

WHEN THE BRAIN FAILS

Huda Zoghbi unravels the genetics of Rett syndrome and other disorders

BY NANCY ROSS-FLANIGAN

Gowned and gloved, Huda Zoghbi holds a squirming mouse in her hand and peers through a plastic hood. “Did you see that?” Zoghbi asks as the mouse flails. “He just had a seizure.”

The wriggling laboratory mouse could have major implications for human health. “Through studies of this animal model,” Zoghbi says, “we hope to gain insight into autism and into one of the most common causes of mental retardation in females, namely Rett syndrome.”

With the mouse safely back in its cage, Zoghbi, an HHMI investigator at Baylor College of Medicine in Houston, turns her attention to another one. Its crooked spine and unsteady gait are tip-offs to its condition, a mouse version of the human neurodegenerative disorder called spinocerebellar ataxia type 1 (SCA1). The mouse and its mates are helping to reveal the chain of glitches that ultimately cause specific groups of neurons to degenerate in people with SCA1.

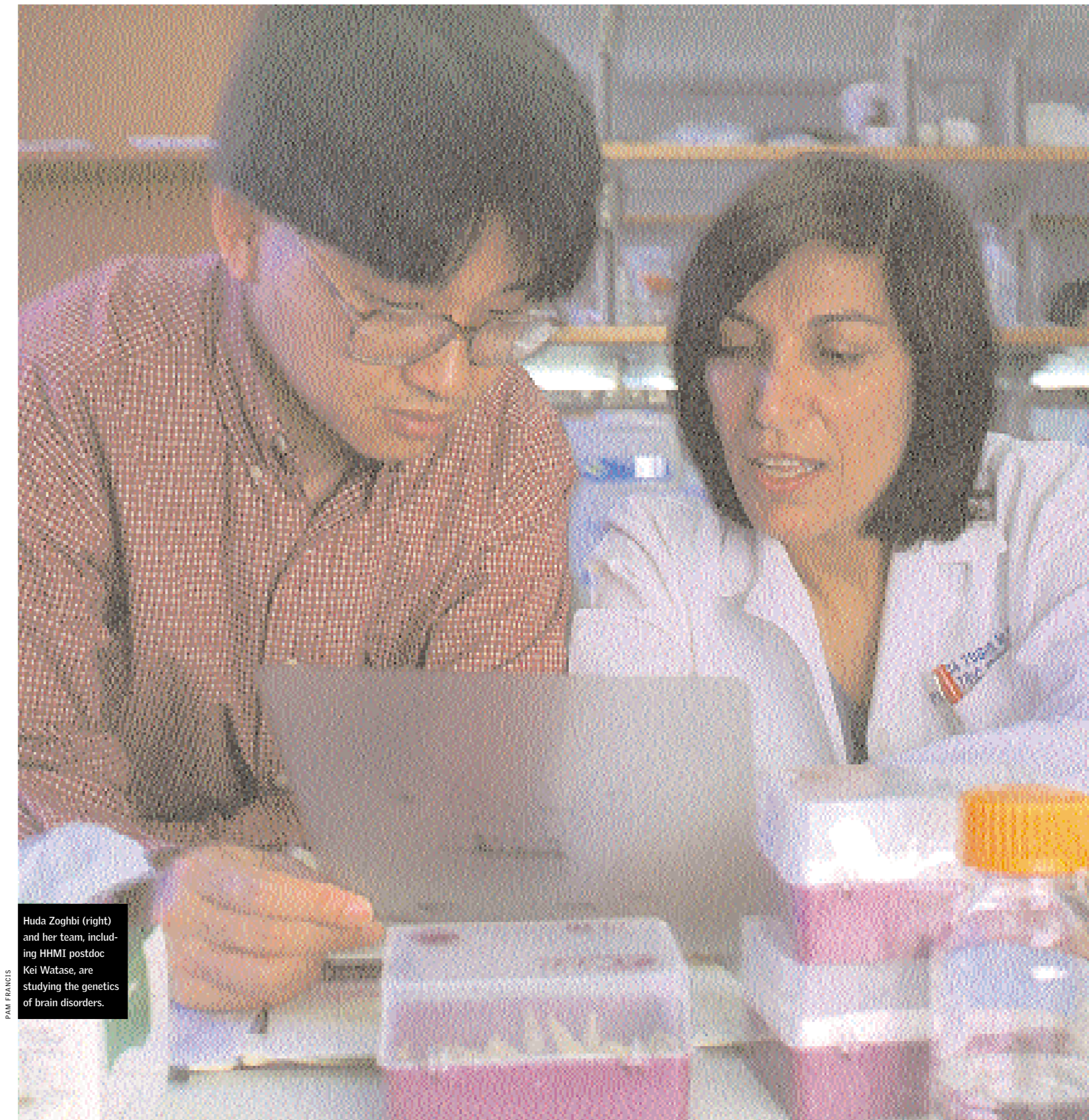
In recent years, Zoghbi and her collaborators have racked up an impressive list of accomplishments in these and related areas of research: discovering the gene for Rett syndrome (*RTT*); identifying the mutation involved in SCA1 and beginning to understand how it wreaks havoc on neurons; and advancing knowledge of the sensory components of coordination. Their work could provide insights into disorders such as autism and Huntington’s disease.

A DISEASE’S DEVASTATION

It all started when Zoghbi saw her first patient with Rett syndrome; nearly 18 years later, the memory remains fresh. Beautiful, brown-eyed Ashley, who began life like any healthy baby and made her parents proud as she learned to crawl, walk, babble and sing “ee-i-ee-i-oh,” had suddenly changed at around 18 months of age.

Instead of gaining skills, Ashley seemed to be losing them, becoming uncoordinated and uncommunicative. She stopped playing with her toys, coloring with crayons and running to the door to greet her daddy when he came home from work. Instead, she just rocked back and forth, staring vacantly and grinding her teeth. When Zoghbi saw her at age three and a half, she was struck by the heartbreaking contrast between Ashley’s beauty and the bizarre behaviors that characterized her Rett syndrome.

“She was constantly wringing her hands, having trouble with balance and staring past all of us,”



Huda Zoghbi (right) and her team, including HHMI postdoc Kei Watase, are studying the genetics of brain disorders.

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Zoghbi recalls. “She would alternate rapid breathing with holding her breath.” Zoghbi, then a pediatric neurology fellow, felt compelled to devote herself to research.

“Many basic scientists go into research because of curiosity about certain biologic problems, but I was driven into science because it was so devastating to see patients with these neurogenetic diseases,” Zoghbi recalls. “Most of the time we could do very little for the kids, and that was very hard on me.”

Having children of her own—Roula, now 16, and Anthony, 14—also fueled Zoghbi’s fervor. “Once I became a parent,” she says, “I realized what pain the children’s parents must be going through, and I thought, ‘I just can’t sit there and watch; I have to do something.’”

Little did she know that “to do something” about Rett syndrome would entail such a long and tedious process. Finding the gene responsible for a disease is tricky enough when the disorder runs in families and researchers can study large groups of affected kin. With this condition, however, fewer than one percent of cases are inherited; most are sporadic, meaning that the mutation can show up anywhere in the population.

Because Rett syndrome occurs mainly in girls, who generally have two X chromosomes instead of an X and a Y, the X chromosome seemed a logical place to start looking for the gene. Still, it took 14 years to pinpoint the gene’s exact location—on the far end of the chromosome’s longest arm—and then to begin deciphering its role. Finally, in 1999, Zoghbi and Uta Francke, then an HHMI investigator at Stanford University School of Medicine, reported the discovery of several mutations in a particular gene known as *MECP2* in patients with Rett syndrome.

Normally, *MECP2* produces a protein that “silences” other genes, preventing them from being expressed. Such inhibition can be critical during development, when various genes must crank out their protein products for precise periods, then stop and remain inactive. Though it’s not yet clear exactly how mutations in *MECP2* cause Rett syndrome, Zoghbi and Francke speculate that the defect allows genes to stay busy when they should be still, leading to overproduction of certain still-

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unidentified proteins, which somehow disrupt neural development.

Discovery of *MECP2* led to a diagnostic test for Rett syndrome, and once researchers began testing patients, it became obvious that the gene’s importance extended beyond this one disease. Several *MECP2* mutations have been found in female patients with autism or with mental retardation alone, and even in some boys with severe mental retardation and seizures, or with features of autism. “So we see now,” says Zoghbi, “that the spectrum of phenotypes that results from mutations in this gene is quite broad, way beyond Rett syndrome.”

The next step is to learn which genes are normally silenced by *MECP2* and which proteins those genes produce. To that end, researchers in Zoghbi’s lab will use the mouse model of Rett syndrome they’ve developed to home in on the genes.

A GENETIC STUTTER Zoghbi and her colleagues are also studying SCA1, a disorder that, unlike Rett syndrome, shows no early neurological symptoms. Patients with SCA1 are usually asymptomatic until adulthood, when they begin to stagger and stumble. Eventually, muscle control degenerates to the point that patients cannot talk, swallow or, finally, even breathe. In 1993, Zoghbi and collaborator Harry Orr of the University of Minnesota cloned the *SCA1* gene and found that the disease-causing mutation is a kind of genetic stutter. In the normal gene, the nucleotide sequence cytosine-adenine-guanine (CAG, the DNA code for the amino acid glutamine) is repeated about 30 times. But in patients with SCA1, CAG is reiterated 40 to 100 times. These extra repeats cause the gene to produce an abnormally long polyglutamine tract in the resultant protein, ataxin-1.

Somehow, these polyglutamine proteins do devastating damage to neurons, and not just any neurons. In patients with SCA1, the

mutant proteins prey mainly on the Purkinje cells of the cerebellum and certain brainstem neurons. Just how does the damage occur? And why are some neurons singled out? These are questions that Zoghbi and colleagues are continuing to explore.

They’ve learned that the number of repeats affects the disease outcome—the more there are, the earlier in life the disease strikes, and the more severe the symptoms. And they’ve found that the abnormal protein forms clumps in the nuclei of affected neurons, perhaps because the cellular machinery that normally breaks down and clears out excess protein doesn’t work efficiently on the polyglutamine chains.

“If it is not cleared sufficiently from the cell,” says Zoghbi, “the protein is free to do things in a neuron that it shouldn’t be doing,” such as interfering with the expression of important genes. To explore these possibilities, the researchers are studying proteins called chaperones, which help other proteins fold properly or hold them in configurations that can be degraded. In work with the mouse model, published in the February 2000, issue of *Nature Neuroscience*, they also found evidence that ataxin-1 interferes with normal gene expression very early in the disease process.

“Early on, before you see aggregations of protein, before you see signs of the disease, we found that the protein alters the expression of genes that are essential for the normal function of the neuron,” says Zoghbi. Several of those genes are involved in regulating calcium, which plays a critical role in normal neuron function.

This picture of how the SCA1 mutation leads to the loss of neuron function is supported by results from a related line of Zoghbi’s research. In that work, she and Baylor colleague Juan Botas are using a fruit fly model of SCA1.

“The power of the model in flies is that we can now screen thousands of genes to see which ones, if they are lost or overexpressed, will make the disease better or worse,” Zoghbi explains. In work recently published in the November 2, 2000, issue of *Nature*, the researchers identified a number of genes that suppress or enhance the neurodegenerative activity of SCA1.

As expected, those genes that impair the protein-degrading pathway make the disease worse. “But we also identified a new molecule that we had not suspected before to be a key player,” says Zoghbi. “Now we will take what we’ve discovered in the fly and try to discover in the mouse model and in human cells which of these components are directly or indirectly working with ataxin-1, so we can understand how they are contributing to neuronal dysfunction and death.”

The eventual goal, of course, is to figure out how to manipulate the process—by enhancing the clearance of ataxin-1 from the cell, for example—to slow the progression of SCA1 or prevent it altogether. To families plagued by SCA1, that would be a great payoff, but the benefits could be even broader. SCA1 is just one of eight neurodegenerative diseases caused by mutant polyglutamine proteins; Huntington’s disease is another. Though different populations of cells are affected in different polygluta-

THE SENSORY SIDE OF COORDINATION

While its SCA1 studies are providing insights into the motor control involved in balance, Zoghbi’s group is also exploring the sensory side of coordination. “When you walk down a hallway, you don’t have to look at your feet; you know exactly where they are,” says Zoghbi. “Even if you close your eyes, you know where your hands and feet are, because there is continuous sensory feedback to your brain about the position of your limbs.” Sensory receptors in the muscles, tendons, joints and inner ear detect motion and position, and they signal the brain through a route known as the proprioceptive pathway.

To better understand that pathway, Zoghbi followed up on a lead from geneticist Hugo Bellen, another HHMI investigator at Baylor, who called her attention to a line of fruit flies that are uncoordinated because they lack a gene called *atonal*. This gene controls the development of the chordotonal organs, major sensory organs in the peripheral nervous system of the fly. Collaborating with Bellen, Zoghbi’s group found the mouse version of *atonal*, which is named “Mouse *atonal* homolog 1” (*Math1*), and began studying its function.

“What we have found is that *Math1* is essential for many components of this pathway, controlling multiple neurons,” says Zoghbi. “These neurons are quite different and diverse in their functions, but they’re all components of a pathway that does the same thing as the chordotonal organs in the fly.”

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mine disorders, the underlying mechanisms seem to be the same, so pinning down the molecular basis of one should shed light on the others.

STRIKING A BALANCE As Zoghbi teases apart problems of genetic expression, she struggles with expression issues of an entirely different sort, such as how to nurture students while still insisting that they do their best. “It’s a fine line to walk,” she says. “You want to demand excellence, but you don’t want to destroy someone in the process.”

When students get discouraged, Zoghbi sits them down and confides that she spent three years working on the wrong region of the SCA1 chromosome. Then she reminds them: “That’s why we call them experiments—if they’re all going to work, we don’t need to do them.... When things don’t work out, we pick up the pieces, we think of an alternative way, we ask another question and we go on.”

Zoghbi tries to strike a balance, too, between family life and professional responsibilities, and she finds an invigorating synergy in the combination. “Having kids helped me be more compassionate and understanding with graduate students and their ups and downs—thinking of their needs and listening to them,” says Zoghbi. “And having graduate students helped me become a better parent, because I learned that they leave you and fly off on their own. You want to see them go into that independent world, and you adjust, and you expect it and you’re happy for it.”

There’s one more important area where Zoghbi reminds herself of the need to accept frustration and stay steady. Like many researchers, she must regularly balance her desire to find cures with the knowledge that the work often proceeds slowly. “In science,” she says, “you have to set some really great goals and realize that you’re going to have to take minuscule steps toward achieving them.”

Another aid to maintaining balance is the firm belief that her research, even when it occasionally comes to a dead end, contributes to the overall efforts and their ultimate products. “Whether it’s our lab or someone else who will discover therapeutics based on some of the work that we do,” says Zoghbi, “it *will* happen.”

THE JUNE FLOOD

Tropical storm Allison dumped roughly three feet of rain on Houston in early June, killing 20 people and causing property damage estimated at \$1 billion. Huda Zoghbi and other HHMI investigators at Baylor College of Medicine did not escape the storm’s wrath.

More than 30,000 of Baylor’s research animals died when basement and sub-basement animal facilities flooded. “We lost about 40 percent of our mice,” Zoghbi says. The manager of HHMI’s administrative office in Houston, Randal Condit, estimates that other investigators who work with mice experienced similar losses. Power failures destroyed many cell cultures, but investigators and their teams used more than 1,500 pounds of dry ice a day to save what they could. “We preserved the things that were irreplaceable, such as patient cell lines and donated tissue,” Zoghbi says, adding that “this was hardest on the graduate students and fellows. A few months’ or a year’s setback is enormous for them.”

Although it is on the first floor of a building near a bayou, HHMI’s administrative office at Baylor sustained no permanent damage. The lab of Richard Gomer, an HHMI investigator at Houston’s Rice University, was untouched. Only a block from Baylor, Rice is on slightly higher ground, and all of its research animals and labs are above ground level.