



AUTHORS at their laboratory for researching prion disease at the Broad Institute in Cambridge, Mass.

ANTISENSE THERAPY

# Preventing *Prions*

Treating susceptible individuals  
while they are still healthy  
offers the best hope for warding off  
a deadly brain disease

*By Sonia Minikel Vallabh and Eric Vallabh Minikel*

**Sonia Minikel Vallabh** and **Eric Vallabh Minikel** run a research laboratory at the Broad Institute of MIT and Harvard dedicated to developing a treatment or cure for prion disease. The couple changed careers to become medical researchers after they learned that Vallabh is at high risk of developing the fatal illness.



**N**O ONE EXPECTS TO LIVE A BEFORE-AND-AFTER KIND OF LIFE, DIVIDED into the moments before and the moments after a single defining event. When the two of us met, fell in love and got married in Sonia's backyard in Hermitage, Pa., we had no idea we were in our "before" life. We had no intention of quitting our careers in law and engineering and taking entry-level jobs in a different field. We could not have imagined the scramble to learn an entirely new discipline from scratch nor a day when we would defend back-to-back our doctoral theses in biomedical research—our presentations intercalating to form a vision for a first-ever treatment for a fatal neurodegenerative disease.

We abruptly entered our "after" life on October 9, 2011, when Sonia learned that she was at risk for a rare DNA mutation that would make her all but certain to die young of a rapidly progressive brain disorder: prion disease. This illness occurs when a protein called PrP that is normally present in our brains changes shape into an abnormal form, called a prion. (Confusingly, the normal version of the protein—PrP, or prion protein—was named *after* the deformed version, the prion, was discovered and named.) A prion causes other copies of PrP that it touches to also warp into prions. This cascade of protein misfolding spreads across the brain, killing brain cells at a rate that outstrips that of any other neurodegenerative disease.

By the end of the year, we knew that Sonia had indeed inherited the dreaded mutation. Since then, we have been on a mission. Success means keeping Sonia's brain, and those of others like her, healthy and fully functional for years or decades, hopefully for a lifetime. Failure means that in her prime, Sonia will be struck down almost overnight. Within weeks of her first noticeable symptom, she will have suffered devastating brain damage and ceased to be the person she was.

Because a single—and apparently an expendable—protein, PrP, is responsible for this disease, we have hope that current technologies can reduce its amount in the brain, depleting the fuel that enables deadly prions to spread. The trouble is the stunning speed with which prion disease progresses: our best chance of winning this battle is to act before catastrophe strikes. But prevention of disease—as opposed to intervening only after disease is underway—is not business as usual. Eight years on,

we are waging, every day, an uphill struggle to forge a new paradigm in drug development: for testing a promising drug not only for its ability to slow the progression of disease but also for its ability to keep healthy brains healthy for longer.

#### **A YEAR OF CRISES**

MONTHS BEFORE WE GOT THE NEWS, we had witnessed the progression of prion disease in Sonia's mother, Kamni. In February 2010, still in her usual good health and with high cognitive function, she went to see an ophthalmologist because of blurry vision. On March 17, when Sonia called to wish her mom a happy 52nd birthday, Kamni was unable to finish a single sentence without losing her train of thought. In May she spoke in tongues, recognized family members less than half the time and forgot that she could no longer walk—which meant that despite our best efforts, she repeatedly got up, fell and hurt herself. From June onward, she became wheelchair-bound and underwent several hospital stays. She was still able to make eye contact but began to recoil from touch, her comfort in the company of loved ones replaced by constant fear of the poking, prodding and endless needlesticks that human presence had come to imply. By July she was unable to speak, eat or sit up. Her face reflected only agony and her eyes only fear as she struggled continuously against the restraints the nurses had used to tie her hands to the hospital bed to keep her from pulling out her feeding and colostomy tubes. In August she was permanently intubated and ventilated, mute and motionless. She still had no diagnosis.

During that year, radiating outward from the primary crisis



VALLABH and Minikel with their daughter, Daruka (1). Sonia inherited a mutation for prion disease from her mother, who died of the illness—but the couple hope to develop a drug that can fend it off indefinitely. Daruka, who was screened for the mutation as an embryo and is free of it, holds a photo of her maternal grandmother (2).

were the second- and third-order crises. What do you do when a person requires more care than one person, or even one entire family, can provide? Hospitals, it turns out, are not responsible for answering this question. After the tests have been run and all possible diagnoses rejected, the patient is discharged to her home until the next inevitable complication—a head injury, pneumonia—justifies a return. Constant crisis mode, and the sudden loss of all household logistics expertise, meant that bills went unpaid, accounts were suspended, electricity turned off. And to be clear, we were the lucky ones. Of the approximately \$1 million in medical bills Kamni incurred that year, her health insurance paid for nearly everything.

In December she passed away, and we felt an emotion we had never imagined we could associate with a loved one's death: relief. It was not a saying of goodbye but a realization that we had already said goodbye. This is what dementia robs us of—not just the person we love but the present-tense goodbye.

After Kamni died, we slowly tried to put the worst behind us—but the worst was one step ahead. When we came home for a family friend's engagement party that October, we attributed Sonia's father's long silences and distant stares to heartbreak, loneliness and the long tail of exhaustion. But as we were loading our bags into the car to go to the airport, he pulled Sonia aside and delivered the news that broke our lives in two. An autopsy had revealed that Kamni's illness had been fatal familial insomnia, a type of genetic prion disease. She had had a defect in the gene for producing PrP, and Sonia was at a 50–50

risk. At the close of 2011, we learned that Sonia had in fact inherited her mother's mutation—which meant that she was all but certain to also develop prion disease. She was 27 years old.

Almost right away we decided to devote our lives to finding a cure. We enrolled in night school to learn biology, abandoned our former professions to take entry-level positions in research laboratories and in 2014 enrolled in a Ph.D. program at Harvard Medical School. Now at the Broad Institute in Cambridge, Mass., we run a prion research lab. It goes without saying that we would not go to such lengths just to keep Sonia alive in a state of profound dementia for 12 months instead of six. The goal was—and is—to keep Sonia's brain healthy for additional years or decades, if possible indefinitely. The goal is prevention.

### A LETHAL FOLD

PRION DISEASE MANIFESTS ITSELF in a variety of ways, described as Creutzfeldt-Jakob disease (CJD), fatal familial insomnia, bovine spongiform encephalopathy (BSE or “mad cow” disease) and others. Many of its names were assigned long before neurologist Stanley B. Prusiner made his Nobel-winning discovery in 1982 that a single causal agent—a protein—unifies them. Though most infamous for the fewer than 1 percent of human cases that are acquired by infection (such as via contaminated meat), most cases of prion disease arise randomly. A PrP molecule in someone's brain spontaneously assumes an abnormal configuration or folding pattern, setting off a rapidly escalating chain reaction. In contrast to such “sporadic” prion disease, about 15 percent of

cases are caused by mutations in *PRNP*, the gene that encodes PrP. For reasons we do not fully understand, these mutations make the protein far more likely to misfold. Whereas a person with two normal copies of *PRNP* has a chance of about one in 5,000 that the PrP proteins in his or her brain will spontaneously deform in his or her lifetime, someone with Kamni's mutation has a risk of more than 90 percent.

The *PRNP* gene is located on the short arm of chromosome 20 in humans. It comprises 15,000 base pairs, of which 762 encode the protein—which, in its final form, is a chain of 208 amino acids. Most variants that give rise to genetic prion disease are changes of a single base in *PRNP*, which alter just one amino acid in the resulting PrP molecule. Sometimes a repeating segment of the gene expands, leading to a longer version of PrP.

In its normal conformation, about half the length of the normal protein is well ordered, consisting mostly of “alpha helices,” spiraling structures common in proteins. At the far end of this section, PrP has a sugar anchor that links it to the outer surface of a cell membrane, its native habitat. (One pathogenic variant of the gene generates a foreshortened PrP, lacking an anchor to the cell membrane.) The other half of the protein is disorderly, forming a floppy tail that hangs off the cell surface and into the space between cells.

Although researchers do not fully understand the shapes of prions, we do know that the misfolded form generally has more “beta sheets”—stacked and pleated strands of amino acids—than alpha helices. In this form, the protein is more resistant to being broken down by enzymes. What makes this shape a prion (proteinaceous infectious particle) is that it can serve as a template, prompting other copies of PrP to also link up and misfold. A cascade of prions spreads through the brain, forming fibrils and aggregates and killing nerve cells by mechanisms that remain unclear.

Prions also come in different strains with different properties—such as which animal species are susceptible to them and how they present themselves clinically. Adding to the complexity, it appears that each strain may actually consist of a range of different misfolded conformations of PrP—analogous to how a population of a given bacterium, in the context of an infection, may harbor genetic diversity that gives some members a leg up if circumstances change. This variability may explain why one drug strategy that researchers have pursued—looking for compounds that reduce the number of prions in cells—has failed. For example, the antimalarial drug quinacrine is effective against prions in cell cultures, but studies in humans, including a randomized double-blind clinical trial in 2013, have found it to be ineffective in patients. Further experiments with quinacrine and other compounds at Prusiner's lab at the University of California, San Francisco, now suggest that even if a drug depletes one of these misfolded configurations, others can rebound to yield drug resistance.

#### THE PREVENTION PARADIGM

ANOTHER SIGNIFICANT CHALLENGE is finding people on whom to test potential drugs. Typically clinical trials of a new drug re-

cruit sick patients to see whether those who receive the medication feel better, function better or survive longer than those who receive a placebo. But in such a rapidly progressive disease, by the time symptomatic patients are identified, they are profoundly debilitated. In the largest reported clinical trial of prion disease, which tested the compound doxycycline, an estimated half of patients were already on life support before being treated. (The doxycycline did not help.)

The core problem is the explosive tempo of the disease. Prions replicate exponentially. Even before symptoms show up, billions of prions have already filled the brain. And once they begin killing brain cells, the rate is blistering; at this point, even an effective antiprion drug may have limited ability to help. Future trials might try to screen for “early symptomatic” patients, but catching the disease early is incredibly difficult. Doctors do not even suspect prion disease until an average of three months from a patient's first symptom—by which time Kamni could no longer speak. Even a drug that halted the disease at that stage would not undo any brain damage already sustained.

## We need a new paradigm in drug development: testing promising drugs not only for their ability to slow the progression of disease but also for their ability to keep healthy brains healthy for longer.

Thus, a drug that could keep Sonia healthy might do nothing in advanced patients at a symptomatic stage of illness. Tests of antiprion compounds in mice suggest that might be the case for many, even most, drugs we could develop for prion disease. One small molecule developed in Prusiner's lab, called IND24, can quadruple the life span of prion-infected mice if given prophylactically, but it does less good if given later—and it loses even a whiff of efficacy as the mice approach the symptomatic stage. The three other chemical compounds that have shown compelling efficacy against mouse strains of prions are also more effective the earlier treatment is begun.

Smart people have grappled with these questions for years when confronting Alzheimer's disease, which also features protein aggregation. Candidate drugs targeting the accumulation of beta-amyloid, the malformed protein found in Alzheimer's brains, have failed, in trial after trial, to benefit patients, leading observers to wonder if the therapeutic hypothesis is wrong or if the time of intervention is simply too late. Two approaches are being employed to test whether anti-amyloid drugs do, in fact, delay Alzheimer's if given earlier. One is to randomly assign still healthy people at high genetic risk of early-onset Alzheimer's to groups receiving drugs or placebo and follow them for years to see who develops cognitive decline. The other ap-

proach, sometimes dubbed “secondary prevention,” recruits cognitively healthy people in whom molecular evidence of the disease process can already be detected, to see whether a drug delays the progression into symptomatic disease. These molecular markers show up decades before the onset of the disease.

Neither approach appears likely to work for prion disease. Following genetically susceptible individuals to the onset of disease turns out to be infeasible because of the highly variable age of onset and the small population of patients. We and others have studied people at risk for prion disease but have not found consistent evidence of the kind of progressive pathology that precedes Alzheimer’s. Prion disease appears to be basically undetectable before dementia ensues: it is less the rumble of a freight train approaching and more the split-second glance upward as the asteroid strikes.

### DEPLETING THE FUEL

WHERE DOES THIS LEAVE US? If trials in symptomatic patients may mislead and trials for prevention are infeasible, how will we show that a drug could save Sonia’s life? We have come to believe that the answer was handed to us at the very beginning of our quest, embedded in the genetic test report that changed our lives. We already know the single gene that causes this disease and the single protein fated to go wrong. The key is to target normal PrP before it ever misfolds.

If we can lower the amount of PrP produced in the brain, all evidence suggests that we will delay the disease. For example, mice producing half the normal amount of PrP take more than twice as long to develop prion disease if infected. With less PrP around, it takes much longer for the prions to replicate. Fortunately for us, PrP does not appear to be essential to brain function. Mice, goats and cows that have the gene for producing PrP “knocked out” are healthy, and so are people with one inactivated copy of the gene.

Targeted lowering of PrP in the brain may now be achievable using antisense oligonucleotides, or ASOs. These are short, chemically modified pieces of DNA, with sequences designed to target an RNA molecule of interest—and they can trigger its destruction so that it no longer produces proteins. Recently Ionis Pharmaceuticals in Carlsbad, Calif., has figured out how to develop and dose ASOs for the human central nervous system. Partnering with Ionis, we have found over the past five years that ASOs that reduce PrP levels keep prion-infected mice healthy for longer. These preclinical results, combined with clinical, genetic and other data we have gathered and the patient registry we have launched, have convinced Ionis’s leadership to undertake development of an ASO-based prion disease drug, with a goal of reaching first-in-human trials in the coming years. For the first time, a major industry player has committed to developing a rational, targeted therapy for prion disease.

If ASOs that lower PrP turn out to help patients with symptomatic prion disease, we will be thrilled. But we need to find a way for such a drug to benefit patients who are at risk, even if it only works on a preventive basis. We propose that PrP concentration in spinal fluid can serve a pharmacodynamic biomarker—a molecular measure of whether a drug has its intended effect. And that this readout can, in turn, serve as a surrogate biomarker: the outcome measured in a clinical trial when one cannot directly gauge whether patients improved. That is, we

propose to treat people who are still healthy and show that the protein that causes the disease is lowered. The U.S. has a framework for such clinical paths, called Accelerated Approval, and there are precedents—including the use of “viral load” to approve HIV/AIDS drugs.

In 2017 we took this proposal to a meeting with the Food and Drug Administration and found great enthusiasm for our preventive approach. We left with a list of homework and a new team of allies. Two years on, we have learned how to precisely measure PrP in spinal fluid and have gathered evidence that it is originating from the central nervous system. We also know that its levels are stable enough over time that we could measure a drug-dependent decrease.

### FORGING AHEAD

WE STILL ENCOUNTER CONSIDERABLE RESISTANCE. At what age should we begin treating people? How will we ultimately confirm that the drug delays disease? These are important questions, and we have the tools to devise rational answers. But the level of anxiety surrounding these issues reflects just how little precedent there is for therapeutic intervention to keep brains healthy. Perhaps the biggest pushback that we get is: Will insurers pay for this kind of drug? And behind it, the larger question: Will society pay for a prescription drug for years and years for people who are not yet sick and who, if the drug works, may never get sick?

For once, the rarity of our disease may work to our advantage. Prion disease patients are rare, genetic ones more so, and those who know they are at risk before onset are yet rarer still. Our impact on an insurer’s bottom line is nothing compared with a new drug for heart disease or diabetes that millions may take. But there is a larger picture, too. We as a society need to ask what we want for our brains. If you were one of the 20 percent of people for whom neurodegenerative disease lies ahead and if you had a preventive drug, when would you take it? Would you wait until after the onset of dementia? Until mild cognitive impairment? Until an MRI showed your brain shrinking? Or would you take it before any of that happened?

In prion disease, we may have no choice. But that also means that we have an opportunity to forge a path toward the goal of prevention. For all the progress in modern neuroscience, every human brain remains unspeakably and unknowably complex, an interconnected network of almost 100 billion neurons we do not understand, cannot fix and cannot possibly replace. If you ask what you want for your brain—and the few brains that you love most in the world—you may find that your answer is the same as ours: prevention. ■

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#### MORE TO EXPLORE

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#### FROM OUR ARCHIVES

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