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It stands to reason that the human body's largest internal organ — the liver, responsible for myriad functions necessary to our survival — is correspondingly complex. Ancient Greek philosophers, including Plato, thought it housed greed, jealousy and other dark emotions. It's considered vital for the free flow of *qi*, or life energy, a key tenet of Chinese traditional medicine.

"Strangely subtle and underappreciated" is how Steven Weinman, M.D., Ph.D., describes the liver. "Its importance is often realized only in the wake of liver failure," he says. "You don't kick off suddenly, like you might with a massive heart attack. You waste away — it's a living death and terrible to watch."

Weinman is the director of the University of Kansas Liver Center, founded in 2007 to stimulate collaborative research on various liver-themed mysteries: for instance, why only 15 percent of alcoholics suffer from cirrhosis, or liver scarring, while the majority are unscathed.

Another puzzle is why, in patients with hepatitis C who have undergone a liver transplant, the disease frequently recurs and is much more aggressive the second time around. Instead of being a stealthy, decades-long process, cirrhosis — also a hepatitis C trademark — can destroy a transplant patient's supposed new lease on life in as little as three years. A recent, intriguing observation from one of the Liver Center's researchers may shed new light on this particular question.

DNA matters

Thanks to today's powerful medical technology, tens of thousands of genomic sequences can be rapidly scanned for minute variations — usually single DNA base changes — that might correlate with specific diseases. In 2008, the Dallas Heart Study uncovered one such variation: a single switch from C to G in a gene called PNPLA3. Individuals with this form of the gene are more likely to have higher

amounts of liver fat.

"It's associated with more severe non-alcoholic fatty liver disease and, for patients whose liver problems stem from heavy drinking or hepatitis C, more advanced cirrhosis," says Winston Dunn, M.D., a transplant hepatologist at the Liver Center. Intrigued, he decided to find out if this form of PNPLA3 had anything to do with the aggressive return of hepatitis C and accelerated liver scarring often observed post-transplant. To do so, he compared 103 pairs of tissue samples — matching donor with recipient — from KU's Liver Bank. All the recipients had received a liver transplant for hepatitis C.

Dunn found that the recipients whose new liver contained the wildtype, or original, form of PNPLA3 (without the single base change from C to G) were considerably protected from developing advanced fibrosis or requiring a second transplant due to failure of the first. Those whose new liver came with the altered version of PNPLA3, however, were not so fortunate.

"I expected to see a difference between the two genotypes, but not quite as marked," Dunn says. "When it comes to liver transplantation, this could impact how organs are allocated. If you're getting a transplant for liver disease *not* related to hepatitis C, current indications are that the genotype of the graft you receive doesn't matter - which means it might be possible for us to save grafts with the wildtype form of PNPLA3 for patients where, because they have hepatitis C, genotype actually matters."

The challenge to overcome, Dunn adds, would be logistical: genotyping donor livers — currently not a standard procedure — for information on PNPLA3, in a reasonable time frame that wouldn't hold up organ allocation. Meanwhile, his colleagues at the Mayo Clinic in Rochester are conducting a similar analysis, albeit with a larger number of tissue samples, to corroborate Dunn's findings. A peer-reviewed publication is also in the works.

"Here at KU, 40 percent of our transplant patients have hepatitis C — the very subset that fares worst post-surgery," Weinman observes. "What Dunn has found, once confirmed, could dramatically alter their survival curves. It's one of the first mass uses of the Liver Bank to address a clinical question, and definitely research with exciting potential.

Liver library

A direct outgrowth of The University of Kansas Hospital's active liver transplant practice, the Liver Bank currently houses more than 2,000 tissue samples from the liver biopsies of over 1,000 patients. It allows researchers to carry out prospective studies and rapidly test ideas in ways that wouldn't be possible otherwise.

The bank had its genesis in a simple request: in 2008, Yvonne Wan, Ph.D., then director of the Liver Center, asked Richard Gilroy, M.D., the liver transplant program's medical director, if she could acquire

spare liver cells for her laboratory research.

"All she wanted were some cells," Gilroy says, "but I decided that, instead, this could be a great opportunity to begin building a collection of tissue samples that would enable more researchers to study what happens to livers over time."

Financially supported by both the hospital and the University of Kansas Medical Center, the Liver Bank serves as a multi-year repository for tissue specimens from excess surgical material in liver transplants, resections and biopsies. The samples are carefully categorized and a separate database, with extensive clinical and pathological data, is also maintained.

"Think of the bank as a library," Gilroy says. "Each patient can be represented as a book, and pages get added with every biopsy. If we had multiple books but each was a thin volume, it would be a useless library; ditto if we had just a handful of heavy tomes. What we want — and what I think we're achieving, over time — are multiple books *and* pages."

There are many questions Gilroy wants to explore, utilizing the Liver Bank. He'd like to use its database, which documents the "chronic rejection cohort" — patients unable, for whatever reason, to tolerate a transplant — to determine how and if immunosuppressive drugs might be modified to address the problem of organ rejection. Then there's what he describes as the $64,000,000 question: why some patients have perfectly normal liver enzymes but remarkably abnormal biopsies.

"We've tended to be somewhat siloed, but the bank is providing great opportunities for translational research," Gilroy says. "The ability to work together is an important measure of the marriage between clinicians and basic scientists. If a flourishing relationship can be built around this bank, our academic prowess will accelerate exponentially."

Weinman agrees. "I think the Liver Bank has reached a critical mass when it comes to tissue collection; its main mission now is to get these samples out to investigators so important questions can be answered," he says. "We've refined the process by which samples can be obtained — it used to be pretty laborious and many people simply gave up. We're also focused on serving KU investigators first, because this resource was created with financial and human capital from here, and we want to promote our liver research."

Porcine beginnings

The liver transplant practice at KU has come a long way since its inception in 1990. Founder and transplant surgeon Jameson Forster, M.D., clearly recalls the program's earliest days where, working with colleague Romano Delcore, M.D., he established a routine surgical procedure — by first practicing on pigs.

"It was a pretty acceptable training model because, when you think about it, we already eat pigs,"

Forster says. How it worked: the liver of a pig was removed and flushed, and the procedure repeated with a second pig, which then received the donor's liver.

"We spent some time figuring out pig intubation, because their throat is different," Forster says. "This was the training ground for getting our act together as a team — surgeons, nurses, anesthesiologists and others — so we could gain experience in working smoothly and efficiently before ever laying hands on a human patient."

"While not set in stone, I'd say the third pig who managed to live for at least a couple of days — instead of dying on the operating table — was a marker of proficiency for a transplant surgeon in training," he adds, before bringing up an image on his computer of the program's original "third pig," Celeste, wandering along a corridor in Wahl Hall, where her ilk were once housed.

Over the last two decades, Forster has seen major changes not only in KU's liver transplant program but, more generally speaking, the field of organ transplantation. "There used to be relatively little federal oversight, but that's no longer the case," he observes. "Which I think is lousy. They're very concerned about how many people we have dying on the wait list. But while having this be a low number is good as far such close scrutiny goes, it may also mean we can't serve patients who are really sick and might make our numbers look bad."

Since Kansas City native Timothy Schmitt, M.D., was recruited from the University of Virginia in October 2011 to serve as the director of transplant surgery, a new Center for Transplantation service line — which Schmitt also heads — has begun taking shape. It encompasses KU Hospital's liver, kidney and pancreas transplant programs, getting everyone within these different disciplines "on the same page with a common mission of care," Schmitt says.

"It puts patients at the center of the wheel instead of drawing a line between departments like Surgery and Medicine," he explains. "We have transplantation specialists within the various academic divisions — hepatology, cardiology, infectious diseases and the like — helping to spearhead this model, so patients aren't shuffled in different directions for the clinical care they need."

Through the Center for Transplantation, protocols for patient work-ups, post-transplant immunosuppression and care are refined and reinforced. "Outcomes data is our measuring stick," Schmitt says. "If the data aren't good, we'll lose patients, transplant contracts and face federal trouble. So we watch over compliance issues and make sure everyone's informed about what's going on. It's mainly quality assessment and improvement, but it also provides accountability."

The factors that determine whether or not a patient with a problematic liver qualifies for a transplant are largely related to age and comorbidities, or the presence of additional disorders on top of the primary disease. "There are complex issues to work through, even for a patient whose liver is clearly failing," Gilroy notes, "because they could be morbidly obese or addicted to drugs, to name two

examples — and any good they might derive from a new liver would be short-lived."

"Ethically, everyone with decompensated liver disease benefits from a transplant," he adds. "But in terms of hard economics, having a patient die post-surgery with a healthy liver that would have survived in someone else really means two deaths. It's not your chance of living that's the question at stake in a liver transplant; it's your chance of dying."

Ultimately, when talking about the transplant program that he built from scratch, Forster is proud that it got off the ground and pleased at its burgeoning growth, especially over the last decade. "Our success needed an initial patient," he says. "23 years ago, Kristine Brees was the person who trusted us and really helped get things going."

Decoding liver disease

In his first year as the Liver Center's director, Weinman has focused on facilitating crosstalk between clinicians and basic scientists, to beef up translational research.

"We have the pieces to build a great liver research program, but they've been in separate orbits," he says. "Given the quality and success of our clinical transplant practice, its research component has been underdeveloped. That's what we're trying to change."

On February 4, 2013, Jody Olson, M.D., successfully applied for the center to become a full-fledged member of the Acute Liver Failure Study Group (ALFSG). This multi-center collaboration, based at UT Southwestern Medical Center in Dallas, is funded by the National Institutes of Health - specifically, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Olson is one of the Liver Center's newest members and specializes in caring for critically ill patients waiting to receive a new liver.

Unlike its chronic counterpart, acute liver failure occurs abruptly — often within weeks of a person having perfectly normal liver function — and its mortality rate, at 80 percent, is alarming. Certain viruses, drugs and autoimmune disorders can trigger this problem, but in the U.S., the biggest single cause is overingestion of acetaminophen, or Tylenol.

Prior to the Liver Center being invited on board, two of its faculty were already collaborating with the ALFSG: Hartmut Jaeschke, Ph.D., on the mechanisms of acetaminophen-induced liver injury, for which research he's internationally known; and Udayan Apte, Ph.D., on liver regeneration post-injury and how this influences patient survival. Apte is also a past recipient of the Liver Scholar Award, granted yearly by the American Association for the Study of Liver Disease to a small number of young investigators whose basic research careers are deemed most promising.

"We demonstrated experience with banking tissue and blood samples, outstanding basic science, and we had Olson — both a clinician-investigator and critical care expert — at the helm," Weinman

says. "These things helped get us over the bar. The acute liver failure patients we see here will now be part of a national registry, with their clinical data and biological samples stored in a central repository. We'll get first dibs on initiating clinical trials and proposing new studies. It's the first time we've had a major NIH-funded network like this at KU, for liver research."

In 2012, Kenneth Dorko joined KU as technical director of the Biospecimen core laboratory. Dorko's specialty is isolating human hepatocytes, or liver cells, from pieces obtained during resections, when part of the liver is surgically removed. These hepatocytes are then distributed to investigators for research purposes.

"It's particularly important for drug studies, because human hepatocytes behave differently than those from mice or rats," Weinman observes. In February 2013, the *Proceedings of the National Academy of Sciences* reported that — contrary to popular belief — the genes used by white blood cells in response to sepsis, trauma or burns were completely different in mice versus humans. The study's unexpected findings generated considerable buzz and made the front page of the *New York Times* .

"It's called into question many other studies based on mouse models," Weinman says, "which is why having Dorko here to make human hepatocytes available for liver research is both timely and therapeutically important."

The Liver Center has "kind of struggled as to its exact purpose," he adds - but changes are afoot, with fresh emphasis on building up and sustaining a culture of academic research.

"There may be better known liver research groups at, say, UC San Francisco or Yale," Weinman says, "but based on the number of NIH-funded investigators we have here and the quality of their work, I'd still rank us as one of the finest liver research groups nationwide. We just need to create a better forum for the intellectual development of ideas between basic scientists and clinicians so, for both communities, collaboration rather than non-interaction is the name of the game."

Note: This story first appeared in the Summer issue of KU Medical Center's magazine Kansas Medicine + Science. You can download the entire issue here.

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