



TO YOUR HEALTH!

Guests toast San Franciscan Isaac Donner in celebration of his 100th birthday.



Secrets of the Centenarians

In certain families, small genetic variations bring good health and long life. Can researchers apply this knowledge to benefit us all?

BY MAYA PINES

IS THERE A FORMULA FOR LIVING TO the age of 100 or beyond? HHMI investigator Louis M. Kunkel believes there is, and he's working hard to define it.

Besides a healthy dose of good luck (Kunkel says it helps to not be killed in a war or a traffic accident), one key to longevity is a highly unusual combination of gene variants that protects against the customary diseases of old age. Several research teams are now in the process of uncovering these genes.

Kunkel, director of the Genomics Program at Children's Hospital in Boston, and his associates recently identified a genetic variant that is particularly prominent among sibling pairs in the New England Centenarian Study, perhaps the world's largest pool of centenarians. They are seeking additional genetic variants that might retard—or perhaps even prevent—many of the diseases that debilitate the old. "People with this rare combination of genes clearly age more slowly," Kunkel says. "When they reach 90, they don't look any older than 70."

Hundreds of centenarians around the world are now contributing their blood and medical histories to the search for these precious genes. They have become a key resource for researchers who hope that as these genes are

revealed, their good effects may be reproduced in other people with the help of new drugs.

CLUSTERED IN FAMILIES

Kunkel was drawn to the hunt for longevity genes about six years ago, through a chance encounter with Thomas T. Perls, a Boston University Medical School geriatrician who had enrolled a large group of centenarians for his New England Centenarian Study. Kunkel's own research was focused on a deadly genetic disorder called Duchenne muscular dystrophy, which affects mostly boys. In 1986, he discovered a mutation that causes this muscle-wasting disease, and he is still working on a therapy for it (see box on p. 16). But he could not resist the opportunity to also apply his knowledge of genetics to what he heard from Perls.

The two men were acquainted through Perls's wife, Leslie Smoot, who happened to be a postdoc in Kunkel's lab. When they met on a street in Cambridge, Massachusetts, in 1997 and started talking about their work, "Tom told me that many of the centenarians whose lineage he was examining were clustered in families," Kunkel recalls. "I realized that's just got to be genetics. We soon started a collaboration."

For his part, Perls remembers that at the

Photographs by Ed Kashi

The black-and-white photographs that accompany this article are from the book *Aging in America: The Years Ahead*, which explores in photos and text the social impact of longevity. Photojournalist Ed Kashi and his wife, writer Julie Winokur, worked eight years on the project. Their book was published last year by PowerHouse Books.



SENIOR BIKER

Wong Wah Po, 104, (center) immigrated to America when he was in his late 80s; he became a U.S. citizen at age 103. He is part of a growing trend of seniors who come to America to be close to their children.

beginning of his study he thought the centenarians had little in common except for their age. But he soon realized that many of them had an unusually large number of equally aged relatives. “We had a 108-year-old man who blew out his birthday candles next to his 102-year-old sister,” Perls recalls. “They told us they had another sibling who was 103, and yet another who was only 99. Two other siblings—also centenarians—had passed away. Four siblings had died in childhood. So here was an incredible clustering, 5 or maybe 6 siblings out of 10! We’ve since found about 7 families like that.” This implied that all these families carried especially protective genes. Shortly after the two scientists met, a new postdoc arrived in Kunkel’s lab—Annibale A. Puca, a young Italian neurologist who wanted to work in genetics—and Kunkel suggested he take on this new project. “I warned him it was going to be a lot of work and high risk, but he said okay,” Kunkel says, “and he spearheaded the whole program.”

Puca and Perls rapidly expanded the group of centenarians, recruiting them through alumni associations, newspaper clippings, and state census lists. After taking samples of the centenarians’ blood, the researchers extracted DNA from it and started looking for genetic markers—specific stretches of DNA that might occur more frequently among these extremely old men and women than among a group of younger people who were the study’s controls. Most scientists believed that human longevity is far too complicated a trait to be influenced by only a few genes. There are so many independent mechanisms of aging that “the chance that only a few major genes control longevity in man is highly unlikely,” wrote a self-styled “pessimist” on this issue, George M. Martin of the University of Washington in Seattle, in the journal *Mechanisms of Ageing and Development* in 2002. But Kunkel’s lab took a different view. “In lower organisms, such as nematodes, fruit flies, and yeast, there are only a few genes that need

to be altered to give a longer life span,” Kunkel says. “My feeling was that there were only a few genes, perhaps four to six, in humans that would do the same.”

The team proceeded to examine genetic markers for the entire genomes of 308 people, selected because they belonged to 137 sibships (sets of siblings) in which at least one member was over 98 and the others were over 90. “From early on, we saw a blip of a peak on chromosome 4,” says Kunkel. “Eventually, in 2001, we found a linkage between one region of this chromosome and longevity.”

SEARCH FOR A SNP

It was “phenomenal” to get a real linkage from such a slight hint in the original data, Kunkel declares. But that didn’t mean further research would be easy. This stretch of DNA was so large—12 million DNA base pairs long—that it seemed it could contain as many as 200 genes. Furthermore, the researchers knew that within

these genes they would have to look for variations in single bases of DNA—"single-nucleotide polymorphisms," or SNPs (pronounced "snips"). "SNPs really represent the difference between individuals," Kunkel explains. "Everybody's DNA is 99.9 percent identical—it's the SNPs that make us unique and allow certain people to live longer. Even though most of our DNA is alike, the 0.1 percent variation means that we have more than 10 million SNPs across the genome. And we're on the verge of being able to map them." For Kunkel, the critical question was "how would we find the one SNP in a single gene that might help a per-

son to live much longer than average?"

The groundbreaking work of the Human Genome Project had not yet been completed at that time, and Kunkel realized that finding this particular SNP would be both expensive and time-consuming. It would also be quite different from zeroing in on a missing or severely garbled gene, as had been done for cystic fibrosis, muscular dystrophy, and other single-gene disorders. The widespread diseases of aging—heart disease, stroke, diabetes, cancer, and Alzheimer's disease—are much more complex and are triggered by subtle gene variations that

produce only slightly altered proteins, Kunkel says. These proteins may either work a little better or be less active than those in the normal population, and several of them may work in concert. Searching for a single SNP would require doing thousands of genetic analyses on each of his subjects (now numbering 653) and comparing the results with the control group. "We estimated it would cost at least \$5 million," Kunkel said. "It finally cost \$8 million and took one-and-a-half years."

Ultimately, all that painstaking work paid off. The paper announcing the discovery of a SNP

Who Are These Centenarians?

"Centenarians tend to be independent, assertive, funny, and gregarious," says Boston University Medical School geriatrician Thomas T. Perls, who at 43 has probably met more people over the age of 100 than anybody else. "They also seem to manage stress very well, which makes sense, since we know that not handling stress predisposes you to cardiovascular disease and high blood pressure."

During a fellowship in geriatrics at Harvard Medical School in the early 1990s, Perls took care of 40 patients at Boston's Hebrew Rehabilitation Center for the Aged. Two of his healthiest patients, who looked as if they were in their seventies, were actually over 100 years old. "They were in really terrific shape," he says. "It was so different from what I expected! This sparked my interest."

As a result, Perls founded the New England Centenarian Study in 1994, becoming one of only a few researchers studying the very old at that time. He started out by looking for people over 100 in eight towns around Boston, using census records, voter registration files, and the media. Later, he expanded the study by adding centenarians from all over the United States. Now it includes 1,600 centenarians and 500 of their children. About 20 percent of the centenarian women in his study had given birth after the age of 40, Perls found, compared to a national average of only 3 percent of mothers. "It showed that these women were aging very slowly," he says.

He also studied the centenarians' siblings and concluded that their chances of living to their early nineties were four times greater than average. More recently, Perls examined the centenarians' children. At the age of 70, he found, they had a 24 percent reduction in mortality compared to the general population, as well as about a 60 percent reduction in the risk of heart disease, hypertension, and diabetes.

More than 90 percent of the centenarians had been in good health



Thomas Perls (right), director of the New England Centenarian Study, with Nelson McNutt, who was born on June 11, 1899.

and completely independent until their early to mid-90s, Perls says. "They lived the vast majority of their lives with good function," he emphasizes.

"So it's not a matter of 'the older you get, the sicker you get' but rather 'the older you get, the healthier you've been.' This is a different way of thinking about aging."

By the time people reach the century mark, however, the healthy ones are in the minority. "We found that 25 percent of the centenarians were doing well, but the remaining 75 percent had mild to severe impairment," Perls reports. "In the end, they die of cardiovascular disease or something that's related to frailty, such as pneumonia."

This fits in well with the theories of Leonard Hayflick, of the University of California, San Francisco, who showed in 1961 that there are limits to the number of times a normal human cell can divide. Even under the most favorable conditions, he said, noncancerous human cells die after about 50 cell divisions (this is now called the "Hayflick limit"). Eliminating the leading causes of death in old age—cardiovascular diseases, stroke, and cancer—"will only result in an increase of about 15 years in human life expectancy," Hayflick declared in the November 9, 2000, issue of *Nature*. Although these 15 years would be a great gift, assuming that people remained healthy during that time, nothing could stop "the inevitable increase in errors and disorders in the cells of vital organs" that results from age, he pointed out. Even the cells' repair processes would become disordered, leading to extreme vulnerability and death.

Then would it be a good thing for more people to live to 100? "Absolutely," says Perls. "Centenarians are sentinels of the health of older people. Our goal is not to get a bunch of individuals to be 120 or 130, but to discover which genes are most protective and then use this information to get a majority of people living almost all their lives in good health, as centenarians generally do."

that contributes to longevity was published in the November 25, 2003, issue of the *Proceedings of the National Academy of Sciences*.

NOW FOR THE OTHERS

The long-sought SNP turned out to lie within the gene for microsomal transfer protein, or MTP, which had been known since the mid-1980s to be involved in cholesterol metabolism.

“It’s quite clear that to live to be 100, you’ve got to maintain your cholesterol at a healthy level,” says Kunkel. “It makes perfect sense. We know that

increased LDL (the ‘bad’ cholesterol) and lowered HDL (the ‘good’ cholesterol) raise your cardiovascular risk and that cardiovascular diseases account for a large percentage of human mortality. So variations in the genes involved in cholesterol packaging will influence your life span. It’s as if these centenarians had been on Lipitor [a cholesterol-lowering drug] from birth!”

This discovery might lead to drugs that are tailored to intervene in the cholesterol pathway. Because the MTP gene was already in the public domain, however, it could not be patented,

much to the disappointment of the former Centagenetix Corporation (founded by Puca, Perls, and Kunkel and now a part of Elixir Pharmaceuticals of Cambridge, Massachusetts), which had bankrolled most of the study.

In any event, this SNP “cannot be the whole story,” Kunkel declares. “There must be other gene variations that enable people to avoid age-related diseases. Some of our original families did not show linkages to chromosome 4.” Nor did a group of centenarians who were tested in France.

Cures for Muscle Diseases?

Ever since Louis M. Kunkel discovered the cause of Duchenne muscular dystrophy (DMD) in 1986, he has been laboring to find a cure for this muscle-wasting disease. DMD—the result of an error in a single gene—attacks 1 out of every 4,000 newborn boys, progressively crippling and then killing them at an early age.

Kunkel saw that patients with DMD lacked a protein, dystrophin, which this gene would have produced if it were functioning normally. So he knew he had to replace the protein somehow. He and others tried many methods—gene therapy to deliver a normal gene to the defective muscle cell, drugs to help restore the missing protein, and cell therapy to inject normal cells into muscle or blood—but despite some partial successes in animals, nothing really worked.

Kunkel’s lab worked mostly with *mdx* mice, a naturally mutant strain that lack dystrophin. When he and his colleagues attempted to cure these crippled mice with injections of muscle stem cells from normal mice, “some of the donor cells did go into the damaged muscles,” he recalls, “but we never got more than 1 to 2 percent of the muscles repaired. Part of the problem was that when you inject cells into a mouse’s tail vein, which is the most accessible part of its circulation, the donor cells go through all the organs—the lungs, liver, heart, and so on—and out through the arterial system. Most of the cells get filtered and lost, and don’t contribute to the therapy.”

Today, however, Kunkel feels he is on the verge of success. The big breakthrough came last summer when a team of Italian scientists headed by Giulio Cossu of Milan’s Stem Cell Research Institute announced it had found a new route for the injection of stem cells into dystrophic mice directly into an artery. The cells seemed to lodge within the capillary system near the injection site. From there, about 30 percent of them migrated to the diseased muscles. “Not only did the cells get there,” he says, “but at later time points, you could see a larger number of donor cells than at the earliest point, as if they were trying to divide.”

“Can we improve on this?” asks Kunkel with a glint in his eye. “If we can get the stem cells into 50 percent of the dystrophic muscles, that’s basically a cure.”

They had trouble at first because “the mouse artery was 10 times smaller than our smallest injection needles—it was like trying to hit it with a hammer!” Kunkel says. “Though a tail vein is even smaller than an artery, it can be hit much more easily because it is right under the surface of the skin and can be made to swell up by warming it. In the new system, the mouse had to be anesthetized and opened up to expose its artery, which was lifted out—a complex procedure.

“It wasn’t until we started collaborating with some vascular surgeons who had been doing heart transplants in mice that we were able to get the stem cells into the mouse arteries efficiently,” he says. In humans, of course, reaching an artery would not be a problem given that human arteries are so much larger.

Getting the stem cells into the muscles was just the first step. Unless these cells supplied enough dystrophin, the diseased muscles would not be repaired. So Kunkel also tried to find different stem cells that could do the job more effectively. In 1999 his lab and that of his colleague Richard Mulligan announced they could restore some of the missing dystrophin in *mdx* mice with the aid of a new kind of stem cells called “side population” (SP) cells, which seemed to work much better. These SP cells had to

be taken from muscle tissue, however. Last year Kunkel’s lab succeeded in deriving similar SP cells from adult skin, which is easier to obtain. Since they originate in adult tissue, both kinds of SP cells will be much less controversial than embryonic stem cells.

“It’s my belief that you can do a lot of therapeutic intervention with adult-derived cells,” says Kunkel. He notes that the new stem cells seem ready to differentiate into every type of muscle tissue, which implies that they have the potential to treat many forms of muscle disease.

The combination of a new cell type and a new delivery system “may revolutionize how one does therapy for muscle diseases,” Kunkel suggests. “When we get it perfected in mice, we’ll go to humans.” He thinks this might happen “in a couple of years.”

Louis Kunkel looks for genetic markers for aging.



Determined to find some of the other SNPs that produce longevity, Kunkel says he's going back to his sample and will redo the whole study. "We now have 310 sibships," he says. "Our genetic markers are much denser. I believe we can get 10 times the power in our next screen than we had in the first."

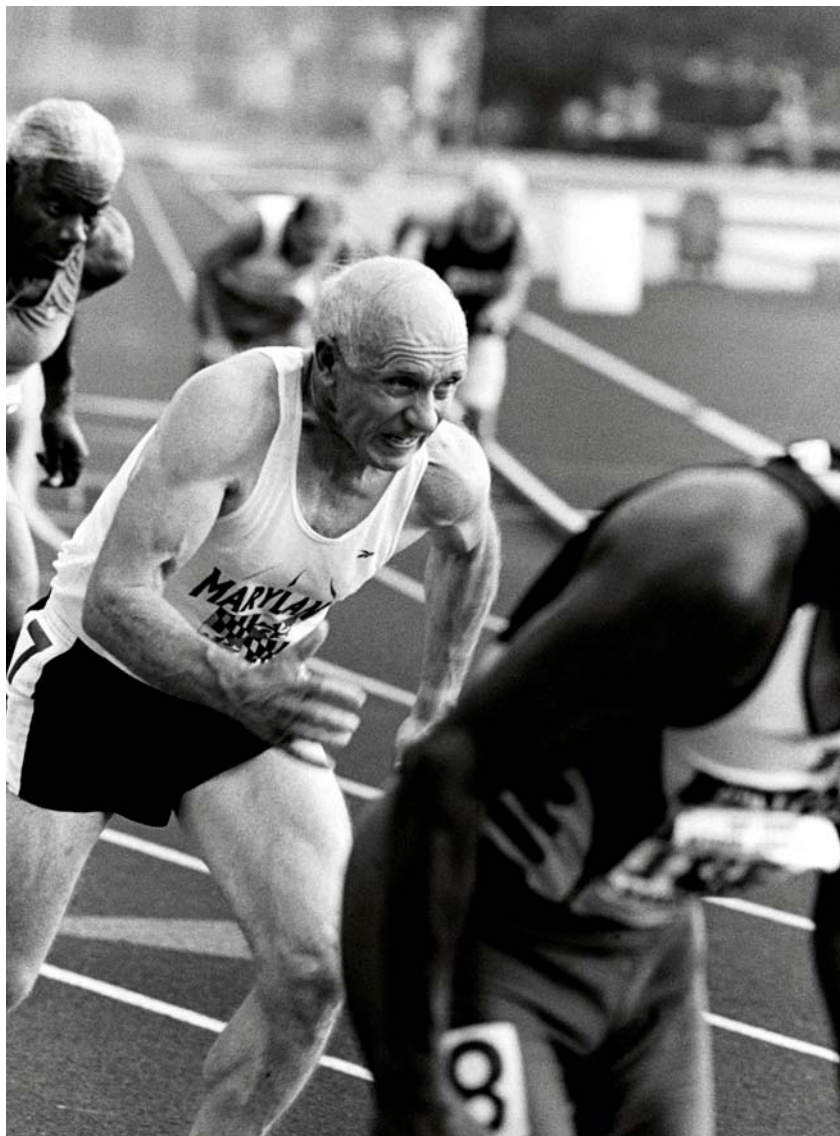
Moreover, the work can be done much more rapidly and inexpensively than last time, he notes, given the giant strides that have been made recently in human genetics. Not only has the entire human genome been sequenced, but many of the errors in the original draft have been corrected. Equally important, all the known genes in the genome are now available on a single Affymetrix DNA chip, allowing researchers to promptly identify which genes are activated and which are damped down in any given situation. In addition, as many as 10,000 different human SNPs have been placed on a single chip.

Similar tools have already turned up new gene variants in yeast, worms, and flies. But Kunkel will use the chips to analyze the DNA of humans. Once his lab gets started on the new longevity project, he believes, it will not take very long to get some definitive answers. He hopes these will lead to drugs that could mimic the protective effects of the centenarians' genes.

GOLD STANDARD

In fact, these studies foreshadow a far-reaching attack on all complex diseases—not just those of the aged but others, such as autism and hypertension. None of these ills could be tackled efficiently in the past. "The centenarians are the ideal control group for such research," Kunkel says. "To reach 100, you must have good alleles [versions of the genes] at all points. So if one wants to find the genes that are connected with hypertension, for instance, one can look across the genome for genes that are highly active in the hypertensive population but down-regulated in centenarians. Ultimately, that's what the centenarians' genes will be used for."

He believes that in the future, "every person who comes to our genetic clinic—or goes through any type of care system—with what appears to be a complex disease should be analyzed in detail. I mean that we should gather all the information we can about each patient's symptoms, the family history of these symptoms, any environmental insults the patient suf-



GO!

More than 12,000 athletes, including these runners, competed in the 2001 Senior Olympics in Baton Rouge, Louisiana.

ferred, any learning disability—anything that would allow us to categorize the patient and [the patient's] family into subtypes of the disease which could be more related to one another and thus more likely to involve the same gene." To make this happen, Kunkel has just appointed a director of phenotyping (the Greek roots of this word mean "classifying phenomena into specific types") who will collect, categorize, and catalogue such patient information.

"We will also analyze the patients' genes but only in the context of the category of symptoms they exhibit," he says. "The samples we col-

lect—under appropriate protocols—will be available to the national groups of patients and researchers that are organizing to find the underlying genetic bases of specific diseases." Eventually, he hopes, many complex disorders such as heart disease, diabetes, and autism will be broken down into more specific categories, which in turn may lead to more precise treatments or ways of preventing the disorder. Kunkel expects this process to accelerate in the near future as more patients' genes are compared with those of the gold standard for humans—the centenarians. **H**