INSIDE STANFORD MEDICINE

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Scientists changed the spatial organization of DNA in cell nuclei, showing how physical relocation altered cell function.

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Battling ear, nose, throat disease in Africa

By Tracie White

PETER KOLTAI

Titus Dzongodza meets with a young patient and her mom at the newly established pediatric otolaryngology clinic at Harare Children's Hospital in Zimbabwe.

Titus Dzongodza, MD, was at the end of a long work day, throwing on his jacket to go home, when an 8-year-old girl gasping for breath walked through the doors of the new pediatric otolaryngology clinic at Harare Children's Hospital in Zimbabwe.

"Immediately, I knew we were in trouble," Dzongodza, who was director of the clinic at the time, said in a Skype interview. "She looked obviously stressed and tearful." Anoona (not her real name) had traveled all day with her mother by bus from their home in a rural village hundreds of miles away to reach the nearest hospital. It was spring of 2018, a year after the opening of the pediatric otolaryngology clinic, which treats disorders of the ear, nose and throat. "I could hear the grating sound in her shaky voice indicating the return of the viral warts on her larynx," Dzongodza said. "I knew we'd have to gather the whole team together in a matter of minutes."

After two years of planning, building, fundraising, training staff and scrounging for medical equipment, the new clinic opened its doors in March 2017. Within its first year, thousands of new patients were making daylong trips by bus to get treatment for neglected conditions. It was only the second such clinic in Africa. (The first was in neighboring South Africa.)

In a country of 14 million people, there are only eight otolaryngologists, also known as ear, nose and throat doctors. Many consider the subspecialty of pediatric ENT unnecessary because of Zimbabwe's many other unmet health care needs.

"This was a bold dream for a full-scale clinic with audiol- See ZIMBABWE, page 6

Juul e-cigarettes pose addiction risk for young users, study reports

By Erin Digitale

Teens and young adults who use Juul brand e-cigarettes are failing to recognize the product's addictive potential, despite using it more often than their peers who smoke conventional cigarettes, according to a new study by researchers at the School of Medicine.

The findings, from an ongoing Stanford project addressing the use and perceptions of tobacco products by California youth, was published Oct. 19 in *JAMA Network Open*. See JUUL, page 7

STEVE HEAP/SHUTTERSTOCK.COM

Upward of 100 patients with undiagnosed diseases find answers with network's help

By Hanae Armitage

More than 100 patients afflicted by mysterious illnesses have been diagnosed through a network of detective-doctors who investigate unidentified diseases, reports a study conducted by scientists at the School of Medicine and multiple collaborating institutes.

The long-awaited diagnoses are the fruits of the Undiagnosed Diseases Network, a program created by the National Institutes of Health in 2014.

"Our goal is to take on the hardest cases in medicine — to find patients and families with conditions that no one has been able to solve," said Euan Ashley, MD, professor of medicine at Stanford. "We wanted to provide a place that these people could come, so the Undiagnosed Diseases Network came together to try to answer that need."

The group, made up of hundreds of doctors across the United States, has so far sleuthed out 132 of 382 previously unknown ailments — roughly 35 percent. "Some of these patients had been waiting decades to put a name to their illness. They tell us how much of a relief it is simply to know what they were up against," Ashley said. But what's most exciting, he said, was that for 80 percent of the network's diagnoses, they distilled actionable information, such as changes to patient therapy, adjustments to future diagnostic testing and recommendations for family screening.

STEVE FISCH

Euan Ashley of Stanford is among the hundreds of medical scientists nationwide who make up the Undiagnosed Diseases Network.

"Our findings underscore the impact that establishing a clear diagnosis can have on clinical decision-making for previously undiagnosed patients," said Kimberly Splinter, associate director of research operations for the network's coordinating center and a genetic counselor at Harvard Medical School. "We hope that the results of this analysis will provide a com- See NETWORK, page 7

Anti-inflammatory works for treating lymphedema symptoms

By Tracie White

For more than three decades, Lisa Hanson did her best to hide the unsightly fluid retention in her left leg that caused uncomfortable swelling and made her skin taut and thickened. At 17, when she was first diagnosed with lymphedema, she threw out her shorts and dresses and began a lifelong journey of wearing compression hose up to her thigh and using an electric sleevelike pump every night to control the swelling.

Now, with a new treatment in hand, she's actually excited to tell people about this chronic condition, which before, she said, left her feeling like "a freak."

"For a long time I couldn't talk to people about my lymphedema without crying because it's something weird and obscure," Hanson said. "Now there is hope for people like me with this disease."

Hanson took part in one of two small clinical trials led by researchers at the School of Medicine which showed that ketoprofen, an inflammation-reducing drug available by prescription and currently approved by the Food and Drug Administration, can effectively treat symptoms of lymphedema and help ease the daily burden of care.

"Ketoprofen restores the health and elasticity of the skin," said Stanley Rockson, MD, professor of cardiovascular medicine at Stanford. "I believe it will reduce recurrent infection. It can also reduce swelling."

ELLIE HANSON

Lisa Hanson took part in a Stanford clinical trial of a drug she said helped reduce the swelling in her left leg from a condition called lymphedema.

A paper describing the findings of the two clinical trials was published Oct. 18 in *JCI Insight*. Rockson is the lead author. Mark Nicolls, MD, professor of pulmonary and critical care medicine at Stanford, is his principal collaborator. They both served as corresponding authors for the manuscript.

"So many patients have gone through decades being told there is no medical treatment," said Rockson, who holds the Allan and Tina Neill Professorship of Lymphatic Research and Medicine. "Now, they can go to a drugstore and get a pill with a doctor's prescription. This new treatment doesn't cure lymphedema, but our studies show it has the capacity to make the illness more livable, more workable."

Painful swelling

Mark Nicolls

Lymphedema is a common but often ignored condition that stems from a damaged lymphatic system and results in unsightly swelling in one or more parts of the body, usually the legs. It can be hereditary or it can occur after a surgical procedure, infection, radiation or other physical trauma. The swelling, caused by a buildup of lymph fluid within the various layers of the skin, increases the risk of infections and can cause debilitating pain and a thickening of the skin that can restrict movement. There is no cure, and there has been no drug therapy available.

Ever since Hanson was diagnosed in her teens, the only available treatment has been to wear compression garments; use the electric pump, which moves the excess fluid from her leg back into the bloodstream; or get massage therapy to suppress the swelling, which can occur throughout the body. She has done all of this religiously for decades.

"It's been a lot of work and a lot of burden putting the compression socks on daily," Hanson said. "It's hard to get them on and off. They're tight and they're heavy. I've used the pump every night sometimes for up to four hours."

As many as 10 million Americans and hundreds of millions of people worldwide suffer from the condition, many from the aftereffects of cancer treatments. Thirty percent of women treated for breast cancer get lymphedema, usually as a result of radiation treatment and lymph node removal, according to the American Cancer Society.

Years ago, Rockson, a physician-scientist who has treated thousands of patients with lymphedema, began to suspect that inflammation was a root cause of the disease. To test his theory, he created a mouse model for lymphedema — the disease would manifest in the animals' tails — and treated it with ketoprofen, a nonsteroidal anti-inflammatory drug, or NSAID.

"It reversed the lymphedema," Rockson said. "We saw tremendous improvement in the structural abnormalities in the skin."

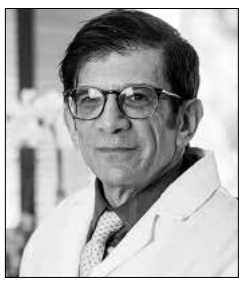
To test ketoprofen in humans, Rockson conducted two pilot trials, which are both discussed in the paper. The first trial had 21 participants who knew they were getting the drug and took it orally for four months. Researchers performed skin biopsies at the beginning of the trial and then four months later at the end of the trial as a measurement of disease severity.

"That was an extremely positive trial," Rockson said. "We saw a tremendous reversal in the disease process in the skin and dramatic reductions in skin thickness." This led to the second double-blind, placebo-controlled study with 34 participants. Hanson, who participated in the second trial, didn't know at first whether she was taking ketoprofen or a placebo. But she felt fairly certain after two months that she was getting ketoprofen.

"After a couple of months, I remember going home one day and taking my compression stockings off and looking at my leg thinking, 'Wow my skin is wrinkly, that's so weird.' The skin wasn't so taut or thick. It was more like normal," Hanson said.

Thinner skin

The second trial further validated that the drug can reduce thickening of the skin. Researchers also examined the anatomy of the skin cells and confirmed that ketoprofen worked by unblocking the molecular pathway that was causing the inflammation and restricting the body's ability to repair its own lymphatic system.



Stanley Rockson

"When you look at skin from lymphedema patients under the microscope, you see a dramatic increase in cell density and increase in connective tissues and fluid around the cells," he said. "What we saw in skin biopsies after the four months of ketoprofen was a reduction in that thickness. All that cell density went away."

Results showed that ketoprofen made the skin healthier and more elastic, Rockson said.

"Anecdotally, we also got the impression that the patients who were treated saw a dramatic decrease in infections, although this analysis wasn't part of the study," Rockson said.

After the four months, the patients in the second trial were "unblinded" and given the option to continue using the drug by prescription, Rockson said. All chose to continue taking the drug, including Hanson, who has now taken the ketoprofen for several years.

"Over time, the swelling has gone down," she said. "It's not a cure. It doesn't make it go away, but it has been easier to take care of my leg." She still wears the compression stockings, but they're much easier to tug on, and the nightly pumping now takes just a fraction of the time it used to.

Hanson, like other participants in the trial, was warned by researchers that past studies have shown gastrointestinal and cardiovascular side effects from long-term use of ketoprofen in some patients, but she still decided to keep taking the drug.

"For me, the choice of being comfortable and not having so much burden in terms of care is a much greater benefit and outweighs the risk," she said.

An inflammatory response

Just how ketoprofen was working at a molecular level, though, remained unclear early on. To further examine this while continuing his ketoprofen trials in humans, Rockson joined forces with Nicolls, whose lab had been studying the molecular pathways of inflammation in pulmonary hypertension.

"We were excited to finally figure out that the drug worked by blocking an inflammatory molecule called leukotriene B4," said Nicolls referring to a study published in May 2017.

The researchers found that the buildup of lymph fluid is actually an inflammatory response within the tissue of the skin, not merely a "plumbing" problem within the lymphatic system, as previously thought. They discovered that the naturally occurring inflammatory molecule LTB4 is elevated in both animal models of lymphedema and in humans with the disease, and that at elevated levels it causes tissue inflammation and impaired lymphatic function.

Further research in mice showed that using ketoprofen to target LTB4 induced lymphatic repair and reversed the disease processes. This indicated that perhaps other therapies could reverse the negative impact of inflammation on lymphatic repair by targeting LTB4.

Other Stanford authors are postdoctoral scholars Wen "Amy" Tian, PhD, and Xinguo Jiang, PhD, who are also affiliated with the Veterans Affairs Palo Alto Health Care System; François Haddad, MD, clinical associate professor of cardiovascular medicine; Leslie Roche, RN, clinical research coordinator at the Stanford Center for Lymphatic and Venous Disorders; and Jinah Kim, MD, PhD, a dermatological pathologist. **ISM**

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STANFORD MEDICINE

Researchers reveal new mechanism for how animal cells stay intact

By Taylor Kubota

Almost eight years ago, Stanford bioengineer Manu Prakash, PhD, was looking for a way to watch every cell in a living adult animal in elaborate detail. He searched the catalog of life and happened upon the simple marine animal Trichoplax adhaerens — or Tplax, as Prakash has come to call it.

This ultra-flat creature lacks both muscles and neurons, but still moves and navigates through its watery world. The Prakash lab found Tplax manages this feat through surprisingly fast contractions in its two skinlike layers — contractions strong enough that they would ordinarily rip apart such seemingly delicate tissues.

In their first paper based on years-long study of this organism, published online Oct. 11 in Proceedings of the National Academy of Sciences, the researchers describe the ultra-fast contractions and propose a hypothesis for how this creature withstands internal and external forces in a marine environment.

The findings could help inform not only how complex animals evolved, but also the creation of an advanced material, called an active solid, that could dramatically and quickly modulate its own physical properties.

Moving without muscles

"Much of the rules of biology that we read in textbooks have been, so far, dictated by a few sets of 'model' organisms," said Prakash, associate professor of bioengineering and senior author of the paper. "If we intend to be the generation that will unravel laws of biology, it's extremely important to understand and appreciate the diversity of what has evolved on our planet and think much more holistically **See TPLAX, page 3**

With gifts, Stanford poised for major advances in neurosciences

By Lisa Trei and Nathan Collins

Stanford will accelerate the pace of discovery about the human brain and advance innovative, interdisciplinary brain science thanks to nearly $250 million in recent gifts from philanthropists from the United States, Asia and Europe.

In recognition of the lead gift from alumna Clara Wu Tsai and her husband, Joe Tsai, the Stanford Neurosciences Institute is changing its name to the Wu Tsai Neurosciences Institute.

The institute's goal is to better understand how the brain functions, both in health and disease, and to pave the way for new treatments for neurological and psychiatric diseases such as depression, anxiety and Alzheimer's. The funds, raised since the institute was established in 2013, provide support to scale up research initiatives; expand resources for faculty, students, postdoctoral scholars and new technologies; and complete an interdisciplinary research complex where scientists from across campus can meet and collaborate.

Stanford President Marc Tessier-Lavigne, PhD, a neuroscientist himself, said the gifts come at a pivotal time.

"We are on the threshold of a very promising era, as we make discoveries about the living brain that were previously unimaginable," Tessier-Lavigne said. "Nearly 450 faculty members from a range of fields are already engaged in the neurosciences and brain-related research at Stanford, making it one of our most vibrant areas of inquiry on campus. These foundational investments uniquely position our scientific community and universitywide institute to advance new breakthroughs. I am deeply thankful for the vision and generosity of our donors, including Clara Wu Tsai and Joe Tsai, who understand the potential of this research to improve brain health and human well-being."

Gift to advance brain discoveries

Clara Wu Tsai served on a presidential task force that helped shape the Stanford Neurosciences Institute and Stanford ChEM-H (Chemistry, Engineering & Medicine for Human Health). She said the concentration of experts from many fields within walking distance of one another on one campus and the university's ability to harness this collective knowledge were key factors that she and her husband took into account when deciding to invest in the neurosciences at Stanford.

"Joe and I believe Stanford is uniquely positioned to drive breakthrough discoveries about the brain, translate them into effective therapies and train future scientists of the world," Wu Tsai said. "Ultimately, we hope the research undertaken at the Wu Tsai Neurosciences Institute will lead to cures and treatments that impact millions of people by prolonging their lives and making them more fulfilling and productive."

Wu Tsai earned a bachelor's degree in international relations and a master's degree in international policy studies at Stanford, both in 1988, as well as an MBA at Harvard. She then pursued a career in business and finance as an executive at American Express and at Taobao, China's largest online shopping site.

The future of Stanford neuroscience

At Stanford, Clara Wu Tsai is an active member of the advisory council that helps guide university institutes focused on the interdisciplinary life sciences. She also co-chairs the advisory cabinet for the neurosciences institute and serves on the university's Global Advisory Council. Previously, she served on the advisory council for Stanford Bio-X.

Joe Tsai is executive vice chairman and one of the founders of Alibaba Group, a global Internet technology company based in China. Alibaba has businesses in digital commerce, entertainment, cloud computing, logistics and financial technology.

Together, Joe and Clara pursue philanthropic investments that advance knowledge, innovation, equality of opportunity and creative arts. Their work with global partners focuses on the translation of new knowledge into practical applications with human and social impact.

Platform for campuswide collaborations

In its role of fostering universitywide initiatives related to brain science, the Wu Tsai Neurosciences Institute will leverage ongoing contributions from the schools of Medicine, of Engineering and of Humanities and Sciences. These partnerships will be further enhanced when a new interdisciplinary research complex opens next year. The complex comprises two intersecting buildings: One will serve as the hub for the Wu Tsai Neurosciences Institute and the other for Stanford ChEM-H.

The 235,000-square-foot research complex is located a short walk from Stanford's basic sciences departments, engineering and medical schools, as well as the children's and adult hospitals. Also nearby is the James H. Clark Center, the home base of Stanford Bio-X, a cross-disciplinary venture launched in 1998 that has become a model for biosciences programs worldwide.

The Wu Tsai Neurosciences Institute is led by William Newsome, PhD, the Vincent V. C. Woo Director, who jointly led the working group for President Barack Obama's BRAIN research initiative. Newsome said that advances in the neurosciences have the potential to transform the 21st century in the way that quantum physics and breaking the genetic code transformed the 20th century.

"Technologies invented in the last decade are making it possible for neuroscientists to acquire new kinds of information about the brain that, until recently, was the stuff of scientists' dreams," said Newsome, who also holds the Harman Family Provostial Professorship. "The sequencing of the human genome, new imaging techniques, optogenetics, discoveries in nanoscience and physics — all of these advances are providing critical insights into our minds and ourselves. Clara and Joe's generous gift will help us get closer to a world where we can effectively diagnose and treat brain injury, diseases and disorders, as well as enhance brain functions to improve lives."

Big Ideas in Neuroscience

When the neurosciences institute was launched five years ago, Newsome asked faculty to form cross-disciplinary teams around what they considered the boldest, most visionary research projects tackling challenges in brain science. More than 230 Stanford scientists and scholars presented dozens of "Big Ideas in Neuroscience" that focused on three research directions: NeuroDiscovery, NeuroEngineering and NeuroHealth. The institute selected projects with tremendous potential that were less likely to secure federal funding at this early stage of research, especially since the projects require substantive teamwork among diverse faculty experts. A second round of Big Ideas was announced last month.

Ongoing projects are looking into brain rejuvenation, addiction, neuro-technologies, brain-machine interfaces, nervous system disorders and psychiatric illness.

In addition, the institute funds modest grants and awards that support high-risk, high-reward collaborations. These include research into cognitive aging and stroke recovery, and treatments for obsessive-compulsive disorder and chronic pain. The institute also fosters teamwork through shared laboratories and collaboration spaces, as well as workshops, seminars and symposia.

STEVE CASTILLO

Joe Tsai and Clara Wu Tsai made a gift to the Stanford Neurosciences Institute, which has been renamed in their honor.

Kathryn Moler, PhD, vice provost and dean of research, said the Wu Tsai Neurosciences Institute will accelerate these types of innovative and interdisciplinary campus endeavors and support collaborations with SLAC National Accelerator Laboratory, the Veterans Affairs Palo Alto Health Care System and biotech firms in Silicon Valley.

"One of the challenges of interdisciplinary research is finding effective ways for experts from different fields to work together," Moler said. "What we are learning from Big Ideas and other cross-campus collaborations is helping to set a new standard for how to do science in the 21st century."

New era for neurosciences at Stanford

The neurosciences institute marked its fifth anniversary Oct. 11 with a symposium titled "Natural/Artificial Intelligence." Future plans include launching an initiative in neuro-translation to help teams of scientists move discoveries from the lab into practical applications. It will also support neuro-theory collaborations involving computer scientists, statisticians, applied physicists and engineers who want to identify fundamental principles of nervous system computation, understand how the brain's neural network of interconnecting neurons operates and also make sense of the vast quantities of new brain data.

Newsome said there has been an explosion of interest from young scientists in collaborative brain research and that the institute will need to raise funding for more fellowships to ramp up cross-disciplinary graduate and postdoctoral training programs. The institute also aims to launch research centers that specialize in highly promising areas such as sensory neuroscience, computational neuroscience, and molecular and cellular approaches to brain disease.

"Breakthroughs in the neurosciences have the potential to not only improve human well-being but to guide future policies and practices that affect the criminal justice system, drug control, national defense, social welfare and education," Newsome said. "I believe that the work of the Wu Tsai Neurosciences Institute will be truly transformative." **ISM**

Tplax

continued from page 2

about what is actually possible in biological systems."

In the early days of studying Tplax, the creatures would move repeatedly out of view under the microscope. But over time, the researchers learned to track and quantify the animals' every cellular squeeze and squirm. Prakash remembers when their efforts first began to pay off.

"There was literally a day where, for the first time, I had some of the stains that label Tplax cells working, and under the microscope we saw an explosion of cellular contractions," Prakash said. "It looked like fireworks under a microscope, and that was the moment that told us there is something very special about this animal and we needed to understand it."

Those fireworks were Tplax's quick contractions, which occur in its flat layer of what are known as epithelial cells — essentially the equivalent of skin. Although these kinds of cells have long been known to contract — in embryos, for example — Tplax's contractions were 10 times faster than any epithelial cell contraction ever reported. This would tear apart the network of cells in any other biological tissue as thin as this animal, which is only about 25 microns thick, or one-quarter the thickness of a sheet of paper.

The researchers think the tissue's strength lies in the fact that while some cells contract strongly, others soften — a hypothesis they call "active cohesion." In many tissues, contracting in reaction to a force would cause a tear, and relaxing would cause the animal to be at the mercy of that force. By doing both simultaneously and in a coordinated manner, the cells involved in Tplax's active cohesion distribute the stress, letting the animal remain whole and in control.

The discovery of an ultra-fast contractile epithelial cell poses new questions for the role of epithelial contractions in coordinating cellular activity across the tissue.

"We look at this simple creature and we see it make decisions and move and hunt," said Shahaf Armon, PhD, a postdoctoral scholar and the lead author of the paper. "It's a huge evolutionary question, how single cells merged to become multicellular organisms and how such a minimal tissue made of identical cells is able to then perform complex behaviors."

Now, the researchers are exploring what other organisms might use active cohesion and are creating artificial material that replicates this mechanism to build an active solid. Key to the speed of these contractions is the unusual geometry of Tplax's epithelial structure: T-shaped cells with a very thin top sheet and a hanging nucleus at the bottom that line up side-by-side like a single layer of bricks. That geometry, which they share with sponges, could inform the development of new materials.

Mysterious beasts

Working with **See TPLAX, page 6**

Scientists modify CRISPR to reorganize genome in cell nuclei

By Hanae Armitage

Stanford researchers have reworked CRISPR-Cas9 gene-editing technology to manipulate the genome in three-dimensional space, allowing them to ferry genetic snippets to different locations in a cell's nucleus.

The new technique, dubbed CRISPR-genome organization or simply CRISPR-GO, uses a modified CRISPR protein to reorganize the genome in three dimensions. If CRISPR is like molecular scissors, then CRISPR-GO is like molecular tweezers, grabbing specific bits of the genome and plunking them down in new locations of the nucleus. But it's more than just physical relocation: Displacing genetic elements can changes how they function.

The research shed new light on how the genome's spatial organization in the nucleus governs the function of the cell overall.

"The question of why spatial organization in a cell matters is an important one, and it's also not one that scientists agree on," said Stanley Qi, PhD, assistant professor of bioengineering and of chemical and systems biology. "CRISPR-GO could provide an opportunity to answer that question by enabling us to target, move and relocate very specific stretches of DNA, and see how their new placements in the nucleus change how they function."

Most mammalian cells contain a nucleus that houses more than 6 feet of DNA, if stretched out in a line. This genetic material determines the fate of the cells and, if out of place or damaged, can lead to disease. Previous studies have shown that DNA tends to clump in certain areas in the nucleus. How that placement affects the DNA's function, however, is still unclear.

In the proof-of-principle study, Qi investigated three distinct subregions of the nucleus using CRISPR-GO, testing an overarching hypothesis: Do genes and other genetic elements behave differently in different zones of the nucleus?

So far, their data show that specific compartments and some free-floating bodies of proteins in the nucleus can sway the function of repositioned DNA. Depending on where the genetic materials are located, some nuclear regions repress gene expression and some accelerate telomere growth, and subsequently cell division. One protein body may even hold the power to suppress tumor formation.

A study detailing this research was published online Oct. 11 in *Cell*. Qi is the senior author. Postdoctoral scholar Haifeng Wang, PhD, is the lead author.

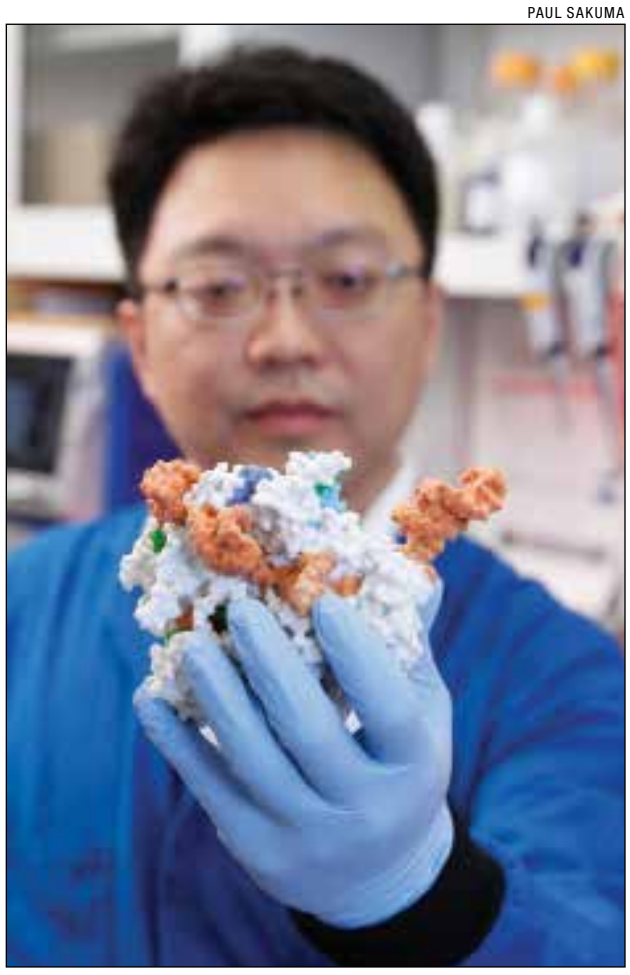
Bridging the gap

Demystifying the physical details of the genome has proved to be a tedious task, but there are some existing technologies that allow scientists to peer into cells and see how their guts are physically organized. What's been missing is a way to tamper with this organization. CRISPR-GO is the first to offer researchers a means to do so.

By decommissioning the "cutting" mechanism of CRISPR-Cas9, the editing tool becomes more of a delivery system, which Qi used to deliver small stretches of DNA via a programmable guide RNA to a new location in the nucleus.

There are three essential parts of CRISPR-GO. First, there's what Qi calls the "address" of the genetic target that you want to relocate — a stretch of DNA that's targeted with a complementary strand of binding RNA. Then, you need the destination's address — the specific portion of DNA in a nuclear compartment to which you want to move the chromatin. Finally, there's the "bridge," which, in this case, is a catalyst that sparks the congealing of the target DNA to its new home in the nucleus.

PAUL SAKUMA



Stanley Qi holds a 3-D printed model of a modified version of the cas9 protein, engineered to deliver DNA to various parts of a cell.

"Kids often like to build little railroads to help trains get from one station to another," said Qi. "It's not so different from what we're doing here."

Different room, different function

Qi describes the functionalities of the nuclear compartments like the spaces of a house. In every room of your home, you do different things — in the kitchen, you cook; in the bedroom, you sleep. In the nucleus of a cell, the same concept applies. There are multiple compartments in the nucleus that all have specific roles in upholding cell functionality overall. Qi and his lab investigated three distinct areas of the nucleus, testing whether they could somehow shift the function of chromatin depending on where they moved it.

By using CRISPR-GO, the researchers observed that genes relocated to a part of the nucleus called the Cajal body, an amorphic and somewhat mysterious blob of proteins and RNA, stopped expressing proteins.

"We were super-excited to see this; it's the first time that researchers have evidence to show the Cajal body can have a direct gene-regulation effect, in this case repressing gene expression," Qi said. "It suggests that the Cajal body has some unexpected role in controlling transcription." That could be big, as transcription is an important process that synthesizes the "code" for protein production.

When Qi used CRISPR-GO to move the DNA of telomeres — the molecular caps of chromosomes that are associated with longevity — from the middle to the edge of the nucleus, the telomeres stopped growing, halting the cell cycle and reducing cell viability. The opposite, however, happened when telomeres were moved closer to the Cajal body: They grew and, in doing so, increased cell viability.

The third application used CRISPR-GO to form a promyelocytic leukemia body. This glob of proteins is known to suppress pro-tumor genes. By positioning it next to cancer-causing genes in the nucleus, Qi plans to test if it can help curb tumor formation.

"Another unique advantage of CRISPR-GO is that we can track the interactions between chromatin DNA and nuclear compartments in real time under a microscope," Wang said.

While the evidence shown by CRISPR-GO is exciting, the research is still in a pilot stage, and there's more work to be done before the findings can be confirmed, Qi said.

"We're very excited about the potential here and, while we've answered a couple questions, we've opened up about 20 more," Qi said.

It will be even more important to decipher why these location-based effects take place in specific nuclear compartments, and what the underlying cause is, he said. One day, Qi hopes, this line of research will come to bear on human health.

Other Stanford authors of the paper are postdoctoral scholars Xiaoshu Xu, PhD, Yanxia Liu, PhD, Xueqiu Lin, PhD, and Timothy Daley, PhD; undergraduate student Cindy Nguyen; graduate students Yuchen Gao and Nathan Kipniss; and research scientist Marie La Russa, PhD.

Qi is a member of Stanford ChEM-H, Stanford Bio-X, the Stanford Cancer Institute and the Stanford Neurosciences Institute.

The research was supported by the Pew Scholar Foundation, the Alfred P. Sloan Foundation, the National Institutes of Health and a gift from the Li Ka Shing Foundation.

Stanford's Department of Bioengineering, which is jointly operated by the schools of Medicine and of Engineering, also supported the work. ISM

Visible and valued: Stanford Medicine's first-ever LGBTQ+ Forum

By Julie Greicius

A "chosen family" is how some of the speakers described their colleagues here during the first-ever Stanford Medicine LGBTQ+ Forum.

Too often those letters — which stand for lesbian, gay, bisexual, transgender and queer/questioning — describe individuals whose sexual orientation or gender identity is kept hidden because it can be personally and professionally risky to be "out." But the visibility-themed event on Oct. 10 was an unmistakable declaration that LGBTQ+ individuals are a seen, treasured and essential part of the Stanford Medicine community.

In his opening remarks, Lloyd Minor, MD, dean of the School of Medicine, emphasized Stanford Medicine's dedication to being a supportive environment for its LBGTQ+ members, and a leader in LGBTQ+ medical education, research and care. He also noted his personal support.

"I want to personally commit to each of you, to all of you, that I'm going to stand beside you. I'm going to make sure that we change the fabric and the culture not only of our institutions, but also together we can really have an impact on the fabric and culture of our society," Minor said. "I think we have an innate responsibility — as an academic medical center, as a great research university — to celebrate and embrace diversity and inclusion in all of its aspects."

The event was founded by MD-PhD student Timothy Keyes, who spent more than a year organizing it with fellow members of the LGBTQ-Meds organization and many other sponsors. Faculty supporters like Yvonne "Bonnie" Maldonado, MD, senior associate dean for faculty development and diversity, recognized the importance of the event, because the LGBTQ+ community is what she called an "invisible minority." Keyes' effort was also championed by faculty sponsors Marcia **See FORUM+, page 5**

VALERIE BERLAND

Attendees gather at a rainbow balloon photo booth during the LGBTQ+ Forum on Oct. 10.

Researchers study head motion in high school football hits

By Erin Digitale

PAUL SAKUMA

(Top) Research assistant William Mehring (right) hands a mouthguard to Menlo School sophomore J.P. McKenney. (Right) Mehring with the mouthguards, which local high school football players will wear during practices and games. The mouthguards contain sensors that measure motion in three directions.

Scientists at the School of Medicine are collaborating with football teams at three Bay Area high schools to understand how hits to the head cause concussions in young players.

In a study launched last month, a research team led by concussion experts David Camarillo, PhD, and Gerald Grant, MD, is outfitting the players with mouthguards that measure the motion of the head during impacts sustained in practices and games. About 100 football players from Menlo School and Sacred Heart Preparatory, which are in Atherton, and Archbishop Mitty High School, in San Jose, are participating in the first year of the study.

Camarillo's team has previously studied concussion in Stanford athletes and NFL players, but never in younger players.

"This will be the first study in kids where we'll be measuring rotation and full motion of the head during impacts," said Camarillo, assistant professor of bioengineering and the co-principal investigator for the study. "It's important to expand our research to the high school level and younger because that's where there are the most athletes."

While the NFL and college football comprise several thousand players, high school and children's football programs account for around 4 million players nationwide, he said.

"Our goal is to focus first on high schools in the Bay Area and to deepen our level of understanding about how concussions may occur in one player but not another," said Grant, professor of neurosurgery and the principal investigator for the study. "We're going to gather prospective data on the players in the preseason, as well as during the season and postseason." Grant is also a pediatric neurosurgeon at Lucile Packard Children's Hospital Stanford, where he treats young athletes with head injuries.

High-tech mouthguards

The researchers are collecting preseason neurocognitive data that they will use as a baseline for comparison with neurocognitive data collected after any suspected injuries. During practices and games, players will wear special mouthguards equipped with sensors that measure motion in three directions — up/down, left/right and front/back — as well as three types of rotational acceleration: roll, pitch and yaw.

Practices and games will be filmed so that researchers can confirm collisions and assess players' speeds prior to impact. Eye tracking data will be collected as part of an effort to understand whether erratic eye motions after a head impact indicate a concussion.

The researchers hope the data will illuminate exactly what types of collision lead to concussion so that coaches can better evaluate when players who have had collisions are at risk. Simply watching collisions from the sidelines often does not provide an accurate sense of whether a player's brain could be damaged.

"It's entirely possible that something that looks really dangerous may produce accelerations that are not very high, and may not be dangerous," Camarillo said. "Conversely, hits that don't look scary may be high-acceleration. It's difficult to see."

Understanding which hits are dangerous might also help players and coaches learn how to prevent risky collisions, the researchers said.

"It's very exciting to think about using this feedback for coaching," Grant said. "We suspect some players may be at greater risk for concussion than others because of the way they tackle. We hope to use the data to give coaches the tools they need to teach an individual athlete how to play using the safest possible technique."

The study will continue through the 2018 football season and will expand in 2019.

The research is being funded by local philanthropists Tad and Dianne Taube through the Taube Stanford Concussion Collaborative. ISM

Forum

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Stefanick, PhD, professor of medicine and of obstetrics and gynecology, and James Lock, MD, professor of psychiatry and behavioral sciences.

Recalling a talk with Ben Barres

In his remarks at the event, Keyes described a visit to Stanford before he had decided to attend. He interviewed with the late neuroscientist Ben Barres, MD, PhD, an openly transgender man, who asked Keyes an unexpected question.

"He said, 'Oh, Tim, by the way, are you gay?'" Keyes recalled. "And I said, 'Yes, of course.' He had noted something on my CV that was LGBTQ leadership-related. And he said, 'Well, …I just want to let you know this is a very supportive community here. …You could come here and be as out as you'd like to be and not have to worry about anything.'"

It was the first time, Keyes said, that anyone had asked him about his sexuality in a positive way with regard to his career. "It was so meaningful," Keyes said. "It made me feel really, really special and … really seen. And that's why I connected that experience back to visibility, because I felt that Ben saw me in a way that I didn't even realize I needed to be seen to feel at home and like I belonged. Of course, I came here to Stanford because of that and because of him."

"A place like Stanford is Stanford because of the community that we bring together."

Several speakers, both live and in a video, told of their experiences in academic medical environments, and why visibility is so important. Keynote speaker Arturo Molina, MD, MS, president-elect of the Stanford Medicine Alumni Association, described how he kept his sexuality secret for years during medical school and after. He and his husband are now the proud fathers of two. Leslee Subak, MD, professor and chair of obstetrics and gynecology, recalled her efforts at community-building during her residency at UCSF in the late 1980s. Today, Subak is developing Stanford Medicine's new Sexual and Gender Medicine Program, which will include clinical care, research, education and advocacy.

'Competent and compassionate care'

Benjamin Laniakea, MD, clinical assistant professor of medicine, described how sorely lacking the LGBTQ+ health training was when he was in medical school from 2009-13. In all four years, he said, it amounted to only a single HIV/AIDS diagnostic question on one exam. Today, Laniakea said, "All I want to be able to do is to have medical students feel armed with the knowledge and skills to provide appropriate LGB and transgender care, to provide this competent and compassionate care. That's it."

About 350 people attended the afternoon, on-campus event, which included LGBTQ+ individuals and their allies and colleagues from the School of Medicine, Stanford Health Care and Stanford Children's Health. Guests were treated to giveaways and food.

As much as the gender and sexual minority members at the event were there to be part of a chosen family, their allies and colleagues had chosen to be part of that same family, too.

"In order for each of us to fulfill our potential, we have to feel a part of the fabric of our community. We have to feel, every day, that who we are, inside, is a vital part of who we are as a member of our community," Minor said. "At its heart and core, a place like Stanford is Stanford because of the community that we bring together."

The journey toward visibility and inclusion may not be easy, the dean concluded, but it's one of the most important for Stanford, whose accomplishments can be an example for the country and the world. ISM

Zimbabwe

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ogy and speech therapy services, as well as two operating rooms with a recovery room and beds for overnight care," said Peter Koltai, MD, professor of otolaryngology and of pediatrics at Stanford, who was recruited three years ago as a volunteer adviser for the project. "Now we believe that this new clinic can be used as a role model to be duplicated across all of Africa."

The problem

The clinic was the vision of Clemence Chidziva, MD, an ENT surgeon and professor of otolaryngology at the University of Zimbabwe. Chidziva knew firsthand the effects of malnutrition, poor medical care and uncontrolled viruses on his pediatric ENT patients. He also knew these problems reached far beyond Zimbabwe into other parts of Africa and the developing world.

"I wanted to build a clinic that could provide high-quality care for children and proper training for pediatric ENT surgeons as well," Chidziva said in a Skype interview.

Harare Central Hospital, in the country's capital, comprises the children's hospital and an adult hospital, maternity hospital and psychiatric hospital. Conditions are poor. Prior to the opening of the new clinic, Chidziva's pediatric ENT patients received care at the adult hospital. (They still undergo surgeries there.) When Koltai first traveled to Zimbabwe in 2015, he stayed in the background, listening and learning about the problems he and Chidziva's staff were going to try to fix.

"When I first arrived, I saw the fragility of this medical system," Koltai said. "The lack of supplies, questionable water and electricity, the marginal cleanliness outside of critical areas in the hospital. There were no fiber-optic capabilities — that is, medical equipment used for internal examination of the body — and no record-keeping for patients. But I also saw the dedication of these doctors, who were working under conditions we would find almost intolerable at Stanford."

The types of ENT problems Chidziva routinely treated — and that Koltai would eventually assist with during his repeated visits to Harare over the years — were far more serious than the general population understands, Chidziva said. There's a common misperception in Zimbabwe that ENT problems in children are trivial. Parents think that continually running noses in their children, constant snoring and painful ear infections are just a way of life.

But, in fact, the list of serious problems is long: untreated ear infections that lead to perforated eardrums and often deafness; HIV infections that cause repeated ear and throat disorders; congenital neck masses; ingested button-cell batteries lodged in airways; leeches that crawl into the ears of babies left to play in the grass, causing uncontrollable bleeding.

"Many of these things are no longer problems in the modern world, but big problems in the developing world," Dzongodza said. He is now on a fellowship in Melbourne, Australia, where he is training to become certified as a pediatric otolaryngologist. He will be the first physician with the certification in Zimbabwe when he returns to lead the clinic in July.

One of the most serious and common medical problems treated by the Zimbabwean physicians is called recurrent respiratory papillomatosis. It's a disease caused by the human papilloma virus, or HPV, that causes growths in the upper respiratory tract. The growths can cause difficulty breathing, damage the vocal cords and become life-threatening. The condition often gets misdiagnosed as asthma, delaying treatment. Children first lose their voices and then struggle to breath until, as in the case of Anoona, the growths threaten to block respiration completely.

"By the time they get to us, they can't sleep, they're not growing, their breath is raspy and they are struggling to get in air," Dzongodza said. "Usually they're about 3 years old when they first show up, then they return maybe three to five times for surgery as the warts keep growing back. It's a challenge for us, especially when much of the equipment we had been using was quite archaic."

> "I can tell you that this accomplishment was no small feat."

This was the case for 8-year-old Anoona, who was rushed into emergency surgery when she arrived on that spring evening struggling to breathe. It would be her eighth surgery to remove the viral warts from her larynx. As a toddler, she had been misdiagnosed with asthma and appeared at the hospital for the first time when she was 3 years old, gasping for breath. This time, though, she would be initially seen at the new clinic, with staff better trained to treat children, and operated on at the adult hospital with new equipment and advanced new imaging technology designed for use with children.

"All the surgeons on the unit had met her one way or the other over the years," Dzongodza said. "Often the senior colleagues would dig into their pockets to get her bus fare for the next journey back to the hospital."

The clinic

To make his vision a reality, Chidziva started by raising funds for construction of the clinic from the Christian Blind Mission International, a charity committed to improving conditions of those living in some of the poorest communities in the world. Next, he invited Koltai to join his team. Koltai's prior experience in helping to set up several pediatric ENT clinics in the United States and working for 10 years as the director of the pediatric otolaryngology program at Stanford, would prove invaluable, Chidziva said.

"Clemence had a vision, and I bought into it," Koltai said. "This project resonated with my goals of seeing the footprint of pediatric otolaryngology spread far and wide. I would supply some of the experience, and Clemence supplied the leadership."

Koltai has spent endless hours scanning eBay in an effort to scrounge up reusable medical equipment at affordable prices. He brought two decommissioned surgical microscopes from Lucile Packard Children's Hospital Stanford to Harare and has been instrumental in plans for the delivery of an ultrasound machine. The Jenks family of Menlo Park, who had supported Koltai's research work in the past, helped pay for the eBay purchases and their shipping fees. Early on, he secured funding from Stanford's Department of Otolaryngology-Head and Neck Surgery to fly the physicians from the clinic, including Dzongodza and Chidziva, to the Bay Area, where they stayed with Koltai and observed him for a month at Stanford Medicine.

NABOTH MATINHIRA

Peter Koltai examines a child in Zimbabwe, where he helped establish a pediatric otolaryngology clinic.

"Peter's work, together with his Zimbabwean counterparts, helping to stock the clinic with instruments and develop a novel training program for the surgeons was a terrific example of an equity partnership," said Michele Barry, MD, director of Stanford's Center for Innovation in Global Health. "Having worked on and off in Zimbabwe for almost 30 years, I can tell you that this accomplishment was no small feat."

> Scanning eBay for medical equipment.

Over the years, Koltai has returned repeatedly to Harare to teach advanced surgical techniques, hold seminars and set up the previously nonexistent record-keeping program. The record-keeping will be essential for Chidziva's long-range plan of creating a training ground at the clinic for future pediatric ENT surgeons, along with a research program to help advance academic appointments at the University of Zimbabwe. The first research project on the docket, he said, will be a clinical trial to provide evidence of what appears to be the widespread scourge of the HPV disease.

"We feel that with scientific evidence to support us, we can get our government to vaccinate for HPV to prevent this disease," Chidziva said.

Still, the clinic remains a work in progress. Plans are moving ahead to open two operating theaters adjacent to the clinic and dedicated to treating children with ENT problems. Fundraising efforts have been amped up to fill gaps in care caused by the tripling of the patient load following the opening of the clinic. Due to constant funding shortfalls, much of the equipment considered essential at Stanford, such as MRI or CT machines, remains out-of-reach luxuries in Harare.

"When we created this clinic, we did it to improve care for our patients," Chidziva said. "But within the first year of opening, we saw 3,500 patients, three times the average caseload. The struggle now continues to get them all onto an operating table in time."

The future

In May, the team organized the first international symposium to advance pediatric otolaryngology across Africa, called PENTAfrica, held in Victoria Falls in Zimbabwe, with the goal of advancing pediatric ENT across Africa. Attended by otolaryngologists and other health care professionals from Africa, Europe and North America, the event launched the organizers' long-range plan to use the new program as a model to provide great access to care across the continent.

"We're hoping our new clinic will plant a seed in each and every country in Africa," said Chidziva. By July, with the return of Dzongodza from Australia and with the opening of the clinic's operating wings, Chidziva will be well on his way to achieving his goals.

For Dzongodza, training thousands of miles away in Melbourne, he still worries about his patients back home, including Anoona. Dzongodza remembers taking off his jacket that evening, then scrambling, like usual, to track down equipment being shared by other staff at the hospital, including the oxygen tank and the pulse oximeter, before operating on the child's airways. But the new surgical equipment and surgical training from Koltai made a difference that night.

"Surgery went on successfully, but our nightmare remains of how to offer social support given her circumstances," Dzongodza said. "I saw the child once more before leaving for Australia, and she was doing well. We can only hope that she continues to make the long journey back to the hospital when she needs us again." **ISM**

Tplax

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laboratory lineages and animals they caught themselves in Monterey, the group grew Tplax in a wide variety of sizes and shapes, creating animals that are hundreds to millions of cells. This variation in size provides a powerful window into understanding how cellular coordination varies as the number of cells increase or decrease.

"Tplax are really mysterious beasts," said Matthew Bull, a graduate student in the Prakash lab and co-author of the paper, "but we use that to our advantage to find where our understanding of what it means to be part of the animal kingdom bends and then breaks."

Graduate student Andres Aranda-Diaz is also co-author of this paper.

Prakash is a member of Stanford Bio-X and of the Stanford Center for Innovation in Global Health, an affiliate of the Stanford Woods Institute for the Environment and a fellow of Stanford ChEM-H.

Stanford's Department of Bioengineering, which is jointly managed by the schools of Medicine and of Engineering, also supported the work. **ISM**

Network

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pelling case for adopting some of the network's diagnostic approaches more broadly in an attempt to clarify diagnoses and refine treatment for patients with rare conditions."

A paper describing the study was published online Oct. 11 in *The New England Journal of Medicine*. Ashley is the senior author and Splinter is the lead author.

Cracking the cases

The effort sprung to life four years ago when the NIH tapped Ashley to co-chair a consortium of cross-disciplinary doctors who would work to crack some of medicine's most perplexing cases — at no charge to the patient. Of the 1,519 applications from patients, 601 were accepted based on the likelihood that the network would be able to help them, given their past medical records and available data. Now, Ashley and the team of physicians have seen more than half of those patients, combining traditional medicine with increasingly cutting-edge diagnostic tests. The network continues to accept applications.

"We do this Sherlock Holmes-like detective work-up by carefully observing, gathering information, and asking pointed questions, but we're also pairing that with the most advanced genomic technologies to try to solve their case," Ashley said.

Every patient had their genome sequenced, even those whose genomes had been previously sequenced. The field of genetic and genomic testing moves so quickly, Ashley explained, that even patients who've had their genome sequenced six months ago benefit from another look. In coordination with genome sequencing, the physicians looked at patients' RNA profiles, analyzing precursor molecules to the proteins found in their bodies. They also broke down a collection of molecules called metabolites, which form as a product of metabolism and can hint at where metabolic processes go wrong.

"Some cases are solved simply because we know more today than we did a year ago," Ashley said.

Among those diagnosed, most exhibited rare versions of known diseases, broadening the symptomatic information doctors can look for when evaluating patients for those particular diseases in the future. But in 31 patients, the network identified previously unknown syndromes.

One that sticks out to study co-author Matthew Wheeler, MD, assistant professor of medicine at Stanford and executive director of the Stanford Center for Undiagnosed Diseases, is the case of a patient who the network followed for multiple years. The patient had mysterious and life-threatening episodes of something called lactic acidosis, a dangerous buildup of lactic acid in the body.

"It's sort of like an extreme version of when you exercise intensely, and you feel that burn from the lactate buildup — only it's your whole body that feels that way," Wheeler said. "Lactic acidosis can also cause your acid-base balance to be out of whack, and when people have severe acid-base disturbances, they're at high risk for arrhythmia or death."

It wasn't clear why the patient was experiencing these symptoms, which seemed to be prompted by a cold or flu. After giving the patient the full gamut of tests and analyzing sequencing information, a team of Stanford scientists found the culprit: a single mutation in the gene ATP5F1D, which is involved in the function of mitochondria, the cell's powerhouse. The genetic oddity and symptoms had never been classified together officially, but from connections within the network and in some instances word of mouth, the scientists found that other doctors around the world had patients plagued by this syndrome. In verifying that the mutation causes the syndrome — called mitochondrial complex V deficiency, nuclear type 5 — network collaborators on the study developed animal models to show causality.

Continuing the search

"This is a new type of scientific odyssey," Ashley said. "We're learning about biology in a way that could help not just one family, but potentially dozens, even hundreds, of families who suffer that same rare condition. That's the biggest benefit of this network effect — the impact of identifying one patient's disease could end up being global."

Even the patients who did not receive a diagnosis benefit from knowing that a team continues to investigate their conditions and that the future may hold an answer even if the present does not.

"We've had patients tell us that just knowing that there is a team looking into their condition, that there is someone in the world who has not given up on them, scientists continuing to keep an eye on the literature — that provides hope," Ashley said.

Now, Ashley and his colleagues are moving into the second phase as they expand network sites and continue to accept applications and see patients.

"Let's face it, solving a third of these cases in the first phase was great — when they came in the door it was 0 percent. So to get to more than 30 percent — we are happy with that, but that still leaves the majority of cases unsolved and many patients still suffering, so we need to do better," Ashley said.

Other Stanford authors of the study are Jonathan Bernstein, MD, PhD, professor of pediatrics; and genetic counselor Chloe Reuter.

Ashley is a member of the Stanford Cardiovascular Institute, Stanford Bio-X and the Stanford Child Health Research Institute.

Researchers from Harvard University, the NIH Clinical Center, Baylor University, University of Maryland, Vanderbilt University, HudsonAlpha Institutes for Biotechnology, University of Oregon, Brigham and Women's Hospital, Pacific Northwest National Laboratory, the University of California, Los Angeles, Duke University, Massachusetts General Hospital and the National Human Genome Research Institute contributed to the study.

The study was funded by the NIH.

Stanford's Department of Medicine also supported the work. **ISM**

Juul

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"I was surprised and concerned that so many youths were using Juul more frequently than other products," said the study's senior author, Bonnie Halpern-Felsher, PhD, professor of pediatrics. "We need to help them understand the risks of addiction. This is not a combustible cigarette, but it still contains an enormous amount of nicotine — at least as much as a pack of cigarettes."

The data show a worrisome disconnect between teens' perceptions of their Juul use and actual addiction, the researchers said. "We ask, 'Do you feel addicted?' And they say no, but a series of questions on a validated scale for assessing loss of autonomy over nicotine show that they're dependent," said the study's lead author, Karma McKelvey, PhD, a postdoctoral scholar.

More nicotine

Juul e-cigarettes first went on sale in 2015 and now account for two-thirds of the U.S. e-cigarette market. They deliver more nicotine than competing brands of e-cigarettes, and produce a throat hit that is more comparable to conventional cigarettes than their predecessors. Juul's design, using flavored nicotine-containing liquids inhaled from colorful pods that resemble USB flash drives, also appeal to the youth market. The Food and Drug Administration recently launched a campaign to warn youths of the dangers of e-cigarettes and attempt to stop Juul sales to young people. However, little scientific research has been done on the impact of Juul use on teenagers and young adults.

The Stanford researchers decided to ask about Juul as part of a tobacco-use study they have been conducting in 10 California high schools. In the first phase of the study, completed in 2014 and 2015, more than 700 students in ninth or 12th grade answered questions about their use and perceptions of tobacco products. The new findings come from follow-up questionnaires completed by 445 participants from this study. They were in 12th grade or a few years out of high school when the new data were collected.

Participants answered questions about whether they had ever heard of Juul; if and how often they used conventional cigarettes, Juul or other types of e-cigarettes; their use of flavored e-cigarette products; their perceptions of the social acceptability of the various products; and their perceptions of the products' risks and benefits. Participants who used any form of e-cigarette also completed a standardized questionnaire to assess their degree of nicotine dependence.

Bonnie Halpern-Felsher

About half of the participants had heard of Juul, and 15.6 percent had used the brand. Other e-cigarettes were used by 30.4 percent of participants, while conventional cigarettes were smoked by 24.3 percent of participants. About two-thirds of the participants who used these products used more than one type of product: some combination of Juul, other e-cigarettes and conventional cigarettes.

The participants reported using Juul about twice as often as smoking conventional cigarettes when asked about use of tobacco products over the past seven or past 30 days.

Believed to be less harmful

Participants thought Juul e-cigarettes were less harmful or addictive than other products mentioned in the survey. However, among the participants who had tried Juul, 58.8 percent reported that they had used Juul within the last 30 days. Among participants who had tried other e-cigarettes or conventional cigarettes, 30.1 percent and 28.3 percent, respectively, reported use within the last 30 days. This was the most striking difference between Juul users and users of other e-cigarettes and conventional cigarettes, and it raises concerns about higher rates of addiction among Juul users, Halpern-Felsher said. Answers to the validated questionnaire about loss of autonomy over nicotine use suggested similar levels of nicotine dependence between Juul users and those using other e-cigarettes, she noted, although the sensitivity of the questionnaire may have been limited by the relatively small number of participants.

The study's results emphasize the need for clear public-health messages about the risks of new types of e-cigarettes, including Juul, the researchers said. "The absence of clear messaging is interpreted as safety among adolescents," McKelvey said. Nicotine-containing products are particularly risky for teens, she added. "The earlier you're exposed to nicotine, the higher the likelihood that you'll be addicted throughout your life."

Teachers and parents also need better information, Halpern-Felsher said. "We need to get in front of identifying and explaining new and different nicotine-containing products so that we can regulate them and protect youth from using them," she said. "It took quite a while for teachers to start realizing that this product [Juul] existed and that what they were seeing in classrooms were not USBs."

Halpern-Felsher and her team have developed a free tobacco prevention toolkit. It is available online for educators, parents and others working with young people at http://med.stanford.edu/tobaccopreventiontoolkit.html.

Mike Baiocchi, PhD, assistant professor of medicine at the Stanford Prevention Research Center, was also an author of the paper. Baiocchi is a member of Stanford Bio-X, and Halpern-Felsher is a member of the Stanford's Child Health Research Institute and the Stanford Cancer Institute.

The research was funded by the National Cancer Institute, the U.S. Food and Drug Administration Center for Tobacco Products, and the Child Health Research Institute.

Stanford's Department of Pediatrics also supported the work

Other research on e-cigarettes

Also publishing Oct. 19 is a commentary by Halpern-Felsher about a tobacco-prevention curriculum developed by Juul. The commentary, which will appear in the *Journal of Adolescent Health*, expresses concern about several aspects of Juul's curriculum. Juul provides schools with a financial incentive of $10,000 to use its curriculum and does not follow best practices in adolescent tobacco education, according to the commentary.

For example, the curriculum does not discuss the role of industry in marketing tobacco- or nicotine-containing products to youths, makes little mention of Juul by name, and does not discuss why young people use e-cigarettes or mention that flavored products such as Juul may be especially appealing to them. The co-author of the commentary is Jessica Liu, a graduate student at Yale University who completed a summer internship in Halpern-Felsher's lab.

Earlier this month, the researchers also published a study in *Addictive Behaviors* exploring teens' perceptions of advertising for flavored e-cigarette liquids. E-cigarette manufacturers, including Juul, claim that their flavors are not marketed to teens. Yet when asked to view ads for flavored e-cigarette liquids, most of the 255 teenage participants in the study said they believed the ads were targeted at their age group. McKelvey is the lead author of this study and Halpern-Felsher is the senior author. Other Stanford co-authors are Baiocchi; research associate Divya Ramamurthi; and Sheila McLaughlin, a program coordinator in pediatric and adolescent medicine. **ISM**

After compassionate-use exemption, girl undergoes unusual heart surgery

By Erin Digitale

When Lizneidy Serratos' mother took her to the hospital at 1 a.m. Aug. 4, she thought wildfire smoke near the family's Reno home was why the 12-year-old was struggling to breathe. But after a series of tests, doctors said Lizneidy's heart was failing.

"Her heart function was only at 10 percent," said Lizneidy's mom, Maricela Alvarado-Lazarit. "It was a shock."

COURTESY OF MARICELA ALVARADO-LAZARIT

Twelve-year-old Lizneidy Serratos in the Dunlevie Garden at Lucile Packard Children's Hospital Stanford.

On Aug. 12, Lizneidy underwent surgery at Lucile Packard Children's Hospital Stanford, becoming the youngest and smallest person in the country — and one of the smallest in the world — to receive the type of heart pump now keeping her alive. She was saved by heroic behind-the-scenes work by her doctors and nurses, who petitioned the Food and Drug Administration for permission to use a medical device that had not yet been approved for children. They got a compassionate-use exemption in about 24 hours.

"When Lizneidy came to us, she was very, very sick," said Katsuhide Maeda, MD, associate professor of cardiothoracic surgery at the Stanford School of Medicine. Lizneidy had dilated cardiomyopathy, a leading cause of heart transplants in children. "She was vomiting and nauseated, and could not eat at all," said Maeda, who performed her surgery. "And she couldn't walk because her heart was so weak."

Identifying the problem

In late July and early August, as wildfire smoke drifted into Reno, Lizneidy's family noticed she was breathing less easily. Then, just after midnight on Aug. 4, Alvarado-Lazarit found Lizneidy sleeping in a strange position.

"I was criss-cross applesauce, with my head down at my feet," Lizneidy said. People with heart failure can often breathe better if they sleep sitting up, but Alvarado-Lazarit did not yet know about the diagnosis. Alarmed, she woke her daughter and took her to the nearest emergency room. Doctors transferred Lizneidy to the pediatric intensive care unit at another Reno hospital, Renown Children's Hospital, which has a pediatric specialty care partnership with Stanford Children's Health. Then, Lizneidy was transferred by an emergency medical flight to Packard Children's.

Lizneidy received medications that stabilized her for a few days. But her heart was not recovering. As in most cases of dilated cardiomyopathy, the physicians did not know why her heart failed.

"It appeared she was getting worse and was going to need a ventricular assist device," said Christopher Almond, MD, associate professor of pediatric cardiology at the School of Medicine. A ventricular assist device is a surgically implanted pump that helps a patient's failing heart move blood through the body.

Almond told the family that Lizneidy would probably need a heart transplant, and that she would receive surgery to implant a pump that could keep her alive until a donor heart became available.

The best pump

The Packard Children's cardiology team wanted to give Lizneidy a pump called the HeartMate 3, which is small enough to implant in the chest. Patients with the pump must wear an external battery pack, but can leave the hospital, walk freely and perform many normal activities.

However, the HeartMate 3 had one drawback in Lizneidy's case. To implant it, Maeda needed to create a hole in the girl's left ventricle, the largest pumping chamber in her heart, and suture a washerlike device called a sewing ring onto the heart to anchor the pump. But the sewing ring that had been approved by the FDA was too big for Lizneidy. There was a smaller ring, but it was approved only in Europe.

"The problem with the larger sewing ring is that Dr. Maeda would have had to sew across one of her most important coronary arteries," Almond said. In rare cases, heart pumps allow children's hearts to regain enough function to avoid a transplant. Closing the artery would have permanently severed the blood supply to part of Lizneidy's heart muscle, cutting off this possibility.

"Because she had a chance of recovery, we didn't want to sacrifice the main artery supplying blood to her heart," Almond said.

So on Aug. 9, Almond began the process of asking the FDA for a compassionate-use exemption. He wrote a letter to the FDA and the device manufacturer, Abbott, requesting permission to use the smaller, unapproved sewing ring; contacted Stanford's ethics team to get its permission for the unusual surgery; and arranged for another cardiologist to provide an independent second opinion to the FDA. On Aug. 10, as hours ticked by and paperwork stacked up, people in several locations across the country — including FDA staff — stayed late at work to help.

"The process for getting compassionate-use approval is a bit complex," Almond said, noting it can take days or weeks.

By 9 p.m. Pacific time on Aug. 10, the approval was complete. In one more bit of serendipity, Almond learned that the small sewing rings — which were commercially available only in Europe — are manufactured 30 miles from Packard Children's, in Pleasanton, California.

The sewing ring arrived at the hospital the following morning. That evening, Lizneidy was getting worse again. It was time for surgery.

Becoming a medical pioneer

On Aug. 12, Lizneidy's family came to the hospital early. She was calm as she was wheeled into the operating room, listening to favorite songs on her phone. The small sewing ring worked just as the doctors had hoped: In the five-hour surgery, Maeda kept Lizneidy's coronary arteries intact. Lizneidy became the first person in the United States to receive a small sewing ring and, at the time of her surgery, the youngest and smallest person in the country to get the HeartMate 3 ventricular assist device.

The pump made an enormous difference. Lizneidy's breathing tube was removed the next day, and she soon began eating again.

"Having her just talking and laughing and asking for things was great," Alvarado-Lazarit said. "When she started being able to get up, it felt like, 'She's going back to normal.'"

Since the surgery, the smaller sewing ring has received FDA approval for commercial use, enabling more patients to benefit from the device.

Lizneidy's family is now staying at the Ronald McDonald House at Stanford. Her medical team plans to monitor her for a few months before deciding whether to add her to the waiting list for a heart transplant.

In the meantime, Lizneidy is relaxed and cheerful, attending seventh grade at the hospital school and taking in stride new challenges, like swallowing lots of pills, which are intended to give her heart every opportunity to recover, if it can. The battery pack for her heart pump is always at her side, housed in a tote bag she wears over her shoulder.

"It's like an annoying best friend, always there," Lizneidy said. "But I'm OK with it." ISM

OF NOTE

reports on significant honors and awards for faculty, staff and students

SHIRIT EINAV, MD, was promoted to associate professor of medicine, effective Aug. 1. Her research focuses on infectious diseases, especially on understanding the roles of virus-host interactions in viral infection and pathogenesis. She is also working to develop broad-spectrum, host-centered antiviral approaches to combat and diagnose emerging viral infections.

ERIC GROSS, MD, PhD, assistant professor of anesthesiology, perioperative and pain medicine, received a grant from the National Heart, Lung and Blood Institute to study the cardiopulmonary effects of e-cigarettes in rodents. The grant provides $1.9 million over four years. The study will examine whether the aldehydes produced in e-cigarettes negatively affect the cardiovascular system.

ODETTE HARRIS, MD, MPH, professor of neurosurgery, was named to the 2018 Ebony Power 100 list compiled by the editors of *Ebony* magazine. The list includes leaders in their fields who have had a positive impact on the African-American community.

JOHN IOANNIDIS, MD, DSc, the C. F. Rehnborg Professor in Disease Prevention and professor of medicine and of health research and policy, was elected to the National Academy of Medicine. Members are selected for their accomplishments and contributions to the advancement of the medical sciences, health care and public health.

Shirit Einav

Eric Gross

Odette Harris

John Ioannidis

Teri Klein

Elsie Ross

Ansuman Satpathy

TERI KLEIN, PhD, professor of biomedical data science and of medicine, along with a colleague at St. Jude's Children's Research Hospital, will receive $5 million over the next five years from the National Institutes of Health to continue the Clinical Pharmacogenetics Implementation Consortium. The consortium provides guidelines to translate pharmacogenomic knowledge to help clinicians understand how genetic test results should be used to optimize drug therapy.

ELSIE ROSS, MD, was appointed assistant professor of surgery, effective July 9. Her research uses advanced data mining techniques and big data approaches to predict outcomes in patients with vascular disease.

ANSUMAN SATPATHY, MD, PhD, instructor in pathology, was named a 2018 STAT Wunderkind. Wunderkinds are selected by editors and reporters of *STAT,* a publication that covers health, medicine and scientific discovery. His research combines cancer immunology and single-cell genomics to determine why immunotherapies work in some patients but not others. ISM