

THE GENE HUNTERS

Closing in on the origins of Alzheimer's disease.

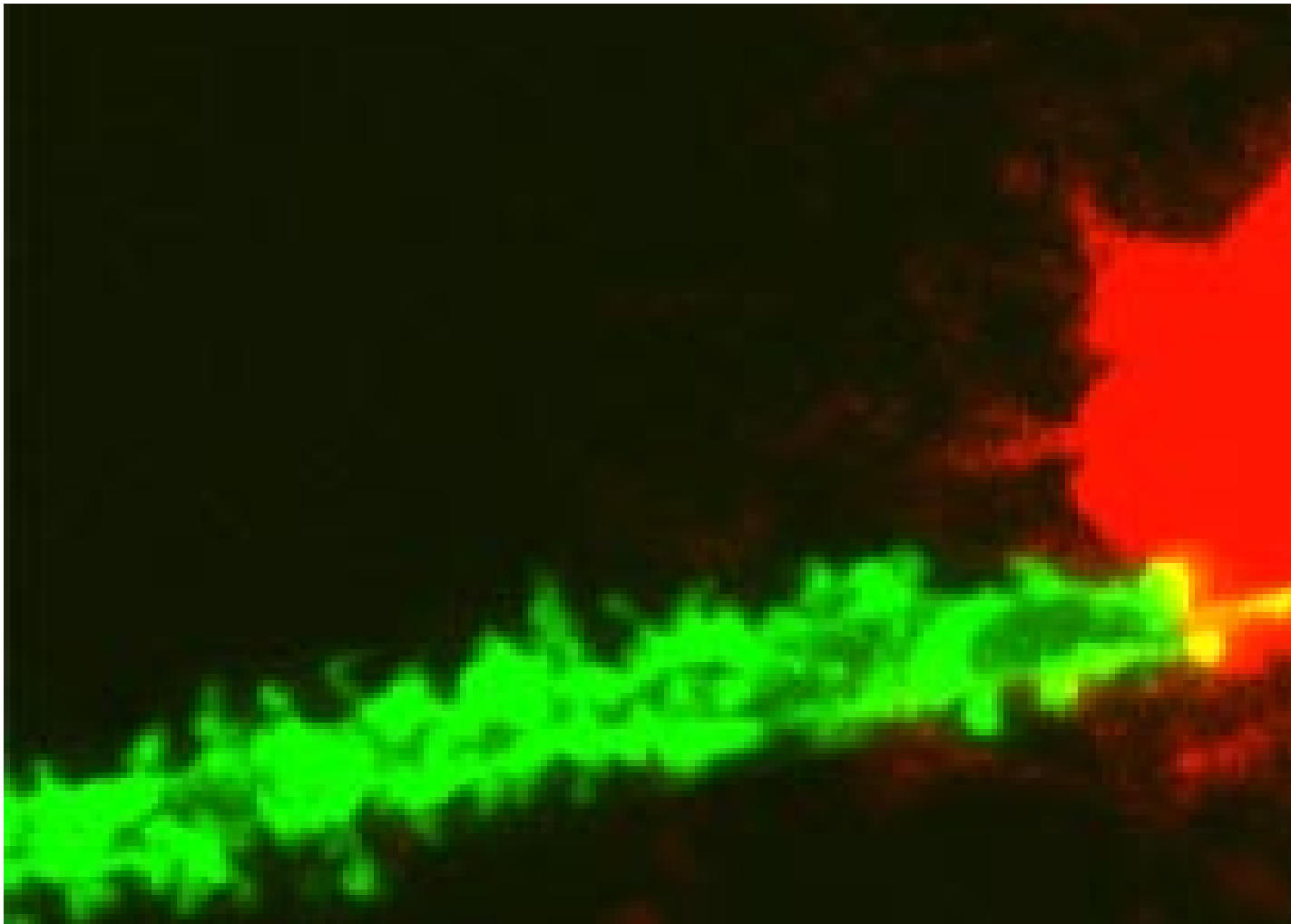
BY SUE HALPERN

One morning last May in the Dominican Republic, two white S.U.V.s left the parking garage at the Gran Almirante Hotel and Casino, in Santiago, just as the gamblers and prostitutes were calling it a night, and headed half an hour north, to the town of Navarette. The lead vehicle was driven by Angel Piriz, a thirty-seven-year-old Cuban doctor who lives in New York. Beside him was Rosarina Estevez, a recent graduate of medical school in Santiago. Both were working as research physicians at Columbia University under

the supervision of Richard Mayeux. For nearly twenty years, Mayeux, a neurologist, epidemiologist, and co-director of the Taub Institute for Research on Alzheimer's Disease and the Aging Brain, has been compiling the world's most comprehensive genetic library of families with Alzheimer's, in an effort to uncover the biological origins of a disease that affects 4.5 million Americans. The family members are predominantly residents of the heavily Dominican neighborhood of Washington Heights, where the Taub Institute is based, or, like the

family that the Columbia researchers were hoping to see, from the Dominican Republic itself.

Navarette isn't much of a town—a strip of concrete shops on either side of the road, and street vendors selling pineapples and mangoes and fresh goat meat—and the family didn't have much of an address. "It's called Ginger Alley," Vincent Santana, the driver of the second vehicle, said, turning sharply into a narrow dirt track patrolled by chickens. Santana, who is in charge of the researchers' field work, gathered the notebooks



In Alzheimer's, research suggests, a protein fragment known as beta-amyloid attacks the brain's synapses. When a neuron's axon

and questionnaires they would need to administer the neuropsychological tests that, along with a medical exam, would determine who would be given a diagnosis of Alzheimer's disease.

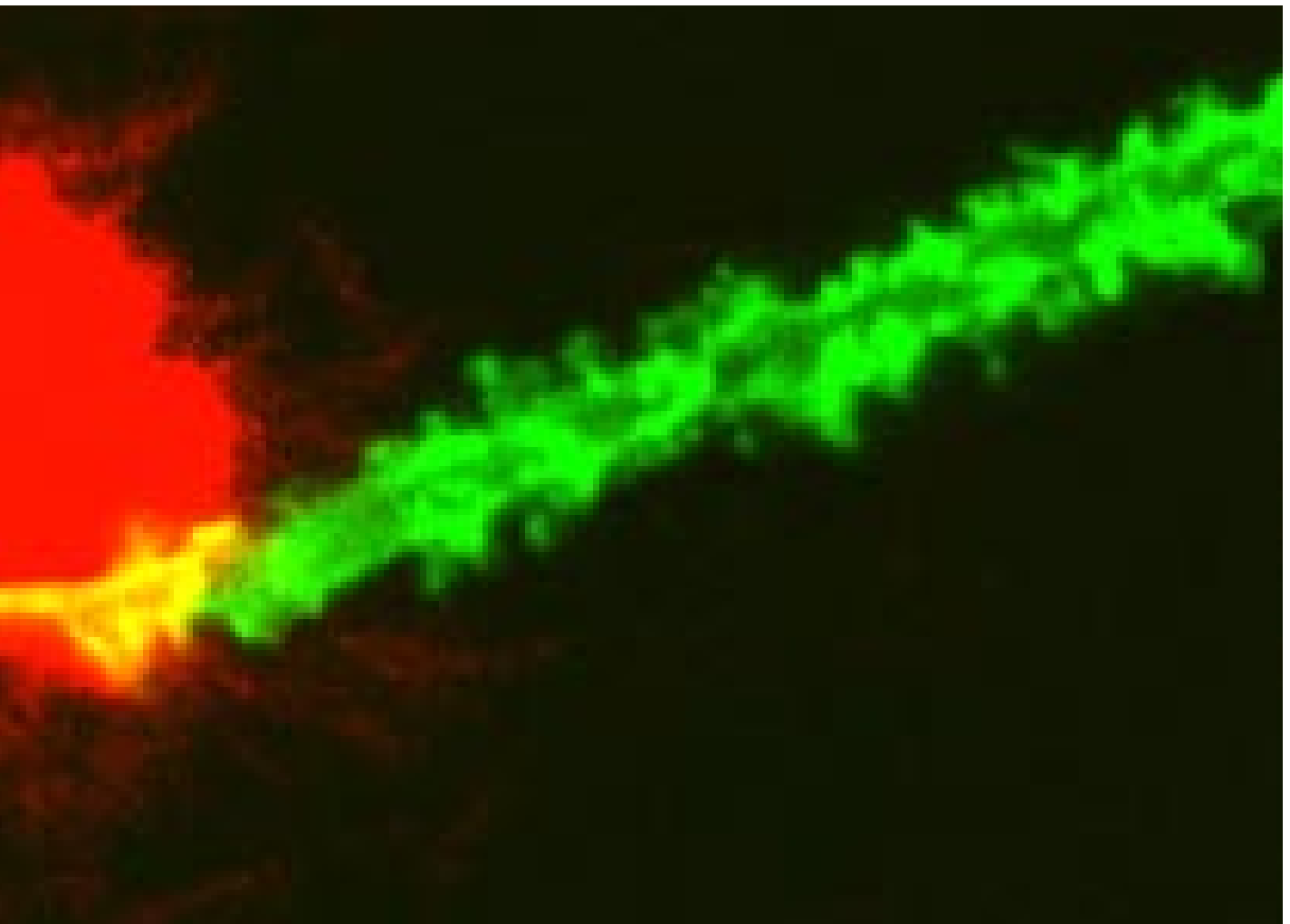
Alzheimer's can be divided into two categories. One is known as early-onset Alzheimer's, which is rare, and tends to strike between the ages of thirty and sixty. Almost half of early-onset Alzheimer's is genetic, and follows the simple laws of Mendelian inheritance: if you are born with the mutated gene, you get the disease. Much more common is the late-onset disease, which tends to afflict people who are sixty-five and older. Because the prevalence of late-onset Alzheimer's increases as the population ages, the number of cases is expected to double in the next twenty-five years. Late-onset Alzheimer's is thought to be genetically influenced, too, but in a much less predictable way: it appears to involve perhaps half a dozen genes that,

individually or in combination, increase one's risk of dementia. Researchers all over the world have spent the past decade hunting for these risk-factor genes, spurred by the impending public-health crisis and the daunting insufficiency of available treatments. They believe that working out the genetics of late-onset Alzheimer's, and thus finding molecular pathways that influence the course of the disease, was the best—and possibly the only—hope for finding a cure. So far, only one of those risk-factor genes has been conclusively identified. In May, though, as the Columbia researchers travelled through the Dominican Republic, drawing blood that was sent by FedEx each day to New York, it looked as if Mayeux's library might soon yield a second.

"This is a branch of the original family we saw here last year," explained Santana, a soft-spoken Dominican-American whose face was often knot-

ted with worry. (Were the directions good? Would the subjects be home? Would they still be willing to volunteer? Would the data be useful? Would the blood spoil? And what about the Red Sox?) He directed Estevez to interview an elderly couple who lived across the way, then headed down the alley with Piriz, past houses made of concrete and tin, cutting through someone's kitchen and into a narrower alley and a warren of houses, asking for a man named Vargas. The proband—the first person in the family that Mayeux's team saw—died in February, at age ninety. "She's confirmed with the disease," Santana said. "In her generation, a couple of cousins and siblings have A.D. There's some first-cousin intermarriage. These people we're seeing today are her cousins. If we can find them."

As the researchers walked through the neighborhood, they attracted a parade of young boys, who eventually led



(displayed as green in the photograph above) encounters deposits of the beta-amyloid (displayed as red), they break apart and wither.

them to Vargas's house, a spare, three-room dwelling. Vargas, a gaunt eighty-three-year-old who was tanned from a life growing bananas and tending rice fields, was lying, bare-chested and wearing blue shorts, on a bed with yellow smiley-face sheets. Surrounded by two of his five wives, four of his fourteen children, and an assortment of other relatives, he wasn't saying much. A few months earlier, he had been told he had pancreatic cancer and he wasn't expected to live out the year. Even so, he had consented to Santana's request to participate in the study.

Santana had known about Vargas for almost a year. In his notes from an interview with the proband in the spring of 2004, there was a reminder to identify and track down all her cousins and their siblings, in order to determine how they were related. Constructing accurate genealogies, which is what Santana does, is fundamental to figuring out how a disease travels among kin, which is what Richard Mayeux does.

"What day of the week is it?" Santana asked Vargas. A series of questions followed: "What is the date?" "What year?" "Where are we?" This was the warmup, and Vargas was doing O.K. He knew that he was in the bedroom, not the kitchen; he knew the year; he knew the season.

Santana leaned in close. "I'm going to read you a list of twelve words, and when I'm done I want you to repeat them back to me. *Huevo*," he began. "*Lava*." Vargas fingered a religious medal he wore around his neck and looked lost. "I can't remember," he said, pointing to his head. The verbal test is one part of a forty-five-minute battery of exams that was developed for the Dominican Republic study by Yaakov Stern, a neuropsychologist who has worked with Mayeux for more than twenty years. If someone, given the opportunity to repeat any of the twelve words six times, for a top score of seventy-two, can't get to twenty-five, he might be considered for "case status." When the test was over, Vargas's score was well below that.

In the next room, Piriz was going through the same routine with one of Vargas's wives, a short seventy-year-old woman in a faded housedress and flip-flops, who was eyeing him warily. "Do you ever find yourself getting lost?"

Piriz asked. "Hell no," she said. He took her blood pressure, looked into her eyes, tested her reflexes. Then he put on green latex gloves, took out a syringe, and prepared to draw her blood.

"I started off thinking Alzheimer's was not a genetic disease," Mayeux told me the first time I visited him, last November, in his unusually tidy office, on the nineteenth floor of the Presbyterian Hospital Building, on West 168th Street. "I thought it was environmental, associated with aging. But the accumulating data convinced me. It doesn't always follow a pattern, but it does also track in families, so that if you have family members with the disease you have a much higher risk of getting it, and siblings with the disease give you an even higher risk. The evidence was very hard to counter."

Mayeux selected one of a series of thick bound volumes that took up most of a wall, and began flipping through the pages as he spoke. It was an encyclopedia of family trees, which showed who in his study was related to whom, which ones had the disease, which ones were disease-free, and which ones were living in the border town between lucidity and dementia.

"What we wanted to do was find a population where we thought the rates were higher, because the thing about genetics is that if you try to identify people who carry the gene you are looking for unusual people," Mayeux said. "It's not like epidemiology, where you try to get random samples of random people. Genetics is just the opposite. You want a biased population. You want families where there is more of the disease, because you have a better chance of figuring out what the gene is.

"That's how we stumbled into this study of people in the Dominican Republic," he went on. "We noticed, when we were doing a general population study of elderly people who live around the hospital, that Dominicans had about three times the rate of Alzheimer's disease compared to the whites in the community. So you have to ask yourself why that would be. Then it starts to explain itself—that, at least in the Dominican Republic, Dominicans tend to marry other Dominicans, and you don't have different populations moving in there. You have a smaller genetic pool, and the

gene pool tends to stay enriched." Mayeux pointed to a page in the book. "Here's one. These two people are twins. Look at how many people who are related to them are affected and how many are beginning to experience symptoms."

Mayeux, who was born in Louisiana, has a nasal drawl and a deceptive air of someone with time on his hands. He is fifty-nine, tall and fit, with a full head of brown hair going slowly gray and a mostly unlined brow. His colleagues joke that he is a graduate of the Dick Clark School of Aging. He is constantly in motion, typically late, and driven: in addition to his role at Taub, he is the director of Columbia University's Sergievsky Center, which conducts epidemiological research on neurological diseases; a professor of neurology, psychiatry, and epidemiology; a practicing physician at Columbia's Memory Disorder Center; and the coordinator of a nationwide effort to collect, store, and make available to researchers genetic material from families with late-onset Alzheimer's disease. (The project was initiated in 2002 by the National Institute of Aging, which also funds the bulk of Mayeux's research.) Still, he is so ready to credit colleagues and collaborators that his own ambition can sometimes seem evanescent.

Since getting his medical degree, at the University of Oklahoma, in 1972, Mayeux has studied diseases of the central nervous system: epilepsy, Parkinson's, Huntington's, Alzheimer's. He is also an adroit administrator, overseeing a staff of a hundred and eighty-five neurologists, geneticists, psychologists, epidemiologists, data-entry clerks, cell biologists, biochemists, genetic counsellors, and animal modellers spread over five floors of the hospital building, as well as a clinic at Columbia's Neurological Institute.

Here they were pursuing the medical version of "big science," drawing on a dozen separate disciplines, each with a distinct vocabulary, methodology, and way of seeing, in an effort to understand diseases that are widely feared because they seem inexplicable and random and so common as to be nearly inevitable. With Alzheimer's, everyone has a stake in the outcome, and for those who have had a parent or close relative succumb to dementia there's particular urgency

to the question: "Are we next?" My visits to the Taub Institute over the past year opened a window on the collaborative—and competitive—nature of much scientific innovation. Forget about the image of the solitary genius hunched at a lab bench; forget about the eureka moment when everything is explained. The members of Mayeux's team had no illusions about what real progress would require. They were looking for mutated genes, for corrupted molecular pathways, for predictors of disease, for effective therapies. The search was painstaking, fraught, and more prone to failure than to success; it was also exhilarating.

Many of the team members—like the geneticist Joe Lee, who sifts through the thirty thousand genes that make up the human genome, seeking a genetic quirk that could account for the neuropathology associated with Alzheimer's disease, and the neurologist Scott Small, who has developed a new way of using magnetic-resonance imaging to look deep into the brain—took the conventional academic route of medical degrees and doctorates. But a surprising number never intended to chase what Mayeux calls "the great white whale of neuroscience." There is on staff a former Wall Street accountant who analyzes M.R.I. data and a German particle physicist who develops diagnostic-imaging techniques. Angel Piriz, the Cuban doctor, had spent three years working for a Manhattan construction company, and he found the Columbia job on the Internet. Vincent Santana was a nineteen-year-old security guard in the Presbyterian Hospital emergency room when he was offered extra hours to escort Mayeux's field researchers on their interviews in Washington Heights, his own neighborhood. Eventually, Santana became one of the researchers, expert in administering and scoring complex neuropsychological tests, and then the research coordinator, and now, at thirty-five, a co-author of four of Mayeux's scientific papers.

Not far from the Taub Institute, in the basement of the New York Brain Bank, there is a room with industrial freezers containing thirty thousand samples of plasma. In 1988, when Mayeux inaugurated what is known as the WHICAP study, a sweeping investigation of the health and habits of twenty-five

hundred elderly residents of Washington Heights, he instructed the researchers to collect blood in addition to recording demographic and medical data. The field of Alzheimer's genetics was in its infancy, and putting blood on ice to look for genes was either capricious or prescient. It had been only four years since scientists at the University of California,

And that chromosome is where, in 1991, geneticists at the University of London found the first Alzheimer's gene. It was called APP, an acronym for amyloid precursor protein, and was associated, in mutated forms, with early-onset Alzheimer's. The mutations caused the overproduction of beta-amyloid in the brain; without exception,



Scott Small, Joe Lee, and Richard Mayeux. Photograph by Martin Schoeller.

San Diego, succeeded in sequencing amyloid, the key constituent of the plaques that accumulate in an Alzheimer's brain, and about two years since it was established that amyloid was a peptide—a protein fragment that came in different lengths. The researchers called the toxic peptide beta-amyloid, and proposed that a genetic mutation causing its overproduction would be found somewhere along chromosome 21, an extra copy of which produces Down syndrome. The amyloid they had sequenced was identical to the amyloid found in the brains of Down patients, and, by middle age, most people with Down syndrome have developed Alzheimer's-like symptoms, so it seemed logical that chromosome 21 would be implicated.

people who carried the mutations developed Alzheimer's disease. The gene gave scientists a way to begin to understand what was happening in an Alzheimer's brain, and a rudimentary hypothesis: Alzheimer's disease was caused by clumps of beta-amyloid that strangled neurons and synapses.

"When it was first discovered, we thought that it was *the* Alzheimer's gene," Mayeux recalled. But the math didn't work: there were millions of cases of Alzheimer's disease, and the APP mutations were estimated to occur in fewer than two hundred of them.

The search for additional genes gained momentum during the nineteen-nineties, and within a few years three more had been found and confirmed.

Two, called presenilins, were discovered by the University of Toronto neurologist and geneticist Peter St George-Hyslop, and were also associated with early-onset Alzheimer's. They caused beta-amyloid to accumulate in the brain by affecting the way enzymes break up the amyloid precursor protein into amyloid fragments—which gave further credence to the idea that too much beta-amyloid precipitated dementia. Still, the presenilin genes couldn't account for the usual form of the disease, either; they were carried by only a few hundred families worldwide.

The fourth Alzheimer's gene, ApoE, was different. It wasn't Mendelian and deterministic, it didn't cause early-onset Alzheimer's, and it wasn't rare; we all carry it. A common variant of this gene is ApoE4; it significantly increases the risk of getting Alzheimer's, but it doesn't cause it. Many who have the ApoE4 gene (about a quarter of the population) never get Alzheimer's disease, and many people who do get Alzheimer's don't carry it; twelve years after its discovery, no one can say why that is.

In the lectures that Mayeux gives to people who may be unfamiliar with the simplest facts of genetics—that genes, which code for proteins, are made

up of sequences of chemicals called bases, and, if one base is out of sequence, the protein may be dysfunctional—he often ends up talking about cops and robbers.

“Our colleagues at the genome center in the United States tell us that there may be three billion base pairs in the entire genome and that there are something like one hundred and twenty million base pairs in each of the chromosomes, and about two thousand to two hundred thousand base pairs in a gene, and what we are doing is looking for a single base pair that is different or out of sequence,” he told a group of Dominican professors and students at the Universidad Tecnológica de Santiago, after his field researchers completed nine more interviews the previous day in Puerto Plata and four more that morning in Jicome.

“So we're looking for that one base pair, which is like having the police know that someone has committed a crime somewhere, but they don't know where, and they have to start looking for him all over the universe,” Mayeux went on. “That's basically where we are. The goal of genetic family studies is to try to get down to the earth, and then into the neighborhood, and eventually to find the culprit.”

If mutations are the robbers, and scientists are the cops, then since the discovery of ApoE4 the cops had made something like a hundred false arrests. In one peer-reviewed paper after another, research teams all over the world claimed to have identified about a hundred unique genes that in some way triggered late-onset Alzheimer's disease. Yet none of those findings had been replicated consistently by other researchers, if at all.

There's a simple reason that no one has found a new Alzheimer's gene in more than a decade, and another reason that is less simple, and they both come down to the same thing: statistics. Risk-factor genes, the genes that will explain late-onset Alzheimer's, are inherently elusive, because carrying them does not automatically presage disease. More challenging, there may be many risk-factor genes, each with a potentially minute effect. To detect such genes requires a large, geographically isolated family study like Mayeux's, with reams of information about each individual's health issues, eating habits, and work and leisure pursuits, as well as genealogies that show the genetic path of the disease. Mayeux's thick books of pedigrees and his database of DNA allow researchers to define a person's genotype (what genes she carries) as well as a phenotype (what traits she embodies) and then to subdivide the phenotype according to which traits specifically correlate with the kind of dementia that characterizes Alzheimer's disease. On the nineteenth floor of the Presbyterian Hospital Building, the crucial diagnostic marker seemed to be a person's performance on certain memory tests, and the researchers were seeing a pattern in performance and disease which they hoped would show up in the genes.

“Age of onset is a wimpy phenotype,” Mayeux said to no one in particular at one of the team's weekly genetics meetings in his office earlier this year. Mayeux thought that relying on family members to identify when a subject began showing signs of the disease was too subjective. “Memory is better. It's quantifiable.”

“Delayed recognition is the most sensitive test we have for A.D.,” Joe Lee, the geneticist, told him. “That and another test that I can't recall. I may be



“I'm only going to the party because the suicide attempt failed.”

a subject for this study soon myself.”

“We all may be,” Mayeux said, laughing. It is a measure of the disease’s prevalence that the seven people sitting around the table had a mother, a father, an aunt, a grandmother, and a grandfather with Alzheimer’s.

A few months after that genetics meeting, Mayeux gathered together in his office a number of his researchers, including the neuropsychologist Yaakov Stern and the research physicians Angel Piriz and Rosarina Estevez. Soon, Santana rolled in a cart piled three feet high with files of first-time participants from the Dominican Republic and follow-up examinations of subjects who had been seen on previous visits, as well as files of their relatives in Washington Heights.

Santana, who was a college dropout when he started working for Mayeux and is now close to completing an M.B.A., handed out score sheets—officially called “clinical core diagnoses”—that looked rather like an I.R.S. 1040 short form, with various sections and schedules and subtotals, all leading to a bottom line: did the participant have Alzheimer’s disease or not? To get there, the researchers had to rule out Parkinson’s disease, prion disease, alcohol dementia, dementia with Lewy bodies, frontotemporal dementia, and anything else that might mimic the symptoms of Alzheimer’s.

“We’ll just get through as many as we get through, and then get the data to Joe Lee, so he can put it into the computer,” Mayeux said, pulling a dozen folders off the cart. “Here’s someone you saw,” he said, waving a folder at Estevez, who was sitting next to him. “What was his blood pressure?” He gazed at her intently, not letting on that he was joking—in fourteen days in the Dominican Republic, the researchers had examined ninety-eight people, and Estevez could not possibly remember, nearly a month later, any individual’s vital signs. She seemed a little stricken. As the newest member of the team, Estevez had not yet grown accustomed to Mayeux’s ability to tease, compliment, and assert his authority, all in the same sentence. “One-seventy-five over ninety,” she shot back. Mayeux looked stunned. “That’s amazing. How did you do that? Did you know or did you guess?” But there was no time for an answer. “O.K., what else

do we know about this guy?” he asked.

Accurately diagnosing a subject’s condition is critical to the gene hunt, and the design of Mayeux’s field studies, with follow-up visits every eighteen months, increased the odds that they would get it right. “The way most studies are done is that a person is seen once and diagnosed as either having Alzheimer’s disease or not having Alzheimer’s disease, and then not seen again,” said Peter St George-Hyslop, who had used Mayeux’s Dominican cell lines to identify some of the presenilin genes’ mutations. “In the Washington Heights and Dominican studies, people are followed up again and again”—enabling pre-symptomatic Alzheimer’s patients to be distinguished from genuinely normal control subjects. A few too many erroneous diagnoses, the researchers knew, could blur the subtle genetic patterns that they were looking for.

To refine its diagnostic powers, the Columbia group convened a meeting once a month to see how close its assessment of a person was to the incontrovertible pathological truth yielded by an autopsy. The meetings, in a crowded conference room, were run by the neurologist Lawrence Honig, another associate of the Taub Institute. The neurologists, pathologists, and psychologists typically sat at a long seminar table that dominated the room, and the rest squeezed in around them. Honig presented a patient’s history and tentative diagnosis, then projected slides of the brain, first whole, then in slices, stained in pink and blue to show its dominant features. Then the doctors decided, based on that one piece of evidence they had been missing, if they had been right.

A week after the August meeting, I stopped by Honig’s nineteenth-floor office, and he showed me slides of a woman whose case had been discussed that day. It was a good illustration of how difficult it could be, even with years of data, to be sure of a diagnosis. Honig, though in his early fifties and going bald, has the boyish affect of someone who has always been the smartest kid in the class, and he started out by walking me briskly through an abridged version of the woman’s medical history: a clerical worker with a year of college, she had been first seen in 1992, at the age of

sixty-eight. An alcoholic and a former smoker, she had numerous ailments—cirrhosis, gallstones, pulmonary disease, and facial-nerve palsy among them. In later years, she was found to carry the ApoE4 gene, and a brain scan showed some atrophy. But she had done well on all her medical and neuropsychological tests, both that first year and at every interval until 2000, when there was a decline in some of her memory scores. Two years later, there was a further decline in memory, and the clinicians discussed whether to move the woman from the non-affected category to a diagnosis of early-stage Alzheimer’s. The neurologists, led by Honig, were pretty sure, based on the fact that the woman’s test scores had been stable for more than a decade, that her recent memory problems were the result of her various physical ailments. The neuropsychologists were sticking by their norms. Unable to agree, they left the diagnosis unchanged, waiting to see what would show up the next time around. But by late 2004, when the woman was scheduled to be seen again, she was dying of congestive heart failure.

On his computer monitor, Honig called up a couple of slides of the woman’s brain. There was nothing in the slices that looked like measles, which is how plaques show up when they’ve been stained, nor did the woman’s brain appear especially shrunken, as Alzheimer’s brains tend to be.

“We couldn’t even find a single plaque,” Honig beamed. “There were no signs of A.D. So I can crow that I was right. But we’re not always right, so we have to be modest.”

Neurologists have spent the past hundred years waiting for pathologists to prove them right, ever since 1906, when the German psychiatrist Alois Alzheimer autopsied the brain of a fifty-one-year-old woman who had exhibited the kinds of behavior that most of us now would reflexively call Alzheimer’s disease, and found it riddled with something that looked like discarded wads of gum (plaques) and matted strands of hair (tangles). Pathologists, for their part, had waited almost as long to find out whether the plaques and tangles caused disease or were just an artifact of some other biological process.

What were they to make of the people who died with all the pathological evidence of Alzheimer's but suffered no dementia? And how to account for the presence of both plaques *and* tangles? Were the plaques, which are made of beta-amyloid, more important agents of disease than the tangles, which are composed of a protein called tau, or were the tangles the prime suspect, or were the two accomplices in fleecing memory?

These questions consumed researchers for decades. But what Professor Rudy Tanzi, of Harvard, has called the debate between "the Baptists and the Tauists"—those who believed in the supremacy of beta-amyloid and those who favored tau tangles—was becoming more civil all the time. The tauists, many of whom had felt marginalized, have seen their research money grow, which in science is a show of respect, and few were disputing the central role of beta-amyloid—especially a form of it called a-beta 42—in making an Alzheimer's brain, though what the plaques were doing in that brain could still rouse a heated discussion. Geneticists were the inadvertent arbiters of the dispute, for the answers were coming not from the examination of slices of tissue but from investigations at the molecular level.

"So much of the work that we've done, going forward, is asking, 'How do these genes cause disease? What are the biological pathways involved?'" Tanzi observed. "What was controversial was whether the plaques, where the toxic a-beta 42 eventually makes its home, are the cause of Alzheimer's disease. And the answer is probably not." Plaques are a problem because they cause inflammation, which can make things worse, but more recent data have directed attention to the way the peptide—long before it has formed into plaques—interferes with the synapses. "For all we know, plaques may be the result of a beneficial attempt by the brain to sequester a-beta 42 away so you don't have it in synapses anymore," Tanzi said. "It's probably the newly made a-beta 42 that is relentlessly attacking the synapses, and probably this is why an Alzheimer's

patient has trouble remembering what happened five minutes ago. When you impair the synapse, eventually it starts to break down."

This point was illustrated, last fall, in a paper in the journal *Nature Neuroscience*. The title of the paper, "Fibrillar Amyloid Deposition Leads to Local Synaptic Abnormalities and Breakage of Neuronal Branches," was not exactly sensational, but the accompanying photographs, showing an Alzheimer's-like condition in genetically altered mice, were vivid, and chilling. In the pictures, dendrites and axons, the parts of a neuron that carry information to and from a cell, highlighted in a radiant green, start out as long, robust, motile tentacles. Then they encounter the amyloid, which shows up as tomato-red clumps. The tentacles break apart, wither, and disappear.

One of the authors of the article, Karen Duff, was also the "author" of one of the original transgenic Alzheimer's mice. Duff is a young British molecular neuroscientist who developed the first mouse model of the presenilins mutation when she was a postdoctoral fellow. (Mice don't naturally develop Alzheimer's, and must be genetically modified to do so.) She continues to make models of neurodegenerative diseases, at the Nathan Kline Institute, in Orangeburg, New York, on the grounds of the Rockland Psychiatric Center, and has supplied Mayeux's team at Columbia with senility-prone rodents. Her office is incongruously cheery, with a bobble-head doll of James Watson on the desk, a cuckoo clock on the wall, and a stuffed-mouse toy perched on a bookshelf. The real mice are across the hall, in clear-plastic cages that resemble neonatal incubators. Duff, who recently received a \$7.5-million grant from the National Institutes of Health to work on tangle diseases, was raising a special line of mice that were prone to developing various frontal-lobe dementias.

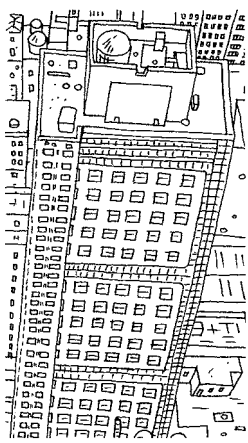
To model a human disease in a mouse, Duff uses a tiny needle to microinject select bits of human DNA into

a mouse egg. ("You're going from the outside to the inside and sometimes the eggs burst.") The introduced DNA integrates with the mouse DNA and is passed along to offspring. The first mouse born with this engineered DNA is called the founder mouse. Breeding the founders produces the "models" that are then used to observe the progression of a disease or to test therapies.

"When I was in school, I wanted to study physics, but I couldn't do the math," Duff said as she picked up a paraplegic mouse and gently stroked its back. "Then, when I was sixteen, I went to a lecture about genes and learned that you could change one thing in three billion and have an effect on the whole organism, and I said, 'That's it.' I wanted to make that one change and see what it did, what pathways were involved, and then go on to treat it. My postdoctoral project was making transgenic mice. Making mice is very hard. You have to be very specific with what you've done. If I have a demented mouse that can't get around a water maze, I know that it is because I changed one gene. The hard part is figuring out the steps in between." She ran her finger along the sick mouse's spine, then laid it back in its cage.

Duff doesn't create new mouse models for other researchers anymore, but she'll make an exception for Mayeux, as soon as he has a new Alzheimer's gene to give her. "It's a symbiotic relationship," she explained. "The geneticists want their findings to be more than a gene on a piece of paper. They want to see that it really does cause disease by putting it into animals. Richard needs me to put the genes in the animals, and I need someone like him to give me the genes to put into my animal models to see what they do. It's a multipart process."

On the morning I visited the neurology mouse lab of the Taub Institute, a lab technician was taping a breathing mask over the snout of an inert black mouse, dosing it with anesthetic gas. Once sedated, the mouse was wrapped in plastic and laid on a tiny bed that fit into a long tube, and its head was strapped in place. Then the technician inserted the tube into a miniature magnetic-resonance scanner that had been designed to look inside the bodies of small animals. On



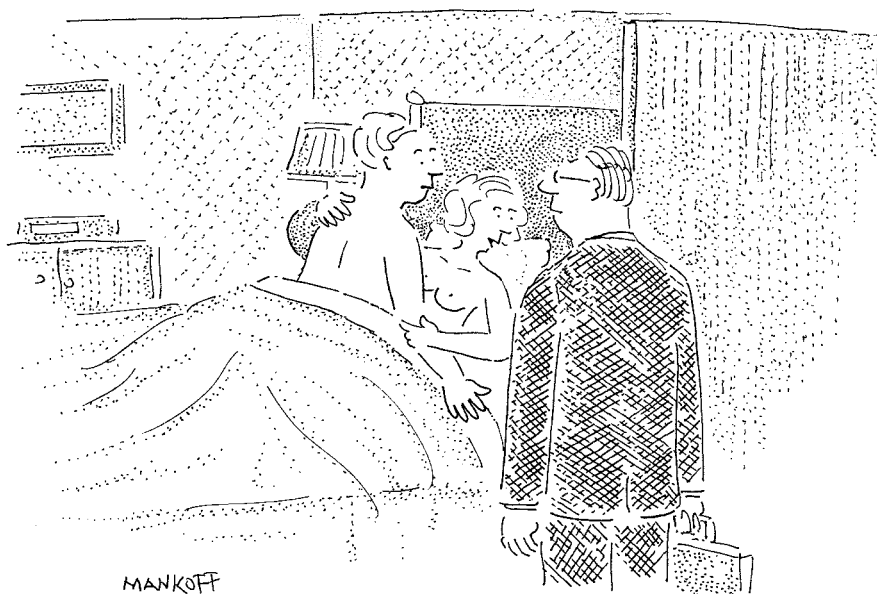
the screen were images of the mouse's brain and its beating heart. The mouse looked peaceful, unperturbed by the percussive hydraulic noises issuing from the machine.

The mouse nursery, where this mouse was raised, was one flight up. There were white mice, black mice, wild mice, sick mice, genetically altered mice, and, though I didn't know it then, a mouse that would later provide tangible support to a hypothesis being worked out by the neurologist Scott Small, about the origin of late-onset Alzheimer's disease.

Small, who is forty-four, was in medical school when the mice were being developed. American by birth, he had grown up outside Tel Aviv and served in the Israeli special forces during Israel's 1982 war with Lebanon, before moving back to the States to attend N.Y.U. He is an intellectually nimble scientist, able to jump effortlessly between cell biology, physiology, electrical engineering, pharmacology, and philosophy. He is also a dedicated if hamstrung clinician. I had been in the examining room when he gave a diagnosis of Alzheimer's to an eighty-six-year-old woman, a diagnosis that was more devastating to her son, a muscular New York City police officer, than it was at that point to her. Small talked to the son with tremendous compassion, but, as soon as he and his mother were gone, slammed his fist on the desk in anger. He was furious—with the universe, with science, with himself—that the best he could offer were free samples of a drug that might help for a couple of months at most.

In his pathophysiology lab, Small had developed a way to use functional magnetic-resonance imaging to see into specific regions of the brain at work—to look at actual cerebral circuits, the brain's moving parts. Using both the imaging and a gene-sifting technique called microarray, he examined discrete sections of the hippocampus, the part of the brain that controls the recording of new memories and is known to deteriorate in Alzheimer's patients. Ultimately, as Small explained in a paper published in the *Annals of Neurology* last week, he determined that something called the retromer complex might be involved in the disease.

"Scott is one of a new generation of what are called 'translational scientists,'"



"Believe me, it's not what it is."

Mayeux told me one day, explaining Small's role at Taub. "He can translate what's happening in a cell in a lab to a human who is sick—from the bench to the bedside and back again. My focus is on characterizing the human disaster. Scott visualizes what I'm saying and brings it down to the cellular level, tests it in a mouse, and brings it back to the human level."

When I went to the Presbyterian Hospital Building to talk to Small in his artfully underfurnished workspace, he was soon standing in front of a classroom-size chalkboard, drawing diagrams. He was trying to explain how the retromer complex works. One diagram had a big circle with two smaller circles inside it, and a Lego-like multi-decker bus alongside it. "God solved the problem of having different kinds of molecules that didn't like each other by compartmentalizing the cells," Small said, tapping the small circles with a piece of chalk. "You have a cell, which is the big circle, and you have different compartments called organelles, which are the small circles. You need a way to take something from one to another." He pointed to the multi-decker bus. "That's what the trafficking molecules do. They act like a shuttle bus, taking cargo from one place to another."

One of the mechanisms for moving proteins from one organelle to an-

other is the retromer complex. Until five years ago, it had been observed only in yeast. Two years ago, after cell biologists found it in humans, a number of retromer molecules showed up in Small's microarray analysis of hippocampal cells. Among them was a protein known as VPS26. Investigating further, Small found that, fifteen years earlier, the protein had been of interest to researchers studying developmental defects. In a journal article about VPS26, he found a reference to a mouse bred without the protein. By chance, the modeller was at Columbia—and the mouse was still being bred. Small and his associates ran the mouse through a battery of cognitive and biochemical tests and, as they reported last month at the annual meeting of the Society for Neuroscience, in Washington, D.C., found that in the absence of VPS26 the mouse had memory defects.

"So how is the retromer complex related to Alzheimer's disease?" Small asked, getting back to his diagram. "We believe that the cargo being moved around the cell by the retromer is the amyloid precursor protein, APP, and that when there's a retromer dysfunction APP builds up. If our model is right, we've uncovered something completely novel that contributes to late-onset Alzheimer's. What we've found is primary to the disease process. We've shown it



"You were a stray before and you can be a stray again."

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in a petri dish, and now we're working with the mouse, and the results look very promising. I can't say more than that right now."

By the summer of 2004, the Columbia team, working with research groups at the University of Toronto and Boston University, had a hunch about where a rogue risk-factor gene resided. Having searched through the entire genome for places where shared patterns of DNA showed up in people with Alzheimer's disease, having pored over many thousands of genes, some of them four spreadsheets long, they began scrutinizing a number of genes that had appeared in their random-association studies, some of which had also showed up in Small's microarray analyses.

It was standard work for Joe Lee, the Columbia group's unassuming statistical geneticist, who could reliably be found staring at his computer monitor. He began examining blocks of DNA, looking for variations in the genetic code that were associated with the disease. Each block—what the scientists call a haplotype—was like a sentence in a paragraph

from a chapter of a book. By comparing the length of that particular sentence in every copy of the book, the researchers could see in which copies the sentence was the same and in which ones it was garbled. If the misconstrued sentence tended to show up in people with Alzheimer's, then there might be a connection between those two things. The scientists would examine the sentence more closely, looking for the precise character, the one extra consonant, the repeated verb—something—in that particular sentence which was consistent in the copies owned by the people who were sick.

While the Toronto group, led by Peter St George-Hyslop, examined the DNA of a few hundred families of European descent, and the B.U. team, led by Lindsay Farrer, looked at the genetics of siblings, Lee focussed on the thousands of samples gathered from the Dominican Republic and Washington Heights studies. By its size alone, Mayeux's genetic library, with its branching trees of large extended families that often spanned two or more generations, was a powerful instrument for detecting

which haplotypes were associated with the disease. But the design and the scope of the complementary Columbia studies gave Lee another advantage. Where the large families from the Dominican Republic enabled him to find disease-linked haplotypes among symptomatic subjects and their relatives, he was also able to see whether those same chunks showed up in the Dominicans enrolled in the Washington Heights study.

Meanwhile, as Lee and the researchers in Toronto and Boston systematically moved along the chromosomes, they began to converge on the same gene. It was as if, having read the same book cover to cover, the teams had each come to focus on a single sentence. Yet the exact place in the gene where something went askew was different for different populations; it was as if, among a group of African-American siblings studied by the Boston University group, the sentence began with an extra article; among the people of European ancestry studied by the Toronto group, it was missing the question mark at the end; and, among the Caribbean-Hispanics studied by the Columbia group, a word in the middle of the sentence was misspelled. So there seemed to be at least three ways that the gene in question could go wrong.

The three lead scientists were growing more confident that the gene they were studying was one of the undiscovered late-onset risk-factor genes. The association was strong, not just in one sample population but in five, and not in one racial group but in three, scattered around the globe. Each researcher had replicated the findings of the others, and in their line of work replication was rare. By nature, the three were skeptics, but, as the evidence mounted, they found it harder to dismiss.

"The 'Aha!' moment happens to different people at different times, and sometimes it never really happens," St George-Hyslop said. "We are aware of little bits of data as they come out that say, 'Yes, it's real,' but not very strongly, so what you get is not really a eureka moment but something that is incremental. It starts out as 'Uh-huh, but it's probably a fluke,' to 'Maybe it's not a fluke,' to 'This could be real, let me see what I can do to make it go away,' to 'Well, it seems pretty robust, but there are still prob-

lems,' to 'We've taken this as far as we can and we concede that there are many things to be done on this story, but before we do too much more it needs to be put in the hands of some other people, with totally different data sets and totally different ways of analyzing things, and see if they get the same results.'

For months, the name of the promising new gene was written on a whiteboard near the door in Richard Mayeux's office, but I had no idea that it was the gene we were talking about, since no one would utter its name in my presence. There was some concern that if I knew it I might inadvertently tip off another research group, which could claim the finding as its own. In genetics there is only one winner, Karen Duff had said in the spring, describing how, during the race to make the first presenilin mouse, she had been reluctant to spare the time to get a broken arm set, for fear that she would lose too much ground to her competitors. When Scott Small had finished explaining his work on the retromer complex, he said, "If tomorrow someone publishes the whole story I just told you, I can cry till I'm blue in the face, but they will have published first." It was only at the end of the summer, when I was sitting with Mayeux in a cabin on a lake in the Adirondacks where he was vacationing with his wife, taking forty- and fifty-mile bike rides almost every day, that he told me the name, and I realized that it had been in front of me for half the year.

By then, though, I had come to understand and appreciate the gene not for what it was called but for what it might do—for how it might add to the amyloid story, and how it could give researchers a new way to treat Alzheimer's. Scientists have already launched clinical trials on vaccines that aim to mobilize antibodies against amyloid plaques. At an earlier stage of development, a number of geneticists—Karen Duff and Rudy Tanzi among them—have been working on drugs that exploit the molecular pathways described by the original, early-onset Alzheimer's genes. These novel molecular therapies are meant to intervene long before plaques form.

"Any way of getting at a molecule—whether it's from microarray, or looking at genes, or God whispering in your ear

to look at a particular protein—when you can pinpoint the primary molecular defect, you're more likely to develop effective treatments," Small explained. "That's Pharmacology 101."

Small, in his own lab and also in collaboration with researchers at Brandeis University and the University of Wisconsin, has already begun to look for a biochemical way to take advantage of his microarray and mouse findings in order to regulate the buildup of amyloid. Together, the scientists are developing a screening assay to test potential compounds, and expect to be able to turn over to chemical engineers a handful of potential drugs in a couple of years; the engineers would modify the drugs' structure to make them more amenable to entering the brain. From there, the compounds would be tested in mice, and, two or three years after that, if all went well, human trials would begin. "Right now, we're two years into a ten-year process," Small said.

Mayeux thinks that, a decade from now, your doctor will look up your gene profile and decide whether you have a high risk for Alzheimer's, and then give you a prophylactic treatment of some sort. "Right now, you don't know what the hell to do," Mayeux said that day in the Adirondacks. "You don't know whether you should take vitamins, whether you should take ibuprofen, and, if you do, if you'll get a stroke, whether you should take estrogen, and if that will give you a stroke. People tell you to use your brain, to use your body, and those are all well and good, but you don't know if it's a lifetime of doing those things, you don't know if it's starting to do crosswords when you're ninety. If we can solve some of these genetic puzzles, we'll know how to treat the disease."

It was three years since he, St George-Hyslop, and Farrer had begun their active collaboration, and despite their successes Mayeux remained circumspect. Having converged on a gene, they now had to find the mutations—the specific changes in the base sequences—and they had to show how these changes influenced the course of the disease. With the possibility of multiple Alzheimer's-linked mutations among them, it might be another few months of intensive effort at the very least before they could be sure.

"Let's say this is a real finding," Mayeux said. "You can bet there will be a ton of work on how this particular gene fits into the big picture. It's really a jigsaw puzzle with five hundred pieces. You can look at it and see some of the key pieces and you can tell that there is a brain on a brown background, but you can't figure out where all the other pieces go. Then you get one piece in there that fits and it helps you get a whole section together. We think we got a piece—it may be more—but you don't know till you nail it. The question is: 'How does a normal protein get misdirected and altered in such a way that it then becomes toxic to nerve cells?' The important point is not that it's this particular gene, but that it's part of a system that is important in the processing of beta-amyloid under normal circumstances and perturbations in that system are serious and could lead to disease. And that this is a modifiable system. Finding the gene is one thing, but once you have a gene it becomes a potential target, something to aim at. That's what you do this for."

Still, Mayeux knew as well as anyone that the road from gene to drug was linear only on paper. In a petri dish, in a mouse, in a person, almost anything—or nothing at all—could happen. Initial attempts to make an Alzheimer's vaccine had progressed to human efficacy trials when, in 2002, twenty participants inexplicably developed encephalitis, and the trials were halted. The first risk-factor gene, ApoE4, had been found in 1993, and more than a decade later nobody had figured out how to take advantage of it therapeutically.

"What I feel best about is that the collections I'm making are going to be around for a while," Mayeux said. "Collecting these Dominican families, putting the data together, having them very well characterized, having the cell lines—if it's not us who find the gene, then someone will find the genetic variant eventually and that will help."

"It's a lot like the movie 'The Maltese Falcon,'" he went on. "You look for, you look for, you look for, and you find something—and then you realize it's not the right thing, and by that evening you're booked on another ship to begin the next search. If this fizzles out, we'll be on that boat." ♦