# QnAs with Huda Y. Zoghbi

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When neuroscientist Huda Zoghbi first came face-to-face with Rett syndrome, she was well on her way to becoming a pediatric neurologist. Impelled by the plight of the patients and intrigued by the bizarre mix of symptoms that mark the syndrome, Zoghbi boldly decided to change course, setting aside her clinical career to seek training in molecular genetics. Over the years, Zoghbi's efforts to unravel the molecular basis of Rett syndrome—a neurodevelopmental disorder characterized by loss of language, cognitive, motor, and social skills that mainly affects girls—have borne fruit. She pinpointed the gene responsible for the vast majority of cases, paving the way toward genetic diagnosis, counseling, and improved disease management. Along the way, Zoghbi's work has paid surprising dividends on other neurologic and psychiatric disorders, such as autism and intellectual disabilities. More recently, her team demonstrated that electrical stimulation of neuronal circuits implicated in Rett syndrome partly reversed learning and memory deficits in a mouse model, raising a glimmer of hope for deep brain stimulation as a therapeutic approach. For her wide-ranging work on Rett and other neurodevelopmental disorders, Zoghbi, now a professor at Baylor College of Medicine, Houston, Texas, has garnered a wealth of accolades, not least of which are membership in the National Academy of Sciences and the 2016 Shaw Prize in life sciences. PNAS caught up with Zoghbi on her decades-long work on Rett syndrome.


**Huda Y. Zoghbi and trainee Hsiao-Tuan Chao. Image courtesy of Paul Kuntz (photographer).**

**PNAS:** In the late 1990s, you discovered the gene implicated in Rett syndrome, and the discovery was more than a decade in the making. Can you take our readers down memory lane?

**Zoghbi:** In fact, it was 16 years from seeing my first patient to identifying the gene responsible for the disorder. It was during my residency as a pediatric neurologist that, together with attending faculty Alan Percy and Vincent Riccardi, I encountered my first patient with Rett syndrome. Back in the 1980s, Rett syndrome was not a well-recognized disorder in the United States; it was first described 50 years ago by Andreas Rett and later in 1983 by Bengt Hagberg and colleagues [that paper (1) led us to recognize the syndrome]. When I saw my first patient, I was struck by the unusual course of the disease. The girl was born healthy and remained so until two years of age, and gradually lost her communication and motor skills. She also wrung her hands incessantly. A week later, another girl with the same symptoms came into my office. I thought if I had encountered two cases in one week there had to be more, so I started combing through medical records for more patients and found half a dozen, all girls. But at the time, many physicians dismissed the idea of the syndrome being a unique clinical entity; many people thought it was a form of cerebral palsy.

**PNAS:** How did you home in on methyl-CpG-binding protein 2 (*MECP2*) as the culprit gene?

**Zoghbi:** I felt certain there was a genetic basis for the disorder. By this time, I had genetic material from over 100 patients to analyze. But these cases were all sporadic, not familial. This was in the mid-1980s, before the human genome was sequenced, and finding a gene for a sporadic disease was no easy

task. I decided to focus on the X chromosome because the cases were all girls, but that didn't narrow the search enough. Over time, we were fortunate enough to find two families who each had two affected individuals, which helped us narrow the candidate region to about one-third of the X chromosome. We were selecting neuronally expressed genes and sequencing them to no avail. A collaboration with Carolyn Schanen and Uta Francke on a third family also validated this region, and a fourth family studied by Eric Hoffman and colleagues helped narrow the region to Xq28. From that point on, we went gene by gene, using a brute-force approach, sequencing each one. Two years later, in 1999, we zeroed in on a methylcytosine-binding protein.

**PNAS:** Did you expect to pinpoint a protein that binds to a chemical modification on DNA?

**Zoghbi:** Not really; this was a surprise. The protein itself had been discovered by Adrian Bird in the 1990s, but nothing was known about its function in the brain.

**PNAS:** Is *MECP2* the only known gene implicated in Rett syndrome? How prevalent is the mutation among the afflicted?

**Zoghbi:** This is the only gene known to cause classic Rett syndrome, and the gene accounts for about 95% of all such cases. Among the classic hallmarks of Rett syndrome are the early normal development and regression after one to two years, the gradual loss of hand use, development of tremors and constant handwringing, an unusual breathing pattern in which the children alternately hold their breath and hyperventilate, among other features. It is the combination of these symptoms that is unique to classic Rett (individual symptoms may appear in other neurologic and psychiatric diseases). But there are several Rett-like syndromes for which a handful of genes have been implicated.

**PNAS:** How did the gene discovery change the clinical diagnosis and management of Rett syndrome?

**Zoghbi:** The diagnosis of Rett syndrome was typically made at around age five to seven (or later), not at two years of age, mainly because the early signs are mistaken for other neurodevelopmental disorders, such as autism, and it can take time for the full range of symptoms to appear. The discovery of the gene enabled early genetic diagnosis of classic Rett syndrome, which can be managed using physical and occupational therapy, among other ways. Also, almost 1% of cases are familial, which means a genetic diagnostic test, which has been available since 1999, can help families get counseling.

From a scientific perspective, the test has revealed that the type of mutation in the gene influences the severity of the condition, and patients can have widely varying severity of symptoms. We also learned that males with severe forms of the mutation died (because they have only one X chromosome), but those with milder mutations, which only slightly disrupt the protein's function, survive but develop severe neuropsychiatric symptoms. What's more, we were able to identify a new class of disorders tied to increased dosage of the gene. Shortly after the gene's discovery, we created a mouse model with an extra copy of the gene, and found that it resulted in neurological symptoms. Before long, similar symptoms were reported in humans with gene duplications spanning the *MECP2* gene.

**PNAS:** If the manifestation of Rett syndrome depends on whether or not the mutant allele is on the inactivated X chromosome, shouldn't the same be true of all X-linked disorders? Are there other examples of diseases in which severity is a function of X inactivation?

**Zoghbi:** Disease severity in Rett is indeed influenced by patterns of X inactivation; some girls with a very favorably skewed X inactivation pattern may exhibit hardly any symptoms at all, but these cases are unfortunately rare. For some X-linked disorders, we don't see the clinical effects of mutations because often having half of the cells express a normal allele is enough to protect normal function. But there are some disorders in which we do see the effect strikingly. For example, incontinentia pigmenti is one such X-linked disorder in which affected females show a mosaic pattern of skin pigmentation defects depending on whether or not the X with the mutant allele is inactivated.

**PNAS:** What function does the MeCP2 protein serve in normal cells? What goes awry in patients?

**Zoghbi:** MeCP2 clearly affects transcription, but exactly how it does so is an area of intensive investigation. We have systematically studied the protein's function in neurons using mouse models, which recapitulate many of the disease symptoms. We and others established that the protein's critical function relevant to Rett is in the brain, not in peripheral tissues. We then found that the function of neurons lacking the protein is partially compromised and that this partial loss of function seems to be sufficient to produce a number of the disease symptoms. Using the mouse models, we have also learned about subsets of neurons that are relevant for specific traits, such as anxiety, motor skills, social behavior etc.

**PNAS:** So what exactly is the protein acting on? What are its target genes?

**Zoghbi:** It has been a challenge to pinpoint those genes. MeCP2 binds broadly throughout the genome, wherever it finds methylated cytosines. We have found using mouse models of Rett and *MECP2* duplication that the expression levels of thousands of genes are altered in an inverse way in the two models. Many of the genes implicated in autism and intellectual

disability are among those misregulated in these models. But the precise molecular cascade leading to the symptoms remains to be worked out.

**PNAS:** Given the protein's broad action in the genome and the spectrum of disease manifestations, what is the best therapeutic approach for Rett syndrome?

**Zoghbi:** Conceptually, the best therapeutic target for Rett syndrome appears to be the MeCP2 protein itself. Ideally, we would want to restore the function of this protein in all brain cells to reverse the symptoms. But doing that is not easy. So we have been looking at downstream effects, such as boosting levels of neurotransmitters implicated in the disorder, manipulating the two main groups of neurons—excitatory and inhibitory—known to be affected in the disorder, and boosting levels of growth factors. The *MECP2* duplication syndrome is relatively more tractable because it is easier to lower levels of a protein than to replace it. We have used antisense oligonucleotide approaches to lower the levels of MeCP2 in symptomatic duplication syndrome mice and see reversal of symptoms, for example.

**PNAS:** In a recent PNAS article (2), you explored the reasons for the delayed onset of the syndrome. Can you explain the findings?

**Zoghbi:** Previous studies have shown that a form of DNA methylation, called non-CpG methylation, becomes more abundant as the brain matures in mice and humans. We had been studying the DNA-binding patterns of the MeCP2 protein in the brain. When the non-CpG data came out, we started overlaying our findings on the data, and we found some intriguing correlations. We selected a few genes—and we don't really know if these are the target genes in the syndrome—to study. One was *Bdnf* (bone-derived neurotrophic factor), and it turned out that MeCP2 binding to this gene happened as the brain matures and when non-CpG methylation occurs in *Bdnf*, suggesting a potential explanation for the delayed onset of symptoms tied to MeCP2 deficiency. That might be part of the story.

**PNAS:** What is your outlook on this work?

**Zoghbi:** One of my main goals for the next five years is to identify modulators of the level of MeCP2 in neurons as a way to identify potential drugs for Rett syndrome and *MECP2* duplication syndrome. Second, given the broad effects of this protein on the genome, we want to continue exploring the strategy of neuromodulation as a therapeutic approach. Third, we want to continue to explore the excitatory and inhibitory neuronal pathways implicated in the disease with a long-term view toward therapy. Finally, we are developing and characterizing humanized mice, which have two human alleles of *MECP2* instead of the mouse alleles, to study the effect of antisense oligonucleotides on the protein levels and disease symptoms and to ensure that we can lower the levels to a normal range but not beyond.


**1** Hagberg B, Acardi J, Dias K, Ramos O (1983) A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use: Rett's syndrome: Report of 35 cases. *Ann Neurol* 14(4):471–479.

**2** Chen L, et al. (2015) MeCP2 binds to non-CG methylated DNA as neurons mature, influencing transcription and the timing of onset for Rett syndrome. *Proc Natl Acad Sci USA* 112(17):5509–5514.