n=4 Editing Alzheimer's genes with CRISPR/Cas9

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SPEAKERS

Speaker 2, Scott LaFee, Speaker 3, Heather Bushman, Speaker 4, John Steele, Speaker 1

Speaker 1 00:03
View a powerful technique that will transform science, medicine, and perhaps the human race in the whole planet. This is the gene editing technology, known by the acronym CRISPR.

Speaker 2 00:14
You go--you cut out the bad things, you replace them with good things. And we're all happy.

Speaker 3 00:19
That's what this technology is—CRISPR technology. It allows you to cut bad parts out of genes and replace them with good ones. And that's great for diseases—

Speaker 4 00:28
—this is a new technology for editing genomes. It's called CRISPR/Cas9. The CRISPR technology allows scientists to make changes to DNA in cells that could allow us to cure genetic disease.

Scott LaFee 00:43
Hi, I'm Scott LaFee for N Equals One, a podcast about science and discovery at UC San Diego.

Heather Bushman 00:53
And I'm Heather Bushman.

Scott LaFee 00:55
In each episode, we bring to you a story of one project, one discovery or one scientist. Today on N Equals One, we're talking about editing genes with a hot new technique, known as CRISPR/Cas9. Heather, my first question. CRISPR, what happened to the E? What does it mean?

Heather Bushman 01:15
I know. I know. It's a long, crazy name. But CRISPR is actually an acronym. It stands for Clustered Regularly Interspaced Short Palindromic Repeats. And that just actually refers to when a what the genome looked like in the bacteria when they first discovered it. So just go with CRISPR for now.

Scott LaFee 01:41
So we hear a lot about CRISPR/Cas9. It's in the news. It's even been on a mentioned in X Files. What is it exactly, Heather?

Heather Bushman 01:50
Well, it's this hot new thing that scientists are kind of tripping all over themselves about recently, because it can be used in so many different fields. So what it is, is a way that scientists can very easily introduce just a few molecules into a cell, and very specifically, edit genes of interest. So I went and talked to John Steele.

John Steele 02:15
I am John Steele, I'm a fifth-year postdoctoral fellow in Larry Goldstein's lab at the Sanford Consortium for Regenerative Medicine at UC San Diego.

Heather Bushman 02:25
John is one of these very excited researchers, because he and his lab have immediately seen what the CRISPR/Cas9 system can bring to their studies. So let me back up for a second. It's new in that scientists are just applying this to all kinds of new applications and just study different cellular behaviors and look for treatments for new diseases. But bacteria have actually been using it for a long, long time. I'll let John explain.

John Steele 02:57
The CRISPR/Cas9 system was originally discovered in the 1980s. But it took a long time to understand what it really was doing. It was discovered as a form of immunity in bacteria and other microorganisms, where essentially, these bacteria will chop up a virus that infects them, and integrated into their own genome behind these repeats, they sort of clustered repeats of DNA, and then package those into RNA guides that allow the Cas9 enzyme to find and bind to foreign DNA and degrade it. So in the case of viruses infecting a cell, viruses find a way to insert their DNA into a cell and replicate themselves that way, this is a way for a very simple cell like a bacteria to fight off those viruses.

Scott LaFee 03:52
So the bacteria have been using it a long time for millennia. How is this old bit of nature revolutionizing biomedical research?

Heather Bushman 04:00
Right, so bacteria use CRISPR/Cas9 to chop up viral DNA and get rid of viruses in the lab researchers are using it to introduce into mammalian cells like human cells and change the DNA. And so that means changing the code for various proteins in the cell. The most basic kind of example to try to understand this is in rare diseases where someone has inherited a single defect in a gene. So here's what John says.

John Steele 04:34
You could imagine, in the case of a childhood neurodegenerative disease that we study, Niemann pick disease, it's a disease that typically affects children in their first decade of life. Typically, children will die by the time that they're 10. They develop neurodegenerative disease that looks pretty similar to

Alzheimer's disease just very rapidly. And it's caused by mutations in The single gene, in this case, Niemann pick disease type c one is caused by mutations in the NPC one gene, that if you had a patient line with a specific mutation in that sequence that caused the disease, we can now program these crispers and target them along with the Cas9 to that specific gene, that specific mutation in the gene and change it back to the wild type sequence.

Scott LaFee 05:29
This all sounds really complex; how does it work exactly?

Heather Bushman 05:32
It is complex. But in a nutshell, the way I think about it is you've got these molecules that you put on a cell, they go inside the cell, and essentially a little piece of guide RNA, RNA is kind of similar to DNA, it's genetic material. And it goes and binds the complementary strand of DNA, whatever piece you're interested in. So you would make the sequence of your guide RNA match up to or complementary to your target gene to that DNA. So then when the RNA gets in there, it immediately goes in pairs up with that target DNA, the gene of interest, and then the Cas9 is coming along.

John Steele 06:14
So the Cas9 enzyme binds to this RNA Guide, which has found its target on the DNA and will essentially act like a pair of scissors, binding to the DNA and then cutting it through both strands.

Heather Bushman 06:32
And then cells just on their own will try to repair that because broken DNA is a bad thing. And cells see it as a danger and immediately want to fix it. But they tend to fix it not very well. So imprecisely fix that gene. And then it's now a repaired strand of DNA, but the sequence isn't quite correct. So now you have essentially inactivated that gene. So that's one strategy. If you just want to turn that gene off. The other strategy is to introduce a different or healthier version of that gene.

Scott LaFee 07:06
Okay, I can see how the CRISPR/Cas9 system might make a good tool, but can you give me an example Heather Howard, John, and the Goldstein lab using CRISPR/Cas9?

Heather Bushman 07:16
The lab actually brings together several hot, newer technologies to bear on Alzheimer's disease research.

John Steele 07:25
So the work that we do in our lab is primarily focused on understanding how neurons die in the case of neurodegenerative disease. In this case, we typically study as our primary disease, Alzheimer's disease, pretty much everyone knows somebody in their family, or immediate circle who has or had Alzheimer's disease, I have at least one grandparent who died of Alzheimer's disease. My wife's family has at least one grandparent who died of Alzheimer's disease, as well as several others who are affected by it. It's a pretty devastating disease, but it's a disease that a lot of people have seen with their own eyes, we are trying to understand how this disease happens at its earliest point. And to do that, we need to be able to look at how certain genes function in human neurons.

Heather Bushman 08:15
So that's CRISPR/Cas9 for editing genes. They're also doing that in a special type of stem cells called induced pluripotent stem cells.

Scott LaFee 08:25
What are induced pluripotent stem cells?

Heather Bushman 08:27
So iPS cells are not embryonic stem cells. And they're not just native adult stem cells in your body. Instead, there's something created in the lab. And they do this in a really cool way, they can start with any cell type. So say, a skin sample from a healthy person or a person with Alzheimer's disease. Start with this, this skin cell, add a few different molecules that tell the skin cell to kind of dial itself back developmentally speaking until it becomes an embryonic stem cell like, and what that means is, it's no longer defined as a skin cell. It's now a type of cell that is pluripotent, meaning it can develop into any other kind of cell so they can use a skin cell, dial it back into an IP s cell and then tell it to drive forward and become some other type of cell like a brain cell, like a neuron if they want to study Alzheimer's or study other diseases that affect the brain. So they have this personalized disease in a dish that they can study.

Scott LaFee 09:33
And so when I did be that you have these IPs sees, you've reduced them back to their stem cell like nature, and then you go in there with CRISPR/Cas9 and you you're basically monkeying around with that elemental structure fixing something or changing the nature that stem cells so that when it develops forward again, it's different than it would have been before.

Heather Bushman 09:56
Yeah, so that could be one type of application. So in step one, they just kind of want to better understand how does Alzheimer's disease develop and there are many different ways there are different forms of Alzheimer's.

John Steele 10:09
So we have a series of skin biopsies that were collected from people with a familial form of Alzheimer's disease. So in this case, it's a little different from what your 85-year-old grandmother might have, these patients tend to get the disease anytime between 30 and 65 years of age they inherited from their family, and the heritability is very, very clear. But they and they will have mutation in any of four different genes. And those mutations we know cause the disease, we just don't really understand how it causes the disease and what happens to the cells. So we, we use CRISPR based genome editing in order to introduce those mutations into the genome of somebody who aged successfully without developing Alzheimer's disease. So this would be a control line, or we'll go into one of these hereditary forms of the disease and correct their disease-causing mutation and show that that mutation really did cause the phenotypes that we were looking at.

Scott LaFee 11:04
And as I had mentioned earlier, you know, it's in the news a lot people are whispering about some future Nobel laureate winning work. But we're obviously not all the way there. What are the biggest challenges?

Heather Bushman 11:16
A couple of things, one, the technology is changing so fast. So it was only a few years ago that scientists realized that we could apply this to mammalian cells.

John Steele 11:26
From a personal note, the field is moving so quickly, that often by the time we're ready to publish a paper, showing how we employ the some of these tools, the tools have changed so much that what we did was almost irrelevant. What took you two years, now maybe you could do in six months.

Heather Bushman 11:46
And another challenge is the ethical concerns, like all new technologies, there's sort of a reluctance or a fear. In this case, there's probably a lot of reason for that. And people are discussing this issue a lot. And what can we do? What should we do? How should it be regulated?

John Steele 12:05
From my perspective, in terms of translating these tools into the clinical space, and using them to do these types of things that that we just talked about, for example, correcting mutations in an embryo before it's used for IVF, securing the regulatory environment and having, in depth ethical conversations and moral conversations as a community is probably a major hurdle. And I think we have done a good job of addressing this, but it should be a priority.

Heather Bushman 12:37
A third challenge to advancing CRISPR/Cas9 as a potential future therapeutic is knowing for sure that you're only hitting the specific genes you're aiming for.

John Steele 12:49
There are still a lot more questions that we haven't answered yet about how specific these genome editing systems are for the one gene that you're trying to target. And we're getting better at making new, second and third and fourth generation versions of these genome editing systems that are really, really specific to their target and have very, very few off target effects. But until we've done the appropriate studies, to show that there are no off-target effects, you can't know for sure that you're not say, correcting a mutation that would cure Niemann pick disease, say in an embryo, while at the same time introducing a mutation that might cause cancer that we just don't know yet.

Scott LaFee 13:36
Given all the excitement, I mean, it sounds like this is something that is completely novel and original and never seen before Was there nothing before this that served the same kind of purpose.

Heather Bushman 13:48
There were a few techniques that scientists could use before CRISPR/Cas9 if they wanted to tinker with genes in a cell or in an organism. But it wasn't easy by any means.

John Steele 14:00
Before the CRISPR/Cas9 system was discovered and developed into a tool for genome editing. We had other forms of genome editing, called talons are zinc fingers, and talons and zinc fingers because they are proteins that bind to the DNA and then recruit an enzyme to break it. They are a little more challenging to design in your standard lab. They require a lot of bioinformatics capacity and understanding of how they work. There are licenses that you have to buy in order to use them and develop them. They cost a lot of money in order to develop and the turnaround time is a bit lower.

Heather Bushman 14:44
Now CRISPR/Cas9 is cheap, it's easy to do. John told me that he can teach you know a first-year undergraduate student to use this within their first week in the lab. It doesn't really take a whole lot of expertise. If it works quickly, it's cheap. Which means they can try on all kinds of things they can. They can use trial and error and see what's interesting, and they're not really so concerned with conserving resources.

John Steele 15:13
If you have to spend months waiting for something that's being made by another company to arrive and you spent $10,000 on it, you hope that it works for what you want, but it might not. And then you spend time finding out that it didn't. And then you have to start all over again. Here, if we design these in-house, we can design 234 different guides to a specific area, order all of the things we need for really under $100 and have them ready to go into cells within a week. And that that's truly amazing. That really speeds up the pace of research.

Scott LaFee 15:51
Wow, this all really sounds exciting. And it sounds like there's this is a tool that addresses a lot of different needs a lot of different diseases and questions and it will be really fascinating to watch the science develop and to see applications down the line. And I think we're on the sounds like we're on the cusp of a new era in bio medicine.

Heather Bushman 16:13
And we really are.

Scott LaFee 16:14
Thanks, Heather.