Marie: Hello and welcome to *The Parkinson's Research Podcast: New Discoveries in Neuroscience*. I'm your host, Dr. Marie McNeely, and I've partnered with The Michael J. Fox Foundation for Parkinson's Research to bring you to the forefront of the field of neuroscience to discuss the latest advances and discoveries with leading experts.

The Michael J. Fox Foundation created this podcast for researchers, clinicians, and industry professionals with the hope that these conversations and the resources we share will advance your efforts and partnerships to improve brain health. We're welcoming guests with a range of experiences and viewpoints. The views expressed belong to the guests themselves.

And today we are thrilled to be welcoming our guest, Dr. Judith Steen. Listeners, Judith is Associate Professor of Neurology at Harvard Medical School, a member of the Harvard Stem Cell Institute, and Director of the Neuroproteomics Laboratory in the F.M. Kirby Neurobiology Center at Boston Children's Hospital. Today, we'll be talking more about her research using proteomics and transcriptomics to better understand neurodegeneration and neuroregeneration. So, Judith, welcome to our show today. How are you?

Judith: Good, thank you. How are you, Marie?

Marie: I'm doing quite well, and I appreciate you taking the time to be here to talk more about your work. But, so our listeners can get to know you a little bit better, Judith, can you start by telling us a little bit more about your background and the path that you took to get to your positions there?

Judith: One of the things that I want to emphasize is that having a broad set of expertise if you're starting out in science is a cool thing to have because it allows you to traverse across fields and allows you to do new things that other people haven't done before. So, I have an honors degree in chemistry and biochemistry for my undergrad. And then I received an NSERC Fellowship, which is a Canadian National Grant, to Study Genetics and Biochemistry during my PhD. And then I moved to the University of Southern Denmark, which was the hotspot or one of the leading proteomic centers in the early 2000s, to learn mass spectrometry. And shortly after that, I moved to Harvard Medical School to do a second postdoc between Harvard Medical School and Sciex, which is then a leading Canadian mass spec instrument company.

And again, this was supported by the Canadian Government with an industrial fellowship. And this extensive training enabled me to run a multidisciplinary lab where we do mass spec data analysis, genetics, cell biology, and drug discovery. So, we have people from various disciplines that join our lab with computational experience, analytical chemistry experience, biology, and also chemistry. And this

allows us to think together in ways that are novel and prevent the silos that happen between different disciplines in our fields. And I really think that helps us to think out of the box.

Marie: Absolutely. And I know your work bridges these different disciplines. As you alluded to, can you share maybe an overview of the scope of the different kinds of research or the main areas of interest your lab has right now?

Judith: Right now, we're focusing mostly on neurodegeneration diseases, trying to understand what allows neurons to survive despite stressors. The way that we think about neurodegeneration is that over a lifespan, you suffer a number of insults, and these can be caused by your lifestyle, by eating junk food, or smoking, alcohol consumption, environmental pesticides or toxins. And all of these can have an effect on your biology and ultimately affect your brain, including trauma to the brain, car accidents, or if you're a Veteran, or if you suffer from injury during sports.

So, the way that we think about it is, how do we enable the brain, and neurons, and all of the cells in the brain to overcome some of these stressors as we age — apart from living a healthier lifestyle? So, we try to study neurodegeneration diseases over the progression of disease, starting as early as possible, in cohorts that have been collected longitudinally or in patients where we know they have a genetic disposition for a particular disease. And then we can understand what are the pathways that are disrupted when you're early in disease, such that we can abrogate the disease early rather than once it happens. And of course, we are also trying to do as much as possible to prevent progression. However, I strongly believe the earlier we can intervene, the better.

Marie: Absolutely. And I know your work looks at a number of different neurodegenerative conditions, so Parkinson's is one of them, Alzheimer's, can you mention some of the others?

Judith: Frontotemporal dementias, we also worked in spinal muscular atrophy. And as you know, there's been a lot of wonderful work in spinal muscular atrophy that allowed children to survive and actually thrive with the new therapeutics, the ASOs (antisense oligonucleotides), that have been used in that disease.

Marie: Excellent. And Judith, I know the work that you're doing is valuable in a lot of different fields here. Can you describe why is this process of quantitative proteomics, the approaches that you use in your laboratory, important and valuable for Parkinson's disease research, specifically, and the therapeutic development that's going on in the field?

Judith: So, right now, if you want to understand how a disease is progressing, if you measure the presence or absence of a protein, it doesn't tell you as much as if you measure the amount of a particular form of a protein that changes over the disease. And this is because, for example, in some of the synucleinopathies or tauopathies, we understand now that there are more pathologic forms of proteins that can make the disease spread throughout the brain.

So, for example, in Parkinson's, one of the things that we're trying to understand is why do we get synuclein aggregation? What exactly is causing that? What are the upstream pathways that we can interfere with that cause the chemical changes in the synuclein? So, not only are we looking at the chemical signatures on the synuclein and quantifying them, we're also quantifying what other proteins are changing that cause these changes. So, if you go as far upstream in terms of the pathways, you can drug the disease earlier and then also monitor the downstream effects of those pathways on the synuclein.

And by measuring these different changes on synuclein, we can tell you which changes happen earliest and which ones are the most important for the disease. So, often we see chemical changes that are not associated with disease on all of our protein markers. However, unless you do the quantification, you do not know whether or not a chemical change is a result, or is associated with disease, or is just happening in the background.

So, when we measure the frequency of the PTM (post-translational modifications) across the population, we look at the stoichiometry, which means the percentage change in that chemistry, and we measure the absolute amount of the synuclein that's showing the pathology. We can then target those forms using drugs or even reagents that allow us to see it in the brain by, say, PET imaging. So, this is a form of imaging that we can use to see molecules that are changing over time in a live patient.

So, unless you know which type of molecule is causing disease and how much of that molecule you have, you cannot drug it effectively. We need to know absolute amounts of molecules per cell or per fresh weight of tissue in order to target the toxic forms of the synuclein with the right chemical, or therapeutic, or ligand to see the toxic forms in the brain in a live patient.

Marie: Well, Judith, I think this is a really exciting area of research, and I know your lab has been developing these approaches, and tools, and workflows to get valuable proteomics and transcriptomics data. And I'd love to go into an example of one of your recent papers published in *Nature Protocols* on profiling post-translational modifications of pathological tau. So, Judith, can you walk us through your rationale for pursuing this particular project?

Judith: We pursued this particular project because there were so many questions about the role of tau in disease. And over the last five to seven years, it's become really evident that pathologic tau has a role to play in Alzheimer's disease. There were questions about what exactly tau was doing. But now we know that certain forms of tau can cause the spreading of the disease throughout the brain. And one of the ideas in the field is to target these toxic forms of tau.

And again, if you want to develop a therapeutic that can target that form of tau, you need to know what that form of tau, the pathologic form of tau, looks like. So, we mapped different modifications on that pathologic form. We showed that it can cause seeding in our cell models. And we then use either antibodies or small molecules to prevent these modifications from occurring, or from allowing the tau to spread through the brain. And we are pursuing the development of therapeutics in this area. But the reason we measured the tau with absolute quantities is to know how much of this drug or antibody to dose a patient with. Without knowing the amount of the tau, often we're just unable to develop the therapeutic properly and measure the affinity of the therapeutic to the tau or the dosage of the drug that we would need for the tau to be removed or to prevent that toxic form from occurring.

Marie: That makes sense. And can you go into some of the detail about the workflow that you used for this particular discovery assay, as we'll call it?

Judith: What we do is we use a standard that's heavy isotope labeled. So, it's not radioactive, it just is heavier than the endogenous tau that you have in your body. And what we do is we spike this standard into our sample. It's the full-length tau. It's completely unmodified. And we know exactly how much of this tau we spike into the sample. And we are able to tell which parts of the tau in the brain are affected by modifications, and then are then able to target those parts of the tau and find the chemical changes by doing this. Furthermore, we can tell using the whole platform how and what enzymes can affect this particular chemical change and then try to prevent that chemical change from occurring by using small molecule inhibitors.

And the idea is to use this assay from drug development and target engagement during clinical trials of the tau. But we wanted to share the details of the methodology so that other people can take advantage of the methodology. So, it was open to the public. And the paper is available on PubMed. And anybody who wants to do the assay can do the assay with a particular type of mass spec.

Marie: I think that's remarkable. And can you share perhaps what are some of the benefits of this particular approach, and how do you anticipate that others in the field may use it?

Judith: So, for example, we're using it not only for studying the human brain tissues that we get from the NIH Brain Banks. We also use it to screen for drugs. So, for example, we have cell assays where we can induce certain stress pathways or certain changes in pathways and measure how those pathways affect the tau. We then can look for small molecules to prevent those tau modifications from occurring.

Marie: That makes sense. And if you had to summarize, perhaps, some of the benefits are kind of what makes this approach different — or the outputs from this approach different — from previous approaches that people have used in this area of research.

Judith: So, we can measure every single peptide on tau. We get about 95 percent sequence coverage of the whole protein. And this is true for synuclein. Right now, we've developed it for synuclein such that we can see 100 percent of the protein. And most of the time when people are measuring tau or synuclein, they don't understand why they don't see certain parts of the tau or certain parts of the synuclein. But with our method, we can see every single peptide from the protein because we have a standard as well. And if we see that a certain peptide is missing, we can go and look for that peptide in its modified state.

Whereas most people will just ignore the fact that they don't see it. If you use a standard core that most of the academic institutions and even the pharmaceutical companies have. But with this particular method, they can then understand which parts of synuclein are being chemically modified and then go and try to understand how that chemistry is different in the disease form versus the healthy form.

Marie: Very interesting. Well, Judith, I think this was a really cool paper. Like we said, listeners can find it out there if they're interested. And I'd love to talk about another kind of approach or platform that you've developed in your laboratory. The FLEXI Platform was developed in your lab. Can you share what this FLEXI Platform is and what prompted you to maybe start working on developing this platform in the very beginning?

Judith: When I was at Harvard Medical School in Marc Kirschner's lab, we were looking at the cell cycle and cancer bio. And what happens during the cell cycle is that you get this mechanism where there seems to be sort of a clock where you get an increment of phosphorylation as you enter cell division — the state of mitosis. And these post-translational modifications occur in a serial fashion, much like they do in the Alzheimer's disease progression. And you can learn a lot about this type of change by looking at the order of the phosphorylation on the cell cycle proteins.

So, that sort of prompted us to thinking about how can we measure these modifications or chemical modifications better? And the use of an unmodified standard was the way to go. And so, the FLEX system can be applied to any protein. So, we developed the FLEXITau. Now we're developing the FLEXISyn. We have a FLEXITDP-43 as well in progress. And we can do this for any protein that is of interest for therapeutic use or of interest in the pathology of any disease, including cancer or any other disease that you're looking at.

Marie: Certainly. And we'll definitely come back to the FLEXISyn. But just to talk more about this platform in general, can you describe what were some of the challenges, Judith, that you had to overcome in developing this FLEXI platform?

Judith: There were a lot. So, basically, the first thing you want to do before you start developing the FLEXI standard is to really know the isoforms and the proteoforms of a particular gene. So, the alpha-synuclein gene or the tau gene has six isoforms in the adult patient. And we did not understand which one of these isoforms was the most prevalent in disease.

There were some papers that used antibodies that suggested that there were particular forms that were disease-associated. But the overall chemistry of those forms was not understood. And what we can do is to give you an idea of the whole chemistry of the whole molecule. And once we have the information, we can then target those forms.

So, for example, in Alzheimer's disease, the whole N-region of the protein is missing from the aggregated form of Tau. So, the 2N-region, for example, is not found to be associated with tau aggregation. And in synucleinopathies, we're seeing similar things where certain parts of the molecule have to be cleaved or modified in order for it to aggregate. And these are usually the disordered regions of the protein. So, understanding that holistically is super important to moving forward for therapeutics that target those tau forms or synuclein forms that spread through the brain.

Marie: That makes sense. And this platform that you developed, you sort of based it on existing tools. Is this something that you think if someone is interested in adopting the FLEXI platform or starting to do studies in this area, is it easy to implement in kind of the traditional lab in your field or is this something where they would need special equipment?

Judith: Well, you need to have multiple expertise in the lab to do this kind of work. So, we basically use an in-vitro expression system in order to obtain unmodified tau or synuclein and develop a FLEX tag that flies really well so we can quantify the protein in very low amounts and then build the actual construct, which includes the peptides from each isoform. So, you need mass spec knowledge, you need

to have molecular biology knowledge and biochemistry knowledge to purify and test the standards. And I think one of the things that would be great is if we could develop kits so that people can use it more widely without having to have all the up-front expertise to do the work.

Marie: Well, that makes sense. And coming back now to, you mentioned of course, FLEXISyn — you are working to develop a workflow called FLEXISyn to examine changes that result in dysfunctional alpha-synuclein. And this work has been funded by The Michael J. Fox Foundation. So, can you tell us why did you decide to apply this FLEXI platform to alpha-synuclein next?

Judith: So, we have been interested in the synucleinopathies and have been dabbling in the area prior to applying. So, we first do global proteomic studies to understand how the differencing synucleinopathies result in aggregation. And then once we know that there are differences between the synucleinopathies and there are pathologic forms — and the way that we figure out what pathologic forms are is to fractionate the synuclein in very different ways. So, we do a lot of biochemistry up front to see if we can isolate the toxic forms of synuclein. For example, in MSA (multiple system atrophy), there are some forms of synuclein that spread much faster than anything in Parkinson's disease. And what we did was to do biochemistry to isolate those types of synuclein from MSA, or even Parkinson's, or dementia with Lewy body. And once we isolate those forms of synuclein, we then do seeding assays.

We developed an in-house seeding assay called FRET assays to measure how much aggregation is caused by these types of synuclein that we'll see in disease. And if we know then that this is something we want to target because of the activity of the synuclein, we then develop a FLEXI assay — and in this case, FLEXISyn — to examine the changes of the synuclein that is causing or increasing aggregation in our cell models or assays. So, if we didn't see this, we wouldn't see the point in targeting the synuclein or using this assay. We would more likely go upstream to study maybe the proteins where you see the most genetic risk factor or the genes that have the highest risk for disease.

Marie: Well, I appreciate you going through some of the background. I think right now we don't have a great understanding of what is happening with alpha-synuclein in neurodegenerative diseases like Parkinson's to make it become this toxic form. So, what is your hypothesis in this case, Judith?

Judith: So, what we've been doing in parallel — first we do pilot experiments — is to look at pathways that are changing in patients that don't have the fully-fledged disease but are moving towards getting the disease. And these tissues are extremely rare because the subjects are normal. But you need to have those longitudinal samples collected at different stages of disease in order to better

understand the disease. And so, we look at the global changes quantitatively in those samples and then look for a particular master regulator that can induce that particular pathway and cause changes in synuclein downstream in cell models. And we have these cell models that we test.

And by doing that, we have to see the change in synuclein. Otherwise we don't move forward with that particular pathway. And we use the FLEXISyn to determine if those changes actually occur within our cell models. And then of course we also look at mouse models and see if they reflect the human disease. And then we can use those also to study whether or not a therapeutic target is affecting the synuclein in mouse models. And if it does, we can target it more effectively and do preclinical studies in those mice.

Marie: Certainly. And Judith, I think there's a lot of work to be done in this area still. Can you explain the scope or perhaps the aims of this particular project that's funded by The Michael J. Fox Foundation and what is your approach for further developing this FLEXISyn platform?

Judith: So right now, we are working to develop an assay that's robust and can be used across a large number of tissues in order to map the changes from stages that are pre-disease all the way to fully-fledged disease in the synucleinopathies — in Parkinson's in particular. And then we will try to develop a cell model on the side for this to understand which pathways are changing in another project. But the goal of this particular project is to have an assay that everybody can use and do multiple types of studies with the findings that they have using the FLEXISyn. It's basically to develop this tool so that it can move the field forward to do whichever drug discovery or biomarker monitoring that can be done to determine how synuclein is changing in disease.

Marie: Excellent. And you mentioned that you are developing this assay to work with various tissues. What are the tissue or sample types that you are potentially working with?

Judith: We usually work first on human disease to understand it better. We use human post-mortem tissues that are donated by families of subjects who have had disease, or are not diseased but they want to further the science of medicine. And we're very grateful to all of these families and subjects that donate these tissues to the NIH Brain Banks. And together with other members of the consortium, who are using other methods in other proteomics methods, we are trying to ensure that every lab is able to see the same changes with their different assays in order to validate each other's work. So, I think that's super important for this particular project.

Marie: Absolutely. And I think the brain tissue is a logical place to start. Do you anticipate that you'll perhaps eventually be able to use tissues or samples that can be collected less invasively — things like skin samples, or blood, or CSF?

Judith: Yes. So, for Alzheimer's disease, one of the markers we found in the brain is also found in blood and in CSF in Alzheimer's disease patients. And it is the number one marker that has been used as a biomarker for monitoring the drug trials that are ongoing in Alzheimer's disease.

Marie: Very interesting. Well, I think there's a lot of exciting work in this area. After this particular project ends — I know it was just funded this year in 2024 — but do you have your next steps planned out?

Judith: Absolutely. As I mentioned, once we understand the changes in the synuclein over the progression of disease in Parkinson's, we will try to go as early as possible and look at changes in pathways for the disease and try to ameliorate disease really, really, really early on. Because for me, targeting the disease as early as possible in people who have a genetic predisposition for the disease is our goal, such that the people who have this disposition do not get to the stage of progression. It is also important to try to stop progression, and we are also looking at the pathways in mid-stage disease and the late stage.

Marie: Wonderful. Well, we are excited to see where this work takes you and the discoveries that come out of it. And I know working in this area of Parkinson's disease, there are a lot of challenges as we alluded to earlier, but there can also be a lot of surprises. So, have you had any surprise findings or unexpected outcomes, Judith, in your work in this area of Parkinson's disease?

Judith: I think a lot of us focused on the spreading of synuclein and synuclein itself, but I think looking at the upstream pathways is something that I'm really focused on now, because of what we're seeing in some of the other diseases that we started studying much earlier. We're seeing similar patterns in Parkinson's. Another thing that has been surprising is that there are some subjects we observed to have both synucleinopathy and tauopathy, and we just submitted a manuscript describing this. It's a descriptive paper where we see that the two molecules can affect the pathways, or there are pathways that are different from Alzheimer's disease or dementia with Lewey body disease in the patients with both aggregates.

So, there are people with mixed pathologies that show this neurodegeneration, but in a different way. So, I think for those types of patients, we might have to use a combination of drugs to target their disease, and understanding the whole spectrum of these neurodegenerative diseases will have to include the patients that have both types of pathology, as well as those who have one type. And

being able to test whether these patients have one or two pathologies will have to be an important goal for our team.

Marie: Definitely. And Judith, I think these are really interesting findings. And we hinted previously that there are some tools or resources or things that are happening in the field that are really helping advance research or move the field forward. So, what do you see as some of the prime examples — whether it's in neuroscience or even drawing from other fields — that are making a big difference or accelerating research in Parkinson's disease?

Judith: One of the things that has been striking recently is the technology development in mass spectrometry and also analytical workflows. So, for example, our lab has also been instrumental in making plasma proteomics — for biomarker discovery — high throughput. One of our papers that we published in *Science Advances,* which was initiated during COVID by the NIAID who funded the lab, and my husband and I worked very closely together. He works mostly on the diagnostics, and I work mostly on the neurodegenerative diseases.

But what we have done is to make plasma proteomics much cheaper and get more robust quantitative data from blood samples. And we completed the largest study ever published, and the second paper was published this year. The first paper was downloaded 15,000 times because we published the methodology in detail, so that other people can use it too. So, I hope that the technologies that we and others are developing in this area are really moving forward the field of proteomics. And the idea is to use mass spectrometers in the future to do these measurements in a high-throughput fashion, because mass spec just provides more accurate and quantitative data than any of the ELISAs.

Marie: Definitely. And Judith, I must say, I really admire your willingness to share and put all those details out there and really take this open science approach to the work that you're doing in the hopes of trying to lift everybody up and really advance and move this field forward. Can you maybe comment specifically on the impacts of The Michael J. Fox Foundation — some of the resources and initiatives that the organization has — whether it's on your research specifically or even just the field as a whole?

Judith: One of the aspects that a lot of these foundations are championing is the fact that a lot of science is not as open. There are large studies that are published, but the data is not available openly. And if the data is of good quality, it can be used by people over and over again to find new things, particularly mass spec data is so rich that you can mine it in many different ways. And not everybody has the same way of mining data. And having the data open source is really important. And I know that The Michael J. Fox Foundation and Gates Foundation are

championing the idea of opening tools, open resources, and collaborations to move the field forward. And I am super excited about this aspect of the research.

Marie: Well, Judith, I know as we spoke about throughout our conversation, there are a lot of unanswered questions that remain and a lot of exciting research being done in the field. What do you see as the most promising, perhaps future directions or areas of opportunity in Parkinson's disease research?

Judith: So, one of the things that I believe we should be working on is cell models to reflect the human disease. Because before we can get into a mouse or a model that is in vivo, we have to be able to have cell models that reflect the human disease. And currently, there are no funding opportunities to develop those cell models. And I think that it's going to be super important if we're going to do high-throughput screens to really understand disease.

Marie: Well, Judith, I agree that developing these cell models, these tools that can be used by many different labs can have huge impacts on the field. So, I'm really glad you mentioned this. And I've enjoyed our conversation. To wrap it up here. I'd love to end with just talking about how your work is bringing us closer to finding a cure for Parkinson's or contributing to improved therapies for people with Parkinson's today.

Judith: I think the most important aspect of our work is that we do quantitative biology in human disease. And we have robust methods that can be used across large patient cohorts, such that we can get information about how the disease progresses, how the proteomic pathways change during the disease progression in order to understand the disease. And then we can use that information to build cell models for high-throughput drug screening and use the assays that we developed for the human tissue characterization to study the mouse models and see which ones reflect the human disease. And then have models that we can do preclinical studies in to move forward the therapeutic aspects.

And then use the assays like FLEXISyn or FLEXITau or any other FLEXI assay to monitor large plasma or CSF in the human patients such that if we are testing a particular drug, we can see target engagement. We can see disease progression changes in the blood of the patients that we are treating so that we can understand how patients are responding to the targets and the drugs that are being used in the clinical trials and understand whether or not the disease is regressing or still progressing using particular assays.

Marie: That makes sense, Judith. And we truly appreciate all of the work that you're doing in the area of Parkinson's research. It's been such a pleasure to chat with you today. So, thank you so much for joining us on the show.

Judith: Thank you, Marie, for all the questions and for your interest in our work.

Marie: Well, Judith, it's been great to chat with you. And listeners, it's been great to have you here with us as well. If you want to know how The Michael J. Fox Foundation can help your research, please visit michaeljfox.org/researchresources. And you can find new episodes of this show each month on the MJFF website or on your favorite podcast platform. When you have a moment, listeners, please subscribe to our show to make sure you don't miss our outstanding lineup of upcoming episodes. We look forward to connecting with you again in our next episode of *The Parkinson's Research Podcast*.