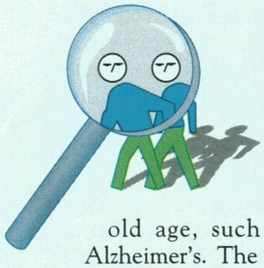
GENETICS

Aging Twins Offer Clues to Late-Onset Diseases



A little-known database, containing a half-century of records on thousands of twins, may turn out to be a gold mine for investigators seeking to understand diseases of old age, such as Parkinson's and Alzheimer's. The database contains extensive medical records on nearly 16,000 pairs of white male twins who served in the U.S. military in World War II. Since then, the National Academy of Sciences (NAS) has kept tabs on the twins' whereabouts, periodically updating their medical histories and recording any deaths, thereby creating one of the world's largest registries of aging twins. Although the academy did not hide this resource—indeed, the cohort has been tapped for studies in the past—it made no concerted effort to publicize it either. In fact, the last time the registry was actively promoted in print was in a book on twins research published in 1978, though it has been mentioned in several research articles since then.

Now, as the 21,000 surviving members of this unique cohort reach their late sixties and seventies, several investigators have stumbled upon this database for the first time and they are thrilled. Alone among the world's twin registries, they say, this one offers a unique opportunity to tease apart the genetic and environmental influences of late-onset diseases because of the registry's size, the broad selection of the subjects, the twins' age, and the fact that two-thirds of them are still alive and available for detailed physical examinations. "It's a once-in-a-lifetime [opportunity]," says Caroline Tanner, a neurologist and epidemiologist at the California Parkinson's Foundation. Tanner first learned of the registry just 3 years ago when a colleague—searching for a useful database—struck gold, she recalls. Now Tanner is heading a new multimillion-dollar collaborative study to probe the causes of Parkinson's, Alzheimer's, stroke, and cancer. It is the largest investigation yet conducted on these twins and, indeed, one of the largest direct-contact twin studies ever carried out.

Among Tanner's collaborators are some of the few scientists already well aware of the registry and the secrets it may unlock. Indeed, "people had been waiting for the [twins] to develop the diseases they're getting now," explains Lawrence M. Brass, a Yale University neurologist who is leading the stroke study. True, the cohort does have limitations, chief of which is that it consists of only white men. Moreover, twin studies themselves have a habit of starting more arguments than they settle because of the methodological difficulties they present. But such problems pale in comparison to the insights the cohort may reveal, say the collaborators, who also include John Breitner, a geriatric psychiatrist at Duke University Medical Center, and Neil Caporaso, a genetic epidemiologist at the National Cancer Institute (NCI).

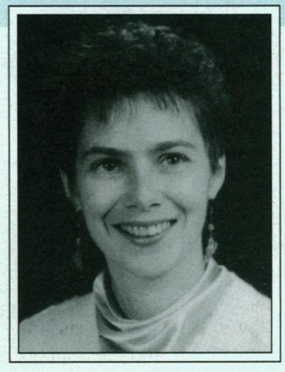
The DeBakey connection. The twin registry is a direct outgrowth of a 1946 campaign by heart surgeon pioneer Michael DeBakey—then in the Army Surgeon General's office—to ensure that the medical research community would have access to World War II records. After a blue-ribbon panel endorsed DeBakey's call that same year, the National Research Council at the academy created what has come to be called the Medical Follow-up Agency (MFUA), which now employs about 10 people coordinating medical studies of veterans and is supported by funds from the National Institutes of Health, the Veterans Administration, and the Department of Defense. Then in the early 1950s, a pathologist studying heart disease suggested to the staff at the new agency that a study of the twins of veterans who had suffered a heart attack might yield insight into heart disease. The MFUA, in particular statistician Bernard M. Cohen, seized on the idea in 1955 and then set about designing and compiling such a registry, which evolved into a mammoth cross-referencing effort involving two dozen staff workers at the MFUA along with officials and clerks in dozens of state and federal agencies.

Cohen first asked the 48 states to search their vital statistics offices for the birth certificates of all white male twins born between 1917 and 1927. About 40 states complied. With that list—54,000 pairs of Mervins and Marvins, Floyds and Boyds—clerks at the Veterans Administration then sought each twin among the several million index cards in their files. They found 15,924 pairs who had served in World War II and whose military medical records were already on file. Yet more clerks pulled their military service records to abstract any medical and physical information.

Cohen died in 1962, about the time the registry was completed. Then MFUA statistician Seymour Jablon, now at NCI, and James Neel, a University of Michigan geneticist, turned to another chore—finding an inexpensive method for determining whether each pair was identical or fraternal, a crucial fact if the registry were to be useful. They tried FBI fingerprint records, physical measurements, and a battery of blood tests. It turned out to be easiest, and 95% accurate, simply to ask the twins whether as children they were "alike as two peas in a pod" and were frequently mistaken for each other. From that they ascertained that about 45% were identical and 55% fraternal.

Research on the twins has already yielded dozens of papers with insights into heart disease, mental illness, and "Type A" and other behaviors such as alcohol consumption. In the largest longitudinal study of adult twins ever done in the United States, Richard Fabsitz of the National Heart, Lung, and Blood Institute and colleagues conducted a series of physical and laboratory examinations on a subset of the cohort to search for genetic influences on various risk factors for heart disease. The conclusion? Genes play a role in smoking, cholesterol levels, obesity, and especially blood pressure. Most recently, Dorit Carmelli, a genetic epidemiologist at SRI International in Menlo Park, analyzed two earlier surveys of the twins' smoking habits. In a report published last September in the *New England Journal of Medicine*, she concluded that genetic factors have a "moderate" influence on whether people ever smoke and on the ability to quit.

A database of World War II veterans offers "a once-in-a-lifetime [opportunity]."
–Caroline Tanner



From the start, however, findings based on this registry have been controversial, as are those of many twin studies. The problem boils down to an assumption used to calculate genetic factors. Typically, twin studies attempt to determine the relative contribution of genes to a disease by comparing the prevalence of a trait in fraternal and identical twins. Any excess concordance—the percentage of pairs where the twins have the trait in common—among identical twins suggests the trait is influenced

ILLUSTRATION: D. DEFRANCESCO

What the Twin Studies Might Tell Us

In this ambitious collaborative study just now getting under way, the four principal investigators have turned to the veteran twins to sort out the most fundamental questions about the origins of several diseases of old age.

■ **Parkinson's:** For Parkinson's disease, the focus of the study headed by Caroline Tanner of the California Parkinson's Foundation, the etiology is unclear, and over the past 70 years sentiment has swung from the environment—the encephalitis virus—to genetics and back again to a near-consensus that both genes and environment play roles. Past studies, however—including 10 twin studies—were muddied by overbroad diagnoses of the disease or by cohorts that were either too small or not randomly selected, says Tanner.

A new lead came in 1982, when neurologist William Langston, Tanner's co-investigator and founder of the California Parkinson's Foundation, discovered drug addicts who had given themselves instant Parkinson's disease by injecting a bad batch of synthetic heroin that destroyed the same area of the brain that is involved in the disease. Now Tanner, Langston, and their team want to pin down whether natural Parkinson's also has an environmental cause. Her hunch is that the disease requires both a genetic susceptibility, such as a mutant liver enzyme, and an encounter with a toxin that kills neurons.

■ **Alzheimer's:** The picture is nearly as hazy for Alzheimer's disease. Some forms clearly run in families, and the disease is sometimes associated with Down's syndrome. But that doesn't necessarily mean that the primary cause of most forms is genetic. Likewise, reports of associations with environmental factors such as head trauma or not smoking are varied and often contradictory. John Breitner of Duke believes genetic factors lie behind Alzheimer's, though the environment obviously plays a role. The parents, siblings, and children of Alzheimer's patients appear to have a 50% chance of developing the disease themselves if they live to age 90, says Breitner. That's the pattern one would expect if Alzheimer's were a dominant trait, like Huntington's disease, but was expressed at different ages depending on environmental cues, he says.

Breitner, who first studied the twins 3 years ago, intends to test this model by studying the timing of the disease in pairs of identical twins. Men with early signs of memory difficulties—and their twins as controls—will be quizzed about 120 environmental variables, including smoking, head injury, depression, and use of aluminum-containing antacids. Most of these have been posited before as either risk factors or protective factors, but the large number of people in this study and the use of co-twins as controls should provide stronger clues, Breitner maintains.

■ **Stroke:** The potential genetic etiology of stroke has never been well explored, according to Lawrence Brass of Yale University, even though such questions have been probed in heart-disease, which is often related. Fewer than a dozen studies have examined genetic risks for stroke, he says, and nearly all were done before CAT scans were available to help distinguish the several different types. Brass, who treats stroke patients at Yale and at the West Haven Veterans Affairs medical center, did find a suggestion of a hereditary factor in stroke when he analyzed questionnaires from some of the twins 8 years ago. But the number of cases recorded at that time was too small to be conclusive. He hopes the new study will locate enough subjects—about 800 men with strokes—to tell whether that pattern holds. Brass also will query the subjects and their twins about their diets and personalities to see whether those are associated with stroke risk. Finally, he hopes to improve clinical management of patients by studying how some established risks—such as hypertension and diabetes—affect a patient's health when they occur together.

■ **Cancer:** In the cancer study, the twins will be asked two questions: whether they've had it, and what kind. The last to join the new study, the cancer team is still working out the details, says Neil Caporaso, senior investigator in the genetic epidemiology branch of the National Cancer Institute. Some of the questions are clear, however, says Caporaso, who wants in particular to see if the twins can help resolve whether genetic factors are involved in lung cancer. The medical literature suggests that in family members of people with lung cancer, the risk of lung cancer is doubled or tripled. But critics say that these studies don't fully take into account the effect of passive smoking—something Caporaso hopes the twin study will remedy. The team also plans to analyze the twins' DNA to look for a correlation between lung cancer and the inheritance of genes that code for enzymes suspected of activating carcinogens in tobacco smoke.

–D.A.

by their shared genes. But this assumes that identical twins live in environments no more similar than those of fraternal twins. Not only is that an obvious oversimplification, but skeptics argue that it's probably wrong, since parents and society treat identical twins far more similarly—when feeding, dressing, and educating them, for instance—than they do fraternal twins. That argument has been raised as recently as February, when critics asserted that Carmelli may have overestimated the genetic influence on smoking.

More widely accepted is a class of twin studies that deliberately exploits the differences in the lives of identical twins. These studies have paired up identical twins as case and control subjects to examine such topics as the effects of vitamin supplements on learning, and of vitamin C on colds. Any differences between these pairs, the reasoning goes, can't be the result of genetic factors and must be caused by environmental ones. "It's like raising two rats in the same lab and giving one a cigarette," explains Brass, who will be using such a case-control analysis as part of his study on stroke.

Although the new studies in Tanner's collaboration are also likely to be challenged for using such techniques, the investigators say that they're undeterred. Information about inherited factors in these diseases is so scarce, they say, that they will take any clues they can find. "I just want to know if [the answer] is black or white. I'll worry about shades of gray later," says Brass.

The collaboration was born about 3 years ago, when Jonas Ellenberg of the National Institute of Neurological Disorders and Stroke (NINDS) was scouting around for any databases of the elderly. He and Tanner are part of a loose network of Parkinson's epidemiologists who want to nail down what kills certain neurons, leading to the tremors and rigidity characteristic of the disease. At a meeting Ellenberg heard someone mention the twin registry, which he and Tanner didn't even know existed. It turned out to be so unusual, and time so precious because most of the twins are now in their seventies, that Tanner and her team quickly put a proposal together, which NINDS approved last fall. The project was planned in collaboration with Breitner of Duke, who was already quite familiar with the registry; he had recently screened the twins for early signs of Alzheimer's disease and was planning a follow-up survey (see box above).

Last fall Tanner discovered that Brass and Caporaso also hoped to survey the same twins within the next couple of years to root

out the genetic factors in stroke and cancer. Realizing that the research community could quickly wear out its welcome by calling the vets too often, Tanner proposed that the four groups collaborate on an initial phone survey that would simultaneously seek likely subjects for each study. The collaborators first met one another in late November. In her initial telephone survey just now getting under way, Tanner is attempting to reach all the survivors from the original cohort—the 16,000 men still in "intact" pairs, and the 5000 others whose twins have died.

The collaborators suspect that the twin cohort could be invaluable for studying other diseases of old age, such as arthritis and pneumonia, once investigators learn of its existence. But with the veterans' average age now above 70, time is running out. "We're getting rather concerned about that," says Eldon Sutton, a University of Texas geneticist who heads a committee that reviews proposals to study the twins. To address that, the review committee hopes to enlist several hundred twins in one additional project: to establish permanent cell lines so that their DNA can be tested in the future as new ideas about the genetic and environmental bases of disease arise.

–David Ansley

David Ansley is a medical reporter at the San Jose Mercury News.

ADDICTION RESEARCH

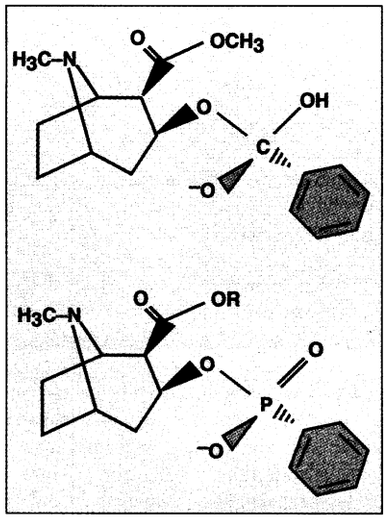
Enzyme May Blunt Cocaine's Action

Of all the common drug dependencies, addiction to cocaine, particularly in its smokable crack form, has proved the most difficult to treat. Now, in what researchers are hailing as the most promising approach in decades, a team led by physician and organic chemist Donald Landry of Columbia University's College of Physicians and Surgeons has created an enzyme that may break down the drug in the bloodstream, thus reducing its addictiveness. Although still in the test tube stage, Landry hopes that his enzyme—which may one day be administered as a vaccine—will add a new weapon to the arsenal of drug treatment. If the enzyme does indeed blunt cocaine's effects, he says, it might enable patients to forego the drug while participating in rehabilitation programs.

Experts in drug addiction say that such a treatment is badly needed. While researchers have searched for years for drugs that could block cocaine's effects or diminish a person's craving for it, their efforts have not panned out. "What we desperately need is an agent that would help a patients stay drug-free for 6 to 12 months while they're undergoing psychological and social rehabilitation," says psychiatrist Herbert Kleber of Columbia, who was the country's deputy drug czar in the late 1980s. Landry's approach, he adds, "is a totally new way of looking at cocaine addiction, and, if successful, will revolutionize the treatment."

What Landry and his colleagues have done is create a catalytic monoclonal antibody that can bind specifically to cocaine and then break it into two inert byproducts, ecognine methyl ester and benzoic acid—the same byproducts that are produced naturally when cocaine is broken down in the human body. "What I'm doing is essentially mimicking the natural degradation pathway in humans," says Landry.

To make the antibody, the researchers first immunized mice with a compound whose structure resembles that of the "transition state" for cocaine breakdown. The transition state is the high-energy, unstable intermediate through which chemicals undergoing a reaction must pass. The Landry team intended to speed up cocaine breakdown by finding an antibody that could bind to the drug and stabilize it in the transition state. The Columbia group then used antibody-producing cells from the immunized animals to hybridomas, cells that make monoclonal antibodies, and screened for those that could break down cocaine. They found one that is comparable in its activity to the enzyme that breaks down cocaine in the body. Such antibodies could be administered, he suggests, as a form of passive immunization to prevent cocaine from reaching the brain and causing the powerful "rush" that makes cocaine so addictive. The native enzyme on its own isn't active enough to do the job, but he hopes that the catalytic antibodies will improve on nature.



Antibody target. The compound used to make the catalytic antibody resembles the cocaine transition state *(above)*.

Landry began looking into the possibility of using catalytic antibodies to treat cocaine addiction in 1990 after hearing George Bush make a plea for researchers to find a way to immunize people against drug addiction. Landry then discovered that in the 1970s, Charles R. Schuster, now a senior research scientist at the Addiction Research Center of the National Institute for Drug Abuse (NIDA) in Baltimore, had actually shown that antibodies to heroin could block its effects in addicted rhesus monkeys. The problem was that once heroin bound to the antibodies, it couldn't be released again. So while the treatment works for low doses of heroin, higher doses sop up all the circulating antibodies and the treatment is no longer effective. And that's where catalytic antibodies have an edge, Landry says. Since they release the cocaine breakdown products, the catalytic antibodies themselves aren't depleted but can continue to bind and destroy more cocaine molecules.

Still, when Landry began his research, he feared that it might fall too far out of mainstream approaches to cocaine addiction to be seriously considered. "There is always that danger in trying something new," he says—although in Landry's case, instead of finding himself "off on [his] lonesome," he was able to anticipate a new trend. Indeed, at about the same time that Landry began his research, other biochemists, including Richard Lerner, president of the Scripps Research Institute and one of the inventors of catalytic antibodies, had launched similar investigations. And last fall, the National Institute on Drug Addiction issued a request for proposals for research using monoclonal antibodies and similar agents to attack cocaine addiction.

Although Landry beat Lerner to the punch, Lerner has only praise for the younger man. "It's a lovely piece of work" and has the potential to "move catalytic antibodies into the treatment of human problems for the first time," he says. But while Landry's research holds great promise, it is still in the test-tube stage. He is currently making additional, more active antibodies and hopes to begin testing them in an animal model of cocaine addiction in the next few months. "That will give us some indication if this treatment is actually effective," he says. In the best of possible worlds, human trials might begin in 5 years.

In the meantime, Landry's research has "demonstrated that what was originally just a concept—using monoclonal antibodies to treat drug abuse—is a feasibility," says Frank Vocci, director of the office of medication development at the NIDA. Far from being outside of the mainstream, Landry's work is now the leading edge.

–Virginia Morell