INSIDE STANFORD MEDICINE

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Two genes that defend against many viral infections have been rendered nonfunctional in toothed whales, a new study says.

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Use science to make world better, grads told

By Tracie White

Developmental biologist Lucy Shapiro, PhD, told 2015 graduates of the School of Medicine how, as a basic scientist who spent most of her life studying single-celled bacteria, she stepped out of her laboratory and onto the global stage to try to help the world avert a potential disaster.

"About 15 years ago, I sat up and looked around me and found that we were in the midst of a perfect storm," said Shapiro, the Virginia and D. K. Ludwig Professor, speaking at the school's commencement June 13 on Alumni Green. "There was a global tide of emerging infectious diseases, rampant antibiotic and antiviral resistance amongst all pathogens and a poor to nonexistent drug pipeline.

"For me the alarm bells went off, and I was convinced that I had to try and do something. Let me tell you the story of how I stepped out of my comfort zone. I launched a one-woman attack."

She took any speaking engagement she could get to educate the public about antibiotic resistance; walked the corridors of power in Washington, D.C., lobbying politicians about the dangers of emerging infectious diseases; and used discoveries from her lab on the single-celled *Caulobacter* bacterium to develop new, effective disease-fighting drugs.

Bench-to-bedside for a better world

A recipient of the National Medal of Science, Shapiro exhorted the graduates to be unafraid of breaking out of their comfort zones and to use science to improve the human condition. Bridging the gap between the lab and the clinic can make the world a better place, she said.

Lloyd Minor, MD, dean of the School of Medicine, also emphasized the importance of bench-to-bedside work in his remarks to the graduates. There has never been a better time for shepherding advances in basic research into the clinic, he said.

"You are beginning your careers at an unprecedented time of opportunities for biomedical science and for human health," he said.

This year's class of 195 graduates comprised 78 students who earned PhDs, 78 who earned medical degrees and 39 who earned master's degrees. It included Katharina Sophia Volz, the first-ever graduate of the Interdepartmental Program in Stem Cell Biology and Regenerative Medicine — the first doctoral program in the nation focusing on stem cell science and translating it to patient care.

Volz, whose work in the lab has opened the doors to improvements in clinical care for heart patients, said Stanford Medicine is the place to be for scientists who want to make a difference in the world.

"Everybody here is reaching for the stars. We can do the best work here of See GRADUATION, page 6

NORBERT VON DER GROEBEN

Kristy Red-Horse, assistant professor of biology, hoods Katharina Sophia Volz, the first-ever graduate of the Interdepartmental Program in Stem Cell Biology and Regenerative Medicine, during the School of Medicine's diploma ceremony June 13 on Alumni Green.

Departments in emergency medicine, biomedical data science to be created

By Ruth Schechter

In a move that reflects the changing landscape of health care and biomedicine, the Stanford University Board of Trustees has unanimously approved the creation of two new departments in the School of Medicine.

The Department of Emergency Medicine and the Department of Biomedical Data Science will be the school's first new departments since the Department of Otolaryngology-Head and Neck Surgery was established in 2003.

"Bringing emergency medicine to full department status, and uniting biostatistics and bioinformatics under the new Biomedical Data Science Department gives us the opportunity to leverage our existing strengths in these disciplines and position ourselves for continued success," said Lloyd Minor, MD, dean of the School of Medicine.

TYLER OLSON / SHUTTERSTOCK

An emergency medicine department will be one of two new departments established at the School of Medicine.

Creating departments dedicated to these disciplines will position Stanford as a leader in these fields, which will help to attract and retain the finest faculty, students and trainees, he added, as well as expand research and clinical programs that will bring exceptional care to patients.

The two new depart- See DEPARTMENTS, page 7

Some heartburn drugs may boost risk of heart attack, according to study

By Bruce Goldman

A large data-mining study carried out by investigators at the School of Medicine has linked a popular class of heartburn drugs to an elevated risk of heart attack.

Proton-pump inhibitors, or PPIs, are among the world's most widely prescribed drugs, with $14 billion in annual sales. They are effective at lowering the acidity of the stomach, in turn preventing heartburn, a burning sensation in the chest that occurs when stomach acid rises up into the esophagus. In any given year, more than 20 million Americans — about one in every 14 — use PPIs such as omeprazole (trade name Prilosec) for heartburn, also known as acid reflux or gastroesophageal reflux disease.

"The association we found with PPI use and increased chances of a subsequent heart attack doesn't in and of itself prove causation," said the study's lead author, Nigam Shah, PhD, MBBS, an assistant professor of biomedical informatics and assistant director of the Stanford Center for Biomedical Informatics Research. But, he said, the study combed through electronic health records of nearly 3 million people and crunched trillions of pieces of medical data, raising concerns that should be taken seriously, especially now that PPIs are available over the counter.

ALICE DAY / SHUTTERSTOCK

Researchers found an association between using a kind of hearburn drug and the likelihood of incurring a heart attack down the road.

More than 100 million prescriptions are filled every year in the United States for PPIs, a class of drugs long considered benign except for people concurrently taking the See HEARTBURN, page 7

In Kenya, program changes male attitudes about sexual violence

By Erin Digitale

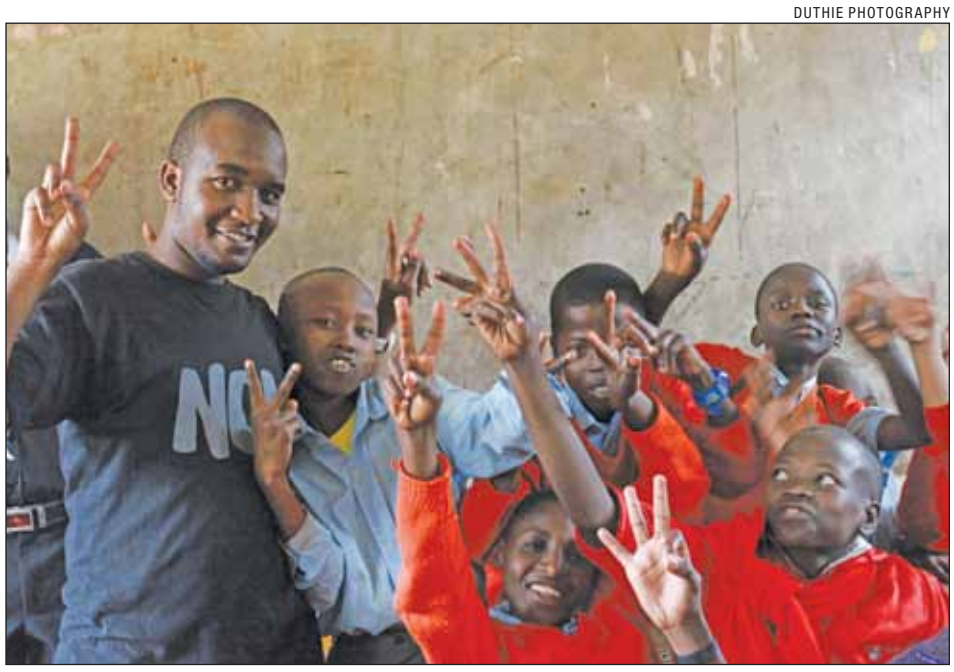
In Kenya, where rape and violence against women are rampant, a short educational program produced lasting improvements in teenage boys' and young men's attitudes toward women, a study from the School of Medicine has found.

The boys and men in the study also were more likely to try to halt violence against women after participating in the program.

The study was published online June 9 in the *Journal of Interpersonal Violence*.

The program was developed by No Means No Worldwide, a nonprofit, nongovernmental organization that works in the slums of Nairobi to prevent sexual assault on girls and women. Prior Stanford studies have shown that the group's empowerment training for adolescent girls produces large reductions in the rate at which these girls are raped. The curriculum for males aimed to change attitudes that lead adolescent boys and young men to think it is acceptable to assault or rape their female peers.

DUTHIE PHOTOGRAPHY



Boys in Nairobi, Kenya, participate in an educational program designed by the nonprofit organization No Means No Worldwide to help them reevaluate their attitudes about violence against women.

"The curriculum for these young men is centered on getting them to think about what kind of people they want to be," said lead author Jennifer Keller, PhD, clinical associate professor of psychiatry and behavioral sciences. "It's about really getting them invested in why they need to step up and care about violence toward women: It affects their mothers, sisters and girlfriends."

Understanding consent

The study included 1,543 males, ages 15-22, who were from Nairobi slums. At 29 high schools, 1,250 of them received six two-hour educational sessions from No Means No Worldwide. The intervention curriculum, called "Your Moment of Truth," focused on helping them recognize the cultural normalization of violence against women, and gain skills and courage to stop it. Topics of discussion included myths about women, negative gender stereotypes, when and how to safely intervene if you see someone else acting violently toward a woman, and what constitutes consent to sexual activity.

"If you think that when you take a woman out to dinner, she owes you something, you may believe that consent is different than it actually is," Keller said. "The instructors and young men talked about understanding what true consent is and how to get that consent."

The comparison group of 293 boys and men at seven other high schools received Kenya's usual two-hour life-skills class.

The researchers used anonymous surveys to ask the participants in both the experimental and control groups about their attitudes toward women; their endorsement of rape myths; whether they had witnessed verbal harassment, physical threats or physical or sexual assault of women; and whether they had successfully intervened to stop such harassment, threats or assault. The boys and men in the experimental group completed surveys before the educational program began, immediately after it ended, and 4½ and nine months later. The participants in the comparison group completed surveys before receiving life-skills training and nine months later.

At the start of the study, participants in both groups reported negative views of women and agreement with myths about sexual assault, although the views of the control group were slightly better than those of the experimental group, possibly because they were slightly younger. After the classes, the experimental group had more positive views toward women and less belief in rape myths, and the improvement persisted 4½ and nine months later. The comparison group had unimproved or worsened attitudes toward women at the nine-month follow-up.

Jennifer Keller

Making an impact

Similar numbers of participants in both groups witnessed verbal harassment and physical or sexual violence against women, but those in the experimental group were at least twice as likely as those in the comparison group to successfully halt such assaults. Within the experimental group, participants with the most positive attitudes toward women were the most likely to step in.

"It's very exciting that this was done in Kenya, that even in this setting with high levels of violence toward women we were able to make such an impact," Keller said.

Future studies will examine how boys' and young men's attitudes relate to their behavior in their own relationships, she said. "It's harder to do," Keller said, noting that research participants may not always admit, even anonymously, that they have behaved violently toward a girlfriend.

The study's success with a relatively young group of males dovetails with prior research showing that it is easier to change negative gender stereotypes in younger groups, she added. In the United States, efforts to improve young men's attitudes often occur in college, but earlier intervention might work better, Keller said.

Other Stanford co-authors of the study are Neville Golden, MD, professor of pediatrics and chief of the Division of Adolescent Medicine at Lucile Packard Children's Hospital Stanford; and Cynthia Kapphahn, MD, professor of pediatrics and an adolescent medicine specialist at Lucile Packard Children's Hospital Stanford. Other collaborators included Jake Sinclair, MD, and Lee Paiva, co-founders of No Means No Worldwide; and collaborators at United States International University, in Nairobi; at the nonprofit NGO Ujamaa-Africa; and at Edgework Consulting in Boston. The study was funded by Ujamaa-Africa.

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

Gene discoveries may lead to regeneration of cells needed for hearing

By Jeffrey Norris

School of Medicine scientists have discovered biological mechanisms that appear to play a role in the regeneration of cells in the inner ear.

Over a lifetime, these cells often are damaged or die due to oxidative stress, excessive noise exposure or toxic drugs. The accumulated loss can significantly compromise hearing. Nearly one in four people ages 65-74, and half who are 75 or older, are candidates for hearing aids because of disabling hearing loss.

The discoveries could lead to new ways of evaluating, in animal models, experimental drug treatments intended to prevent hearing loss or restore hearing, and might even lead to methods for regenerating vital cells that have been lost, said Stefan Heller, PhD, professor of otolaryngology.

A paper describing the findings, as well as new methods to quickly link changes in cell function during development to molecular changes within cells, was published June 9 in *Cell Reports*. Heller is the senior author of the paper. Postdoctoral scholars Jöerg Waldhaus, PhD, and Robert Durruthy-Durruthy, PhD, share the lead authorship.

Sound waves striking the eardrum cause vibrations that are transmitted through tiny bones in the middle ear to fluid within the snail-shell-shaped cochlea of the inner ear. Specialized cochlear cells in a region called the organ of Corti use hairlike sensors to detect the vibrations in cochlear fluid and then trigger nerve signals that are sent to the brain.

"Compared to other senses, we know very little about how hearing works," Heller said. "The cells are rare. We have to crack open a bone to get to them. They perish quickly, so we must work fast." There are 120 million retinal cells in a mouse eye, Heller said, but only 3,200 hair cells in a mouse ear.

By using new techniques to rapidly and deeply probe individual cells, Heller's team has begun to close the knowledge gap.

Molecular mysteries

Many of the biophysical properties of hair cells are understood. Different hair cells along the cochlear spiral are tuned to respond to distinct ranges of sound frequency based on differences in their electrical properties. Frequency is encoded by the place and the properties of the cells' locations in the cochlea. This understanding has led to the development of cochlear implants to restore hearing in deaf people.

STEVE FISCH

Discoveries by Stefan Heller and his colleagues could lead to new ways of evaluating, in animal models, experimental drug treatments intended to prevent hearing loss or restore hearing.

However, little is known about the molecular biology that determines how hair cells develop at specific locations and how different electrical properties arise among hair cells specialized to detect different frequencies. This makes it difficult for scientists to envision strategies to regenerate the specialized cells or to prevent their death, particularly in the high-frequency region of the cochlea, where cells are more susceptible to injury.

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STANFORD MEDICINE

Discovery of molecular pathway could lead to pancreatitis treatments

By Tracie White

The pancreas is a tricky organ for researchers and surgeons alike because of its sensitivity. Tucked away in a hard-to-reach spot behind the stomach, it's in charge of secreting enzymes to help digest everything you eat. Even slightly puncturing the pancreas during surgery can cause it to begin digesting itself.

That may be in part why there is so little understanding of what causes pancreatitis, a fairly common and quite painful disorder.

"We try not to touch the pancreas," said Aida Habtezion, MD, assistant professor of gastroenterology and hepatology. "That's one of the reasons the field has not progressed much. We don't have much access to the pancreas. We especially don't want to touch it when it is inflamed with pancreatitis."

By working with animal models and cells retrieved from the few surgeries involving the human pancreas, Habtezion has spearheaded new research that provides insight for the first time into the molecular pathway that leads to chronic pancreatitis, the debilitating, long-term inflammation of the organ.

In a study published May 18 in *Nature Communications*, Habtezion and her colleagues found that blocking this pathway stops the progression of the uncontrolled growth of scar tissue, or fibrosis, that's the hallmark of chronic pancreatitis.

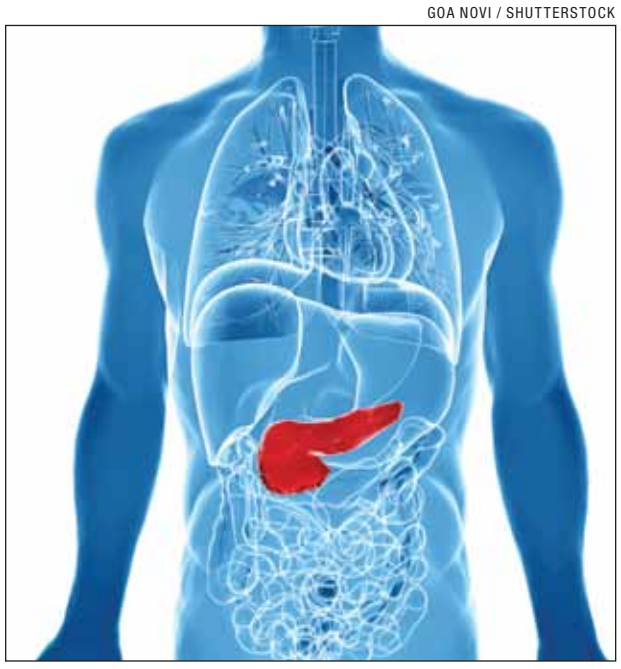
"This is the first step to showing that you can alter the progression of this disease," said Habtezion, senior author of the study. The lead author is postdoctoral scholar Jing Xue, PhD.

Habtezion, a gastroenterologist with a background in immunology, splits her workdays between the lab and the hospital. Her interest in these patients has crossed over into her lab.

Aida Habtezion

"Acute pancreatitis is one of the most common gastrointestinal admissions-related illnesses," she said. "Some people just have one or two episodes, and we never see them back." Others go on to develop chronic pancreatitis, which is a risk factor for pancreatic cancer.

GOA NOVI / SHUTTERSTOCK



New research provides insight for the first time into the molecular pathway that leads to chronic inflammation of the pancreas.

No known cure

Chronic pancreatitis is marked by constant, severe stomach pain. There is no known cure and little treatment beyond narcotics to help control the pain. The disease destroys the ability of the pancreas to absorb nutrients, leading to nutritional deficiencies and malnutrition, along with the crippling nausea and diarrhea caused by the abdominal pain. Key contributors to the disease include excessive alcohol consumption, gallstones and genetic factors.

"My lab has been interested in the inflammatory responses associated with pancreatitis and in understanding the molecular pathways that may be targeted to alter the progression of the disease," Habtezion said.

It's generally understood that chronic pancreatitis is marked by the uncontrolled growth of scar tissue in the pancreas, slowly destroying the organ's ability to function. Just how this happens is less clear.

In previous research, Habtezion's lab has shown that macrophages, a type of immune cell in the body, play a role in the acute form of pancreatitis. The goal of the new study was to determine the role of macrophages in the development of chronic pancreatitis from the acute form of the disease. Previous research has also shown that pancreatic stellate cells may play a role in fibrosis. These cells live in the pancreas and travel to injury sites when activated.

"Our most important finding was that there is cross-talk between macrophages and stellate cells," Habtezion said. "We identified this pathway."

Next, they set out to determine if blocking this pathway would slow or stop fibrosis. This is where colleagues from Cedars-Sinai Medical Center — co-authors Stephen Pandol, MD, director of basic and translational pancreas research, and Ramachandran Murali, PhD, associate professor of biomedical sciences — helped out.

"Dr. Murali said, 'Oh, I have this agent that can block this receptor,'" Habtezion said. "He was developing the potential drug as treatment for another disease. We used this blocking peptide in both the animal models and the human cells."

When applied to the pathway, the pharmacological agent successfully slowed the fibrosis, she said.

"For the first time we can show that macrophages interact with pancreatic stellate cells via a particular immune pathway, and by targeting this pathway we show a decrease in chronic pancreatitis/fibrosis progression," she said. "This has great implication in a disease that has no active therapy with no known agents that can alter its natural devastating course."

Other Stanford co-authors of the study are research assistant Vishal Sharma and Michael Hsieh, MD, PhD, a former assistant professor of pediatric urology.

The study was funded by the National Pancreas Foundation, Department of Veterans Affairs and the National Institutes of Health.

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

School's magazine draws top awards in national competition

By Susan Ipaktchian

Stanford Medicine magazine earned six awards, including top prize in the category of "best articles of the year," in a national competition.

In all, the magazine took home a platinum, three golds, a silver and a bronze in the 2015 Circle of Excellence Awards Program, a contest held by the Council for the Advancement and Support of Education, or CASE. The magazine is produced by the medical school's Office of Communication & Public Affairs.

Writer Tracie White earned the sole platinum award in the best-articles category for "Almost without hope," a look at the heartbreakingly scarce medical resources on an Indian reservation in South Dakota. The judges wrote that they "admired the author's handling of a subject ripe with standard conventions and hackneyed writing. The author never fell into this trap, capturing the story and delivering it creatively. With a strong fact/narrative balance, the author got this one right. Job well done."

Stanford Medicine earned a gold award in the special-constituency magazine category. One judge described herself as "drooling over the stories." The judges added that "the writing makes the technical and medical topics understandable by lay readers." The magazine is edited by Rosanne Spector.

The magazine also earned its fourth consecutive gold award for periodical staff writing. Following are the five stories submitted in this category:

- "Immune system disruption," by Kris Newby, tells the story of a young woman felled by chronic fatigue syndrome who is now recovering and taking part in a trailblazing Stanford study.
- "Fresh starts for hearts," by Krista Conger, describes the potential for stem cell research to help patients, such as the children in one family stricken by a life-threatening heart disease.
- "Her left hand," by Tracie White, recounts the experience of a Stanford surgeon who temporarily lost the full use of her hands.
- "Rethinking Alzheimer's," by Bruce Goldman, captures the advances in research into the disease.
- "Brain attack," by Erin Digitale, focuses on a controversial psychiatric disease that devastates children's lives.

JASON HOLLEY

This illustration from the spring 2014 issue of *Stanford Medicine* magazine won a silver award from CASE.

The judges said they were "particularly blown away by the depth of the reporting and the degree of access the reporters had to their sources."

The magazine earned a gold award for periodical design for its spring 2014 issue, whose theme was mysteries of the heart. The judges said the theme "was carried through the entire magazine in an exceptional way, and we especially loved the variety of interpretations of the theme seen in the illustrations, each of which was compelling, a wonder to look at and a strong partner to the editorial in terms of conveying the subject." The magazine's art direction is provided by David Armario Design.

The illustration for "Fresh starts for hearts," a story in the spring 2014 issue, earned a silver award. The artist who created the image is Jason Holley. "The illustration for this article was beautiful in an artistic way, yet told a story that complemented the article completely," the judges wrote.

The cover for the spring 2014 issue, also drawn by Holley, earned a bronze award. Judges praised the cover as "a beautiful, artful, understated cover."

CASE is a professional organization for those in the fields of communications, alumni relations and development at educational institutions. It includes more than 3,600 colleges and universities, as well as independent elementary and secondary schools in 77 countries. To recognize the best work in these fields, CASE sponsors its annual Circle of Excellence Awards. ISM

Magazine article nabs award in health-care journalism contest

"Opening up: The evolving world of surgery," an article published in the summer 2014 issue of *Stanford Medicine* magazine, won an award from the Association of Health Care Journalists.

Written by Ruthann Richter of the medical school's Office of Communication & Public Affairs, the story describes the experience of a sixth-year surgical resident at Stanford to illustrate changes in surgical practices and training, and the greater acceptance of women in the field.

The article won third place in the "consumer/feature (small)" category, which includes stories in magazines with a circulation of less than 500,000, in the association's 2014 contest. The annual contest is open to print and broadcast reporters throughout the country and recognizes the best health reporting. More than 420 entries were received for this year's contest. ISM

Toothed whales have survived millions of years without key antiviral proteins

By Jennifer Huber

Researchers at the School of Medicine have determined that toothed whales lack functional Mx genes — a surprising discovery, since all 56 other sequenced mammals in the study possess these genes to fight off viruses like HIV, measles and flu.

Modern toothed whales, including dolphins, orcas and sperm whales, have inherited defunct copies of the Mx1 and Mx2 genes, profoundly altering their immune systems. The basic role of these Mx genes is to make proteins that fight viral infections. The researchers hope that understanding this newly discovered mysterious genetic anomaly will help preserve these cetaceans as they face extensive die-offs.

"Given how important the Mx genes seem to be in fighting off disease in humans and other mammals, it's striking to see a species lose them both and go about its business for millions of years," said Gill Bejerano, PhD, associate professor of developmental biology, of computer science and of pediatrics. "It's hard to determine if this is related to the die-offs. We hope that our observations will provide particular targets to go after when carcasses wash ashore, so we can better understand what is happening."

Bejerano is the senior author of a paper, published online June 15 in the *Proceedings of the National Academy of Sciences*, that describes the work. The lead author is graduate student Benjamin Braun.

Gill Bejerano

Double-pronged strategy

Bejerano said his lab began investigating Mx genes because, in primates, they are engaged in an arms race against rapidly evolving viral proteins. In order to determine the state of the Mx genes in mammals, he and his colleagues compared the genomes of 60 mammals.

"We compared the whole-genome sequence of four toothed whales, a baleen whale and dozens of related mammals like cows and humans," said Bejerano. "When we looked carefully at the genome sequences, it was very clear that the Mx genes are completely messed up only in the toothed whales."

The scientists identified a variety of Mx1 and Mx2 mutations, including deleted sections of genes and DNA sequences that prematurely truncate protein synthesis, in the toothed whales' genomes that would prevent the genes from making functional proteins. Strikingly, all other mammals screened had healthy-looking Mx genes.

In addition to using comparative genomic analyses, the researchers performed transcriptomic analysis to validate that these mutations prevent Mx1 and Mx2 genes from producing functional proteins.

"Genes are used to make RNA and, from them, to make proteins," Bejerano said. "So we obtained RNA samples from two toothed whales, a baleen whale and two closely related species. When we looked at the output — the RNA that the genes were trying to make — there was nothing like a functional Mx RNA coming out of these messed up gene loci in the toothed whales."

He added, "Our double-pronged approach allows us to say with confidence that Mx proteins simply do not exist in toothed whales anymore."

Intriguing hypothesis

The investigators were particularly intrigued by the genomic difference observed between the toothed and baleen whales, because they share a common ancestor. Instead of teeth, baleen whales have baleen plates, which they use to filter food from water.

"The simplest, most likely scenario is that the common ancestor of the toothed whales lost both Mx genes shortly after the baleens and toothed whales split about 33-37 million years ago," Bejerano said. "It's tempting to think that this common ancestor was subjected to a very nasty virus that was exploiting the Mx1 and Mx2 genes. Their option was to lose both genes or die. We can't know for sure, but it's a tempting hypothesis based on how some viruses seem to exploit Mx genes today."

ROBERT PITMAN / NOAA

Toothed whales, including orcas, lack certain proteins that help other mammals fight viral infections.

Bejerano hopes these observations will inspire other researchers to collect samples and do in vitro experiments to determine whether toothed whales' immune systems are compromised or whether they've instead developed intriguing compensatory mutations. Ultimately, this understanding may help scientists fight human diseases, such as autoimmune disorders.

"It's likely that the toothed whales' immune system is very different from ours," Bejerano said. "I think this will open up very exciting research avenues, either to better protect the compromised whales, or to study their different viral defenses, and someday add them to our own arsenal. We're putting the genomic discovery out there, and we hope immunologists will follow up on it.

"Every single genome sequenced is a treasure trove of secrets. This is an amazing time to be a thoughtful genomicist. And hopefully, we've helped make it a slightly better time to be a toothed whale."

Other Stanford co-authors of the study are postdoctoral scholar Amir Marcovitz, PhD, former postdoctoral scholar Gray Camp, PhD, and former undergraduate student Robin Jia.

The research was supported by a Stanford Center for Computational, Evolutionary and Human Genomics postdoctoral fellowship, a Stanford School of Medicine Dean's postdoctoral fellowship, a PhRMA Foundation Informatics postdoctoral fellowship, a Microsoft Research Faculty Fellowship, the David and Lucile Packard Foundation and the National Institutes of Health.

Stanford's Department of Computer Science, Department of Developmental Biology and Department of Pediatrics also supported the work. ISM

Jennifer Huber is a freelance writer.

Genetic underpinnings of brain networks seen in imaging study

By Bruce Goldman

A new study by researchers at the School of Medicine found that synchronized physiological interactions between remote brain regions have genetic underpinnings.

The research was performed at Stanford but was made possible by collaborations with the Seattle-based Allen Institute for Brain Science and the IMAGEN Consortium, a multicenter European project, said the study's senior author, Michael Greicius, MD, an associate professor of neurology and neurological sciences.

The study was published June 11 in *Science*.

An emerging consensus among neuroscientists is that cognitive operations are performed not by individual brain regions working in isolation, but by networks consisting of several discrete brain regions — anatomically connected either directly via white-matter tracts or indirectly through intermediary nodes — that share "functional connectivity," meaning that activity in these regions is tightly coupled.

Michael Greicius

Any given functional network is normally most active during the performance of the task associated with that network, as in the case of autobiographical memory ("What did I eat for dinner last night?"). But the synchronous activity of component regions persists when networks are idling. Well over a dozen functional networks have been identified via a technique called resting-state functional magnetic resonance imaging, said Greicius, who is the medical director of the Stanford Center for Memory Disorders.

In resting-state fMRI scans, the individual is asked to simply lie still and relax for several minutes. The results of these scans indicate that even at rest, the brain's functional networks continue to hum along at their own distinguishable frequencies and phases, like different radio stations playing simultaneously, but quietly, on the same radio.

Digging into fMRI images

However, whether resting-state fMRI-derived images, which measure local blood flows in different places throughout the brain, actually reflect neuronal activity has been controversial.

"There's been some skepticism regarding the validity of resting-state network activity," said Greicius. "We wanted to dig deeper and get to the molecular underpinnings of these imaging results, which indicated that the brain maintains its exquisite functional-network architecture even at rest."

To start, Greicius and his colleagues computationally blended resting-state fMRI data they'd obtained from eight-minute scans of 15 healthy adults whose sole instructions were to lie still and relax. This enabled them to pinpoint numerous well-delineated functional networks.

Hoping to find genes that might promote or at least be involved in functional connectivity, the investigators next sought gene-expression profiles — measurements of activity levels of each of the human genome's approximately 20,000 known genes — of regions within corresponding functional networks.

There's no noninvasive way to obtain gene profiles of brain tissue in living humans. But Jonas Richiardi, PhD, a postdoctoral scholar in Greicius' lab now at the University of Geneva in Switzerland, made use of massive amounts of carefully annotated and meticulously archived data derived from the Allen Institute's collection of six post-mortem human brain samples. The institute's scientists have obtained gene-expression profiles of several hundred tissue samples excised from specific locations throughout the brain. Richiardi shares lead authorship of the study with neurology instructor Andre Altmann, PhD, who was also a postdoctoral scholar during the study's duration.

Greicius and his colleagues narrowed their focus to cortical areas associated with four functional networks that are all well-characterized in the imaging literature; consist of discrete, noncontiguous regions in both hemispheres; and are well-represented in the Allen Institute's human-brain database. Along with the default-mode network associated with autobiographical memory, they looked at gene-expression profiles in component regions of the brain's sensorimotor, visuospatial and salience (emotion) networks.

Zeroing in on gene activity

The researchers were hunting specifically for a set of genes whose expression rose or fell in a more synchronized fashion from region to region within a given network than between networks or outside any network. Using sophisticated statistical methods, they identified a set of 136 genes that showed a correlated pattern of gene expression in regions within each network.

These 136 genes weren't specific to any single network, Greicius noted. Rather, "any one of these genes that was being expressed at a high, intermediate or low level in one region of any network, regardless of which network you'd picked, was also being expressed at corresponding levels in the other regions of that network," he said.

The Stanford team validated their findings by turning to another database. The IMAGEN Consortium has conducted widespread See NETWORK, page 5

5 QUESTIONS

an occasional feature in which an expert answers five questions on a science or policy topic

A three-month checkup of MyHeart Counts

In March, the School of Medicine launched MyHeart Counts, a first-of-its-kind iPhone app that enables users to participate in a large-scale study of the human heart. The app assesses each user's cardiovascular health and provides information on how to improve it. Researchers involved with the MyHeart Counts study will investigate, among other things, what factors motivate people to improve their heart health.

MyHeart Counts is one of the first apps to use Apple's ResearchKit framework, which is designed to make it easier for scientists to study health and disease by allowing them to gather more real-world participant data through the iPhone. The app collects data about participants' physical activity using the smartphone's built-in motion sensors. Participants also are asked to respond to surveys and questions related to their cardiac risk factors. They get feedback on their chances of developing heart disease and an estimate of their "heart age."

Recently, writer Tracie White checked in with Michael McConnell, MD, a professor of cardiovascular medicine at Stanford, about how things with the app and the study are progressing. He is the study's principal investigator.

1 Since the MyHeart Counts app was released, almost 40,000 users have downloaded it and consented to participate in the study. How does this compare with recruitments for medical research trials in general?

MCCONNELL: There have been larger research studies, particularly national efforts to study their populations, but we believe enrolling this many participants in such a short time frame is unprecedented. We very much appreciate the interest of the public for participating in medical research and are excited to offer this new approach that clearly facilitates participation. For the future, we hope to learn from our research participants — from both their feedback and their data — on how best to engage with them and learn the most from our research.

Michael McConnell

2 Participants are asked to keep their iPhone with them as much as they can during a seven-day period. What happens during activities such as swimming when they can't carry a phone?

MCCONNELL: The iPhone has a motion chip and sensors that measure your activities without draining the battery, but only if the phone is with you. The MyHeart Counts app captures these data, plus checks with you daily during the seven-day assessment to enter any activities that were not captured by the phone, such as swimming. While you only need an iPhone to participate in MyHeart Counts, the app does collect activity data from wearable activity devices that are linked to the Health app on the iPhone, including the Apple Watch.

3 Have you been happy with how the consent process is working?

MCCONNELL: We have been very happy with the consent process and have received positive feedback from both users and other researchers. We greatly appreciate the efforts of John Wilbanks from Sage Bionetworks in working with all the institutions involved in ResearchKit to develop this "e-consent" process, as well as the input from our own bioethics group and institutional review board. We believe this new consent process gives the participant study information in a more user-friendly way, as well as more time to review and decide. These are critical elements to informed consent, but we will need to understand in more depth how participants respond to this format. The goal is to broaden the ability for people to participate in medical research in which they are interested.

4 When the app was first released, it was announced that behavior modification methods would be studied. When do you expect this phase of the app to be rolled out and what types of methods will be used?

MCCONNELL: Yes, healthy behaviors are critical to preventing heart disease and stroke, so the MyHeart Counts app will study which motivational tools are most helpful. This will follow the second activity and fitness assessment, which occurs after three months. The initial approach will be empowering participants with more personalized feedback about their individual behaviors and risk, based on the American Heart Association's "Life's Simple 7" guidance.

5 How are you ensuring that participants stay anonymous and that data won't be hacked or fall into the wrong hands? Also, have there been bumps along the road as the app has been rolled out?

MCCONNELL: We follow the best data security protocols, such as encrypting the data as it leaves the phone and using a secure server to receive the data. Also, personal identifying information is separated from the research data so that only anonymous data goes to a special secure server for analysis. While we employ all these security measures, we simply cannot guarantee that any system is 100 percent foolproof.

There have been technical bumps in using a smartphone app for research. This is clearly one of the challenges faced by the first group of ResearchKit apps. We actively listen to user feedback and have made improvements to the app; future researchers will benefit from what we've learned. The main advice I would give to other researchers is to be straightforward with your participants that there may be technical issues, even for the next generation of ResearchKit apps, and to ask for feedback to help improve the technology as this new approach to research continues to evolve. **ISM**

High physician-group density could increase medical costs

By Becky Bach

As physician groups grow larger, their increased market share may drive costs up, rather than down, as they gain bargaining power with insurers, according to a new study by researchers at the School of Medicine.

The study was published in the June issue of Health Affairs.

"When physician groups and health systems merge, a key justification is that patients will benefit from better coordination between physicians and expanded access to specialists. Even if these benefits materialize, a potential side effect is that the larger group gains stronger footing when negotiating prices," said the study's lead author, Eric Sun, MD, an instructor of anesthesiology, perioperative and pain medicine.

Laurence Baker

Sun and senior author Laurence Baker, PhD, professor of health research and policy, examined the fees orthopedic surgeons billed for knee replacements between 2001 and 2010. They correlated these fees with the concentration of physician groups using a measurement known as the Herfindahl-Hirschman Index, which is commonly used by regulators, Sun said.

Even though the overall number of markets considered "moderately" or "highly" concentrated remained about the same during the study period, the nationwide average cost of knee replacements fell $261. However, physicians' fees in the most concentrated markets ended the study period $168 higher than fees in the least concentrated markets — a jump of 7 percent.

This finding could have implications for the Affordable Care Act, which encourages physicians to coordinate care in an effort to improve patient health. But such collaboration could also allow physicians to bargain for higher fees from insurers, Sun said.

"The point is not to say that consolidation is a bad thing," Sun said. "But as we think about encouraging these kinds of mergers, we really want to weigh the costs against the benefits."

In the future, Sun said he and Baker plan to investigate whether a higher concentration of physician groups improves patient outcomes.

The study was funded by the National Institute of Health Care Management and the Foundation for Anesthesia Education and Research. Stanford's Department of Anesthesiology, Perioperative and Pain Medicine and the Department of Medicine also supported the work. **ISM**

Network

continued from page 4

imaging, cognitive and genetic tests on 14-year-olds in an effort to predict who's at high risk of encountering problems such as substance abuse by age 16. Among other things, the IMAGEN database contains detailed information on tiny variations from the norm in subjects' genomic sequences. Altmann spearheaded an analysis of the variants present in the 136 genes of interest in 259 healthy adolescents.

Additional experiments using tissue samples obtained from two additional data sets, the Allen Institute's mouse-brain and mouse-connectivity atlases, confirmed and amplified the findings from research on human brains. The reliance on large, shared data sets was another important feature of the study and, Altmann said, "highlights the value of making scientific data freely available. We had an idea and found collaborators willing to share their painstakingly collected data."

The identification of functional-connectivity-associated genes sets the stage for targeted clinical applications, such as finding out how neurodegeneration propagates within a network.

"Our work holds potential implications for a number of neuropsychiatric disorders," said Richiardi.

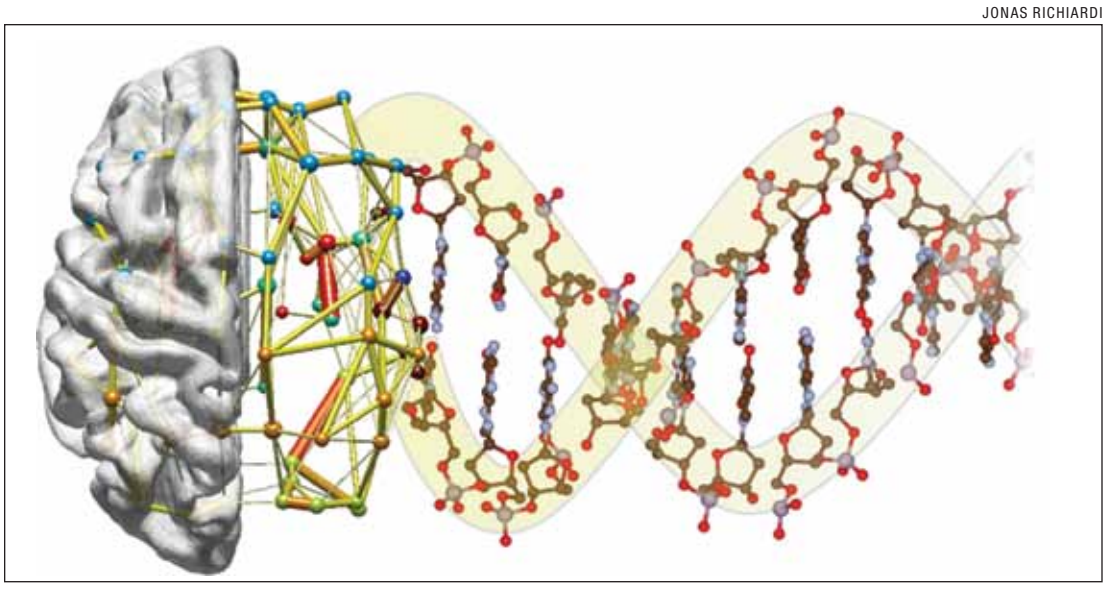
Evidence suggests, for instance, that Alzheimer's disease spreads from one brain region to the next within the brain's so-called default-mode network, which is activated when a person is recalling recent autobiographical events. Resting-state imaging holds exceptional potential in cases where task-based fMRI isn't applicable. Alzheimer's patients, for example, have difficulty focusing on memory-based tasks. Future work will focus on genes whose expression is correlated within one network, but not in other networks. Focusing on default-mode network-specific genes, for example, may lend novel insights into Alzheimer's disease.

Another Stanford co-author of the study is postdoctoral scholar Anna-Clare Milazzo, PhD.

The experiments reported in the study were funded by the National Institutes of Health, the Allen Institute, the Feldman Family Foundation, the IMAGEN Consortium and a Marie Curie Fellowship from the European Union.

Stanford's Department of Neurology and Neurological Sciences also supported the work. **ISM**

JONAS RICHIARDI



Past neuroimaging studies have defined several "functional networks" in which remote regions of the brain appear to operate in synchrony. A new study provides the molecular underpinnings for this theory.

Graduation

continued from page 1

anywhere," said Volz, 28, a native of Ulm, Germany, the birthplace of Albert Einstein. She has worked in 10 different labs across the globe. Her father and mother, Johannes and Luise Volz, traveled from Germany to celebrate with her.

"I've never been in a more supportive environment," said Volz, who discovered the progenitors to the muscle layer around the coronary arteries, a finding with implications for regenerative medicine and finding treatments for coronary artery disease.

Well-wishers, garlands and fussy babies

Some in the crowd of well-wishers, seated under a giant white tent, held garlands of flowers for the graduates, while toddlers ran around the lawn and babies fussed and cried. The two student speakers added humor and pathos to the occasion, with memories of their years of hard work and discovery.

"I'd like to run one last experiment," said Francisco Jose Emilio Gimenez, a PhD graduate in biomedical information. "Who here had serious doubts they would ever finish their PhD?"

The dozens of hands shooting up from the stage were followed by laughter from the crowd.

Meghan Galligan, a medical degree graduate, said she was both nervous to be in front of the crowd and concerned about whether her puffy black graduation cap would stay put. "I'm wearing a French pastry hat and worried it's going to fall off," she said.

Her years of education to become a physician changed the day she entered clinical care training. "From the day we started clinics, we would really never be the same as those bright-eyed individuals who gathered here for orientation," she said. "How could we be after gaining such privileged access into the human condition?"

NORBERT VON DER GROEBEN

Lucy Shapiro, left, addresses graduates at the School of Medicine's diploma ceremony on June 13. Monica Eneriz-Wiemer, who earned a medical degree, hugs her mother Gloria Eneriz.

NORBERT VON DER GROEBEN

Lloyd Minor, dean of the medical school, with Sarah Osmundson, who earned a master's degree in epidemiology and clinical research, and her two children, Liam (left) and Ethan.

Role as government adviser

Shapiro's desire to improve the human condition led her out of the lab to the nation's capital. She has since served in advisory roles in the administrations of Bill Clinton and George W. Bush on the threat of infectious disease in developing countries. Now director of the Beckman Center for Molecular and Genetic Medicine at Stanford, Shapiro has been a faculty member since 1989. She was founding chair of the Department of Developmental Biology and also started a biotech company in Palo Alto to test and develop antibiotics and antifungals.

Her lab at Stanford made breakthroughs in understanding the genetic circuitry of simple cells, setting the stage for the development of new antibiotics. Shapiro told the audience that over the 25 years she has worked at the School of Medicine, she has seen a major shift in the connection between those who conduct research in labs and those who care for patients in clinics.

"We have finally learned to talk to each other," said Shapiro. "I've watched the convergence of basic research and clinical applications without the loss of curiosity-driven research in the lab or patient-focused care in the clinic."

This new connection, she said, is key to the future of global health.

"This is no ordinary time, from shattering political unrest in the Middle East and North Africa and the consequent flood of immigrant populations that serves as a petri dish for infectious pathogens, to global shifts in urban environments, to climate change, which is having substantial impact on health ... all contributing to the appearance of old pathogens in new places and new pathogens for which we have no immunity.

"We here must care about an Ebola outbreak 8,000 miles away in West Africa; we here must care about a cholera outbreak in Haiti; we wait for the consequences of the earthquake in Nepal. We live in a global village."

This is your time to shape the future, Shapiro told the graduates.

"Step out of your comfort zone and follow your intuition," she said. "Don't be afraid of taking chances. Ask, 'How can I change what's wrong?' " **ISM**

Awards recognize exceptional work in education, patient care

At the School of Medicine's commencement June 13, more than two-dozen faculty members, staff and trainees, as well as a student, were recognized for outstanding work in education or patient care, or both.

In addition, **JOSEPH LIPSICK**, MD, PhD, professor of pathology and of genetics, received one of the four 2015 Walter J. Gores Awards, the university's highest teaching honor, at the university's commencement June 14.

AWARDS IN MEDICINE

STEVEN LIN, MD, clinical instructor of medicine; **KALPANA NATHAN**, MD, clinical assistant professor of psychiatry and behavioral sciences; and **IAN NELLIGAN**, MD, MPH, clinical instructor of medicine, received the Arthur L. Bloomfield Award in Recognition of Excellence in the Teaching of Clinical Medicine.

GERALD GRANT, MD, associate professor of neurosurgery, received the Franklin G. Ebaugh Jr. Award for Excellence in Advising Medical Students.

JOANNA BADGER, MD, clinical associate professor of dermatology, received the Alwin C. Rambar-James BD Mark Award for Excellence in Patient Care, which recognizes a member of the medical faculty for compassion in working with patients and their families, excellence in providing medical treatment, and effectiveness and pleasantness in interactions with patient-care staff.

ANDREW CONNOLLY, MD, associate professor of pathology, received the Lawrence H. Mathers Award for Exceptional Commitment to Teaching and Active Involvement in Medical Student Education.

NEIL GESUNDHEIT, MD, MPH, professor of medicine, received the Award for Excellence in Promotion of the Learning Environment and Student Wellness.

VUONG VU, an administrative associate in the Department of Pathology, received the Medical Education Staff Service Award.

DARREN SALMI, MD, clinical assistant professor of surgery and of pathology, received the Outstanding Lecture/Presentation Award.

NANCY CUAN, MD, received the Outstanding Community Clinic Preceptor-Clinical Instruction Award.

ERIKA SCHILLINGER, MD, clinical associate professor of medicine, received the Henry J. Kaiser Family Foundation Award for Outstanding and Innovative Contributions to Medical Education.

GILBERT CHU, MD, PhD, professor of medicine and of biochemistry; **TINA COWAN**, PhD, associate professor of pathology; and **SAKTI SRIVASTAVA**, MD, associate professor of surgery, received the Henry J. Kaiser Family Foundation Award for Excellence in Preclinical Teaching.

YOSHI MITARAI, MD, clinical assistant professor of surgery; **REBECCA SEEKAMP**, MD, clinical assistant professor of medicine; and **MONICA STEMMLE**, MD, clinical assistant professor of pediatrics, received the Henry J. Kaiser Family Foundation Award for Excellence in Clinical Teaching.

Medical residents **KEVIN CHI**, MD, pediatrics; **VICTORIA KLYCE**, MD, emergency medicine; **JOZEF LAZAR**, MD, dermatology; **RUSTIN MASSOUDI**, MD, urology; **FELIPE PEREZ**, MD, anesthesiology, perioperative and pain medicine; and **NINA VASAN**, MD, psychiatry and behavioral sciences, received the Arnold P. Gold Foundation Award for Humanism and Excellence in Teaching. The award is given to residents based on their commitment to teaching and the compassionate treatment of students, colleagues and patients and their families.

AWARDS IN BIOSCIENCES

KEVIN GRIMES, MD, MBA, associate professor of chemical and systems biology, and **JULIE THERIOT**, PhD, professor of biochemistry and of microbiology and immunology, received the Faculty Award for Excellence in Teaching.

MIRIAM GOODMAN, PhD, associate professor of molecular and cellular physiology, and **JOSEPH LIPSICK**, MD, PhD, professor of pathology and of genetics, received the Faculty Award for Excellence in Diversity and Inclusion. This award recognizes faculty who make distinguished contributions toward enhancing diversity, equity and inclusion in the biosciences.

SEUNG KIM, MD, PhD, professor of developmental biology, and **GAVIN SHERLOCK**, PhD, associate professor of genetics, received the Faculty Award for Excellence in Mentoring and Service. This award recognizes faculty who make distinguished contributions toward enhancing the quality of training and the educational experience for biosciences graduate students.

STEVEN SLOAN, an MD/PhD student, received the Teaching Assistant Award.

GORES AWARD

Lipsick, who received a Walter J. Gores Award on June 14, was honored "for his deep commitment to improving the Cancer Biology Program curriculum as director of the program, revamping core courses in his sabbatical year, and including undergraduate as well as graduate courses."

He was commended "for his dedication to undergraduate education – he was one of the first faculty to volunteer and develop a new course for Thinking Matters" and "for his generosity in mentoring his graduate teaching fellows, providing them opportunities to design and give lectures on topics of interest."

Lipsick also was honored "for his thought-provoking courses that students described as 'challenging, but SO fun. They are like puzzles! … Do your brain a favor and TAKE THIS CLASS!'" and "for simply superb teaching that can transform a large lecture hall into an intimate setting for learning." **ISM**

Departments

continued from page 1

ments will bring the school's total number to 30. School officials said more information about plans for the departments will be shared in the coming weeks.

Biomedical data science

Biology and health care are being transformed by large-scale data analysis. The Department of Biomedical Data Science will build on the medical school's strengths in developing and applying information technology to prevent disease, deliver more efficient patient care, streamline applications in translational research and improve access to biomedical data. The field has grown rapidly and has proven to be an invaluable tool for ensuring clinical objectives and best practices.

"Stanford is already a world leader in innovation methodology in biostatistics and biomedical informatics," said Russ Altman, MD, PhD, professor of bioengineering, of genetics and of biomedical informatics research. "Uniting these disciplines under the auspices of a single department will allow us to approach data and information in a whole new way." Altman, a member of the core planning group that prepared the proposal for departmental status, added, "Big data holds unbelievable opportunities to change how medicine is explored and delivered.

Quantitative modeling and qualitative modeling have been traditionally distinct practices. The department will establish a unified discipline to address computational challenges in biomedicine and an expanded graduate training program that will attract top students and faculty working in these formerly disparate applications. It will focus on scientific leadership, faculty development and training new leaders.

"Leadership in this area is fundamental to Stanford's vision of leading the biomedical revolution in precision health," Minor said. "Stanford is already the world leader in innovative methodology in biostatistics and bioinformatics, and bringing together these two synergetic areas allows us to approach data and information in a whole new way — creating the new discipline of biomedical data science."

The two new departments will bring the school's total number to 30.

Altman noted that "a unified department will allow Stanford to take a quantum leap in application and methodology innovation, and in defining the discipline. It will allow us to apply concentrated expertise to every aspect of medicine and biomedicine, and bring us closer to precision health in providing care."

Emergency medicine

The decision to establish emergency medicine as its own department — it has been a division in the Department of Surgery — reflects its importance in evaluating and managing complex and high-acuity patients. It serves as the major point of entry to inpatient care as more than 40 percent of all patients admitted to the hospital are first evaluated and treated by emergency medicine physicians.

A recent study by Rand Health, an independent health research group, found that emergency physicians act as the major decision-makers for approximately half of all hospital admissions in the United States and are used with increasing frequency to conduct complex diagnostic workups. Emergency medicine is recognized as an autonomous department in 85 percent of the nation's medical colleges, and Stanford was the last medical school in California without a department devoted to emergency medicine.

"Emergency medicine coordinates and collaborates with virtually every specialty in the hospital, interfaces directly with the community, maintains a sizeable training program of students, residents and fellows, and conducts research in a nationally important area of scholarship," said Robert Jackler, MD, professor and chair of otolaryngology-head and neck surgery, who chaired the faculty task force that evaluated the transition proposal. "It's an essential component of Stanford Medicine that will serve an even more vital role as a department."

As a field, emergency medicine has emerged as an independent academic medical specialty with unique applications in triage, pandemics, humanitarian outreach and community service. Stanford is the only level-1 adult trauma center between San Francisco and San Jose and was verified as a level-1 pediatric trauma center in 2013 — the only one in the Bay Area recognized by the American College of Surgeons.

"Transitioning from a division to a department will allow emergency medicine to reach its full potential in health care and training," Jackler said. "It's an essential aspect of Stanford Medicine and now has the opportunity to take a leadership role in setting strategic goals and coordinating care." ISM

Ruth Schechter is a freelance writer and editor

Heartburn

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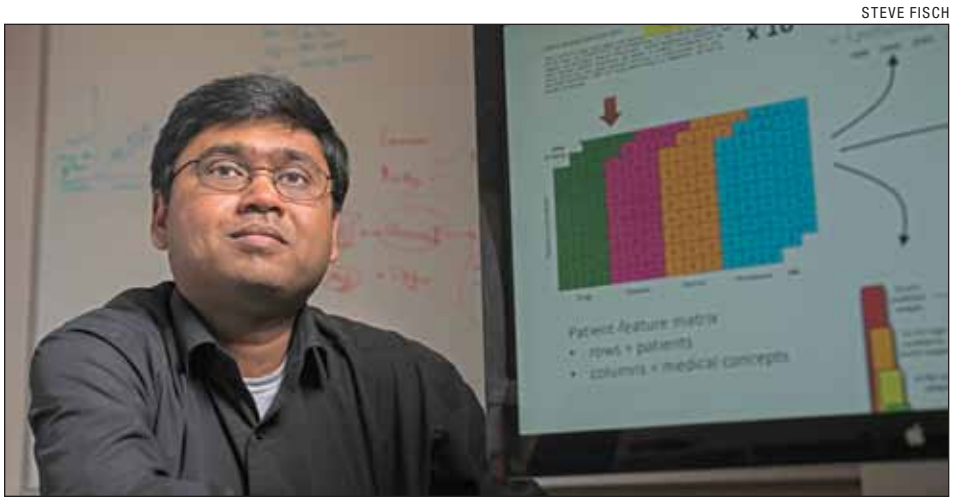
blood thinner clopidogrel (Plavix). However, the new study upends this view: It indicates that PPI use was associated with a roughly 20 percent increase in the rate of subsequent heart-attack risk among *all* adult PPI users, even when excluding those also taking clopidogrel.

A paper describing the findings was published June 10 in *PLOS ONE*.

"These drugs may not be as safe as we think," said Nicholas Leeper, MD, the study's senior author and an assistant professor of vascular surgery and of cardiovascular medicine.

STEVE FISCH



Nigam Shah has pioneered the use of data-mining techniques to capture sometimes elusive but medically important phenomena.

No elevated risk linked to H2 blockers

Interestingly, another commonly prescribed heartburn drug class called H2 blockers showed no association with elevated heart-attack risk. H2 blockers, which have been around longer than PPIs, are reasonably effective against heartburn and are the second-largest-selling class of drugs used to treat it.

The study's findings lend support to an explanation for an untoward effect of PPIs on heart-disease risk proposed by Stanford scientists a few years ago. Research done then showed that PPIs impede the production of an important substance, nitric oxide, in the endothelial cells that line all of the nearly 100,000 miles of blood vessels in an average adult's body.

Shah has pioneered the use of data-mining techniques to capture sometimes elusive but medically important phenomena. His methodology makes it possible, for example, to scour huge numbers of electronic health records — not only their structured portions, but also free-form notes entered by attending clinicians — for hints of an association between use of a drug or drug combination and unanticipated health outcomes, whether good or bad.

This kind of work is promoted by Stanford Medicine's Biomedical Data Science Initiative, which strives to make powerful transformations in human health and scientific discovery by fostering innovative collaborations among medical researchers, computer scientists, statisticians and physicians.

At Stanford, all clinical notes dating back to 1994 have been entered into a database known by its acronym, STRIDE. Shah, Leeper, and their colleagues sifted through data on 19 million encounters between Stanford physicians and 1.8 million patients and identified more than 70,000 who were age 18 or older and had been diagnosed with heartburn. To this number, the scientists added more than 227,000 adult heartburn patients culled from another privately held health-record database containing data on 1.1 million patients. They compared subsequent heart-attack frequencies of those who either had been prescribed PPIs, or said they were using them, with those of heartburn sufferers who were not using PPIs. The researchers saw a 16-21 percent increase in the rate of heart attacks, depending on which statistical approach they used, among members of the PPI group. This higher heart-attack frequency could be seen even in otherwise healthy PPI users under age 45.

Other bad outcomes

To further validate the association, they turned to an ongoing prospective, longitudinal study of 1,500 patients with chest pain, shortness of breath or abnormal stress-test results, conducted by Stanford in collaboration with Mount Sinai Medical Center in New York City. As a routine part of this study, patients are asked whether they are using PPIs.

To ensure that they would spot adverse drug effects if there were any, Shah, Leeper and their colleagues looked for not only heart attacks but cardiac arrest, stroke and other bad outcomes. They found that in this study population, PPI use more than doubled the risk of a patient's suffering a subsequent major adverse cardiovascular event.

Several hypotheses have been advanced to explain the increased cardiovascular risk attributable to PPI use among clopidogrel users, who in the past were often placed on PPIs because clopidogrel can increase gastric distress. But those hypotheses haven't held up well under scrutiny.

A new hypothesis was born in 2013, when a study in *Circulation* by John Cooke, MD, PhD, then a professor of cardiovascular medicine at Stanford, and his colleagues, including Yohannes Ghebremariam, PhD, implicated PPIs in igniting a cascade of biochemical reactions that led to diminished nitric oxide levels in endothelial tissue. (Cooke and Ghebremariam, both now at Houston Methodist Research Institute, are co-authors of the new study.)

"That study implied that PPIs' cardiovascular-risk effect had nothing to do with clopidogrel but was, instead, a direct effect on blood vessels themselves," Leeper said. "That could mean everybody on PPIs, not just people with coronary disease, is at increased risk from these drugs."

Those findings inspired Shah and Leeper to undertake the new study. "We looked at cardiovascular risk for different PPI drugs," said Shah. "And we found that the degree to which the use of any particular PPI was associated with a subsequent heart attack mirrors the degree to which the drug inhibits nitric oxide in the vasculature."

A small pilot trial led by Leeper and recently published in *Vascular Medicine* showed a trend between PPI use and increases in a chemical known to impair the function of an enzyme that produces nitric oxide. But the study population of 21 subjects was too small to show a conclusive link.

"This association needs to be tested in a large, prospective, randomized trial," said Leeper. "The truth will come out when we randomize several hundred people, give half of them PPIs and put the other half on H2 blockers, and see what happens."

Neither Shah nor Leeper recommends that people now on PPIs simply stop taking them without first talking to their doctors about alternatives.

Meanwhile, they both said, the study results should give clinicians and patients pause when deciding whether to take these medications — particularly because they're so often taken for far longer time periods than the label recommends.

Research scientist Paea LePendu, PhD, shares lead ≠≠authorship with Shah. Other Stanford co-authors are postdoctoral scholar Anna Bauer-Mehren, PhD; research assistant Srinivasan Iyer; and medical resident Kevin Nead, MD.

The study was funded by the National Institute of General Medical Science and the National Human Genome Research Institute.

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

Molecular cause of heart condition identified by researchers

By Krista Conger

In 2012, researchers at the School of Medicine showed that heart muscle cells made from the skin of people with a cardiac condition called dilated cardiomyopathy beat with less force than those made from the skin of healthy people. These cells also responded less readily to the waves of calcium that control the timing and strength of each contraction.

Now, the same research team has teased apart the molecular basis for these differences and identified a drug treatment that at least partially restores function to diseased cells grown in a laboratory dish. They also observed how a key cardiac signaling cascade, called the beta-adrenergic pathway, develops as heart muscle cells mature, and identified key aspects about how it functions in both normal and diseased cells.

The researchers hope that the findings will help clinicians better hone treatments for a variety of cardiac conditions, which are now often treated with a one-size-fits-all approach.

"Right now, nearly all patients with cardiomyopathy are given drugs to modulate the beta-adrenergic pathway in the heart, which is known to be dysfunctional," said Joseph Wu, MD, PhD, director of Stanford's Cardiovascular Institute. "But until now, we've not known what exactly is going wrong with this pathway at a molecular level."

A paper describing the research was published online June 18 in *Cell Stem Cell*. Wu, a professor of medicine and of radiology, is the senior author of the paper, and postdoctoral scholar Haodi Wu, PhD, is the lead author. (Joseph Wu and Haodi Wu are not related.)

Using skin-derived cells to study disease

The research relies on what's known as induced pluripotent stem cells, or iPS cells, to make heart muscle cells from skin. IPS cells can be coaxed to develop into nearly any tissue in the body. The technique gives researchers access to a variety of human cell types, such as brain and heart muscle cells, that are typically difficult to obtain for study.

This study adds to others suggesting that heart muscle cells made from skin cells accurately incorporate the minute details of diseases that afflict those from whom the skin cells were derived.

"We wanted to characterize the mechanisms that underlie the functional impairment of the cells," said Haodi Wu. "Until now, we've used iPS-cell-derived heart muscle cells as a disease model for cardiomyopathy, but it's not been known how precisely these cells recapitulate the disease phenotype. Now we see that although diseased and healthy cells undergo a similar developmental and maturation process, the mutation carried by the diseased cells causes them to respond differently to signaling by the beta-adrenergic pathway."

Joseph Wu

Dilated cardiomyopathy occurs when a portion of the heart muscle enlarges and begins to lose the ability to pump blood efficiently. Eventually, the enlarged muscle weakens and fails, requiring either medication or even transplant. Dilated cardiomyopathy can be due to restrictions in blood flow (a condition known as ischemia) that can cause a heart attack, or to nonischemic causes such as viral infection. Although many cases of nonischemic dilated cardiomyopathy occur sporadically and without an apparent cause, dilated cardiomyopathy can also be inherited through a variety of genetic mutations. One of these mutations affects a protein called TNNT2, which is located on the muscle fibers of the heart and helps to regulate their contraction.

> **"As a cardiologist, I feel this basic research study is very clinically relevant."**

The researchers were building upon a 2011 study from the Wu lab published in *Science Translational Medicine* showing that stem-cell-derived heart muscle cells from people with cardiomyopathy differ in obvious ways from those derived from healthy people. They contract less forcefully and respond less strongly to the beta-adrenergic signaling pathway that increases heart rate and stroke volume and force in response to stress or exercise.

Molecular messengers degraded

Haodi Wu and his colleagues showed that mutated TNNT2 in the cells from patients with dilated cardiomyopathy travels into the cells' nuclei and stimulates chemical tags like methyl groups to attach to DNA and DNA-packaging protein complexes called histones. This process is called epigenomic modification. These modifications work to increase the expression of two genes encoding proteins called phosphodiesterases, which degrade small molecular messengers essential to the beta-adrenergic signaling pathway.

To test their findings, the researchers stimulated iPS-cell-derived heart muscle cells with isoproterenol, which activates the beta-adrenergic pathway. Healthy cells responded vigorously, contracting about 80 percent more quickly and with about 60 percent more force. In contrast, the contraction rate of the diseased cells only increased by about 37 percent, and the force of the contraction remained roughly the same.

"We saw a very dramatic effect in normal cells, but a much smaller functional change in cells made from patients with dilated cardiomyopathy," said Haodi Wu. "This is very similar to what we see in human patients. They can have a high blood adrenaline level, but the output of their heart remains weak."

When the researchers treated the cells with molecules that blocked the function of the phosphodiesterase proteins, diseased cells responded more strongly to isoproterenol treatment, and their contraction rate and force approached that of healthy cells.

"As a cardiologist, I feel this basic research study is very clinically relevant," said Joseph Wu. "The beta-adrenergic pathway is a major pharmaceutical target for many cardiac conditions. This study confirms that iPS-cell-derived cardiomyocytes can help us to understand biologically important pathways at a molecular level, and can aid in drug screening. The ability to make a patient's own heart cells for study is the epitome of personalized medicine."

Other Stanford co-authors are postdoctoral scholars Mingxia Gu, PhD, Feng Lan, PhD, and Jared Churko, PhD; cardiovascular medical fellow Karim Sallam, MD; instructor Elena Matsa, PhD; graduate student Arun Sharma; and senior research scientist Joseph Gold, PhD.

The work was funded by the American Heart Association and the National Institutes of Health.

Stanford's Department of Medicine and the Stanford Cardiovascular Institute also supported the work. ISM

$9 million grant establishes open-access autism database

Dennis Wall, PhD, an autism researcher at the School of Medicine, is leading a new project to establish the largest-ever collaborative, open-access repository of bioinformatic data on autism.

The Hartwell Autism Research and Technology Initiative, known as iHART, will provide the scientific community with a centralized repository of data to benefit biomedical research on autism and help children affected with the developmental disorder, which hinders social and communication skills. It is funded by a $9 million grant from the Hartwell Foundation, a charitable organization whose mission is to support early-stage biomedical research projects that improve the health and well-being of children in the United States.

Dennis Wall

Wall, an associate professor of systems medicine in the Department of Pediatrics, will direct the iHART cloud-based computing and communications technology platform. The initiative seeks to assemble a comprehensive scientific repository of data on autism spectrum disorder through collaboration with researchers. The bioinformatics effort will deploy state-of-the-art computational tools of systems biology, machine learning and inference algorithms to inspire users to exploit the full potential of available data related to autism.

"Our goal is for iHART's easily accessible computing and analytics platform to enable complex queries that may refine the definitions of autism," Wall said. "We hope iHART will lead the way toward clinical translation of various biomarkers for early detection and therapeutic intervention, and provide a multitude of solutions that help families and their children."

The platform will enable researchers to ask questions that simultaneously draw on many kinds of data on autism spectrum disorder, including phenotypes, proteomics, metabolomics, genomics, measurements and imaging of brain activity, information on the gut microbiome, blood-based biomarkers, physician narratives, diagnostic test results and treatment protocols. The platform will include a portal to enable data integration, as well as experimental design and validation. The initial repository integrates genetic, phenotypic, genomic and other data on nearly 5,000 individuals affected with autism spectrum disorder.

In addition to the School of Medicine, the Hartwell Foundation will collaborate with the Simons Foundation, the University of California-Los Angeles and the New York Genome Center to accelerate the addition of autism data to the database. ISM

Hearing

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Once hair cells die in a mature mammal, they are not replaced. But scientists have recently determined that a supporting cell type, called the inner pillar cell, has the potential to regenerate hair cells in newborn mice.

In its new study of 2-day-old mice, Heller's lab team measured the activity of 192 genes. The researchers determined which genes were turned on, or "expressed," in each of 808 hair cells and supporting cells from either the apex or base of the organ of Corti. They quantified this gene expression by measuring the amount of RNA produced from each gene.

> **"Compared to other senses, we know very little about how hearing works."**

The researchers identified patterns of gene expression that may determine whether inner pillar cells can give rise to new hair cells. Similarly, they discovered gradual changes in the expression of specific genes across cells that span the organ of Corti from its base to its apex that may be crucial for the establishment and maintenance of a population of hair cells that responds to a range of sound frequencies.

Crunching the data

Using powerful number-crunching software to analyze the large amount of genetic data, Heller's lab team accurately identified the two known types of hair cells and the seven known types of supporting cells and created a computer-generated map of their locations within the organ of Corti. They did this using only the genetic data, but then used other previously known DNA sequences to independently verify the accuracy of the cell identification and mapping.

The strategy the researchers used to predict the spatial location of cells within the organ of Corti from gene-expression data also should prove useful to biologists who study other types of cells in different organs, Heller said.

Rapid advances in single-cell gene-expression analysis are likely to supplant a standard technique called in-situ hybridization, according to Heller. The standard technique relies on labeled genetic probes to target individual genes one by one in order to identify specific cell types. The new approach of measuring hundreds of genes in parallel and reconstructing the organs in the computer appears to be more accurate and powerful.

"Molecular gradients play a key role in developmental biology, but in the past researchers depended on identifying gradients in one molecule at a time," Heller said. "With these new techniques, we are identifying cells that, for example, have molecular characteristics of stem cells, by analyzing the expression of many genes all at once, and we know precisely where they are located."

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