



SOLVING

BY DENNIS MEREDITH


It's an insidious, silent killer that can surface suddenly to trigger heart attack, kidney failure or stroke. With every heartbeat, chronic high blood pressure overworks the heart and distends arteries, slowly causing them to thicken and clog. But exactly how and why the disease arises has been a subject of confusion.

Before Rick Lifton and his colleagues began studying hypertension at the molecular level, most doctors viewed it as an impossibly intricate puzzle whose pieces consisted largely of environmental influences such as diet and exercise. In recent years, however, Lifton's group has made a series of important findings establishing the powerful role of genes—in particular, mutations that alter salt metabolism—in this devastating disorder that affects one in four Americans.

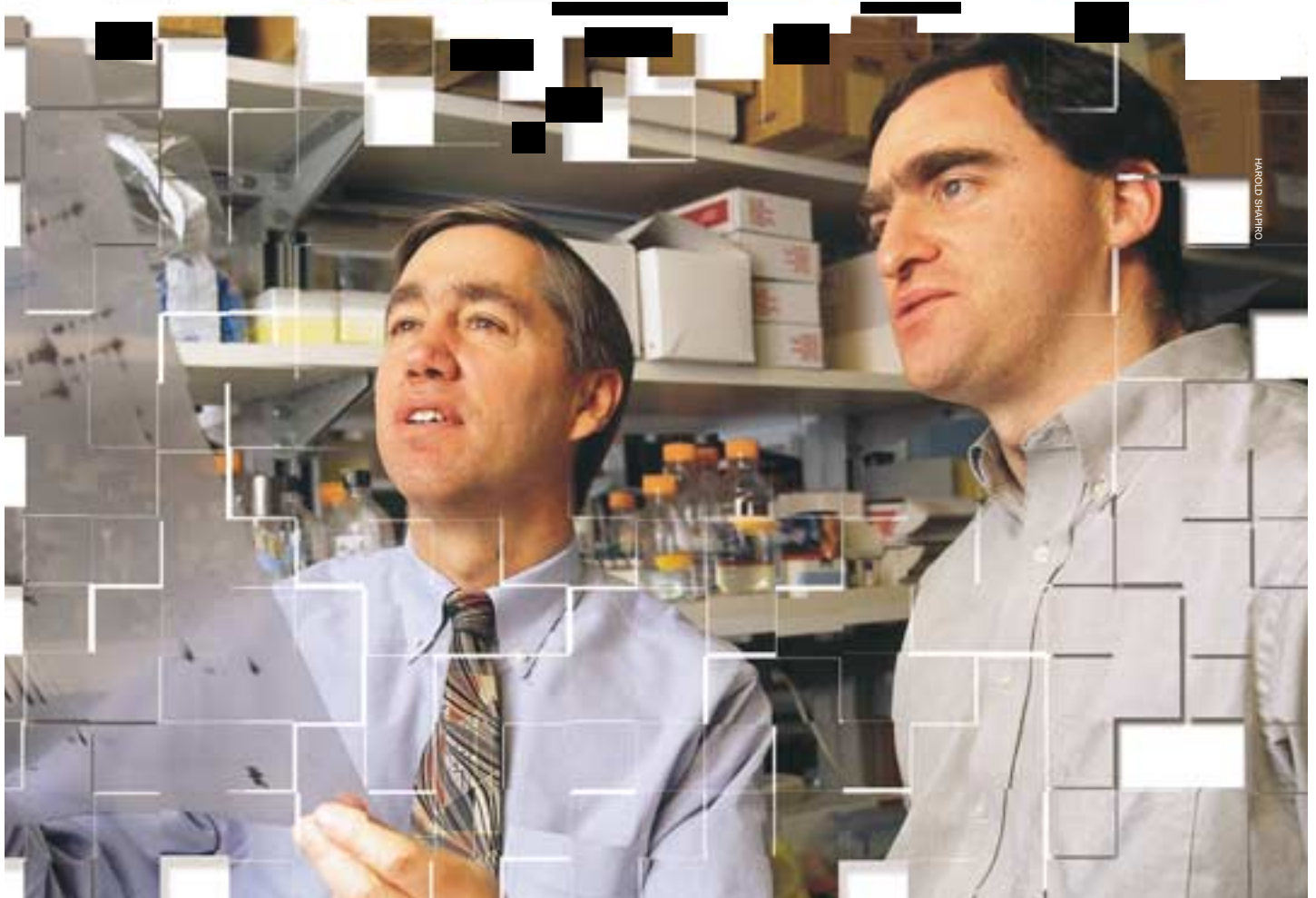
The change in perspective resulting from Lifton's genetic studies, carried out by an energetic team of clinician-researchers at Yale's Boyer Center for Molecular Medicine, is reverberating throughout the biomedical community. "Thanks to Lifton's landmark research, hypertension is now in the mainstream of molecular medicine," says Joseph L. Goldstein, professor and chair of molecular genetics at the University of Texas Southwestern Medical Center and corecipient of the 1985 Nobel Prize in physiology or medicine for his research on cholesterol metabolism. Goldstein also heads HHMI's medical advisory board.

It was, indeed, the prospect of overturning conventional wisdom that tantalized Lifton 15 years ago when he emerged from a residency

Rick Lifton (left) studies hypertension with team member David Geller.



hypertension's DEADLY PUZZLE



HAROLD SHAPIRO

DEVELOPING the **HOLISTIC** SCIENTIST

Rick Lifton's brightly lit maze of laboratories and offices, arrayed along a gracefully curved corridor of Yale's Boyer Center for Molecular Medicine, is a deceptively low-key place. The few sounds—the delicate clink of glassware at lab benches, the clicking of computer keys, the subtle murmur of consultations over data—give little hint of the dynamic discoveries emerging from within.

Lifton is naturally proud of those accomplishments, but he's equally proud of the scientists he mentors here, most of them physicians with purely clinical backgrounds. These young scientists are mastering the full range of talents they'll need to see their own projects through from start to finish and to assume leadership roles in their fields. Given Lifton's holistic perspective, team members come away not only equipped to pin down a disease in the clinic but also capable of rigorously analyzing its genetics and then figuring out how a genetic mutation causes disease.

"Most of the people trained in his lab have been physicians who came here not even knowing how to run a gel and then left as really good scientists," says Murat Gunel, a neurosurgeon who joined the lab eight years ago and is now establishing his own research group. "They learned to ask the key questions that enable translation of basic research to answer clinical problems."

The need to master both the tools of the profession and the strategy of using them to attack scientific questions was a lesson Lifton learned early on. "I was fortunate to have some spectacular mentors who gave me extremely good training and insight into how to approach problems," he says, citing in particular Lawrence Kedes and David Hogness, with whom he worked at Stanford. Just as those scientists encouraged him to identify his own project and follow wherever it led, so Lifton does for the young scientists in his own laboratory.

"I realized that to be successful in science, one needs to be intellectually flexible and not just wedded to one set of tools," he says. "That's not going to give an individual a broad perspective on science or the confidence to start their own lab."

Many labs don't allow young researchers to identify a disease and take full responsibility for

studying it, he admits. "I think there are a lot of laboratories that operate more as factories," he says. Among such labs, Lifton has observed two particular syndromes.

"One is where the lab is built around a particular technology, and that's it. It's like the old saying that to a child with a hammer, everything is a nail. This laboratory's leaders say, 'We won't think about doing problems that can't use this same hammer and the same nail,'" he explains.

"And secondly, there's the phenomenon of team science. In some areas, including genomics, it's relatively popular to develop, either within or across labs, big consortiums in which you divide up the problem. As a result, one person will only do, say, the analysis of linkage, and one person will only do the physical mapping, and one person will only do the gene identification and mutation detection."

Such an approach may seem efficient, but ultimately it does not produce fully capable scientists, Lifton contends. "I think in the long run it takes away a lot of the zest of doing science—which is driven by the fact that you've got an interesting problem. The reason you want to do science is that you're passionate about solving problems."

Researchers with holistic training are also prepared to adapt to changing scientific tools and to lead the way in developing them. "Anyone working today who thinks that 10 years from now they're going to be using the same tools is sadly mistaken," Lifton warns.

Ali Gharavi, a physician in the laboratory, relishes the environment Lifton has created. "There are no walls, no barriers, in this lab. I walked in and Rick said, 'Pick a project and you can work on it.' And having an M.D., I could identify certain problems that were important in my field and try to address them."

For Murat Gunel as well, Lifton's encouragement has been crucial.

"As I had to make the important choices, he helped me and guided me," Gunel says. "At those times when I felt I was only going to be a mediocre scientist, it was Rick who kept pushing me, supporting me."

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at Boston's Brigham and Women's Hospital pondering where best to concentrate his energies. The young physician-scientist was already well equipped scientifically, having done graduate research in the Stanford laboratory of molecular biologist David Hogness. There, isolating genes in fruit flies, he learned new techniques of genetic manipulation and analysis. He also established an indefatigable, independent-minded research style, often working until three in the morning to chase down a result, grabbing a few hours' sleep and then returning to the bench at dawn to launch his quest for the next piece of data.

"I came out of residency and saw that there were a lot of smart people doing very good work on the molecular genetics of cancer, cholesterol disorders and diabetes," the soft-spoken HHMI investigator recalls. "Yet, here was this very common trait, hypertension, that affects 50 million Americans and is one of the leading causes of morbidity and mortality. And we knew almost nothing about the primary determinants of this disease." When he proposed to explore the genetic basis of hypertension, Lifton immediately encountered skeptics. He remembers people telling him, "Hypertension is just much too complicated to try to apply these tools to, and you should think about something else." Lifton recalls that such remarks "just made me all the more convinced that this was the right thing to be doing."

Rather than tackle the massive puzzle of hypertension in the entire population, he decided to narrow his focus to genetic disorders affecting blood pressure—choosing just those distinctive medical puzzle pieces that he could most readily fit together to begin building a picture of the disease. The approach echoed that of Goldstein and fellow Nobelist Michael Brown, who in the 1970s tackled the complexities of cholesterol metabolism by tracing the cause of familial hypercholesterolemia. Their discovery that people with the dis-

order lack receptors for low-density lipoprotein and thus cannot remove this form of cholesterol from the bloodstream led to a radically new molecular understanding of the illness and set the stage for the multibillion-dollar market for cholesterol-lowering drugs.

"I figured the genetic approach might allow us to get our foot in the door in terms of understanding some of the fundamental pathways that affect blood pressure," Lifton says. So he decided to search for rare, single-gene forms of high and low blood pressure, using them to gain clues to the overall pathways involved in blood pressure regulation.

Lifton recalls that there was also doubt that even the targeted approach would prove fruitful. "The leading textbook on hypertension at that time made almost no mention of single-gene disorders that related to blood pressure, and there was a lot of skepticism that some of the diseases reported as single cases even existed as distinct entities," he says. "So it was a pretty murky start."

At that time, Lifton had taken a visiting faculty post at the University of Utah, but Brigham and Women's Hospital had also invited him to retain his clinical position there. This affiliation proved crucial when a colleague at the hospital, Robert Dluhy, encountered a patient with a rare form of hypertension called glucocorticoid-remediable aldosteronism, or GRA. Lifton immediately began a genetic study of the patient and her family.

"We began an investigation of this patient's family and fairly quickly acquired evidence that the disease was caused by a mutation in a particular gene," Lifton says. "However, the nature of the mutation proved elusive. We finally suspected that the mutation might be an unusual gene duplication that fused pieces of two normal genes to create a new gene with a different function. We devised an experiment to test this hypothesis. It was one of those seminal moments—coming back into the lab at three in the morning

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to look at the results. The autoradiogram was unequivocal and clearly showed that the head of one gene had been fused to the body of the other. This resulted in abnormal regulation of a critical steroid hormone. These are the rare moments of discovery and insight that we thrive on."

With this success under his belt, Lifton launched studies of every inherited form of high or low blood pressure for which he could recruit patients. After moving to Yale in 1993, he and his colleagues began studying a form of hypertension known as Liddle's syndrome, finding it to be caused by mutations affecting the renal epithelial sodium channel. Specifically, they focused on a defect that allowed a flood of salt into the bloodstream, raising blood pressure. Another key target for study became the gene encoding the mineralocorticoid receptor (MR) for the steroid hormone aldosterone, which regulates those sodium channels.

The first clue to MR's key role in salt balance came with the team's studies of a disease that had little apparent connection with high blood pressure. David Geller, a physician-scientist in Lifton's laboratory, was exploring a genetic disorder called pseudohypoaldosteronism type 1, which produces life-threatening loss of salt from the bloodstream at birth, along with other metabolic abnormalities. Geller discovered that the underlying cause of this "salt wasting" was a loss-of-function mutation in MR.

The scientists reasoned that if mutations causing loss of MR function produced salt wasting, perhaps mutations that increased the receptor's activity might cause salt retention and hypertension. Sure enough, when Geller began to study patients with early onset of severe hypertension, he



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became the first to pinpoint the specific mutations that overactivated MR, producing hypertension.

In one striking discovery, Geller, Lifton and colleague Paul Sigler reported last July in *Science* that a mutation in MR makes the receptor exquisitely sensitive to progesterone. They proposed that this finding explains why some pregnant women experience dramatic spikes in blood pressure: their mutant, progesterone-sensitive MR is activated by the 100-fold progesterone increase that occurs during pregnancy. Geller is now probing the molecular details of this mutation and scouting the possibility that mutations in other “nuclear receptors” resembling MR might cause entirely different metabolic disorders. Such mutations might affect the receptors for hormones, such as glucocorticoid, androgen and progesterone itself.

Researchers in Lifton’s laboratory have also pinpointed mutations that lower blood pressure by impairing salt reabsorption in the kidneys. They have shown, for example, that diseases known as Gitelman’s and Bartter’s syndromes, which feature low salt retention and low blood pressure, can arise from mutations in any of four genes. These include genes that encode cotransporters that mediate reabsorption of sodium and chloride ions, as well as genes that encode specific potassium and chloride ion channels involved in this same process.

Studies of the salt-regulation pathway by Lifton and his colleagues have revealed mutations in four genes that raise blood pressure and in eight that lower it. That body of work has unequivocally established the genetic contribution to hypertension, says Oliver Smithies, an Excellence Professor in Pathology and Laboratory Medicine at the University of North Carolina at Chapel Hill and a leading hypertension researcher. “Dr. Lifton’s research papers are a joy to read,” says Smithies. “They go right to the heart of the problems he investigates.”

Lifton is now zeroing in on a new blood pressure pathway.

BEYOND Hypertension

While pursuing the genetics of blood pressure regulation, Rick Lifton has encouraged scientists in his laboratory to follow their interests in other disorders whose secrets might yield to genetic exploration.

Kidney malfunction—end-stage renal disease, in particular—is a major problem that some team members are beginning to tackle. “If you take a broad view of big public-health problems,” Lifton says, “in the last 30 years we’ve made substantial strides in preventing stroke by treating hypertension, and a lot of progress in lowering the incidence of heart attack by reducing smoking and cholesterol levels. And yet, the incidence of end-stage renal disease has continued to go up, doubling every 10 years. So, we’ve been looking around for genetic approaches to end-stage renal disease, one of the most interesting of which has been the most common form of glomerulonephritis, called IgA nephropathy.”

IgA nephropathy affects up to 1 percent of the population worldwide and 100,000 people in the United States alone. It first shows up as blood in the urine, progressing to kidney-clogging deposits of the immune-related protein immunoglobulin, or IgA. Many patients develop kidney failure and need dialysis or a transplant to survive.

A number of scientists have assumed that IgA nephropathy sprang from multiple factors. But when physician Ali Gharavi, a fellow in Lifton’s lab, studied the disease in 30 U.S. and Italian families, he found, surprisingly, that the disease in most of the families was attributable to a single genetic locus on chromosome 6. Gharavi reported the results in the November 2000 *Nature Genetics*. “This falls into the category of a disease about which we know almost nothing of its fundamental pathophysiology,” Lifton says. “This finding demonstrates that genetic approaches to this disease will likely reveal its underlying biological mechanisms.”

In another promising foray, Lifton has launched studies of how pH and magnesium levels are controlled in the kidneys. “Our work on magnesium has taken us in some unexpected directions,” he says. “We’ve ended up discovering that mutations in a particular class of molecules called the claudins mediate the flux of electrolytes through a novel pathway called the paracellular pathway.” The surprise, says Lifton, is that this pathway consists of selective pores in the tight junctions between cells that allow certain ions to pass between the cells but not through the cell membrane. “These aren’t simple holes in the gaskets; they’re highly selective and specific pores,” he explains.

Working in Lifton’s laboratory on a study of families with a rare magnesium-wasting disorder, Yin Lu, a physician-scientist, and Keith Choate, an M.D., Ph.D. student, have pinpointed a culprit gene named *paracellin-1*. This gene mediates the selective flux of magnesium across the tight junctions of a specific segment of the kidney’s epithelium. The implication is that other members of the claudin family mediate the selective flux of ions, nutrients and even cells across body membranes.

In other genetic studies, lab member Murat Gunel, an assistant professor of neurosurgery, is focusing on a common neurological disorder called cavernous malformations, in which blood vessels in the brain become abnormally enlarged, causing seizures and paralysis. He is identifying genes whose mutations can cause the disease and exploring the underlying molecular mechanisms.

“All these studies represent our efforts to create an environment where young physician-scientists and postdocs can come and learn human genetics and laboratory methods and then pursue problems on their own,” says Lifton. “While we certainly remain absolutely committed to our core studies of hypertension, we also believe it important to explore promising new directions. The limitation, of course, is how many we can actually juggle simultaneously.”

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Lifton’s group is now zeroing in on an entirely new pathway that regulates blood pressure. Lifton says that he and his colleagues “have just started to crack one of the last remaining single-gene forms of hypertension.” This pathway “looks like it’s going to be more interesting than any of the others,” he says. “It’s almost impossibly exciting because we

know the genes involved and the clinical consequences of the mutations. However, we have none of the lines to connect one to the other. It’s like a skier seeing, after a fresh snowfall, nothing but virgin powder as far as the eye can see.”

Clearly, Lifton still delights in scientific exploration. “What I find immensely exciting and satisfying about science,” he

says, “is just learning something new—figuring out the way nature has been working for millions or billions of years, the way a particular human disease has worked ever since people have been on the planet. Those rare moments of crystallization where you suddenly see into the problem—those are priceless. Those are the moments that drive you.”