

**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 are welcoming guests with a range of experiences and viewpoints. The views expressed belong to the guests themselves.

And today, we are excited to welcome our guest Dr. Antonio Strafella. Listeners, Antonio is the Krembil-Rossy Chair in Molecular Imaging of Neurodegenerative Diseases and Professor in the Department of Medicine within the Division of Neurology at the Toronto Western Hospital, which is part of the University Health Network at the University of Toronto. He also serves as Director of Clinical Research and Translation with the Temerty Faculty of Medicine at the University Toronto, and he is a movement disorder neurologist in the Edmond J. Safra Program in Parkinson's Disease and Morton & Gloria Shulman Movement Disorders Clinic at the Toronto Western Hospital.

Now today, we are going to be talking more about Antonio's research on current and emerging imaging biomarkers for determining the diagnosis, prognosis, or susceptibility for Parkinson's disease and other movement disorders. So Antonio, welcome to the show today. How are you?

**Antonio:** I'm very well. Thank you. And thank you for having me on the podcast.

**Marie:** Well, we are excited to learn more about you and your work, and perhaps we can start with your background first. So, Antonio, can you tell us a little bit more about yourself and how you found your way to your current positions there?

**Antonio:** Well, thank you. Yes, it is a very long road that took me here after so many years in the field, and I'm originally from Italy, and I did my medical training at the University of Bologna. And after my medical school and residency in neurology, I had an opportunity to move to Canada (University of Toronto), and this was in the middle 90s when I had an opportunity to join the team of the movement disorders group at the Toronto Western Hospital. And at that time, I started doing research in relation to Parkinson's disease, and particularly doing neurophysiological recordings plus neuromodulation, including transcranial magnetic stimulation and deep brain stimulation, combined with neuroimaging techniques. So, this was my first experience back then in the middle 90s with an amazing group of colleagues in movement disorders at the Toronto Western Hospital.

And after this first fellowship, I had the opportunity to join right after the McGill University at the Montreal Neurological Institute, where I did the second fellowship, and then I gained more experience in the field of movement disorders with neuroimaging combined with neuromodulation. And after this fellowship, I joined the faculty as an assistant professor in the early 2000s. And since then, I've been focusing mostly as a clinician-scientist in movement disorders with a focus of Parkinson's disease and neuroimaging, developing different neuroimaging approaches, including MRI and molecular imaging.

And after joining the faculty at McGill, I had an opportunity then — an offer to move to Toronto at University of Toronto, again at the Toronto Western Hospital, University Health Network, to join the movement disorders team, expanding my experience in the field of neuroimaging, combining with movement disorders. And so, everything else is history for me because since then I've been focusing and working mostly on movement disorders, Parkinson's disease, atypical parkinsonisms, trying to develop different radiotracers and molecular imaging techniques to investigate this challenging disease.

**Marie:** Well, Antonio, thank you for that background. And I'm excited to chat more about the work that you've been doing, and particularly the imaging approaches and biomarkers. So perhaps to set the stage, we can talk just a little bit about the current state of things. So, can you describe, Antonio, what is currently used clinically today to facilitate diagnosis and monitoring of, whether it's Parkinson's disease, or parkinsonism, or other movement disorders, in terms of these imaging approaches and biomarkers?

**Antonio:** This is indeed a very hard topic at the moment in the field of biomarkers and molecular imaging. I have to say that so far, there's been always a major challenge in trying to have a reliable biomarker for diagnosis and prognosis of Parkinson's disease and atypical parkinsonism, especially when it comes also to differential diagnosis. So, at the moment, I would say the only really reliable and also widely distributed around different centers in North America, South America, Europe, and so on is what we call DAT scanning, imaging with dopamine transporter.

So, the only reason why we are using now this tracer is because indeed it is easily accessible, has been proved to have some potential validity in terms of detecting changes in the dopaminergic system in terms of degeneration. And of course, because we need sometimes a biomarker, an imaging biomarker for clinical trials, and DAT scanning is at the moment the one that is widely distributed. So, at this point, certainly it is the most used imaging technique, although I don't think it's probably the most ideal because the aim is to find and identify a biomarker actually that it would be not just important for early diagnosis,

but also to be able to monitor disease over time. And also eventually have a use of internal prognosis, which, of course, in this case, DAT scanning is not probably the best biomarker. So, we're trying to work around this problem and hopefully we're going to be successful in identifying other biomarkers.

**Marie:** Absolutely. And as you mentioned, there's been a lot of research being done in this area to really advance imaging approaches and try to develop these new biomarkers. So, can you tell us more about what are some of these emerging imaging approaches and biomarkers that you're seeing right now as really promising for determining whether it's diagnosis, prognosis, or susceptibility for Parkinson's or movement disorders?

**Antonio:** Of course, in this case, we need to start thinking first in terms of biomarkers using MRI first or molecular imaging. So, for MRI, probably I think the most used metrics at the moment is probably neuromelanin. In terms of changes in neuromelanin, sometimes it can be quite early. And the acquisition with MRI, of this, let's say "biomarker", even though at the moment, I wouldn't say it is a real biomarker because that's not been validated, but many centers have been using neuromelanin because, of course, everybody has an MRI.

The challenge is, of course, that this type of acquisition is to be done at least with a 3T MRI, which sometimes is not possible. The majority just, they will have the classic 1.5T MRI (Tesla), and only a few centers that do also research, especially — let's say North America, of course, Europe, and Australia — they would be able to do also 3T MRI, but maybe in other parts of the world where 3T MRI is not easy to be accessed, and that would be not feasible for these centers. So, DTI is also another approach that MRI can use in order of measuring changes in the basal ganglia — the striatum. So, DTI is Diffusion Tensor Imaging, and again, this is something that is not validated entirely, but there's also promising data for DTI acquisitions.

When it comes to molecular imaging, certainly apart from what I already mentioned before in terms of dopaminergic imaging, which, besides dopamine transporter, we have also, of course, the classical, old-fashioned fluorodopa. We also have other radiotracers called dihydrotetrabenazine or VMAT imaging. And VMAT is the vesicular monoamine transporter — and again, that is, imaged with dihydrotetrabenazine — is a radiotracer for the presynaptic dopaminergic terminal. But now the focus in the last five years has been, for example, two other interesting approaches. One is synaptic density, for example, where it is possible to measure changes in the synapse at the subcortical and cortical level. And these changes might be sensitive to differentiate between Parkinson's and parkinsonism, which is actually very important for a clinical point of view.

Another very exciting development is in the field of tau imaging. And in the last five years, there has been many, many, let's say, radiotracers applied to imaging the tau in Alzheimer's disease, but there is still some problems and challenges in imaging in atypical Parkinsonism like PSP, for example, or corticobasal degeneration, which are different forms of tau, which we call 4R tauopathies. And for this atypical parkinsonism, there is a lot of work going on in the field, and we are hoping that actually in the next couple years to come up with a better radiotracer to image these atypical parkinsonisms, which would be extremely useful now with all the clinical trials that are currently ongoing. And we need definitely a biomarker for tau for major changes implemented or also associated with these new clinical trials.

**Marie:** Absolutely, Antonio. I think that's really exciting that there's all this new research coming out in this area. And I'd like to dive into the synaptic density work specifically. I know you recently published a review article in the *Journal of Neuroscience Research* that was really synthesizing evidence surrounding use of this synaptic density PET imaging. So, can you tell us more about your work looking at this as a biomarker?

**Antonio:** Synaptic density, this new radiotracer that has been used for measuring the synaptic density, actually started initially with work done in the US at Yale University with the first publication in the field of epilepsy. So, it was actually exciting to see changes in the terminals that were associated with, of course, the focus of epilepsy. And since then, in the last five years, there has been an explosion of interest to apply this biomarker for Alzheimer's, for example, where you can detect changes in the middle part of the temporal lobe (the typical area that you in patients with abnormality in Alzheimer's disease), and as well in other diseases, including of course Parkinson's and parkinsonisms.

So now, in the last couple years, and actually it is an ongoing project, we are trying to image patients with Parkinson's disease and atypical parkinsonism like multiple systems atrophy, PSP, and also other movement disorders in order to see if this biomarker, this radiotracer, is able to detect differences in terms of synaptic density across these different movement disorder pathologies. So, certainly we know that for Parkinson's disease and atypical parkinsonisms, synaptic density is very important because we know that there is degeneration of terminals in these diseases. And so, the point is, how much of this degeneration can be quantified across different cortical regions and also at the level of subcortical regions?

So, for example, it's not just important to have a measure of degeneration in the synapse. It's also very important the spatial distribution across the brain, because that also is going to give us an indication of how diffuse is the degeneration across different cortical areas. And that can be also very important for prognosis

when it comes to progression of the disease or different diseases associated with movement disorders. So, this is the current focus at the moment. And it is exciting because it's a work in progress, and hopefully we'll be able to come up with better approaches to measure the evolution of Parkinson's and atypical parkinsonism across time, because that's the challenge at the moment.

**Marie:** Absolutely. And I think when we're talking about biomarkers, having something that you can sort of quantitatively assess and have these cutoffs or thresholds is really valuable. So, can you talk about how something like synaptic density can be quantified?

**Antonio:** Certainly. Synaptic density can be quantified using different approaches when it comes to PET imaging. So, most of the studies that we've done so far, because we're trying to study the kinetics of the radiotracer, we actually do also arterial sampling, we collect blood from the patient. And of course, we change measures across time over two hours in order to use that as an input function to measure eventually the kinetics of the radiotracer across time.

But this can be very complicated because it can be done only in specific centers with a major infrastructure like ours, for example. And the point is actually trying to have a way to measure these tracers in a way that can be applied also in other centers where there is not a very sophisticated infrastructure. And so, this is what we call, for example, using a reference region model. Let's say, we have a specific part of the brain that we can use as a reference region. And so, we don't need the blood withdrawal in this case. And so, this approach would allow other centers to, of course, be able to measure this radiotracer over time.

And so, it would be less complicated in order to see changes in Parkinson's or atypical parkinsonisms. And so, when it comes to PET imaging (positron emission tomography), the methodological approach is very heavy, very complicated. Sometimes you need not just physicists, but also mathematicians, in order to come up with this kinetic modeling that you need to understand in order to see how the radiotracer is working.

And another challenge, for example, I would like to bring to your attention is that sometimes when you have a tracer and you see a nice image that is provided in a paper or during a poster presentation, the first thing you have to ask yourself — is what we are seeing is actually what we're aiming to measure? And here is something that we learned very recently with tau imaging. Tau imaging applied to atypical parkinsonisms, just an example.

So, we add these beautiful pictures showing a signal, for example, in the basal ganglia. And the first sight, of course, everybody was extremely excited to see something that everybody thought was tau binding. But then over time, we

learned actually that what we were seeing was also what we call “off-target binding”. So actually, the tracer was binding to something else. And the fact that we were able to measure this binding also in normal subjects that normally do not have tau deposition. So, it was an indication for us that the image and the signal that we were measuring in this patient was not just tau, but was also off-target to something else. So, this is something that you have to be aware of when you use new tracers, especially when you have a new potential radiotracer that you want to test.

So, you want to make sure that there is a very good binding to the target that you intend to measure and that your signal is not coming also from other targets that you do not intend to measure on. So, this is something that we need to be aware of. And so, at this point, there is always an important team of colleagues that work around these types of problems. That's why the image that you see is sometimes the work of many people, not just one researcher, because you need to deal with the many aspects and many other complex, let's say, methodological elements that you need to keep in mind when you do these types of studies.

**Marie:** Absolutely. And I think, Antonio, you brought up some important points about just the process of developing a novel radiotracer. It is a long and difficult process. So, can you walk us through an example of maybe one of these tracers that you've worked on? I think obviously these start in most cases in preclinical models of some sort. What is that process like in terms of troubleshooting, getting it to work, and then moving it down the pipeline to get it closer to, whether it's research use in humans or ultimately use in the clinic?

**Antonio:** It is extremely, extremely challenging to have a radiotracer available for human injection and extremely costly as well. So, the major problem when it comes to molecular imaging is because, of course, we're dealing with radioactive material. So, you need to first do lots of work dealing with the different regulatory bodies across provinces and across, of course, countries in order to deal with the limitations when it comes to radioactive material. So, let's imagine that we are developing a new tracer, and we want to eventually use this in patients.

So, of course, you start with preclinical work in small animals. And the first challenge is, does this biomarker bind to your target? So, that's the first basic question that you want to ask yourself. And of course, you will do all this in vitro and in vivo preclinical work to make sure that there is a target engagement. So then, let's say you are successful, and that is already three years of your work, gone with this in vitro/in vivo work, pre-clinical work.

**Marie:** Right.

**Antonio:** So, after these first three years of work, then you're excited. And then of course, you say, okay, now I want to start imaging, for example, non-human primates. And so, then the next question will be does this tracer — will it cross the blood barrier, right? So, the question will be does it reach the target? Does it enter the brain? And at this point, that's the next major barrier you want to make sure that you're going to be successful. So, once you get to this next level of the challenge, which is another couple years of studies, so you know that the tracer is binding to the target and it's crossing the blood barrier. So then, you want to submit all this paperwork to FDA or Health Canada. And so, they will review all your work and say, okay, you are ready to inject for the first time in humans. So, five years have gone through, and you're going to inject your first, I would say, "normal subject". Once you inject your normal subject, then you will see again, how differently it will behave from non-human primates, because that's possible, right?

So, you have to deal with a senior normal subject. And then, of course, you have to deal with other comorbidities. Maybe this patient has diabetes, maybe this patient has cardiovascular problems. And so, that can affect also the distribution of the tracer somehow in the brain. And this is going to take another year or so just to measure the kinetics of the tracer in normal subjects. Then finally, after six years or so, if you're lucky, then you're going to inject also for the first time a patient. So, that is a long process and very costly in the meantime. So, at the end of the, let's say if you're successful with the tracer — which is not always the case, but if you're successful — you can say that the first publication on a patient will come out almost after seven, eight years from the first studies.

So, we're saying 10 years easily. So, this costs a lot of time, a lot of money in terms of investment. And of course, it is very charged because what you start will not necessarily lead to a successful avenue. And this is the example we're experiencing, for example, with the alpha-synuclein imaging. Alpha-synuclein, which is the holy grail of Parkinson's, so far hasn't been extremely, extremely successful, even though The Michael J. Fox Foundation has already invested a significant amount of money to develop a radiotracer for alpha-synuclein with little success so far. So the more I think, there is still some way to go in order to identify a very good tracer for alpha-synuclein. So, it is exciting, but extremely, extremely challenging to come up with good biomarkers.

**Marie:** Absolutely. Are there any biomarkers in the works or radiotracers from either your work or others that you're feeling really optimistic about or really excited about the results that you've seen so far?

**Antonio:** Definitely. At the moment, for example, the good biomarkers that have some promise, and I think people are very positive, is definitely tau imaging at this point. So, we are moving towards the third generation of tau. So, five years ago was first generation, then the second generation of tau. Imaging for atypical

parkinsonism was actually added in the first one, but now the third generation I think will be the one that will allow to actually reach the target openly and probably a better biomarker to quantify the protein deposition in PSP.

**Marie:** Very cool. Now, if these tau imaging biomarkers are successful, if we can make it through to that final stage, how do you envision them being used, whether it's clinically or in research?

**Antonio:** Well, I mean, the aim is always, of course, besides research, which is of course, really important, but the aim is always clinical, right? So, how we can use this biomarker for tau for early diagnosis, but also for measuring the trajectory of the disease over time, or prognosis. And also, how we can use this for clinical trials. So, the point when it comes to clinical trials is that, again, unfortunately, maybe you have this biomarker available in certain centers, but not everyone would be able to produce or acquire these type of imaging in many other centers around the world.

So, that is actually the main challenge. So, for a clinical trial, of course, besides clinical metrics, you need also other biomarkers. And so, imaging certainly is one of those. And it's something that from a quantitative point of view can be very precise when it comes to measuring changes in the brain locally, but also across different parts of the brain. So that, I think, will be the major challenge in the future. Once we identify a good biomarker, how we can implement those around the world in different centers in order that we can actually have a consistent measure across the centers, right? So, that's the other challenge that we need to deal with now — not in the future — now. Because it's actually something very complex to implement. And it takes years sometimes.

And sometimes you have to also deal with barriers that sometimes are not easy to overcome. So, that is probably one of the most challenging aspects I've encountered so far when it comes to implementation of research or translational research into clinical applications.

**Marie:** Well, Antonio, thank you so much for talking about some of this exciting new research in the field. And I think you've also recently published a review article in *Parkinsonism and Related Disorders*, looking at the potential role of AI and machine learning in diagnosing things like Parkinson's disease. And I think there has just been an explosion of interest in this area. So, what are your thoughts?

**Antonio:** There's been an explosion of AI applied to healthcare in general. And certainly the future will be focusing on the use of AI and machine learning to improve diagnosis and also for prognostication as well. But the challenge at the moment is that we don't really know how this AI or machine learning really works. So, it's kind of a black box. For scientists, a black box is actually the most challenging



thing that you have to deal with, because you want to know what is in there in order to understand what is coming out as a result. So, the current learning curve is that we need to understand how these algorithms really work first.

So, as you know, AI is a big umbrella. Machine learning is a section of AI. And then we go also into deep learning approaches, right? So, this potentially can be extremely useful when we want to identify biomarkers for brain disorders in general. But at this point in time, all these different models that this machine learning uses, they have not really been validated in, for example, Parkinson's or atypical parkinsonisms. So, unless we do a very methodological approach in terms of trying to validate these models that are out there in the field to movement disorders and neurological disorders in general, I don't think we can really extrapolate those results, understand how this can be applied for, let's say, Parkinson's or atypical parkinsonism to improve diagnosis and prognosis. Certainly, it is exciting, but first, I think there is a huge amount of work that has to be done in order to really be reliable when it comes to clinical trials.

Because I don't think at the moment, we can use this for clinical trials, if we don't really understand the different aspects of how these algorithms really work. Of course, the more data you have to use these machine learning techniques is of course, the better. But again, we also need to understand how these algorithms really use these data in order to come up with some results and with an output. So, personally, I'm trying to engage more and more in this field because you cannot escape machine learning in the next five years, especially with the large databases of data available out there that we want to use. It's probably the only way to come up with some answers. But for me, in order to start using these machine learning or deep learning techniques, I need to really understand more and more how they really work before I want to engage in terms of diagnosis and prognosis.

**Marie:** Absolutely. And I think you brought up some really important points relevant to this artificial intelligence (AI) machine learning kind of work because the size of the data set, the nature of the data set, the complexity that you're working with, I think is critical to consider because I think a simple algorithm is probably easiest to understand what it's doing, but probably not sufficient to capture the complexity of what's going on in a neurological population, something like Parkinson's disease. So, kind of finding that balance of something that you can easily interpret, but also that captures the level of complexity that's needed.

**Antonio:** Exactly. Correct. So, and again, this machine learning, then you go more into what we call deep learning, which is different layers of complexity, not just one layer. Then I don't really understand how these results are coming out — [what] these outputs from these techniques kind of measure or quantify. So, for example, when I told you before, using PET imaging and the kinetic modeling

applied to measure the kinetics of a tracer, we know exactly each step of the kinetic modeling now in order to understand that what we're seeing is actually reliable or not.

But when it comes to machine learning and deep learning, hard to say. I don't feel confident at the moment to say, oh, I know that what I see is what I want to see, and then not just noise. And it takes time to learn all this. I mean, the more you use it, the more you learn. So, there is a learning curve that will require at least five years in my experience to have a better idea how this can be implemented in our research.

**Marie:** Absolutely. And I think as you're learning, the field is continuing to change and evolve too. So, it's a constant game of catch up.

**Antonio:** Right. Exactly.

**Marie:** Well, Antonio, we've talked about some of the work that you've been doing. And I think some of the results that are most exciting are the ones that are surprising or unexpected. So, Antonio, do you have an example of one of these unexpected outcomes that you've seen in your research?

**Antonio:** Unexpected results sometimes actually do the rule in research, rather than the exception. Because as in all, sometimes when we start a new study, a new, you know, we submit a grant, right? We have a specific hypothesis that we want to test. And I have to say, sometimes these hypotheses, yes, guide us very well for what we want to test. But sometimes we get completely different outcomes. And then we don't really understand why.

And then you say, okay, it is not clear if it's the biomarker that is not engaged properly, or it's actually the way we're measuring something that is not appropriate, or is actually that's the way it should be. So, when it comes to molecular imaging, it's not very uncommon to have these kind of challenges that what you see is not really what you expected in the first place. And again, this comes again, exactly with what I mentioned before, when it comes to off-target, for example. And off-target is one of the major challenges that you have to keep in mind every time that you measure something with the molecular imaging or neuroimaging in general, because maybe what you're measuring is just noise.

And another very, very important aspect that we learn in neuroimaging, but it can be in any type of research, we're trying to measure changes in the brain. Most of the time, what we are measuring is actually just a compensatory mechanism. And it is not actually the disease itself that is responsible for the, let's say, the degeneration, but what we're measuring is the way the brain is compensating to

the first insult, the first trigger. So, we're just having an indirect measure of the disease.

And that's actually when sometimes you see things that do not apply or maybe are completely different than what you were expecting. Because as an example, sometimes when you measure a dopamine transporter in the synapse, it's not just the degeneration that you're measuring, but sometimes you're measuring just the tau regulation of dopamine transporter in the synapse as a compensation to the trigger, to the disease. So, it is just an example of many that is a challenge because then you don't really know if this is the disease or just the compensatory reaction of the brain to the disease.

**Marie:** That makes sense. And I think as more of these new molecular imaging tools and approaches are developed, I think it's really highlighted some important issues surrounding access and implementation of molecular imaging in clinical practice. So, I'd love to touch on this issue as well. Antonio, can you talk more about some of these maybe challenges or considerations related to implementation?

**Antonio:** Certainly. Implementation of these techniques requires a town to be successful. That is never, never to work with one person. And again, in my own experience, I learned that if I have very good collaborators — and the collaboration here is a key to be successful — if you have very good collaborators, the chance to succeed is very high. And again, given the assumption that you're able to interact with other bodies outside of your institution, which is in this case, the government, right? Because the government, of course, wants to monitor the use of radiotracer when it comes to radioactivity or radioactive material. So, you have to be able to provide the evidence that what you want to do is safe for patients, of course.

And so, this is a major barrier. Also, just a simple MRI sometimes can be very challenging because, as you know, as we get more and more into the sophisticated high-field imaging like 7 Tesla, you can imagine that even a very little metal in your body can be a problem. So, in that case, you need to make sure that you have an infrastructure that can help you to overcome all these problems in terms of how to make the environment safe for the patients, especially when you come to 7T MRI, because it is potentially extremely useful for us, but at the same time, it is extremely challenging when it comes to safety.

So, this implementation, of course, is challenging for a center which has a very good infrastructure, but then you can imagine for a center that does not have a very strong background in research. So, and that's totally unfeasible in that case. How you can do clinical trials using this sophisticated approach in the center where there is no tradition for such type of biomarkers or also for an

infrastructure that requires a significant amount of expertise and knowledge in order to be successful.

**Marie:** Well, that makes sense. And I think this idea of disparities and access to nuclear medicine studies, thinking about things clinically, as well as, like you said, the infrastructure can be a barrier to widespread adoption of some of these new biomarkers, even if they're developed. How do you see ways that we could potentially overcome this?

**Antonio:** These disparities now become more and more evident as we try to be more inclusive in terms of clinical trials for different centers around the world. And we know that access and implementation of molecular imaging in clinical practice — it is important, but at the same time, we have to deal with these limitations. So, sometimes, of course, there has been an attempt actually to use remote imaging to do analysis of certain images acquired somewhere else. So, we're trying to use, let's say, the network across different centers in order to bypass the challenges. For example, you can acquire data in the center with a certain scanner, but then the analysis is conducted somewhere else to avoid the complexity of implementing the analysis for certain biomarkers. And so, that could be an approach that can be potentially useful. And that's probably the only way that we can do that.

Of course, still, you need an infrastructure to be able to acquire the data, and that can be done, for example, in a few centers nearby of a certain capability, But at the moment, I have to say there are different strategies that our colleagues are trying to implement, especially in collaboration with industry and pharma, in order to be more inclusive across different countries for the clinical trials and applications of different biomarkers. But there is an interest to try to do this, for sure, because we know that. But of course, the cost is probably one of the challenges, especially nowadays, where finding resources and funding for research is not that straightforward.

**Marie:** Certainly, that makes sense. And I really love that you highlighted the importance of collaboration in this response, as well as in some of our previous parts of the conversation. And I think these collaborations are critical for moving the field forward, as well as development of useful tools, and resources, and other things. So, do you have examples of — whether it's, like I said — collaborations, tools, resources, etc. that you see are really having an impact on the field?

**Antonio:** Well, I have to say, of course, we know very well The Michael J. Fox Foundation has done an amazing job to work in this field to move the classical needle forward. Again, the PPMI, as you know, it is one of the major databases that we have for Parkinson's. And again, I think this is something that started a number of years ago, and is moving forward, I have to say, I've been surprised by the fact

that The Michael J. Fox Foundation has been behind this for so long, and it keeps staying behind this project because it requires a huge amount of time and funding in order to sustain something of this kind of size, and also determination.

And again, personally, what The Michael J. Fox Foundation is for Parkinson's, of course, the same is applied to Alzheimer's and other neurological conditions. I think the field, in general, of neuroscience is moving towards large databases in terms of the data collection. I don't think we can just rely on the data that are acquired in one center. That's not appropriate anymore. That probably was okay 20 years ago. But now the future is open science. And now we can implement open science, which means large data collection into machine learning and AI in general.

So, these are the two main areas that will move forward in the next five years. Therefore, this will change the landscape of research in neuroscience. But again, we need to be very careful because we need to really understand how machine learning works in order to input all this data and all these databases from collaborations from around the world in order to come up with some results that make sense to the field and actually will allow us to really come up eventually with a cure for Parkinson's. That is the final holy grail.

**Marie:** Definitely. So, we talked about then some of the things, in terms of tools and resources and collaborations that are helping advance the field, but I think there are still many unanswered questions in the world of Parkinson's research today. So, Antonio, what do you see as some of the most important unanswered questions or maybe the biggest area of opportunity for Parkinson's research?

**Antonio:** Parkinson's is a huge umbrella, right? We learned that Parkinson's is not just one disease. So, we can say that Parkinson's can present in many different forms. Certainly, that is the main question, the main challenge. The main question that we need to answer is, when it comes to clinical trials, how we can stratify patients first, how we can bring together participants that will allow you to measure the large variability that we have under this condition. And so, until we really understand and we find a way to really stratify patients differently from what has been done so far, I don't think we'll be able to move the needle forward in order to have a different outcome.

So, the approach has been always so far, how we can, at least in the last 20 years, classification, stratification of patients has been always based on the classical symptoms of Parkinson's disease. But given the overlap of presentations, sometimes with atypical parkinsonisms, how we can instead try to collect or identify participants based, for example, on certain biological aspects, rather than just symptoms. And so, if we are successful on this, then of course,

we might be able to have different outcomes in terms of implementing new drugs using different biomarkers.

So, the question is complex, the approach must be different, we cannot use the same approach for clinical trials as it has been done in the past 20 years. It has to be different, also because we learned over the years that the results were not successful. And so, we need to change the approach, and also the way we do research. Of course, with the large databases, and eventually using also AI. But at first, we need to go back to the basics of how we can stratify these patients in order to really have a large representation of the different aspects of the disease.

**Marie:** Certainly, I definitely agree with you. I think these are really important questions to be answering in the field. And I think these imaging biomarkers are a potential viable pathway for improving this stratification process. So, this sort of answers the question, but could you summarize for us, Antonio, how you see your work really bringing us closer to finding a cure for Parkinson's or contributing to these improved therapies for people with Parkinson's today?

**Antonio:** Well, definitely my type of work in the field of biomarkers would be important in order to quantify the changes that are obtained with these new drugs or clinical trials. So, certainly, adding good reliable biomarkers for diagnosis, prognosis, accuracy, and so on definitely will bring you a step closer in order to say with the certainty that whatever you are trying to measure is actually important. I believe that as we get more of these biomarkers, including potentially the alpha-synuclein biomarker with imaging, with molecular imaging. So, I think this will definitely have a significant impact in the field, because then we can say, okay, there is a target engagement, there is a change in the target induced by this drug.

So, that's actually the only way we can do if we want to measure the disease over time in terms of not just diagnosis or prognosis, but also in terms of a drug effect.

**Marie:** Certainly, I think this is definitely something to be looking forward to, and we really appreciate all the work that you're doing in this area. And we appreciate you joining us on the show today to share your insights and expertise. It's been a pleasure to chat with you.

**Antonio:** Thank you, Marie, for inviting me. It was a pleasure.

**Marie:** 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](https://www.MichaelJFox.org/researchresources). And you can find new episodes of this show each month on the MJFF website or on your favorite podcast platform. And 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*.