INSIDE STANFORD MEDICINE

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High levels of diversity among natural killer cells may strongly predispose people to infection by HIV.
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Iron seen in brain immune cells of Alzheimer's disease patients

By Bruce Goldman

Examining postmortem brain tissue from Alzheimer's disease patients, School of Medicine investigators identified what appear to be iron-containing microglia — specialized scavenger cells that sometimes become inflammatory — in a particular part of the hippocampus, a key brain structure whose integrity is critical to memory formation.

In postmortem brain tissue from people not diagnosed with Alzheimer's, neither the iron deposits nor the scavenger cells engulfing them were present in that brain region.

The findings, recounted in a study now available online in *Neurobiology of Aging*, suggest that high-field magnetic resonance imaging, in particular an advanced version called 7T MRI that uses a powerful 7-Tesla magnet, could someday be used to diagnose and monitor Alzheimer's patients earlier than is currently possible.

The findings also add a new suspect to the Alzheimer's disease lineup. A long-held hypothesis holds that the most notorious feature of Alzheimer's disease, amyloid plaques, is the main cause of the disorder. These plaques are extracellular aggregations of a small protein called beta-amyloid that are prominent in diseased patients' brains, as well as in mouse models of the disease. The other most cited key player is tau, another Alzheimer's-associated protein that abnormally aggregates into threadlike tangles inside nerve cells. Surprisingly, in the brain region of interest there was no consistent overlap between the iron-laden microglia and the amyloid plaques or tau.

"Microglia are the brain's immune cells," said Michael Zeineh, MD, PhD, assistant professor of neuroradiology and the study's lead author. In their resting state, they're like police officers in the doughnut shop, sitting down and relaxing, their guns holstered, but keeping their eyes open while placidly munching on whatever cellular debris or stray

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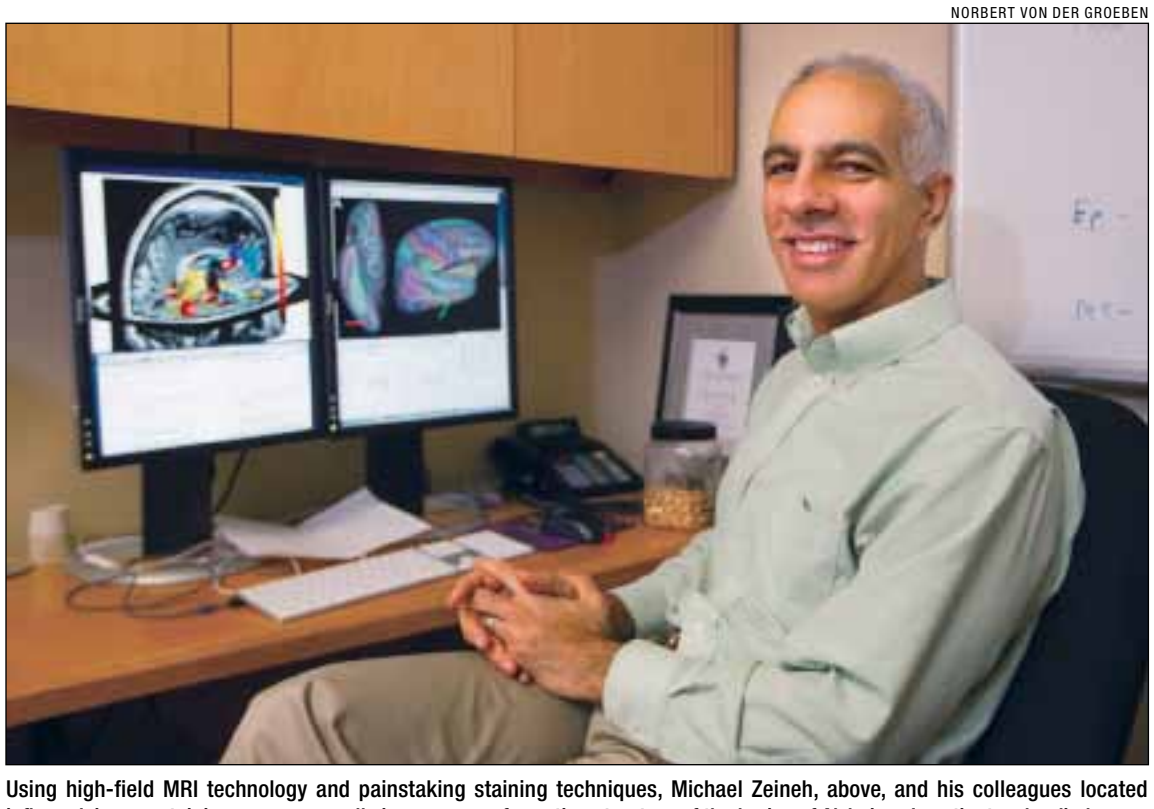
NORBERT VON DER GROEBEN



Using high-field MRI technology and painstaking staining techniques, Michael Zeineh, above, and his colleagues located inflamed, iron-containing scavenger cells in a memory-formation structure of the brains of Alzheimer's patients who died.

Juvenile inmates hospitalized more for mental health

By Erin Digitale

Juvenile inmates are much more likely to be hospitalized for mental health problems than children and teenagers who are not incarcerated, according to a new study from the School of Medicine.

In addition, the hospital stays of these inmates are longer, suggesting that their underlying mental health problems are worse.

The new study, which was published online July 21 in the *Journal of Adolescent Health*, examined almost 2 million hospitalizations of California boys and girls over a 15-year period. Mental health diagnoses were responsible for 63 percent of hospital stays by young people in the juvenile justice system, compared to 19 percent for those not in the system.

Although mental health problems have been previously documented in juvenile inmates, the study's large size and assessment of hospital stays gives new insight into the widespread nature and severity of their mental health diagnoses.

"We know young people in the juvenile justice system have a disproportionate burden of

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RUIGSANTOS / SHUTTERSTOCK

Antioxidants help treat skin-picking disorder in mice, researcher says

By Ruthann Richter

Two antioxidant supplements are effective in treating skin-picking disorder in mice, according to a study led by a School of Medicine researcher.

The finding suggests that people with the potentially serious disorder might benefit from this therapy.

An estimated 4 percent of the population — or about 1 in 25 — suffer from skin-picking disorder, in which repeated, compulsive picking or scratching of the skin can lead to severe disfigurement and life-threatening infection. Skin picking is also common among laboratory mice, which may develop potentially fatal ulcerative dermatitis, skin lesions, caused by excessive grooming.

The condition is the single leading avoidable cause of death among laboratory mice, said Joseph Garner, PhD, associate professor of comparative medicine and senior author of the study, which was published online July 13 in *PLOS ONE*. The lead author is Nneka George, DVM, of the University of North Carolina at Chapel Hill.

In the study, the researchers experimented with two antioxidant supplements — N-acetylcysteine and glutathione — to treat mice with skin-picking disorder. NAC is used by cells to make

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Cellular damage seen after CT scanning

By Tracie White

Using new laboratory technology, scientists have shown that cellular damage is detectable in patients after CT scanning, according to a new study led by researchers at the School of Medicine.

"We now know that even exposure to small amounts of radiation from computed tomagraphy scanning is associated with cellular damage," said Patricia Nguyen, MD, one of the lead authors of the study and an assistant professor of cardiovascular medicine at Stanford. "Whether or not this causes cancer or any negative effect to the patient is still not clear, but these results should encourage physicians toward adhering to dose-reduction strategies."

The study was published online July 22 in the *Journal of the American College of Cardiology: Cardiovascular Imaging*. Won Hee Lee, PhD, and Yong Fuga Li, PhD, both postdoctoral scholars, are the study's other lead authors.

"The use of medical imaging for heart disease has exploded in the past decade," said Joseph Wu, MD, senior author of the study. Wu, a professor of medicine and of radiology, is director of the Stanford Cardiovascular Institute. "These tests expose patients to a nontrivial amount of low-dose radiation. But nobody really knows exactly what this low-dose radiation does to the patient. We now have the technology that allows us to look at very subtle, cell-level changes."

Along with the burgeoning use of advanced medical imaging tests over the past decade have come rising public health concerns about possible links between low-dose radiation and cancer. The worry is that increased radiation exposure from such diagnostic procedures as CT scans, which expose the body to low-dose X-ray beams, can damage DNA and create mutations that spur cells to grow into tumors.

NITHINRAU

Computed tomography scanners are used for medical imaging.

'Legitimate concerns'

But there has been limited scientific evidence to date that shows the effects of this low-dose radiation on the body, according to the study. Currently, there is a bill winding its way through Congress to fund more research on the health effects of low doses of

See CT, page 6

Doctors testified for tobacco companies against cancer patients

By Tracie White

Despite scientific evidence to the contrary, a small group of otolaryngologists have repeatedly testified, on behalf of the tobacco industry, that heavy smoking did not cause the cancer in cases of dying patients suing for damages, according to a study by a School of Medicine researcher.

"I was shocked by the degree to which these physicians were willing to testify, in my opinion in an unscientific way, to deny a dying plaintiff — suffering the aftermath of a lifetime of smoking — of a fair trial," said Robert Jackler, MD, professor and chair of otolaryngology-head and neck surgery, referring to the physicians cited in the study as a "pool of experts willing to say over and over again that smoking didn't cause cancer."

The study was published online July 17 in *Laryngoscope*.

Jackler, who holds the Edward C. and Amy H. Sewall Professorship in Otorhinolaryngology, conducted a year and a half of research, which included reading through thousands of pages of publicly available, expert-witness depositions and trial testimony. He then reviewed the scientific literature to see if testimony by expert witnesses for the tobacco industry was supported by evidence. Jackler said that a physician serving as an expert has an ethical obligation to interpret the scientific data in a fair and balanced manner. The literature, he found, repeatedly repudiated the testimony. "My study found they used scientifically invalid methods to support their testimony," he said.

NORBERT VON DER GROEBEN

Robert Jackler reviewed thousands of pages of expert-witness depositions and trial testimony for his study.

Salted fish, mouthwash — but not tobacco?

The study reports that six board-certified otolaryngologists were paid by one or more of the tobacco companies R.J. Reynolds, Phillip Morris and Lorillard to serve as expert witnesses. These physicians gave testimony that indicated a multiplicity of environmental factors, ranging from exposure to cleaning solvents to the consumption of salted fish to the use of mouthwash, were more likely to have caused the plaintiff's head and neck cancers than years of heavy smoking. The cases occurred between 2009 and 2014. One physician said he was paid $100,000 to testify in a single case. Another admitted that her opinion was written by tobacco company lawyers and then approved by her. Still another rejected reports from the Surgeon General as authoritative sources.

Together, the six otolaryngologists in this study helped to defend the tobacco industry in more than 50 cases.

"Evidence shows that this testimony, which was remarkably similar across cases, was part of a defense strategy shaped by tobacco's law firms," the study said. "By highlighting an exhaustive list of potential risk factors, such as alcohol, diesel fumes, machinery fluid, salted fish, reflux of stomach acid, mouthwash and even urban living, they created doubt in the minds of the jurors as to the role of smoking in the plaintiff's cancer."

The study said the physicians were "well-coached" by tobacco lawyers, and their testimony was "faithful to the tactical narrative that there are many, many causes of head and neck cancer — and that factors other than smoking must have caused the plaintiff's disease."

'Obvious fallacy'

The study said: "An obvious fallacy of this argument lies in the fact that literally billions of nonsmoking people are exposed regularly to gasoline fumes, use cleaning solvents, eat salted fish or live in urban environments. Were these causative factors for head and neck cancer, with even a minute fraction of the potency of tobacco, the rate of head and neck cancer among nonsmokers would be much great than what has been observed."

Jackler has for years conducted scholarly research focusing on the tobacco industry's influence on public health. He has published multiple studies on the impact of the tobacco industry's advertising, marketing and promotion.

In this study, he reviewed nine cases that resulted from a 1999 Florida class-action suit (Engle v. Liggett) in which an award of $145 billion was reversed on appeal. The Florida Supreme Court decision in 2006 that upheld the Engle jury decision of widespread wrongdoing on the part of the tobacco industry enabled individual cases to proceed.

"The addictiveness of nicotine, the dangers of tobacco and the track record of industry deception and misconduct are considered factual in subsequent trials," the study said. "This has resulted in thousands of individual Engle progeny cases. Because the cases primarily focused on whether tobacco caused the plaintiff's diseases, expert testimony was crucial."

Plaintiffs' cancers

For the study, Jackler examined a small fraction of these progeny cases. Patients in these cases had cancer in sites such as the larynx, the mouth and the esophagus. All of the plaintiffs in these cases were long-term, heavy smokers — more than a pack a day for many years. The key issue in these lawsuits was whether it was more likely than not that smoking caused the individual plaintiff's cancer (greater than 50 percent probable is the legal standard). Although since the 1990s tobacco companies have admitted that their products cause cancer, in litigation they vigorously argue that smoking did not cause an individual plaintiff's cancer.

"Otolaryngologists in this study routinely expressed the opinion that, more likely than not, tobacco did not cause the smoker's head and neck cancer," the study said. "It is not credible that even a lengthy list of these causes come even remotely close to approaching the greater than 50 percent cause."

In contrast, the scientific literature demonstrates that tobacco directly contributes to head and neck cancers at a greater than 50 percent likelihood, Jackler said.

"[The tobacco industry identifies] the best experts that money can buy, [trains] them in their well-honed narrative to manufacture doubt in the minds of the jury and [makes] use of them over and over in case after case," the study said.

Given the ethical traditions of medicine, it seems likely that these physicians believe their well-compensated testimony on behalf of tobacco companies occurs in the shadows, out of view of their families, friends and professional colleagues, Jackler said.

Stanford's Department of Otolaryngology-Head and Neck Surgery supported the work. ISM

Low levels of hormone linked to social deficit in kids with autism

By Erin Digitale

A brain-chemistry deficit in children with autism may help to explain their social difficulties, according to new findings from the School of Medicine.

The research team found a correlation between low levels of vasopressin, a hormone involved in social behavior, and the inability of autistic children to understand that other people's thoughts and motivations can differ from their own.

The research was published July 22 in *PLOS ONE*.

"Autistic children who had the lowest vasopressin levels in their blood also had the greatest social impairment," said the study's senior author, Karen Parker, PhD, associate professor of psychiatry and behavioral sciences.

The findings raise the possibility that treatment with vasopressin might reduce social problems for autistic children who have low vasopressin levels, a hypothesis that Parker and her team are now testing in a clinical trial. However, the new research also showed that children without autism can have low vasopressin levels without displaying social impairment, Parker noted; in other words, autism is not explained by a vasopressin deficit alone.

Investigating vasopressin

Autism is a developmental disorder that affects 1 out of every 68 children in the United States. It is characterized by social and communication deficits and repetitive behaviors. The new study examined a social trait that psychologists call "theory of mind": the ability to understand that others have different perspectives. Poor "theory of mind" makes it harder for people with autism to empathize and form relationships with others.

Vasopressin is a small-protein hormone that is structurally similar to oxytocin. Like oxytocin, it has roles in social behavior. Vasopressin also helps regulate blood pressure.

In the new study, the researchers first verified that vasopressin levels in the blood accurately reflected vasopressin levels in the brain by measuring the hormone's levels simultaneously in the blood and cerebrospinal fluid of 28 people who were having the fluid collected for medical reasons.

They then recruited 159 children ages 3-12 for behavioral testing. Of these children, 57 had autism, 47 did not have autism but had a sibling who did, and 55 were typically developing children with no autistic siblings. All of the children completed standard psychiatric assessments of their neurocognitive abilities, social responsiveness, theory of mind, and ability to recognize others' emotions, which is known as affect recognition. All children gave blood samples that were measured for vasopressin.

In all three groups, children had a wide range of vasopressin levels, with some children in each group having low, medium and high levels. Children without autism had similar scores on theory of mind tests regardless of their blood vasopressin level, but in children with autism, low blood vasopressin was a marker of low theory of mind ability.

NORBERT VON DER GROEBEN

Karen Parker and her colleagues found that low levels of the hormone vasopressin in children with autism predict a social deficit that affects their ability of to empathize with others.

Testing the hormone's effects

Parker and her collaborator, Antonio Hardan, MD, professor of psychiatry and behavioral sciences, are now investigating whether vasopressin treatment improves social ability in children with autism. They are interested in whether the hormone is beneficial only for autistic children who start with low vasopressin levels or whether it might benefit all children with **See AUTISM, page 3**

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STANFORD MEDICINE

Women's immune system genes operate differently than men's, study finds

By Jennie Dusheck

A new technology for studying the human body's vast system for toggling genes on and off reveals that genes associated with the immune system toggle more frequently, and those same genes operate differently in women and men.

Some genes are virtually always on, like the clock light on a microwave; others sit unused for years at a time, like some regrettable appliance you bought, stuffed into the back of the closet and forgotten. Some genes can be always on in one person and always off in another. A minority of genes switch on and off, like a favorite cell phone app. A new technology, which makes it possible to study the molecules that regulate all of that switching in living people as they go about their lives, has revealed some intriguing surprises, according to a study from the School of Medicine.

One of those discoveries is that the genes that switch on and off differently from person to person are more likely to be associated with autoimmune diseases. Another is that women and men use different switches to turn on many immune system genes. It's too soon to be sure, but that difference in activity might explain the much higher incidence in women of autoimmune diseases such as scleroderma, lupus and rheumatoid arthritis.

"Part of why this is possible is a new technology that was invented at Stanford for measuring the accessibility of the genome to regulatory elements," explained the study's senior author, Howard Chang, MD, PhD, professor of dermatology.

The new technique, called ATAC-seq and developed by Chang's team, lets researchers sample living cells in real time to see what they are up to. "In the past," he said, "people needed a huge number of cells to do this kind of measurement. You'd actually need a pound of flesh to get certain rare cell types. So you can't get that out of a live person — and certainly not more than once, right?"

Examining the source

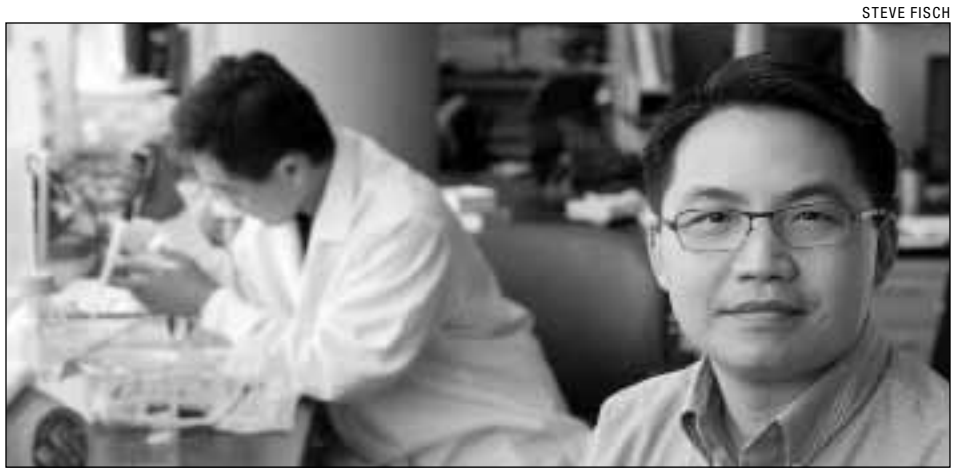
Researchers coped by growing cells in the lab so they had enough cells to study. "But now," continued Chang, "you are studying copies of copies; you aren't studying the original cells anymore. Those months of being grown in the lab completely changes how the cells are behaving and so you are no longer looking at the personal. How the laboratory cells behave has nothing to do with what the person just ate, whether they had a fight with their girlfriend or whether they had an infection," said Chang. With lab-grown cells, the cells haven't experienced any of those things, all of which can alter the regulation of individual genes.

The new study, which was published July 29 in the new journal *Cell Systems*, took ordinary blood samples from 12 healthy volunteers to measure how certain genes are switched on and off, and how that measure varied from individual to individual. Chang's team also looked at how much change occurred at different times in the same volunteers. The researchers looked exclusively at specialized immune cells called T cells, which are easy to isolate from a standard blood test and easy for volunteers to supply and which are an important component of the immune system.

One goal of the study was to establish a baseline measure of how much this gene-switching activity varies among healthy people. That way, when other researchers make similar measures in people who are ill, they'll have an idea of what is normal. Another goal was to refine the new technique for measuring gene activity in standard blood samples.

"We were interested in exploring the landscape of gene regulation directly from live people and look at differences," said Chang. "We asked, 'How different or similar are people?' This is different from asking if they have the same genes." Even in identical twins, he said, one twin could have an autoimmune disease and the other could be perfectly well. And, indeed, the team reported that over a third of the variation in gene activity was not connected to a genetic difference, suggesting a strong role for the environment. "I would say the majority of the difference is likely from a nongenetic source," he said.

STEVE FISCH



Howard Chang is the senior author of a study revealing that immune system genes switch on and off differently in women and men, and that the source of that variation is not primarily in the DNA.

The sex factor

Across the 12 volunteers, 7 percent of the genes were switched on in different patterns from person to person. For each person, these patterns persisted over time, like a unique fingerprint. "But the single greatest predictor for genes' tendency to turn on and off was the sex of the person. In terms of significance," said Chang, "sex was far more important than all the other things we looked at, perhaps even combined."

When the team measured gene activity levels from 30 of the top 500 genes the researchers expected would show gender-influenced activity, they found that 20 of the 30 genes showed significant differential activity between men and women.

Chang directs the Center for Personal Dynamic Regulomes at Stanford University, which aims to map the "regulome" — the complete set of all the switches that turn genes on and off in real time.

Other Stanford-affiliated authors of the paper are Kun Qu, PhD, senior research associate; Lisa Zaba, MD, PhD, instructor of dermatology; Paul Giresi, PhD, former postdoctoral scholar; Rui Li, life science research assistant; Michelle Longmire, MD, clinical instructor of dermatology; Youn Kim, MD, the Joanne and Peter Haas Jr., Professor for Cutaneous Lymphoma Research; and William Greenleaf, PhD, assistant professor of genetics.

This work was supported by the National Institutes of Health, the Howard Hughes Medical Institute, the Stanford Cancer Center, the Scleroderma Research Foundation and the Haas Family Foundation.

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

Autism

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autism. More information about the team's vasopressin clinical trial is available at http://med.stanford.edu/clinicaltrials/trials/NCT01962870 or by calling Robin Libove at 736-1235.

Other Stanford-affiliated authors of the paper are Dean Carson, PhD, postdoctoral scholar; Joseph Garner, PhD, associate professor of comparative medicine; laboratory managers Shellie Hyde and Raena Sumiyoshi; Robin Libove, social science research assistant; Sean Berquist, former social science research assistant; Kirsten Hornbeak, medical resident; Lisa Jackson, graduate student; Christopher Howerton, PhD, former postdoctoral scholar; Sadie Hannah, RN, nurse practitioner in pediatric oncology; Sonia Partap, MD, clinical assistant professor of neurology and neurological sciences and a pediatric neuro-oncologist at Lucile Packard Children's Hospital Stanford; Jennifer Phillips, PhD, clinical associate professor of psychiatry and behavioral sciences and co-director of the Autism and Developmental Disabilities Clinic at Lucile Packard Children's Hospital Stanford; and Hardan, who is division chief of child and adolescent psychiatry, and director of the Autism and Developmental Disabilities Clinic.

Parker and Hardan are members of Stanford's Child Health Research Institute, the Stanford Autism Center, Bio-X and the Stanford Neurosciences Institute. Garner is a member of the Child Health Research Institute and Bio-X.

The research was funded by a Simons Foundation Autism Research Initiative Pilot Award, the Katherine D. McCormick Fund, the Mosbacher Family Fund for Autism Research, Stanford's Bio-X NeuroVentures Program, the Weston Havens Foundation, Stanford's Child Health Research Institute, an Autism Speaks Meixner Fellowship in Translational Research, a Stanford School of Medicine Dean's Postdoctoral Fellowship, and the National Institutes of Health.

The Department of Psychiatry and Behavioral Sciences also supported the research. ISM

Heart-health app launches in UK, Hong Kong

By Tracie White

MyHeart Counts, an iPhone app that allows users to learn about their own heart health while also participating in a large-scale study designed by cardiologists at the School of Medicine, became available in Hong Kong and the United Kingdom on Aug. 6.

MyHeart Counts is the first of the initial handful of apps designed using ResearchKit, Apple's open-source software platform for creating medical-research apps, to expand overseas.

"The idea is to move into one country at a time until we go global," said Euan Ashley, MD, a professor of cardiovascular medicine at Stanford and co-investigator for the MyHeart Counts study. "We hope to add more countries every few months."

The app collects data about physical activity and cardiac risk factors for Stanford scientists studying the prevention and treatment of heart disease. They aim to make MyHeart Counts the largest study of measured physical activity and cardiovascular health to date.

A new version of the iPhone app was also launched Aug. 6. Researchers are encouraging the more than 41,000 users who have agreed to participate in the study to upgrade to the new version.

"Now, we're actually giving data back to the participants," Ashley said. "For example, the participants can see where they fall in relation to others on their 6-minute walk test."

The MyHeart Counts app, which is free, was launched in March as a way for users to learn about their heart health while helping advance the field of cardiovascular medicine. Built on Apple's ResearchKit framework, the app uses the iPhone's built-in motion sensors to collect data on physical activity and other cardiac risk factors for a research study.

"We are ready to take the study as far as it will go. We would like to build a new Framingham heart study for the ages," Ashley said, referring to the long-term cardiovascular study that has followed three generations of participants in Framingham, Massachusetts. "We would like millions of participants."

Once every three months, participants are asked to monitor one week's worth of physical activity, complete a 6-minute walk fitness test if they are able to, and enter their risk-factor information. The app now also delivers a comprehensive summary of each user's heart health and areas for improvement.

The new version of the app focuses on empowering participants with more feedback about their individual behaviors and risk, based on the American Heart Association's "Life's Simple 7" recommendations. "We'll now be providing feedback about physical activity, diet, blood pressure and cholesterol levels," said Michael McConnell, MD, professor of cardiovascular medicine and principal investigator for the study.

Researchers are reporting that they have collected the most data ever on the 6-minute walk fitness test for a single study. The new version of the app will include information comparing the user's data to others participating in the fitness test, as well as a newsfeed section that provides updates on heart health news.

For information about the app, visit https://myheartcounts.stanford.edu. ISM

NORBERT VON DER GROEBEN

The MyHeart Counts app has garnered more than 41,000 users who have agreed to participate in a study of cardiovascular health.

Elusive liver stem cell identified in mice, solving longtime mystery

By Krista Conger

Researchers at the School of Medicine have identified a cell type in the liver of mice that can both self-renew and make new liver cells. The discovery solves a long-standing mystery as to how the organ, which is responsible for many metabolic processes, maintains itself when liver cells, most of which are called hepatocytes, grow old and die.

"There's always been a question as to how the liver replaces dying hepatocytes," said professor of developmental biology Roel Nusse, PhD. "Most other tissues have a dedicated population of cells that can divide to make a copy of themselves, which we call self-renewal, and can also give rise to the more-specialized cells that make up that tissue. But there never was any evidence for a stem cell in the liver."

Researchers have assumed instead that mature hepatocytes would themselves divide to replace a dying neighbor. However, these cells have an abnormal amount of DNA, which would make cell division extremely difficult.

Nusse is the senior author of the work, which was published Aug. 5 in *Nature*. He is also a member of the Stanford Cancer Institute, the Stanford Institute for Stem Cell Biology and Regenerative Medicine and a Howard Hughes Medical Institute investigator. First author Bruce Wang, MD, an assistant professor of gastroenterology and hepatology at the University of California-San Francisco, led the research as a visiting scholar in Nusse's lab.

The liver is a large, multi-lobed organ that plays a vital role in filtering toxins from the blood. It also makes digestive enzymes and is involved in many important metabolic processes. A central vein carries blood through each lobe of the organ; the stem cells identified by Wang and Nusse are found adjacent to these veins.

Roel Nusse

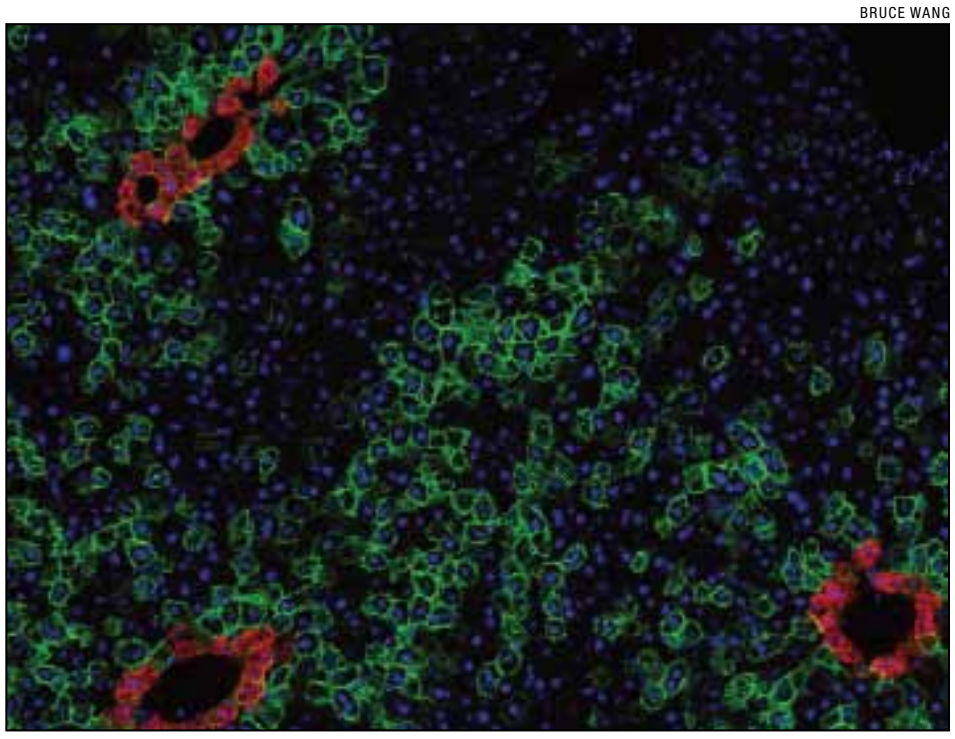
Abundance of chromosomes

Until now, it's been thought that there was just one class of hepatocytes in the liver. Most of these mature cells are polyploid, meaning they have more than the normal two copies of each chromosome. Although this abundance of chromosomes makes it difficult, if not impossible, for the cells to divide normally, it may confer other benefits.

"If it's not necessary for a cell to maintain the capacity to divide, it can do whatever it wants with its genome," said Nusse. "Red blood cells, for instance, have no DNA. Muscle cells have many copies of each chromosome." An advantage of making extra copies of chromosomes could be the enhanced ability to quickly make large amounts of particular proteins, for example.

In contrast, the cell population identified by Wang and Nusse in the mice is diploid, with a normal complement of DNA. They can divide to make others like themselves, or to make cells that start as diploid but then acquire additional copies of their genome as they move outward from the central vein into the main body of the liver.

BRUCE WANG



Liver stem cells (red) from mice surround the central veins of the organ. Their descendants (green) have spread outward over a period of one year to populate much of the liver.

"People in the field have always thought of hepatocytes as a single cell type," said Wang. "And yet the cell we identified is clearly different from others in the liver. Maybe we should accept that there may be several subtypes of hepatocytes, potentially with different functions."

Wang and Nusse identified the liver stem cells around the central veins by looking in mice for cells anywhere in the organ that expressed a protein called Axin2. The protein is produced by cells in response to the presence of members of the Wnt signaling protein family. Years of research in the Nusse laboratory have shown that the Wnt proteins play a critical role in embryonic development, and also in the growth and maintenance of stem cells throughout the body.

Wang and Nusse further found that, in the liver, the endothelial cells that line the interior surface of the central veins make Wnt2 and Wnt9b. These Wnt proteins, in turn, confer stem cell properties on the neighboring hepatocytes.

Finally, the researchers learned that a portion of the descendants of the Axin2-expressing cells move outward from the central vein over time. These cells become polyploid and begin to express other, hepatocyte-specific genes. After one year, these descendants had replaced about 30 percent of the entire mouse liver, and made up about 40 percent of all hepatocytes in the organ.

The newly identified liver stem cell also expresses genes associated with very early embryonic development, which may give a clue as to when and where they arise.

"Perhaps these stem cells in the adult liver actually arise very early in development," said Nusse, "when the embryo sets aside a certain population of cells to maintain the organ during adult life."

Potential aid for drug testing

Although the current research was conducted in mice, the possibility that there is more than one kind of hepatocyte in humans could transform the study of liver biology, the researchers said. For example, hepatocytes have proven notoriously difficult to grow in laboratory culture for study or for use in drug testing.

"The most common reason that promising new drugs for any type of condition fail is that they are found to be toxic to liver," said Wang. "Researchers have been trying for decades to find a way to maintain hepatocytes in the laboratory on which to test the effects of potential medications before trying them in humans. Perhaps we haven't been culturing the right subtype. These stem cells might be more likely to fare well in culture."

There's also an opportunity to better understand human disease.

"Does liver cancer arise from a specific subtype of cells?" said Wang. "This model also gives us a way to understand how chromosome number is controlled. Does the presence of the Wnt proteins keep the stem cells in a diploid state? These are fundamental biological questions we can now begin to address."

Other Stanford-affiliated authors of the paper include MD/PhD student Ludan Zhao and research associates Matt Fish and Catriona Logan, PhD.

The research was supported by the Howard Hughes Medical Institute and the Reed-Stinehart Foundation.

Stanford's Department of Developmental Biology also supported the work. ISM

Team links gene expression, immune system with cancer survival rates

By Krista Conger

Physicians have long sought a way to accurately predict cancer patients' survival outcomes by looking at biological details of the specific cancers they have. But despite concerted efforts, no such clinical crystal ball exists for the majority of cancers.

Now, researchers at the School of Medicine have compiled a database that integrates gene expression patterns of 39 types of cancer from nearly 18,000 patients with data about how long those patients lived.

Combining the data from so many people and cancers allowed the researchers to overcome reproducibility issues inherent in smaller studies. As a result, the researchers were able to clearly see broad patterns that correlate with poor or good survival outcomes. This information could help them pinpoint potential therapeutic targets.

"We were able to identify key pathways that can dramatically stratify survival across diverse cancer types," said Ash Alizadeh, MD, PhD, an assistant professor of medicine and a member of the Stanford Cancer Institute. "The patterns were very striking, especially because few such examples are currently available for the use of genes or immune cells for cancer prognosis."

In particular, the researchers found that high expression of a gene called FOXM1, which is involved in cell growth, was associated with a poor prognosis across multiple cancers, while the expression of the KLRB1 gene, which modulates the body's immune response to cancer, seemed to confer a protective effect.

A paper describing the research was published online July 20 in *Nature Medicine*. Alizadeh shares senior authorship with Sylvia Plevritis, PhD, professor of radiology. Postdoctoral scholar Aaron Newman, PhD, and senior research scientist Andrew Gentles, PhD, share lead authorship of the paper.

The new database, which will be available to physicians and researchers, is called PRECOG, an abbreviation for "prediction of cancer outcomes from genomic profiles."

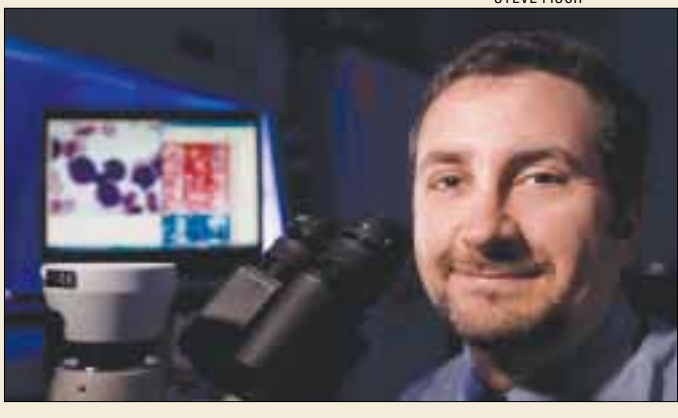
In addition to identifying potentially useful gene expression patterns in cancers, the researchers also used Cibersort, a recently published technique developed by Newman in Alizadeh's laboratory, to determine the composition of white blood cells that flock to a tumor. Cibersort assesses the relative levels of specific immune cells from a mishmash of cancer and normal cells and deduces the cell types from genes expressed in the bulk tumor — a process that Newman likens to analyzing a smoothie to identify its component fruits and berries.

"We were able to infer which immune cells are present or absent in individual solid tumors, to estimate their prevalence and to correlate that information with patient survival," said Newman. "We found you can even broadly distinguish cancer types just based on what kind of immune cells have infiltrated the tumor."

Putting it all together

Researchers have tried for years to identify specific patterns of gene expression in cancerous tumors that differ from those in normal tissue. By doing

STEVE FISCH



A database compiled by Ash Alizadeh and his team links gene expression patterns and immune system response to patient survival rates in nearly 18,000 cases of 39 types of cancers.

Researchers link HIV susceptibility to class of immune cells

By Bruce Goldman

High diversity among certain cells that help fight viruses and tumors is strongly associated with the likelihood of subsequent infection by HIV, School of Medicine researchers have found.

Natural killer cells, or NK cells, are lymphocytes, a type of white blood cell. NK cells' increased diversity, the scientists learned, may stem from prior exposures to viruses.

The findings, described in a study published July 22 in *Science Translational Medicine*, could spur the development of blood tests capable of flagging individuals' susceptibility to viral infection. The study also offers insights into the workings of NK cells, a somewhat poorly understood but crucial group of immune cells.

"This puts NK-cell diversity on the map as a metric of immune function," said Catherine Blish, MD, PhD, assistant professor of infectious diseases and geographical medicine and the study's senior author. "But it was a first foray. Before we can say definitively that NK-cell diversity predicts a person's susceptibility to infection, we need to validate these findings by looking at large numbers of individuals in a different population.

"NK cells are particularly suited to detecting and demolishing virally infected or cancerous cells," Blish added. "They arrive on the scene quickly, and they act quickly. An NK cell can kill an infected cell in 10 minutes."

Unexpected finding

Unexpectedly, it was higher, rather than lower, diversity in this immune-cell population that turned out to be associated with increased HIV susceptibility in the study. The investigators had figured that, as is the case with B cells and T cells — the two other, better-known types of lymphocytes — diversity in NK cells would be a strength, not a detriment, Blish said.

"Our hypothesis was wrong," she said. "We didn't think NK-cell diversity would be a bad thing, or that NK cells' diversification would occur to the extent that it does with viral exposure."

Using a cutting-edge, single-cell analytic technology called mass cytometry, Blish and her colleagues, including the study's lead author, graduate student Dara Strauss-Albee, showed that overall diversity in people's NK-cell repertoires is low at birth and steadily accumulates over the course of a lifetime.

An individual T cell has surface receptors that recognize unique protein snippets, called peptides, on other cells' surfaces. The structures of these receptors, which can discern "healthy" versus "suspect" peptides, differs from T cell to T cell. So a healthy person's legion of T cells can surveil and sort out hundreds of millions of different peptides representing possible invaders.

Unlike their T cell cousins, NK cells don't have surface receptors that recognize unique peptides. Instead, these lymphocytes harbor various combinations of generic receptors. Some receptors recognize signs of other cells' normalcy, and others recognize signs that a cell is stressed — due, say, to viral infection or cancerous mutation. On recognizing their targeted features on other cells' surfaces, an NK cell's "normalcy" receptors tend to inhibit it, while its stress-recognizing receptors activate it.

All told, NK cells can have many thousands of different combinations of these receptors on their surfaces, with each combination yielding a slightly different activation threshold. An NK cell's surface features also vary depending on its degree of maturation.

Mass cytometry analysis of NK cells exposed in a dish to HIV — as well as to West Nile virus, which differs substantially from HIV in its makeup and its modus operandi — showed that exposure to virus-infected cells leads to differentiation of NK cells and to an increased diversity among them. But diversification in the NK-cell population, the experiments indicated, was associated with a diminished ability of these cells' ability to replicate and kill.

The researchers also showed that while healthy human adults differ considerably from one another in the diversity of their NK-cell populations, a given adult's NK-cell population remains quite stable, changing little over periods of many months. An examination of NK cells extracted from umbilical-cord blood showed that newborns' NK-cell population is much less diverse.

HIV link

Blish said that she believes that viral exposure during one's lifetime is the driving force behind the maturation, differentiation and diversification of NK cells.

In order to assess the impact of NK-cell diversity on adult humans' viral susceptibility, Blish and her associates turned to blood samples that had been drawn during the Mama Salama Study, a longitudinal study of just over 1,300 healthy pregnant or postpartum Kenyan women. In that study, 25 of the women were found to have HIV. For 13 of these women, blood drawn both before and after infection was available.

> **"This puts NK-cell diversity on the map as a metric of immune function."**

Using mass cytometry, the researchers carried out a precise analysis of NK cells in the women's blood and observed a strong positive correlation between the diversity of a woman's NK cell population and her likelihood of becoming infected with HIV. This correlation held up when the scientists controlled for age, marital status, knowledge of sexual partners' HIV status and history of trading sex for money or goods. The two groups of women were also statistically indistinguishable with respect to their sexually transmitted disease status or their reported frequency of recent unprotected sex.

The NK-diversity-dependent difference in these women's likelihood of HIV infection was huge. Those with the most NK-cell diversity were 10 times as likely as those with the least diversity to become infected.

A 10-fold risk increase based solely on NK-cell diversity is far from negligible, said Blish. "By way of comparison, having syphilis increases the risk of contracting HIV two- to four-fold, while circumcised men's HIV risk is reduced by a factor of 2.5 or 3," she said.

The observations could have clinical potential, most immediately by spotlighting people who need to be closely monitored for possible viral infections and, perhaps, prophylactically treated. But Blish cautioned that the study remains preliminary.

Other Stanford co-authors are professor of statistics Susan Holmes, PhD; statistics graduate student Julia Fukuyama; research assistant Emily Liang (now a medical student at UCLA); and immunology graduate student Justin Jarrell.

The study was funded by a Beckman Young Investigator Award, a National Institutes of Health New Innovator Award and a National Science Foundation training grant.

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

JUAN GAERTNER / SHUTTERSTOCK

Natural killer cells, illustrated here in white, attack viruses and tumors in the body.

Catherine Blish

s; information could help pinpoint potential therapeutic targets

so, it may be possible to learn what has gone wrong in the cancer cells, and give ideas as to how best to block the cells' destructive growth. But the extreme variability among individual patients and tumors has made the process difficult, even when focused on particular cancer types.

"There are many more genes in a cell than there are patients with any one type of cancer, and this makes discovering the important genes for cancer outcomes a tough problem," said Gentles. "Because it's easy to find spurious associations that don't hold up in follow-up studies, we combined information from a vast array of cancer types to better see meaningful correlations."

Gentles and Alizadeh first collected publicly available data on gene expression patterns of many types of cancers. They then painstakingly matched the gene expression profiles with clinical information about the patients, including their age, disease status and how long they survived after diagnosis. Together with Newman, they combined the studies into a final database.

"We wanted to be able to connect gene expression data with patient outcome for thousands of people at once," said Alizadeh. "Then we could ask what we could learn more broadly."

Seeing the forest

By looking at the forest, rather than the trees, the researchers made some surprising findings. They observed that prognostic genes were often shared among distinct cancer types, suggesting that similar biological programs impact survival across cancers. They were able to identify the top 10 genes that seemed to confer adverse outcomes, and the top 10 associated with more positive outcomes. Many of these genes are involved in aspects of cell division or are associated with distinct types of white blood cells that flood a tumor.

They were also able to identify combinations of white blood cells that appear favorable. In particular, the presence of elevated numbers of plasma cells, which secrete large amounts of antibodies, and certain types of T cells correlated with better patient survival rates across many different types of solid cancers, including lung and breast cancers. Conversely, a high proportion of neutrophils, also known as granulocytes, were associated with adverse outcomes.

> **"We wanted to be able to connect gene expression data with patient outcome for thousands of people at once."**

The researchers hope that PRECOG and Cibersort will increase our understanding of cancer biology and aid in the development of new therapies for cancer patients. In addition, the researchers are applying these tools to better predict which patients will respond to new and emerging anti-cancer therapies. This is especially important given recent advances in the development of drugs that engage immune responses in cancer patients, but work well only for a subset of patients, said Alizadeh.

Other Stanford-affiliated authors are research associate Chih Long Liu, PhD; former postdoctoral scholars Scott Bratman, MD, PhD, and Dongkyoon Kim, PhD; lab manager Weiguo Feng, PhD; instructor Viswam Nair, MD; senior research scientist Yue Xu, MD, PhD; laboratory assistant Amanda Khuong; senior medical technologist Chuong Hoang, MD; assistant professor of radiation oncology Maximilian Diehn, MD, PhD; and professor of pathology Robert West, MD, PhD.

The research was supported by the Doris Duke Charitable Foundation, the National Institutes of Health, the B&J Cardan Oncology Research Fund, the Ludwig Institute for Cancer Research, the Department of Defense, the Siebel Stem Cell Institute and the Thomas and Stacey Siebel Foundation.

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

CT

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radiation, Wu said. This study's findings point to the need for more research, he said.

"I think there are legitimate concerns about the exposure to low-dose radiation, but the problem is that it is difficult to prove a causal relationship with cancer," Nguyen said. "Even though we show some damage is occurring at a cellular level, this damage is being repaired. It is the damage that escapes repair, or the cells that are not eliminated and are mutated, that go on and produce cancer. We can't track those cells with current technology."

In this study, researchers examined the effects on human cells of low-dose radiation from a wide range of cardiac and vascular CT scans. These imaging procedures are commonly used for a number of reasons, including management of patients suspected of having obstructive coronary artery disease, and for those with aortic stenosis in preparation of transcatheter aortic valve replacement.

A CT scan, which is used for imaging and diagnostic procedures throughout the body, exposes patients to at least 150 times the amount of radiation from a single chest X-ray, the study said.

In 2007, the National Cancer Institute estimated that 29,000 future cancer cases could be attributed to the 72 million CT scans performed in the country that year.

Increase in DNA damage, cell death

But the reliability of such predictions depends on how scientists measure the underlying link between radiation and cancer in the first place, Nguyen said.

"Because we don't know much about the effects of low-dose radiation — all we know is about high doses from atomic bomb blast survivors — we just assume it's directly proportional to the dose," said Nguyen. "We wanted to see what really happens at the cellular level."

Researchers examined the blood of 67 patients undergoing cardiac CT angiograms. Using such techniques as whole-genome sequencing and flow cytometery to measure biomarkers of DNA damage, researchers examined the blood of patients both before and after undergoing the procedure.

Joseph Wu

Results showed an increase in DNA damage and cell death, as well as increased expression of genes involved in cell repair and death, the study said. Although most cells damaged by the scan were repaired, a small percentage of the cells died, the study said.

"These findings raise the possibility that radiation exposure from cardiac CT angiography may cause DNA damage that can lead to mutations if damaged cells are not repaired or eliminated properly," the study said. "Cumulative cell death after repeated exposures may also be problematic."

"We need to learn more because it's not a benign effect even at these low dosages," Nguyen said. "Our research supports the idea that maybe physicians shouldn't just use the best image quality in all cases. We shouldn't eliminate CT scans because they're obviously important, but you can make it safer by reducing the doses, by getting better machines and technology, and by giving patients something to protect them."

Nguyen added: "It is important to note that we did not detect any DNA damage in patients receiving the lowest doses of radiation and who were of average weight and had regular heart rates."

Other Stanford authors are postdoctoral scholars Shijun Hu, PhD, Charles Chan, PhD, Jared Churko, PhD, and Sang-Ging Ong, PhD; medical student Wan Xing Hong; research assistants Grace Liang and Ivy Nguyen; Jia Wang, PhD, CT physicist; Russ Altman, MD, PhD, professor of bioengineering, of genetics and of medicine; and Dominik Fleischmann, MD, professor of radiology.

This work was supported by the American Heart Association, the National Institutes of Health and the Stanford Cardiovascular Institute.

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

Juvenile

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mental illness, but I was really surprised by the magnitude of the problem, because hospitalizations typically occur for very severe illness," said the study's lead author, Arash Anoshiravani, MD, clinical assistant professor of adolescent medicine. Mental-health hospital stays were even more common in detained girls than boys, he noted. "If you just looked at girls, 74 percent of their hospitalizations were for mental illnesses," he said. "That's pretty sobering."

Anoshiravani is also an adolescent medicine specialist at Lucile Packard Children's Hospital Stanford and medical director of the Santa Clara County Juvenile Custody Institutions.

The study examined all California hospital discharges between 1997 and 2011 for 11- to 18-year-old kids. Data on non-California residents were excluded, leaving 1.9 million hospitalizations. Of these, 11,367 were for patients who had come from or were being discharged to a juvenile detention facility.

Types of mental-health diagnoses

Hospitalized juvenile inmates were older, more likely to be male, to be African-American, to be from larger urban counties and to have public health insurance than their nonincarcerated counterparts.

Median hospital stays were about one day longer for inmates than noninmates (six compared with five days). However, for certain categories of hospitalization, the gap in length of stay was much greater: Teens and children transferred to substance-abuse treatment facilities had a median stay of as long as 71 days if they were in the juvenile justice system, versus 28 days for nondetained young people. Because more juvenile inmates are publicly insured, these longer stays increase public expenditure, too.

The types of mental health diagnoses did not differ much between the groups: Depressive disorders, substance abuse and conduct disorders were the most common mental health problems in both groups, with conduct disorders occurring somewhat more often in detained youth. The types of diagnoses suggest that

NORBERT VON DER GROEBEN

Arash Anoshiravani and his colleagues found that children and teens in the juvenile justice system are more likely to be hospitalized for mental health problems than those not in the system.

many incarcerated teens' mental health problems developed in response to stressful and traumatic childhood experiences, such as being abused or witnessing violence, Anoshiravani said.

"They're regular kids who have had really, really horrible childhoods," he said, adding that he hopes the new data will motivate social change around the problem.

"We are arresting kids who have mental health problems probably related to their experiences as children," he said. "Is that the way we should be dealing with this, or should we be getting them into treatment earlier, before they start getting caught up in the justice system?"

Other Stanford-affiliated authors of the paper are Olga Saynina, social science research assistant; Lisa Chamberlain, MD, associate professor of pediatrics; Lynne Huffman, MD, associate professor of pediatrics; Ewen Wang, MD, professor of surgery; and Paul Wise, MD, professor of pediatrics.

The work was funded by an early career investigator award from Stanford's Child Health Research Institute, of which Chamberlain, Huffman and Wise are members.

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

Skin

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glutathione, which is the brain's main, naturally occurring antioxidant. NAC has been used experimentally in people with a variety of conditions, including Parkinson's disease, autism and cystic fibrosis, and reports of individual cases suggest it could be useful in treating skin picking.

Potentially fewer side effects from glutathione

"With NAC, almost every mouse got a little bit better," Garner said. "But there is a huge variability in response, anywhere from a slight improvement to complete cure."

NAC also took a long time to show an effect: If an animal was cured, it took six to eight weeks. With glutathione, the pattern was very different. Fifty percent responded, but in these animals it was a rapid, all-or-nothing response: If they got better, they were completely cured in two to three weeks. The animals that didn't get better were worse off to start with, the researchers found

"What's exciting is that we have a compound that works. It works as well as NAC. It's clearly working differently, or at least more directly. This different response profile gives us some hope that there may be some nonresponders, or people who can't tolerate NAC, who may be helped by glutathione," he said.

Joseph Garner

Skin-picking disorder is a surprisingly common condition, yet many patients avoid seeking help because of the shame and embarrassment, Garner said.

"People suffer in complete silence," he said. "They think they are the only one who has it, despite the fact that it's very common, and it kills people."

By the time people do seek help, about 35 percent have required some kind of antibiotic treatment, and 5 percent have required intravenous antibiotics to treat potentially life-threatening infections, he said. Some are referred for psychiatric help. Although cognitive behavioral therapy can be an effective form of treatment, there are few practitioners equipped to do this form of therapy, Garner said.

People with the condition also may be prone to compulsive hair pulling, another body-focused, repetitive-behavior disorder.

A clinical trial among patients with the hair-pulling disorder, also known as trichotillomania, showed NAC to be an effective treatment in 56 percent of cases.

Study methods

For the study, the researchers selected 16 mice with lesions on the face, neck and limbs — signs of skin picking. Because the condition is painful and potentially fatal, the researchers treated all the mice with a thin film of topical antibiotic and steroidal ointment to relieve their discomfort. A third of the mice received a high dose of NAC in their drinking water. Another third were given drops of glutathione on the nose. (Because mice are nose breathers, they easily inhaled the compound.) A control group was given neither of the compounds.

Almost all the animals treated with NAC showed some improvement, though the improvement was slow. By the end of the study, 40 percent were fully cured. Among the animals treated with glutathione, results were more rapid: Within two to four weeks, about half were cured; the other half did not respond. There was no change among the control animals.

Garner believes that NAC works by combating oxidative stress that causes certain cells in the brain to die or become inactive. "Our thought is maybe NAC works because in the brain it is the precursor to glutathione, and the brain has to make glutathione to protect itself against oxidative stress," he said.

NAC, however, isn't easily tolerated by many people, causing gastrointestinal distress, he said. Intra-nasal glutathione, on the other hand, may avoid these potential side effects by bypassing the gut and liver, delivering the compound directly to the brain. He first presented the results in April to a patient advocacy group.

"The sense of excitement from patients, advocates and researchers was palpable," he said. "This is the first new potential drug for this disorder in years."

Garner said his next step is to plan a clinical trial in patients to test the value of intra-nasal glutathione.

Another Stanford co-author of the study is research associate Jerome Geronimo. The experiments were conducted by researchers at the University of North Carolina Chapel Hill.

The study was funded by the Timothy Foundation, a private nonprofit supporting research in body-focused repetitive behaviors, and by the Division of Laboratory Animal Medicine at UNC-Chapel Hill.

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

Alzheimer's

continued from page 1

substances might come their way. If they encounter anything suspicious, though, they whirl into action. Activated microglia are like officers with their guns out and firing, Zeineh said.

Microglia inflamed

The bulk of microglia found in association with iron in the study were in an activated, inflammatory state. Alzheimer's is increasingly understood to involve brain inflammation, and groups led by Stanford researchers such as neurologists Katrin Andreasson, MD, and Tony Wyss-Coray, PhD, and neurobiologist Ben Barres, MD, PhD, have previously fingered microglia as potential suspects in the early inflammatory pathology of the disease. This study adds the new finding that inflamed, iron-associated microglia are present in the hippocampus in Alzheimer's and are observable by 7T MRI, which could advance the scientific community's understanding of the disease.

The researchers noted that this was a preliminary study performed on a small number of human brain specimens, which are generally difficult to obtain. In this case, the specimens were supplied by the study's senior author, Brian Rutt, PhD, professor of radiology.

"Some imaging studies using mouse models of Alzheimer's disease had revealed the presence in these mice's brains of tiny, mysterious black dots that could signal the presence of iron, an element that shows up dark under MRI and, in certain chemical forms, can be highly reactive and inflammation-inducing," Rutt said. These mouse studies had raised the possibility that this iron might be tightly associated with amyloid plaques.

Rutt teamed up with Zeineh to scrutinize the human brain specimens for iron particles. "We wanted to see if there was an association of iron with Alzheimer's plaques in humans," Rutt said.

In a series of steps combining 7T MRI, computational analysis and painstaking laboratory staining techniques, the scientists probed slabs of tissue taken from several places within the brains of each of five Alzheimer's and five control brain specimens. "We weren't sure where to look," Rutt said.

> "We didn't consistently find the iron associated with plaques as we were expecting."

These slabs were scanned via 7T MRI, which can provide hair's-thickness resolution in three dimensions. In images from four out of the five Alzheimer's brains — but in none of the control brains — the researchers observed black dots in the subiculum, a component of the hippocampus. The hippocampus is known to incur some of the earliest and most severe ravages of Alzheimer's disease.

The Stanford scientists then carefully sectioned the tissue slabs into several hundred ultrathin sections; incubated them with stains that pinpoint the location of iron, microglia, amyloid plaques and tau; and analyzed the resulting stain patterns.

Amyloid plaque, tau not consistently near iron

What emerged was evidence that iron, frequently engulfed by microglia, was occupying the same spots in the subiculum of Alzheimer's brains where 7T MRI had found black dots. Those microglia were mostly in an activated state.

As notable was the relative absence of amyloid plaques in these spots. "We didn't consistently find the iron associated with plaques as we were expecting, despite our best efforts to do that," said Rutt. Tau was more often nearby — but, again, not consistently.

"Amyloid is found all over the brain in Alzheimer's disease, and often in the brains of people who've died with no complaints of memory loss at all," Zeineh said. "Tau is also found throughout the Alzheimer's brain. This iron-microglia complex, in contrast, really seems concentrated in the subiculum — and, so far, it's showing up only in brains from Alzheimer's patients."

Zeineh and Rutt said they don't know how the iron gets into brain tissue, or why it accumulates where it does. Micro-injury to small cerebral blood vessels there was one possibility, they speculated.

The researchers cautioned that the stains used in the study wouldn't have been able to visualize soluble clusters of beta-amyloid, now increasingly believed to be the protein's toxic form, as opposed to the aggregated plaques. Soluble amyloid may yet be playing a major if still poorly understood role, they said.

Zeineh, Rutt and Hannes Vogel, MD, a professor of pathology and co-author of the study, plan to explore these findings further in collaboration with Edward Plowey, MD, PhD, assistant professor of pathology. They intend to examine more wide-ranging areas of the brain and to stain for more cell types within larger numbers of postmortem brain specimens.

They also plan to hunt for iron-filled microglia in the brains of living patients during the early stages of neurodegeneration and memory loss that precede the onset of Alzheimer's disease. Their ultimate goal is to translate these imaging findings into clinical tools to help in the fight against dementia.

The study was carried out in collaboration with researchers in Canada and Germany. It was funded by the Radiological Society of North America and by General Electric Healthcare.

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

Medicaid's dental coverage may not prevent tooth-related ER visits

By Beth Duff-Brown

More than 2 percent of all emergency department visits are now related to nontraumatic dental conditions, according to a study by researchers at Stanford, UC-San Francisco, Truven Health Analytics and the federal Agency for Healthcare Research and Quality.

Although the expansion of Medicaid under the Affordable Care Act has made millions of low-income and rural Americans eligible for health insurance, many states don't provide dental coverage for adults under their Medicaid programs. Paying for dental insurance on the individual market or paying for dental services out of pocket is cost-prohibitive for Medicaid beneficiaries, many of whom are at or beneath the federal poverty level. So many have turned to EDs for such care.

The researchers said Medicaid dental coverage could help reduce the need for many low-income Americans to visit emergency departments for dental conditions that may have otherwise been prevented with adequate access to basic dental care.

Kathryn McDonald

"It is likely that EDs will continue to provide care to individuals without adequate access to community-based dental care unless new dental service delivery models are developed to expand access in underserved areas, and unless more dental providers begin to accept Medicaid under the ACA," the researchers wrote in their study, which was published Aug. 3 in *Health Affairs.*

Stanford is the prime contractor for the AHRQ for this multi-institutional research. Kathryn McDonald, executive director of Stanford's Center for Health Policy/Center for Primary Care and Outcomes Research, is a co-author of the paper and principal investigator of the study.

Difficulty finding dental care

From 2001 to 2008, emergency room visits for routine dental conditions — such as cavities, tooth pain and gingivitis — increased by 41 percent in the United States, while emergency room visits for all conditions rose by only 13 percent, the study said.

This is partly due to the lack of dental coverage under Medicaid in some areas, the shortfall of dental providers in rural communities and the dearth of dentists in urban areas willing to take on new Medicaid patients.

"Past research has shown that many dentists do not accept Medicaid," said study co-author Kathryn Fingar, a researcher at Truven Health Analytics in Sacramento. "Therefore people with Medicaid may find it difficult to get dental care in an office-based setting, even if they have dental insurance and even if there is an adequate supply of dentists in their community. In these instances, patients may need to use emergency rooms for dental problems, which generally can do little for patients seeking dental care except prescribe pain medications and antibiotics."

According to the American Dental Association, an estimated 8.3 million adults on Medicaid are eligible to gain expanded dental coverage under the Affordable Care Act.

"If these newly insured individuals cannot find a provider that accepts their insurance, emergency department use for dental conditions may not be reduced, even though access to dental insurance through Medicaid has increased," Fingar said.

The study examined county-level rates of emergency room visits for nontraumatic dental conditions in 29 states in 2010. They found that an adequate supply of dental providers was associated with lower rates of emergency room visits for dental care by patients with Medicaid in rural counties, but not in urban counties, where some 90 percent of dental emergency room visits occurred.

In urban areas, expanded Medicaid dental coverage did not appear to reduce dental emergency room visits despite an adequate supply of dentists. These findings suggest that even in states whose Medicaid programs offer expanded dental coverage, patients may have difficulty locating dentists who accept Medicaid. The rate of dentists who accept Medicaid has been reported to be as low as 11 percent in Missouri, 15 percent in Florida and 20 percent in New York.

"Faced with pressure to cut costs, some states have lowered Medicaid reimbursement rates for dental services, which reduces the incentive for dentists to participate in the program," the study said.

The recession that began in 2007 led to budget cuts and increased Medicaid enrollment. In response, many states cut Medicaid benefits in order to reduce expenditures, including expanded dental coverage for adults.

Some states, including California and Washington, have since reinstated nonemergency dental services, but access continues to be limited in other states. In 2012, fewer than half of the states provided expanded dental coverage to Medicaid patients who were not pregnant or disabled.

Value of preventive care

As of January 2013, some 45 million Americans were living in regions with shortages of dental care providers, particularly in rural areas. In coming years, the national supply of dentists is expected to decrease further as many current dentists retire.

ROMMMA / SHUTTERSTOCK

"The large number of visits to emergency rooms for dental conditions that could be treated in outpatient settings is indicative of the fact that our health-care system treats dental care differently than other preventive care when, in fact, dental care should be considered part of a person's overall health and well-being," said Maria Raven, MD, MPH, associate professor of emergency medicine at UCSF and the study's senior author.

"It should not be considered a luxury; it should be considered a necessity," she added. "Inadequate dental care has downstream consequences, including infection, need for costly extractions and important cosmetic consequences for patients."

The authors suggest several possibilities to reduce the number of emergency room visits for dental problems:

- Establish on-site dental clinics in emergency rooms.
- Expand dental coverage using less-expensive telehealth and mid-level dental providers who are not dentists, but trained to perform preventive and restorative care, such as fillings and minor extractions.
- Incentivize payers or providers to offer or refer patients to preventive dental care, similar to colon and cervical cancer screenings routinely offered today.

"To implement these types of solutions, which may require alterations in the way dental services are bought and paid for in the United States, dental care must be viewed not as an optional add-on but as an integral part of an individual's overall health care," the authors wrote.

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

Beth Duff-Brown is communications manager at the Center for Health Policy/Center for Primary Care and Outcomes Research.

El-Sayed appointed associate dean for maternal and child health

By Erin Digitale

Maternal-fetal medicine expert Yasser El-Sayed, MD, has been appointed as an associate dean for maternal and child health at the School of Medicine, effective immediately.

El-Sayed will focus on obstetrics and related women's issues, ensuring that pre-conception and pregnancy-related care are fully integrated into the portfolio of services at Lucile Packard Children's Hospital Stanford and into the school's strategic planning. He joins three other associate deans for maternal and child health who represent faculty affairs, research and global affairs.

"Yasser is known as balanced in his approach: He is good at considering both hospital administrative and School of Medicine perspectives," said David Stevenson, MD, senior associate dean for maternal and child health, who appointed El-Sayed to the new role. "He is perceived as very responsive, and he's also a really good doctor, as well as a very strong and well-established clinical investigator."

El-Sayed came to Stanford as an intern in 1990. He is the Charles B. and Ann L. Johnson Professor and director of the Division of Maternal-Fetal Medicine and Obstetrics at the School of Medicine. He is also co-director of the Johnson Center for Pregnancy and Newborn Services and obstetrician-in-chief at Lucile Packard Children's Hospital Stanford. He will retain these roles with his new position. Over the course of his career, El-Sayed has been instrumental in developing and directing the division's extensive clinical and research programs.

"I think the challenges we face for perinatal care are profound and exciting," El-Sayed said. "The hospital and university have to work closely together to continue to prove that academic, tertiary medical centers are critical for providing excellent health care to the population. The new team of associate deans speaks to how faculty members in the medical school, with leadership roles at the hospital, can facilitate that kind of productive, visionary dynamic." ISM

Yasser El-Sayed

OF NOTE

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

Steven Asch

Sang-Ick Chang

Marion Buckwalter

Stephen Felt

Norman Lacayo

Jason Lee

Alison Marsden

Marco Perez

Maria Polyakova

Manu Prakash

ALISON MARSDEN, PhD, was appointed associate professor of pediatrics and of bioengineering, effective July 1. Marsden specializes in pediatric and congenital heart disease, using simulations of blood flow to improve medical device design and imaging and to study the progression of heart disease. She also works with clinical researchers to develop tools for personalized medicine and treatment planning.

MARION BUCKWALTER, MD, PhD, was promoted to associate professor of neurology and neurological sciences, effective April 1. Her research examines how inflammation affects patient outcomes after strokes. Her team recently discovered that autoimmunity may play an important role in the development of dementia following stroke.

STEPHEN FELT, DVM, MPH, has been promoted to associate professor of comparative medicine, effective April 1. He researches imaging models for laboratory animal species, and ways to improve the health and welfare of laboratory animals.

NORMAN LACAYO, MD, has been promoted to associate professor of pediatrics, effective April 1. His recent research focuses on gene and protein expression in the leukemia cells of children diagnosed with acute leukemia, with an aim to improve diagnosis and therapy for each patient.

JASON LEE, MD, was promoted to professor of surgery, effective April 1. His research focuses on the development of techniques and devices to repair complex aortic aneurysms. He is analyzing the performance of a variety of devices to see which result in the best patient outcomes. He also teaches physicians worldwide how to use these devices.

MARCO PEREZ, MD, was appointed assistant professor of medicine, effective May 1. Perez is the director of the Stanford Inherited Arrhythmia Clinic, and his research, which focuses on rare and inherited arrhythmias, uses genetics and epidemiology to investigate the causes of cardiovascular diseases.

MARIA POLYAKOVA, PhD, assistant professor of health research and policy, has received the 2014 Geneva Association's Ernst Meyer Prize. This award was given in recognition of her research on risk and health insurance economics. Based in Switzerland, the Geneva Association focuses on insurance economics research.

MANU PRAKASH, PhD, assistant professor of bioengineering, was named a 2015 National Geographic Emerging Explorer. As one of 14 honorees, he will receive $10,000. Prakash specializes in developing low-cost scientific tools, such as the Foldscope microscope and a small-scale chemistry kit.

LAURA ROBERTS, MD, the Katharine Dexter McCormick and Stanley McCormick Memorial Professor and chair of psychiatry and behavioral sciences, will be awarded $50,000 as the recipient of the 2015 MacLean Center Prize in Clinical Ethics from the MacLean Center for Clinical Medical Ethics at the University of Chicago. Roberts specializes in ethics, suicide prevention and careers and leadership in academic medicine and medical education.

Laura Roberts

IVAN SOLTESZ, PhD, was appointed professor of neurosurgery, effective May 1. He is also the vice chair of neurosurgery. His research focuses on inhibition in the brain and the mechanisms of circuit dysfunction in epilepsy. His team has created virtual networks of brain regions using supercomputers, and developed methods for the control of epilepsy.

JUNAID ZAMAN, MA, BMBCh, MRCP, has received the UK-U.S. Fulbright British Heart Foundation Research Scholar Award. Zaman is a postdoctoral research fellow at Imperial College London and a postdoctoral scholar at the School of Medicine. He will receive about $109,000 to do research at Stanford for one year. He plans to examine treatments for sudden cardiac death. ISM

Researchers awarded $14 million from NIH for two precision-health projects

By Jennie Dusheck

The National Institutes of Health has awarded School of Medicine researchers Teri Klein, PhD, and Russ Altman, MD, PhD, $14 million in funding for two projects that will advance the practice of precision health.

Altman, a professor of bioengineering, of medicine and of genetics, and Klein, a senior research scientist, are the principal investigators for a four-year, $10 million grant from the National Institute of General Medical Sciences to expand their premier precision-health resource, PharmGKB knowledge base, now in its 15th year. PharmGKB provides comprehensive information about how genetics affects drug response in individuals.

People can react very differently to the same drugs. For example, the enzyme CYP2D6 is involved in metabolizing hundreds of prescription drugs. One drug that CYP2D6 metabolizes is the opiate painkiller codeine, which CYP2D6 converts into morphine — the active form of the painkiller. Most people have just two copies of the CYP2D6 gene, but some of us have more. Extra copies of the gene pump out so much of the enzyme that codeine and other drugs are metabolized far more rapidly than prescribing physicians expect. People with more than two copies of the gene can convert a standard dose of codeine to morphine so rapidly that they may overdose.

Klein and Altman created PharmGKB in 2000 — collecting, curating and disseminating information about the many ways that humans vary in how they respond to different drugs. The knowledge base includes, for example, gene/drug associations, dosing guidelines and drug labels.

PharmGKB has organized genetic information derived from nearly 10,000 scientific papers and documented nearly 13,000 associations between specific alleles and drug response phenotypes.

With the new $10 million grant, Klein, director of PharmGKB, and Altman plan to expand the repository; tackle drug responses involving multiple genes; and reprogram the knowledge base so it is accessible to users with different levels of scientific sophistication.

Genotype-based recommendations for clinicians are essential to bring pharmacogenomics into the clinic.

The second grant is a three-year, $4 million grant from NIGMS and the National Human Genome Research Institute to Klein and co-principal investigator Mary Relling, PharmD, chair of pharmaceutical sciences at St. Jude Children's Research Hospital, for the Clinical Pharmacogenetics Implementation Consortium.

CPIC publishes clinical guidelines that teach health-care providers how to use information about their patients' genetics to guide and optimize drug prescriptions. ISM