An energy shortage in the cells causes the heart to pump poorly.

PROBLEM AT THE PUMP

NEW HOPE FOR A DANGEROUS CHILDHOOD HEART CONDITION

BY CRISTINA ROUVALIS

ILLUSTRATION BY MICHAEL HIRSHON

For biologist Cecilia Lo, every day at work revolves around medical mysteries. But there was one particular diagnosis that was so confounding and devastating, she couldn't rest until she figured it out. In the United States, an average of one-third of babies born with hypoplastic left heart syndrome (HLHS) die before their first birthday. What made the condition fatal for some, while others grew up to live fairly normal lives?

Lo, Distinguished Professor and F. Sargent Cheever Professor, who chairs developmental biology at the University of Pittsburgh, has spent four years trying to pinpoint the mechanism that leads to cardiac failure in certain babies born without a functional left ventricle, the heart chamber responsible for pumping oxygenated blood to the rest of the body. Some children struggle even after major surgery to reconfigure the organ—in which a surgeon creates a one-chamber heart that allows the right ventricle to pick up the slack—and even when the operation is technically successful.

Although outcomes are better at UPMC Children's Hospital of Pittsburgh and are improving generally with advances in surgical options and medical approaches, for a subset of children with HLHS—"It's heartbreaking," says Lo. "There is interest to see if we can rescue these babies and to develop therapies to treat them if they go into acute heart failure."

Many speculated that the poor outcomes were because the surgically enhanced right ventricle becomes overworked to the point that it just gives out.

But based on a study of the condition in mice, Lo theorized the problem was at the cellular level. If she could prove her hypothesis, it might allow doctors to identify the highest-risk patients and move them to the top of the long waiting list for a heart transplant. "If we don't understand what causes the heart failure, there is no way to identify who is at risk."

Medical researchers expect surprises—behind many medical breakthroughs are hypotheses that don't exactly pan out and force scientists down a new path. But when Lo peered into the microscope to examine the heart cells, she tingled with excitement. The surprise was that there were no surprises. Her theory held up—the mitochondria of people with severe HLHS were damaged.

"It was very gratifying, very exciting. What we predicted actually happened in a very clear-cut fashion. A lot of the time your hypothesis doesn't necessarily go exactly the way you think. But this just came out beautifully."

She likens the heart to a car and mitochondria to the gasoline that fuels it—if the mitochondria are defective, the car won't move.

To test her hypothesis, Lo and her team started with research on mice, looking for genetic mutations that cause congenital heart disease. "We proved that you could get HLHS in mice." And by studying the animals, they discovered the problem was the mitochondria in the diseased mouse cells.

To test the theory on humans, they collected fibroblast cells (which build connective tissue) from three healthy children and 10 with HLHS. They divided the HLHS cells into two groups—those from patients with severe HLHS who either died or had a transplant before their first birthday and those from a mildly affected group of patients who had lived past age 5 without a transplant.

They converted the fibroblast cells into pluripotent stem cells, which have the capacity to develop into any kind of cell in the body. By adding a specific mixture of nutrients and growth factors, the researchers were able to turn the stem cells into human heart cells that actually beat in the dish.

Just as a heart pulsates as it pumps blood, so do individual heart cells. (See them in motion at pi.tt/LoFindings.)

Peering at the heart cells through a microscope at the John G. Rangos Sr. Research Center, Lo noticed differences between the two study groups immediately. The heart cells of the healthy patient pulsated robustly. The heart cells of patients who had mild HLHS were similar to the healthy heart cells, pumping a little slower but still steadily. In contrast, the cells from patients with severe HLHS pumped slowly, their labored movement similar to what doctors saw in the hearts of the sickest patients.

Lo discovered that both groups of HLHS cells had defective mitochondria, but the damage was more significant in the group with severe disease. These cells were also unable to use natural defenses to compensate for the damaged mitochondria. The languid pumping could be fatal for a baby, even after a procedure to reroute the anatomy of the underdeveloped heart. At the root of this energy shortage in HLHS is a process known as oxidative stress.

Typically, when the body works as it's supposed to, mitochondria produce the energy required for cells to function. This happens through a series of steps in which electrons are passed along by protein molecules and then accepted by oxygen. But when there's oxidative damage, the electrons get lost before they reach the oxygen molecule, holding up the energy production process. The result is damage to the mitochondria, resulting in damage to the cell's ability to function and, ultimately, a severely damaged organ. "The heart is not going to work very well if you have cells that die and can't pump blood," says Lo. Her team uncovered a metabolic marker that may one day be accessed with a simple blood test.

"There's a lot of data that shows that these patients also have poor neurodevelopmental outcomes such as autism or cognitive impairment. The genes that regulate heart development also regulate brain development."

Having figured out what caused the problem, Lo and her team set out to test various drugs in search of potential therapies to remedy the mitochondrial defect. They found two promising candidates—sildenafil, which is commonly known by the brand name Viagra, a drug that increases blood flow. The other one is a supplement called TUDCA, a "molecular chaperone" that restores the folded structure of protein molecules that have fallen apart because of oxidative stress. Xinxiu "Cindy" Xu was a lead author on a May 2022 Cell Stem Cell paper that described the findings.

Both of these drugs are already known to be safe in adult humans and won't have to go through a lengthy approval process. "We don't have to do safety studies like you would if the drug had never been used clinically." TUDCA is sold over the counter in health food stores and is often used by body builders. Whether either would be safe and effective for babies with HLHS, we don't yet know.

First, clinical studies have to be conducted in this patient population. But that's difficult with a relatively rare condition. "Any one medical center might not have enough patients," says Lo. "You might only get 10 or 15 a year." So clinical trials would require multiple centers to participate, which would require more funding.

Nationally HLHS affects about 1,000 babies born each year.

Mary Sanderson (not her real name), a new mother in Harrisburg, had never heard of HLHS until she was pregnant with her first child and had a prenatal screening.

The first doctor she saw told her that surgery wouldn't be an option and that her baby wouldn't survive. That was agonizing.

Then a second doctor, a cardiologist, referred her and her husband to UPMC Children's Hospital, which has some of the best HLHS patient outcomes in the country. Children's cardiology and surgery teams

are led by Jacqueline Kreutzer, professor of pediatrics who is the Peter and Ada Rossin Professor of Pediatric Cardiology, and Victor Morell, surgeon-in-chief who is the Eugene S. Wiener Professor of Cardiothoracic Surgery.

Sanderson's baby boy was born on May 7 and had his first major operation on the 12th. "He is recovering well," she says.

Sanderson says the condition made the entire birth process incredibly stressful: "I couldn't enjoy any part of the pregnancy. I was always worried. I went into labor in Harrisburg, and I worried I wouldn't get to Pittsburgh on time."

She is relieved that doctors at Children's could do surgery—"Science is amazing," she says—and gladly allowed researchers in Lo's lab to take cell and blood samples from her son. "It is so important that they learn different ways to treat it."

"Despite major advances in pediatric cardiology over the last few decades, there continue to be major challenges and unsolved life threatening conditions that affect children with congenital heart disease," says Kreutzer. "Dr. Lo's groundbreaking research is critical to improve their quality of life and shift traditional care approaches to a new level."

Even if all goes smoothly with Lo's studies, it's difficult to predict how long it will take to finish them and get approval for treatment. Lo and her team plan to test other drugs, as well, to see if they can reverse the effects of mitochondrial damage safely.

A biomarker might come sooner, perhaps within five years, if funding is available. Her research opens the possibility of a simple blood test that will let doctors know which babies are at the highest risk of going into cardiac failure, bumping them to the top of the long transplant list. "There aren't enough to go around," Lo says.

"This will tell us who should have priority to get the transplant." ■

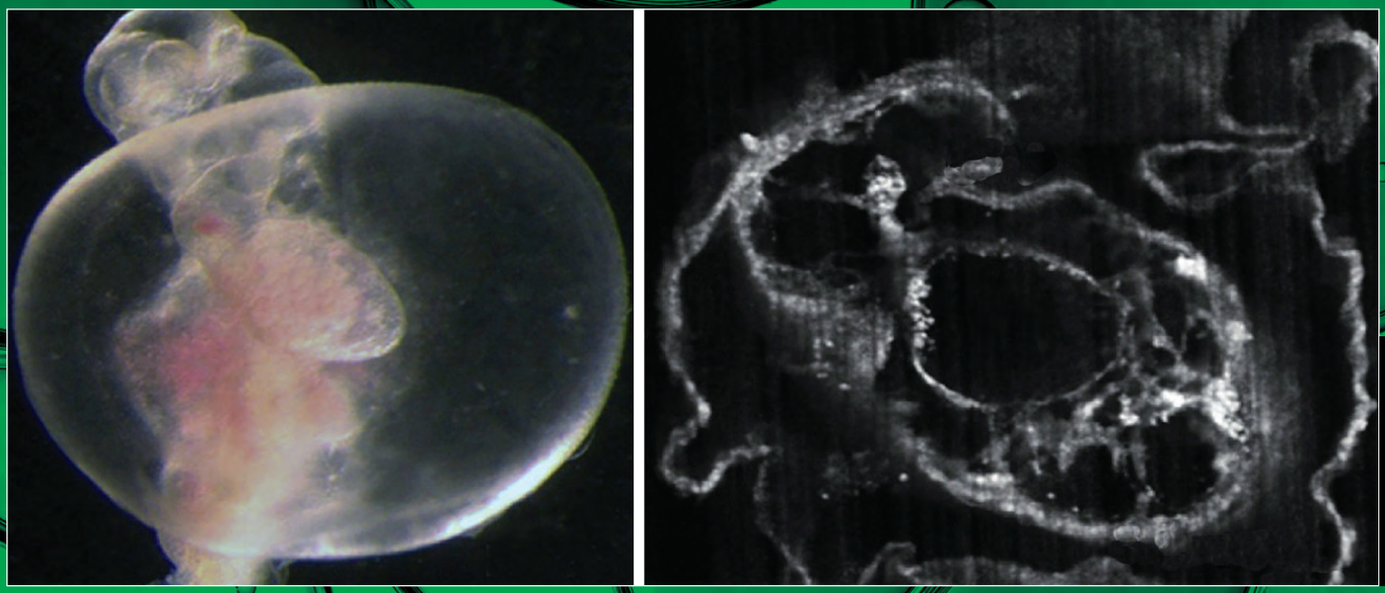
Two major breakthroughs from Cecilia Lo's lab this year are likely to save the lives of children with heart conditions.

THIS FAMILY CARRIED A RARE MUTATION THAT SHOULD HAVE BEEN LETHAL.

WHAT WAS KEEPING THEM ALIVE?

BY ERIC BOODMAN
FROM STAT NEWS.

The result didn't make sense. The researcher kept scanning the mouse's pregnant belly, back and forth, back and forth. They could see the embryos on the ultrasound screen, cocoon-like, in grayscale—but there were no heartbeats. It should've been visible by now, at eight and a half days, a rhythmic flashing, a clue that the organ was on its way. Instead, each outline was utterly still, dead before it had come to life.



After the lethal mutation was edited into mouse eggs using CRISPR, the early stage mouse hearts developed severe abnormalities, as shown here.

Image: Cell Reports Medicine

That couldn't be right. These mice carried the exact same genetic mutation as a family who lived about an hour away, in the county outside Pittsburgh, near the West Virginia line. The humans definitely had beating hearts. Nicole Burns worked at an assisted living facility, hoisting residents in and out of bed, guiding them as they shuffled to dinner with a firm hand on the back. Her daughter Peyton loved hunting. Her son Cameron loved playing basketball. Their genome couldn't contain something so lethal: They were all very much alive.

The researchers tried again, inserting the family's genetic variant into the DNA of a mouse egg, injecting that into the coils of a mother's oviduct. The same thing kept happening, sound waves bouncing back and revealing [mouse] embryos dead in the womb.

"This is basically not a survivable mutation," said Cecilia Lo, chair of developmental biology at the University of Pittsburgh, who led the research. "If you have it, you're pretty much dead."

So how had Burns and her relatives survived? Lo wondered if, against all odds, they'd inherited not just one but two ultra-rare mutations, the second shielding them from the deadly effects of the first. It seemed stunningly improbable—but that is, in fact, what she, postdoctoral researcher Xinxiu Xu and their colleagues showed in a paper [published in February in Cell Reports Medicine].

The idea wasn't new. In 2016, some geneticists had hypothesized that people were walking around with this sort of good-gene, bad-gene drama going undetected on their chromosomes. The researchers had combed through over half a million genomes, looking for patients who should have inherited a severe childhood disease but hadn't gotten any serious symptoms. As Stephen Friend, now a visiting professor at the University of Oxford, put it, "Clinicians would say, 'If anyone with that mutation were alive, I wouldn't believe it.'"

Out of 589,306 people, 13 were medically unbelievable. But the data were anonymized; there was no way for them to go back to those patients and find the protective variants they suspected were lurking in their cells.

Now, almost by accident, Lo found herself analyzing eight genomes that seemed to tell the story Friend had been speculating about. Her team dove back into the code, searching for the mutation that might be responsible for the rescue. There were a handful of other variants shared by all eight relatives—but only one of those genes was called into action in the heart. Bingo. Sure enough, when the researchers bred new genetically modified mice, this time with both variants, the [mouse] embryos survived. Some also showed the same heart defect as Burns and her kids. In other words, imperfect as it was, the second variant was protective.

It felt like a scientific triumph. Often, the narrative arc in biomedicine is a quest for disease-causing genes. Understand harmful alterations, the thinking goes, and you can hopefully reverse them—and there are reams and reams of papers about these "bad genes." There are "good gene" storylines, too. In 1996, a genetic variant was described that could stymie HIV—an observation that became a class of drugs. But Lo's paper was genre-bending, in a way. The "good gene" they'd pinpointed wasn't safeguarding against something extraneous, like a virus; it was softening the damage of another gene. "That's the untold story of human genetics," she said.

To Friend, the paper could provide a road map to look for other, similar instances. But it was also a technical feat. "It's almost like, 'Here's what you could do today that you couldn't do 10 years ago.' It's like, whoa! Just beautiful," he said.

But that wasn't exactly how Burns felt about what was going on inside her genome.

Nicole Burns lives in her great-grandfather's house, in Amity, Pennsylvania, about an hour south of Pittsburgh. He'd had over 100 acres of rolling Appalachian foothills, with sheep, horses, cows, pigs, though some of the land has since been sold off. What's left from the farm is mostly scrap, a rusted plough, an old wheel sitting at the edge of the yard where it fades weedily into woods. …

The land wasn't the only thing he'd passed down. Her other less appealing birthright was a hole in the heart. For her, actually, it was several, the biggest the size of a quarter. Normally, blood would pass from the heart's right side to the lungs, from the lungs to the left side and from the left side to the rest of the body. But these openings allowed some to burble backward, oxygen-rich blood leaking from left to right, to get resent to the lung. Sometimes, those holes close up on their own. If they don't, the inefficiency can slowly, over decades, stretch out the tissue, changing the heart's shape, potentially shifting its rhythm. In some cases, a doctor can thread a patch in through an artery or vein, but that doesn't always work.

In her family, heart surgery is a kind of ritual, a rite of passage. Her great-grandfather was the exception, diagnosed too late to operate, and he died of a massive heart attack. After that, everyone ended up under the knife. Her grandfather, a metallurgist at a steel mill, got surgery as an adult. Her mom, aunt and uncle all got it around their teens. She and her sister got it, both her kids had to have it, and it seemed like

if they were to have children themselves, they'd be destined to need heart surgery, too.

But it only came to a geneticist's attention because of Burns' son Cameron. He was born six and a half weeks early, by C-section. "I knew something was wrong, because they wheeled me down in the bed so I would be able to see him," she said. He was already hooked up to too many machines for her to hold him. A minute later, they rushed him to the children's hospital.

She discharged herself early …. Cameron's team kept calling her in her hospital room .... His body kept filling up with fluid. This wasn't because of a hole. His heart just wasn't pumping right.

The cardiologist said she had a choice to make. They could take him off all the machines and medications—nothing seemed to be working anyway—and see if he could pull through. Or they could try … inserting a drain tube, to see if they could siphon away some of his swelling. She chose the drain. "It was selfish on my part, because I was thinking, you know, any time I can get with him—God forbid, before something happens—that's what I want," she said.

It worked. But during those discussions, the cardiologist also heard something that intrigued him. Often, inheritance patterns of congenital heart defects were murkier, weirder, harder to predict. "Like, not everybody in the family has it. That's what struck me," said Brian Feingold, director of the pediatric heart failure center at University of Pittsburgh Medical Center. "That's why I said to Dr. Lo, 'You have the expertise to help unravel what's going on here; there's something different than what we typically see going on in this family.'"

The researchers drove out on a snowy day in 2011. Four generations of the family were there: Eight biological relatives who had holes in their hearts and three relatives by marriage who didn't. The kitchen became a makeshift lab for swabbing noses and collecting blood. Burns' grandmother had bought pop and a tray of sandwiches. The snow was picking up. Once they had their samples, the scientists didn't linger. After they left, the house quieted. Burns was glad that part was done. Now, she thought, maybe they'll be able to start figuring out some of these mysteries. …

For Friend and his colleagues, the idea of looking for mutations that suppress bad counterparts began with lab animals. Experiments in the classic creatures of genetics—yeast and roundworms, for instance—showed how much interplay there was between different parts of DNA. A complex choreography of many genes is often the backstory to a particular trait.

That gave them an idea. So much of the search for drugs involved looking for disease-causing variants. Repairing the damage is often tough—and Friend wondered if there might be cases where toying with a different gene could make it easier to stop some of the first one's troublemaking. Imagine a broken machine on an assembly line, twisting a piece unusably out of shape. Getting that device back to normal is an intricate fix. But if there were another, earlier point on the conveyor belt where you could reroute around that station, maybe you could save some of the damage and keep things rolling. …

[One] challenge is finding the people who have unidentified protective variants. "They're not going to be coming into clinics, because they're not sick," explained Friend. "They're not under the microscope, so to speak."

Even if they are sick, they're exceedingly rare. …

The rarity itself can create a methodological problem. … As [Eric Minikel, a grad student at the Broad Institute] explained, "… So if you look at a person who has supposedly-lethal mutation X but is mysteriously alive, there's no quick way to know which, if any, of their rare genetic variants kept them alive."

In the case of Burns' family, it seemed possible, but slow. At the time, sequencing whole genomes was prohibitively expensive. The team could read only short snippets of DNA and then try to piece together the part of the code that contained instructions for making proteins—a technique in which tiny alterations might be missed.

They were looking for a mutation shared by the eight affected relatives that might explain their heart issues. They found nothing. Years passed. Burns wondered whatever happened to the blood and saliva she'd given away. Her doctors had no news when she asked. Her mom died. Then her aunt. Both had heart attacks.

But, by five years ago or so, sequencing methods had become fast, accurate and cheap enough for the team to go back into the freezer and get out the family's vials. When they found mutations, they had CRISPR gene-editing tools, allowing them to easily and quickly insert the alterations into mice. When they took bits from a family member's samples and chemically coaxed them into heart muscle cells, they saw only a mild structural defect, and the cells could still beat, could still help generate a pulse.

When they spliced the first, lethal variant into African clawed frogs, another common lab animal, the embryos had a heartbeat that was reduced but not eliminated. And there were questions the study didn't answer. How exactly this pair of mutations was giving rise to the strange set of symptoms in Burns' family remains unclear. The first gene was already known to play an important role in the growth of heart tissue. But why would this combo so insistently give rise to heart holes?

… For now, the idea [of developing a therapy from the Burns' case] retains an aura of what-if, a garden of forking paths that is the germ of all research, at once captivating and dismaying. [This winter], Lo called Burns to share what she'd found in the family's genomes. "I told them, 'You know, you're actually very blessed,'" Lo recalled. "'Think about it. None of you would have been born if you didn't have the second mutation.'"

… When [Burns] finally heard about the results from Lo, she felt … conflicted. On the one hand, it felt good, to have some sort of closure, some kind of answer. On the other, she wished her grandfather and mother and aunt could have been alive to hear it. It was cool to know you were so scientifically unique. She'd told some of her friends at work. "They just kind of look at you like, holy cow. Really?"

But there were also downsides to being such a mystery. She knew there wouldn't be a therapy anytime soon. The biggest change was that the cardiologist would want to follow up more, to keep an eye on their hearts, powering them through work shifts and classes and basketball games and hunting trips, all normal but utterly improbable. ■