



COVER IMAGE. NATURE. OCTOBER 4, 2001. IMAGE BY YURI VEKLIICH AND JOHN W. WEISEL. UNIVERSITY OF PENNSYLVANIA.

# Tracking a Perpetrator Gene

*A single mutation can lead to a devastating disorder of the circulatory system.*

ON MAY DAY 1999, JENNIFER CHAMBERLIN,\* A 43-YEAR-OLD secretary in the Midwest, stayed home to recuperate from back surgery and spend time with her three daughters, on spring break from school. Walking into her kitchen, Jenn fainted and fell to the floor. She soon regained consciousness but never returned to her normal self. “It was like she was in a trance,” says Jenn’s husband, Tim. “She was always lying down and talked just when talked to.” Doctors could-

\*The names of the patient and her husband have been changed.

n’t detect any physical ailments. “She might need to see a psychologist,” a physician told Tim. Over the next 3 weeks, Jenn plunged deeper into mental darkness, crying out in delusional outbursts.

At the end of May, Jenn’s platelet count had dropped precipitously to 16,000 per microliter—the minimum for a normal count is 150,000. Follow-up tests revealed that her red blood cells were breaking into shards. With those tell-tale symptoms, doctors finally diagnosed her with thrombot-

OPPOSITE \_ A BLOOD CLOT CONSISTS OF A PLUG OF PLATELETS ENMESHED IN A NETWORK OF INSOLUBLE FIBRIN MOLECULES. IN THIS COLORIZED SCANNING ELECTRON MICROGRAPH OF A BLOOD CLOT THAT FORMED IN VITRO, THE TEAL STRANDS ARE FIBRIN, THE PURPLE CLUSTERS ARE ADHERENT PLATELETS, AND THE RED OBJECTS ARE TRAPPED RED BLOOD CELLS.

ic thrombocytopenic purpura (TTP), a rare disorder of the blood-clotting system that was almost always fatal until the 1980s and 1990s, when doctors developed a crude but effective blood plasma transfusion treatment.

Scientists understood relatively little about the cause of Jenn's illness until recently. Researchers knew that platelets in TTP patients form spontaneous clots, or thrombi, within the narrowest blood vessels. As a result, circulating platelets become depleted, and red blood cells become shredded as they squeeze through the occluded vessels. The restricted circulation and anemia leave tissues starved for oxygen, leading to strokes, heart attacks, and failure of other critical organs. Jenn's May Day attack, her doctors suggest, was probably the first of several strokes resulting from TTP.

Doctors ordered plasma-exchange therapy, the only known treatment that might save her life. For three and a half hours every day Jenn lay in a hospital bed, hooked up to a machine via a large catheter in her arm vein. The machine filtered Jenn's blood, saving the cells and replacing the plasma portion with about one and a half volumes of donor plasma. The treatment worked. Jenn's platelet count rebounded, and her condition improved over 5 days. But on the sixth day her platelets dropped again, and she endured another plasma exchange. Then another. Ultimately, in just over 2 years, she underwent more than 145 exchanges.

One of Jennifer Chamberlin's hematologists is HHMI investigator J. Evan Sadler, from Washington University School of Medicine in St. Louis. Sadler and HHMI investigator David Ginsburg, at the University of Michigan, were both drawn to investigate TTP by their prior research on a key blood-clotting protein called von Willebrand factor (VWF). In the mid-1980s, the two physician-scientists independently cloned the gene for VWF and since then they have been studying the protein's roles in blood clotting. One of those roles, Sadler explains, is to make platelets adhere to the walls of injured blood vessels.

In 1996, two research groups, one led by Han-Mou Tsai at the Albert Einstein College

of Medicine in New York and one by Miha Furlan in Bern, Switzerland, separately discovered that an unidentified enzyme in blood could cleave VWF but only if the protein was slightly unfolded. Such unfolding might occur when VWF, tethering platelets to a blood vessel, gets stretched in the current. Why VWF might get cleaved was anyone's guess, but an important clue soon followed.

The next year, Furlan's group made the crucial finding that children with a rare hereditary form of TTP lacked the VWF-cleaving activity in their blood, suggesting a link between VWF, the enzyme, and the disease. Then, in 1998, Tsai and Furlan, in separate studies, bolstered that notion by showing that most adults with the acquired form of TTP (including, as it turned out, Jennifer Chamberlin) produce antibodies against the still-unidentified enzyme.

The finding shifted researchers into high gear in their efforts to identify the VWF-cleaving activity. "We were then madly working to try to purify, to clone this protein," Sadler recounts. In collaboration with Dominic Chung at the University of Washington, who purified enough of the protein to determine some of its amino acid sequence, Xinglong Zheng, then a postdoctoral fellow in Sadler's lab, used a combination of bioinformatics and DNA sequencing to identify the gene.

Meanwhile, Ginsburg, Tsai, and colleagues were following a different strategy to identify the gene encoding the VWF-cleaving enzyme. Analyzing plasma samples from people with TTP in their families, the researchers found that, although plasma from children with the disease had no VWF-cleaving activity, plasma from their parents and some of their siblings (none of whom had TTP) showed about half the normal activity. This suggested that TTP is caused by a single recessive mutation in the gene responsible for the VWF-cleaving activity—that is, a mutation in one of an individual's two copies of the gene cuts down the enzyme activity, but for an individual to contract TTP, both gene copies must be mutated.

Gallia Levy, an M.D.-Ph.D. student in Ginsburg's lab, used this information in conjunction with genetic data from family members' DNA samples to determine the chromosomal location of the gene. Levy traveled to Cincinnati, to the lab of William Nichols, a former student of Ginsburg, who had the equipment and expertise for conducting the complex

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genetic analysis. “Gallia spent a week in his lab running all the genotypes. She came back with the data and sure enough, we had mapped the gene!” Ginsburg recounts. “We were pretty excited about it. She started analyzing more markers and narrowing the genetic location. When we got down to a manageable region of the genome, she went through the genes there, one by one, sequencing them in the blood samples from the TTP patients to see if we could find a mutation.” Levy finally pinpointed the gene and found it to be mutated in all the children who had TTP. The gene, called *ADAMTS13*, encoded a protease—an enzyme that cleaves protein. At practically the same time, Sadler’s team identified *ADAMTS13* as the blood protease that cleaves VWF in particular.

Sadler and Ginsburg have learned much about *ADAMTS13* since their discoveries 4 years ago. The general idea, says Sadler, is that *ADAMTS13* regulates the activity of VWF in the blood. VWF recruits circulating platelets into a thrombus around a broken blood vessel. “If you don’t have *ADAMTS13*, or if your own immune system destroys your *ADAMTS13*,” Sadler explains, “these platelet thrombi grow out of control and you end up with TTP.”

Sadler’s group is currently trying to understand why some people produce autoantibodies to *ADAMTS13* and to figure out what causes the vast differences in the severity of the disease in different patients. “We’ll treat many of our patients with plasma exchange and they get better,” he says. “Their antibody goes away, their *ADAMTS13* comes back, and they’re fine. But other patients may return to the hospital in a week or a few months with another episode. And each episode can be devastating. If we can identify patients with a high likelihood of relapse, and give them more intensive therapy up front, then I think we can save some lives.” Ginsburg’s lab is working toward a better understanding of childhood TTP. They recently developed a “knockout” mouse, lacking the *ADAMTS13* gene, which mimics the human condition.

A commonsense treatment for TTP would be to administer recombinant *ADAMTS13*, Sadler explains. “That would be perfect for kids with con-

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genital *ADAMTS13* deficiency.” It might also help adults with the acquired form of the disease.

Tragically, both researchers concede, the ultimate obstacle to a better treatment for TTP may not be scientific, but economic. “It’s one of these rare examples where we have an opportunity to take a basic research finding straight to the bedside,” Ginsburg says. “And it just isn’t going to happen because of the financial realities of the pharmaceutical industry.”

“We’re talking about a disease that, at most, strikes maybe 1 in 100,000 people,” Ginsburg says. “And pharmaceutical companies are not interested in developing a drug unless the market is a bare minimum of half a billion dollars a year. Otherwise, it’s not worth their effort.”

Meanwhile, the multiple strokes Jennifer Chamberlin suffered as a result of TTP took her sight and hearing, but her illness has been in complete remission for more than 4 years. Her doctors, including Sadler, credit rituximab, an immunosuppressive drug that kills antibody-making white blood cells and has shown remarkable effectiveness in treating autoimmune diseases. “We may be able to apply what we have learned about *ADAMTS13* to identify high-risk patients like Mrs. Chamberlin, who could benefit from immunosuppression with rituximab at the time of first diagnosis,” Sadler says. Ginsburg concurs: “That would be a real improvement.” ■