

NOVEMBER/DECEMBER 2014

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Pioneering Three-Gene Signature Test for Predicting Kidney Transplant Rejection Receives Top Research Honors

In July 2013, *The New England Journal of Medicine* published a study by **Manikkam Suthanthiran, MD**, Chief of Nephrology and Hypertension at NewYork-Presbyterian/Weill Cornell, and his research team on a breakthrough noninvasive test that can detect whether transplanted kidneys are in the process of being rejected, as well as identify patients at risk for rejection weeks to months before they show symptoms. The innovative discovery was recognized by the National



Dr. Manikkam Suthanthiran

Institute of Allergy and Infectious Diseases as one of its top 20 research advances for 2013, and in April 2014, the Clinical Research Forum selected the work as one of the Top 10 Outstanding Clinical Research Achievements in the United States. Most recently, Dr. Suthanthiran was selected to receive the prestigious Jean Hamburger Award of the International Society of Nephrology, which

recognizes outstanding research in nephrology with a clinical emphasis.

The recognition bestowed upon Dr. Suthanthiran and his team is clearly well deserved. For more than a decade, they have pursued an alternative method to the invasive percutaneous needle core biopsy procedure traditionally used to determine kidney allograft status. The solution came in the form of a urine-based test. "By measuring just three genetic molecules in a urine

sample, the test accurately diagnoses acute rejection of kidney transplants, the most frequent and serious complication of kidney transplants," says Dr. Suthanthiran. "We were told we would never be able to isolate good quality mRNA from urine."

Since his earliest days of training at Harvard, Dr. Suthanthiran has always followed the maxim,

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A New Perspective on IgA Nephropathy

Immunoglobulin A nephropathy (IgAN) is the most common form of primary glomerulonephritis. It is characterized by the deposition of the antibody immunoglobulin A in the filtering unit of the kidney, resulting in glomerulonephritis, glomerular sclerosis, and progressive loss of kidney function. In fact, IgAN is a significant cause of end-stage renal disease worldwide, and the most common cause of kidney failure in China and Japan. More than 20% of patients eventually require a transplant.

A better understanding of the etiology of IgA nephropathy and its complex genetic structure has been a major focus of **Ali G. Gharavi, MD**, Chief of Nephrology at NewYork-Presbyterian/Columbia, and his research team. Their work has produced important findings, including the identification of multiple regions of the genome that confer risk of IgAN and novel insight into the mechanisms of kidney injury underlying the condition.

"When I was in medical school, the explanation for the higher prevalence of IgA nephropathy in East Asians was attributed to a higher rate of kidney biopsies performed by physicians in China and Japan," notes Dr. Gharavi. "Conversely, it was thought that IgAN was uncommon in Africa because of the few number of kidney specialists available, so the disease went undiagnosed. We now know that this is not necessarily the case. For example, if you look at Asian populations who have immigrated to America, there's a higher prevalence of IgAN, suggesting an inherited factor, as well as an ethnic variation."

Dr. Gharavi, along with Columbia nephrologist **Krzysztof Kiryluk, MD**, and collaborators at some 35 medical centers around the world, undertook a genome-wide association study of more than 20,000 individuals to determine the validity of this theory.

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Pioneering Three-Gene Signature Test (continued from page 1)

“never say never.” And while a number of researchers had tried to develop blood or urine-based tests to measure genes or proteins that signify kidney organ rejection, Dr. Suthanthiran and his research team were the first to create a gene expression profile urine test – an advance that was reported in *The New England Journal of Medicine* in 2001, with an update in 2005.

The research team – using a number of sophisticated tools they developed – measured the absolute levels of 13 prespecified messenger RNA (mRNA) molecules in urine samples collected from kidney graft recipients at the time of for-cause biopsy for acute allograft dysfunction, investigating whether differential diagnosis is feasible using urinary cell mRNA profiles. They found that increased expression of three mRNAs could determine if an organ will be, or is being, rejected. The mRNAs indicated that killer T immune cells are being recruited to the kidney in order to destroy what the body has come to recognize as alien tissue.

The signature test consists of adding levels of the three mRNAs in urine into a composite score. “Tracked over time, a rising score can indicate heightened immune system activity against a transplanted kidney,” says Dr. Suthanthiran. “A score that stays the same suggests that the patient is not at risk for rejection. This allows us to monitor kidney transplant patients using urine samples rather than an invasive biopsy. You cannot carry out invasive biopsies monthly to see what’s going on with a kidney. With urine, we can test monthly, or even weekly, making the monitoring process much easier for the patient.”

The composite score also enables physicians to tailor a patient’s use of multiple immunosuppressive drugs over time. Any increase would suggest a somewhat higher dose of therapy is needed to keep the organ safe. “This is akin to monitoring blood glucose in a patient with diabetes,” says Dr. Suthanthiran. “Because different people have different sensitivity to the two-to-four immunosuppressive drugs they have to take, this test offers us a very personalized approach to managing transplantations.”

The promise of the test first developed in Dr. Suthanthiran’s laboratory at Weill Cornell and previously reported more than a decade ago in *The New England Journal of Medicine*, led to an NIH-sponsored multicenter clinical trial in 2006 that would include nearly 500 kidney transplant patients at five major transplant centers in the country. In this first-of-its-kind blinded study, researchers collected 4,300 urine specimens during the first year of transplantation, starting at day three post-transplantation. Dr. Suthanthiran’s laboratory processed and measured the samples for 13 different genes, using the assay they developed in their lab. Their results were sent to the NIH statistical core for further analysis. The three-gene-based biomarkers signature was used to derive a composite score and identify a threshold value indicative of rejection. This score accurately detected transplant rejection with a low occurrence of false-positive and false-negative results.

“Such a test was sorely needed to help improve the longevity of kidney transplants and the lives of patients who receive these organs,” says **Darshana M. Dadhania, MD**, a transplant nephrologist at Weill Cornell and co-author of the NIH study. “The creatinine blood test used to help identify rejection is much less specific than the three-gene signature. Creatinine can go up for many reasons, including simple dehydration in a patient, and

when this happens we then need to do a highly invasive needle-stick biopsy to look at the kidney and determine the cause.”

“The three-gene signature test is about 85% accurate, much higher than the creatinine test,” adds Dr. Suthanthiran. “To achieve 100% success following transplantation, to achieve tolerance of the transplanted organ, that is our primary goal – because when tolerance is achieved, a transplanted organ truly brings the gift of life.

“The three-gene signature test has moved us from the one-size-fits-all drug treatment model to a much more personalized treatment plan,” says Dr. Suthanthiran. “We have also developed additional biomarkers to detect fibrosis, a common feature of kidney transplants destined to fail, to diagnose BKV viral disease, a common viral disease in kidney transplant recipients, and to determine why kidney transplants are not functioning well. The noninvasive tests we have developed, together, provide us with an opportunity to manage transplant patients in a more precise, individualized fashion. The progress we have made with these tests transcend transplantation and have broader implications for designing therapies to prevent the progression of native kidney disease.”

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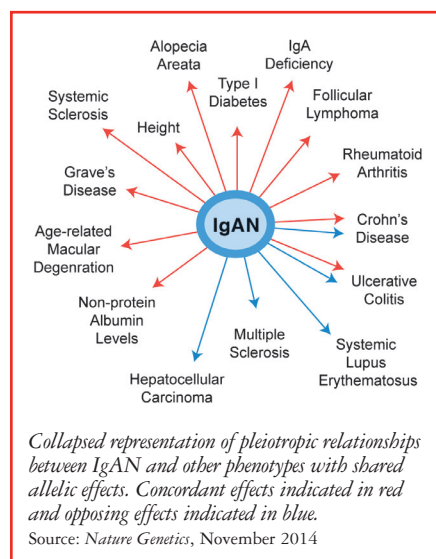
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A New Perspective on IgA Nephropathy (continued from page 1)

“We collected DNA samples from subjects in Asia, Europe, and North America,” says Dr. Gharavi. “When we did the analysis, we identified 15 regions – six new regions and nine previously reported genes – that were associated with the risk of developing IgA nephropathy and the age at which the disease develops. To our surprise, each one of these genetic susceptibility regions harbored a gene that is involved in the maintenance or protection of the mucosal lining of the intestine and had been identified in the context of genetic studies of inflammatory bowel disease. This study really changed our thinking about IgA nephropathy. It suggests that it is an inflammatory disease involving, or perhaps initiated in, the intestine. It’s not simply an intrinsic disease of the kidney.

“IgA nephropathy has traditionally been clustered with other kidney-type diseases; diseases that cause kidney problems such as lupus,” continues Dr. Gharavi. “Many of the drugs that have been tried to treat IgA nephropathy have been applied and developed for lupus. We have not had a lot of success with these. Interestingly, IgA nephropathy is a common cause of nephritis among patients with inflammatory bowel disease. This connection had previously been described in the literature, but no mechanism had been identified. If inflammatory disorders involving different organs have the same underlying mechanism, they may have a common treatment. This has led us to alternatively consider drugs that target pathways involved in intestinal inflammation.

Perhaps, we should repurpose drugs that are used for IBD, which share some genetic risk factors with IgA nephropathy or that may be developed by targeting the genetic risk factors identified in the study as a way of treating IgAN.”



The genome-wide association study also looked at the frequency of the 15 genetic risk factors, revealing that Asians had the greatest number of genetic risk factors; Africans had the fewest, with Europeans somewhere in between. “This told us that there are, in fact, different levels of genetic predisposition in different populations and that may be the reason why this disease is more common in Asians, less common in Africans, and in-between in Europeans,” says Dr. Gharavi. “It may be that Asians, on average, are just more genetically predisposed to this disease.”

The observed geographic pattern suggested that the genes that increase the risk of developing IgA nephropathy might also be beneficial in some way. Dr. Gharavi and his colleagues went on to compare the distribution of various environmental factors with the frequency of the genetic risk factors. “The strongest correlation was with the diversity of parasitic worms called helminths, which often infest the intestine,” notes Dr. Gharavi. “We hypoth-



Dr. Ali G. Gharavi

esized that high genetic risk of IgAN in East Asian populations might represent a consequence of protective adaptations against endemic intestinal worm infections. Connecting the disease with a pathogen is very helpful. Now we can go back and look at the pathways that fight intestinal worm infection to see if we can find ways to fight IgA nephropathy.”

Published in the November 2014 issue of *Nature Genetics*, the study was funded by the National Institute of Diabetes and Digestive and Kidney Diseases. It is the largest genome-wide association study of IgAN to date.

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