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Rett Syndrome Traced to Defective Gene "Silencer"

A 14-year search for the cause of a rare genetic disease that strikes young girls has uncovered the first example of a human disease that is linked to a defective gene silencing mechanism.

Rett syndrome (RTT) is a neurodevelopmental disorder seen in young girls that causes a sudden and permanent decline in mental capabilities. In 1985, HHMI investigator Huda Zoghbi, who was then a neurology fellow, published a clinical research report on Rett syndrome. Her initial encounter with the disorder had a lasting impact on her career. Shortly after seeing her first RTT patients, Zoghbi decided to change her career plans, switching from clinical medicine to a research position. Shortly after switching to research, she started the long, tedious search for the genetic causes of RTT.

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"Finding that gene is the hardest thing I've ever worked on," Zoghbi said in an interview. "It's the best case for the rewards of perseverance that I can think of."

The gene, called *MECP2*, resides on the far end of the longest arm of the X chromosome. *MECP2* encodes a protein named methyl-CpG-binding protein 2, or MeCP2, that binds to genes that a cell has methylated. During development certain genes that are to be silenced, or prevented from being expressed, are physically marked by the addition of methyl groups. When bound to a methylated gene, MeCP2 facilitates gene silencing by attracting other proteins that prevent access to the cell's DNA transcription machinery.

Previous work by other researchers had shown that inactivating the mouse version of *Mecp2* caused a lethal defect in male mouse embryos. Zoghbi's group at Baylor College of Medicine, in collaboration with researchers led by Uta Francke, an HHMI investigator at Stanford University School of Medicine, found several mutations in this gene in patients with Rett syndrome. The researchers report their discovery in the October 1999 issue of the journal *Nature Genetics*.

Girls with RTT are born without any sign of the disease and appear to develop normally for the first six to 18 months of life. Then they begin to regress, sometimes abruptly, losing their burgeoning skills in muscle control and communication. Eventually the disease stabilizes and the girls grow up with extensive mental retardation and a propensity to sit and wring their hands.

"I saw my first RTT patient as a pediatric neurology fellow in October 1983, though I didn't yet know what it was," said Zoghbi. "I was amazed by two things. First, her history of normal development followed by a period of regression. Second, I can't think of another neurological disease in which patients will sit there and endlessly wring their hands."

A week later, Zoghbi was scheduled to see a young girl who had been diagnosed with cerebral palsy. This patient too was wringing her hands. "I thought that since I saw two in one week, there must be more," Zoghbi said. Examination of patient records revealed six more girls with similar signs: hand-wringing, ataxia and spasticity.

RTT has been her biggest challenge, Zoghbi said, largely because only one percent of recorded cases are inherited. Researchers have a much easier time homing in on a defective gene when they are working with large families with numerous affected members. Francke, who has also been searching for the RTT gene independent of Zoghbi's efforts, agrees. "We couldn't use any of the standard techniques available for finding such genes. We basically had to pick candidate genes and see if they were the gene involved in Rett syndrome," she explained.

While 99 percent of RTT cases occur randomly in the population, both Zoghbi and Francke who began collaborating instead of competing five years ago had a logical starting hypothesis: If the syndrome strikes only girls, whose sex-determining chromosomes are XX, and not boys, whose sex is determined by the X and Y chromosomes, then the defect must reside in a gene carried on the X chromosome. Furthermore, the two investigators reasoned that the gene was both dominant meaning one mutant copy of the gene could trigger the disease and lethal.

Such properties would explain why only girls develop RTT. A female fetus could survive the effects of the otherwise lethal mutation because it has two X chromosomes — one that harbors a normal copy of the gene and the other a defective copy. The normal gene can compensate partially for the defective gene, allowing the fetus to survive. In a male fetus, however, there would be no opportunity to inherit a compensating, functional copy of the gene since the male fetus has but one X chromosome. As a result, development is so out of kilter that the male fetus dies either before or shortly after birth.

Zoghbi and Francke, along with members of their laboratories, narrowed the search for the RTT gene by analyzing shared and unshared DNA sequences in a small number of Rett families. By 1998, other scientists helped to further narrow the search to about 200 candidate genes. Postdoctoral fellows Ruthie Amir of Zoghbi's laboratory and Mimi Wan of Francke's laboratory split up

the work of analyzing those 200 genes.

A quarter of the way through a set of 50 candidate genes, Igna Van den Veyver, a member of Zoghbi's lab, suggested to Amir that *MECP2* would be an excellent candidate gene based on some unexpected experimental results from an unrelated project. Sure enough, Amir found mutations in *MECP2* that were similar in about 30 percent of their RTT patients.

"This was an absolute surprise, the last gene I would have ever expected to be involved in Rett syndrome," said Francke. "The fact that knocking out the gene in mice led to developmental arrest and death of the fetus seemed to rule it out as a candidate for RTT. We also thought that MeCP2 may be involved in regulating a great many genes in different tissues and therefore would be unlikely to be involved in a disease that primarily affected the nervous system."

Zoghbi said that it is not yet clear exactly how *MECP2* defects cause RTT. One hypothesis, she said, is that the defective MeCP2 protein allows genes to remain active that should have been silenced at points along the precisely timed process of nervous system development.

"The exciting part of this discovery is not just what it may mean for RTT patients," she explained. "Now we know about a whole genetic pathway involved in neural development, and that human disease can result from its destruction. Many other disorders could be caused by defects in other components in this pathway perhaps autism, for example."

Though the scientists have identified a fraction of the mutations affecting their RTT patients, Zoghbi believes that they will find the other mutations in the DNA sequences of the *MECP2* gene that have yet to be analyzed. And while the discovery of the mutations may someday suggest possible therapies for this tragic disorder, the more immediate result should be a test for the early diagnosis and prenatal detection of RTT in the rare families where the mutation is inherited.