Growing a Cure

WHRY Blazes a Path to Leave Hypoparathyroidism Behind

“What do you think?”

It was eight years ago. Dr. Julie Ann Sosa and Dr. Sanziana Roman, two leading endocrine surgeons, approached Dr. Diane Krause about growing new parathyroid cells in the lab. They wanted to treat patients — mostly women — suffering from a lack of the hormone these cells produce.

Dr. Krause, an internationally recognized stem cell researcher, was immediately intrigued.

“I thought, ‘You know what — that sounds like a good idea,’” Krause said. “But was it even feasible?”

Thanks to a pair of pilot grants from Women’s Health Research at Yale, we now know the answer is, “Yes, it is feasible.” Moreover, while the team has not yet reached their ultimate goal, they have successfully achieved critically important steps in guiding a stem cell down the path to becoming a functional parathyroid-like cell. And with new funding from WHRY this year, Krause and her collaborators continue to move closer to a cure for this condition, known as hypoparathyroidism.

“Patients contact me every week,” Krause said. “They want us to succeed. They need the breakthroughs. And that’s what keeps us going.”

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Growing a Cure (Continued from front cover)

What Causes Hypoparathyroidism, and What Is It?

Causes of hypoparathyroidism include autoimmune diseases and hereditary conditions that lead to depleted levels of a key hormone. But the chain of events leading toward this condition most often starts with cancer of the thyroid gland. This is an organ located in the neck that releases hormones controlling metabolic functions such as breathing, heart rate, cholesterol levels, body temperature, and more.

Women in the United States suffer from thyroid disease at rates up to eight times higher than men. Women develop thyroid cancer at a rate three times higher than men.

Krause, Professor of Laboratory Medicine, Cell Biology, and Pathology and Associate Director of the Yale Stem Cell Center, regularly hears from women who have had their thyroid glands removed to treat this cancer. To be sure that no malignant tissue remains, such surgery often involves removing the parathyroid glands, four rice-sized structures that are normally located behind the thyroid and that are responsible for maintaining the body’s calcium levels.

Without these glands and the hormone they produce, called parathyroid hormone (PTH), bones do not release this important mineral into the bloodstream when calcium levels drop. In addition, the intestines receive no signal to absorb more calcium from food, and the kidneys do not increase their ability to hold on to calcium before releasing it in urine.

Calcium deficiency can lead to serious health problems, including painful muscle spasms in the hands and feet, seizures, an irregular heartbeat, or even heart failure. Patients also suffer on a daily basis due to fatigue and “brain fog,” a less well understood aspect of the disease. Current treatments require regular calcium supplements that can be short-acting and trigger unwanted side effects, making it difficult for patients to sustain a proper balance.

A New Way Forward

In 2010, WHRY awarded a pilot project grant to Krause and Sosa, now Chair of Surgery at the University of California, San Francisco (UCSF), to take the first steps toward transforming stem cells into parathyroid cells that can serve the same function as intact parathyroid glands.

Stem cells are the body’s basic, convertible building blocks. Either through natural human development or laboratory manipulations, stem cells have the potential to become any of the body’s specialized cells that serve a specific function, such as blood cells or cells that form bone or make up heart tissue.

Krause, Sosa, and Roman, also now a professor of surgery at UCSF, recruited Dr. Betty Lawton, a research associate in Krause’s lab with the technical expertise needed to complete the team. With WHRY’s grant, the team reached the first, difficult step.

Building on earlier published efforts, the team discovered the completed process will need to encompass five stages of specialization from an undifferentiated stem cell to a fully functioning parathyroid-like cell. Each step involves inducing the cell to express the specific genes necessary to guide the process in the desired direction.

In achieving the first step, the researchers labored 24 hours a day, seven days a week to manipulate chemical stimuli, timing, the growth medium, and temperature to eventually steer stem cells into becoming what is known as anterior definitive endoderm, a type of cell that makes up the inside layer in very early human development and gives rise to internal organs, including parathyroid glands.

“This is intricate, delicate, arduous work,” said Dr. Carolyn M. Mazure,
Director of WHRY. “Every step along the way provides benchmarks and creates a model for stem cell researchers to follow in pursuit of treatments for diseases and conditions that diminish human health and well-being.”

With additional funding from WHRY in 2014, Krause began to tackle a separate challenge needed to produce mature parathyroid cells from stem cells: how to test their function by injecting the newly created cells into the body so they attach, survive, and perform the necessary calcium-regulating work of the missing parathyroid glands.

Using an animal model, Krause successfully injected preserved human parathyroid cells into muscle tissue and demonstrated that single cells are a sufficient long-term substitute for the intact three-dimensional architecture of a parathyroid gland. This was an essential step forward, proving that once the stem cell-derived cells are created and purified, they will be able to function upon transplantation.

The Goal: Starting with a Patient’s Own Cells

To date, Krause has worked with stem cells from government-approved banks. But using a patient’s own cells — likely skin cells — will minimize the potential for that person’s immune system to reject the cells. Krause expects these tailored cells, once achieved, to work just as well as the missing parathyroid glands in regulating calcium.

The team continues to make progress toward using a patient’s own cells, walking them back in development so they become what are known as induced pluripotent stem cells (iPSCs), leading them into becoming parathyroid cells, and injecting them back into the patient.

“Our goal is nothing less than personalized regenerative medicine for parathyroid function,” Krause said.

Krause leveraged the findings of the WHRY-funded pilot projects to obtain a pair of larger state grants to advance both arms of this work, teaming with endocrine surgeon Dr. Glenda Callender and genetics and computer science professor Dr. Smita Krishnaswamy, while maintaining her collaboration with Drs. Sosa and Roman.

In January, Krause, who helped establish Yale’s dedicated stem cell center, attended a meeting of 30 researchers from across the country to share notes and reassert their commitment to pursuing stem cell therapy to treat hypoparathyroidism.

“Dr. Krause and Dr. Sosa exemplify the approach to interdisciplinary collaboration and translational research that has been the hallmark of Women’s Health Research at Yale since the beginning,” Mazure said. “These are leaders who embody the endurance and perseverance necessary for intensive research. Even while on opposite sides of the country, they understand the value of sharing expertise, data, and materials to overcome critical health problems that affect women in their daily lives.”

Discovering the Keys to Decode an Unwritten Recipe

So far, no one has successfully proven the ability to use iPSCs to effectively treat a disease in human subjects. The process of growing the cells involves much trial and error, coupled with careful monitoring in the lab — creating something from scratch that the body normally does automatically. Then there is the challenge of getting the cells to survive and function in a patient.

“If we succeed in our work on hypoparathyroidism, it would certainly be relevant to developing other needed therapies,” Krause said. “This is new territory for everyone. Each time we make progress, it becomes clearer what the next steps are.”

In one significant development, Dr. Krishnaswamy conducted a computer analysis to map how the cells within a normal parathyroid gland relate to one another, and she uncovered a diversity of cell types. Some make PTH, others are highly diverse in their makeup and serve different functions entirely, such as providing blood vessels for the gland.

One type is a parathyroid progenitor cell, which is more specialized than a basic stem cell in pushing development toward the target of a
The NIH Policy on Sex as a Biological Variable: A Check-Up

By Rick Harrison

Two years have gone by since the National Institutes of Health began requiring grant applicants to consider sex as a biological variable (SABV) in the design and analysis of laboratory studies using animals, tissues, and cells.

Judging from a study published in the Journal of Women’s Health in December, we are moving forward but have more work to do.

The authors, Dr. Nicole C. Woitowich from the Women’s Health Research Institute and Dr. Teresa K. Woodruff from Northwestern University, surveyed members of standing NIH study sections who both evaluate grant applications and often submit applications of their own to receive funding. One survey was conducted shortly after the policy was first put in place in 2016, followed by another survey one year later.

Of the researchers who responded to the 2017 survey, 68 percent said it was important for NIH-funded research to consider sex, up from 63 percent in 2016. Only 58 percent agreed that such a policy improves the rigor and reproducibility of the work. That’s an improvement from 54 percent who saw the scientific value of the policy in 2016.

The responses to both of these questions reveal a need to better educate researchers about the benefits of considering sex in every study. Notably, only 61 percent of respondents in the latest survey said that SABV was consistently weighed into the application scoring system.

All this, despite the fact that carefully conducted science continues to demonstrate sex and gender differences in human health. Because basic laboratory studies form the necessary foundation to conduct human studies, it is essential to include SABV to understand human health.

Just as we are doing at WHRY.

mature parathyroid cell that secretes PTH. The data indicate such cells are the ones most likely to attach successfully after injection and may prove to be key to giving a patient long-term parathyroid function.

Recently, the team has successfully tested a method to make stem cells glow red if they “turn on” the gene to make parathyroid hormone. This will allow the researchers to more efficiently produce enough and isolate the right type of cells for implantation.

The latest funding from WHRY, thanks to The Werth Family Foundation, will allow Krause’s team to perform in-depth investigations of normal parathyroid glands to identify the specific composition and function of different compartments within the individual cells.

“Our research is now at a critical point where we need to better understand the basic mechanisms of adult parathyroid gland maintenance and regeneration,” Krause said.

“This is the practical knowledge necessary to reach our goal of using these cells to restore normal parathyroid function for patients suffering from hypoparathyroidism.”

Krause thanked WHRY for the center’s longstanding support.

“This work couldn’t be done without WHRY,” she said. “The NIH does not fund science for an idea. They need established concepts, ready to publish. But how do you start?”

Krause recalled her meeting with Sosa and Roman eight years ago.

“We didn’t have any data,” she said. “We had an important idea to help patients and just had to start. For that, we needed a pilot funding source like WHRY for us to take the first steps.”

The Women’s Health Research at Yale Pilot Project Program is supported in part by The Rice Family Foundation, The Werth Family Foundation, The Community Foundation for Greater New Haven, the Maximilian E. and Marion O. Hoffman Foundation, the Seymour L. Lustman Memorial Fund, The Seedlings Foundation, and The Eppley Foundation for Research.
What Do You Get When You Teach Students to Ask New and Important Questions?

A Promise for a Better Future

Women’s Health Research at Yale's Undergraduate Fellowship offers our students the opportunity to work alongside and learn from Yale faculty members who study the influence of sex and gender in biomedical research and translate findings into medical practice.

Read on to meet our current class of fellows and learn what they are doing to advance the health of women.

Nafeesa Abuwala

**WHRY Mentors:** Dr. Megan Smith, Associate Professor of Psychiatry in the Yale Child Study Center and Yale School of Public Health and Founder and Director of the Mental health Outreach for MotherS (MOMS) Partnership, and Dr. Andrea Diaz Stransky, a clinical fellow in the Child Study Center

**Fellowship:** Understanding the value of community-based interventions on childhood mental health in the New Haven area’s Hispanic community. Abuwala is helping to implement a culturally sensitive MOMS program for immigrant mothers and their children to overcome barriers to care.

**Interests:** Abuwala has tutored and mentored in New Haven through Students for Autism Awareness at Yale and helped to establish medical clinics in rural Peru. Abuwala also volunteers at HAVEN Free Clinic, providing language interpretation for doctor-patient consultations.

Cecilia Crews

**WHRY Mentor:** Dr. Njeri Thande, Assistant Professor of Medicine (Cardiology)

**Fellowship:** Assessing the integration of sex and gender-based findings into the traditional medical school curriculum. Crews is working on an academic paper for a scientific journal that links the importance of studying women to policies for improving care.

**Interests:** Crews is a certified EMT who completed an internship with Doctors Without Borders and delivered health care in Haiti and Rwanda. At school, she has served as an Advocacy Coordinator for Reproductive Justice Action League at Yale (RALY), an undergraduate group for which she led a drive to gather menstrual products for the New Haven homeless community.
SECOND YEAR IN OUR FELLOWSHIP

Kaveri Curlin

**WHRY Mentors:** Njeri Thande and Dr. Carolyn M. Mazure

**Fellowship:** Last year, Curlin analyzed surveys of medical students assessing whether or how sex and gender was covered in their classes. Her work provided evidence to support conversations with course directors, who have since agreed to address the issue. In addition, Curlin presented this work at the 2018 Sex and Gender Health Education Summit in Utah, and the conference's organizers selected it as one of the four best submissions. She is a co-author of a recently published article on this work. This year she is completing a paper using a national database to determine how a physician's number of years practicing medicine might affect the health outcomes of patients with heart disease.

**Interests:** Curlin has worked as a student researcher in the lab of Dr. Valerie Horsley, Associate Professor of Molecular, Cellular, and Developmental Biology, investigating the mechanisms of how specialized fat storage cells called adipocytes contribute to the injury and healing process. She also performs and serves as the student rush coordinator for Coup de Brass, the school's premier French horn choir, and as a Chaplaincy Fellow, offering spiritual support for her classmates.

“*This has been one of the most rewarding experiences in my college career. As I get ready to leave Yale, I will be forever grateful for my ‘scientific home’ at WHRY.*”

— Kaveri Curlin

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**Jillian Eckroate**

**WHRY Mentor:** Dr. Kimberly Yonkers, Professor of Psychiatry, of Epidemiology (Chronic Diseases) and of Obstetrics, Gynecology, and Reproductive Sciences; Director, Center for Wellbeing of Women and Mothers

**Fellowship:** Conducting a qualitative study, including focus group methodologies, to identify the challenges that women face when deciding whether or not to use alcohol during pregnancy and what type of messaging motivates abstaining from alcohol consumption. The ultimate goal of the study is to design motivational text messages that can be regularly sent to women in order to reduce their alcohol use during pregnancy.

**Interests:** As a sexual health researcher for Student Partnerships for Global Health, Eckroate designed and conducted a mixed-methods public health study to understand how an increase in sexting and social media usage has affected the sexual health and decision-making of young adults in Nicaragua.
**Devyn Rigsby**

**WHRY Mentor:** Dr. Lisa Freed, Assistant Professor of Clinical Medicine, Director of Yale New Haven Hospital’s Women’s Heart and Vascular Program

**Fellowship:** Hands-on shadowing of a highly experienced cardiologist in an active clinical environment while participating in gender-based research on how physicians and clinical providers decide on appropriate care. In one project, Rigsby is contacting cardiology patients to study patient compliance with taking prescribed lipid medications. In a second project, she is assessing whether physicians at various points in their training and careers exhibit gender bias when treating individuals who present with chest pain.

**Interests:** Rigsby serves as the Chief of Staff for the Yale College Council, and in her junior year, she worked on the research team of Dr. Kevin Schuster, Associate Professor of Surgery, contributing to studies on opioid addiction and ventricular assist device outcomes.

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**Suyeon Hong**

**WHRY Mentor:** Dr. Carolyn Mazure

**Fellowship:** Working with Dr. Mazure and WHRY’s communications staff to build on the student-run blog. Titled “Why Didn’t I Know This?” the blog features Hong’s personal and informative take on the history and current state of women’s health research while advancing the center’s messaging goals through multimedia tools.

**Interests:** Hong has assisted with endometriosis research in the lab of Dr. Hugh Taylor, Chair of Obstetrics, Gynecology and Reproductive Sciences, and helped develop a curriculum to teach English as a Second Language in a New Haven elementary school. She has served as a board member of Yale Queer + Asian, Secretary of the LGBTQ Student Cooperative, and Policy Co-Chair of Yale Medical Professions Outreach.

“WHRY is teaching me how to communicate about my experiences in research and health care. I’m excited to continue developing these skills as I advance in my career as a pre-med student.”

— Suyeon Hong

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**Year:** Senior

**Residential College:** Pauli Murray

**Major:** Molecular, Cellular, and Developmental Biology

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Scan the code above with your smartphone or visit bit.ly/whryblog to read the latest posts from Suyeon on the blog Why Didn’t I Know This?
Where Medical Research Begins

In the United States, funding sources for medical research include the federal government, primarily the National Institutes of Health, and private industry, often pharmaceutical companies seeking to discover a profitable new drug.

At universities like Yale, researchers are expected to generate external funding for their projects. Grant awards can be used for certain types of equipment, salaries to cover tasks directly related to the funded research project, and other research-related expenses.

Unfortunately, there is far more demand for public and private grants than available funds. For example, the success rate of applications for research grants to the NIH hovered at around 35 percent for decades. Over the last 10 years, that rate plunged to the high teens. Adjusted for inflation, the purchasing power of those funds dipped by an estimated 25 percent.

Less funding means fewer research studies. But it also affects the type and quality of the work. In a 2014 paper in the Proceedings of the National Academy of Sciences, the authors wrote that “hyper-competition for the resources and positions that are required to conduct science suppresses the creativity, cooperation, risk-taking, and original thinking required to make fundamental discoveries.”

A 2016 survey in the journal Nature found that the demands of competition for grants and the attendant administrative tasks allow researchers to spend only 38 percent of their time on actual research. Competition is particularly difficult for less-experienced researchers. And, as a reminder, an NIH grant covers only part of the total cost of research and requires that salary support apply only to the time investigators are spending on the experiment.

This is why we are so lucky to have Women’s Health Research at Yale. WHRY provides and pays for many essentials necessary to plan and conduct research not covered by NIH grants. It also provides seed funding for innovative studies in women’s health that would not otherwise reach that first NIH threshold. These are ambitious and carefully crafted studies aimed at achieving major health advances.

Over the 20 years of WHRY’s existence, 73 percent of the center’s funded researchers have been junior or mid-level faculty. And 61 percent of investigators have gone on to obtain subsequent external grants to advance their vital work, three times the success rate of new NIH investigator-initiated applications. That’s money, for example, to help women better detect and receive treatment for heart attacks, breast and ovarian cancer, and autoimmune diseases; develop a vaccination strategy for sexually transmitted disease; support safer pregnancies; and help devise gender-specific interventions for addiction.

As a self-supporting center, WHRY’s annual operating expenses are in part paid with interest income from a modest endowment and foundation grants. But one third of our funding comes from private donations.

This is why WHRY needs supporters committed to women’s health: so that we can continue to fund new studies. So WHRY can translate results into action that benefit women and sex difference research that benefits both women and men. And, so the center can educate the public and policymakers about the best ways to improve public health.

In summary, when it comes to the question of where we find the resources needed to achieve equity in health research and practice, the critical answer is: you.

With great appreciation for your thoughtful generosity,

Barbara M. Riley
Philanthropy Chair
Why We Need to Study Sex and Gender

Count to eight, and somewhere in the United States a baby will be born.

You might imagine that this newborn has an equal chance of being female or male. But that’s not the case.

It’s true that at conception, scientists believe there is a 50 percent chance any given fertilized egg will be female. But a study published in the Proceedings of the National Academy of Sciences in 2015 found that the total mortality of females during gestation outnumbers males. The specific biological causes of this discrepancy remain unclear.

When it comes to this and so many aspects of biology, psychology, and overall health, answers remain elusive. Which is why we must continue asking the right questions. Thankfully, scientists with Women’s Health Research at Yale and our partners around the world are learning more about the influence of sex and gender on health. And we are finding not sameness, but difference.

“As we study women, data show sex and gender differences in the prevalence, risk factors, presentation, and course of diseases and conditions,” said Dr. Carolyn M. Mazure, Director of Women’s Health Research at Yale. “We are also learning that responses to prevention strategies and treatments can vary for women and men. We must continue to explore these differences if we are to improve the health of everyone.”

Historically, women were not widely studied, partly under assumptions — now proven false — that results found in male subjects represent an accurate reflection of what results would be in women.

Now entering our third decade, Women’s Health Research at Yale has driven medical science forward with a focus on studying women and examining health differences between women and men to improve the lives of everyone. The center’s mission is one of scientific inclusivity.

“When some of our core differences — grounded in biology and affected by environment and experience — can significantly impact our health,” Mazure said. “And so we need to study sex and gender in order to better detect and treat diseases that affect women and men differently.”

The health-related differences affecting females more than males stretch from head to toe. For example, women are more likely to:

- suffer from chronic diseases and disability
- have acute and chronic pain
- die following a heart attack
- develop depression and anxiety
- develop autoimmune diseases
- develop Alzheimer’s disease
• and more rapidly escalate from use to a substance or behavioral addiction with a higher likelihood to relapse after a quit attempt

There’s more. Women with diabetes are more likely to develop cancer than men with diabetes. Women experience stress differently — psychologically and physically. And women are more likely to be blind, even when accounting for the longer average life expectancy of women. Women also:

• wake faster than men following general anesthesia
• are more likely to need a repeated hip replacement surgery
• are more likely to be diagnosed with a more advanced bladder cancer and respond worse to treatment
• are three times more likely to suffer from migraines
• are more likely to have shingles and experience a recurrence of this disease’s painful rash
• are more often affected by anemias such as iron deficiency
• and are more likely to develop osteoporosis and suffer broken bones

“There are so many aspects of health and disease affected by sex and gender,” Mazure said, “that it might well be the case that areas unaffected by sex and gender are a rarity.”

However, despite the clearly demonstrated need for sex-and-gender-focused research, women remain under-enrolled in various studies of cardiovascular disease and cancer — the top two causes of death for women. In addition, women are underrepresented in clinical trials exploring causes, prevention, and treatment of HIV, the virus that causes AIDS. A recent WHRY-led study found that companies applying for government approval to market medical devices rarely determine if or how sex and gender might influence safety and effectiveness.

And even when women are included in sufficient numbers, studies are not always analyzed by sex or gender to uncover any significant differences. It was only in 2016 that the National Institutes of Health, the world’s largest single funder of biomedical research, required the inclusion of female animals, tissues, and cells in laboratory studies, which serve as the necessary basis for human trials.

These unexamined differences have serious consequences for the health care women receive and their outcomes. For example, even though cardiovascular disease (CVD) is culpable for about one in every three female deaths, studies have shown that women suffering heart attack symptoms arrive at a hospital later than men. Moreover, treatment of women for CVD, including heart attacks, is less aggressive than for men and less likely to achieve the best possible results.

It is because of such life-diminishing repercussions that Women’s Health Research at Yale exists. We are funding researchers and training others to overcome this gap in knowledge and to make sure everyone has the best chance possible to live a long and healthy life.

WHRY’s pilot projects and interdisciplinary research partnerships have focused on sex and gender differences in heart attacks, chronic urinary tract infections, skin cancer, autism spectrum disorder, neurological aging, cognitive dysfunction, autoimmune disorders, addiction, and depression, among many other diseases and conditions.

And there is so much more to do.

“Even when women are included in sufficient numbers, studies are not always analyzed by sex or gender to uncover any significant differences.

Even when women are included in sufficient numbers, studies are not always analyzed by sex or gender to uncover any significant differences.

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**Definitions of Sex and Gender**

**Sex** is the classification of living things, generally as male or female according to their reproductive organs and functions assigned by chromosomal compliment.

**Gender** is a person’s self-representation as male or female, or how that person is responded to by social institutions based on the individual’s gender presentation. Gender is rooted in biology and shaped by environment and experience.

Source: The National Academy of Medicine
Women’s Health Research at Yale

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Women’s Health Research at Yale is changing the landscape of medical research and practice by ensuring the study of women and examining health differences between women and men to improve the lives of everyone.

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IMPRESSING THE HEALTH OF PREGNANT WOMEN

More than six million women are pregnant in the United States in any given year. Over the course of their pregnancies and while breast feeding, more than 90 percent of these women will take at least one medication.

But clinical studies of medications, diseases, and conditions often exclude pregnant and lactating women. This leaves women and their caregivers guessing about how the biological changes caused by pregnancy and childbirth affect the development of diseases and the efficacy and safety of particular treatments.

Read the full story of a new federal report on this topic online by visiting our website: yalewhr.org