Speaker 1:

Welcome to the Eye on the Cure Podcast, the podcast about winning the fight against retinal disease from the Foundation Fighting Blindness.

Ben Shaberman:

Welcome everyone to the Eye on the Cure Podcast. I am your host, Ben Shaberman, with the Foundation Fighting Blindness. I'm very excited today to have as my guest Rando Allikmets. Rando is a PhD, and he's the William and Donna Aquavella Professor at the Department of Ophthalmology and Pathology and Cell Biology at the Harkness Eye Institute at Columbia University in New York. He's also director of research there. Rando, welcome to Eye on the Cure.

Rando Allikmets:

Thank you very much, Ben. It's a pleasure.

Ben Shaberman:

The pleasure is mine. Much of the podcast we're going to focus on Stargardt disease and other macular dystrophies because that's really Rando's wheelhouse. He's been working on identifying and understanding the genetic factors that cause macular conditions, like AMD, Stargardt disease, Best disease, cone-rod dystrophies for much of his career. But Rando, I'd like to start out hearing about how you got involved in retinal genetics because you came over from Estonia. I know you studied in the USSR a few years ago, but you came over to study cancer gene, if I'm correct.

Rando Allikmets:

Yeah. My career has been developing through many continents. Again, I'm an Estonian. At that time, and this was the seventies and eighties, we couldn't go West, so the best school I had was Moscow University, that I attended from '78 to '83. I graduated actually as a virologist and molecular biologist. Then my studies five years, studies in Moscow, after which I got my PhD and went back to Estonia, were mostly dedicated towards genome library constructions and gene cloning, new methods. At that time, this was all hard form. Nowadays, you'll sit at the computer and that's it. Then it was heavy manual labor. My PhD thesis was in developing methods of genomic library construction and gene cloning. Soon thereafter, I started looking into cancer genes, specifically at tumor suppressor genes.

Our one main goal at that time was to find the tumor suppressor gene for lung cancer that was on chromosome 3. Again, at that time people were looking at separate chromosomes, not at the genome, and so we were trying several cloning methods. I did a lot of that work in Stockholm, Sweden at Karolinska Institute in the tumor biology department with legendary leader, and now deceased, George Klein. That's when I was doing mostly cancer work. That work got me invited to National Cancer Institute, which I joined in the fall or pretty much before Christmas in 1991. My main goal or topic of my doctoral research at NCI was to study the ATP-binding cassette transporter superfamily. Why was that, that this superfamily of genes, proteins have several examples of transporters that oppose or contradict chemotherapy?

So meaning that they pump chemotherapeutic drugs out of the cells. Therefore, making chemotherapy less effect. Also, the first major gene other than multi-drug resistance that was cloned was the gene for cystic fibrosis. This was cloned in collaboration with my NCI boss, Mike Dean, with former long-term NIH director. That study was published in '89. So that's where my work started to be in a ABC genes, ATP-binding cassette transporters. What I had to do, we started from model organisms, but in mid-nineties

we were able already, since the tools became available, to move into human genes. I was told or tasked to clone all ABC transporters in the human genome that had not been yet identified. When I started, there was about a dozen genes known. When I finished, it's now 48. I cloned most of them, not all of them, in their entirety. I cloned pieces of genes out of genomic libraries and then trying to map these genes to human chromosomes and also look at the expression of those genes.

Then it was done by standard commercially available northern blots, that included only a dozen or so main tissues, like brain, muscle, and liver and so on. So that what I was doing. We cloned several interesting and published disease genes that other people have now continued working on, but one of these pieces of the gene turned out to be kind of difficult to work on. When I tried looking at its expression, the northern blots were clean, so there was no trace of its expression in any major tissue. Usually the ABC transporters are so-called housekeeping genes. They are expressed in many tissues and sometimes in all tissues. But this one I couldn't see where it's expressed. I even thought maybe it's a contamination of the library and not a real gene. And then also the mapping, I had some issues, but I had a couple of breakthroughs.

When we looked where that piece of the gene came from that appeared in the database that was publicly available, it appeared to have come from the retina library that Jeremy Nathans was, and still is at Johns Hopkins, had deposited to the database. So then we said, "Aha, it probably has to do with some specificity." So we contacted him and he put it on a northern blot containing retina RNA, and it lit up like a candle. So it was a direct hit and immediately told us it's a very tissue-specific gene, that it was expressed in the retina. So at the same time, I was able to map it on human chromosome 1. When we looked at the literature a year before my now group collaborators, Jim Lapovsky from Houston and Paul Bernstein and Mark Leppert from University of Utah, they had mapped the Stargardt region on chromosome 1, and this gene mapped straight in the middle of that region. So once we knew that it mapped into the Stargardt region and also that it is retina specific, it was a slam dunk.

So we did the mutation analysis, I cloned the full gene. And so this was how the Stargardt disease gene was discovered. An interesting remark is that often people don't know the history of that. For example, the protein had been described 20 years before that in late seventies by Dr. Papermaster who was really excited. He said that I described the protein, now finally the gene has been found 20 years later. And this opened a lot of venues for research, although I wasn't maybe extremely excited because I said, "Okay, this is just another eye disease gene or any disease gene that we have discovered. It is a single gene disorder." Stargardt is a recessive disease, meaning you need two mutations. Both alleles have to be mutated. Usually one comes from the father mutation, the other from the mother, and so disease appears in the family out of nowhere, and so this is what recessive diseases due.

So then we said, "Okay, Stargardt is a macular dystrophy. Why not to try and see if it is also a ABCA4 variation?" At first we called the gene ABCR. Some people were joking that it's Rando. No, it was retina, ABCR. And then with my then boss, Mike Dean, again, I was still at National Cancer Institute, we renamed the entire superfamily, and so that's why the genes got those numbers. Yeah, that now we have ABC8 subfamily and B and C, and in every subfamily there is A1, A2, A3, and this gene is now A4. So we screened this gene in AMD patients, age-related macular degeneration patients, and to our great joy, we did detect that variation in ABCA4 was associated with age-related macular degeneration.

Again, at that time, and this was 1997, people didn't have or study groups, didn't have too many patients, either Stargardt patients or AMD patients collected. So we did the initial study in a pretty small number of cases, and the way you do it, you look at the number of variants in cases and then you look at the number of variants in matched controls. It's age matched, ethnically matched. And then you see if there is more in cases or less than in the general population. This is how these association studies are done. And so yes, we published this paper, and it actually happened to appear in Science, but it caused

really a huge splash of interest. And most people said that Allikmets et al are wrong and this is a spurious finding. And we have figured this out later, much later what happens in AMD, but today I will stick with Stargardt discussion and yes, and I thought, "Okay, yeah, we found the gene."

Then, of course, I learned since I knew nothing about the eye before I joined the department of ophthalmology at Columbia, then I learned that Stargardt is really the most frequent Mendelian, meaning single gene disorder, retinal disorder. Again, the estimations have usually been around one in 10,000 people in the world are affected. I think these numbers are actually much larger because as we soon found out, and also colleagues of ours, is that mutations in ABCA4, they cause not only Stargardt phenotype, they cause corneal dystrophy, some even said retinitis pigmentosa-like phenotypes. They cause early onset disease. They cause, now we know, very late onset disease. And actually, that's why oftentimes Stargardt patient, if the onset is over 60 years of age, so they can be confused with AMD.

Ben Shaberman:

Right.

Rando Allikmets:

Because these diseases both have the same thing, that you have the central atrophy or geographic atrophy as it is known.

Ben Shaberman:

Right. So when did you come to Columbia? When did you make that switch out of the cancer world into the retinal world?

Rando Allikmets:

I started at Columbia March 1st, '99.

Ben Shaberman:

Wow.

Rando Allikmets:

So it's been... Now it will be 23 years at Columbia. And I really like it here a lot. I had a very great chairman, Stanley Chang, who was a regional surgeon himself who really was building up the department, because basically when I was interviewed they say, "Can you establish a retina genetics, or let's say, eye genetics laboratory?" Because there was none in the whole tri-state area, and actually still is not. And I said, "Okay, let me try." And because I had worked only for governments before, so I had no practice with writing grants and I was already in my late thirties. And so I said, "Okay, let me try." And that actually did work out fine, and I did get all the help needed to start the-

Ben Shaberman:

And you got some grants from us, too, right?

Rando Allikmets:

Yes. And that's another interesting story that I will tell right now.

Ben Shaberman:

Okay.

Rando Allikmets:

But what happened here was that since my so called to pain was disputed, so I didn't try to push the ABCA4 research as much in my earlier years here at Columbia. But I did more of a general research into age-related macular degeneration and this. And as I mentioned, my chairman, Stanley Chang, he assembled all the retina clinicians, said, "You'll find all the AMD samples for the controls," this and that. And Stargardt kind of came along the same way. So I have these protocols now over 20 years for studying both early onset Stargardt disease and then late onset diseases, mostly age-related macular degeneration. And yes, I was lucky to get some funding early. Specifically I did get the RPB Career Development award and the connection to FFB started also a pretty curious way.

So I had the visit of then FFB top officials and they said, "You have to do gene therapy for Stargardt disease." I said, "I have no clue what gene therapy is." And they said, "Well, you found the gene, didn't you?" I said, "Yes, I did." "And now do the gene therapy." So then it was, again, a totally new thing for me. I asked, luckily we had here, since most gene therapy at that time, and even until now, mostly it's done in viral vectors. Yeah, you deliver the gene in the viral vector to the tissue, in this case photo receptors, where we found out it was expressed only. Now they also say that it may be expressed in the original pigment epithelium. That's still under discussion. But basically we have to deliver the normal working gene to the subretinal space so the virus then can go into photo receptors and RP at the same time.

We made the mouse model of Stargardt disease by knocking out the ABCA4, so it was knocked out. There was no ABCA4 expressed, and so we started testing different gene, I mean different vectors for gene therapy. Now, the problem was, and still is with ABCA4, and some other very important eye disease genes, that it is a really big gene. So it is big, it has a lot of mutations in it that cause the disease. Now we know, I think, over 2,000 already, most of them are very rare. But since the gene was so big, so the viral vectors based on adeno-associated virus that were and still are the most popular. And for example, the Leber congenital amaurosis caused by mutations RPE65. That gene, that was done in AAV vectors, but we couldn't use AAV vectors since they just didn't fit the entire big ABCA4 gene.

So we chose lentiviral vectors as a model of delivering. And luckily, we have here some well-known scientists who have worked on lentiviruses. HIV, for example, is one of lentiviruses. But for obvious reasons, vectors based on HIV were not used much. So we looked at different possibilities, and so we started slowly working on this. And this study was funded by FFP for almost a decade, I would say, maybe a little less. And during that, we learned that Oxford Biomed, they had developed their own vectors based on equine immunodeficiency virus, meaning equine that is a horse virus. And so therefore these were considered better option than HIV-based in humans. And also, they had done several studies in terms of the viral envelopes that in their studies showed better transduction rates, meaning that the gene was delivered at much better efficacy to the eye tissue.

So then we contacted them and we collaborated for a few years, and we published the preclinical results that were really pretty good in even the system that was mouse, whose eyes are not exactly like humans. And then we also showed that we can get the expression of the gene in the non-human primate eyes. And we tried several models, and we published these studies in late two thousands, 2008, and some later, our vectors with ABCA4 went into clinical trials in, I think, 2011 or around that time. So yes, unfortunately, those trials were stopped. This is just one example that has frustrated me, that there are many good preclinical studies for treating Stargardt disease. And now there are several clinical trials

happening, but the pace of moving from the trial to the approved drug or treatment modality has been extremely slow. And of course, there are many reasons for that.

Then with Stargardt disease, the issues are, and also the same with age-related macular degeneration, that is mostly the disease is slow progressing, or relatively slow progressing, except the very early onset form. And so in reasonable timeframe, like two years or so, it is difficult to detect the effect just factually. So that has been an issue here also. So currently, I'm personally not working on any new therapy. I am really fine-tuning the genetic cause of Stargardt disease. We have made many interesting discoveries that I did not expect. I thought it's just a simple monogenic disease. So you have two mutations, you have the disease. Now we know there are a lot of different kinds of mutations that some express under certain conditions. We know there are modifiers, both in the ABCA4 gene and in the genome. And so all of them cause different kind of disease, different phenotype.

And we are, A, trying to figure out specifically what can we predict from genetic data. Can we tell the patient what to expect and what is the course of their disease? Can we tell them what is most important or tell the companies that are running clinical trials what group of patients with Stargardt disease would be informative in their study depending on again what the modality is? Yeah, because we discussed gene therapy, I still think that for recessive disease this would be the best option. But then now there are several other therapies in the pipeline, in clinical trials. Most of them modulate visual cycle. ABCA4 works in the visual cycle. It just flips the vitamin A derivatives in the rod and cone outer segments, and so if it's not working properly, these derivatives accumulate and form toxic compounds. What is generally called lipofuscin or A2E is one of the main component of lipofuscin.

They are toxic to the cells, and specifically they're toxic to photo receptors. But A2E is in the retinal pigment epithelium, so in Stargardt disease we have different forms. In some cases photo receptors die first. In some cases RPE, retinal pigment epithelium, dies first. But the outcome is really kind of the same. You get dead retina, usually in the middle. So it's the macular disease, you lose the phobia, you lose your central vision. In this way it is the opposite to retinitis pigmentosa, where disease starts from periphery and the vision is restricted to the middle after many years, and then it's also lost there. In this case, you lose your central vision, but most Stargardt patients still retain pretty good peripheral vision. And depending on the severity of the disease, some patients can refocus their vision so they can actually have a very decent visual acuity.

And so there are many therapies out there, and I hope that some of them will sooner rather than later get approved by FDA. Again, my opinion about those therapies, again is my opinion as a scientist, so in gene therapy I said if it works would be the best because you really do not replace the defective gene, but you add the working gene and it's called gene augmentation therapy. And the other good thing about Stargardt is that if you are a carrier of ABCA4 augmentation, you have one of them, like in Stargardt parents, they each have one mutation, your vision is fine. You do not develop any issue. So this is what we are trying to do. We are trying to really help the patients. We recently published a very good study by my best student as a first author, Winston Lee, that we really constructed a matrix of phenotypes depending on genotypes.

And even people who don't know specifically ABCA4 or Stargardt disease as well, they can really look up what mutations does the patient have and then they can advise the patient of progression of the disease, whether it's rapid onset and very bad form of the disease. And this is usually maybe in 10% of all Stargardt patients, but that means that you have no functional Stargardt disease. So that's the case. Most cases have some Stargardt disease, sorry, ABCA4 function left. And so therefore, their disease progression is much slower and sometimes not that bad as it could have been.

Ben Shaberman:

In talking about gene therapy, earlier you mentioned that the ABCA4 gene is relatively big, doesn't fit in the standard viral container. And I just wanted to comment that there are newer versions of these delivery systems that are moving toward clinical trials where you can either split the gene up so it's delivered in two containers or you can try to take elements out of the gene to shrink it down to fit in the container. There's protein splicing, so there's a lot of great work going on. It's not in clinical trials just yet, but a lot of great gene therapy work going on for ABCA4 so we can get some newer Stargardt disease gene therapies.

Rando Allikmets:

Yeah, this is absolutely correct. Yeah, because I do advise some of these companies that are working on it. As you mentioned, yes, there is the protein splicing option, putting two different... I mean reducing the size of the gene, I don't believe in that really, although ABCA4 contains two very similar halves. And the studies in mice, again, have been very successful in injecting two vectors that each one half and then they either, by recombination in the cell, or by some other means, form a functional protein. So these studies have shown real promise in mice, but I am not so sure about how they will work in humans, because you need to have two different vectors going into the same cell so that if with a higher titers can, of course, be accomplished. But I still prefer a single vector idea. Now this said, as you said, Ben, you're absolutely right that technology has really shown incredible development.

Then there are nanoparticles that are used to deliver that have no limit in size, or at least they will definitely take ABCA4. And then there are some other delivery methods that people are trying, and even splicing from two could work. I'm not saying it will not, it could. And then the other line of work is mostly the modulators of the vitamin A, visual cycle or the vitamin A uptake. And I also say that you could call gene therapy a cure, but I would not tell patients that they shouldn't try other things because, again, if they slow down the disease and if you start taking those compounds or drugs, and again, most of these are oral drugs, that is another big benefit because they don't involve operation really, what is the injection, subretinal injection to.

And so yes, I think that probably the future could be a combination of the two, that you have the visual cycle modulator and then at least it slows down the disease until the gene therapy becomes available. But it's still then you have more cells left to be injected, and I see no reason why some of these therapies that are currently close or in clinical trials are not still completely approved, because I think it's very important to get to the patients quicker than we are doing now.

Ben Shaberman:

And the foundation and other research groups are really focused on moving these through the clinic. And you're right, they're moving faster than they have been. And what I love about your story is back when you got involved in this space... Well, you didn't even know you were getting involved. It happened very serendipitously, but it really was an art form because we didn't have the technology, the computers, the sequencers. And today, it's just a whole different landscape where you can use technology to find things so much more quickly. But I really appreciate your sharing the stories of your early work and how science can sometimes be a serendipitous thing. And it changed the landscape for patients with Stargardt disease, it changed your career, and we're at a much more hopeful juncture now, thanks to-

Rando Allikmets:

Yes. I always tell my students or anybody who wants to listen when they ask me what career advice you can give, I say that science is unpredictable. You don't know. And there are two things, what you need to

do. You need to work really hard and be ready for suffering, because this is how it is. Although some people call me finding the Stargardt gene by accident, it was not an accident, because I was systematically looking into ABC transporters and finding out what they do and what diseases they could be involved in because some of the known genes were involved in the diseases. So the surprise may have been that it turned out to be an eye gene, a retina gene. But other than that, yeah, I think that it kind of... Because as I said, we cloned and published many genes that are now ABC transporters that are now widely used in cancer research and stem cell research and some other disease genes.

So yes, so it is really... But you do have to have some luck also. In addition to working hard and then really not expecting much, you have to get lucky at some times. And in this case turned out to be. But I think that some of this so-called luck was because of ABCA4, the Stargardt gene being so interesting and so complicated. That has really changed, not even eye genetics, but genetics in general. I mean, I wouldn't say that we were trailblazers or so, but again, Mendelian diseases, this is a model of really complex Mendelian disease. People were usually saying, "Oh, monogenic disease and polygenic," like age-related macular degeneration, complex disease, simple disease. I always say that actually ABCA4, Stargardt disease is the good example of simple disease being very complex, genetically and also clinically. And so that's why... That is also probably one of the reasons why the treatment has been a little slow to get there, because it is so heterogeneous.

But we have learned a lot and certainly it really made my career. I remember that at those times when this was art and cloning the genes. So people always said, "You have to get your gene. That will make your career." And I can say that ABCA4 definitely has been that gene for me, but it really has made my scientific life really interesting and I like it. And so I always now want to really get quicker results for patients in terms of treatment. But at first it was really an investigation into unknown.

Ben Shaberman:

Right. But through your hard work, your passion, your commitment, and a little funding from various groups, you made it happen. And Rando, I really appreciate you taking time to kind of tell the Stargardt disease, ABCA4 story. It's a long history, and I think even for our constituents out there who don't have Stargardt disease or a macular condition, it's a great example of how science can work. It's not always a straightforward, simple journey. But the good news is we've gone from not even knowing what the gene is to having a lot of clinical trials underway. And we appreciate, again, your hard work and focus to understand the gene, the disease, and help science move forward.

Rando Allikmets:

Yeah, anytime. I'm always happy to explain these things so people will understand. Sometimes they say genetics is so complicated. It actually is, but it's not that complicated, so you can always explain it to the patients and to everybody who is interested. I'm often in touch with parents of Stargardt kids because they are worried and they are asking what to expect. And as I said, a few years ago, I couldn't predict much anything. So I said it's a complicated. Now we have the manual, it is not... But we have that for the doctors, for genetic counselors so that they can advise patients. And of course, the work goes on and there is still plenty to find, but I think we know 90%, I would say.

Ben Shaberman:

That's awesome. That's awesome. Again, Rando, thank you so much for your time and your reflections and your perspectives. It's been a lot of fun, and it's been wonderful to know you over the years. I know when I started with the foundation back in 2004, 2005, you were one of the first investigators I met, and it's always been great to learn from you. So thank you.

Rando Allikmets:

And I still get good support from FFB, and I'm very happy with that. So now we are doing this fine-tuning of Stargardt clinical trials with FFB grants and we're trying to figure out especially how we can really make clinical trials more efficient by selecting patients and by predicting the outcomes. And yeah, this has been... So yeah, as I said, it's 20 years at least we've been interacting with Foundation Fighting Blindness.

Ben Shaberman:

Well, it's been a great relationship, a good investment on our part. So thank you, Rando. And thank you to all our Eye on the Cure Podcast listeners. We're glad you could join us for this episode, and stay tuned for our next installment of Eye On the Cure.

Speaker 1:

This has been Eye On the Cure. To help us win the fight, please donate at foundationfightingblindness.org.