawl the body looking for potential invaders, like a virus, bacteria, or a new organ. Once they sense a problem, the cells get excited, extending thousands of tiny protrusions, or dendrites (dendron is Greek for "tree"), to snatch up loose bits of the offending newcomer. The cells then cart their bundles off and present them to the body's T-cells, which attack the intruder.

Since his initial discoveries about dendritic cells, Thomson, director of transplant immunology and associate director for basic research at the Pitt-UPMC Thomas E. Starzl Transplantation Institute, has continued to explore the pathways through which these cells can induce tolerance. In 2002, his group found that exposing dendritic cells to the immunosuppressor rapamycin helped keep them immature. The cells could then be reinjected into the body to regulate immune responses to a transplanted organ.

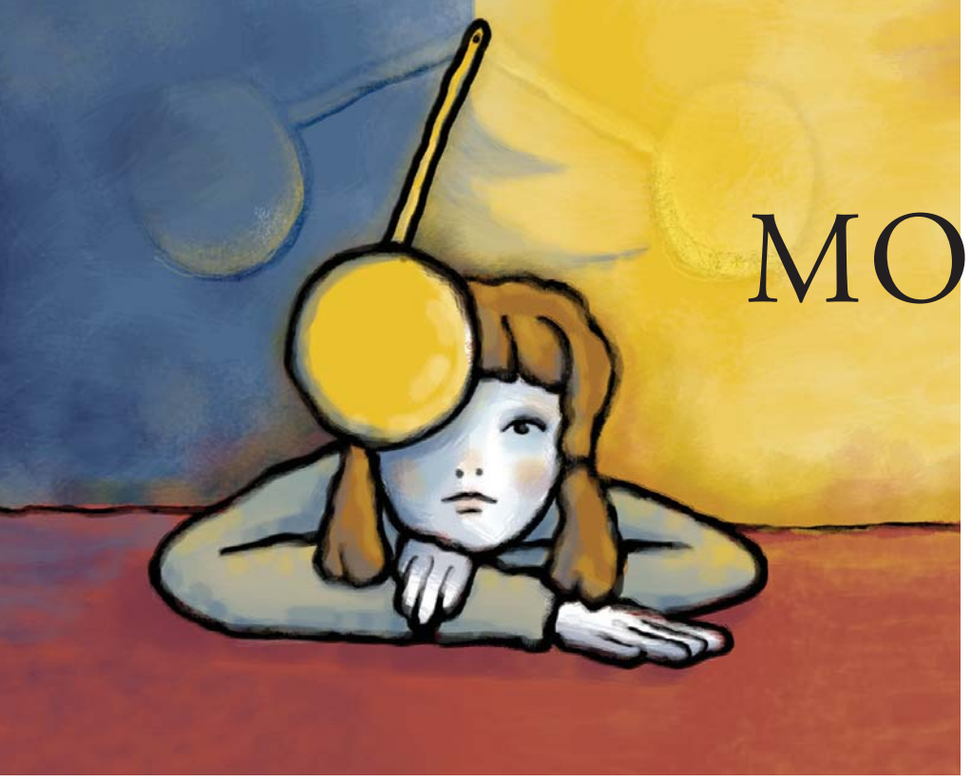
Thomson and his collaborators recently found several molecular signatures for these "tolerogenic" cells. "That's a big question for us and the rest of the field," the immunologist says. "What is the hallmark of a tolerogenic dendritic cell?" And, otherwise "how do you know your negative vaccine cell is doing any good?"

His group found high concentrations of two molecules (ST2 and IL-10) in cells with tolerogenic properties. Thomson is hopeful the FDA will approve human safety trials on tolerogenic dendritic cells in transplantation within the next few years. ■



COURTESY DONNA STOLZ

Thomson has found a way to keep dendritic cells "stably immature" (right image) so they don't attack transplanted tissue. On the left is a mature dendritic cell which is normally highly effective in stimulating the body's immune response.



MOLLY FEUER

MOOD PENDULUM

HELP FOR CHILDREN WITH BIPOLAR DISORDER

BY ELAINE VITONE

Adults living with bipolar disorder—that pendulum of moods that swings from stability, to mania, to depression, and back again—struggle with a range of devastating symptoms. Depending on the severity of the disorder, they might undertake reckless behavior they cannot control, endure strained interpersonal relationships, and even harbor suicidal thoughts.

"If you develop the illness as an adult, it's very hard, but at least you already have your foundation in place," says the University of Pittsburgh's Boris Birmaher, an MD who holds an endowed chair in Early Onset Bipolar Disease and is a professor of psychiatry. "But if you get this as a child, it's much worse. Unless you treat these kids, they don't have the opportunity to develop normally—emotionally, cognitively, socially, or academically."

Unfortunately, diagnosing bipolar disorder is especially challenging. On average, it takes adults 10 years to get an accurate diagnosis (that's from the time they first seek help to the time they arrive at the answer). And for children, the challenge is even greater. After all, it's normal for kids to be fanciful, to believe they can fly. But in adults, the same behavior would be a red flag. And the symptoms of bipolar disorder overlap with those of several other disorders common in children—ADHD, for example—further confounding diagnosis.

To address these challenges, 10 years ago Birmaher founded the Child and Adolescent Bipolar Services Clinic at Western Psychiatric Institute and Clinic. He believes it is the only clinic exclusively devoted to the treatment and study of bipolar disorder in children and adolescents.

"In psychiatry, all you have is the assessment," says MD Associate Professor of Psychiatry David Axelson, the bipolar clinic's current director. "There aren't any blood tests, X-rays, or genetic tests to help with diagnosis. It's all based on the history and mental status that people report to us."

From day one, the clinic has used an exceptionally thorough diagnostic process. It begins with a 20-minute phone interview with the child's caretaker—this step rules out a bipolar diagnosis for about 40 percent of callers. (These callers are then assisted with referrals.) Next, the child and caretaker come in for a detailed assessment with an experienced psychiatric nurse or a master's level clinician. Based on this interview, an attending psychiatrist at the clinic will confirm the diagnosis and meet with the family. All told, the process of diagnosing a single patient can last as long as six hours. "We call it the Cadillac of interviews," Birmaher says.

In the last decade, the clinic has assessed and/or treated many more than 1,000 children and adolescents. Its patient load—which averages around 260 at a given time, many of whom are study participants—offers singular insight into this often-misunderstood disorder. Ongoing studies are investigating early symptoms, long-term outcomes, treatment efficacy, potential biomarkers, and genetics, among other areas.

Perhaps most notable among the clinic's research projects is Course and Outcome of Bipolar Youth (COBY), a study led by Axelson and Birmaher that follows 440 children at UPMC, UCLA, and Brown University. The study is in its seventh year; analyses from COBY have been widely published. It is the largest study of its kind.

COBY helped Axelson elucidate the subtle differences between children with bipolar 1 (the most severe form of the disorder) and children with subthreshold bipolar (in which episodes with two or more symptoms last four or more days at a time). It also allowed Birmaher to explain how mood shifts in bipolar children can differ from those in adults. Mood swings in children with subthreshold bipolar disorder are more frequent than in adults with the same disorder.

Axelson is quick to point out that the clinic has seen a lot of success stories. With proper treatment, many patients have managed their symptoms and done well in college and beyond. Yet doctors need better diagnostic criteria so they can help kids early on—when it counts most. Accurate diagnosis is a major focus of the clinic's studies.

"That's why we have to keep working," says Axelson. ■