

SETTINGS FOR A MEDICAL THRILLER:

The lagoon of Venice // The countryside of England // The jungle of Papua New Guinea // The labs of researchers worldwide trying to understand a deadly protein.

In Pursuit of Prions

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In November of 1765, a physician from a respected Venetian family died in the Campo Santi Apostoli, near the Jewish ghetto. The cause of death, according to a contemporary parish account, was “an organic defect of the heart’s sack,” though this was probably just guesswork. But priests ordinarily wrote detailed descriptions only of diseases that caught their eye, and this report is one of the longest in that year. In addition to suffering from the symptoms that were recorded (breathing difficulties, eventual paralysis), it is likely that the doctor had been extraordinarily anxious, like a horse at full gallop, sweaty and prone to shaking. And that he had been exhausted, falling in and out of a light, dream-wracked sleep. Many of the doctor’s descendants, down to the present decade, have shown similar symptoms in the course of dying from a disease we now call fatal familial insomnia, or FFI, suggesting that the doctor may have been the earliest recorded case.

FFI is an autosomal dominant mutation, which means that a child of a parent with the disease has a 50% chance of getting it too. This Italian family is one of a handful in the world afflicted with FFI. The family members have a carefully charted family tree: They can trace their disease back maybe to the Venetian doctor and with certainty to a relative of his, an aristocrat named Giuseppe who lived in the Veneto in the mid-nineteenth century. From him the disease goes down to Vincenzo in the 1880s, Giovanni in the 1910s, Pietro in the







1940s and Assunta, Pierina and Silvano in the last three decades of the twentieth century.

Although obscure, FFI is an important disease, a key constituent of a cluster called prion diseases. The first known prion disease was scrapie, initially chronicled in sheep in eighteenth-century England. With scrapie, the affected sheep, suffering from an itch caused by neurological alterations, rubs its back against objects until it rips its wool off. The disease is always fatal. In the 1950s, researchers in Papua New Guinea came upon another prion disease, kuru, that nearly wiped out a primitive tribe. Then in the late 1980s, Britain awoke to mad cow disease, which killed hundreds of thousands of cattle and jumped species to humans who had eaten the infected meat. The human form of the disease, known as variant Creutzfeldt-Jakob disease (vCJD), has killed roughly 160 people.

Prion diseases are difficult to diagnose because they confound basic epidemiology. For one thing, the time from infection to first symptom is extremely long—there are still members of the New Guinea tribe dying of kuru 50 years after being infected. And unlike other infectious diseases, prion diseases leave no detectable trace of infection in the body—no increased white-blood-cell counts, no fever. Odder still is that the agent behind them seems not to be a virus or a bacterium but a protein that replicates itself without DNA or RNA.

Yet as small as the family of prion diseases is, understanding them has deepened our knowledge of a variety of more

common diseases. Prions generally form malignant protein plaques in the brains of their victims, a pathology that also characterizes Alzheimer's and Parkinson's diseases. Prion researchers believe that if they can cure prion diseases such as FFI, they may have an excellent point of entry into curing those others.

The Italian family with the rare sleeping sickness is an educated one: Its members were nobles in the old Venetian Empire and, more recently, doctors, high-ranking military officers and businessmen. But with public success has come hidden suffering and fear. In 1770, Giuseppe (who was probably the doctor's nephew) was born in the Veneto. He had eight children, five of whom died in infancy and one, Costante, who fell gravely ill in his teens. Costante likely had trouble sleeping and hallucinated, a symptom of the disease when it strikes younger victims. His death was followed in short order by Giuseppe's own and then through the decades by dozens of relatives. The disease unleashed a long chain of misery down through the centuries. Because FFI usually strikes late in life, it does not, like many other lethal genetic defects, prevent reproduction, so each generation passes on the mutation to the next.

Doctors thought they knew what was killing the family. Diagnoses ranged from "nervous gastric fever" to meningitis, presenile dementia, ictus and alcoholic encephalopathy. Yet this was all speculation, a grasping-about in the dark.

The modern era of prion research began on the morning of March 8, 1957, when 33-year-old pediatrician Carleton Gajdusek from Yonkers, N.Y. appeared in the office of the public health director in Port Moresby, New Guinea. Gajdusek had chosen Port Moresby as the entry point for a multinational study of child development, but what he learned from the director changed his life.

In the early 1950s Australian officials had discovered that members of the Fore tribe, in New Guinea's remote Eastern Highlands, were being stricken with a mysterious ailment. The Fore thought that sufferers of the disease, whose chief symptom was uncontrollable laughter, were under a spell, and they called the sorcery that afflicted these shivering victims kuru ("shaking").

Gajdusek, who had trained at Harvard Medical School and the California Institute of Technology, took a more scientific approach, investigating everything the Fore ate, drank and touched. He suspected the smoke in their huts and the copper in their water. He sent patients to Port Moresby and brain



This story is adapted by the author from his book *The Family That Couldn't Sleep: A Medical Mystery*, recently published by Random House, and includes additional reporting.

tissue to a top laboratory in Melbourne, Australia and to the National Institutes of Health (NIH) in Bethesda, Md. for pathology studies.

Melbourne saw nothing wrong with the tissue Gajdusek had sent, but the NIH was more thorough. What they saw was eye-opening: “very spectacular neuronophagia...very intense gliosis...striking changes affecting Purkinje cells...and bizarre deformities of Purkinje-cell dendrites,” in the words of pathologist Igor Klatzo, who studied them. Klatzo also noted that the disease reminded him of one “described by Jakob and Creutzfeldt” in the 1920s that struck a handful of elderly people, leaving holes in their brains.

Gajdusek never did discover how the Fore were getting kuru. In the early 1960s the anthropologist Shirley Glasse and her husband Robert would trace it to mortuary cannibalism. But by that time Gajdusek had left the Fore region for the NIH to continue working on the disease. Gajdusek thought the disease was hereditary, but he was not sure. Then in 1959 he got some important new information.

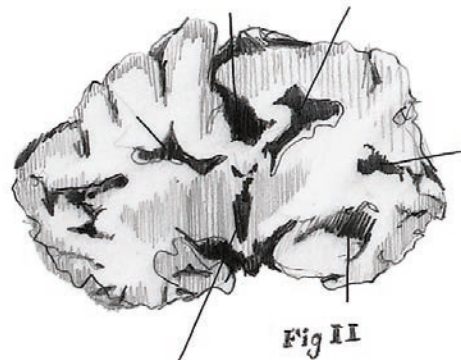
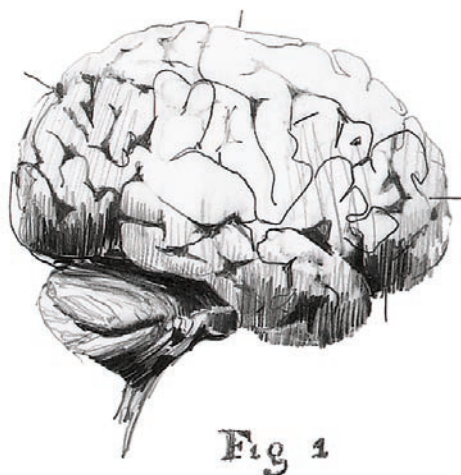
An American veterinary pathologist working in England named William Hadlow happened to see an exhibit on kuru at the Wellcome Medical Museum in London that included photographs of the victims’ brains. He felt as if he were seeing the same scrapie slides he had in his lab and wrote a note to the *Lancet*, the British medical journal, about the coincidence. He also sent a copy of his letter to Gajdusek.



Two things were known about scrapie by then: It could be transmitted, and it took a long time for the symptoms to show up. Those findings gave Gajdusek a clue. He threw himself into trying to prove that kuru was infectious, injecting homogenate made from the brains of kuru victims into everything from mice to hamsters to chimpanzees. Finally, in June 1965, Georgette, one of the chimps who had been injected with kuru tissue 21 months before, began to show the signs, “dragging herself around, shaking, looking for all the world like a kuru patient,” in the words of Michael Alpers, the head of the research program.

After the chimp died, Gajdusek asked a British pathologist expert in scrapie to examine the chimp’s brain. What the pathologist saw reminded her of what she had seen in kuru victims. The chimp appeared to have died of the disease that killed the Fore. It was a moment of triumph for Gajdusek and his lab. Here was proof that kuru was an infectious disease, though Gajdusek had no idea what the nature of the infection was—bacterium, virus, protozoan or some other infectious agent.

At a museum exhibit on kuru in London, a veterinary pathologist saw photographs of the victims’ brains that looked just like the scrapie slides he had in his lab.



Gajdusek was intrigued by Klatzo's comparison of kuru to CJD, so he began inoculating healthy chimps with tissue from sufferers of CJD as well. Ultimately, these chimps also grew sick. When the animals died, pathologists examined their brains and saw that the sponginess and the holes resembled those of both kuru and scrapie victims.

One, as the saying goes, was a novelty; two was a coincidence; three—scrapie, kuru, CJD—was a theory. These long-ignored diseases, Gajdusek declared in the late 1960s, shared a cause, some sort of hard-to-destroy, slow-acting virus. The key sticking point was the word virus. Viruses are essentially bits of nucleic acid covered by protein; they insert themselves into cells and trick the cell's reproductive apparatus into copying them. They generally provoke an immune reaction in their unwilling host, and it is these reactions that usually reveal the strain of the virus. Though they are not technically alive, viruses do contain bits of DNA or RNA, so they can be deactivated by the same techniques that kill living things—everything from washing with soap and water to heating to irradiation. And most viruses, once they are removed from the cell that they are parasitizing, die on their own, often in a few hours.

Gajdusek's "odd, slow viruses," as he called them, didn't fit this description: Heat, formalin and disinfectants did not harm them. Even stranger, radiation did not diminish their virulence. But the agent seemed to have strains—some sheep with scrapie had a lot of trembling; others had an unrelenting itch. These facts added up to a very strange infectious agent: an indestructible strain-specific particle that didn't die under conditions that would kill a virus and provoked no immune system response.

What was this particle? Gajdusek did not go as far as other researchers who posited that it was no virus at all. Experiments with centrifuges had turned up a lot of protein but no nucleic acid. Was it because there wasn't any in the infectious agent? Could an infectious agent be entirely made of protein?

The conventional wisdom said no. Proteins are building blocks and engines and messengers of the body, but they are not alive. And to suggest, as some researchers began to, that a protein could make copies of itself in the victim's body—



that a nonliving agent was somehow replicating itself or being replicated in the victim's body without DNA and causing disease—pushed up against the impossible.

During the late 1960s and 1970s, Gajdusek gradually committed his prestige to the protein-only point of view. In 1976 he was honored with a Nobel Prize in Physiology or Medicine for discovering "a completely new type of infectious agent." Certainly he was being rewarded for doing the key experiments in the field (showing the agent was transmissible) and for the expert framing of a question (Was infection possible without a living agent?) rather than for answering it. All that could be said with certainty was that there was a group of diseases out there that were not like conventional ones.

Stanley Prusiner, a professor of neurology at the University of California at San Francisco (UCSF), entered what Gajdusek named the "slow virus" field in 1974, when it was stuck somewhere between theory and particle. Prusiner had trained as a chemist and thought the only way to be sure about the nature of the scrapie particle was to pull it out of the muck of the scrapie or kuru homogenate. He set to work and by the mid-1980s had separated out an agent 5,000 times more concentrated than in nature. Prusiner's measurements indicated that it was small even for a protein and much smaller than the smallest known viruses. He mixed the agent with enzymes that dissolve proteins and with enzymes that dissolve nucleic acid, and found that chemicals that destroy proteins destroyed the particle's infectivity, whereas chemicals that destroyed nucleic acids actually increased the infectivity, strongly hinting that



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the infection resided in a protein and not in a virus.

In 1982 Prusiner coined the word prion, an acronym (for “proteinaceous infectious particle”) that didn’t silence the skeptics. To do that, Prusiner had to synthesize a prion in a test tube and show it was sufficient to cause a prion disease. Then no one could say there was a virus hiding behind the protein. But prions had another surprise in store: Once Prusiner, working with other labs, purified the prion enough to determine part of its amino acid sequence, he found that prions were ordinary proteins manufactured by a healthy gene in the host’s own body. In other words, prions were not something that entered the victim—they were something the body itself produced.

Working over the next decade, Prusiner developed a theory to explain the apparent impossibility of an infection caused by the host itself. The body manufactures proteins as ribbons, which then fold into three-dimensional shapes that allow them to fulfill whatever functions they perform. Most proteins, because of the arrangement of their atoms, have only one such shape, but prions—Prusiner’s theory goes—have two: one normal, the second infectious and lethal. When a disease-causing prion is placed beside a normal prion in a test tube, the abnormal one can sometimes even convert the nor-

mal one to the former’s shape.

After one prion becomes altered, its new shape allows it to bond with adjoining healthy prions, and the bonding causes those prions, in turn, to fold wrongly and bond with others, making them fold wrongly too. The process is called conformational influence. Infectious prion disease, then, would result from the introduction of a misshapen prion from outside, and inherited prion disease from faulty manufacture by the genes. A third kind of prion disease, sporadic, would come from the chance misfolding of a healthy protein in the body.

There were (and are) many scientists who disagree with this theory, and they challenged Prusiner to synthesize a prion in a test tube and show that it could cause infection. In the meantime, though, a prion plague came along that moved the center of the field back from molecular biology to epidemiology.

In the late 1980s, cows in England began shaking, falling over and sometimes charging their handlers. The first veterinarians to see the disease suspected that scrapie had jumped species—the symptoms and the pathology were quite similar—and after some opposition from the British government, which feared the economic consequences, a consensus formed



that this was the case. Unfortunately, with that consensus came the assumption among many in government and the sciences that infected beef would be safe to eat, just as scrapie-infected sheep is. In fact, some manufacturers preferred the skinnier scrapie-infected sheep for pies. (The lack of infection presumably is based on the difference in shape between the sheep and the human prion protein.)

By the mid-1990s a more-than-ordinary number of Britons were coming down with CJD, the rare prion disease that is either inherited or occasionally simply develops by chance. And the victims were teenagers and young adults, whereas CJD is a disease of old age. The British government had gotten it wrong: Humans could “catch” mad cow disease (this type is now called variant CJD). There was suddenly a new pool

F of prion disease victims clamoring for an explanation of what was happening to them and, even more, for a cure.

or the Italian family living in the Veneto, mad cow disease was just something they read about in the newspapers. Fatal familial insomnia had by now spread throughout the various branches of the family, where it was known as “the family disease.” Hospitals could do little more than poke and prod the family members committed to their care in a vain attempt to understand their condition.

Then finally there was progress. In 1983, Silvano, the great-grandchild of Giuseppe, began to sweat and lose the ability to sleep. He was in his early fifties. His niece’s husband, Ignazio, a newly minted internist, grew intrigued. Ignazio took Silvano to the sleep clinic at the University of Bologna, where researchers observed an unprecedented EEG: a mixture of brain waves that was typical of neither sleep nor wakefulness. Silvano had no illusions about his fate. “I assume you’ll want the brain when it’s time,” he told the admitting neurologist. After Silvano died, the autopsy, conducted by Pierluigi Gambetti at Case Western Reserve in Cleveland, revealed that the disease, whatever its cause, ate away portions of the thalamus, setting off the insomnia and unregulated body functions.

A second key piece of the puzzle fell into place when, in

the 1980s, Ignazio and his wife urged a distant family member to bring her sister to Bologna. The young woman, named Teresa, had a slower form of FFI (the difference would later be traced to a polymorphism on the prion gene). Teresa’s EEG was distinct from Silvano’s, and the sharp-eyed neurologist who headed the clinic, Elio Lugaresi, saw a resemblance between it and CJD. After Teresa’s death, Gambetti observed that the pattern of spongiform tissue and holes in her brain also resembled CJD. Antibodies he borrowed from Prusiner confirmed the presence of prions. Prusiner, in turn, used some of the family’s tissue to show that each kind of prion causes a distinct, specific disease in mice. He was now another step forward in establishing that the prion protein and only the prion protein was responsible for prion disease. In 1997 he was awarded the second Nobel Prize in the field. And at last FFI

P was not just a single family’s dreadful fate; it was one of a group of diseases that, because of the effort being expended to understand mad cow disease, might one day be cured.

Prion researchers never thought their work was just about a group of rare conditions. They hoped to shed light on a range of non-prion diseases that have always eluded medical understanding. This idea was already on Carleton Gajdusek’s mind in 1957, when, newly arrived in Papua New Guinea to study kuru, he promised the NIH that if he could “crack” the disease, “parkinsonism, Huntington’s chorea, multiple sclerosis, etc., etc.” might follow.

The list that Gajdusek drew up and that Prusiner elaborated on also includes such neurodegenerative disorders as Alzheimer’s disease, such immune system disorders as Crohn’s disease and rheumatoid arthritis, and metabolic diseases like adult-onset diabetes.

Besides many having overlapping symptoms—diminishing coordination, stress-induced worsening, late onset—all these diseases also leave misfolded proteins behind. This shared characteristic is not something researchers who specialized in these ailments had paid much attention to historically. That all these diseases caused the buildup of the same gunk in the body was seen as incidental.

By contrast, prion researchers thought the misfolded proteins might be the cause of the disease. Strikingly, most of the diseases produced not just misfolded proteins but misfolded proteins of a particular kind, called amyloid plaques or amyloid fibril sheets, that developed when atoms bonded across

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the frames of damaged proteins, like the rungs of a fabulously strong ladder. Enzymes couldn't digest them, and water didn't dissolve them. There was nothing in biology as tough; it seemed safe to say that no cell could survive their presence.

The amazing thing is how long it took us to see all these things that were right in front of our face," says Fred Cohen, a molecular chemist at UCSF whose lab works with Prusiner's. "The parallels were obvious."

through the decades the Italian family has kept on dying: 14 since 1973, eight in the past decade. In 2003, Vittorino, a Venetian living on the southern end of the lagoon, developed insomnia and uncontrolled sweating. His family read of the disease when the FFI family announced a foundation for research, and got in touch. Vittorino turned out to be a distant cousin, related through Giuseppe, the likely nephew of the original patient, the Venetian doctor. Though Vittorino's death was no less brutal, at least the family knew they were not alone. Outside the church his daughters put out an offering receptacle with "For the Victims of Fatal Familial Insomnia" written on it.

Among the current generation, chance dictates that 25 more carry the gene that causes the disease. The question they face every day is, What can be done? There are two prion drug trials under way: one with quinacrine, an antimalarial synthetic; the other with pentosan, originally prescribed for bladder-lining infections. Though a victim of mad cow disease in Britain has remained alive now for six years thanks to pentosan treatments delivered with a shunt, scientists remain only cautiously optimistic about both drugs.

Meanwhile, the past few months have seen three discoveries. The genetic information for the prion protein resides on a gene on chromosome 20. It has long been known that mice whose prion genes have been removed live more or less normal lives, but no one knew whether this observation would also be true for larger mammals. Recently a pharmaceutical research company in Sioux Falls, S.D. successfully created eight cows with no prion proteins. The cows, which U.S. Department of Agriculture researchers have followed for a year

and a half, seem to behave normally. While gene therapy in humans remains a hope, if it ever becomes a reality, prion disease, with its simple genetic basis, could be one of the first experimental targets.

At the same time a group of researchers in England has found a molecule called L13 that binds to the prion protein. In theory the blood of infected individuals could be purified in a filter containing the molecule. It could prevent the accumulation of malignant prions in the brain and the onset of the disease, assuming that scientists can find a way to filter them out of the central nervous system too.

And a third method of combating the disease has also recently been shown to have some success. Researchers in Germany have used a type of RNA that silences gene activity to prevent the prion gene from producing proteins. In tests on mice infected with scrapie, they found that mice genetically altered to produce this "interference RNA" for prion proteins lived significantly longer than the control group.

The Italian family has not consented to be a part of either drug trial, though they closely follow developments in the field. Perhaps one day gene therapy will allow them to live without a prion gene, or they will have their blood regularly cleansed. Maybe the science of interference RNA will advance far enough to allow for a drug to block the action of the prion gene. Meanwhile, the family members grow older, wondering what will happen to them. Some refuse to even have the genetic test to



→ DOSSIER

1. *Prion Diseases of Humans and Animals*, edited by Stanley Prusiner, John Collinge, et al. (Chichester, England: Ellis Horwood, 1992). The definitive history of prion research.
2. *Kuru: Early Letters and Field-Notes from the Collection of D. Carleton Gajdusek*, by D. Carleton Gajdusek, edited by Judith Farquhar and D. Carleton Gajdusek (New York: Raven Press, 1981). This colorful compilation includes excerpts from Gajdusek's journals and his correspondence with other researchers looking for kuru's origins.
3. "Virus Paper Reignites Prion Spat," by Heidi Ledford, *Nature*, February 2007. An overview of challenges to the prion orthodoxy, including the recent finding by prion doubter Laura Manuelidis, a Yale neuropathologist, that viruslike particles were present in cultured cells infected with the human form of mad cow disease.