

Learning From Patients: The Science of Medicine (2003)
Lecture Three—A Healthy Nervous System: A Delicate Balance
Huda Y. Zoghbi, M.D.

1. Start of Lecture 3 (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 third lecture is titled "A Healthy Nervous System: A Delicate Balance." And now, to introduce our program the president of the Howard Hughes Medical Institute Dr. Thomas Cech.

2. Introduction by HHMI President Dr. Thomas Cech (01:00)

Good morning, and welcome to the second day of our 2003 Holiday Lectures on Science. Today Dr. Huda Zoghbi is going to talk about two diseases that for a long time were tragic mysteries. One of them is called spinocerebellar ataxia and the other is Rett syndrome. The good news is that through the research done both in Dr. Zoghbi's laboratory and in other labs these mysteries have been largely solved and by understanding them we're able to really make an impact on the treatment of these neurological disorders. How do you go about solving these sorts of mysteries? Well, clearly, it takes careful observation and listening to patients. In the case of the spinocerebellar ataxia SCA for short the work was helped by the knowledge about a small rural town that was having trouble understanding why so many of their citizens were being devastated by this health problem. It was really afflicting the entire community. Dr. Zoghbi speaks of her first visit to the town where many older homes have large porches and on those porches, there were many wheelchairs. And in talking to the citizens she found that the people inhabiting those wheelchairs seemed to be younger and younger every year. So these clues led eventually to the identification of the gene SCA that is involved in the progression of this disease. Today's talk is entitled "A Healthy Nervous System: A Delicate Balance." Let's start out with a video about Dr. Zoghbi.

3. Introductory interview with Dr. Huda Zoghbi (02:51)

I actually got interested in science later in my medical career than most in the sense that I initially thought "I will finish medical school "and do residency in pediatrics "and actually become a pediatric cardiologist." But during my pediatric residency I got exposed to neurology and I found it a fascinating field where you think a lot about the anatomy of the region and you try to reconstruct the pathway that causes the problem. So a lot of patients with devastating genetic disorders and particularly the young ones such as those with Rett syndrome that truly made a big impression on me. I was inspired by the patients and made the decision to get into scientific training based on these exposures. Most of the diseases that sort of affected my decision were diseases where someone starts off being healthy and then either a degenerative process happens or, as in the case of Rett this later developmental process is interrupted. So Rett is one of the diseases we study in the lab. The other class of diseases we study in the lab are a class of neurodegenerative diseases collectively called the spinocerebellar ataxia. And what ataxia is is loss of balance and coordination. So in my lab, what we do is basically mostly use genetic and molecular biology and some biochemistry tools to try to understand some devastating brain diseases. And our goal is to really understand the mechanism how these diseases affect the brain function and why are these patients suffering from all these problems. And our hope, if we understand these mechanisms maybe we can modulate them and offer something to the patients. What I would like my lecture to accomplish is to show the students how nowadays we have fantastic tools at our hands

where we can really make a big difference for science and medicine. Now we have a genome that's sequenced. We know so much more about the brain. And I want to let them know that science is really fun. You wake up in the morning and you come to work it's very exciting. You're never bored because you never know what the new day is gonna bring. And that's really wonderful, and especially if one day you can take that back to a patient or to the clinic. So I hope that they will see that and they will seize the opportunity.

4. The importance of balance and coordination in everyday life (05:27)

Good morning, and thank you for being here. Today's lecture is just like yesterday. We'll have two components to each lecture and there will be a period for questions. I would very much like to hear from you. Your questions yesterday were fantastic and sometimes they might even inspire a research idea. So please think about questions after each module. As you've heard I will be telling you about two different disorders one that affects balance and one that's a developmental childhood disease called Rett syndrome. And I thought perhaps before we talk about the disease that affects balance is to get you to think a little bit how important are balance and coordination in our everyday life. Maybe we can first think of the most beautiful way we can admire and think about balance and coordination watching Michael Jordan get that basket into the net with a perfect shot. When he spins around and throws it he just makes it right where it's supposed to be. It takes balance exquisite balance and coordination to do that. Michelle Kwan skating she does a beautiful landing. She has to have a very healthy nervous system and perfect balance to do that. And even those of us who are not talented to do something so beautiful but we do our usual thing in everyday life we do need balance. As you hear me speak, my speech is smooth. It has a beautiful rhythm that you can understand it. That's because of balance and coordination. There are nerves that coordinate the speed of my speech and its rhythm. As I walk on this stage I'm not tripping all over the place. I don't have to look at my feet because my brain knows exactly where I should put my feet to go in whatever direction I like to do. The same thing if I was to pick a cup of water. I bring it straight to my mouth. I don't hit my face with it. I know exactly where to get it because of balance. You're taking notes. When you put your dots on the "i's" this little, simple task it takes a lot of balance and coordination to do.

5. Cerebellum is important for coordination and balance (07:30)

So that's really what balance is important for. And you might wonder well, how does this all happen? What does it take to be balanced? It takes a lot of neurons talking to each other in the nervous system. But within the nervous system there's a part of the brain and that's the smaller part the green, nice part that we call the little brain also known as the cerebellum. That's where the center of coordination of movement and balance takes place. And basically, the information comes to the cerebellum from the periphery. If I pick a cup of water the nerves in my hand will send the information through my spinal cord into the cerebellum and then the neurons within the cerebellum will talk to each other will send the information back to the cortex where the neurons there motor neurons will send the information back through the spinal cord to the muscles and tell my hand how much force to put on that cup. So it's a very exquisite system, really for us to perform these tasks. And we do so many of them all at the same time so effortless, without much effort and that's because of balance.

6. Cell types found in cerebellum, particularly the Purkinje cell (08:40)

So let's look at the cerebellum now and see who are these neurons the major players that coordinate the movement. If we were to take a section through the cerebellum and look at these neurons you will see that there are many types of them. But the little blue cells in the cerebellum are the first ones to receive the information from the periphery and these are called granule cells and there are many, many of them in the cerebellum. There are 70 billion granule cells in the cerebellum. This is in fact the largest

population of neurons in our central nervous system. These neurons send their axons to another neuron that's called the Purkinje cell. The Purkinje cell is sort of the biggest powerhouse within the cerebellum. It's really a very important cell. First, you'll notice it has many elaborate dendrites and these dendrites make tens of thousands of contacts with other neurons with the granule cells and other neurons. And this Purkinje cell is the busiest neuron in our nervous system. It fires at fifty times a second. So that tells you that there's always activity going on in the cerebellum to modulate everything you do. What the Purkinje cell does is send the information, via another neuron back to the cerebral cortex so that your movement is nicely coordinated. So what how does the Purkinje cell know what to do? This is the beautiful Purkinje cell again. You can see its elaborate dendrites. How does it know what to do? How does it know what to tell its neighboring cells and how much to coordinate a movement? It does that through, of course the proteins within that cell that instruct it what to do that instruct all this activity that keeps it healthy and alive since it's spending so much energy. And the information in these for these proteins is encoded within the DNA and its nucleus. So it comes down to the DNA. So it's not surprising, then that genetic diseases of the brain where the DNA may have an alteration will have such a devastating effect on such a cell. And this was my introduction to this class of diseases My introduction was through seeing patients that have had some changes in their DNA that really caused them to have big problems with balance and coordination.

7. The story of an extended family with ataxia (11:01)

So in the next videotape I'm gonna share with you the story of this family from Montgomery, Texas which is about fifty miles north of Houston where I have encountered some of the patients at our hospital in Houston and then went back and visited this family. And the story started with a young child who was having trouble in school. And if we can have the first video we'll see how this went. This young boy was falling at the age of six years of age had difficulty holding a pen and doing any of his classwork. And it turned out that his father at the age of 30 had problems with his balance and coordination. and his two brothers at 9 and 11 had problems with balance and coordination and then became wheelchair-bound. As we dug deeper into the history of this family we found there were actually hundreds of individuals affected with this disease. And as you've heard from Dr. Cech unfortunately, they did not know many of them in the older generation they did not know they had genetic disease. They knew they couldn't walk. They knew they needed a wheelchair and they thought maybe they had rickets. That was the idea. So they were quite happy to see someone go by and visit them in their own town and begin to evaluate them clinically and pursue the cause of their disease.

8. Video: A patient with spinocerebellar ataxia type 1 (SCA1) (12:25)

And I thought also, perhaps it would be helpful for you to see what does an adult patient affected with this disease look like. And one of my patients, Milan, who is from Louisiana he's from another large family just like this family. He is from Louisiana. He has spinocerebellar ataxia. And Milan and his wife were very kind to allow us to film them so that hopefully you can learn something about this disease and one day join the research effort on it. When you watch the video of Milan I want you to pay attention to some of the things we've talked about. We talked about balance important for speech. Listen to his speech. We talked about balance important for fine movements. Watch how he's missing the target. He doesn't quite have that fine movement. And we talked about it affecting the way to walk and Milan is now in a wheelchair. He is midcourse in his disease. So he hasn't really, you know advanced as much as some patients where they may become bedridden. So watch for these things as you watch the video of Milan. Let's have the video. -Good morning, Mr. Cloud. -Hi. -Good to see you. -Thank you. Thank you very much for coming. -We appreciate it. -You're welcome. -How are you feeling today? -All right. -Doing all right? -Yeah. So I was gonna ask you to perhaps touch my finger OK. and then touch your nose the tip of your nose, right. And then again back to my finger and the tip of your nose. OK. Let's do it with the left hand see how similar or different they are. OK. The tip of your nose. You ready? Take

your time. OK. Just stabilize yourself. Excellent. You're doing, actually So Milan and many members of his family have this disease, spinocerebellar ataxia type 1.

9. Cellular, clinical, and genetic facts about SCA1 (14:43)

And what happens in this disease The first thing that happens is that those Purkinje cells that we were talking about earlier that are really important in sending this information about coordination unfortunately degenerate. And as you've heard by now the disease is called spinocerebellar ataxia type 1 and we're gonna call it SCA1 for short throughout the course of this disease. By the way, it's called spinocerebellar ataxia because neurons in the spinal cord degenerate in the cerebellum degenerate and the final picture is this balance problem, or ataxia. And it's type 1 because it was the first clinical entity to have its gene mapped, so therefore it's called type 1. So what happens in this disease those Purkinje cells degenerate and then, eventually, additional neurons degenerate and the patients have multiple problems. As we trace this family history this is the first family we followed the one in Montgomery, Texas we've learned that multiple individuals are affected. And as you can see by the fact there are affected individuals in every generation and there is transmission from males to males and females to males all sorts of transmissions it's clear that this is a dominant inheritance pattern. This disease is progressive. After the patients go through this problem with their balance and coordination other problems emerge. Breathing is also relying on balance and coordination so their breathing becomes disordered. Swallowing you need balance to really swallow smoothly to coordinate the movement of your food through your esophagus. That also fails. So eventually these patients will have a problem with choking on their food. They have a problem with breathing. They get secondary infections, and unfortunately often that's the cause of their death.

10. Anticipation: Later generations have earlier onset of disease (16:36)

And as we traced this family history and learned that many of these patients are affected something interesting was observed and that's the importance of clinical observation. That's where I really learned something very important from my patients. I was able to examine individuals in the last four generations. The ones in the earlier generation, of course have passed away before I made contact with this family but I was able to talk to the grandchildren and great-grandchildren. And I asked about these older individuals and I was told that Grandma and Great-grandpa were perfectly healthy till they were about 70 years of age. And really they lived with their disease for about twenty years and some of them died in their 80s and some into their 90s. So that's a pretty good that's a really good lifespan. But what I've learned is those individuals in the following generation had disease when they were in their 50s and those individuals lived till they're about 65 or 70Ñ still not too bad. It was really mostly my patient group that I began examining that I noticed that their onset was in their 40s and those individuals were wheelchair-bound by their 50s and quite ill somewhere in their late 50s. And then the following generation individuals were affected in their 30s and their disease was devastating by the time they were 40 or 45. And the child that presented to us in the hospital that young boy I told you his story at the beginning he actually had onset when he was 6 years of age and one of his siblings was a teenager. So now the onset is childhood and teen. And finally, our youngest patient she had the disease onset at 4 years of age and she passed away when she was 10 years old. So you might wonder and we've talked a little bit about this yesterday about what really might explain this and this was a very important clinical observation and we needed to understand it. Well, it turned out that clinicians in the past have described this in the medical literature. They described the phenomenon not necessarily for this disease but for another neurologic disease called myotonic dystrophy that when a disease occurs earlier in a family we call this anticipation. And the idea was, you're calling it anticipation because now you know there is an inherited disease in the family and you are thinking their child is at risk of being affected. Therefore, you look for symptoms of the disease. You recognize all the symptoms of the disease so you diagnose it earlier and earlier. Well, that may have been true if the disease was diagnosed earlier but the patients had

the same disease course. But in this case, that's not the case. As you've learned Grandpa lived into their 70s or 80s but the great-grandchildren really died by 10 years of age. So you can't really anticipate early death. That's something that has to be biologically explained.

11. CAG trinucleotide repeat expansion causes SCA1 (19:40)

So what might explain anticipation? And that was the thing that was intriguing us and that's where the patients really got us to think of a biological phenomenon that will explain that. So the only thing that we figured can explain this was a mutation that's changing over generations. The disease-causing mutation must be changing. It must be a dynamic mutation that gets worse as the disease is passed and then these patients will have a worse disease course. So what do we mean exactly by a dynamic mutation? We mean that a mutation where the DNA changes as it's passed from one individual to the other. And this is exactly what happens in this disease. This is a disease that's caused by the expansion of a trinucleotide repeat. The trinucleotides are C, A, and G and those are illustrated as a little pink block here. And in most of us in this room these repeats range from about 6 to 35. And in this case the normal is shown as 10. And we're looking here at a strand of DNA and the loop is just to show you a block of 10 repeats. So this is what you see in a normal, unaffected individual. But in the patients what you're seeing is a repeat of 40, 50, 60 and 70. Now, you might wonder why does this happen? What can cause a repeat that in many of us in this room is ranging somewhere between 10 and 30 to go to 40 or to 50 or to 60? Well, it turns out that after a certain threshold of these repeats when the repeats are about 35 or so they get a little bit bigger. During the process of DNA replication as the DNA is unwind the double helix is open, the two strands are separated the DNA polymerase is trying to replicate that DNA the DNA polymerase relies on some unique sequences which here are shown in blue to anchor itself and to know precisely where it might be. And sometimes when the repeats get slightly long it might get lost in the process and will lose where its placed because all the sequences are the same C-A-G, C-A-G, C-A-G and will lose its position. And now you might miss a site and get a little bit extra of DNA and on the second cycle that DNA will get bigger. And I think the following animation that you're gonna see now is a cartoon that shows you how this might happen. Let's roll the film.

12. Animations: Trinucleotide repeat expansion (22:07)

So imagine this yellow portion is the portion where the trinucleotide repeat is. And as you know helicases open the DNA, and DNA polymerases copy it. So we're gonna watch the repeat now get through the helicase the single strand as it's shown. The DNA polymerase slips on that repeat and a hairpin is formed. And as this now will go through you'll watch as it'll go through the second cycle next round of replication. This longer repeat will now get replicated and incorporated in our DNA. And you're gonna end up with an expansion. And here you've seen a small expansion but imagine this going on again and again and again. So with time, this will continue to get bigger and that's why the disease will keep getting worse.

13. Number of CAG repeats influences the age of disease onset (23:15)

So having now discovered that this is really the basis of the worsening of the disease you can go back and visit all the patients and ask about exactly when did they notice their disease onset and see what the size of the repeat in each particular patient is and see if that's really true if it's the repeat expansion that's explaining the phenomena of anticipation and disease worsening. And what we find is exactly that. The longer the repeat the earlier is the age of onset. And those shorter repeats in the range of 40 or so you can see we have some individuals that had their disease in the 60s.

14. CAG repeat produces polyglutamine, causing many diseases (23:55)

So SCA1, then is caused by the expansion of this trinucleotide repeat CAG. What does the CAG repeat do? It turns out that the CAG which, as I mentioned, in you and I is somewhere between 6 and 38, and in SCA1 goes to 39 to 82 resides within a gene and it actually resides within the part of the gene that makes a protein. So what the CAG repeat does it encodes for the amino acid glutamine. And when you have multiple glutamines such as 70 or 80 glutamines we call that a polyglutamine tract. So this disease got named polyglutamine disorder for short just because this is the mechanism at least at the protein level that we know of at this point that causes this disease. And the protein within which this repeat resides is a novel protein. We didn't know much about it so for lack of a better name we'll call it ataxin-1 again, because it's the first one involved in ataxia. But what's interesting and what I would like you to remember is that this phenomena is not unique to SCA1. It turns out that there are now many more polyglutamine diseases. There are actually, to date 9 polyglutamine diseases. And I'm sure some of you heard of Huntington disease. This is probably one of the most famous and more common polyglutamine diseases. And there are many other spinocerebellar ataxias and some other neurodegenerative diseases. What these diseases share in common is that all of them are caused by an expansion of this unstable triplet repeat trinucleotide repeat CAG that in each case this repeat encodes for the amino acid glutamine. Although the genes are different the phenotype eventually is neurodegeneration. And in each disease a different group of neurons is affected and those are shown in yellow and the ones that are affected later on in the course of disease are shown in blue. You'll see that there are many SCAs on this cartoon and that's because many spinocerebellar ataxia imbalance disorders are caused by this disease mechanism. And in fact, in all of these Purkinje cells that you just learned about are the first one to go first ones to go.

15. Benefits of identifying disease-causing genes (26:14)

So what are the implications of discovering the SCA1 gene or any of these genes? What I want to tell you is if you were to encounter ten different patients with balance disorder to you and even to us neurologists they will look very similar. It's very hard to tell them apart. And some of them may have deafness. Some of them may have blindness. Some of them may have peripheral nerve problems. So it's very hard to tell them apart. And in the past these patients had to go through a lot of tests to see if we can tell them apart. They had to have CAT scans MRI scans lots of blood tests and sometimes a nerve biopsy or a muscle biopsy to see if their nerves or muscles are affected. So they went through a lot and sometimes you couldn't even come up with a diagnosis until these genes were identified. Now you actually can do a single test a blood test, a DNA test and you can tell if a person has SCA1 or SCA2 or 3. And what was surprising as this genetic data came along we now know of almost 22 genetically distinct spinocerebellar ataxias. The genes have been identified for some but not all but the others, the genes have been mapped. So this has been a quite important way to distinguish these disorders. The other thing that's very valuable is obviously the families now can know who is affected and can have the choice of being tested. In this scenario You have an affected woman who has a daughter who is in her 30s. And she would like to have a baby but she doesn't know if she's affected. She's at risk. By at least having the option of genetic counseling and testing she might choose to adopt a child or she might choose to have fetal testing. Families have this option at this point in time. And lastly and very importantly is once we know what the disease gene is and what is the mutation what alteration we have we can begin to ask why does the neuron degenerate? And here the interesting question is why is it when you have 35 or even 38 glutamines the Purkinje cell is healthy but when you have 39 or 40 or 45 glutamines that Purkinje cell is sick and dies? And that's something we hope to address in the next section.

16. Q&A: How does the change in protein affect the cell? (28:37)

Now we have time for questions so I would like to hear from you. Yes. I'm from Montgomery Blair High School. How does then the change in the protein, actually, like physically affect what's going on inside the cell? That's a very good question and that's gonna be the topic of the second lecture. That's such an important question you get a T-shirt for that. And we'll address that in the next component.

17. Q&A: Does the disease affect other involuntary functions? (29:06)

Yes. When you were talking about the disease the SCA1Ñ and you said, like, it disrupts the breathing which is often, like, the cause of death since breathing is usually, like, involuntary does death ever result from, like, irregular heartbeat since that's also involuntary? That's a very good question. So the involuntary control of breathing is controlled by certain neurons that are the nuclei for cranial nerves like the vagus nerve and other nerves. And what happens in this disease eventually as the disease progresses, many of these neurons degenerate. So at the very end you might have irregularity in heartbeat because at the very end really many neurons die and communication between neurons isn't healthy anymore. But earlier on, the choking comes because as you swallow there is a certain rhythm to the movement of the muscles and that is perturbed due to the loss of neurons in the cerebellum. I like this question, too.

18. Q&A: What are the differences among the 22 types of SCAs? (30:07)

You said you've identified 22 different kinds. What's the difference between, like, each kind? So clinically, they're very hard to differentiate. They really all look very similar. As I mentioned, some of them have loss of retinal cells. So, like, SCA7 there's loss of neurons in the retina and these patients are blind. Some of them may have problems with rigidity. So the part of the brain the basal ganglia that control the smoothness of a movement is affected. So there are subtle differences among some of them but other than that, they're all degeneration of Purkinje cells. *They're about the same things?* Yeah. And it's different proteins. And we're gonna talk a little bit about why is it that they look similar perhaps in the next lecture. But you can imagine this Purkinje cell. It's a very active cell. It's very vulnerable. So you can see, since it depends on the integrity of the DNA maybe any subtle change in any gene although the genes are different is gonna be detrimental to the cell. Thank you. Yes.

19. Q&A: How do the nerve cells express specific genes? (31:10)

I'm Mary Abbey from Edison. My question was that I have a hard time visualizing what nerve cells are composed of and how they really function. Like, how do they know what part of DNA to take advantage of? Well, that's a really good question. Because the DNA is in every cell, right? And what happens, though although the DNA is in every cell because of where the cell is present because of the program of development it has gone through from the time it used to be just a little epithelial cell to the time it became a very highly specialized neuron there have been a lot of other proteins other molecules talking to the environment of the cells and regulating which parts of that DNA are going to be expressed and which parts are gonna be silent. So now you have only a repertoire of genes that are expressed specifically in that cell and therefore make the proteins that specify its function. So that's something that happens over the course of development as the whole brain is put together. OK.

20. Q&A: Are African-American families more vulnerable to SCA1? (32:17)

I'm Jimmy Merinlow at Churchill High School. I couldn't help but to notice that all your examples there -were African-American. -Yes. I was wondering if there's some sort of These two families happen to be African-American families but these diseases affect multiple ethnic groups. I have many Caucasian families. I even have Hispanic families. My largest two families happen to be African-Americans and perhaps I got to notice them first because unfortunately both of them were in parts particularly the one in

Montgomery in rural Texas where they didn't see a doctor they didn't know they had an inherited disease did not have access to as much health care so the families were huge and allowed us to study a big portion and go after the gene. But there are all ethnic groups all sorts of ethnic groups are affected by this disease. Hi. My name is Crystal Shaver, and I attend Margaret Mary Washington Senior High School. And I was wondering, as the expansion gets larger do they have different effects or do the symptoms just get worse? The symptoms get worse but sometimes you get even more neurons to degenerate. So that's a good question.

21. Is SCA1 caused by a loss-of-function mutation? (33:23)

Well, I wish we had more time but we need to move on to our second part of this lecture. And now the question that all of you are quite interested in which is, why is it that this slight change in the protein is causing neurodegeneration? So on this cartoon, we're now switching to protein. We're not looking at DNA. And this is just a schematic to show you that the short glutamine tract on the left the neuron is healthy the long glutamine tract on the right is changing the fate of this neuron. Well, how does this happen and what happens? One and that's the good thing about having a gene. You can start asking hypotheses and go in the lab and test them. Does the mutation does the expansion affect the function of ataxin-1? Is it possible that ataxin-1 loses its function? A protein in a cell doesn't work in isolation. It works in cooperation with other proteins. And in this cartoon, what we'll try to show you is that these other proteins are the green and turquoise and gray shapes that may be interacting with it. Is it possible, now, that when you have a big expansion the shape of the protein changes one of these some of these proteins can't anymore interact. It's losing its function and that's why the neuron is not happy. Can we test that? And the answer is yes. You can test it by trying to see what happens when you lose the function of the protein in the organism. And how do you do that? Well, in mice, you can do genetic engineering to delete a gene. And if we were to delete the mouse *Scal* gene we're obviously gonna lose ataxin-1 function and if we lost ataxin-1 function we can look what happens to this mouse and does this look anything like SCA1? and that's what we did. and when we did that, we found out that this mouse does not have ataxia. This mouse was very coordinated. To two years of age, lived fine. So that told us that SCA1 is not caused by loss of function of ataxin-1.

22. Is SCA1 caused by a gain-of-function mutation? (35:27)

Well, could it be, then that maybe when this protein gets a large expansion changes its shape, well, now maybe it gains a new function? It gains a different function whereby it's now interacting with a new protein it normally doesn't interact with. And can we test for that? And the answer is again yes. And the mouse is an amazing model to go back and test this hypothesis. What you can do is you can add a human mutated *SCA1* gene to the mouse and see what happens to the mouse. And back to the earlier question. You know, how do certain cell types know which proteins are normally in them? You can also regulate expression when you add a gene. And in this case, we regulated so that this gene is only expressed in the Purkinje cells of the mice. We just wanted to see if we affect the Purkinje cells do we get ataxia? And this is what happens. When we put this mutated human *SCA1* gene in the Purkinje cells of the mouse we of course put it in the DNA of the mouse but now it's only expressed in the Purkinje cells because we directed expression there we got a mouse that has ataxia. And this obviously told us that mutant ataxin-1 must gain a toxic function.

23. How do you tell that a mouse is ataxic? (36:43)

And you might wonder, how can I tell if a mouse is ataxic? Well, it's actually very easy because if you've watched mice they're actually some of the most balanced characters. They can jump and land on their feet and run very fast. So when we watch them in the home cage we do know that they are having balance problems. But sometimes you want to quantify that. Just looking at them is not good enough so

we can quantify how ataxic are they by measuring how long can they stay on a rotating rod because it takes a lot of balance to stay on that rod. And the next film is gonna show you that.

24. Video: Balance test for SCA1 mouse (37:19)

Let's roll the film showing healthy mice and SCA1 mice. So the mouse on the right is having trouble. It does just cannot keep up. And you'll see as one of my post-docs puts it back on the rotating rod it still really can't handle it. These are doing just fine hanging on. So that's how we can tell. We measure how many seconds can they stay on a rotating rod and we can quantify how ataxic are they.

25. Purkinje cells degenerate in SCA1 mice (37:53)

Now that you have a mouse model and now that you know this mutant protein is in the Purkinje cell you can go back and look at these Purkinje cells. And first I just want to show you an actual picture of these Purkinje cells rather than a cartoon just so that you can appreciate how beautiful they are and how elaborate are their dendrites. This is a slice of a mouse cerebellum stained with an antibody to a protein in these Purkinje cells. And you can see each cell body, and you see elaborate dendrites. But when this mouse has a copy of the human gene look what happens to the Purkinje cells. We lose the dendrites and the cells disappear very much like what we see in the human disease.

26. Ataxin-1 accumulates in both SCA1 mice and patients (38:31)

So now we know that we've got a mouse that has ataxia that has loss of Purkinje cells we can get back to our original question. A protein with a long glutamine how does it differ from a protein with a short glutamine? And to do that, you can now use an antibody to ataxin-1. And look. Where is this protein in the Purkinje cell and what does it look like? Let's first look at the left. At the left you see a section from a healthy mouse. Normal ataxin-1. The stain and the brown staining within the nucleus is the immunostaining of ataxin-1. But look what happens when we stain the Purkinje cells of mutant mice, SCA1 mice. What do you see? You see brown staining in the nucleus but what you also see is some additional staining. Very dark accumulation of ataxin-1 within this nucleus. Much darker than what you see in the healthy Purkinje cells. So that told us that mutant ataxin-1 is behaving differently, that there's a lot more of it and it's in these really tight accumulations. Now, you might wonder, if you're paying attention is that we added an extra copy of human *SCA1* to these mice. They already have their own copies of *Scal* so this is extra. And you might argue, "Well, maybe you have more of it "because you're making more of it. "You just added so much more to the Purkinje cells. "They're making more. That's why it's accumulating." Well, to be sure that this is not the reason we're having too much accumulation of ataxin-1 we went back and looked in patient tissue. And what we found when we looked in the neurons of our patients who died from this disease we found that in many neurons we do see these accumulations. So now we're seeing accumulation in spite of the fact there's only one mutant allele and there is one healthy allele. We know now we're seeing the accumulation without making too much of the protein.

27. Mutant ataxin-1 resists protein degradation and accumulates (40:29)

So if you're seeing accumulation and you're not making too much why might you see the accumulation? Anyone has an idea? Volunteer? It's sort of one cell born, one cell dies. You make, you degrade. Right? So it's the same principle. The amount of protein in the cell is very tightly regulated. It's very tightly balanced. And once the protein does its function it's usually degraded, turned over. And then when it's needed again, it's synthesized again finish its function, degraded. And this process is really a very critical process within the cell. And there is a whole complex of proteins called the proteasome. Their job is that very important housekeeping job. They're roaming the cells and seeing which proteins have

finished their functions and when these proteins finish functioning, we degrade them.

28. Ubiquitin acts as a marker for proteins targeted for degradation (41:25)

Now, you might wonder, how does the proteasome, this factory that degrades proteins that have finished their function how does it know when to degrade the protein? Otherwise, it might just go around and degrade every protein, and the cell will be miserable. So how does it know how to do that? It knows how to do that because the cell is very smart. The cell is gonna flag every protein that's ready for degradation by sticking on a small molecule that's very important. It's called ubiquitin, and it's in every cell. That's why it's called ubiquitin. That protein, when it's attached to a protein that's ready to be degraded the proteasome knows to degrade it. And now that I've told you a little bit about this let's watch this process through this coming animation and you're gonna see how this all happens. Let's roll the animation.

29. Animation: Ubiquitin and proteasome (42:14)

So the little pink protein is ubiquitin and our yellow protein here is ataxin-1. For ubiquitin to be carried to proteins, it needs a carrier. It's called the ubiquitin carrier enzyme. Once our ataxin has done its function and it's ready to be degraded another enzyme will come, called ligase ubiquitin ligase. It comes and attaches to it. And the ligase will interact with the carrier and take the ubiquitin from the carrier back to our protein. And now it can put more and more ubiquitin molecules. The protein will have to have multiple ubiquitin molecules to be degraded. And now once it's flagged the proteasome recognizes it's ready for degradation. The proteasome has a cap. The cap opens. The protein unwinds and enters the business end of this proteasome the catalytic core that chops this protein into little pieces. And this protein will be degraded and the peptides and amino acids recycled. So this is really what happens for many, many, many cellular proteins. This is a very important process and these are the players. Now imagine mutant ataxin-1. It has a different shape, and it's getting ubiquitinated. It gets to the proteasome, but its shape is so unusual. It's just not as flexible, perhaps. We think, at least, that's maybe what's happening. It just can't quite unwind and get through that proteasome. It's stuck. And that's what we think is happening because, at least based on some data and looking at tissues and cells we had some evidence in support of this. So here, if you were to look back at the neurons from these patients, you'll see the ataxin-1 accumulation. And if you were now to look is ubiquitin stuck with this ataxin-1 accumulation? The answer is yes. And what we also find sometimes, components of the proteasome stuck there. So it tells us they recognize it needs to be degraded but somehow it isn't quite degraded.

30. Protein accumulation occurs in many neurodegenerative diseases (44:19)

Now, coming back to someone asked "So why is it that all these proteins in such mutations "are giving us a similar picture?" Well, it turns out that from the study of these rare diseases we found the phenomena that's actually more common to even a broader class of neurodegenerative diseases. So if you were to look at this next slide you will see that this idea of protein accumulation and proteins that are resisting degradation is really common to more than just the ataxias and that class of neurodegenerative diseases. We talked about SCA1 and Huntington but in Alzheimer there are proteins that accumulate, as well. There's a protein called tau that's shown accumulating in a neuron here. And in Parkinson disease there is a protein called synuclein that's shown as this dark purple accumulation. And in the middle in ALS or Lou Gehrig's disease there is a protein called S.O.D. that's also accumulating as this dark blob. And many of you have heard of mad cow disease and that again all that red accumulation is the prion protein accumulating. So it turns out that cells are very sensitive especially neurons and that any change, whether it's a sequence change in that protein, just one amino acid change or in the case of glutamine diseases a glutamine expansion or even an environmental toxin as in some cases of sporadic Parkinson disease that protein may change due to that toxin and that would lead to abnormal degradation

of this protein and its accumulation. So what was really important about this, then is the study of these rare diseases even the rare cases of inherited Alzheimer. Most of Alzheimer is sporadic, but the rare familial cases where there were mutations in tau protein or the amyloid precursor protein that led to the accumulation of a protein have really informed us about these more common sporadic diseases that affect about 25 million worldwide. So this was a very important discovery that now can be expanded to other neurodegenerative diseases.

31. Using chaperones to counteract protein accumulation (46:28)

So what can be done about it? Well, we know that the cell is smart. The cell knew that there will be some protein that will take an abnormal shape and the cell has a certain class of additional proteins called chaperones and their job is to find the protein that looks like it has an abnormal shape and somehow maybe changing is not able to do its function or doing some unusual function. and what the cell does is provide the chaperone to contact this protein and help it take back a more functional shape or a shape that's not interacting with many other things it's not supposed to interact with. And the chaperones are smart. If they catch a protein that's misfolded and if they try to get it back to its healthy shape and it doesn't what they will do is "We'll take it "to the proteasome." If you can't fix it, degrade it. So what they do, they'll say "OK, well, you can't be fixed. "We'll chaperone you "all the way to the proteasome." What they'll do then is help it unwind a little bit and get through that cap more easier than our protein perhaps was stuck. So with this information we wanted to know what will happen if we gave our SCA1 mice extra copies of chaperones. And the way to do that is to breed our SCA1 mice to mice that have been engineered to make extra copies of chaperones. And then what we can do is put these mice on a rotating rod and see how does their balance do. So if you look at the pink bars just pink you'll see that if you only have the *SCA1* mutation with training you get better but the best you can do is stay 300 seconds on the rotating rod but now if you have the *SCA1* mutation and an extra copy of chaperones you can stay longer. So that was excellent. That told us that chaperones do indeed that this whole model of protein resisting clearance and that chaperones may help chaperone it and get it cleared is really holding up and we found that this chaperone is an important modifier, then of the disease course. It improves the performance of the mice, and it improved the degeneration, but this was one gene. It took years to do it in mice and a lot of work.

32. Using an SCA1 model in *Drosophila* to find disease modifiers (48:43)

Can we develop a faster system maybe to find more modifiers things that might make the disease better? Of course, modifiers can make it better or worse but we're looking for things that make it better. How do we do that? To do that, we turn to another genetic model *Drosophila*. You might wonder, "You're gonna model a human disease " in the fruit fly?" Well, we can, and we did. What we did is we took the human *SCA1* gene and we put it in the fruit fly and in the fruit fly you can choose where you want that gene to be expressed. In this cartoon, you see the healthy fly on the left. The SCA1 fly has a degenerating eye because in this case we put the mutant *SCA1* gene and made it expressed only in the eye. So now that you have a fly with degenerating eyes what you can do is see what can make it better. And in flies it can be done easily because you have thousands of flies that have been genetically engineered to either have extra copies of gene or to lack a copy of a gene. And those are shown just as a collection with different colors just to tell you each of these carries a change in one gene. So what you do, you breed your SCA1 fly or mate the SCA1 fly with one of each of these flies and just look at the eyes. It's a very simple screen. You can do thousands and thousands and see which ones will make the SCA1 eyes better. And when we did that we're, of course, interested in things that make them better. The things that make them worse we're not interested in those at this point. We want to see what makes them better. We found several that made them better and among those, we found one that we were expecting and hoping for and indeed it was there. You would expect the chaperone to make it better. If you make extra copies of chaperones just like we did in the mice we would expect that to be better. And I'm gonna show you how

these eyes look... when you have extra copies of chaperones. So this is a *Drosophila* eye. On the left, you see a healthy eye from just a healthy fly. No genetic engineering here. In the middle, you see the eye from an SCA1 fly that has the mutant gene expressed in the eye and I'm sure you can notice that the photoreceptive ommatidia are not healthy and degenerating. And on the right, you see now what happens when you have an extra copy of chaperones. We call it suppressed because we suppress the degeneration. So this is a good modifier. But more importantly what this modifier told us is that now we can use the system to find more and more genes and pathways that can make the disease better. And it gave us sort of a proof of principle that this assay can be used to study modifiers of the disease.

33. Inhibiting protein kinase AKT can suppress the effects of SCA1 (51:31)

So we went back and looked at most of the things that make the fly better and we found one, an enzyme, AKT. It's a kinase. It phosphorylates protein. And it turned out that this enzyme phosphorylates ataxin-1. What we found is if we decrease the level of this enzyme by just deleting one copy of the gene that's why it says "plus/minus" these flies are healthy and they have one copy of *AKT* and lack one that was enough to suppress the neurodegeneration. This was a very exciting discovery because this being an enzyme you can now try to find some kinase inhibitors and therefore really go from using this genetic approach from genes that you have to mate and look at the phenotype effect hopefully find a pill because inhibitors can come in the form of a medicine or a pill that you can give to the mice and see if they'll get better. And as I speak to you, we have hundreds of busy mice taking these AKT inhibitors and we hope to see some improvement. And if we do, we call this a preclinical trial. We can then start thinking about some of this class of drugs and see if any of those will actually affect the human disease.

34. Q&A: Are chaperones specific? Do they degrade other proteins? (52:50)

OK. Now we have time for questions. -Yes. -Is the chaperone specific or does it also degrade other important proteins? So the chaperone is actually not specific in the sense there is a very important chaperone in the cell called heat shock protein 70. So if you shock the cell with heat many proteins will become misfolded. They have abnormal shape. You want to try to refold some of them or degrade them. This protein will recognize all of them. Sometimes, it relies on cofactors that may be specific but it is for many, many other proteins.

35. Q&A: Why do cells degrade proteins? (53:31)

Why do proteins degrade? Why wouldn't they just continue doing their function and the cell would just not create more proteins? Proteins have to degrade because it is sometimes if a protein is instructing the cell to divide, and you don't need the cell there's a protein that tells the cell "Go and divide and divide and divide." But you know you don't want to divide anymore. How are you gonna shut that process? If that protein's still there instructing the cell to divide you're gonna the only way you're gonna stop it is by degrading that protein. That's one example. There are many reasons, really. You know, even when you're thinking if you're thinking about something and now you want to listen to music, you know you want to stop certain processes to let certain others so every function of the cells has to turn off some protein and degrade them so that you don't have so much noise all the time within the cell.

36. Q&A: Does mutant ataxin interfere with proteasome function? (54:24)

I'm Corrine Pender from Thomas Jefferson High School and I was wondering when or I was wondering how long the abnormal ataxin would remain attached to the proteasome and if it stays there for a while I was wondering if that could prevent the proteasome from performing degradation of other proteins and then cause more problems in the cell. What a great question. You deserve two T-shirts for that question. But that's a really important question. You're asking a very important question and that question is, when

we get a protein that's just slightly misfolded and not being cleared and it's tying up the proteasome choking up the proteasome it's gonna affect many other proteins in the cell and is this happening? And the answer is we think this probably happens as the disease progresses. As you have more and more ataxin we think that eventually the proteasome gets compromised. And we don't know for sure, so we're actually testing that *in vivo* through looking at the proteasome activity *in vivo* in a special model that degrades other proteins. So that's a great question.

37. Q&A: Does a mutation affect all cells? (55:25)

When you discover that there is the DNA mutation does that affect all the cells that are produced and also, if not, is there is the protein accumulation enough to affect healthy cells in the brain? Right. That's also an excellent question. So this mutation is in every sorry. Bad cerebellum. I told everybody I grew in a country where we don't have baseball or softball so I'm disadvantaged. But basically you're saying this mutation is in every cell. It's a dominantly inherited trait. It's in the DNA of all affected individuals and the protein is made in every cell so is it all cells in the brain that are affected and how about these other peripheral cells? Let's deal with the peripheral cells. What we think is happening because many peripheral cells divide versus brain cells once they differentiate, that's it. They're postmitotic. They're stuck with what they have. So if they have a protein that can't be cleared over time this is not good. But then the question gets more complicated. Well, this isn't all neurons. Why is it that the Purkinje cell and the brain stem neurons and breathing and coordination neurons that are affected and not others? And we think now that this is a function of multiple factors. It's a function how much of that kinase you might have in these neurons. It's a function do you have twice as much ataxin-1 in the Purkinje cell versus another neuron. So there are multiple factors that give you the specific cells that are vulnerable compared to others. Well, I think we're running out of time. We'll talk more at lunch. Thank you.

38. Closing remarks by HHMI President Dr. Thomas Cech (57:05)

Thank you, Huda, for a terrific lecture. We'll break now for half an hour. When we return, Dr. Zoghbi will present our final lecture of this year's series. she will continue with her theme of understanding disorders of the nervous system focusing this time on Rett syndrome a condition that appears to afflict only young girls.