Kennedy's Disease Association
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Good morning. Hi, I am a neuropathologist at the University of Michigan and my lab studies Kennedy's Disease. I split my time between research and some clinical work. About 2 years ago, I finished up my post-doc with Kurt Fischbeck; it's like a family reunion here. And, I'll be showing a few slides about some of the work that's ongoing in my lab.

I just wanted to briefly tell you about one aspect of my clinical responsibilities, which is to direct a brain bank at the University of Michigan which is associated with our Alzheimer's Disease Research Center. In this brain bank we have collected tissue at autopsy from patients who died from a variety of neurodegenerative diseases. Most of those patients were followed at the University of Michigan. The impetus for having a brain bank is that it's a repository for human tissue from patients who were very well characterized clinically when they were alive, and pathologically after they've died. And this can be a very important resource for investigators who study neurodegenerative diseases. Most of the cases that are in our brain bank are from patients who were seen at the University, but on rare occasions we've banked tissue from patients who were not seen at the University. And, such an instance came up this past year when the KDA asked us to bank tissue on a patient with Kennedy's Disease who was autopsied at the Mayo Clinic. We were very happy to help out the KDA with this request. We have been able to bank this tissue and distribute it to those investigators who requested it from the KDA and whose requests were approved from the KDA Scientific Advisory Board and Board of Directors. The brain bank in Michigan is happy to continue to provide this service to the to the community of investigators interested in Kennedy's Disease and to continue to provide this help to the KDA. So, I just wanted to make you aware of the value of brain banking and I'd be happy to answer any questions on it or on autopsy. I don't know if it's an issue that you've thought about at all.

Susanne Waite, KDA President & Executive Director:

The KDA thanks you and the University of Michigan very much for providing these services for us on a pro-bono basis. (Applause)

Andrew Lieberman, M.D., Ph.D.:

Most of my time is spent doing research and I wanted to tell you about one project in the lab which is to make a new mouse model to Kennedy's Disease which is called a knock-in mouse model. I wanted to tell you about this because the KDA has been very enthusiastically led a fund-raising drive to generate pilot support for this project, and it's a cause to which many of you have generously contributed.

Quickly I want to go through what is a knock-in mouse model and how it differs from some of the very valuable transgenic models that currently exist. I'm going to show you what we've done so far and then briefly summarize what we hope to do with this mouse when it's fully characterized.

Basically the difference between the transgenic mice that are already in existence and a knock-in mouse is that in a knock-in mouse we want to insert the Kennedy's mutation directly into the mouse androgen receptor gene. And, by doing so, we expect that the mutant androgen receptor protein is going to be expressed in all the cells in the body of the mouse that normally express the androgen receptor and at the appropriate levels. And because the expression of the mutant protein is at endogenous levels, we expect that the mice are going to very closely model the phenotype of patients with Kennedy's Disease. So, how do we do this?

We use cells called embryonic stem cells. Some of you may have heard of stem cells in the press because of the interest in human stem cells; we're using mouse stem cells. And what we've done is we've taken these mouse stem cells and swapped the first piece of the mouse androgen receptor, which is called the first exon. We swapped it with the first exon of the human androgen receptor. We put in not the normal first exon, but the first axon with 113 CAG repeats.

Our collaborators in this work have done the same thing and put in a normal repeat length of 21 CAG repeats to create a non-pathogenic control. So, these stem cells come from mice that normally grow brown or agouti colored hair –my post-doc tells me it looks a little bit like my own hair color. (Laughter) He has black hair so he can say that. Then you take those brown stem cells and you inject it into a developing mouse embryo. That embryo comes from a black furred mouse. And what you get is on the next slide - you get kind of a chimeric (laughter) mixture. They're actually a little bit smaller than the animals shown here.

The idea is the same – you get a striped animal. The idea is that the brown parts of this mouse are derived from the stem cell that we manipulated and the black parts come from the embryo that we injected into. And we actually were able to get 7 of these chimeric mice that are striped, and on the next slide, we got 2 animals that were very high percentage brown. These mice are very high percentage agouti, while others were about half brown and half black.

What we wanted to do was breed these male chimeric mice to see if we could get transmission of the mutant gene. And – on the next slide – the way you do this is you take your chimeric male animals that are a mixture of brown and black, and breed them with black female mice.

And as you all know, the Kennedy's mutation is on the X-chromosome. So these chimeric males can father both brown and black pups, but it's only going to be the brown female pups that can have the mutant gene. The father can only pass it on to a daughter. We were a little concerned that this was going to be a very rare event and that is because, as you all know, the males with Kennedy's Disease have decreased fertility. We were concerned that this was going to be the same issue with our chimeric mice. And indeed that turned out to be the case.

On the next slide you can see that these 7 chimeric male mice went on to generate over 350 pups. And of those 350 pups, 4 of them turned out to be brown, and to our good fortune, all 4 of those brown pups were females. So we were very excited about this because the fact that the mutation was then transferred to a female mouse meant that we had been able to generate our line of mice and, we should be able to then pass on the mutation to subsequent generations.

So briefly – on the next slide – how do we know that those mice have the mutation? And this is just one bit of data where we did a genetic analysis called a PCR. We do a genetic manipulation to amplify that first exon of the androgen receptor gene. And in the first column you can see the size of the band from the normal mouse androgen receptor. So that's from a male mouse – a male black mouse. And in the far right lane, we did the same test and used DNA from the stem cell that generated one of the chimeric male mice. And then in the middle 3 lanes are DNA from 3 of the brown female mice. And you can see that it has both the upper band, which comes from the mutant gene, and the lower band, which comes from the mouse – the normal mouse gene. Indicating that those 3 brown mice all had inherited the mutation from their father, just as we had hoped.

So once we had these female mice we wanted to breed them to generate additional mice. And just like humans that carry the mutation, we would expect that these female mice would be able to pass that mutation along to 50 percent of the offspring. So half the offspring should get the mutation, and that there should be half males and half females in the subsequent litters. And on the next slide, we found that was exactly the case. So this is from the first 2 litters – we've had 3 subsequent ones. Of those that have been characterized to date, there are 16 wild type and 16 mutant mice. So, exactly what we expected – half of the mice have inherited the mutant gene; and there are 18 males and 14 females now, so there's about a 50/50 sex ratio. So we're very pleased with our progress so far; this indicates that we've been able to make a knock-in mouse, we've been able to put the Kennedy's mutation into the mouse androgen receptor gene and we've been able to get that mutation to be inherited in 2 generations.

At this point, we're working hard to generate lots of these mice to do some additional tests. So what we want to do with these mice – on the next slide –

the first thing to do is we want to see if these mice get sick. Do they get Kennedy's Disease?

And based on the experience of other labs with similar kinds of models of polyglutamine disease, we expect it's going to take about a year for these mice to start showing disease. And then once we characterize the disease in these animals, we want to use these mice to understand how the mutation causes the disease. And then we hope to use them, in pre-clinical trials to test treatment strategies for the disease.

Finally I just wanted to acknowledge people that did a bunch of this work in my lab. And we have generated the mice in collaboration with Diane Robins in Human Genetics at Michigan. So, thanks very much. (Applause)

Audience Question

How long does it take for you to see that the mouse is ill?

Andrew Lieberman, M.D., Ph.D.

Our oldest mice are only about 6 weeks old and they're not, they're not showing any signs of the disease. So we don't know exactly when our mice will become ill. Based on Al's experience with his mice, and other people who have made knock-in mice of other polyglutamine diseases, we expect it's going to take about a year. But we, we don't know for sure.

Audience Question

Do you expect a decrease in the male mice to be able to fertilize the female mice?

Andrew Lieberman, M.D., Ph.D.

Yes, and that's a good question. You know, are these mice, how well will these mice model the unit disease? Are the females, females going to be relatively asymptomatic and are the males going to show signs of decreased androgen function, and in particular are they going to show decreased fertility? We don't know the answer to that yet. We just set up some matings using the male mice that have the targeted mutation. They look like normal males – we had to learn what a male and a female mouse looks like. Which isn't always easy for us to determine. But they look normally male. And then it's just going to be a matter of waiting to see if they're going to be able to breed, which we don't know yet.

Audience Question

I did suggest at the dinner table when you mentioned this that I'd love to see a web-cam that watched the mice and – you know, a following of how they're doing and…I think that'd be wonderful for people who are part of the KDA.

Andrew Lieberman, M.D., Ph.D.

Yeah, I think that's a neat idea for us to put on the web some sort of update on how our mice are doing…