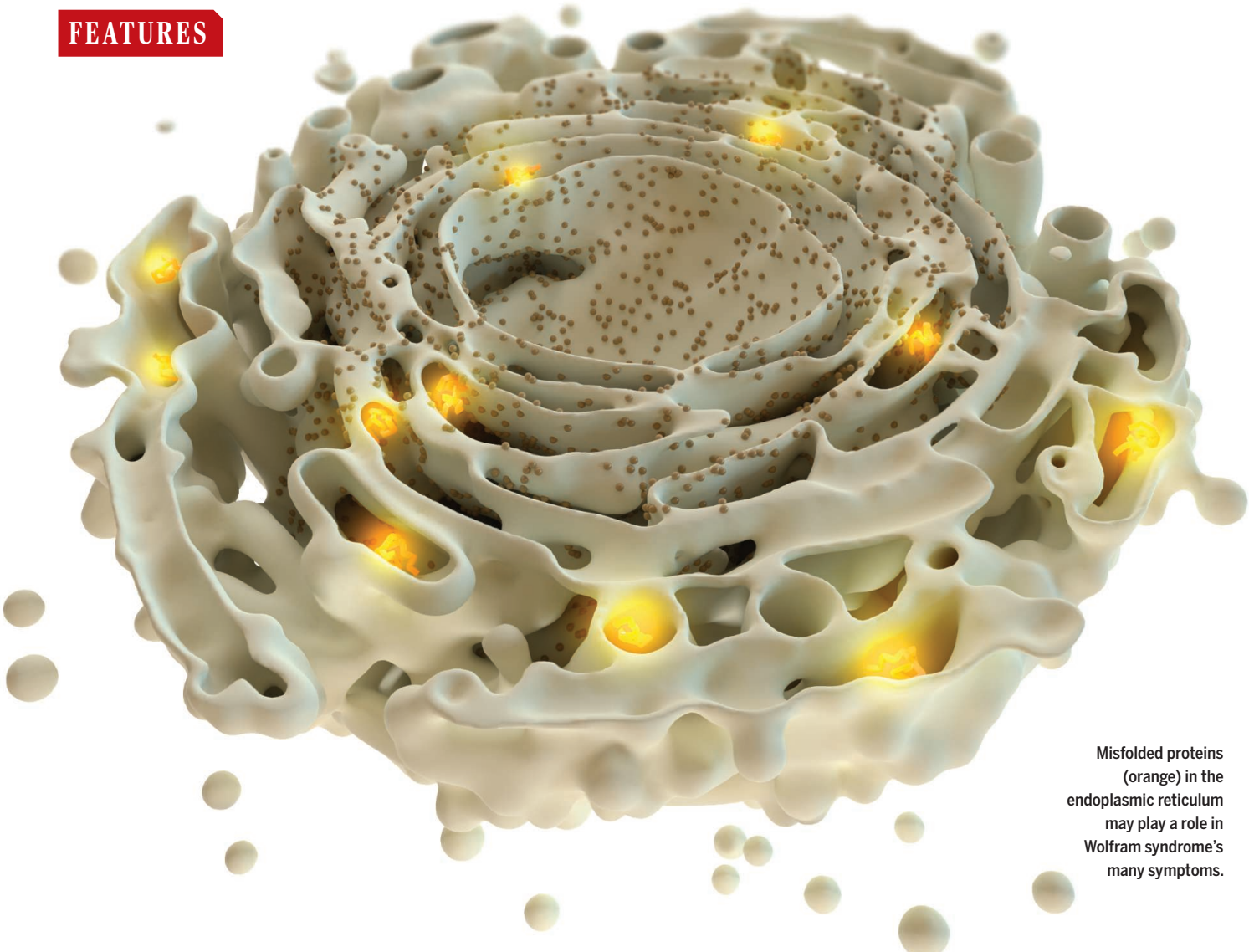


Misfolded proteins (orange) in the endoplasmic reticulum may play a role in Wolfram syndrome's many symptoms.

ILLUSTRATION: V. ALTOUNIAN/*SCIENCE*

A REVEALING FLAW

A rare disease that cripples a key cellular organelle holds clues to treating more common conditions

By **Mitch Leslie**

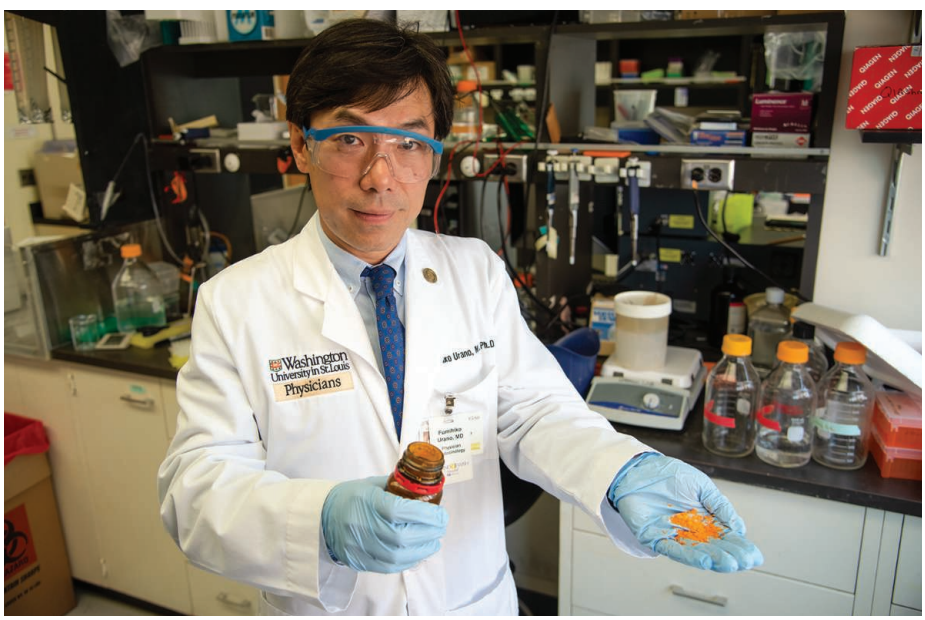
Maureen Marshall-Doss says the first sign that her vision was deteriorating came when she misidentified the color of a dress. At a backyard get-together about 20 years ago, the Indianapolis resident pointed out an attractive yellow dress another woman was wearing. "You see that as yellow? She's wearing a pink dress," Marshall-Doss recalls her husband responding.

Today, Marshall-Doss is virtually blind. With help from custommade eyeglasses that magnify objects 500 times, "I can see shapes," she says. But she can no longer drive and had to quit the job she loved as a school librarian. Along with her dimming vision, she has type 1 diabetes and has lost her sense of taste and smell.

Marshall-Doss is one of 15,000 to 30,000 people around the world with Wolfram syndrome, a genetic disease. For decades, the condition remained enigmatic, untreatable, and fatal. But in the past few years, insights into its mechanism have begun to pay off, leading to the first clinical trials of drugs that might slow the illness and sparking hopes that gene therapy and the CRISPR DNA-editing tool might rectify the underlying genetic flaws. "Here is a rare disease that the basic science is telling us how to treat," says physiologist Barbara Ehrlich of the Yale School of Medicine.

The research could also aid more than the relatively few patients with Wolfram syn-

drome. Driving the disease's many symptoms is a malfunction of the endoplasmic reticulum (ER), the multichambered organelle that serves as a finishing school for many cellular proteins. Known as ER stress, the same problem helps propel far more common illnesses, including type 2 diabetes, amyotrophic lateral sclerosis (ALS), Parkinson's disease, and Alzheimer's disease. "Wolfram syndrome is the prototype of an endoplasmic reticulum disorder," says medical geneticist Fumihiko "Fumi" Urano of Washington University School of Medicine in St. Louis. Because Wolfram syndrome is "simpler," says Scott Oakes, a cell biologist and pathologist at the University of Chicago, researchers think it could illuminate the mechanisms of other ER-disrupting diseases, which affect hundreds of millions of people worldwide.



Fumihiko Urano holds dantrolene, a muscle relaxant drug he helped test as a treatment for Wolfram syndrome.

IN THE LATE 1930s, four children with diabetes were going blind, and doctors were stumped. Like many other people in the United States struggling through the Great Depression, the siblings ate a paltry diet, subsisting on potatoes, bread, oatmeal, and a little milk. But after examining three of the children, Donald Wolfram, a physician at the Mayo Clinic in Rochester, Minnesota, and an ophthalmologist colleague ruled out malnutrition as the cause of their puzzling condition. Lead poisoning and syphilis—though common enough—weren't to blame, either. When Wolfram and his partner wrote up the cases in 1938, they concluded that the symptoms could be "manifestations of an hereditary or acquired cerebral lesion."

The physicians were right that the syndrome eventually named for Wolfram is hereditary. Recessive mutations in the gene for a protein called wolframin are responsible for most cases, with glitches in a second gene causing most of the rest. However, the pair was wrong to think the defect lies only in the brain. Instead, the symptoms stem from widespread cell death. "It's definitely a disease that affects the whole body," Marshall-Doss says.

The first sign of the illness, appearing when patients are children, is usually diabetes mellitus, or faulty sugar metabolism, sparked by the demise of insulin-secreting beta cells in the pancreas. Most patients also develop the unrelated condition diabetes insipidus, in which the pituitary gland doesn't dole out enough of a hormone that helps control the body's fluid balance, causing the kidneys to produce huge amounts of urine.

Ellie White, 19, of Centennial, Colorado, who was diagnosed with Wolfram syndrome 12 years ago, says she hasn't had a full night of sleep since she was 3 years old. She gets up again and again to use the bathroom and monitor her blood sugar.

Yet she and other patients say that as disruptive as those problems are, they are not the disease's most dismaying consequence. "The biggest symptom of Wolfram syndrome that affects me the most is my vision," White says. Because neurons in the optic nerve perish, patients usually go blind within 10 years of their first visual symptoms.

Other neurons die as well. As the disease progresses, brain cells expire, and walking, breathing, and swallowing become difficult. Most people with Wolfram syndrome die before age 40, often because they can no longer breathe. At 57, Marshall-Doss is one of the oldest patients; one of her mutated genes may yield a partly functional version of wolframin, triggering a milder form of the disease, Urano says.

TWO ADVANCES have made it possible to begin to tackle those symptoms. The first was Urano's discovery nearly 20 years ago that linked Wolfram syndrome to ER stress.

The ER is where about one-third of a cell's newly made proteins fold into the correct shapes and undergo fine-tuning. Cells can develop ER stress whenever they are under duress, such as when they don't have enough oxygen or when misfolded proteins begin to pile up inside the organelle.

In test tube experiments, Urano and his colleagues were measuring the activity of genes to pinpoint which ones help alleviate ER stress. One gene that popped up encodes wolframin, which scientists had shown in 1998 was mutated in patients with Wolfram syndrome. Following up on that finding, Urano and his team determined that wolframin takes part in what's known as the unfolded protein response, which is a mechanism for coping with ER stress in which cells take steps including dialing back protein production.

Scientists think wolframin plays a key role in the unfolded protein response, though they haven't nailed down exactly how. When wolframin is impaired, cells become vulnerable to ER stress. And if they can't relieve that stress, they often self-destruct, which could explain why so many neurons and beta cells die in the disease.

Defective wolframin may harm cells in other ways. The ER tends the cell's supply of calcium, continually releasing and absorbing the ion to control the amount in the cytoplasm. Changes in calcium levels promote certain cellular activities, including the contraction of heart muscle cells and the release of neurotransmitters by neurons. And wolframin affects calcium regulation.

Beta cells genetically engineered to lack functional wolframin brim with calcium, Ehrlich and colleagues reported in July 2020 in the *Proceedings of the National Academy of Sciences*. When exposed to lots of sugar, the altered cells release less insulin and are more likely to die than healthy beta cells, the team found. The cells share that vulnerability with beta cells from patients with Wolfram syndrome. "We think that excess calcium is leading to excess cell death," Ehrlich says.

ER malfunctions could hamstring other organelles as well. The ER donates cal-

PHOTO: WASHINGTON UNIVERSITY SCHOOL OF MEDICINE IN ST. LOUIS

cium to the mitochondria, the cell's power plants, helping them generate energy. In 2018, a team led by molecular biologist Cécile Delettre and molecular and cellular biologist Benjamin Delprat, both of the French biomedical research agency INSERM, discovered that in cells from patients with Wolfram syndrome, mitochondria receive less calcium from the ER and produce less energy. Those underpowered mitochondria could spur the death of optic nerve cells, the researchers speculate.

The link between ER stress and Wolfram syndrome has been crucial for identifying potential treatments because "otherwise we would have nothing to target," Urano says. But a second development was also key, he says: the advocacy and support of patient organizations, such as the Snow Foundation and the Ellie White Foundation, headed by its namesake's mother. The foundations have stepped up with money for lab research and clinical trials when other sources, including government agencies, didn't come through.

Scientists, patients, and their advocates say Urano also deserves much of the credit. Besides treating patients, he heads the international registry of cases and has taken the lead in organizing clinical trials, screening compounds for possible use as treatments, and devising potential therapies. "Fumi is clearly the driving force," says Stephanie Snow Gebel, co-founder of the Snow Foundation, who about 10 years ago helped persuade him to forgo a plum job as department chair at a Japanese university and take over the Wolfram program at Washington University.

PATIENTS COULD SOON start to reap the benefits. In 2016, Urano and colleagues started the world's first clinical trial for the disease: a phase 1/2 study of dantrolene, an approved muscle relaxant. The molecule was a top performer when they screened 73 potential treatments for their ability to save cells with terminal ER stress. Dantrolene didn't improve vision in the 22 participants, including White, the scientists reported in an October 2020 preprint. But in some patients, beta cells appeared to be working better and releasing more insulin. The drug is safe, but Urano says it will need to be chemically tweaked to target its effects before future trials are warranted.

Researchers are pursuing other possible treatments targeting ER stress or calcium levels. In 2018, U.K. scientists launched a trial that will include 70 patients to evaluate sodium valproate, a therapy for bipolar disorder and epilepsy that, in the lab, prevents cells with faulty wolframin from dying. Last year, another compound that emerged from Urano's screens, the diabetes drug liraglutide, entered a clinical trial. Also last year, an experimental drug developed by Amylyx Pharmaceuticals for Alzheimer's disease and ALS received orphan drug designation from the U.S. Food and Drug Administration for Wolfram syndrome because it curbs ER stress. That designation offers tax breaks and other incentives, and it will get trials started sooner, Urano says.

Ehrlich and her team have a candidate of their own that they have begun to test in rodents: the drug ibudilast, which is approved in Japan to treat asthma. The researchers found it reduces calcium levels in beta cells lacking wolframin and boosts their survival and insulin output. New screening projects may reveal still more candidates.

But Urano knows that even if a treatment receives approval, it would be only "a Band-Aid for Wolfram syndrome." Hoping to develop a genetic cure, he and colleagues are introducing replacement genes into cells from patients and from mice engineered to replicate the disease. The researchers are endowing the cells with healthy copies of the gene for wolframin or the gene for a protein that reduces ER stress to determine whether they restore cellular function and reduce cell death. At INSERM, Delettre and colleagues are also evaluating whether directing a working gene into optic nerve cells can curtail vision loss in mice with faulty wolframin. The scientists are still gathering data, but early results suggest the treatment can halt the deterioration.

Urano and his collaborators have also turned to the genome editor CRISPR, deploying it to correct the gene defect in patients' stem cells and then growing them into beta cells. When the researchers transplanted the revamped cells into mice with diabetes, the animals' blood sugar returned to healthy levels, the team reported in April 2020 in *Science Translational Medicine*.

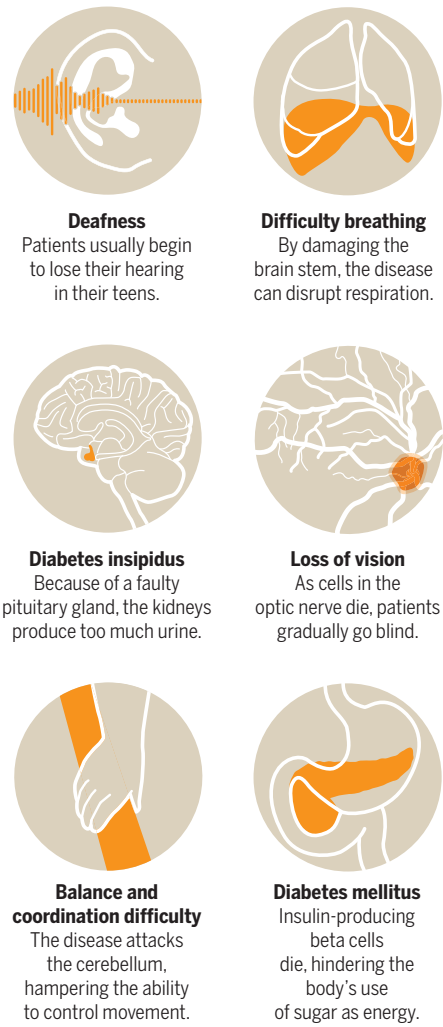
Stem cell biologist Catherine Verfaillie of KU Leuven is collaborating on the CRISPR research. But she notes that because the faulty wolframin gene affects so many tissues, researchers will have to figure out how to deliver the CRISPR components to most cells in large organs such as the brain and liver—a prospect she calls "pretty daunting." Urano agrees, predicting that CRISPR-based Wolfram therapies might take 10 to 20 years to develop. The alternative approach, gene therapy, could reach clinical trials more quickly, in 3 to 10 years, he says, because researchers have more experience with gene therapy and have created several treatments that have already been approved for other illnesses.

Because it stems from a single genetic glitch, Wolfram syndrome could also help scientists tease out the role of the ER in more complex diseases, including neurological conditions, type 2 diabetes, and cancer. The ER also falters in those diseases, causing cells to die, but the mechanism is harder to discern because they stem from myriad genetic and environmental factors. In Alzheimer's disease, for instance, neurons develop ER stress as misfolded proteins accumulate inside and outside the cells.

Besides deepening researchers' understanding of other conditions, the research on Wolfram syndrome might even deliver candidate treatments. "Everyone would be very excited if we can make advances in targeting ER stress in Wolfram syndrome," Oakes says. "It would open up the whole field to doing this in other degenerative diseases." ■

A wide-ranging toll

Mutations in the gene for wolframin disrupt the endoplasmic reticulum and lead to cell death throughout the body, causing a range of symptoms.



Deafness
Patients usually begin to lose their hearing in their teens.

Difficulty breathing
By damaging the brain stem, the disease can disrupt respiration.

Diabetes insipidus
Because of a faulty pituitary gland, the kidneys produce too much urine.

Loss of vision
As cells in the optic nerve die, patients gradually go blind.

Balance and coordination difficulty
The disease attacks the cerebellum, hampering the ability to control movement.

Diabetes mellitus
Insulin-producing beta cells die, hindering the body's use of sugar as energy.

ILLUSTRATIONS: V. ALTOUNIAN/*SCIENCE*

A revealing flaw

Mitch Leslie

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