To better detect heart transplant rejection, scientists test for traces of donor’s genome

Heart transplant recipients and their physicians are likely more concerned with the function of the donated organ than with the donor’s DNA sequences that tag along in the new, healthy tissue. However, researchers at the Stanford Cardiovascular Institute have shown that an increase in the amount of the donor’s DNA in the recipient’s blood is one of the earliest detectable signs of organ rejection.

The finding implies that a simple blood draw may soon replace the regular surgical biopsies that are currently used to track the health of the donor heart. Closely tracking the dynamics of this concurrent “genome transplant” may also allow doctors to avoid the use of high doses of medication required to combat more
Detecting rejection by spotting donor DNA

(Continued from Page 1)

advanced cases of rejection.

“Heart transplant recipients undergo at least 12 tissue biopsies during the first year after their transplant and two or three each year for about four additional years,” said Hannah Valantine, MD, professor of cardiovascular medicine. “Signs of rejection are treated aggressively with large doses of corticosteroids, which can themselves have side effects including diabetes, hypertension and renal problems. The idea that we might now be able to diagnose rejection earlier and noninvasively is very, very exciting.”

“This approach, which we call genome transplant dynamics, or GTD, solves a long-standing problem in cardiac transplantation,” said Stephen Quake, PhD, who developed the sequencing technology used in the study. “It’s so difficult to find and implant a donor heart, and then doctors have to remove pieces of it every few months to test for rejection.” Quake is the Lee Otterson Professor of Bioengineering in Stanford’s schools of Medicine and of Engineering.

In 2010, Valantine pioneered the first blood test to diagnose organ rejection. That test, called the AlloMap, relies on the expression profile of 20 genes in a patient’s blood sample to determine whether the body has launched an attack on the donated organ. In contrast, this new technique uses advanced genome-sequencing technology developed by Quake to measure levels of donor DNA released when cells in the transplanted heart are damaged as occurs early in the rejection process.

The researchers believe using the two methods in tandem will allow the noninvasive monitoring of the health of many transplanted organs, including hearts, lungs and kidneys. Quake and Valantine are co-senior authors of the research, which published online March 28 in the Proceedings of the National Academy of Sciences. Thomas Snyder, PhD, a research associate in Quake’s laboratory, performed much of the DNA sequencing and computational work, and Kiran Khush, MD, an instructor in cardiovascular medicine, compiled the medical records of the patients in the study and documented any episodes of rejection they experienced.

The current study began when Valantine noticed research by Quake in 2008 showing that it is possible to detect fetal chromosomal abnormalities by sequencing cell-free DNA fragments in a maternal blood sample.

“When I saw that, I thought, wow, this technique could probably be used to monitor heart rejection,” said Valantine, noting that cells damaged during rejection also release DNA into the circulatory system.

The results of their research were absolutely confirmatory, said Valantine. “In every case we could see an increase in donor DNA in the patient’s blood before the biopsy itself showed any sign of rejection.” Rejection episodes corresponded to levels of donor DNA approaching 3-4 percent; when the patients were successfully treated with immunosuppressants, the amount of the donor DNA in the blood decreased to less than 1 percent of total free DNA.

“For the first time, we can now use cell-free DNA for practical diagnostic questions in organ transplantation,” said Quake, who noted that the technique is likely to be applicable to many other organs.

Quake, Valantine and Snyder have filed a patent for use of the technique. Before the test can be routinely used, the researchers plan to conduct a prospective study in which participants will be identified and then tracked over time. They recently received a three-year, $2 million grant from the National Institutes of Health for prospective studies to diagnose acute rejection of heart and lung transplants.

The research was supported by the National Institutes of Health and the Howard Hughes Medical Institute.
CVI scientists spot new gene regions identified that predispose people to heart attack

Thirteen new gene regions have been convincingly linked to coronary atherosclerosis in a massive, new, international genetics study involving investigators from the Stanford Cardiovascular Institute.

The results of the study, published online March 6 in Nature Genetics, provide 13 vital new clues on the etiology of this disease, the most common cause of death worldwide. The study doubles the number of gene regions previously known to predispose people to this condition. Coronary atherosclerosis is the process by which plaque builds up in the wall of heart vessels, eventually leading to chest pain and potentially lethal heart attacks.

The study was conducted by an international consortium that pooled resources to analyze data from 14 genome-wide association studies. Consortium investigators examined the complete genetic profiles of more than 22,000 people of European descent with coronary heart disease or a heart attack history and 60,000 healthy people — close to 10 times more than the next-largest whole-genome study to date.

“Out of the roughly 3 billion bases in our DNA code, we’re talking about finding a few bases that are different in some people, and that difference leads to a change in the function of a gene or a set of genes that in turn changes your lifetime risk of having heart disease. That is like looking for a change in one letter in one word in the Encyclopedia Britannica,” Quertermous said.

Combining genetic data from multiple studies is absolutely critical to identifying these needles in the haystack, as the genetic architecture of coronary atherosclerosis has proven to be far more complex than anticipated. “The signals from these gene regions are all rather subtle, making large-scale collaborations a prerequisite for any meaningful progress,” said Themistocles (Tim) Assimes, MD, PhD, assistant professor of medicine at Stanford and one of the study’s 10 lead authors. The results also suggest that it may be worthwhile to determine an individual’s profile of genetic variants for heart attacks as part of routine clinical care in the near future. The ADVANCE study was supported by a grant from the Reynolds Foundation and the NHLBI.
Fridays at Falk  4-5:30 PM - A postdoc-centric afternoon series

In the new year the Fridays at Falk series continues to be a forum for informal talks, discussion, and happy hour. They are intended to provide a venue for postdocs, graduate students, and interested faculty to learn more about who’s doing what, where, and when in the CVI. The planning committee includes Arwen Hunter, PhD, from the Cooke lab, Ildi Toma, PhD, and Elaine Wang, PhD from the Yang lab.

Stanford Institutes of Medicine Summer Research Program (SMIR)

This year, all 5 Institutes in the School of Medicine will be participating in the SIMR Program (Stanford Institutes of Medicine Summer Research Program) which is lead by PJ Utz, Faculty Director. Would you or someone in your lab consider hosting a high school student for this coming summer? The program dates are Mon., June 13th-Fri., August 5th (8 weeks). To remind you, all safety training, lectures, parking, and some meals are provided for all students, as is their full summer stipend. All you need to do is identify a student, fellow, or research assistant to volunteer to mentor them. The students are free!

Stanford Training Course in Pluripotential Stem Cell Culture, organized by Drs. Renee Reijo Pera and Vittorio Sebastiano of the Stem Cell Institute

The Stanford Center for Human Embryonic Stem Cell Research and Education is pleased to announce the course for human Embryonic Stem Cells (hESCs) culture for the PCBC Consortium.

For more info about the program, pls. visit http://hesc.stanford.edu/ or contact Cynthia Klein Email address cklein@stanford.edu or Vittorio Sebastiano Email address vsebast@stanford.edu
FRONTIERS IN CARDIOVASCULAR SCIENCE

TUESDAY, APRIL 12, 2011
Digitizing Man: The Future of Medicine
Eric J. Topol, MD
Scripps Translational Science Institute, La Jolla, CA

TUESDAY, APRIL 19, 2011
Circadian Clock Regulation of Metabolism and Obesity
Brian Feldman, MD, PhD
Stanford University

WEDNESDAY, APRIL 20, 2011
Pluripotent Stem Cells and the Immunobiology of Engineered Heart Tissues: A Translational Approach
Sonja Schrepfer, MD, PhD
University Heart Center Hamburg, Germany

TUESDAY, APRIL 26, 2011
Kawasaki Disease 2011
Jane W. Newberger, MD, MPH
Children's Hospital, Harvard Boston, MA

TUESDAY, MAY 10, 2011
Insulin Resistance; the Link between Obesity and Cardiovascular Disease
Gerald M. Reaven, MD
Stanford University

TUESDAY, MAY 17, 2011
Epidemiology of Heart Failure and LV Remodeling: Insights from Community-Based Studies
Vasan S. Ramachandran, MD, DM, FACC, FAHA
Boston University, MA

TUESDAY, MAY 24, 2011
Microsystems for Cardiac Cell Mechanobiology
Beth Pruitt, PhD, MSc
Stanford University

TUESDAY, MAY 31, 2011
Agnostic Approaches to Cardiovascular Genetics and Genomics
Nicholas J. Leeper, MD
Stanford University

TUESDAY, JUNE 7, 2011
Modeling Oxido-Reductive Stress Signaling Networks Linked to Protein-Induced Heart Disease in Flies and Mice
Ivor J. Benjamin, MD, FAHA, FACC
University of Utah Salt Lake City, UT

FRIDAY, JUNE 10, 2011
Writing The NIH Career Development (K) Award: Strategies for Success
Christopher C. Dant, PhD
Norris Cotton Cancer Center and Dartmouth Medical School, Hanover, NH

TUESDAY, JUNE 14, 2011
The Myosin Family of Molecular Motors: From Single Molecule Analysis to Clinical Trials in Heart Failure
James A. Spudich, PhD
Stanford University
New method allows human embryonic stem cells to avoid immune system rejection

A short-term treatment with three immune-dampening drugs allowed human embryonic stem cells to survive and thrive in mice, according to researchers at the Stanford University School of Medicine. Without such treatment, the animals’ immune systems quickly hunt down and destroy the transplanted cells. The finding is important because it may allow humans to accept transplanted stem cells intended to treat disease or injury without requiring the ongoing use of powerful immunosuppressant medications.

Just as it does with transplanted organs, the human body recognizes foreign cells and rejects them. Embryonic stem cells, or ES cells, and the tissues they become are by definition immunologically different from any potential recipient. Physicians also have to overcome the fact that unspecialized ES cells can form tumors when transplanted into the body.

“We are very excited about the clinical potential of this finding,” said Joseph Wu, MD, PhD, associate professor of cardiovascular medicine and of radiology at Stanford and senior author of the study, published in the March issue of Cell Stem Cell. “The immunological issue is one of the most important biological problems to solve, in my opinion. Clinicians need to make sure there is no tumor formation, and also that the cells are not rejected.” This paper, in tandem with a previous study by Wu published in February in the Journal of Clinical Investigation, helps to recast a scientific debate over the relative benefits of embryonic stem cells as compared with iPS cells, or induced pluripotent stem cells, which can be created from a person’s own skin or other cells.

Some scientists argue that iPS cells can differentiate into other tissue as well as ES cells can — without the problem of immune system rejection. Yet others contend that although iPS cells behave very much like ES cells in a laboratory dish, they are not identical and may not be perfect stand-ins.

Wu’s paper in February sheds new light on the dissimilarities between the two cell types. “When we compared the gene expression patterns between single cells, we saw that they were actually quite different,” he said. That paper is the first to compare the gene expression patterns between iPS and ES cells on a single-cell level. In addition, although using a patient’s own cells sidesteps the problem of immune rejection, generating these tailor-made cells does have drawbacks. “Most people don’t realize that, although it’s possible to generate patient-specific iPS cells, the cost of doing so would likely be prohibitive for all but the most specialized applications,” said Wu. “It also takes time — time that a patient with an acute health problem like a stroke, heart attack, or neurological trauma may not have.”

Wu’s latest paper addresses ES cells’ problem with immune system rejection. In addition to Wu and the paper’s first author, graduate student Jeremy Pearl, other Stanford researchers involved in the study include graduate student Andrew Lee; postdoctoral scholars Dennis Leveson-Gower, PhD, Ning Sun, PhD, and Feng Lan, PhD; former postdoctoral scholar Zhumur Ghosh, PhD; professor of medicine Robert Negrin, MD; and professor of microbiology and immunology Mark Davis, PhD. The research was supported by the National Institutes of Health, the Howard Hughes Medical Institute and the Ellison Medical Foundation.

A longer version of this article can be found at: http://cvi.stanford.edu/
Cardiovascular Institute
APR 2011

Skin cells help to develop possible heart defect treatment in first-of-its-kind study

Using skin cells from young patients who have a severe genetic heart defect, Stanford University School of Medicine scientists have generated beating heart cells that carry the same genetic mutation. The newly created human heart cells — cardiomyocytes — allowed the researchers for the first time to examine and characterize the disorder at the cellular level. In a study published online in Nature, the investigators also report their identification of a promising drug to reverse the heart malfunction — for which there are currently no good treatments — after using these newly created heart cells to check the effects of a plethora of compounds.

The new approach involved converting skin cells to heart cells in a dish by reprogramming them to an embryonic-stem-cell-like state, so that the cells are capable of “differentiating” into a multitude of cell types. The scientists then coaxed these induced pluripotent stem cells to become heart cells. The iPS-cell approach represents a big advance because no good alternative methods for studying human heart malfunction at the cellular level now exist.

“This may be the first time this noninvasive ‘disease-in-a-dish’ technique has been used successfully to screen for drugs in heart disorders,” said Ricardo Dolmetsch, PhD, associate professor of neurobiology and senior author of the study. The study’s first author is Masayuki Yazawa, PhD, a postdoctoral researcher in Dolmetsch’s lab.

Nearly a dozen genetic mutations identified in humans are known to cause disruptions result in a condition called long QT syndrome. (The name reflects an elongated interval between two portions of the waveform typically observed in an electrocardiogram.) People with LQTS suffer from arrhythmias, or irregular heartbeats, and are vulnerable to ventricular fibrillation, an often fatal state in which heart cells contract chaotically.

For their Nature study, Dolmetsch and his colleagues turned to patients with Timothy syndrome, one genetic mutation known to cause LQTS. Patients with Timothy syndrome are highly susceptible to ventricular fibrillation and often die at an early age. Another hallmark feature of Timothy syndrome is autism, which is the primary focus of Dolmetsch’s research.

The defective gene in Timothy syndrome encodes a called a calcium channel protein. Significantly, Dolmetsch’s group found that in the Timothy syndrome-derived ventricular cells, but not atrial or nodal cells, the calcium channels encoded by the mutant gene opened normally to allow calcium flow but stayed open longer than those of normal cells.

With special dyes that mirror calcium concentrations, Dolmetsch and his team were able to visually inspect calcium flow in heart cells prepared from Timothy syndrome patients’ skin.

The investigators examined the response of these irregular-beating cells to different drugs that have been reported to affect heartbeat rhythms. When they added one of these drugs — roscovitine, currently in clinical trials for an unrelated indication — to the cell-culture medium at the right dose, the deficient calcium flow was restored, and so was the regular heartbeat.

Dolmetsch cautioned that at this point roscovitine should not be considered an adequate treatment for LQTS — it hasn’t been tested for this purpose in living animals, let alone humans, and may have pronounced side effects. Still, he said, it’s a promising compound for further drug development. Stanford’s Office of Technology Licensing has applied for U.S. patents related to the discovery, and Dolmetsch is starting a new company that intends to license those patents once they’re granted.

A longer version of this article can be found at: http://cvi.stanford.edu/
Little benefit from electronic health records from 2005 to 2007, study says

Electronic health records did little to improve the quality of health care from 2005 through 2007, even when bolstered by software that gives doctors treatment tips for individual patients. That’s what two researchers at the Stanford University School of Medicine found by analyzing nationwide physician survey data from nearly 250,000 patient visits over that three-year period.

“There’s a lot of enthusiasm and money being invested in electronic health records,” said the senior author of the study, Randall Stafford, MD, PhD, associate professor of medicine at the Stanford Prevention Research Center. The federal government’s economic stimulus package of 2009 invested $19 billion in health information technology, including incentives for adoption of electronic health record systems. “It makes sense, but on the other hand it’s an unproven proposition. When the federal government decides to invest in health-care technology because it will improve the quality of care, that’s not based on evidence. That’s a presumption.”

And based on the new study, that presumption is in doubt, at least when it comes to the current use of electronic health records, even those that offer treatment guidance — a feature called clinical decision support.

The new study builds on a 2007 analysis by Stafford and colleagues showing that electronic records alone had not made an impact. In the new study, Stafford and former Stanford undergraduate student Max Romano (now a medical student at Johns Hopkins) analyzed more current data and looked specifically at whether clinical decision support improved the quality of care.

“Decision support software provides physicians with specific guidance based on best practice,” said Stafford. “For example, the computer system might flash up an alert reminding physicians about something they failed to do, or the software might question a particular choice the physician has made about an order for a diagnostic test or a medication.”

“Most studies before ours focused on how single EHR systems work in a few premier academic medical centers, and some of those studies have found significant benefits,” said Romano. “Our study takes a different approach: We looked at all non-federal outpatient settings in the United States, from solo private practices to community health centers, to see whether EHRs were having any noticeable impacts in the real world, and we found no significant differences in care quality.”

So why didn’t electronic health records translate into better care? “These are complicated systems used by individuals who have received little formal training, at least until recently,” said Stafford. As a result, physicians might not have made full use of them. Some other factors that influence quality of care include physician communication skills, patients’ access to health care, patients’ health literacy, pressures of outpatient practice and whether physician payment rewards good quality care.

“We’ve shown that electronic health records and clinical decision support don’t by themselves improve quality,” said Stafford. “If we want improved quality, we have to look at the whole range of issues that affect quality of care and not put all of our hopes on a single technology.”

A longer version of this article can be found at: http://cvi.stanford.edu/