Fish offers possible clue to evolution of human toes

By Krista Conger

Consider the engineering marvel that is your foot. Be it hairy or homely, without its solid support you’d be hard-pressed to walk or jump normally.

Now, researchers at the School of Medicine and the HudsonAlpha Institute for Biotechnology in Huntsville, Alabama, have identified a change in gene expression between humans and primates that may have helped give us this edge when it comes to walking upright. And they did it by studying a tiny fish called the threespine stickleback that has evolved radically different skeletal structures to match environments around the world.

“It’s somewhat unusual to have a research project that spans from fish all the way to humans, but it’s clear that tweaking the expression levels of molecules called bone morphogenetic proteins can result in significant changes not just in the skeletal armor of the stickleback, but also in the hind-limb development of humans and primates,” said David Kingley, PhD, professor of developmental biology at the University of Alabama.

Butte and his colleagues developed a way to probe and get detailed measurements of living cells through an advancement in atomic force microscopy.

Ambien improves stroke recovery in mice

By Bruce Goldman

Mice that had strokes rebounded significantly faster if they received low doses of a popular sleeping aid, according to researchers at the School of Medicine.

Zolpidem, better known by the trade name Ambien, has long been approved by the U.S. Food and Drug Administration for treating insomnia. But it has never before been definitively shown to enhance recovery from stroke, said Gary Steinberg, MD, PhD, professor and chair of neurosurgery. Steinberg shares senior authorship of the study, which was published online Dec. 18 in Brain, with senior research scientist Tonya Bliss, PhD.

Steinberg, the Bernard and Ronni Lacroute-William Randolph Hearst Professor in Neurosurgery and the Neurosciences, cautioned that the study’s results need to be independently replicated in other laboratories before clinical trials of the drug’s capacity as a stroke-recovery agent can begin.

Every year, Americans incur about 800,000 strokes, the nation’s largest single cause of neurologic disability, exacting an annual tab of about $74 billion in medical costs and lost productivity.

A stroke’s initial damage, which arises when the blood supply to part of the brain is blocked, occurs within the first several hours. Drugs and mechanical devices for clearing the blockage are available, but to be effective they must be initiated within several hours of the stroke’s onset. As a result, fewer than 10 percent of stroke patients benefit from them.

After a few days during which tissue death continues to spread to adjacent brain regions due to repercuSSIONs from the initial damage, the brain begins slowly rewiring itself and substituting new neural connections.

Ambien, which is approved by the U.S. Food and Drug Administration for treating insomnia, has long been shown to enhance recovery from stroke, said Gary Steinberg, the Bernard and Ronni Lacroute-William Randolph Hearst Professor in Neurosurgery and the Neurosciences, who shares senior authorship of the study, which was published online Dec. 18 in Brain.

A paper describing the work was published online Nov. 11 in ACS Nano. Butte, an assistant professor of pediatric immunology, is the senior author. Lead authorship is shared by Andrew Wang, PhD, a former postdoctoral scholar in Butte’s lab, and Karthik Vijayaraghavan, PhD, who was a graduate student and member of the microphononics lab led by Olav Solgaard, PhD, a professor of electrical engineering.

“Mice that were given the drug showed a lot of recovery for the amount of drug they received,” said Butte. “What a cell feels like — its mechanical properties that affect how it makes contact with other cells and tissues — is much more important than what it looks like, but the technology just wasn’t there to allow us to examine it.”

“A lot of work has been done studying the mechanics of a cell and its structures just beneath the surface.”

The way Butte and his colleagues use AFM to measure the mechanical properties of cells is akin to the way a builder taps her knuckles along a drywall, listening for the change in pitch that will tell her a wooden stud is on the other side. When

Test could indicate whether infections are viral or bacterial

By Jennie Dusheck

A team of immunologists and informatics experts at the School of Medicine has identified a distinctive pattern of gene expression that distinguishes people with a viral infection from those with a bacterial infection. The team also identified a second pattern of gene expression that is more specific: It can distinguish people with a viral infection from those with a bacterial infection.

When pathogens infect the cells of the body, the infection sets off a chain reaction involving the immune system that changes the expression of hundreds of genes. Gene expression is the process by which cells extract information from
Study: Overprescribing opioids not limited to a few bad apples

By Beth Duff-Brown

Most prescriptions for opioid painkillers are made by the broad swath of U.S. general practitioners, not by a limited group of specialists, according to a study by researchers at the School of Medicine.

This finding contradicts with previous studies by others that indicated the U.S. opioid epidemic is stoked by a small population of prolific prescribers operating out of corrupt “pill mills.”

The study, which examined Medicare prescription drug claims data for 2013, appeared in a research letter online Dec. 14 in JAMA Internal Medicine.

“The bulk of opioid prescriptions are distributed by the large population of general practitioners,” said lead author Jonathan Chen, MD, PhD, an instructor of medicine and Stanford Health Policy VA Medical Informatics Fellow.

Prescribing patterns

The researchers found that the top 10 percent of opioid prescribers account for 57 percent of opioid prescriptions. This prescribing pattern is comparable to that found in the Medicare data for prescribers of all drugs: The top 10 percent of all drug prescribers account for 63 percent of all drug prescriptions.

The specialties that prescribed the most Schedule II opioids in 2013 were family practice (15.3 million prescriptions), nurse practitioner (4.1 million) and physician assistant (3.1 million prescriptions), according to the study. Schedule II opioids are substances approved by the Food and Drug Administration for medical use and recognized as carrying a high potential of abuse.

These findings indicate law enforcement efforts to shut down pill-mill prescribers are insufficient to address the widespread overprescribing of opioids, Chen said. “Forty to 50 percent of curtail national opioid overprescribing must address a broad swath of prescribers to be effective.”

He added, “Being a physician myself, I am acutely aware of the emotional angst that can occur when deciding whether to prescribe opioids to a patient who may have simultaneously developed a chronic-pain and substance-dependence problem. The public health epidemic of opioid overuse is perhaps not surprising given the tenfold increase in volume over the past 20 years.”

Different findings from different data set

In 2011, a study by the California Workers’ Compensation Institute found that 1 percent of prescribers accounted for one-third of opioid prescriptions, and that the top 10 percent accounted for 80 percent of prescriptions.

The new Stanford study used a different data set: Instead of California Workers’ Compensation prescrip-tions, it looked at prescriber data from the 2013 Medi-care prescription drug coverage claims and investigated whether such disproportionate prescribing of opioids occurs in the national Medicare population.

Prior studies looked at Schedule II opioids, which include the commonly abused drugs hydrocodone, codeine and fentanyl.

The data set created by the Centers for Medicare and Medicaid Services included all prescribers and represented all Medicare prescription drug coverage claims for 2013: 808,020 prescribers and 1.18 billion claims. The researchers focused on the data for Schedule II opioids: 381,575 prescribers and 56.5 million claims.

“This data set indicates no special distinctions in the concentration of opioid prescribing among Medicare prescribers,” said Chen. “The earlier study suggests potentially aberrant behavior among those extreme outlier prescribers, while implying the remaining majority do not contribute much to the problem — and now we know this is not the case.”

The authors attribute the difference in the California Workers’ Compensation data to the traits of specific populations, which perhaps has a greater prevalence of multiple illnesses or employment that put people more prone to injury, while the Medicare population is more generally representative of the population at large.

They found that opioid prescriptions per prescriber were concentrated among specialty services for interventional pain management (1,124.9 prescriptions, on average, per prescriber), pain management (921.1), anesthesiology (604.9) and physical medicine and rehabilitation (348.2).

By sheer volume, however, there are so many more general practitioners that they dominated the total quantity of prescriptions.

Anna Lemke, MD, is the study’s senior author. Other Stanford co-authors are professor of psychiatry and behavioral sciences and associate professor of medicine Nigam Shah, MBBS, MD, PhD.

The research was supported in part by the U.S. Department of Veterans Affairs Office of Academic Affiliations, the VA Health Services Research and Development Service, the National Institute of General Medical Sciences and the Brad and Franz Manucos Charitable Trust.

Stanford’s Department of Medicine also supported the work.

Beth Duff-Brown is the communications manager for Stanford Health Policy.

Interaction of attention networks weaker in kids with ADHD

By Erin Digitale

Interactions between three brain networks — the default mode, salience and executive attention — are weaker than normal in children with attention-deficit hyperactivity disorder (ADHD), a new study suggests.

“At present, diagnosing ADHD is a subjective and unreliable process, not just clinical and parental assessments of behavior,” said Weidong Cai, PhD, an instructor in psychiatry and behavioral sciences and the study’s lead author. “This study also demonstrates that we can develop a very robust biomarker based on functional neuroimaging to reliably differentiate children with ADHD from other kids.”

Menon’s team studied functional magnetic resonance imaging (fMRI) brain scans from 180 children, half with ADHD and half without. The scans were taken when the children were awake but resting quietly.

The children were also assessed for ADHD symptoms using traditional diagnostic methods. All study data were obtained from the ADHD-200 Consortium, an open-source database of fMRI scans and other clinical characteristics of hundreds of children with or without ADHD in the United States.

“Discerning ADHD from non-ADHD

The team scored each brain scan according to the synchronization between the salience network and two other related brain networks: the default mode network, a set of brain regions that directs self-referential activities such as daydreams, and the executive attention network, which manipulates information in working memory. To focus one’s attention, the salience network must turn down the activity of the default mode network while turning up the activity of the central executive network.


In a new study, School of Medicine researchers report finding an easy method to cut laboratory mice of a common, life-threatening skin condition. Millions of laboratory mice suffer from a skin condition known as ulcerative dermatitis; it is a major source of discomfort for these animals and the most common reason for unexplained euthanasia. Between 4 and 21 percent (depending on factors like strain and environment) of laboratory mice experience the condition, in which they develop, ulcerated lesions that become progressively worse with repeated scratching, said Sean Adams, DVM, PhD, a third-year resident in laboratory animal medicine at Stanford and lead author of the new study.

To address the problem, which has long bedeviled veterinarians, Adams and his colleagues designed a simple plastic device that briefly immobilizes the animals so that caregivers can quickly trim the affected animals’ toenails in two minutes or less, saving researchers time and money while alleviating animal suffering. Adams and his colleagues also trimmed the mice’s toenails at the same time that they were treated with topical anti-inflammatory ointments, found. And the results held up even after the animals’ toenails had regrown, as they were unable to continue self-traumatizing the affected area.

“This is a simple, cheap, effective means of treating ulcerative dermatitis, which represents the single most preventable reason for euthanasia,” Adams said. “I think it’s a very surprising find in how simple this technique is.”

The study, the first to systematically look at the impact of toenail trims, was published online Jan. 6 in PLOS ONE.

Saves mice, simplifies care

Adams said the technique not only saves mice from suffering and having to be euthanized as a humane necessity, but also simplifies their care. The Stanford veterinarians were able to clip the animals’ nails in two minutes or less, saving for themselves the discomfort of spending daily anti-inflammatory ointments, which were only minimally effective in curbing inflammation, he said.

“Now we have this mouse with just shreds of fur on the body. They rip themselves apart,” he said. “Veterinarians have tried many approaches to treatment, typically involving application of a topical anti-inflammatory ointment. These have produced variable results, though studies have never shown them to be more than 65 percent effective in healing their flank wounds, as the researchers report. Moreover, these ointments have to be applied daily, causing a major burden for animal care providers.”

Study methods and results

In 2013, the researchers started giving veterinarians at Stanford the freedom to apply either the topical anti-inflammatory Tresaderm mice were uncowed, dermatitis to trim their toenails under anesthesia. The toe-trimmed mice also got an application of Vetericyn, a form of bleach that bacteria can’t grow and helps calm inflammation. The mice were of different strains and were housed in five facilities.

After a year, Adams and his colleagues went back and examined the records of 324 animals, including 98 who had been treated with Tresaderm and 39 who had their toenails trimmed, to see how well they did. They found that animals with trimmed nails did significantly better, with 93.3 percent healing within 14 days. Among mice receiving Tresaderm, 25.4 percent were cured during the same time period.

To determine whether the results held up over time, the researchers followed another 54 animals over a six-week period, both trimming their toenails and applying Vetericyn to soothe the affected area. Mice toenail trimming began to regress within a few days, so the researchers wondered if the animals would begin the destructive scratching cycle again. But to their surprise, the animals refrained from scratching their wounds, which continued to heal.

“It’s a curative treatment. It’s not just palliative,” Adams said. “This really does break the cycle to allow a cure to occur. It is completely different from the other treatments out there.”

He said the toenail treatment was not effective in healing animals who had lesions involving the soles of their feet, as the mice sought relief from their discomfort by chewing the wounds in this area.

Finally, to make sure that the topical treatments were not confounding the results, the research team tested three different ointments — Tresaderm, Vetericyn and the antibiotic Bactercin — together with toenail trimming, and found no difference in results between the three.

Immobilizing mice

While toenail trimming was clearly the superior treatment, Adams said the veterinarians recognized it wasn’t entirely practical when done under general anesthesia. So he devised a simple trimming device, modifying a plastic tube to create two small curous for the animals’ feet. When the mice are fitted into the tube, they are temporarily immobilized with their feet outstretched, making nail trimming simple and relatively stress-free for both caregivers and animals, he said.

He said the mice don’t struggle or resist, and after a day or two of practice, technicians could clip the nails in as little as 30 seconds. “You just give a little pedi-cure and it changes everything,” Adams said.

A lab mouse with ulcerative dermatitis (left) was given a toenail trim. Fourteen days later (right), the lesions had healed and fur had regrown in the affected area.

“I think we’ll start seeing more people help pick up this technique because it’s very easy to do,” he added. “There is definitely interest in finding good techniques for the problem because this is an issue for every institution that employs mice.”

He said it’s especially important to laboratories that use unusual strains, such as transgenic models, that can be very valuable.

Some institutions, including Massachusetts General Hospital and the University of Colorado, also perform toenail trimming while veterinarians at UC-Davis and UCSF have shown an interest in the technique, he said.

David Chu, DVM, a staff veterinarian, is an senior author of the study. Other Stanford-affiliated co-authors of the study are Joseph Garner, PhD, associate professor of comparative medicine, Stephen Felt, DVM, MPH, associate professor of comparative medicine; and Jerome Geromin, a research assistant.

The study was funded in part by the National Institutes of Health, which helps support Adams’ residency, and by private contributions to Garner’s mouse welfare work.
Researchers invent process to accelerate protein evolution

By Ramin Skibba

All living things require proteins, members of a vast family of molecules that nature "makes to order" according to the blueprints in DNA.

Through the natural process of evolution, DNA mutations generate new or more effective proteins. Humans have found so many alternative uses for these molecules — as foods, industrial enzymes, anti-cancer drugs — that scientists are eager to better understand how to engineer protein variants designed for specific uses.

Now, Stanford researchers have invented a technique to dramatically accelerate protein evolution for this purpose. This technology, described in a paper published online Dec. 7 in Nature Chemical Biology, allows researchers to test millions of variants of a given protein, choose the best for some task and determine the DNA sequence that creates this variant.

"Evolution, the survival of the fittest, takes place over a span of thousands of years, but we can now direct proteins to evolve in hours or days," said Jennifer Cochran, PhD, an associate professor of bioengineering, who shares senior authorship of the paper with Thomas Baer, PhD, executive director of the Stanford Photonics Research Center. The lead author is Bob Chen, a graduate student in bioengineering.

"This is a practical, versatile system with broad applications that researchers will find easy to use," Baer said.

By combining Cochran's protein engineering know-how with Baer's expertise in laser-based instrumentation, the team created a tool that can test millions of protein variants in a matter of hours.

"The demonstrations are impressive, and I look forward to seeing this technology more widely adopted," said Frances Arnold, PhD, professor of chemical engineering at Caltech who was not affiliated with the study.

Making a million mutants

The researchers call their tool µSCALE, for Single Cell Extraction Laser Extraction.

The "µ" stands for the microcapillary glass slide that holds the protein samples. The slide is roughly the size and thickness of a penny, yet in that space a million capillary tubes are arrayed like straws, open on the top and bottom.

The power of µSCALE is how it enables researchers to build upon current biochemical techniques to run a million protein experiments simultaneously, then extract and further analyze the most promising results.

"Researchers first employ a process termed "mutagenesis" to create random variations in a specific gene. These mutations are inserted into batches of yeast or bacterial cells, which express the altered gene and produce millions of random protein variants.

A µSCALE user mixes millions of tiny opaque glass beads into a sample containing millions of yeast or bacterial cells and spreads the mixture on a microcapillary slide. Tiny amounts of fluid trickle into each tube, carrying individual cells. Surface tension traps the liquid and the beads into a sample containing millions of yeast or bacterial cells, which express the altered gene and produce millions of random protein variants.

Thus, µSCALE emports the contents of a single capillary onto a collector plate, where the DNA of the protein obtained is sequenced and the lone variant responsible for the protein of interest can be identified.

"One of the unique features of µSCALE is that it allows researchers to rapidly isolate a single desired cell from hundreds of thousands of other cells," said Chen, who wrote the software to examine and detect signs of interesting protein activity within the test tubes.

"This promising variant can then be collected and reprocessed through µSCALE to further evolve and optimize the protein.

"It is an exciting new tool to answer important questions about proteins," Cochran said, likening µSCALE to the way that high-throughput tools for gene analysis have allowed researchers to unlock key features of biology underlying human disease.

Genes and proteins

The project began five years ago when Baer and study co-author Ivan Dimov, PhD, a visiting instructor and Siebel Fellow at the Stanford Institute for Stem Cell Biology and Regenerative Medicine, came up with the first instrument. They showed how to identify cell types in a microcapillary array and extract a single capillary's contents using glass beads and a focused laser.

About three years ago, Cochran and Baer joined forces to develop µSCALE for protein engineering, and the team devised three experiments to showcase µSCALE's utility and flexibility.

In one experiment, researchers sifted through a protein library produced in yeast cells to select antibodies that bound most tightly to a cancer target. Antibodies with a high target-binding affinity are known to be effective against cancer. In a second example, they engineered a bright orange fluorescent protein biosensor. Using µSCALE, they did this almost 10 times faster than previous methods. Such biosensors are often used as tags in a wide variety of biology experiments. In a third experiment, carried out with Daniel Herschlag, PhD, professor of biochemistry and a co-author of the study, used µSCALE to improve a model enzyme.

"This system will allow us to explore the evolutionary and functional relationships between enzymes, guiding the engineering of new enzymes that can carry our medical beneficial reactions," Herschlag said.

Other Stanford co-authors of the paper are graduate students Sungwon Lim and Arvind Kannan, postdoctoral scholar Spencer Alford, PhD, and research assistant Sunny Sundey.

This project was supported by the Stanford-Walace H. Coulter Translational Partnership Award Program, the Siebel Stem Cell Institute and the Thomas and Stacey Siebel Foundation, the Stanford Photonics Research Center, a Hirochi America Faculty Scholar Award, the National Institutes of Health, the National Science Foundation Graduate Fellowship Program, the Howard Hughes Medical Institute International Student Research Program, a Fannie and John Hertz Foundation Graduate Fellowship, the Stanford Bio-X Fellowship Program, the Stanford Graduate Fellowship Program and the Stanford Dean's Fellowship Program.

SRI Biosciences, Stanford Cancer Institute launch drug discovery program

A new collaborative program between scientists at SRI Biosciences, a division of SRI International, and physician-researchers from the Stanford Cancer Institute will pursue development of novel compounds to treat multiple forms of cancer and other conditions.

The SRI Biosciences-Stanford Drug Discovery and Development Program is a spinoff of the SRI-SCI Advanced Therapeutics program that brought together multidisciplinary teams from SRI Sciences and Stanford researchers and investigators and technical experts to advance promising projects. The program will be co-led by Sanjay Malhotra, PhD, associate professor of radiation oncology, and Nathan Collins, PhD, executive director of the Stanford Cancer Institute. The program will make possible the acquisition and development of new compounds and targets, and it will provide access to the critical in vitro and in vivo model systems necessary for disease mechanism understanding and target discovery, and to the clinical trial infrastructure necessary through clinical safety and proof of concept.

"Advances in genomic and molecular analysis of individual patients and their cancers are creating new therapeutic opportunities," said Beverly Mitchell, M.D., director of the Stanford Cancer Institute. "We are excited to work with the skilled SRI Biosciences researchers to enhance our drug discovery and development efforts.

"This program will be co-led by Sanjay Malhotra, PhD, associate professor of radiation oncology, and Nathan Collins, PhD, executive director of the Stanford Cancer Institute. Together they will coordinate and oversee the development of novel compounds and targets, and it will provide access to the critical in vitro and in vivo model systems necessary for disease mechanism understanding and target discovery, and to the clinical trial infrastructure necessary through clinical safety and proof of concept.

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A collaboration between computer scientists and geneticists at Stanford has produced a novel technique for mapping the diversity of bacteria living in the human gut.

The new approach revealed a far more diverse community than the researchers had anticipated. “The bacteria are genetically much more heterogeneous than we thought,” said Michael Snyder, PhD, professor and chair of genetics.

Any two humans typically differ by about 1 in 1,000 DNA bases, whereas bacteria of the same species may differ by as many as 250 in 1,000, Snyder said. “I don’t think people realized just how much diversity there was. The complexity we found was astounding,” he said.

In the past, researchers could only study bacteria that would grow in the lab. But the vast majority of bacterial species will not grow on traditional culture medium. As a result, the true diversity of bacteria — not only in the human gut but throughout the living world — has remained largely unexplored.

In recent years, a genomics approach has begun to reveal diverse communities of new bacterial species growing nearly everywhere biologists have looked. Modern gene sequencing has tantalized biologists with hints of bacterial worlds as biodiverse as any tropical rain forest. Yet the limitations of current technologies have created only a blurry picture and prevented researchers from seeing all that is there.

Of particular interest are the bacteria that live in our intestines. Some communities of bacterial species in the gut have been associated with good health, others with any of a long list of conditions — including obesity, Type 2 diabetes, bowel disease and liver disease. And some bacteria can sicken and even kill, such as certain strains of E. coli that causes cholera. Given their importance to human health, the ecological communities of bacteria that live inside us and on our skin have come under increasing scrutiny.

A Stanford team has overcome some of the limitations of current sequencing technology to create a sharper picture of the bacterial community, or microbiome, of the human gut. The team used new computational approaches and “long-read” DNA sequencing to reveal the diversity of bacteria in the gut microbiome of a single human. As a result, describing their work was published online Dec. 14 in *Nature Biotechnology*. The lead author is Volodymyr Kuleshov, a doctoral student in computer science at Stanford. Snyder, the Stanford W. Ascher-Zoglou, PhD.

Problem posed by short snippets of DNA

Current DNA sequencing technology looks at very short snippets of DNA sequences. If you are looking at just one genome — from a bacterium or a single person, for example — you can assemble the snippets into a whole genome, much as you might painstakingly assemble a jigsaw puzzle.

But when you are looking at snippets from a mass of different bacteria from the human gut, assembling those snippets is like trying to assemble 100 jigsaw puzzles from unique pieces from all 100 puzzles jumbled together, explained Snyder. Any two pieces could be from completely unrelated puzzles — analogous to different species of bacteria — while others could be from multiple copies of the same puzzle — analogous to the same species of bacteria.

If that sounds difficult, the real challenge is being able to tell apart the pieces from puzzles that are almost the same but not quite. And that’s what the researchers’ new technique does. “We have assembled whole genomes from this big gemisch, which has never been done before,” said Snyder.

“We normally sequence 100 DNA bases off a 300-base fragment,” he said. “You just get snippets of information.” But using a new informatics approach, Snyder and Batzoglou’s team stitched together larger segments of the genome. “We have a sophisticated algorithm that lets us put together all these pieces — first assembling the snippets into longer, 10,000-base pieces, then the 10,000-base pieces into still-longer fragments, and then those into whole genomes,” Snyder said.

Great bacterial diversity

Such long sequences of DNA can span hundreds or even thousands of genes that couldn’t be recovered from short-read sequencing; they can help classify bacteria and other organisms by how related they are to one another; and the long sequences also help identify rare bacteria that might be missed by current methods. “We could assemble either entire genomes or at least very, very large chunks of the genome,” said Snyder.

Being able to see such long sections of the genome means being able to distinguish not only different species of bacteria, but different strains of the same species. The team tested the technique on a standardized sample of known bacteria and then took it for a spin on the gut contents of a human male. The result revealed not only lots of species, but many different strains of the same species. One bacterial species, for example, included five separate strains — all from one person.

The consequences of having so many different strains are hard to predict, but some strains may be more or less likely to make people ill. For example, many strains of E. coli bacteria live harmlessly and even helpfully in the human gut, while others are lethal. Being able to tell one strain from another could help researchers determine which strains are dangerous and why.

Right now, researchers who want to study virulence have to isolate that strain and then grow it in the lab. But some bacteria don’t grow easily in the lab. If researchers can study the genes that contribute to virulence directly in the mixture of bacteria from a human gut sample, they don’t need to isolate it and grow it in a pure culture. “When you assemble the whole genome, you have a better idea of what the pathogenic genes are. I think it’s going to be very, very powerful for understanding the generic basis of pathogens,” said Snyder.

The new approach will make it easier to construct the evolutionary history of strains of infectious bacteria or viruses, such as Ebola. And the approach can be used in the field to study microbial diversity in healthy people and other animals, as well as in plants, water and soil. “When we put this together now, using these long reads, it’s like an IMAX movie,” Snyder said. “You can see the whole thing much more clearly than with what we do now, which is like an old black-and-white TV.”

Other Stanford-affiliated authors of the paper are postdoctoral scholars Chao Jiang, PhD, and Wenyu Zhou, PhD, and research associate Fereidoun Jahani, PhD.

This work was supported by National Institutes of Health. Stanford’s departments of Genetics and of Computer Science also supported the work.

Stunning diversity of gut bacteria revealed through technique

By Jennie DuShew

Researchers have overcome some of the limitations of current sequencing technology to better measure the diversity of bacteria in the human gut.

The 92,000-square-foot, five-story Neuroscience Health Center opens to patients today. The center, a part of Stanford Health Care, is designed to serve people with neurological conditions or injuries such as brain tumors, movement disorders, brain aneurysms, spine deterioration, Parkinson’s disease and memory disorders. Located on the Stanford Medicine campus next to Hoover Pavilion, the center offers advanced diagnostic testing and treatments, as well as support services, all available in a single, easy-to-navigate building.

Inside Stanford Medicine

January 11, 2016

Please give blood

Blood type needed:

O+, O-

To request an appointment, call 723-7831 or you can make an appointment online.

3373 Hillview Ave., Palo Alto
440 Busuego Drive, Menlo Park
515 South Dr., Menlo Park
846 Goldfield Rd., Menlo Park

http://bloodcenter.stanford.edu
for those destroyed by the stroke. Within three to six months, at least 90 per-cent of all the recovery a stroke patient is likely to experience takes place. No pharmacological therapy has been shown to improve recovery after a stroke. In fact, no effective treatments during the recovery phase exist, other than physical therapy, which has been shown to be only marginally successful.

Nerve-cell signaling bolstered

Steinberg and Bliss attributed zol-pidem’s effectiveness to an increase in the number of GABA receptors, which play a role in synaptic function. The drug was given to a region of the mouse brain, and the scientists looped in their colleagues’ recordings of the activity of GABA receptors elsewhere on their outer membranes, which they observed was resulting from an effect on a region of the far more common GABA receptor signaling.

To do that, Steinberg, Bliss and their colleagues conducted a series of anatomical, physiological and behavioral experi- ments. Their efforts were assisted by the fact that GABA receptors are small and differ from their extrasynaptic counterparts. So low doses were likely to enhance synaptic GABA signaling without having much of an effect on extrasynaptic GABA receptors.

The team delayed zolpidem adminis- tration until three days after the stroke. This was to avoid any possible effect of the drug on the brain tissue that had already been destroyed by the stroke and is known as a secondary insult. This was to ensure that the observed effect was resulting from an effect on brain recovery, rather than from the drug prolonging initial tissue damage from the stroke.

The researchers subjected these mice to two kinds of tests. One measured the mice’s ability to traverse a horizontal rotating beam. In almost every case, zolpidem-treated mice recovered at a faster rate than control mice did. It took about a month, for example, for mice not given zolpidem to recover their full ability to traverse the rotating beam. On the other hand, those given zolpidem recovered to full ability to navigate the rotating beam in about half the time. Mice given zolpidem recovered at a much faster rate.

To determine whether the transient increase in post-stroke synaptic GABA signaling was beneficial — and, if so, how much it would be needed to help stroke victims — the scientists turned to zolpidem, which works by enhancing synaptic GABA sig- naling. They induced either of two different types of strokes in mice — one type severely damages sensory ability; the other deeply impairs movement. This allowed them to test the drug in a regimen of either zol-pidem or a control solution that did not contain the drug.

Sub-sedative doses

The scientists administered the drug in sub-sedative doses to determine how the mice would perform on tests of sensory ability and motor coordination, so the mice needed to be fully awake. Zolpidem is known to have a much higher affinity for synapse-associated GABA receptors than for their extrasynaptic counterparts. So low doses were likely to enhance synaptic GABA signaling without having much of an effect on extrasynaptic GABA receptors.

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The threespine stickleback is remarkable in that it has evolved to have many different body structures to equip it for life in different parts of the world. It sports a protein in its hind limbs, but not its forelimbs, and a protein that is easy to visualize in mice. Laboratory vertebrates with engineered regulatory information were created for the first time a “transcriptional signature” that can be used as a proxy for whatever immune mechanism is induced by the presence of a pathogen. The signature has been found in human patients with viral infections, which is different from women’s. Other research has suggested that men’s immune response to vaccines was somehow suppressed. In previous work, researchers looked at men’s and women’s responses on the third day after vaccination, when women had no response at all. But Khatri’s group found that men were responding most on the first day after vaccination, when women showed a strong anti-Shigella response. The Khatri lab has funding to develop such a test.

The research was an example of Stanford Medicine’s focus on precision health, the goal of which is to anticipate and prevent disease in the healthy and precisely diagnose and treat disease in the ill.

Is the vaccine working?

Another goal is to more precisely determine whether someone is responding to vaccination. “The goal of vaccination is to generate the same immune response without the symptoms,” he said. “If the immune response is truly virus-specific, we should see the same response in vaccinates.” And, in fact, the Khatri team found that in three independent studies of flu vaccine recipients, all those judged to have responded to vaccination by other measures also displayed the 11-gene signature for all viruses, followed by other infections. “It seems that when there is a viral infection, you turn on the meta-virus signature,” said Khatri. “You can imagine a meta-virus, enterovirus and adenovirus, as well as bacteria such as SARS corona-virus, Bacteroidetes and Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae and Salmonella. In the larger group, the team found the same altered pattern of expression in the same genes in humans with viral infections.

The meta-virus signature not only identified individuals with an active viral infection, but also those who were in- cluencing one but not detecting flu from blood samples taken frequently — every eight hours for five days — the Stanford team discovered the meta-virus gene signature pattern waving in the background. The researchers then discovered the first symptoms. “An individual’s gene expression signature changed before they showed symptoms for the virus, but not until 24 hours before who was going to show symptoms,” said Khatri.

The same high-frequency sampling data also revealed that the meta-virus signature signal, the one indicating any vi- rus, began first, then, a few hours later, the more-specific influenza meta-signature signal began in people with the flu. “It seems that when there is a viral infection, you turn on the meta-virus signature response, and then it turns on, if it’s viral, which virus it is? And then it turns on a specialized response for that virus.”

Theoretically, the meta-virus signature could be used clinically to distin- guish viral from bacterial infections to determine whether antibiotics should be prescribed. The Khatri lab has funding to develop such a test.

The recent findings are an example of Stanford Medicine’s focus on precision health, the goal of which is to anticipate and prevent disease in the healthy and precisely diagnose and treat disease in the ill.

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Until now, said Khatri, no one has found the immune response that turns on in both the vaccination response and in actual infections. This paper demonstrates for the first time a “transcriptional signature” that can be used as a proxy for whatever immune mechanism is induced by the presence of a pathogen. The signature has been found in human patients with viral infections, which is different from women’s. Other research has suggested that men’s immune response to vaccines was somehow suppressed. In previous work, researchers looked at men’s and women’s responses on the third day after vaccination, when women had no response at all. But Khatri’s group found that men were responding most on the first day after vaccination, when women showed a strong anti-Shigella response. The Khatri lab has funding to develop such a test.

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Stanley Falkow to be awarded National Medal of Science

By Krista Conger

Stanley Falkow, PhD, the Robert W. and Vivian E. Cahill Professor in Cancer Research, Emeritus, at the School of Medicine, has been awarded the 2015 National Medal of Science. The honor was announced Dec. 22 by the White House.

Falkow is being recognized for his pioneering work in studying how bacteria can cause human disease and how antibiotic resistance spreads.

“It was a total surprise,” said Falkow, who learned of the award on Dec. 19 in an email from John Holdren, PhD, the president’s chief science advisor. “I always say, ‘In science, it’s not ‘I,’ it’s ‘we.’ And it’s so true. There are hundreds of students and colleagues around the world with whom I’d like to share this honor.”

“Dr. Falkow is deeply deserving of this award,” said Lloyd Minor, MD, the dean of the School of Medicine. “He has made invaluable contributions to the field of microbiology and understanding the effects of bacteria on human health. We at Stanford Medi- cine are extremely proud and honored he has been recognized in this way.”

Falkow, 81, is an emeritus professor of microbiology and immunology and a member of the Stanford Cancer Institute. The award will be presented in a ceremony at the White House later this month.

Falkow is well-known for his work on extrachromosomal elements called plasmids and their role in antibiotic resistance and pathogenicity in humans and animals. As a graduate student in the early 1960s, first at the University of Michigan and later at Brown University, and then as an independent researcher at Georgetown University, he learned the biochemical and microbiological techniques necessary to de- duce how bacteria transmit antibiotic resistance to one another. In particu- lar, he found that some bacteria were resistant to antibiotics to which they had never been exposed, which at first confounded researchers. Falkow subse- quently discovered that bacteria gained their resistance by sharing their genes much more promiscuously than had been thought possible.

When Falkow arrived at Stanford in 1981, he set aside his study of plasmids to concentrate on how organisms as diverse as cholera, plague and whoop- ing cough cause disease in humans.

Falkow is one of nine recipients of the 2015 National Medal of Science, which recognizes individuals for out- standing contributions to the fields of several scientific disciplines. He is one of two Stanford recipients; the other is psychologist Albert Bandura, PhD, the David Starr Jordan Professor, Emeritus.

“We congratulate both emeriti pro- fessors Stanley Falkow and Albert Band- dura on this extremely well-deserved honor. We are so proud that they have been recognized for their contributions not just to our country, but to human- ity,” said Stanford President John Hen- nessy, PhD. “Their lifetime of work in preventing infectious disease, and in learning how we can understand and change behavior, has been instrument- al in helping people around the world lead healthier, more productive and more peaceful lives.”

Falkow’s previous honors include the 2008 Lasker-Koshland Award for Special Achievement in Medical Sci- ence; the 2000 Robert-Koch Award from the Robert-Koch Foundation in Germany; election to the Institute of Medicine; membership in the Na- tional Academy of Sciences and the Royal Society; and a former presidency of the American Society of Microbiol- ogy.