Megagene Unmasked

Huge gene leads to many tumors in the kidneys

By KATHLEEN FACKELMANN

Researchers now have a complete picture of the monster gene thought to cause polycystic kidney disease (PKD), an inherited disorder that strikes about 1 in 1,000 people worldwide.

Last year, a team of European investigators nabbed the gene and provided a partial sketch of the sequence of chemical units that go into it. Now, two groups of international investigators have confirmed and extended that work.

"We don't have a magic bullet today," comments Jared J. Grantham, a nephrologist at the University of Kansas Medical Center in Kansas City. "But I'm absolutely convinced that by the turn of the century we're going to have some very effective therapy for PKD." Grantham also serves as chairman of the Polycystic Kidney Research Foundation in Kansas City.

About 600,000 people in the United States have this disease, in which some of the urine-collecting tubules of the kidneys can balloon into tumorlike cysts. These tubules are part of the nephron, the kidney's urine-making workhorse. The cysts can become infected or stop functioning altogether. People who develop renal failure must opt for a kidney transplant or dialysis, a technique in which a machine filters poisons from the blood.

Kidney disease is the dominant feature of this disorder. Yet people with PKD can develop liver cysts and a dangerous pouching, or aneurysm, of a brain artery. Some people with PKD also suffer from high blood pressure.

The story begins with a landmark publication in the June 17, 1994 CELL. Peter C. Harris of the John Radcliffe Hospital in Oxford, England, and a team of European investigators had homed in on the location of a gene that, when mutated, causes about 90 percent of inherited PKD. Other genetic errors cause the remaining cases.

Harris and the European Polycystic Kidney Disease Consortium had identified the gene, called PKD1, on the short arm of chromosome 16, 1 of the 23 pairs of human chromosomes. The team had published a sequence of about 40 percent of the gene's base pairs, the chemical units that make up DNA.

That achievement lit a fire under a

Tracking a bogus peptide

When Janet S. van Adelsberg set out to find the protein linked to a deadly kidney disease, she joined a pack of investigators all pursuing the same elusive quarry.

Van Adelsberg won the race. Sort of. And therein lies a tale that epitomizes the mercurial nature of scientific endeavor.

When a team of European investigators published the PKD1 gene's sequence in June last year, van Adelsberg jumped into the hunt for the gene's protein product. Genes carry the detailed instructions that a cell's machinery needs to make a protein. Researchers believe the mutant version of PKD1 codes for a flawed protein that underlies polycystic kidney disease (PKD).

For technical reasons, van Adelsberg focused on the last 276 base pairs of the published sequence. After working night and day for 6 months, she finally had a piece of the protein in hand.

What's more, it appeared to be an extracellular matrix protein, a find that fit in nicely with a leading theory of a mechanism underlying PKD. Some researchers believe this disease results from a flaw in the connective tissue that supports the epithelial cells in the kidney's tubules. Extracellular matrix proteins are found in that tissue.

Van Adelsberg and her colleague's report of their findings was accepted by NATURE MEDICINE. She was scheduled to give a talk at a workshop sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, Md. Just days before the meeting, the unthinkable happened.

Van Adelsberg got a call from Gregory G. Germino of the Johns Hopkins University School of Medicine in Baltimore. It turns out that the European group's published sequence contained an error. For van Adelsberg and many other researchers, that error would prove fatal to their findings. She had based her work on a coding sequence that was now in dispute—that, indeed, probably didn't exist.

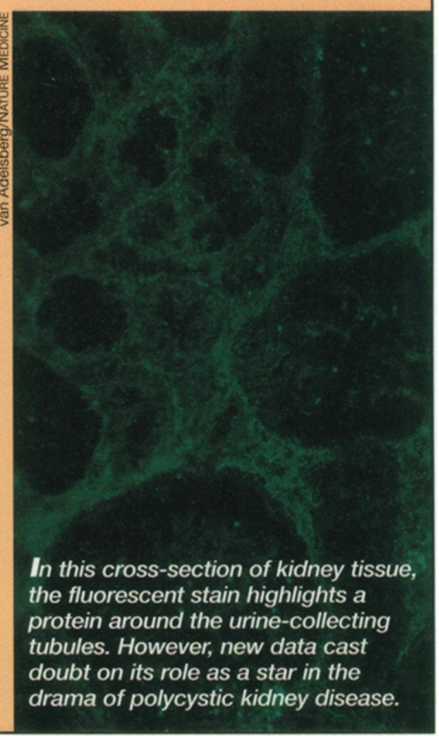
It was too late to stop the presses at NATURE MEDICINE: Van Adelsberg's paper is the cover story in the April issue. Furthermore, van Adelsberg went on to give a talk at the workshop, albeit not the talk she would have liked to have given.

As for the piece of protein, or peptide, she had identified, van Adelsberg says it's an interesting find, but whether it has anything to do with PKD remains an open question.

"I think she did fabulous work, based on the sequence that was published," comments Michael C. Schneider of Brigham and Women's Hospital in Boston.

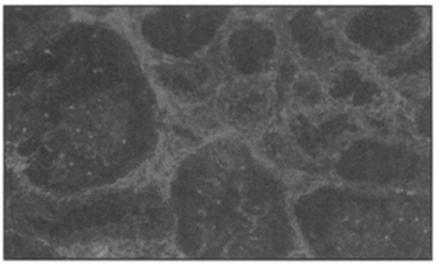
In the end, most researchers agree that the episode illustrates the essence of the scientific process. "This misfortune proves nicely what science is all about, because the truth will always be found," says Jared J. Grantham of the University of Kansas Medical Center in Kansas City.

For patients, this blind alley may even prove a boon. Van Adelsberg and the others who had been chasing the nonexistent peptide have now shifted gears. They're all hot on the trail of the real protein, based on the true sequence of base pairs. —*K. Fackelmann*



van Adelsberg/NATURE MEDICINE

In this cross-section of kidney tissue, the fluorescent stain highlights a protein around the urine-collecting tubules. However, new data cast doubt on its role as a star in the drama of polycystic kidney disease.



team led by nephrologist Gregory G. Germino of the Johns Hopkins University School of Medicine in Baltimore. Germino and his colleagues had been scouring the same neighborhood of chromosome 16 for this gene when the Europeans announced their discovery.

In due course, the Germino group identified the remaining 60 percent of the gene.

When they compared their data to those of Harris' group, they noted a key discrepancy. Germino's team had found an error in the published sequence of the gene.

That misstep, which scientists attribute to the massive size of the gene, had led more than one research team astray (see sidebar). Germino's group detailed their advance at a workshop on March 31 at the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, Md., and in the April HUMAN MOLECULAR GENETICS.

Scientists hope such research will help them understand how this gene functions in people with healthy kidneys.

"The disease process helps you identify a normal part of the [kidney's] hardware," Grantham says, adding that the gene could play a role in regulating the growth of the epithelial, or skinlike, cells that make up the wall of the kidney tubule.

Unraveling the gene's normal function may enable scientists to track down what goes wrong in PKD. Genes give a cell the information needed to build proteins from the correct sequence of amino acids. Harris and other investigators have already identified some of the mutations in the PKD1 gene that they believe cause the gene to make an altered version of its protein product. They speculate that the abnormal protein leads to the disease.

Germino's data hint that the defective protein may lace the surface of a tubule's epithelial cells. Such cells may never get the message to stop growing. As they proliferate, the normally hair-thin tubule starts to expand and retain fluid. Eventually, this urine-collecting duct can reach the size of a lemon.

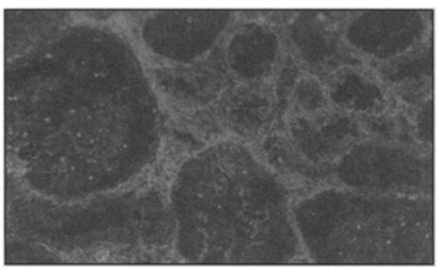
The European announcement inspired another team of researchers who had been doggedly tracking the PKD1 gene.

Sandra Glucksmann of Millennium Pharmaceuticals, Inc., in Cambridge, Mass., and her colleagues also presented their findings at the March workshop. They have put together a more detailed map of the PKD1 gene, including the precise location of the exons, or coding sequences, essential for making the protein.

Her team's map of the gene should speed identification of its protein product, Glucksmann says.

Once she learned about Germino's data, Glucksmann hand-delivered her team's manuscript to the Harvard Square office of CELL. "The competition has been fierce," she notes. The paper, which was accepted in 4 days, appears in the April 21 issue of that journal.

The authors of the CELL report include Glucksmann, Michael C. Schneider of Brigham and Women's Hospital and



Harvard Medical School in Boston, and Anna-Maria Frischauf of the Imperial Cancer Research Fund in London.

Harris and his colleagues now have data very similar to those published in the most recent CELL report. Their full description of the PKD1 gene and its predicted protein product appears in the June NATURE GENETICS.

Such research may lead to a test for PKD. Medical diagnosis of the disorder can be difficult, because symptoms often don't strike until midlife. Even then, only 45 percent of those with the disease go on to suffer renal failure by age 60. For the moment, doctors must ask about family history and then do some detective work in order to identify PKD. If researchers can find the mutations that occur most frequently, however, they may be able to devise a blood test to flag people who have inherited the flawed gene.

Because of the gene's large size, there may be many places in its DNA where flaws can lurk, Schneider says. Multiple mutations would make a simple blood test less likely, he adds. The Polycystic Kidney Research Foundation has set up a consortium to hunt for mutations in this gene, Grantham adds.

A blood test would come in handy for families considering a kidney transplant. In some cases, young persons want to donate a kidney to an older relative who suffers from renal failure. Yet doctors can't always rule out the donor's own risk of developing the disease in the future. With a DNA test, doctors could give potential donors a more definitive indication of risk, Germino says.

Because of the late appearance of symptoms, prenatal diagnosis of fetuses with this flawed gene seems less pressing, Grantham says.

Furthermore, if researchers can figure out how the protein works, they might be able to devise a drug aimed at slowing the progress of PKD. Currently, "there's no pill that will make the cysts go away," Grantham notes. Even treatment that slows cyst growth might make an enormous difference for people whose lives are cut short by this disorder, he notes.

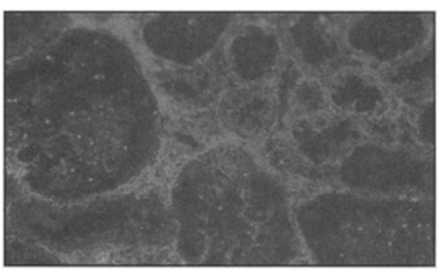
Harris agrees. "We hope we'll be able to understand the basis of this disease," he says. "And from that we hope we'll be able to develop a therapy."

To avoid testing unproven drugs on people, researchers will try to create animals with symptoms similar to those of PKD. By disabling the PKD1 gene in mice, researchers hope to produce rodents with the characteristic cystic kidney tubules.

"That would give us a [more precise] animal model—which we really don't have for this disease," notes Schneider. If they had such polycystic mice, researchers could tinker with various therapies aimed at mitigating or even reversing the course of the disease, he adds.

After more than a decade of research into this black box of a disorder, the discovery of the PKD1 gene represents "an enormous tour de force," Grantham says, noting that researchers in the field now are pursuing a number of intriguing leads.

Finding the PKD1 gene has cleared out an "enormous bottleneck in our understanding of this disease," Germino adds. "There's going to be an explosion in knowledge about PKD over the next 2 to 4 years."



That's not to say that the path ahead will prove smooth. The gene comprises about 14,000 base pairs, making it one of the largest disease-causing genes known to scientists. "It's a big gene," Grantham says. Schneider goes one step further, describing the gene's size as a molecular biologist's "nightmare."

All agree that PKD1 will provide scientists with plenty of challenges in the years ahead.

"I think the complexity of this makes it even more interesting," Schneider says. "We're going to be slogging through this little by little," he says. "I think it's going to be a daunting task for any one group." □