Welcome to Eye On the Cure, a podcast from the Foundation Fighting Blindness, providing science research, news, and insights about the world of vision and blinding diseases.

Welcome everyone to the Eye on the Cure podcast. I'm your host, Ben Shaberman with the Foundation Fighting Blindness, and I'm excited today to talk with Peter Quinn. He's a PhD and principal investigator and associate research scientist at Columbia University in New York. And we're going to talk all about CRISPR gene editing. It's a topic we at the foundation get a lot of questions about and the approach holds promise for treating inherited retinal diseases. And it was just about a year ago that I heard Peter give a really great talk on CRISPR at a pretty large meeting, and I made a note to myself to have him on the podcast. And here Peter is so welcome to the podcast, Peter, it's wonderful to have you.

Yeah, thanks for inviting me, Ben. I've been looking forward to this all week. So yeah, let's get started.

All right, and before we dive into some discussion, I wanted to give you a little more background on Peter. He has a lab at Columbia and his lab is focused on clinically translatable work, and they're applying induced pluripotent stem cell derived retinal organoid models to further the understanding and treatment of inherited retinal diseases. And currently he and his team are developing a gene augmentation and gene editing therapeutics program. Also, Dr. Quinn has won several prestigious awards and grants, including the NIH Outstanding Scholars and Neuroscience Award and the New York Stem Cell Foundation Drunkenmiller Fellowship. And he was recently awarded an NIG RO1 grant. That's a pretty large grant to investigate the use of prime editing to treat CRB1 mediated inherited retinal diseases. And also Peter has a grant from the foundation. And Peter, what is that grant for?

Yeah, so that grant is again using that newer type of gene editing, prime editing, but then to treat patients that have PRPH2 mutations.

And so we may be talking a little more about that in a minute, but Peter, what I'd like to do to start is help our listeners understand the difference between gene therapy and gene editing, that simple concept.

Yeah, so actually it's interesting because people use the word gene therapy, but actually it means two types of things. It means gene augmentation therapy and gene editing therapy. So gene augmentation is also known as gene supplementation or gene replacement. And that refers to when we deliver a healthy version of a mutated piece of DNA, we deliver a healthy version to a cell in hopes that that cell will have the mutated version and the healthy version. We hope that healthy version will replace the function of that mutated piece of DNA. And I guess a good example of that would be Luxturna, that the FDA approved treatment for mutations in RPE65. And yeah, gene addition is really, or gene therapy gene augmentation is really appropriate for mutations that lead to loss or diminished protein function in contrast to, but it's not suitable for all mutations. So there's some mutations, for instance, that we inherit in a dominant manner, so they form a toxic protein, so then gene augmentation would not be suitable for them.

And then gene editing is suitable for those kind of more complicated mutation approaches. So gene editing is also known as genome engineering, and it's an approach where we can disrupt, insert, delete, modify, or replace pieces of DNA in a person's genome. So gene editing kind of allows us to target specific sequence of the DNA to correct errors or to remove a segment in our gene where that segment might make dysfunctional protein and it might be able to allow us to just disable a copy of a gene that's causing a problem.

And yeah, there's been many kind of different iterations of gene editing tools. So there's these mega nucleases and these zinc fingers and these TALENs. But the newest kind of form that I think we're mostly focused on today is this CRISPR-Cas systems. And in contrast to gene augmentation therapy, gene editing is specific not only to a particular gene, but also to a specific cell of mutations. So that's the big

difference between the two, I would say. And gene augmentation is really suitable for treating a larger number of patients because it if it's suitable, it can treat all of those mutations in that gene. Whereas for gene editing, yeah, we have to design an approach for each single mutation at a time.

So in a nutshell, you can say gene therapy, you're really replacing the whole gene. You're delivering a whole new gene. Where with gene editing it's more like you're cutting and pasting, or not always pasting, but you're snipping out a part of the gene. You're not replacing the whole thing, you're just doing a little?

Exactly that.

And so let's talk about CRISPR-Cas9. It's kind of a technical discussion, but it's really cool how this approach actually works in nature and how it's been translated to be a way to do gene editing. And just so our listeners know, CRISPR stands for clustered regularly interspaced short palindromic repeats, but we're not going to use that long string of words any further. Just for due diligence, I wanted people to know what that stands for. So anyway, Peter, tell us what CRISPR-Cas9 is, how it came about?

Actually, funnily enough, the CRISPR system was first mentioned in literature all the way back in the late eighties, where people recognized these kind of repeat array structures in bacteria. And then with the advent of the human genome project, when sequencing became more prevalent, we kind of actually realized that this was a bacterial adaptive immune system.

So how does this system work? So it works in three kind of steps. So there's adaption expression and interference, and basically during an adaption phase, a bacteria recognizes that a virus has attacked it. And this virus, of course is inserting foreign DNA into this bacteria. And there's proteins in this bacteria that can recognize this foreign DNA, and they kind of store small segments of that foreign DNA into the bacterial genome, so into something called a CRISPR array. And you can think of this CRISPR array as kind of like a memory bank. So all of the viruses that that bacteria has encountered before, that bacteria has small segments of DNA against that virus.

So then in step two, that expression phase, that whole kind of CRISPR array is expressed, transcribed. So then all of those short sequences are made, and then these short sequences kind of combine with a scaffold sequence and then they can bind something called Cas9. Cas9 is kind of really the effector of this whole process because it can cause strand breaks in our DNA, so it can cut our DNA. So then in the kind of last phase of this adaptive immune system interference, the next time that virus comes and tries to attack that bacteria and inserts its foreign DNA, that Cas9 can combine with those short sequences. And those short sequences actually guide that Cas9 protein to areas of that virus and allow the Cas9 to cut it up. And so this is kind of how the Cas9 of the bacteria stops that invading viral DNA from causing infection in the bacteria.

And more recently we've adapted the system, so we realized that it's programmable, so we can just change the RNA sequence that tells that Cas9 where to go. And we've been able to allow CRISPR to edit in mammalian cells. So this is pretty important because now we can do all of those kind of disruptions, insertions, deletions and modifications in our DNA in a very precise manner, in a very targeted manner. And that's why in 2020, Emmanuelle Charpentier and Jennifer A. Doudna were given the Nobel Prize for CRISPR gene editing. And now there's new updated versions of this like base in prime editing from David Liu's lab that also holds significant clinical promise.

And again, just to summarize, can we think of CRISPR-Cas9 as like a search or find and destroy or cut mechanism? So you locate the mutation and then you can snip it out or alter it?

Yeah, exactly that. So we can tell that Cas9 where to go. We can tell it to go next to a deleterious mutation, and then we can cut at that site and then we can kind of manipulate that cut site to correct that area.

So CRISPR knows the address, if you will, of the mutation. And then the Cas9 is like the scissors or the editor?

Exactly this. Yeah.

Very cool. So you sort of touched on this a few minutes ago, but why would we want to use CRISPR-Cas9 over let's say a conventional gene therapy? Gene therapies have been in the clinic since late 2007. That's when what became Luxturna first moved into a clinical trial. CRISPR is still pretty new, but there are advantages of CRISPR over gene therapy. Can you talk about those?

Yeah, certainly. So we talked previously about recessive versus dominant diseases. So a recessive disease typically lead to a loss of protein function whilst dominant diseases typically form a toxic protein. So then even if we deliver that healthy copy, that toxic protein going to still interfere. So then what we can do with gene editing is we can turn off that toxic protein so we can stop it from working. And I guess that's one of the major things that gene editing allows us to do is to treat dominant diseases. Of course, gene addition or gene augmentation therapy also has some issues that we maybe didn't realize. So we deliver this healthy copy and to deliver this healthy copy, we have to have this regulatory sequence in front of it called a promoter. And this promoter just drives this expression.

But we didn't realize that that promoter could get turned off in our cells, so go under something called DNA methylation. So that means that even if you deliver that healthy copy and it goes to the right cells and it's helping those cells recover maybe somewhere down the line that promoter can be turned off and then that gene therapy is not working anymore.

And the advantage of gene editing technologies is that we don't need to keep them being delivered for a long time. We just need them to correct the mistake and then we actually don't need them to be expressed or be in our cells anymore. So we just need them to be there transiently. So this is the advantage, one of the major advantages of that therapy system as well is that we just need them there for a short moment in time to do their job and then they don't need to be there anymore.

Interesting. And isn't gene editing potentially better when the gene is large and difficult to deliver through traditional gene therapy?

Exactly. So that's one of the biggest issues in general, is typically delivery. So currently the delivery system that people use is AAV and disadvantages because it can only package up to about 4.7 kb of DNA. So for those bigger genes like CEP290 or EYS that are well over that packaging limit, they just don't fit in that AAV vector. So gene editing then kind of allows us to address those genes that we can't deliver in a typical viral vector.

And I know there are two types of CRISPR or variations on CRISPR, I'll say, that are emerging. There's base editing and prime editing. And can you talk about how those vary from just regular CRISPR, if you want to call it that?

Yeah, so we'll go sequentially. So let's start with base editing. So you gave a good description of what the classical CRISPR system is. It's kind of like a pair of molecular scissors that we can target to a specific place in our genome. Well, base editing has kind of been likened to a pencil, where you can write a single base pair into another. So it's really suitable for correcting those kind of single letter swaps, those point mutations in our DNA that cause genetic disease. And it takes advantage again, of the CRISPR system, but unlike the traditional system, it doesn't cause double strand breaks in our DNA. So that means that one or both of those cleavage domains in that Cas protein that causes the cutting has been deactivated, stop working. So guide RNA, the sequence that tells the Cas9 where to go in our genome still takes it to the correct place in the genome, but then how do we elicit a change if we're not cutting our DNA?

And that's where then the base editing enzyme comes in, and that's something such as deaminase. And together the Cas9 and the base editing enzyme together, they're called a base editor. And this base editing enzyme can carry out specific desired chemical modifications of our DNA in a very defined window to where that guide RNA has taken the base editor. So canonically, there's two kind of different types of base editors, there's CBEs, cytosine base editors, and these can perform specific types of changes. So CTT DNA changes and there's adenine based editors or ABEs, and these can kind of cause adenine, toine based changes.

But actually, there's lots of different DNA based changes that we can make in our DNA. There's actually 12 main different types. So base editing so far is limited in the fact that it can only make those few specific changes, but of course, there's many teams working all around to address this and make base editors that can address more of these different types of letter swaps.

Just a question to clarify. So you said that base editing is more like using a pencil than a scissor, so it's less invasive to the DNA. Would you agree with that?

Yes, exactly. So it doesn't really cut the DNA, and this is one of the main advantages of it. So the fact that it doesn't form these cuts, so these cuts in our DNA, they can be repaired by endogenous repair mechanism, DNA repair mechanism in two main ways, in a precise way and an imprecise way. And typically, that imprecise way is more efficiently to happen. So you can imagine that those DNA cuts, if they don't happen where we want them to happen in our genome, that can cause a deleterious effect. So that's something that we wouldn't want to happen. So for instance, if those guide RNAs, they can recognize somewhere else in our genome sometimes. So they might be very specific for where we want them to go, but sometimes they might also accidentally take that Cas9 somewhere else in our genome, and if they cause double strand breaks there, then we're disrupting the function of a gene that we didn't want to disrupt. And with base editing and also prime editing, that doesn't happen.

So let's talk about prime editing and why that's a pretty attractive approach?

Yeah, I guess let's keep going with a general description first. So prime editing has been likened to a word processor. So it has this kind of search and replace function where you can kind of swap one piece of DNA with another. And again, the prime editor uses that CAS nine, but that CAS nine doesn't cut both strands of our DNA, it just nicks our DNA and that Cas9 Nickases, what it's called, is fused to something called a reverse transcriptase. And a reverse transcriptase, again, is like an enzyme that can basically turn an RNA set of instructions into DNA. So then with prime editing, there's a second component called a pegRNA. And this pegRNA is like that guide RNA. So it takes the prime editors to the right place in the genome, but that pegRNA has this extra piece of sequence that's an RNA set of instructions to tell that reverse transcriptase how to repair that area in your genome. So it's kind of bringing all of the components together to perform the correction.

So in a nutshell, prime editing is also less invasive than traditional CRISPR because you're just nicking DNA instead of doing a double strand break. But can't it also edit a larger piece of genetic material, a larger piece of DNA than typical CRISPR?

Exactly. So prime editing actually can, I was talking about all those different types of letter swaps and prime editing can actually do all 12 of those different types of letter swaps whilst base editing can only do a couple. But what also prime editing can do is it can delete and insert a large number of base pairs. And there's newer versions of prime editing now where we can not just replace small regions, but we can replace maybe even whole exons and eventually even whole genes. And of course, this is pretty exciting because then it won't be a mutation specific approach anymore. It will cover a larger number of mutations at once. And of course, this is very attractive as a therapeutic.

Certainly. That sounds very cool. And what's exciting is the use of this technology is pretty new. It hasn't been in development for that long, but excitingly, a couple of years ago we had a clinical trial underway for a CRISPR-Cas9, emerging therapy for a retinal disease, for LCA10, a specific mutation in the gene CEP290. That trial was being conducted by Editas, and unfortunately, Editas decided to not move further with that trial, but there were some encouraging results, weren't there?

Yeah. So this was a really landmark trial because it was the first CRISPR therapy to move into clinical trials in vivo, and it was to fix a mutation in something called CEP290, which is then again a gene that's too big to fit into an AAV. And I think it was the end of 2022, the kind of last results of this phase one, phase two trial. And what they found is actually three of the 14 patients that they treated responded to the therapy. And actually what they found is that the safety profile, which is the point of a phase one two trial, really was good. But also that they found that in some patients that this therapy could work, which of course is really fantastic. It was the first kind of editing therapy for the eye that was shown to be safe and maybe have some improvements for patients.

But of course, this has a downside to it, which is only three of those 14 patients responded. And of those three patients, two were homozygous for this mutation and one was heterozygous and they couldn't really work out why that heterozygous patient responded to the therapy, was the other heterozygous patients in the trial. And this, I guess led them to decide to discontinue or to pause this trial for now, which of course, is disappointing,

Right? That is. But I think the bottom line, even though the trial was stopped, it was a landmark trial. It was the first really CRISPR trial for a treatment that was applied to the human body directly, and there was some efficacy and it was safe. So I think we need to consider that a success to some extent, and hopefully that will inform future emerging CRISPR-Cas9 therapies, especially for inherited retinal diseases.

Yeah, no, certainly I think that is the case. And there's a plethora of new clinical trials, based editing and hopefully prime editing soon, showing some pretty promising results. So base editing, Verve Therapeutics showed the first in vivo base editing late last year, and that seemed to be safe and effective. And then at the RD Retinal Degeneration meeting not so long ago in Spain, Prime Medicine, so that's the company, the spinout company, about prime editing, they showed some really positive data about how they could treat reduction mutations. So one of the most prevalent reduction mutations in the US, and they showed they could in vivo, in a mouse model that had humanized sequences about it, they could get up to 70% editing efficiency. So I think all of this technology is very promising to use going forwards.

Definitely. And I remember that talk and it's exciting to see that project for Rhodopsin moving forward. But you're doing some CRISPR-Cas9 research in your lab, which we're excited about and some of which we're funding. Can you talk about some of that?

Yeah, certainly. So I'll talk about two projects that I showed at ARVO last year. So one is on Crumbs1, which I've worked on for nearly a decade, and the other is on PRPH2. So I'll start with Crumbs1 and then I'll go to the other one. So mutations in Crumbs1, they cause this kind of large spectrum of different inherited retinal diseases. And again, there's no approved medicine today. And Crumbs1 is this kind of key member of a complex that is involved in apical polarity, and importantly it's involved in cell adhesion. So it's important for how cells kind of stick together. And the crumbs complex or the crumbs members are expressed in the photoreceptor cells and the [inaudible 00:20:58] cells of our eye. And if there's mutations in Crumbs1, these cells don't stick together properly anymore and then go under the degeneration, and that's why they cause blindness, these mutations.

But to complicate matters, Jeremy Kay from Duke University found that there was this new isoform of Crumbs1 called Crumbs1B, and importantly what he found was that the canonical Crumbs1, Crumbs1A

was in mostly miglia cells, and this new one was mostly in photoreceptors, but both of them, mutations affect both of these different isoforms, these different variants. So by isoform I mean just like there's small variations that structurally between these two different versions of Crumbs1.

So that kind of complicates doing a classical gene augmentation therapy. People have done that. And actually my previous supervisor, Jan Wijnholds, has some fantastic papers out last year on doing gene augmentation for Crumbs1 in these retinal organoids, these mini retinas in a dish. But because of that kind of complication with the gene augmentation therapy, because of these different variants, we thought of doing gene editing. And actually that's when I started working with prime editing and we were listening to a talk by David Liu, and I thought, oh, that's just so cool. And we spoke to him and he was really great and he shared some of his resources and helped us really get starting.

And what we've been doing is kind of addressing the most prevalent mutations at the moment in Crumbs1, and basically just trying to repair those in patient stem cells. And we hope to soon start taking those components and then putting them in delivery systems and then also trying to use these patient retinal organoids to then see if we can correct the phenotypes we find in these patient organoids.

That's great. And for our listeners, I wanted to mention that when you say Crumbs1, we often refer to the actual gene as CRB1.

Yes.

So this research can apply to some of the mutations in CRB1. And I know we have a lot of families out there that are affected by that gene and very interested in your work, but it sounds like a promising project. And then what are you doing with the PRPH2 gene?

Yeah, so actually that's been a similar approach, but Crumbs1 is a recessive disease, whereas PRPH2, typically patients who have it, they inherit it in a dominant manner, so they just need one mutation, not two mutations for it to cause a deleterious effect. And PRPH2 is, again, is important in those photo receptors cells, those light sensing cells of our retina. So the mutations in these kind of stop those cells working properly. And what we're doing is because we're doing two things, so one, we don't know what each of these mutations do. So some of these mutations cause loss of function, so that means that they don't express the protein or express less protein, and other mutations then are in a dominant manner, so they're toxic. So some mutations might be addressable with gene augmentation, but others will need a gene editing approach. And actually, that's been the work that I'm doing that the foundation has been funding.

And we kind of started actually working on this because of a patient. So a patient gave a very small donation, and I started working on their very rare splicing mutation in PRPH2 and designing a therapeutic approach for this and designing, making disease models of this in a dish. So there's mini retinas in a dish. And then with the foundation's money, we were able to expand this project. And what we realized is that adjacent to this very rare mutation, there's a very prevalent mutation, the c.828+3A, the ACT mutation in PRPH2, which is actually very prevalent in the US.

So what we're doing there is we're also trying to do these specific gene editing approaches against each separate mutation. But also, as I mentioned earlier, prime editing can treat several mutations at once. So we're kind of comparing if treating all of these mutations as once as one therapy as opposed to the specific strategy, which one is more efficient or are they similarly efficient? Because then of course if they're similarly efficient, we can just move with the one that treats more of those mutations.

That's really cool. It's interesting how you set out focused on one thing, one specific mutation, and in the process learned how to address a lot of other mutations. That's very cool. It sounds like really great work. And I'll add, it's definitely cutting edge. Ha, ha, ha. Anyway, enough of my pathetic humor. Peter, thanks so much for giving us a pretty broad and in-depth overview of how CRISPR-Cas9 gene editing is

being used to treat inherited retinal diseases. It really is an innovative and promising approach for these conditions. And to wrap up, can you tell us how you got into retinal research and got excited about CRISPR?

I always liked biology. I did it at uni. And then I actually, at the tail end of uni, I started working for a company that worked on epithelial stem cells. So actually I was really into stem cell biology. And then at the end of that, I realized if I wanted to progress up the ladder, I should go and get a PhD. So I started off by going getting a master's in London. And during that time, I met someone called Dr. David Tree, and he was this really passionate person. He kind of made everyone very enthusiastic about science. And I ended up doing my thesis project with him actually, for my masters. And he worked on a type of cell polarity called planar cell polarity in stem cells. And that was something that I was really interested at the time. And then with his support, I applied for PhD programs, and one of the ones I applied for was on another type of polarity, the one we just discussed on Crumbs1 with someone called Jan Wijnholds in the Netherlands.

And I, luckily enough was chosen to do my PhD there after the interview. And then Jan kind of was the person who taught me about Crumbs1 inherited retinal diseases and gene therapy and cell therapy and disease modeling in rodents and these mini retina in a dish. And yeah, there's people in Jan's lab that I kind of really got my passion for science and my work ethic. So people like Robert Hook and Lucy Pelosi and [inaudible 00:26:49].

But during my time in Jan's lab, actually two really cool things happened, which was, and both of them revolve around going to ARVO. Was the first time that I'd met a patient that had the disease I was studying and their families. And that was pretty inspiring. And also, I saw Jean Bennett speak, and she was giving her keynote, I think in 2014, and she had Yannick, her patient up on stage talking about the benefits of gene therapy, and that was very inspiring.

So I kind of set then that I wanted to continue working in therapies. And then at the end of my PhD, Jan was like, oh, you should talk to Steve Sang at Columbia. Dr. Steven Sang because I was interested also in gene editing. So again, at ARVO, I bumped into Steve after giving a talk, and he had this whole entourage around him, which was honestly pretty intimidating at the time. But he invited me to have an interview and then invited me to join his group. And then a couple of years later, I did that. And in Steve's lab, I was lucky, I'd already reached out to the CRBF, so the Curing Retinal Blindness Foundation at that time run by Kristin Smedley. And I submitted an application for a project, which was on doing gene editing for Crumbs1.

So then the minute I got to Steve's lab, I started working on this. I learned a lot of other things. I worked on other IRDs with Steve, and he taught me about using gene editing in different ways. Steve's lab invented the ablate replace approach. That's actually where you combine gene editing and gene documentation together, which is particularly important for dominant diseases again.

And then, yeah, during my time with Steve, I guess I did well. And then I was lucky enough that the chair of my department, Jack Chaffey, let me start running my own research lab. And then I got funded by the NIH and you guys, the FFP, and yeah, that's my career so far, I guess.

That's really cool. You've moved up the ladder pretty well from doing the work in Jan Wijnholds' lab. That's somebody we've provided a lot of support for. And now you have your own lab. That's pretty exciting. What is it like to run a lab? How many scientists do you have in your lab?

Yeah, so currently I have three scientists in my lab, so two technicians and a postdoc. And actually, because of course, I was in Steven Sangs lab for so long, I still have some projects ongoing there. So I work with some of his PhD students and I love it. It's really fun. I wish sometimes that I did less admin and I was working at the bench a bit more. But it's still really a lot of fun to mentor other people and

progress projects based also more on their effort sometimes, on the bench than my own. But I do a lot more planning these days.

Well, we're excited about the great work that you and your lab are doing, especially on CRISPR-Cas9. And again, thank you Peter, for taking time out of your day to educate our listeners about the technologies and some of the projects that are underway that you're involved with. So great to have you on the podcast.

Thank you, Ben. You're very welcome. It was great to do it.

And listeners, thanks as always for joining the podcast, and we look forward to having you back for the next episode. See you later.

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