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 that we share will advance your efforts and partnerships to improve brain health. We are welcoming guests with a range of experiences and viewpoints. The views expressed belong to the guests themselves.

Today, we are excited to be welcoming our guest, Dr. John Seibyl. So, listeners, John is Chairman of the Board, Co-Founder, and Senior Scientist of the Institute for Neurodegenerative Disorders in New Haven, Connecticut. He is also a board-certified physician in both Psychiatry and Nuclear Medicine, and he is an Adjunct faculty member in Neurology at Yale University School of Medicine.

Today, we are excited to talk more about his work using neuroimaging and biomarkers to improve diagnosis and assessment in patients with neurodegenerative diseases, such as Parkinson's disease. So John, welcome to our show today. How are you?

John: I'm doing well. Thank you for having me.

Marie: Well, thank you so much for joining us, and we're looking forward to getting to know more about you and the wonderful work that you do in our conversation today. But perhaps we'll start with you. Can you tell us a little bit more about your background, John, and how you found your way to your current positions?

John: That's a long and winding road, I'm afraid. I'm a physician, and I trained in psychiatry at the Yale University School of Medicine, then joined the faculty at the School of Medicine in the Department of Psychiatry. And about a year or two after I was an assistant professor, my chairman came up to me and said, John, we need someone who can do imaging for the department. We have these research studies, and we don't have control over the cameras. We don't know how they work. So, I want you to go back and do a fellowship in nuclear medicine.

Well, I was a young assistant professor and very eager, if not stupid. So, I jumped at the opportunity, and I spent two years in the Nuclear Medicine Department at the Yale New Haven Hospital and learned to do imaging. And after

that training period, a fellowship of two years, I sort of liked the imaging. I liked taking pictures of the brain. I liked diagnosing different diseases using these interesting techniques.

And so I stayed in this department of diagnostic radiology as an assistant professor, and eventually moved up over the course of four or five years to become the chief of the section of Nuclear Medicine at Yale. And then in 2000, my colleague and I, a neurologist with a specialty in movement disorders named Ken Marek, and I decided that we wanted to start something outside the university that would allow us a little bit more freedom to move faster for these large research trials that we were doing. So, we founded something called the Institute for Neurodegenerative Disorders. I know it's a handful to speak, so we just reduced it to IND. And that took off over the years.

And we also started a company called Molecular Neuroimaging. And that was a company that was designed to use imaging trials to help understand how drugs work in the brain to help determine if a patient is eligible for a clinical study because it helps confirm the diagnosis. And those two entities went along for about 15 or 16 years. We grew very much over that time. We had 120 employees. And then the company was bought out by Konica Minolta. And I retired from that company and then continued to work at the Institute for Neurodegenerative Disorders, or IND, where I'm the Chairman of the Board now. One of the interesting twists that happened about 11 years ago is, I developed Parkinson's disease, which is something that has really changed my life dramatically in all sorts of ways, which I'm sure we'll talk about as we go along.

But currently, I'm semi-retired, although my wife doesn't believe that I am. And I'm writing papers, doing the fun stuff, not supervising anyone, but having an enjoyable time really engaged in the research that I've been doing for some 30 years now.

Marie: Absolutely. And I think, John, the Institute for Neurodegenerative Disorders, IND, is a remarkable institute. So, for people who aren't familiar with it, can you paint a picture of what it looks like now?

John: Sure. IND was founded to do research studies, primarily clinical trials in Parkinson's disease. Although we do some — a little bit — of Alzheimer's, but not that much. And the idea was to have a convenient place for research participants to come, where they would be taken care of and treated well, because as researchers, we're entirely dependent on patients to participate in the research endeavor to push the envelope and to really make the new discoveries and to test out the new medications that come along.

So, we tried to create an entity that was very forgiving and comfortable for patients who were considering research. And what we offered is, we don't charge. We offer full evaluation with neurologic exam and imaging if need be to understand the diagnosis, and then offer the opportunity to participate in a trial. You don't have to. But in order to stay with IND over the long run, you'd have to be in a trial. And we had remarkable success in that our patients are extremely loyal, and they participate in these studies.

In Parkinson's disease, you really have to study over the long term. You're looking for changes that take place over years. It's fortunately a very slowly progressing disease, but it is progressing. And we're trying to understand how medications might slow down that rate of progression.

So you've got a slow rate of progression, then you're slowing down the slow rate of progression even slower, which means in order to see a signal change, or to see improvement, or to see any effect of medication, you have to study people for a long time. And that's why we try to make it as easy as possible for people to take advantage of the opportunity to participate in research. We arrange hotels if they need to be. We arrange flights if they're coming from out of town and really have an experience that they more or less enjoy. And we've had great success. People come back. They discovered that, it turns out in Connecticut, there's these gambling casinos up the road from us. And so they come and they make a little holiday of it. They go to Foxwoods and enjoy that, and then come down for the day.

And they also develop relationships with other patients who are participating and happen to be scanned, or treated, or evaluated at the same time that they're being evaluated. So, they arrange to come back together if they're coming from across the United States or Canada. And they develop these long-standing friendships. So, it's very nice. It's like a big family of people who are very motivated to the research, to the clinical trials, and our retention rates are extremely good as a result of that.

Marie: Absolutely. And it sounds like the Institute really keeps the patient or the participants at the forefront. And I think that is so important. And perhaps you have had a role in that as well. So, I'd love to talk about your experiences and just how this Parkinson's disease research is personal for you. As you alluded to, 11 years ago, you were diagnosed yourself. So, from, I guess, an intellectual perspective, how has Parkinson's disease maybe informed your experiences as a patient? And then perhaps on the flip side, John, how has being a patient then informed your work as a Parkinson's investigator?

John: The main point is that having Parkinson's disease has informed my research more than doing research has informed my Parkinson's disease, which is sort of

interesting because I would have predicted the other way if I were ever imagined that I would have this sort of unique position. When I was a fellow in nuclear medicine, my mentor, who was a nuclear medicine physician and Chief of the Section of Nuclear Medicine at Yale, had Parkinson's disease. And we did a scan where one of the first scans with a drug called beta-CIT is very much like DaTscan. It's a little radioactively labeled molecule that is an analog of cocaine. It gets injected into the bloodstream.

It goes up into the brain and produces an image that shows you if there's some loss of cells or loss of the target sites for the radiopharmaceutical to bind to. And he had one of the first scans and we looked at it on the computer together in the basement where our cameras were. And it was extremely abnormal. And he looked at that scan and said, this is really interesting. I looked at him thinking, my God, he's looking at a terrible-looking scan showing the destruction of nerve cells in his brain. And he's looking at it from an intellectual perspective.

But now, he's passed away, but many years later, I'm sort of in the same position where I'm looking at my physical symptoms that are slowly progressing. Fortunately, I have done well over the 11 years. I have a tremor, which is primarily my problem, but I've done reasonably well. And I look at it from an intellectual perspective to try and understand what that symptom is about. Why does it occur now? What do I know from my scientific background that informs me to help me understand what's happening with the particular range of symptoms that I've developed? So, why some come, why some go away?

I wake up in the morning wondering about what will be a challenge today, physically. In terms of the other way around, how have my symptoms or my Parkinson's disease influenced my science? I think they've influenced it a lot. They've made me think of this disease as more than just tremor, rigidity, bradykinesia, gait disturbance, which are the classic signs of Parkinson's disease. To understand it, it's a much more complex and much more engrossing, engaging cluster of symptoms than just the motor symptoms.

In fact, the non-motor symptoms, that is, the non-movement symptoms are sometimes more of a problem than the motor symptoms are. Constipation, sleep disturbances, all sorts of things that I was surprised to have enclosed in the package of Parkinson's disease, as it expresses in me. I think the other thing that it has done is made me think more about life in general and more about how I want to conduct my semi-retirement. It's recharged me. It's energized me. I feel like when I get up in the morning, I'm working to cure the disease that I have. I think that's a great blessing in and of itself. I wonder about my future, and of course, as all patients do, that of my caretaker, my wife, who's an incredible partner in this process and with whom I'm lucky to have her just to help me.

I think of this as something that has been very negative in some ways, but has really charged my energy to devote towards some of the research enterprises that we're undertaking and has also given me a new sense of patience, I guess, because I can't do things as I had done before. If I can do them, I do them much slower. And I just have to realize that this is par for the course. And that's been a little hard for me because I've been a go-getter academic physician who runs around a lot, goes around the world to meetings, that sort of thing. I've realized that I have to have almost spiritual levels of patience in order to cope with the things that I can't do anymore. So, while there's been a lot of bad in having a disease that is progressive, there's also some upside to it in that it has really changed my thinking and has made me a little more of a complete person who thinks in a broader sense because I can't just run and do the things I used to simply do. So, I write poetry about Parkinson's disease as a great outlet to help me sort through what's going on with my body and what will happen over the future. And I write scientific papers. That's a strange combination, but I think it actually works for me because it gives me both a scientific and intellectual outlet, as well as an emotional and psychological outlet as I deal with this problem.

Marie: Well, John, I'm so glad to hear that you found this creative outlet through poetry, and I think that's wonderful. And I know your experiences have brought unique insights, I think, into Parkinson's disease and maybe what some of the most important or most pressing issues are that need to be addressed. So, can you talk about how your experiences seeing things from the patient side of things have informed how you developed your research priorities and maybe how you conduct your research?

John: I guess this made me more mindful of time. It also has made me more focused on getting the papers out, staying up a little late in order to get that little bit of data analyzed. It's sort of given me an energy for the enterprise, which I had to some extent, but I have it much more now because I've got a little bit more skin in the game.

I think that in terms of neurodegenerative diseases in general, and by that I mean primarily Alzheimer's disease, we're in a very good place that there are now treatments that are FDA approved that have shown in Alzheimer's disease to slow down the progression of the disease. The primary target has been a protein called alpha-synuclein that accumulates in the brain in Parkinson's disease. In Alzheimer's disease, it's beta amyloid (Aβ) and tau. And for the Alzheimer's disease treatment regimens that have looked promising and have been approved, these agents actually remove this Aβ protein. And as a result, there's some slowing and even some mild improvement in symptoms. It's pretty modest. But if you were to use the word “disease modification” in Alzheimer's disease 10 years ago, that would be an oxymoron. This just wouldn't exist.

Now we're talking about modifying the disease in Alzheimer's disease. And the imaging has been a very important part of that, just as the imaging has been important in Parkinson's disease, where we have the same sort of idea where there's an abnormal protein that accumulates. It's called alpha-synuclein, as I mentioned. It's a different protein. It's in a different area of the brain. But like the other proteins that are aberrant and abnormal in neurodegenerative diseases, it causes havoc with cells. It makes them dysfunctional, and it kills them. And so, it's the same sort of idea.

And the imaging helps to identify those people who have those changes happening in the brain. Maybe it'll happen a decade or 15 years of changes in the brain prior to the motor symptoms developing, prior to the tremor and the stiffness. And that's because the brain is a remarkable entity. It can compensate for all sorts of things, including a process that is causing neurodegeneration without showing any signs or symptoms until you reach a certain threshold at which point you start developing visible signs, and it progresses from there.

But the imaging picks that up ahead of time. If you can only identify those who will have that abnormal protein, you could theoretically get them on a treatment that is disease-modifying and can interfere with the pathologic process of this protein accumulation in the brain and then even prevent Parkinson's disease. That's the great dream. But it's not that far off, I think.

I think that there are some very promising treatments that will attack this protein abnormality and have the potential for either slowing down or stopping the progression. Unreal until it happens, until it's been demonstrated. But we're really developing all the tool sets now with imaging to line up our ability to track this.

But the idea that we can intervene and identify patients in some form or fashion, maybe with a blood test which picks up the protein or currently there's a very sensitive cerebrospinal fluid test called SAA, seed amplification assay, which really was a dramatic discovery that took place over the last year that can identify, with a high degree of sensitivity and specificity, those people who will have or who have Parkinson's disease because they have this abnormal protein accumulation in the brain, which has been a game changer. It's made us think about how we need to sort of rethink out how we diagnose Parkinson's disease. We don't diagnose it anymore on the basis of motor symptoms, of movement problems.

We diagnose it on the basis of the accumulation of this abnormal protein. It's a biologically based diagnosis, which can happen years before the motor problems happen. And as a result, will allow us to have a much more effective intervention. So, this is what's exciting to me and what's exciting to the field right now, this new amplification assay. The question is, how is it going to be implemented? How are

we going to use clinical trials, and what are the best algorithms combining imaging with this assay to identify those patients who are not patients yet, but are certainly at risk if not they have the pathologic process of Parkinson's disease and how it tracks over time with an intervention of medication, or an antibody, or treatment that is aimed at reducing that protein load in the brain, and therefore reverse the processes of Parkinson's disease progression.

So, it's quite amazing. And as a patient, I don't think I've ever been more hopeful over the decade or more that I've had Parkinson's disease than I am now, knowing that as I progress, that there's less to salvage, but there's still some to salvage. And I would be happy if my symptoms didn't change for the rest of my life. I would take that in a flash. Or if I progressed slower, I would take that in a flash as well. Because up to this point, the symptomatic treatments are great, but they're just symptomatic. They're not perfect, and they have side effects, and all sorts of problems, but they don't alter the course of the disease. And this is what we're looking for — new treatments alter the course of the disease.

Marie: Absolutely. And John, I'm really glad you laid out some of this big picture context. And I absolutely agree with you that seeing the progress in the field in recent years is really inspiring and exciting. And perhaps we'll hone in specifically on the imaging work that you and others in the field have done. So, can you talk a little bit, John, about how research using imaging biomarkers in these neurological conditions, such as Parkinson's, has really changed or evolved over time?

John: Sure, I'm sort of the long haul guy in this process. I've been laboring in this vineyard for a long time — for thirty plus years. And as I mentioned, we did some of the first imaging of the dopamine transporter. That's the little target in the brain that we image with our scan. And the way it works in nuclear medicine scanning is that you receive an injection of a molecule which binds to that particular receptor or that target site, the dopamine transporter, and then it sticks there and undergoes radioactive decay and the camera picks up and makes a picture of where the photons are coming from.

So, it's like a light, but different wavelength of photon coming out of the brain. And the spectra, the PET camera can reconstruct it. And you get a 3-D volume of the brain where material has accumulated. And it accumulates very highly in what's called this striatum. It's a region of the brain that's sort of in the middle, and it helps control and make adjustments to the fine motor movements that we make in walking, or picking up a spoon, or whatever we do. It helps make that a smooth process. And when you start losing cells, you interfere with the function of that. It's called the nigrostriatal circuit. And as a result, develop symptoms.

So, we can tell from the scan, there's a hole in the brain basically where the material doesn't stick, but it should be sticking. And we can tell how severe

someone is. So, for example, in some trials, including the Parkinson's Progression Markers Initiative trial, in the earliest study with the initial cohort, these were patients with a very early symptoms. They had only six months with the symptoms. None of them were on medications, or very few, I think, because they weren't severe enough to be on medications. And when we scanned them with this test, which picks up these dopamine transporters in the brain, they had about 50% loss compared to an age-matched group of patients without Parkinson's disease, or healthy volunteers.

So, very dramatic changes that you can see. So, it turns out that the imaging is much more sensitive for picking up these changes than the clinical examination because when you first present with Parkinson's disease, a great bulk of people have symptoms on one side of their body. I, for example, have symptoms on my right side. Unfortunately, I'm right-handed. My left side is symptomatic, but it always lags behind and is not as severe.

So, I do a lot with my left hand as well as my right because I'm less affected by tremor and some of the problems that my right hand has. But that asymmetry you can see on the scan before you can see it clinically. So, when patients present, they'll have symptoms on one side of their body. When we do a scan with this dopamine transporter agent, it shows changes on both sides, with the side opposite of the symptomatic side most severe (because the right side controls the left, and left side controls the right in the brain at that level). And the side that has no symptoms, we see changes that are not as bad as the other side, but there's still dramatic changes.

So, we knew that imaging was really sensitive for picking up these changes. And when you have a clinical trial, many are done with very early patients because those are the patients who have the most brain function to salvage, if you will, for whom you expect to get a good demonstration of the efficacy of the intervention that's designed to slow the progression of disease. So, these are very early in their disease course. And we consistently show a lot of changes.

The other thing that the imaging has done for us is that it's hard for even a movement disorders specialist to make an accurate diagnosis in some cases of idiopathic Parkinson's disease. In fact, at the earliest stages, the clinical studies have shown that about 90% of patients are diagnosed correctly based on a follow-up of a year or two years, and the gold standard being clinical diagnosis. But 10% are not. And that's not because the neurologists are not good. It's because the symptoms are so subtle and overlap with so many other things that you can't tease them apart very readily.

So, when we did these studies 20 years ago for enrollment to decide who could go into a clinical trial, we made the rule that you ought to have an abnormal scan

in order to be clinically enrolled in the trial because if you had a normal scan, you wouldn't show any changes because you don't have the changes that the medications are designed to fix. So, when we first reported the imaging, many of the movement disorder specialists were a little bit upset with me because I was telling them they're the gold standard. They're the experts which they are. And I was telling them that they were wrong in some 10-14% of the cases that they felt had idiopathic Parkinson's disease.

So, we knew that in order for this to fly, we had to make up a name for these patients who had normal scans, but who movement disorder specialists felt that they actually had Parkinson's disease. So, we called them SWEDDS, that's scans without evidence of dopaminergic deficit, which is a big term that just means their scans were normal. And we didn't say that they didn't have Parkinson's disease because we couldn't say that since we're not the standard against which that diagnosis was rendered. It was the movement disorder guys.

But what we did say, I'd go to meetings and people would say, oh, Dr. Seibyl, your scan is just not sensitive. I'm the movement disorders specialist. And I say that he has idiopathic Parkinson's disease. So, I said, again, being patient, I said, well, let's just follow these patients up over time and see what happens. If they truly have Parkinson's disease, they'll develop changes because it's inexorably progressive. The changes are going to occur.

And we followed them up. And lo and behold, the SWEDDS' scans (patients with normal scans, but initial diagnosis of PD) didn't show changes in their clinical symptoms over time. They didn't need to go on medications. They didn't show changes in their scans over time. The bottom line was they didn't have Parkinson's disease. And so, that was an important study because it now made using dopamine transporter imaging for eligibility in the clinical trial an important component of studies.

So, I think there are very few Parkinson's disease clinical trials that are done now that don't use dopamine transporter abnormality as a criteria for enrollment in the study. Very important because if you threw all those subjects in, including those that have normal scans, you sort of dilute down your ability to find an effect of the medication because you're putting in a lot of noise into the data. And you don't have the ability to tease things apart and identify drug effectors, which may be there. So, this is now de rigueur that it's absolutely normal, if not always done, that dopamine transporter imaging happens as a result of ensuring the appropriateness of the patients for the trial. It's also, by the way, not very ethical if you have a way to identify those who do and don't have the disease that you're trying to treat, and you don't use it, and you treat someone with an investigational drug who doesn't have that disease. It's not very good.

So, I think that for all sorts of reasons, imaging has become really important in that capacity as well. But in addition to identifying those who are eligible for disease for enrollment in the trial, you want to monitor the changes over time. And this has been more challenging because as I mentioned, the changes in the brain are very slow. You lose more dopamine transporters as time goes on, but it's about 8-11% per year, which is a pretty tiny change. And in order to study something with some power, a drug effect that slows down the progression by 50%, say, so instead of 8-11%, you get 4-6% per year. You then begin to get to the limits of the sensitivity of your testing over the course of a year. So, you need to go longer periods of time and/or you need to add participants so that you have more of a statistical power in your sample size to demonstrate the changes that you need to demonstrate.

So, imaging was thought to be a good way to lower the requirements for the numbers of patients, and speed up the trials as a result, or shorten the time that you need to treat a patient because the imaging is more sensitive to changes. And that's true to some extent, but it's been a little bit of a problem because it turns out that the normal variability associated with the test is about — the standard deviations about 100% of the mean. There's a wide variability in patients in terms of the rates of change. And some of that's clearly biological because people change/progress at different rates. And it's not known why, but it's just a fact that clinically they progress at different rates. Some are very, very slow. Some are very fast. And we don't have a good handle on that.

The result is the imaging is probably the most effective way we have right now, of monitoring the changes over time. But it's still not perfect, at least as it currently stands, because one of the things that we pull from the images is not just a pretty picture, but we pull a quantitative number. And that number is what is used to evaluate the change. So we might see a 7% change, 10% change, a 20% change, but it's always expressed in a quantitative fashion.

I'm pretty good at looking at scans and visually reading them, but I would never read a scan for progression because I can't tell a difference between a 15%, or 20%, or 25% decline in signal in the brain from one scan to another. It's just too subtle. And that's why the quantification is important. So, those are the two ways that imaging has been used. Now, as time has gone on and we develop this new alpha-synuclein assay, turns out this assay is more sensitive than the imaging. Because what the assay is pulling up is the presence of these abnormal proteins called alpha-synuclein, which have been distorted in their shape, and as a result, cause destruction of nerve cells.

The alpha-synuclein protein is something that was discovered only about 20 years ago and has been shown to be present in what the pathologists see under the microscope when they look at a postmortem examination of a Parkinson's

disease patient's brain, something called Lewy bodies, which are these little clumps of alpha-synuclein and other things inside cells that are the hallmark or the pathognomonic ideal of what Parkinson's is. If you don't have Lewy bodies (it's a Lewy body disease), you don't have Parkinson's disease. So, we know that Parkinson's disease spreads in a particular manner. There have been some very elegant postmortem studies that have been done by a German investigator named Braak who looked very carefully at where the Lewy bodies were in the brain in patients who had passed away and had different degrees of Parkinson's disease, from mild to very severe.

And he developed this algorithm based on this cross-sectional data that the disease starts, more or less, outside the central nervous system. It goes into the lower parts of the brain stem and into the midbrain, and then it extends up into the cortex over time, which is the main area of the brain. But it follows a pattern, and the patterns may be due to some amazing things that have been discovered in Parkinson's disease. Two observations, one is that Parkinson's disease, the alpha-synuclein abnormal protein may actually come from the GI tract.

So, the gut and the brain may be connected in all sorts of ways. They certainly are connected embryologically because the nerve cells derive from the same source in the embryo who's developing into a fetus. In the brain, you have something called vasoactive intestinal peptide. And in the gut, you have some of the same neurochemicals that are found in the brain because they share original primordial cells.

But the theory is that alpha-synuclein is formed in the gut because of the particular environment, the microbiome that is conducive to causing alpha-synuclein, which is down there, to misinform and become aberrant. It gets taken up into the vagus nerve, which is a nerve that runs from the brain to control the motility of the bowels and other things. And it goes retrograde, it goes backwards up to the vagus nerve into the lower brain stem and subsequently gets into the olfactory area where smell is important. It gets into the substantia nigra, which is the cells that project up to the stratum. And it follows this course of spreading and amplifying itself.

And so what an abnormal alpha-synuclein fibril does is it goes into the cell, and it forms like a template. And so other alpha-synuclein fibrils that are floating around within the cell bind to that template, and they get misfolded. And so, they keep attracting other ones, and they misfold and misfold and misfold until they develop enough of accumulation that they have a Lewey body. But they spread across from one cell to the next through the connection between cells, which is called the synapse. So, you get alpha-synuclein being propagated and formed into Lewey bodies. And some of it goes across the synapse, from one cell to the next, and passes along that abnormality.

So, it makes sense how the spread occurs because of the networks that are fairly well understood. On the other hand, the symptoms that people have and the course of the symptoms also follows from this pattern described by Braak. So, one of the earliest signs of Parkinson's disease is not movement problems, but it's loss of the sense of smell. And olfactory loss is not uncommon. People get it with head trauma, and they get it with chronic sinusitis and other things. But it's pretty clear that most patients with Parkinson's disease have smell loss, making some people think that the initial olfactory areas and even the olfactory bulb, which is right next to your nose and in your head, there may be some alphas and nucleon that crosses over from there and affects the sense of smell.

And so, one of the screening tests, I mentioned earlier that it's hard to identify patients who could be scanned to demonstrate the changes that you would expect to see if they had Parkinson's disease. But one of the screening tools that we've used is a smell test. It's a simple scratch and sniff smell test. It's called the UPSIT, University of Pennsylvania Smell Identification Test. And we've done studies where we've sent literally hundreds of these tests out to people to test their own sense of smell. And you get this little card that has a little scratch area, and you sniff it and you identify, is it bubble gum? Is it gasoline? Is it barbecue? Whatever the token is.

And there's, I think there's some 30 items on the test, something like that. And the test is sent back and scored. And so, based on the smell test alone, we've identified people who have an abnormality. And we've invited them to come to New Haven, Connecticut, for an all-expense-paid, lovely visit to IND where they received a clinical evaluation, a motor examination, and a scan. And what we found was that a certain percentage of those who had no symptoms at all of motor problems of classic Parkinson's disease, but who had an abnormal test, also had an abnormal scan. It wasn't abnormal enough to cause symptoms, but it was clearly identifying them at risk for developing the motor component Parkinson's disease.

And so, that became really a very interesting way to enrich clinical trials for those patients who would be at risk for the motor symptoms and who probably have Parkinson's disease, which is not manifest at the present time. The other very sensitive measure, it turns out, is sleep disturbance as well, because the areas that control sleep are in those lower and mid brainstem regions. And normally when one sleeps, you go through certain stages, stage one through four, and then you enter into what's called REM sleep, or rapid eye movement sleep. And it's in rapid eye movement sleep that you dream. But because of the disturbance in the sleep centers in early patients who may have Parkinson's disease, they dream in stage four. And when you dream in stage four, not in REM sleep, the difference is that it's like the clutch is not engaged in the vehicle. So, you have a

dream, and you act it out with your hands, or your arms, or your feet, or whatever. In REM sleep, you're essentially paralyzed. Your brain is doing all sorts of things, but your body's not responding.

In stage four, your body responds. And so, people have these very tortured nights where they're twisting and turning. And generally they're not affected so much as their bed partners, in fact, who may describe being sometimes kicked out of bed or pushed out of bed as their partner dreams and acts out the dreams. So, those have been two potent ways to identify people who are at risk and who could maybe benefit from having a scan to identify if they do indeed have a dopamine deficit consistent with the Parkinson-like process. The other thing I'd say is that the sleep is not entirely specific to Parkinson's disease. The sleep disturbance is also seen in dementia with Lewy bodies, which is a disorder that presents with both motor symptoms and also with memory deficits. But it's similar to Parkinson's disease in that it is an alpha-synucleinopathy, or it is alpha-synuclein that is the abnormal protein, one of the abnormal proteins, that accumulate in the brain. So, there's all sorts of these tools that have been developed for clinical trials. And imaging is just one of the tools.

And the thing about tools is they're always improving and always changing. And they've changed dramatically in the last two years or three years. As we've looked for new targets in the brain, we'd like to, for example, image alpha-synuclein, because that again is the primary pathology, it's the primary cause of Parkinson's disease. It is, like all neurodegenerative disorders, what's called a proteinopathy. That means that bad protein accumulates in the brain for different diseases like Alzheimer's and ALS or Lou Gehrig's disease. For Parkinson's disease, the proteins are different, but it's the same idea in terms of the identification of patients, the management of patients with protein-affecting treatments and the monitoring of them with biomarkers (imaging and other biomarkers).

So, that's sort of the waterfront as I see it in terms of how imaging is used. And as new tools are developed, like we're very close to getting an alpha-synuclein tracer. There have been some suggested, one originally came from a company two years ago called AC Immune, and it looks like it may be good for multiple system atrophy or MSA, but not so great if you have Parkinson's disease because the alpha-synuclein in MSA is a little different than the alpha-synuclein in idiopathic Parkinson's disease.

So, maybe a marker for that, although the data is still coming in. But the point is we're getting close to getting a target, something that will allow us to measure alpha-synuclein, and more important, quantify it. And even more important than that, identify which regions of the brain it's in. So we'd be able to do what Braak did in staging patients in post-mortem studies, but in a living human because the

imaging gives us that same kind of information, but we don't need a post-mortem sample to do it. We can do imaging and achieve what we hope would be a very similar sort of endpoint or ability to detect whether the patient has the disease and how severe it is, and whether the symptoms that they're having correlate with the areas of the brain that we see abnormality.

So, it's been a struggle to get these new tracers. There's always a development phase, but they're coming along as well. And I think we'll be in a good position with some very powerful sets of tools as the imaging represents when there is a putative potential disease-altering treatment for the disorder.

Marie: Definitely. And John, I'm glad you mentioned this idea of developing a PET biomarker for alpha-synuclein. I think this imaging biomarker has been a hot topic in research. So, can you go into a little bit more detail about why has it been so difficult to develop an alpha-synuclein radio tracer for Parkinson's disease? And maybe where are we in this process? What have been some of the steps going towards it?

John: That's a great question. I ask that often. Where are we? I wrote a piece for the *Journal of Nuclear Medicine* last year entitled, "Alpha-Synuclein PET and Parkinson Disease Therapeutic Trials: Ever the Twain Shall Meet?" because, as you point out, it has been difficult. And it's been difficult for a number of reasons, not the least of which is, it turns out that the density of the target and how concentrated those binding sites are allows you to image more readily if there are just more binding sites versus relatively low density of binding sites.

And for alpha-synuclein, the number of binding sites is smaller than for other proteins. like in Alzheimer's disease for Aβ or tau. And so as a result, you have to have a radiopharmaceutical that has very high sensitivity or very high affinity for binding to that target. So, the affinity is basically, how well does it stick to the tracer? Does it bind really tightly? And if it binds tightly, that's good because you'll always get a little background uptake, a little radioactivity that's in the bloodstream or in the soft tissue that will reduce the signal size because that's not binding to the protein that you want to measure. It's just causing a little bit of noise.

And the lower the density, the lower the target to background ratio is. The higher the density, in general, the higher, unless you have a very high-potency tracer. So, getting these tracers that have high affinity is one thing, but also getting a tracer with high specificity is important as well. And specificity means that a tracer will bind to alpha-synuclein and not much else, or nothing else. But some of the tracers that have come along bind to alpha-synuclein, they bind to a little bit of monoamine oxidase-B, they bind to a little bit of Aβ. So, they haven't had the

specificity that allows you to tease out the targets that you're interested in because of the overlap in terms of their binding.

Again, this is getting better. There's been great effort put in, by particularly The Fox Foundation, in funding the alpha-synuclein projects, which has really moved the field along very quickly. Where backbone structures are identified by chemists, structure-activity relationships are assessed via computer models, and the tracers are tried in initial humans and see how it goes. So, there's a machinery in place to move these candidates through. And every failure, we learn something. And believe me, we've had lots of failures for all the targets that we started out looking for over the years.

But I liken it to the wandering lost in the desert of failed compounds. We're wandering around now looking at different backbone structures, different addendums that you make to the molecule to change its imaging properties to make it more penetrant into the brain. But you don't want it to be too penetrant because then it hangs around in the off-target areas. You want it to be high-affinity. You want it to be very high-specificity. You want to have what's called a good specific activity, which means that the amount of mass dose of the tracer is very low compared to the amount of radioactivity so that it can be safely administered without any pharmacologic effect.

So, there are a number of criteria that you need to fulfill. And alpha-synuclein is a tricky target for the reasons that I mentioned, but I'm optimistic. And I think that we've got some good leads. There's some imaging compounds that are under evaluation right now. And we're going to hit on this because we're getting close. And I've seen this happen with other targets over the years where we wander around a little bit until we kind of hit on something, and then we quickly improve it and end up with something quite good and useful for the particular question that is being asked of the radiotracer.

The other thing that's happening is that there are more MR studies that are looking at functional measures. MRI is different from PET or SPECT imaging in that you don't inject a radiotracer. But what you do is you put the head in a big magnet, and the magnet sort of orients the mostly water molecules in a certain direction. When you turn the magnet off, those water molecules go back to the random organization. And as a result of that, they send out a little radio signal. So, basically it turns your brain into a radio station, or a thousand radio stations, or thousands of thousands of radio stations. With that signal, it creates a very high-definition, high-resolution image of the brain or some functional process like blood flow or a neuromelanin which is accumulated in this nigrostriatal tract that’s important in Parkinson's disease. So, that looks promising as well.

One of the challenges of doing imaging is that you want a quantitative measure, and you want that measure to have good test-retest reliability. It should be reproducible in the research volunteer, and that it should be able to be done consistently across different centers that have different cameras and have different techniques and different ways of doing things. And so, there's been a lot of effort that I made over the last 12 years in standardizing the imaging across centers. We developed some techniques to go to centers with different SPECT cameras for dopamine transfer imaging. And we would take with us something called a phantom, which was basically a model of the head that had two areas in the brain that you could fill up and a background region could fill up. So, it looked like a regular scan when it was filled, and it had the same attenuation properties of bone. So, you had this skull that produced a very realistic or anthropomorphic image of the brain that you're interested in.

So, we use that by imaging at one center versus another center and making corrections so that every scan from different centers produces the same number from the phantom. Just as an aside, when you travel in Europe with what looks like a head, and you put it through the X-ray machine, you get a head, and people always stop you.

Marie: Security's interested. Yeah.

John: Essentially, the different countries had different responses, as you'd predict. In Germany, when we would travel with this phantom, and they'd take it through the X-ray, they'd come and pull you aside. They were very serious. They said, what do you have here? I said, it's a $10,000 piece of plastic that allows us to calibrate special X-ray machines called SPECT cameras. And they would say, they want to look for some documentation, some paperwork, and they would be very officious and very formal and all.

When we traveled in Italy, it was completely different because at the time we were doing it, the prior Pope had Parkinson's disease. So, I would say that this is a phantom. It's called a phantom. It allows us to scan the camera and calibrate it so that we can study the disease that the Pope had. And the face of the security person lighted up. He said, oh, the Pope! He took the head up and he put it on his shoulder and had his friend take a picture of him with two heads. Then he showed it to the other friends. They had a wonderful time just enjoying this silly little piece of laboratory equipment, which is so important for our research. So, we have these ways of calibrating cameras and making sure that we get a robust and reproducible signal that's reliable in multiple centers around the world. And that's what IND basically did for the PPMI study. We had to develop these techniques so that we could use these tools to do the large trials that are necessary to demonstrate the changes that a potential effective treatment may have in the brain.

It took years to do it and great expense, but it's now a fairly mature process. And so, as I said earlier, we're really geared up in terms of our tools, which have been getting better to do these clinical trials and to get an outcome measure, which is going to be robust and help prove whether or not the medication is effective or not. Or the antibody or whatever the entity is that's putatively going to help the patients.

Marie: Absolutely. And I know IND is running some of the largest brain imaging clinical trials in the world, as you alluded to, for assessing neuroprotective drugs for Parkinson's as well as Alzheimer's. So, can you talk about what some of the sort of big achievements or accomplishments have been, or where are these clinical trials at in terms of the process?

John: Yeah, there's a lot happening in clinical trials right now. I mentioned the main issue is we now have tools that can identify pathologic changes in the brain early, but we need algorithms that allow us to do this efficiently. Because we can send smell tests around, and we can do sleep assessments, and that sort of thing. I mean, what we have done to develop what we formerly called “prodromal patients”. I say formerly because we're not calling them prodromal. We're calling them, if they have a positive alpha-synuclein seed amplification assay test, they have Parkinson's diseases, it’s a new algorithm that we've introduced into the field and sort of can't use the word “prodromal” anymore because they're not prodromal. They have the disease.

But developing those algorithms so that we identify, who do we test for a cerebrospinal fluid test? As it's not a super invasive process, but it's pretty invasive, and people don't like to have lumbar punctures. They would rather have a scan, but a scan is expensive, and there's more risk because there's the radiation associated with it. So, it's more complex and more expensive.

So, developing the algorithms are really important. And it may be that as the alpha-synuclein test develops, that we'd be able to use a blood specimen in order to test the positivity of the scan. There have been some blood assays using it, but it's been a little bit difficult to get the same reliability as the cerebrospinal fluid sampling because the blood has all sorts of extra proteins and other things in it that confound the test a little bit. So, that being said, there is some progress that's being made. Or people have looked at tissue biopsies for alpha-synuclein, including the cheek, the skin, the GI tract, different places that are amenable to having a little pinch of tissue taken and the assay done. But again, it's not quite as good as the cerebrospinal fluid measures, and we look for some improvement in those so we do have some easy screening measure.

And the model, I guess, would be in the real world, assuming that these studies hold up, we would, at age 50 or whatever, that you'd get a blood test or some simple test to see if you have a proteinopathy, whether that's the alpha-synuclein accumulation or whether that's tau, which appears in the blood or Aβ for Alzheimer's disease. It'd be an Alzheimer's screening blood test and a Parkinson's disease screening blood test, I would predict. And then from there, you decide if it's positive, who needs a scan in order to stage the disease.

And so, this has all come to reality. It's been talked about for a long time, but it's only been in the last year that people are actually doing something about it. And what we're doing is we're redefining how we refer to Parkinson's disease by describing it as a disease of alpha-synuclein accumulation that is made up based on the diagnosis of abnormal alpha-synuclein in the brain, measured via the cerebrospinal fluid or other way, if that becomes viable. And that it's also an abnormality of dopamine nerve cell deficits. And that those two biological biomarker keys are how we define the disease. And then from there, we have functional assessments, sort of a staging system, where we identify the impact of those changes on the patient's function.

And that came about because the FDA and patients and caregivers, for that matter, don't really care whether your Hoehn and Yahr is two, or three, or whatever. They want to know what's wrong with my body or my spouse's body, and what kind of functional problems are they going to have? What are they going to need me to do? Are they going to have problems falling? Are they going to have problems with tremor? Are they going to have memory problems?

Whatever it is, I need to have some sense of prognosis in order to gather my resources to best help this person. And I think that, by having an integrated biological biomarker and clinical staging system that meets those needs. It's also the needs of the FDA who look at these drug trials and say, okay, so tell me what the effect of your drug is that's relevant to the patient. I mean, you may treat with a drug, and it may change the scan to make it look normal or better, but if it has no effect clinically, then we don't treat scans. We treat patients. It doesn't mean anything. They want a functional measure. How affected is the patient as a result of having Parkinson's disease, and how much improvement or lack of progression they received following the treatment.

And so, we're now in sort of a new era, and it's an era that makes a lot of sense. Instead of having the eponymal names for diseases like Parkinson's disease, or Lou Gehrig's disease, or whatever, to refer to it based on its biology is important. And it's what the rest of medicine has done for years, but we haven't had the ability to do that in neurology until we have some ways to test the inciting pathology in the brain. And now we do.

And as a result, I think it'll make for cleaner diagnoses. It'll give caretakers and patients a better sense of prognosis over time because there's expected pathways that people will take in terms of their functional changes. And it will please the regulators as well. And the pharmaceutical companies where there's more standardization of the outcome measures. This is, we had to standardized how we get those outcome measures with the imaging. So, a very heady time if you excuse the pun.

Marie: I like it. Well, I know we are at this really exciting point, I think, in research as well as care for Parkinson's disease. And John, you mentioned PPMI earlier. Can you talk a little bit more about whether it's PPMI, or other tools, or resources, or research, or even collaborations that you think are really helping your work or maybe the field as a whole in Parkinson's disease research?

John: Well, I am the Co-Chair, the Chief of the imaging core of PPMI. So, I am biased in that sense, have been for 12 years. PPMI is an amazing study. It's really amazing. And it speaks to the power and the foresight of The Fox Foundation to fund this, some 13 years ago, when we were first getting started. I didn't have Parkinson's disease then when we started.

And I didn't understand the value of developing these tools for imaging biomarkers at the time because I was merged in the sexy studies where we're looking at new biomarkers, and new tracers, and other things, and didn't think that a naturalistic study where you enroll a patient in a trial and you wouldn't do anything. You just follow them. Over every six months, they come back and they'd get a scan and they get more frequent motor evaluations to see how the changes are happening.

This seemed a little bit ho-hum to me at the time. But then as the data started coming in, it was like a fine wine as it aged. It got even more and more useful and more remarkable because these patients had tremendous retention rates, number one. So, we were able to get longitudinal data, which is how you study any disease effectively by looking at how it changes over the course of its progression over time.

And that data was really good because people stayed around. The retention was so high, and they also allowed themselves to participate in all aspects of the study, which included the obtaining of cerebrospinal fluid with lumbar puncture over multiple time points. And when we first designed the study, we were worried that people wouldn't participate because they were concerned about getting a lumbar puncture. Who wants to get a spinal tap? It sounds terrible, although it's essentially a fairly benign procedure for the great bulk of people.

And as the data came in, and it was complete and thorough, we began to see certain trends, certain phenotypes, hoping that we'd be able to predict who would develop changes faster than others. And as the data continued to come in, we began to expand to look at genetic components that may predict progression, looking at LRRK2 patients, looking at GBA patients.

These are genotypes or certain genes that patients have that put them at higher risk for Parkinson's disease. And we were able to look at what we formerly called prodromal patients. But most important, we had this great database that was available online to all investigators, including the scans, that they could download themselves and analyze themselves, including cerebrospinal fluid, and other body fluids that they could request samples of and analyze them themselves.

So, like shareware, we got the resources. We had a lot of brain power working on these data sets that we collected so that we could tap into the creativity of the field of investigators to kind of push the bounds a little bit further than we could alone as being the sole investigators on the data that we acquired. And that really paid off this year because we had all this cerebrospinal fluid and patients who are incredibly well characterized because of their fidelity and returning for their repeat examinations. And we were able to take some 1,200 cerebrospinal fluid samples and analyze them all at once, which gave us an answer. If we tried to do that study a priori or without having the PPMI data, it would take years to do the study.

We would not know the answer that we know now. That this is a very exciting and potent assay and process that has great utility for the clinical trial process and, ultimately, for the clinical care process, as many of the techniques that we're developing in clinical trials will be applied to subsequent clinical care, patients once things become approved. So, PPMI has been just a great success. It's been an interesting collaboration between industry, and academia, and the foundation world. And I really applaud The Fox Foundation for their insights and their prescience in being able to fund a study like this and coordinate the great amount of work that's been done. And what we have as a result is this large shared data set, which is pushing along developments in Parkinson's disease much faster than it ever could have occurred if the old funding mechanisms and the old processes were in place.

I think what's good about The Fox Foundation is that they sort of treat the research process like a business where they're very specific as to what their goals are and action plans to achieve those goals, like developing an alpha-synuclein tracer, for example.

So, having that focus and that kind of business background makes for things to move a little bit faster than they might otherwise. So, it's just been a great ride

with those folks. And I'm so grateful for that. They saw that this was something of value because we're now starting to reap the harvest and take advantage of years of data that are now informing trials and processes that allow us to be more efficient and get to a drug that's going to be effective or even maybe a cure that we wouldn't have been able to get to otherwise.

Marie: Definitely. And John, we truly appreciate all the contributions that you have made in this regard and perhaps to wrap up our conversation today. I'd love to pick your brain and just understand what you see right now as some of the biggest unanswered questions, or perhaps areas of opportunity, in Parkinson's disease research today.

John: It's always good to think of areas of deficit as opportunities because they truly are. I think that there are a number of areas that we need to have a better sense of. One thing is, what is the ultimate value of the seed amplification assay going to be? Can we get it to be quantitative, for example? So, it's not right now, but if we look at patients over time, can we get a number that is somehow reflective of the degree and extent of the abnormal proteinopathy in the brain with alpha-synuclein? It's one of the reasons that imaging is complementary to the seeding assay is because imaging gives you location in the brain, and it also gives you quantitation to some extent. So, we can do better in terms of characterizing alpha-synuclein, either with improvements in the assay or improvements in development of an alpha-synuclein tracer.

So that's an area that is of current focus and of current interest. By way of analogy, in Alzheimer's disease, they do have a blood test for Aβ and tau, and imaging with Aβ and tau and FDA-approved tracers for this. So, they have laid a pathway that we can follow to some extent, although we're a little different in some ways, as how to use these biomarkers. But they're using both the serum assays and serum biomarkers and the imaging biomarkers in ways to characterize patients.

And then ultimately, how that gets incorporated into the real world, into real practice patterns of how we treat neurodegenerative diseases. It remains something that will be discovered in terms of who gets screened, what the best algorithms are for assessment over time. Is it going to be like at age 50, you get a colonoscopy and you get one every so many years to look for changes? Or you get a mammography with certain frequencies, and look for changes. And so, it will become a screening tool, and how do we use that screening tool? Will we be able to use imaging biomarkers or other biomarkers as a way to track changes?

It might be, for example, that there are different types of treatments that affect the alpha-synuclein in the brain in Parkinson's disease and that some people are responsive to some treatments, but not others. And so, it would be good to have

a biomarker way of telling who's showing a response and who's not, so that we might switch them to a different medication or different therapeutic approach, which may be more effective for them. So, that remains to be seen, that this connection between the ultimate clinical application and the research use is a very tight one. And I think what we're doing now in the research realm will reflect out in the clinical realm in the real world, because these tools are just necessary to find these patients and treat them in a timely fashion. So, there's some road to go.

Marie: Definitely. Well, I'm excited to see how all of these different developments unfold as well as are implemented in the clinical setting in the coming years. And, John, I know we covered a lot of ground in our conversation. We truly appreciate your insights, and we're so happy you were able to join us on the show today.

John: Well, it's my pleasure. Thank you for having me. And I love to talk about this stuff because it's been my life. And I think this almost has a sort of sacred quality to me because it's so important. There's so many people who suffer in all sorts of ways, and what we're doing now is laying the groundwork to alleviate some of that suffering or prevent it in the future. I'd be happy if after what we do that no one gets Parkinson's disease ever. That it's gone like polio used to be or something like that.

Marie: Certainly. Well, John, I think being part of the solution and a brighter future for so many people is an inspiring note to end on. So, thank you so much for your time today.

John: It's my pleasure. Thank you.

Marie: Well, John, it's been wonderful to hear more about you and your work. 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, 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.