Learning From Patients: The Science of Medicine (2003)
Lecture Four—The Strength of Families: Solving Rett Syndrome
Huda Y. Zoghbi, M.D.

1. Start of Lecture 4 (00:11)

From the Howard Hughes Medical Institute the 2003 Holiday Lectures on Science. This year's lectures "Learning from Patients, the Science of Medicine" will be given by Dr. Bert Vogelstein Howard Hughes Medical Institute Investigator at Johns Hopkins University School of Medicine and Dr. Huda Zoghbi Howard Hughes Medical Institute Investigator at Baylor College of Medicine. The fourth lecture is titled "The Strength of Families: Solving Rett Syndrome." And now to introduce our program the grants program director of the Howard Hughes Medical Institute Dr. Dennis Liu.

2. Introduction by HHMI Grants Program Director Dr. Dennis Liu (01:02)

Hello. In a moment, you're gonna learn about a disease called Rett syndrome. Back when I was in graduate school Rett syndrome was sort of an obscure genetic mystery. No one really understood how it worked and it was a profound tragedy for the families that were affected. In fact, the disease was often mistakenly lumped together with cerebral palsy which is a completely different disorder, of course. The families that were affected by this had another interesting mystery which was a mystery to researchers only girls seemed to be affected. So I actually used this as a teaching example in my own work to point out that not everything was understood in science and in fact even the basic pattern of inheritance of a disorder was often a puzzle that needed to be worked through. Well, fortunately Huda Zoghbi didn't just puzzle over this mystery. She worked for 16 long years to try and solve the mystery and as a result, she has a really fascinating story that she's gonna tell you. We're gonna have another brief video to introduce her and then to close the lectures HHMI president Tom Cech will return.

3. Introductory interview with Dr. Huda Zoghbi (02:21)

I think everybody really works very hard at the lab because they love it. They love what they're doing, they love their projects but at the same time, everybody is interactive and is happy to help each other. We like people who are interactive because we work on big problems. So one person can't solve that big problem whether it's neurodegeneration or Rett syndrome or the role of MATH1 in neural development. These are projects that require teamwork on them. So we have three teams working in these areas and that means the members of the team need to get along. So the culture is, people work together very cooperatively and they really give input to each other and are very interactive to solve these problems and I think the other side of the culture is the passion about these diseases is really just as strong among all the new young people that join the lab as I feel about these diseases. So that's what's really fun. I don't have to motivate them to be excited to do something for these disorders or these developmental questions. They come in very motivated to do something. I find science the most humbling experience because you really you think you've learned a lot, and you think you know a lot and then a new avenue will open up and you'll discover how much you really didn't know. My favorite part of my job is talking and interacting with people in my lab to see what good have we done today have we found something new that's really gonna make a difference? And then translating that or go back in to the patients and tell them. So these are really two very exciting moments being in the lab with the little progress on a day-to-day basis but eventually when a big story happens that impacts the patient, we can tell them that. One of the most rewarding things in science is the relationships you form along the way and for me, that, I think, has enriched my life tremendously those relationships with my patients with my students with my mentors and collaborators and then my friends outside work who support me when things are slow or frustrating or hectic. So I think that, you know, it's a very enriching experience to be working in

medicine and science where you make all these relationships and keep them for a very long time.

4. Rett syndrome introduction (04:53)

Hello again. So, like Bert, I was a pediatrician and a child neurologist and during my child neurology training I saw my first Rett syndrome patient in 1983 and it was actually seeing this patient that propelled me to pursue a career in research and I'm gonna tell you now the journey that we had to take to discover the cause of Rett syndrome and the big emphasis in this lecture is the strength of families. The families have been crucial in moving research forward and you'll appreciate that as we go through the talk. Rett syndrome is a childhood developmental disorder which means these girls don't go through the developmental program as most children do and before you look at a Rett syndrome child I thought it would be very helpful for you to see what a child typically go through during normal development. I know many of you have seen children, been around them but probably, you've never wondered at what age can a child walk or talk or do certain specific tasks. So our first introduction is just a brief video to walk you through a normal course of childhood development the first four years of life. Let's look at that. *"It's Anna's birthday."*

5. Video: Normal child development (06:07)

This is a girl one year old playing well with her hands, running *"Oh, well."* Interacting making some make-believe in her play drinking from a cup now dancing and coloring, grooming and now doing more intricate dances. So this is what typical girls will do and

6. General description of Rett syndrome (06:42)

many Rett syndrome girls actually start doing many of these things and parents, of course, are joyed that their girls are going through that very nice program watching them learn those skills but then something happens and it differs for every girl but somewhere between 6 to 18 months of age these girls will stop learning new skills and gradually will even lose the skills they once have acquired and this process of losing their skills and sort of developing some unusual features happen over the course of two to three years which are very scary and devastating and I thought for you to really get a good appreciation of what Rett syndrome girls look like we're gonna have you watch a video and the video is narrated by Julia Roberts who is passionate about this disorder. She has seen a child with this disorder and was very touched. So she narrates this video and you're gonna see different girls with Rett syndrome. Let's roll the film.

7. Video: Julia Roberts describes Rett syndrome (07:41)

I want to tell you about a developmental disorder called Rett syndrome. You may not have heard of Rett syndrome before now. Many people haven't, but it's highly likely you have encountered a person or a family who's living with it. Also, it's believed that there may be hundreds of thousands of young girls and women worldwide who have Rett syndrome but have not yet been identified or have simply been misdiagnosed. *Rett syndrome is a neurological disorder that affects girls at an early, critical stage in their bodies' development. The word "syndrome" is used to categorize a collection of particular symptoms. For Rett syndrome the symptoms include a regression of skills leading to a loss of the ability to pick up and hold things repetitive hand movements seizures, teeth grinding curvature of the spine, and breathing difficulties. Some girls have the ability to walk. However, many lose this skill as the disorder progresses. Although shortened life is a possibility individuals with Rett syndrome have been known to live into their 50s.*

8. Rett syndrome is typically rare and sporadic (08:49)

So you've seen some of the features of Rett syndrome and many of the girls go through that and develop most of these features, in fact. Rett syndrome affects about one in 10,000 girls girls that have all these features you've seen in the film and in the majority of these cases the disease is sporadic. In 99% of these cases it's just one child in one family and this is what we really mean when we say sporadic. If you were to take about 100 families with a daughter with Rett syndrome and if you look at their pedigree structure about 99 of them or so will look like this where there is one daughter affected with Rett syndrome and the other children are healthy. So it happens typically once in a family and that's really what made studying this disorder challenging because you just have one case, so it's really tough to get a handle on it genetically.

9. Rare cases of families with inherited Rett syndrome (09:46)

Back in 1983 when I saw my very first patient with Rett syndrome due to a wonderful report written by clinicians from Europe in the *Annals of Neurology* there was a description of one family the one shown on the left where there was a small family with two half-sisters affected with Rett syndrome. That was our first clue that this disease could be genetic and shortly after that, in our clinics we became aware of another family where there were also two half-sisters affected with Rett syndrome. So between 1983 and 1986, these really were the two families that were identified and those were the two families that stayed with us for ten years and it was really based on these families that I was convinced this must be a genetic disorder. What convinced me is these very two small families and the fact the disease is always in girls. I felt there has to be a genetic explanation for that but of course, getting at the Rett syndrome gene with very small families like this was a big challenge and here is where the strength of families come to play. This particular family came to our attention in 1996 thanks to the mother, Maureen Woodcock who recognized that something is unusual going on in her family. If you were to look at Maureen's family she had a daughter, shown right here and her name is Erika. I want you to remember the names because you're gonna hear something in the next video that will become important. Erika had classic Rett syndrome. She had another daughter. Her name is Tiffany. Tiffany did not have Rett syndrome. Tiffany just had some learning disability but she was fine. She graduated high school and she actually married and had children herself and this daughter, her name is Paige. You're gonna hear about her in the next film. So what you're gonna hear now is Maureen's experience when she received a terrifying call from her daughter Tiffany. Let's roll the film.

10. Video: Woodcock family's story (11:49)

And I pick up the phone, and it's Tiffany and the first thing she does is, she asks me "Mom, what was Erika like at nine months?" *And I just said, "Why? What's wrong?" Because I hadn't seen Paige for a couple of months.* So she says, "Well, she's sitting up "but she's not getting into a sitting position herself "and she's not starting to crawl" "and so they're a little concerned" "and I told them that Erika had Rett syndrome" "and they don't know anything about it" meaning the doctors and the therapists. "So what do you know about it? What was Erika like?" *If you'd shot me or punched me in the stomach* I don't think it could have hurt as much because that was the worst question I think anyone could have ever asked me. *As Paige developed the familiar symptoms of hand-wringing, tremors, and seizures it became apparent to Maureen and others that Paige indeed had Rett syndrome.*

11. Four family pedigrees shed light on the pattern of inheritance (12:44)

So as you see now it immediately became apparent that Paige also had Rett syndrome. So we have now an aunt and a niece with classic Rett syndrome and Maureen went on the Net and e-mailed all the doctors and said, "In the families "have you seen anything like this?" And was immediately willing to

submit DNA for genetic study and that has been extremely helpful as you will see when I walk you through the data. And then in 1997 another family was identified where there were three affected girls with Rett syndrome. This family was in Brazil. Again, the Family Association brought them here and helped researchers study them. So when you look at these four families now, perhaps, you can refine your hypotheses a little bit and start asking what kind of genetic inheritance is occurring in these families, and perhaps I'll walk you through what we went through. Could this be a recessive disorder? And the answer is no because, really when you see here the two different fathers and knowing that the disorder's happening at the rate of about one in 10,000 the odds will be extremely low that in two families, this will happen twice. Then we think of dominant disorders. Well, this is unlikely to be autosomal dominant because we don't Here is a person who's really unaffected with Rett syndrome but she does have a child that's affected with Rett syndrome and most of these mothers seem to be asymptomatic. So that led us to think that most likely this is an X-linked dominant disorder where the mothers are probably spared because of specific reasons...

12. X-chromosome inactivation (14:25)

and the reason that these mothers spared as we will think about it in the next slide is because the number of X chromosomes matter. As you know, in the males, we have two sex chromosomes the X chromosome and the Y chromosome and the X chromosome is shown as pink and in males, every cell of the body will express all the genes from that X chromosome. That X chromosome is active in every cell of the body. In females, there are two X chromosomes and we put them as one pink and one blue to show that one comes from the mother one comes from the father. In females, only one of these X chromosomes is active in each of the cells. So half of the cells will express the genes from the X that comes from the father and half the one from the mother. Now, do you know why is it that we have that why is it that one of the X chromosomes is active in every cell and the other one is inactive? Any ideas? Well, it could be probably because they're the same chromosome so you don't need both of them turned on. Because you just have genes from one chromosome. That's close. The idea here is, males only have one X and they're gonna have so much of these genes expressing that X. We can't imagine nature wanting females to have an advantage and have twice as much, right? So think of it this way to keep things equal. OK, so in the next video, we're gonna see how does this happen. How does this X chromosome inactivation happen? When does it happen, and what is the outcome of it?

13. Animation: X-chromosome inactivation (16:00)

If you will look at the next animation you're gonna look at fertilized cells and the cells start off in a female with two X chromosomes. The cell divides and the embryo is getting slightly bigger and still each cell has both X chromosomes active but in early embryogenesis each cell will inactivate one of its Xs and one cell will remain with a paternal X as active while the other one, the maternal one and now this process will happen at random and you'll have almost half of the cells with the maternal X active, half with the paternal X. The embryo will continue to grow. Cells divide. All the descendants of that cell if it started with the maternal X active all the descendants will keep the same X active and you've seen this in calico cats, right? The coat color gene is on the X chromosome and that's precisely why these cats look like this a mosaic appearance because of the process of X chromosome inactivation.

14. X-chromosome inactivation can explain asymptomatic carriers (17:02)

So if we were to go back now to the families with Rett syndrome imagine now you have a mutation on one of the X chromosomes and that mutation is causing the Rett syndrome gene. If you happen to be fortunate where the majority of your cells are expressing the genes from the healthy X chromosome because that's the one that's active in the majority of the cells you can see how this mother can escape the disease and will look healthy, whereas in the daughters if both X chromosomes are randomly

inactivated and you have a mosaic that's about 50-50 you're gonna get Rett syndrome and actually, when we went back and looked at the Woodcock family that's exactly what happened. The aunt and the niece that have the full-blown classic Rett syndrome had this mosaic pattern, whereas Tiffany who was healthier and just had mild learning disability Tiffany has a small percentage of her cells only 5%, expressing the X chromosome with the Rett mutant gene as the active one where all the rest were expressing the healthy X chromosome.

15. How sporadic cases of Rett syndrome can arise (18:11)

So this is true. Now, we've talked about the four familial cases and you're still wondering "Well, she told us the majority of Rett syndrome "is sporadic disease" and, "Do these four families have anything to do "with the majority of the cases that are sporadic "and if those other cases are sporadic "are those caused by something environmental "or something else "or is it really also a genetic disease "and how can you have a genetic disease "if it's really sporadic in every family?" And if we were to look at this family this can happen if the mutation actually happened by chance in one of the eggs or one of the sperms of either parent. So imagine that these people are healthy but a random event happens and a mutation happens in the gene in either an egg or a sperm and then that is the one that happens to be fertilized. You will end up with a child with Rett syndrome.

16. Challenge of mapping genes from small number of samples (19:05)

So with this background we have decided that Rett syndrome is a genetic disease and my lab decided we're gonna go and clone this gene although we know we're limited. We have very small families. So when you have very small families you really have to design a sort of specific strategy to get at this gene. And what our first strategy was we're convinced this is X-linked dominant so we're gonna focus on the X chromosome and because the number of families is small we're gonna use an exclusion strategy. We're gonna eliminate regions that these girls don't share and focus on those they share. So let's roll the next animation so that you can see what we did.

17. Animation: Exclusion mapping strategy (19:44)

We rationalize that the girl will get an X from the father and then X from her mother and you know Xs recombine during meiosis so you get an assortment of portions of each of the mother's eggs. The same will be true for the second daughter. Now, we argued, since these two daughters have Rett the only place the gene could be is in the regions of their Xs that are shared the same region they inherited from the mother and the regions that are different are not likely to contain this gene. So that was our idea about how we can get at the Rett syndrome gene.

18. Discovering the Rett syndrome gene (20:20)

So we went on and began to study. The first family we have was this one family as I mentioned to you. We looked at which regions are not shared and the brown region shaded on the X was not shared. Then we studied the second family and we were able to eliminate a bit more and remember, for ten years, that's all we had. So for ten years, we almost eliminated 2/3 of the X and we were searching the rest of the X for the Rett syndrome gene by looking at candidates and sequencing them and then the Woodcock family came along and was an extreme help to us whereby we could eliminate even more of the X chromosome and finally, when the fourth family came along when we looked at all of the X chromosomes from all these affected girls the only region that they all had in common that they shared between them and their siblings was the very tip of the long arm of the X chromosome. So we decided this is where the gene must reside and of course, we looked how many genes are there and back in 1999 when we were looking there were about 200 genes and I am fortunate that there are many wonderful

postdocs who're willing to really keep with it till they find it until Ruthie Amir in our lab went through these genes and upon sequencing them she found a mutation in one of those genes which turned out to be *MECP2* and that is the Rett syndrome gene. So what is this protein? What's this gene? It's a gene that encodes a methyl CpG binding protein. It's on the tip of the X and down below is shown the protein structure. The blue and the pink are two important domains of this protein which you're gonna hear about in the next lecture and we're gonna know a little bit more about what this protein does. Of course, finding the gene enabled now diagnosis in all of these patients that were suspected to have Rett syndrome and the first thing finding the gene affirmed to us that most of these sporadic cases do indeed have a mutation in this gene and, of course, it allows then to embark on trying to understand the disease mechanism which we will hear about in the next lecture but this is one thing to remember from this story is that here's a disease that's sporadic in the majority of the cases but these very rare and precious families who sadly got struck by this disease more than once they came forward. They really helped us to discover this and hopefully you'll see the impact of this discovery in the next slide. Now we have time for questions.

19. Q&A: What happens to the males who inherit this gene? (22:59)

Yes. What happens to the males who inherit the dysfunctional genes? We're gonna hear about them in the next segment. You were very observant to notice there were some males in these pedigrees and those were much sicker and we'll hear about that in the next lecture. Yes?

20. Q&A: Why don’t symptoms appear until 6-18 months of age? (23:18)

Was there a reason why it didn't show up until, like, 6 to 18 months? Is there a reason why it doesn't show? That's a beautiful question. Here you go. That's the reason I went into research because I thought, you know, it doesn't make sense. These girls are learning how to walk how to talk, use their hands and then they're losing this ability and it's really not a degeneration. Their brain cells aren't dying. So why is that? And we're gonna address that in the next section. Yes?

21. Q&A: What causes the mutations in the specific gene? (23:51)

Do you know what causes the mutation in the specific gene, or is that not known? There are hundreds of mutations that have been discovered in this gene in different individuals and these mutations are all sorts of mutations. There are some where a "C" becomes a "T" and there are some where a deletion happens within the gene. There are all sorts of rearrangements that happen within it. So there are all different kinds, and it just it's a gene that's probably a little bit more vulnerable for mutation maybe than other regions. We don't know that, but we don't understand why.

22. Q&A: Why don’t cells inactivate the mutant gene? (24:27)

You said that the two X chromosomes they're randomly represented in different cells within the body. Is the cell not able to tell that one of the X chromosomes is mutated and could then decide not to represent that one or is there no way for That is a very good question. So the question is, why doesn't the cell recognize there's a mutation on this X chromosome? "Let's inactivate it in all the cells." That doesn't happen at the level of the inactivation but what happens if the mutation is severe enough so that it really compromises the function of the cell the cell with the healthy X chromosome early on during life will divide a lot more and most of the embryo will be made from that cell and that actually happens for many X-linked diseases. This is a superb question. I'm George from T.C. Williams. Depending on what mutation occurs in that gene are there different, like, levels of severity of Retts or is it all the same? I think that's another excellent question. I think next time we should just have one lecture and questions and answers because you guys ask all the questions for the next lecture, which is wonderful. That's

something we're gonna be talking about and that's really an important one and the answer is, yes, it does and we'll talk about that briefly. Yes?

23. Q&A: Can you remove genes that cause disease? (25:42)

Hi. I noticed in the earlier lecture you mentioned that in some of the mice you were able to just remove the *SCA1* gene. I was wondering, what are the factors that are preventing us from actually removing certain genes that cause certain diseases in humans? So, umso how but if you remove a gene in a human, you're gonna cause a so every gene has a function. Although we removed *SCA1,* we wanted to see does the loss of function of that protein cause ataxia. Although we didn't get ataxia, we got another problem. These mice could not learn. These mice had very big problem with their memory. So removing genes is not a good thing from any organism. Yes.

24. Q&A: How can you distinguish different neurological diseases? (26:27)

Before we had a genetic way to recognize Retts and know exactly what gene it was I remember that the symptoms were wringing hands and tremors and um, how does one recognize where one disease ends and where the other one starts like the difference between neurologic diseases? Yes. You're asking another very important question. There goes the camera. Basically, what you're asking well, relying on clinical phenotype is a dangerous thing because you're right. You might see a girl that has tremors and that girl actually might be an early patient with an ataxia. You know, how do you know that this tremor is from Rett rather than from an ataxia? The hand wringing, I have to admit that's very unique to Rett syndrome and that's something really very special for this disease. So once you see that you're pretty much confident this is what you're seeing. However, some of the other symptoms, no, they're not. Seizures, mental retardation, autism, tremorsthey're not and that's why many of these girls are misdiagnosed and until we have this test that's exactly why it's an advantage to have the gene. We can now move on and study these patients. OK. We have to move on to the second part.

25. What does methyl-CpG-binding protein 2 (MeCP2) do? (27:44)

So let's try to understand what does this protein do and what does methyl CpG mean. So I told you this is a protein that is called methyl CpG binding protein and you must be wondering what on earth is CpG. CpG is a cytosine followed by guanine and typically when that happens often that cytosine can be methylated and when it's methylated as you can see on this slide this protein methyl CpG binding protein can bind and now the significance of the color coding of the domains on this protein will slowly become apparent. That blue domain of the protein is the specific domain that we call the methyl CpG binding domain. It binds to these methylated cytosines in the DNA through this region and now you're wondering methylcytosine what does that do and is it in our DNA? And the answer is yes. It is in our DNA. It's almost probably, it's gonna be a way for us to think about this almost like a fifth base. It's really a base of DNA that's very important and you can appreciate why I say that if you were to look at this next slide...

26. How MeCP2 regulates gene expression (29:06)

and hear the story of what these methylated cytosines do. Methylated cytosines are present throughout our DNA. Often, we can find them at the beginning of the gene and you know that the beginning of the gene is where the regulation of the expression of this gene happens. Typically, that's where transcription factors can come and bind and regulate expression of that gene. When a gene is actively expressed as shown by this red line on this cartoon typically the DNA is open, and the chromatin is open. So the way we keep the DNA open and transcription factor can access it and come and sit and induce transcription the way that happens is by keeping histones acetylated and these little green balls are basically the acetyl

group on the spiral of the histone. So when histones are acetylated the gene is actively transcribed. Now, there are these little CpG sites at the beginning. These little pink balls on a stick are these methylated cytosine and they're there to serve a purpose because sometimes you don't want a gene to be always expressed. You want to shut it off. You want it to become quiet. How does that happen? Well, that's where this protein will come into play. Through its methyl-binding domain it will bind the cytosine, and through its pink domain which is called the transcription repression domain it will recruit additional protein. Among these proteins are two enzymes called HDAC1 and HDAC2. These are histone deacetylases. You noticed when the histone deacetylases came close to this, uh, chromatin the acetyl group moved off and now the chromatin is tied transcription factors can't come on and the gene is silenced. So essentially, this protein through its methyl-binding domain will come to a site of DNA where it wants to silence this gene and through the transcription repression domain it deacetylates histone and silences these genes. This is an important concept to get and we want you to get it so we're gonna show it all over again in this beautiful animation. Let's roll the film.

27. Animation: MeCP2 regulation of gene expression (31:21)

So here is the gene being transcribed and next to it you see acetylated histones. Nice open chromatin. And now you catch these little methyl cytosines. Methyl groups are appearing. And here comes the protein. It binds and will attract this protein complex along with the enzymes the deacetylases and now acetyl groups are gone and histones become tied. Chromatin closes. No transcription factors can come through and the gene is silent. So this is whatwhat we believe this protein is doing and more and more studies are affirming that.

28. X-chromosome inactivation causes various phenotype in girls (32:11)

So having with this knowledge let's go back now to what happens in the patients. Somebody asked me, you know, what are the phenotypes? Do the mutations matter? And we'll get to this point in this slide. When many patients with Rett syndrome had their DNA sequenced all the majority, I would say of patients with classic Rett syndrome had mutations on this gene. But then clinicians said, "OK, well, my patient "I have a patient "that has mental retardation and seizures" back to your question "but she doesn't have all the features of Rett syndrome. "Could she have Rett syndrome?" Well, it turns out some girls with just mental retardation and seizures had mutations on this gene. Some girls with just autism had mutations on this gene and some girls with very mild mental retardation had also mutations on this gene. We even know of some normal individuals without any symptoms that had carried the mutations like the mothers I told you about in these familial cases. So having understood the principle of X chromosome inactivation you should not be surprised now that you're getting this broad spectrum. You can imagine if half of your cells express the X chromosome that carries the Rett syndrome mutation on it you're gonna end up with classic Rett syndrome. But if only 8% or 10% of the cells will have that X chromosome as the active one and the majority have the healthy X chromosome you'll end up with mild mental retardation or autism. And when the vast majority of the cells carry the healthy X chromosome as the active one you even have an asymptomatic person. Of course, this asymptomatic person is at risk of having a child affected with Rett syndrome so we cannot forget about them.

29. Different MECP2 mutation causes various phenotypes in boys (33:51)

Now back to the families. One other thing that came to be very important from studying these families and paying careful attention to them is you'll now notice we're gonna shift our attention to the boys in these families. In three of these families there are boys that have a line drawn through the square and that tells you these boys have died and actually these boys have died very early on in life. One of them died at six months, one of them at one year and one of them just before his second birthday. And the reason these boys died is because they were very ill. They were born, and they had very low tone in their

body. They couldn't really smile or sit or walk like normal boys would do and some of them required respirator assistance to even breathe normally. These were very sick boys. Nobody thought they had Rett syndrome because this doesn't look anything like that. There is no period of normal development early on here. They're very, very severe. But when we look at their DNAs because their sisters had Rett syndrome it turned out that these boys did indeed have mutation in the same gene so that will tell you if a mutation is severe enough to cause classic Rett syndrome in a girl it's causing the severe phenotype in boys and this is a summary of what we've learned from the boys. Remember the boy has a single X chromosome so if a boy carries a mutation that inactivates the protein like the one shown on the second line where a big domain of this protein is gone this boy will be very sick and this boy will die in the very first year of life. He doesn't have another X chromosome that's active in some of his cells. If this boy has a mutation that truncates the protein but keeps at least the two key domains intact there are a bunch of boys like this and these boys have mental retardation seizures, tremors, and balance problems so they will live and those boys actually some of them are 14 years old and they are in families that have multiple-affected boys so we call this X-linked mental retardation. And even if there are some boys that have a very mild mutation one that just simply changes one amino acid, keeps everything in the protein present but the subtle change in the amino acid is enough to affect the binding of this protein maybe the protein binds but not as well not to all the regions of the DNA. These boys have presented with yet another class of disorders. Bipolar disease. There is a family with multiple boys affected with bipolar disease as well as tremors and mental retardation. So what you see here is again having now this gene in hand and looking at the spectrum of phenotypes you can see with this gene you can see a broad spectrum in girls mostly because of X-chromosomal activation and a broad spectrum in boys mostly because of the type of mutation. So that has instructed a lot of clinicians and this now tells us that the prevalence of the mutations in this gene is probably much higher than the estimated prevalence of Rett syndrome which is one in 10,000. And this is really important for you to remember because back to the same idea whether it's in cancer or neurologic diseases you started with a very rare disease but this rare disease can teach you about a broader class of diseases that affect many, many people in the population.

30. MECP2 gene is expressed during neuronal maturation (37:13)

Now let's go through another question that was asked by the audience. These girls start off normally. They learn how to do things and they go on for a few months maybe a year, maybe a year and a half and then they have problems. Why is that? Why does it take 18 months to get symptoms of this disease? Well, we thought, now that we know what the protein is maybe one way we can begin to understand that is see where is this protein in the brain and when does it come on in the brain and is that important? Does that parallel human brain development? We know that human brain develop continues after birth. We always knew that it continues up to six years of age four to six years of age so we wanted to see is there a relationship between when this protein is on in the cells and when brain development is taking place and we started very early on. We wanted to look the earliest time when we can detect this protein and neurons and the earliest time point we look at when the first brain begins to develop is ten weeks human fetal gestation and at that time point if you were to look at the human brain what you really see is a tube that's your spinal cord and a little bit of the brainstem that's just above the spinal cord. That's the place it first develops because imagine as a fetus and an embryo putting the breathing machinery that's the most important thing to do and the spinal cord is critical early on in development. And the next portion of the brain to develop is the very superficial portion of the brain where there are the earliest neurons. They're called the pioneer neurons. These are the sites where we're finding this protein. These red dots is where we first detect this protein. We find it in these very early maturing neurons. Now, if we're to look at the second time point in development that's 19 weeks now we see it in slightly more neurons. We're beginning to see it in the developing cerebellum we're beginning to see it in parts of the brain that control the smoothness of movement and we're starting to see it in the deep part of the cortex where the second class of neurons to mature is the deep layer of the cerebral cortex. And if we're to look at 26 weeks even more neurons now are maturing so more neurons are positive for this protein. At 35

weeks of age, even more neurons are positive. We see now almost, uh, high density of neurons in the cerebral cortex and in the cerebellum. What this is telling us the minute a neuron matures, this protein comes on so there's a relationship between neuronal maturation during development and when this protein is detected within the neurons. The big surprise came is as we went postnatally and kept looking at brain sections we found that the number of neurons that become positive for this protein continues to increase up to ten years of age. This was a very, really, interesting discovery because for one, it told us that there are neurons that are changing during childhood all the way up to ten years of age. That tells you, really neuronal maturation isn't done by four or five or six years but it also told us that this protein continues to increase in its levels which means there is a relationship between maturation and when this protein is needed all the way through that process but at the same time, it sort of provided us with an insight to why is it that we take a long time to see the phenotype because really early on, it is not very essential because many of the neurons don't have it yet and it takes a while to quite a few neurons are dependent on it and that's most likely why it takes such a long time to see the phenotypic features.

31. Designing a mouse model for Rett syndrome (41:05)

So now that we know where this protein is expressed and we know every way you mutate it you might get a neurological phenotype a developmental neurological phenotype we want to understand why. Why is it when you lose part or all of the activity of this protein you get this disease? And how do you study the pathogenesis of Rett syndrome? I'm sure that by now you appreciate that mouse is a valuable model in our laboratory. They're quite close to us in many ways and they have a nervous system that we can study and understand in a way that we study our own. So in our lab we decided to make a mouse model for this disease but having seen the big spectrum of phenotypes that we see with this disease choosing the mutation was critical because we wanted a mutation that causes classic Rett syndrome in females but at the same time we wanted one that will give us viable males. We don't want it to be severe enough to kill all the males where we're forced to study them in the female and then be facing the issue of X chromosome inactivation and the variability in the female mice from X inactivation. So what we picked is a truncated mutation. We engineered a mutation that preserved the two domains of the protein but it happens right at the amino acid where we know that many female patients have had deletions in that region and beyond that have classic Rett and we hope that by preserving the two domains the male mice will be viable. And this is what happened. We made these mice and we looked at the male mice and as you can see from this chart that many of the features we see in the human patient were present in these mice. These mice looked normal the first four to five weeks of life. We couldn't tell anything was wrong with them which we would predict based on where we see this protein as expressed when and where. Then they develop tremors. They become spastic. They become hyperactive. They get seizures. They get ataxia. We even were quite surprised to see that they get the same this classic hand movement that we see in the humans is replicated in these mice with their forepaws and you're gonna see that in a minute. We also started probing the social behavior. We knew autistic features are present in Rett girls. We wanted to seedo these mice interact socially well and the answer is no. They have social behavior abnormalities and they had abnormal curved spine like we see in the girls.

32. Video: Rett mouse phenotypes (43:42)

So in the next videotapes, you're gonna see two videos. The first one we're gonna show you that these mice do have problems with their balance and the second video, we'll look at the paw movements. Let's look at the balance video. So I'm sure you can appreciate this mouse is also having difficulty and this is typical of the mouse that fell of most of the Rett mice. Now, the next animation you're gonna first see a healthy mouse and see how it keeps its paws separated normally. Let's roll the film. So this mouse is a healthy mouse and you can tell it's really trying to get out of the hand of the handler. It's just curving up, trying to get out. And this is the Rett model mouse. You can see that it's constantly wringing its forepaws and keeping them together. It's not trying to actually fight the handler. It's just very often

wringing its paws and every mouse does this. We pretty much can tell when we have a bunch of mice born. We just pick them up at about five to six weeks and we can see all those that have the mutant gene will wring their forepaws.

33. Rett mouse revealed anxiety as a Rett syndrome phenotype (44:55)

Now, in addition to these studying the mouse model taught us about another phenotype that really we did not maybe catch as well in the patients and that's, um, an anxiety phenotype that we've seen in these animals. So you might wonder "How can you tell if a mouse is anxious? "Do you interview them "and you see that you're making them nervous? "How do you do it?" Well, there are some tasks that you can do and this is one of there's several tasks we can do. I'm just gonna show you one of them. This is one such task. It's called the open field test and what you do you put the mouse in a big open space and the mouse doesn't like this. It doesn't like being in the limelight. It doesn't like all the attention. So what happens when you do this the first ten minutes the mouse will go to the periphery of the space and will just hang around the walls trying to hide away. Doesn't want to see anybody. Very worried about what might happen if the mouse is noticed. But after, uh, ten minutes it starts saying "There is no danger in this room. "This is not a hostile environment" so it starts venturing into the middle of the open space and it feels comfortable. Nothing is alarming so its anxiety will go down and now will spend a lot more time in the center of this open space and this is what happens with a healthy mouse. The Rett mouse, unfortunately doesn't reach that level of calm and will stay at the periphery because it's anxious and we can do additional tests and we can confirm that what we're observing here Is actually due to anxiety and nothing else so this is something we learned from studying the mouse model. Then we went back to the parents and said "Do these girls show any anxiety?" And it turns out that the answer is yes that if you walk in a room you do tend to make them more anxious or if they meet somebody new, that indeed happens.

34. Using DNA microarray to find genes regulated by MeCP2 (46:49)

The other thing we can do now that we have a mouse model is we know that this is what we think this protein is doing. It's binding these methylated cytosines Keep the chromatin tight Genes silenced when they're supposed to be silenced. So perhaps when this protein is mutated some genes cannot be silenced and the answer is which are these genes and how can we find them? By having a mouse model that you can study from the very early onset of a disease rather than very late you can answer these questions and that's what we're beginning to do. So how do you do that? How you can find which genes may be regulated by, uh this protein? The way you do it you can isolate RNA from the Rett mouse because when genes are transcribed they make the RNA and you can collect that RNA and make cDNA from it and you can label that cDNA with a red dot and then you can do the same for a healthy mouse but now you label it with a different dye a green dye and you can put both of these probes on a slide or a chip that has multiple genes throughout, uh, the genome. They're expressed from everywhere. Typically we look at the brain in this case and you want to see which genes have similar levels and which ones have different levels. If the amount of RNA made in the Rett mouse is equal to the amount of RNA made in the healthy mouse green and red together when they're at equal intensity they're gonna give you a yellow so you're gonna see a similar amount of these genes being transcribed. But if there is a gene that's more abundant in the Rett mouse that's not being silenced effectively in the Rett mouse What you're gonna see that the level of the RNA is higher and that's gonna look like Rett. Of course, as the disease progresses some genes will be misregulated as downstream effects. You'll also sometimes find some green ones because some genes are lower in the mutants than the wild type.

35. Finding other genes may explain mechanisms of other diseases (48:48)

So having that in hand, then we can now begin to ask in different brain regions even at the single-neuron level which genes are misregulated in the Rett mouse and why is it important to get at these genes? It is important because you know now the whole phenotypic spectrum of Rett syndrome so if you get a handle at some of these genes you're gonna gain insight about autism, seizures mental retardation and many of the features that we see in Rett syndrome. So I'm sure you can appreciate from having followed this story not only the strength of the families but the strength of the discovery which will allow us to do it in the future. We've gone from studying a very rare disease to now learning about a broad spectrum of human developmental disorders that we will hope to continue to learn from studying the genes that are regulated by this protein. From a practical point of view having discovered that anxiety is a phenotype of this syndrome when this protein loses its function and having a model that displays this anxiety we can now treat these animals With anxiety-reducing drug and seedoes that help? Would that decrease their tremor? Would that decrease their forepaw wringing? And could it maybe enhance their function? We hope to do that in the mice, and if that really works we hope to carry that, then to the patient in clinical trials. So this is the story of Rett syndrome and you've heard earlier the story of SCA1 and in closing I'd just first like to thank all the families and the patients that have helped us along the way. Their effort has been amazing and I'm sure you can appreciate that from having heard both stories and, uh, they continue to help us anytime we really have questions and we hope to bring something back to them. And finally, nothing in the lab happens by one person. It takes a whole group of people to do that and I'm fortunate that throughout the years we've always had outstanding students and fellows and technicians who've helped along the way and I'm very grateful to them. Thank you. We can now take questions.

36. Q&A: Why is MeCP2 not needed until neurons are fully mature? (51:06)

You explained that the the protein is used to silence certain genes which are being expressed. Why is it that this protein is not needed until neurons are fully matured? Right. So we think there are many other proteins some of them from the same family that contain this methyl CpG-binding domain so there probably are other proteins that may be needed earlier but what we think is when a neuron matures it's now responding to activity, you know? This neuron's mature now. It can perform its function and once an activity happens this neuron has to turn on gene expression to accommodate that activity whether it's learning or whatever task the organism is doing and when that is done, now it has to that particular gene that's responsible for that has to be quieted and we think this protein is important for that. When the organism is now doing a lot of activity that may require fine-tuning this is when this protein comes into play and that'syou can imagine a child first they sit around, they're watching and they're learning few things but somewhere between six to eighteen months of age you can see how much this child has to learn more now and do so many simultaneous activity and maybe this fine-tuning is important and it's done by this protein. Yes?

37. Q&A: Does sporadic Rett worsen in each generation? (52:32)

If someone with a sporadic case of Rett syndrome just has a very mild case caused by, say, a slight mutation if they then have children will their children tend to have a greater, uh will they exhibit more symptoms, will they have more mutations or will they just continue to be very mild? Excellent question. So if an individual had a sporadic mutation and they presented, let's say with mild learning disability or mild mental retardation that female, if she was to get pregnant with every pregnancy, she has a 50-50 chance passing on the Rett syndrome mutation, right? Because one of her X chromosomes might do that. Now, once that gene is passed on, that child we cannot predict what their phenotype might be. It could be as great as being totally asymptomatic and that child was fortunate to inactivate or to have the cells with the healthy X active survive but that child could have the random process and end up with classic Rett so we cannot usually predict what the phenotype is gonna be. It's rare to be worse because to be worse that means the cells with the mutant allele have to have the selective advantage to survive

38. Q&A: How far away are you from improving pateints' condition? (53:53)

Hi. I'm Jamie from St. Albans. So you found the gene and the mechanisms responsible for Rett syndrome and you've linked that to other neurodevelopmental disorders. How off do youhow far off do you believe you are from applying this to improving the condition in humans? Wonderful question and it's the one that keeps me up at night sometimes. So as you can appreciate, this is a very complex disease and that's exactly my point about science as a humbling experience because you think you've found a gene you have a protein you're gonna go after doing something about it you're gonna make the disease better but then you're humbled because you discover this is probably gonna regulate many, many proteins and many, many gene functions so it's probably taking many changes throughout the nervous system that's giving you this phenotype and what we decided we're gonna do is really try to dissect these as much as possible reduce them all to some unique problem whether it's in the cerebellum, whether it's in the brainstem whether it's in the cortex and try to find out what are the targets in these specialized cells and work our way that way and we might discover it's gonna take a cocktail of treatments. It might take an anxiety-reducing drug a seizure-reducing drug a behavioral intervention. We don't know yet. So I'm optimistic that hopefully within my life span we can do something for these girls but I cannot tell you exactly when.

39. Q&A: How is Rett syndrome turning some genes off? (55:22)

You mentioned the green genes on the slide representing the Rett genotype and the regular genotype. Would that, umthe showing up of the green genes have to do with the MeCP2 maybe turning off genes that are expected to perform certain functions? Right. So normally we think of MeCP2 as a repressor as a silencer of genes so we would expect maybe its direct targets to be up. So those will show up at red but imagine if you have a gene that's not turned off that's always on and if that one sometimes might repress or do something to another gene the downstream effects could give you these green genes. We can't throw out the possibility that it might normally activate some genes but we think those green downstream are downstream effects. OK. Thank you very much and the last two are for grabs.

40. Closing remarks by HHMI President Dr. Thomas Cech (56:28)

I'd like to thank everyone for this great Holiday Lecture series. The production team the students in the audience here for their terrific questions and especially our two speakers Dr. Bert Vogelstein and Dr. Huda Zoghbi. Clearly it takes a lot of patience to do this sort of work. Before we close I'd like to announce our topic for next year the 2004 Holiday Lectures on Science. From worms to humans feeding is an important behavior in animals maybe especially during the holiday season. In our next series HHMI investigators Cori Bargmann from San Francisco and Jeff Friedman from New York will explore the behavior, physiology and genetics of feeding, weight control, and obesity. We hope to see you then. And now from all of us here at the Howard Hughes Medical Institute have a happy holiday season and a healthy new year.