Marie: Hello and welcome to *The Parkinson's Research Podcast: New Discoveries in Neuroscience*. I'm your host, Dr. Marie McNeely, and I 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. Now, we're welcoming guests with a range of experiences and viewpoints. The views expressed belong to the guests themselves.

Today, we are excited to welcome our guest, Dr. Dario Alessi. Listeners, Dario is Professor of Signal Transduction and Science Director of the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit at the University of Dundee. Today, we're looking forward to talking more about his research on the role of genetics, particularly the LRRK2 gene in neurodegenerative diseases, including Parkinson's disease. So Dario, welcome to the show today. How are you?

Dario: I'm doing very well, Marie, and thank you for the kind invitation to join this podcast.

Marie: We are so excited to have you here with us today. And I'd like to start by talking about your background a little bit. I know you've got a unique story about your path to your current position and your line of work. So, can you tell us more about your background and how you found your way to your current position, Dario?

Dario: I think the journey I took to getting on to working on Parkinson's disease is kind of unusual because the journey started in 2004 when I was sort of really happily researching biology that was relevant to understanding cancer and high blood pressure. And then I happened to come across the brilliant papers of Thomas Gasser and Andrew Singleton describing mutations in an unstudied gene called LRRK2, or L-R-R-K-2, that caused Parkinson's disease. And LRRK2, the gene encoder type of enzyme that I had expertise in working on, and it's called the protein kinase. I just remember thinking at the time that it was really fascinating how such an enzyme could be linked to Parkinson's disease. And if you could study this, it might yield new ideas of how to treat and even prevent Parkinson's.

That was in 2004. And then all my colleagues that I work with told me that it would be, well, they said “madness”, I guess – unadvisable would have been the other way to put it – for me to work on this because I'm not a neuroscientist. I was a biochemist. And there was no one else working in my university, which is located in a small place in the east of Scotland, that could guide me on this

research. But I still felt that it was a really important problem. And I did have one connection with Parkinson's that came from my grandfather, who was, unfortunately, significantly impacted by this condition. So, the first few years were quite challenging working on this project because my colleagues were proven correct at the beginning for the reasons I've just explained.

But the really transforming moment for me was, I got an invitation out of the blue from Brian Fiske, who was a senior director of The Michael J. Fox Foundation. He invited me to a meeting. And I'm still not sure why he invited me or even knew who I was. But that meeting opened my eyes. It gave me an opportunity to meet neuroscientists, and clinicians, and people working in pharmaceutical companies. And this provided me even more inspiration to work in this field. And at the time, I'd also met a lot of people impacted with Parkinson's disease. That motivated me even more. So, that was the start. And then in 2010, Brian asked me to set up a team to study the LRRK2 biology. And that was how I got into the field.

Marie: I absolutely love this. And I love that connection with The Michael J. Fox Foundation. I think the organization really excels in bringing people together and bringing people from different areas in to help address some of these big problems in Parkinson's disease.

Dario: Yes, I think that's really important because then Brian must have realized that maybe someone working outside could offer a different perspective. And that's maybe why he invited me. And I really enjoy working in a collaborative way. So, although I'm not a neuroscientist, I don't work in a pharmaceutical company. I love collaborating with neuroscientists, and pharmaceutical researchers, and clinicians. And I do what I can do. And I like working with them together and leading to synergistic interactions. And it's a way to tackle difficult problems that's very effective.

Marie: Absolutely. I think leveraging the expertise of an interdisciplinary team can really help when working on some of these really interdisciplinary questions like Parkinson's disease, which bring in a lot of different areas. So, I'd love to get into some of the details of your work here.

Much of your work has focused on this LRRK2, which you said has captivated you for quite a number of years. So, can you maybe set the stage here for listeners who might not have a genetics or a sort of biochemistry background? What are the mechanisms by which LRRK2 variants may contribute to Parkinson's disease?

Dario: So, LRRK2 was discovered, as I've said, by genetics, these wonderful geneticists looking for genes in our genome that can cause Parkinson's disease. And it's clear that the mutations in LRRK2 are called dominant. So we always have two

copies of every gene, one from our mother and one from our father. And it turns out that if you have a mutation that causes Parkinson's disease in LRRK2, if you inherit that mutation either from your mother or your father, you have a much greater chance of developing Parkinson's disease. And it's really quite clear that the mutations make the enzyme more active.

So, it is really important for understanding the biology. And the other feature about LRRK2 is it's actually one of the most common genes that causes inherited Parkinson's disease. And worldwide, Parkinson's affects 10 million people, and there's no disease-modifying therapies. And it's thought that at least 2% of the people with Parkinson's have a mutation that increases the activity of the LRRK2 enzyme. And it's also possible that environmental factors, the work of Timothy Greenamyre has shown that environmental toxins that are linked to Parkinson's disease also activate the LRRK2 system. So, the idea is that basically too much of this pathway is leading to the disease. And then the research side of it was to understand how is LRRK2 linked to Parkinson's disease and dissect that biology. But I think that the fact that the enzyme is activated is very favorable for drug discovery, because it's much easier to develop a drug that lowers the LRRK2 activity than activates it. So, this discovery was important because it suggested to pharmaceutical companies that LRRK2 would be a very attractive target to develop new therapies for Parkinson's disease.

Marie: Absolutely, and I'd love to dig into that a little bit deeper. I know there are multiple different mutations or variants in LRRK2 that can, or may potentially, contribute to Parkinson's disease. What are some of these variants of particular interest that might be good targets for drug development?

Dario: So, the most common variant has a technical name G2019S. And I think at least 80 or 90% of people with LRRK2 mutations have this common variant. And this is located right within the enzymatic domain that's called a protein kinase. And then there's other mutations that are very interestingly located in other regions of the enzyme outside the kinase domain. And one of the most studied ones, it has a name called 1441C.

This is actually located in another domain that's got a technical name of a GTPase domain. And then there's other mutations. We're actually working with my clinical colleague Esther Sammler. We've tested, I think, 215 variants that have been reported in people with Parkinson's. And we've demonstrated, I think, that around 45 clearly switch on the LRRK2 kinase activity. And the groups of Andres Leschziner and Ji Sun have recently dissolved the structure of the LRRK2 enzyme. It's a very big protein. And it turns out that the mutations that activate the enzyme are very interesting. They actually destabilize the inactive conformation of the enzyme, making it more active. So, when the LRRK2 enzyme

is made in cells, it's folded into an inactive conformation. And all the mutations seem to destabilize that inactive conformation, thereby making it more active.

Marie: That's very interesting. Now, do they destabilize it in different ways that you would need different drugs to target these individuals who might have different mutations or variants?

Dario: So, it turns out that if you get a drug that targets the kinase domain, and these drugs that have been developed are called type I kinase inhibitors. So, all the companies that I know about who worked on LRRK2 have got these type I kinase inhibitors. And these bind to the kinase domain, and they seem to block the activity of the enzyme, irrespective of the variant, which is a good thing. So, the inhibitors will work with many different variants. But it is also possible that another company called ESCAPE Bio has also generated inhibitors that target just the G2019S mutation, and they have a very different mode of action that they can just bind this active conformation that's induced by the variant. And this is a very interesting approach because it's possible that these inhibitors might have less side effects than the inhibitors that target all the variants. But I think most companies have gone with the pan-kinase inhibitors because they're more likely to benefit more patients.

Marie: Absolutely. And I'm glad you brought up this issue of side effects. I think this is clearly a major problem when developing any kind of drug. Can you talk about some of the side effects that have emerged of potential concern, or things that we just need to keep in mind as we're developing these LRRK2 inhibitors?

Dario: The major side effects that have been reported in the literature are in the lung, essentially, and potentially also in the kidney. But I think the work is more advanced in the lung. So, it turns out when you inhibit the whole of the pathway, either by removing the LRRK2 gene from an animal model, you get sort of hyper-inflamed cells in the lung that have large vacuoles inside them. And then more recently, the pharmaceutical company Merck had published a very important paper where they used non-human primate models and administered these animals actually very high doses of the LRRK2 inhibitors, probably maybe 10- to 50-fold higher than the patients would normally receive.

These doses also induce similar lung pathology. So, I think these results are important because it suggests that certainly if you inhibit the pathway completely, this might not be desirable. But it turns out that one person in 500 lacks one copy of the LRRK2 gene, because the mutation that deletes it is quite common. So, many people are lacking one copy of the LRRK2 gene, so these people, which have 50% of the enzyme, they're completely normal. There's no evidence that they get lung disease. So, we know if you inhibit 50%, you're fine. If you inhibit 100%, that's not good.

So, the key is to work out is it 60, 70, 80%? What's the safe cut off? And then also not only that, but it's their way to monitor the lungs so you can actually catch any issues at a very early stage. I think with many medications, for example, people measure liver toxicity regularly as a good therapy option. So, maybe one would monitor lung function in people. We know the mutations make the enzyme more active. So, you would expect that the drug, you don't need to lower it back to normal levels. And you wouldn't completely wipe it out.

So, I think that's the strategy. The clinical trials are ongoing, Denali and Biogen have a compound that's in a phase II clinical trial called DNL151. And so far, they say that they have not observed any lung issues with the doses that they're using.

Marie: That is tremendously exciting. And I think, really promising to hear. And you mentioned this important step here involved in moving these drugs down the pathway and being able to monitor patients going forward. And I think developing these monitoring or measurement assays is a key part of solving this big problem in Parkinson's disease. So, I'd love to talk about some of these novel approaches that you've contributed to developing in this area. And perhaps we could start with the LRRK2 kinase assay.

Dario: Yes. So, that happened right at the beginning. Actually, before we met Brian Fiske, I was able to persuade the researchers working on other projects to do a few extra hours every day in the lab to work on this project. And what we did is because we didn't know LRRK2 as a technical name as a kinase. And what kinase enzymes do is they attach phosphate molecules onto their target. And this changes the biology of the target. And we have 20,000 or so proteins in our cells. So, the key question is what was the target of the LRRK2 kinase? So, the first approach we did in 2007, we were very naive at the time.

Sometimes being naive is good. We just made the LRRK2 enzyme, and we made it in a 293 cell. And then we thought because Parkinson's disease involves the brain, we started with the brain. We actually separated the 20,000 proteins in the brain into about 100 or so different fractions using something called chromatography. And then we tested each one of those fractions to see whether LRRK2 would put a phosphate molecule onto any one of the proteins in these fractions.

And we found only one target that the LRRK2 worked on in these fractions. And it was a protein called myosin. So this result was good and it was also bad. It was good because we could build an assay. So, we could build technology to assess LRRK2 activity.

We chopped myosin up and we found out where it was targeted by LRRK2. And then we could make a peptide that encompassed the site that was phosphorylated. And we call this peptide LRRKtide. And then this enabled us to develop a very accurate assay for LRRK2. And then you could measure the effects of the variants. So that was quite nice because we could show that this very common G2019S variant did indeed stimulate the LRRK2 activity intrinsically. So, that proved that the mutation was increasing the activity. But, more important than that, it enabled all the companies to use this technology to screen for drugs. This is what companies are very good at. They screen for millions of compounds that they found inhibitors, and they elaborated them using this assay. So, from this point of view, this work that we did was good.

The bad thing was that we thought that myosin might be the physiological target of LRRK2. So, we spent about three or four, maybe more, years trying to prove this. And then we eventually convinced ourselves that this was not a physiological target. And then we had to go and find the real target. But I guess this result was useful.

Marie: Oh, absolutely. I think this LRRK2 kinase assay has been tremendously valuable for moving both the research forward as well as the drug discovery side of things. And in addition to this LRRK2 kinase assay, you also developed this LRRK2 Ser935 dephosphorylation assay. So perhaps you could break that down and talk a little bit about this assay.

Dario: Delighted to do that. So, the first thing we did is we developed this in vitro assay that worked well. So, all the companies came to us, and we helped them set up the assays. But then they came back to us and said, we've got inhibitors, but how do we test these in cells and animal models? Because it turned out that myosin wasn't the real substrate. So, what we did is we did something extremely simple. I mean, for me, it's always the most simple things that are the best. So, what we did is some enzymes like kinases, obviously they have a real target that they regulate in biology. But some kinases are a bit stupid. And they actually put phosphate on themselves at various places. This is called autophosphorylation.

It may or may not have a physiological role. So we wondered whether LRRK2 would do this on itself. I had two brilliant postdocs at the time, Jeremy Nichols and Nic Dzamko, who then went on to study whether or not LRRK2 itself was phosphorylated, and whether this phosphorylation was controlled by the LRRK2 kinase activity. And this led to the discovery of 935. And at the time, because none of the companies would give us their inhibitors, so we had to look for some very pleiotropic compounds that we could just buy from vendors that inhibited many kinases. But using these pleiotropic compounds, we discovered the 935 site. And as soon as you added the LRRK2 inhibitor, it becomes dephosphorylated.

And then we let companies know about this. And they also found the same results with their much more selective inhibitors. And what was really crucial, actually at the time, was The Michael J. Fox Foundation gave us a grant to work with them and a company called Epitomics at the time, but now it's become part of Abcam to make a rabbit's monoclonal antibody that detects this site. And this antibody was the game changer because it works so well. You can really measure this phosphorylation in any tissue or cell that LRRK2 is expressed in. And obviously, it was then commercialized with The Michael J. Fox Foundation. So, everyone could order and benefit from that reagent. I think that was probably quite good because it has enabled companies to verify that the LRRK2 inhibitor was working in a cell or animal model, which is a central next step of the preclinical drug development.

Marie: Definitely. And I think the Rab protein piece of the story is very interesting as well. And you developed this Rab phosphorylation assay. I feel like you've just been an assay-developing machine there, Dario.

Dario: It's good, developing an assay, because you have a basic question. You have to make the tools, and then test them, and you get satisfaction. It can take several years' work, but it's very elegant, simple, and useful. So, I really enjoy doing things like this.

Marie: Certainly. I think the downstream impacts are enormous for this kind of work. And I'd love to talk about this Rab phosphorylation assay as well. This is the third of the trio that you developed.

Dario: That was the biggest breakthrough. So, the stuff I've talked to you about before was good work. It was useful, but it didn't transform our understanding of the biology. Now, if you want, I'm happy to tell you the story that surrounds the Rab. So, in 2010, we're going back to Brian Fiske. He asked me to set up a team. He told me that he wanted to call the LEAPS team. I ended up calling it the dream team – different experts to find out what the real target of LRRK2 was. So, I decided we needed to work very collaboratively on this. And I enlisted the help of three of the world's leading mass spectrometry experts. And I also wanted them to work together with Merck and GSK, who were very involved in this project and had a lot of reagents and expertise. And of course, The Michael J. Fox Foundation were a key part of this team, and also myself.

So, I thought this would go well. I started contacting these team members. But the key problem I encountered was that GSK and Merck and the three mass spectrometry experts, they all told me, oh, no, no, we're fierce competitors. We'll never work with the other team members here. We can't work with them. But it's just one of the times in your life that you can't take no for an answer. This is a

once in a lifetime opportunity. So, I think I managed to persuade them that the race was against Parkinson's and not each other. When I told them about that, in the end, they agreed to work together. And we worked – it was an amazing collaboration.

We had six or seven partners. In each side, there were several people working together. The Fox Foundation invested a huge amount of money that's much more than I'd ever received for any other type of project. And I was optimistic with such a good team that within three years, we could find the target. But the biology turned out to be much harder than we anticipated.

So, by three years when the funding ran out, another very lucky thing happened. Brian Fiske had been promoted to a more senior role, and another scientist, Marco Baptista, came into The Fox Foundation. Now, Marco was a really true gentleman. I remember him telling me that the philosophy of The Fox Foundation is not to leave any stone unturned. And then he went on to add there was a fine line between success and failure. So because of this, we got another three years funding for this project. And yeah, no, it's always my attitude. If you're doing something important, and it doesn't work. Don't give up. Just keep on putting, even if you can, even more effort into the project. Sometimes you do need long term support to address the most difficult moments.

So, I think the Eureka moment came at the end of the second three years. This was in 2016, where we eventually found that the key targets of LRRK2 were these Rab proteins. These are another type of enzyme that control fundamental cell biology. And this biology lies at the heart of understanding Parkinson's disease. When we found this, all of the team members, this will probably be one of the highlights of one's scientific career, because it opened the door to accelerate the understanding of Parkinson's disease. And since then, the pace of progress has been completely staggering. And it's also enabled drug companies to test their inhibitors that they're working in humans and work out how well they're working in humans. It was an incredible time.

Marie: Certainly. And in this story, Dario, you mentioned fruitful collaborations and conversations with individuals in different roles at The Michael J. Fox Foundation at the time, including Brian Fiske and Marco Baptista. And I know you've been working with Shalini Padmanabhan at MJFF in recent years as well. So, can you talk about this collaboration and some of the recent work that you've done together?

Dario: Shalini came on the scene around 2016, just after the Rab breakthrough had been made. And I must say, she's played an amazing role in the next phase of the research and supporting the research, both our group and many other groups, to understand the role that Rab proteins play in Parkinson's disease. And

I think this work has really contributed to our understanding of how the LRRK2 system controls the health of organelles, and how this is disrupted in Parkinson's disease.

And I just would like to say that it's really been a great privilege to work closely with Shalini over the years. And I know at least a dozen other scientists who would say the same thing as me. You know, she has such passion and energy for the research side of things, and she has such high standards. She's always asking very thoughtful questions, and she wants experiments to be done yesterday. In some ways, she's potentially the conductor of the orchestra for Parkinson's research, at least in this LRRK2 Rab biology area.

Marie: Oh, definitely. So, can you describe briefly how this Rab phosphorylation assay actually works?

Dario: So, Rabs are another type of enzyme. And the way I think about the control process in the cell called cellular trafficking, and the way I see this is a bit like your FedEx system. You pick up a parcel from a dispatch, and then it moves through the system — is driven through various trucks and vans — till it goes to the last mile through a small van and is delivered in your office or doorstep. And the Rabs are really key orchestrators of different parts of this process in which the parcel is received and moved through the system to the end product.

And I think this is what happens in Parkinson's disease. If you think about it, there's many things that can disrupt the progress of a FedEx package. And you can have bad weather on the road, one of the trucks could break down, the computer systems could break, you could have potholes in the road, etc. So, there's hundreds of ways you can affect this postal system. And I think it's the same thing in the cell. The cell’s got to take in and move cargos from one part of the cell to another. It's got to move organelles around the cells. And there's lots of ways that you can disrupt this process. And if the process is disrupted in a particular type of way, we think that this could lead to Parkinson's disease.

But the question was how does the phosphorylation of the lab protein affect its function? And for that question, the technical way is that the Rabs are a GTPase, they work in two different conformations. They can be on and off. So, they're not either normally on or normally off. But the way that the LRRK2 works is actually it puts a phosphate molecule in the critical region of these proteins. And this opens up their ability to do a third function. So, not being on and off, but being able to bind to new sets of targets and influence this cell trafficking system.

Marie: Certainly, and Dario, I think understanding better the role of the Rab protein in this system was an amazing scientific discovery. And then developing this assay to be able to measure or monitor it was another critical step. I guess, you've had

a lot of successes along the way. What were some of the biggest surprises or maybe the unexpected turns or outcomes that you've encountered in your work?

Dario: Yes. So, when I thought about the LRRK2 and what it might do, we thought they would work by switching the on off mechanism of the Rab protein. That's what everybody thought. But it turns out, the biology works by enabling them to do this third function to bind to targets.

So, that was also quite unexpected. And then another big surprise, this comes into more recent work that actually my collaborators, Suzanne Pfeffer, working at Stanford University, has really been playing a huge role in. And we've discovered that Rab proteins are clearly the target of LRRK2, but they also regulate LRRK2. So, it turns out that the N-terminal domain of LRRK2 that is actually binding to different Rab proteins, even some of the Rab proteins that it's a target of. So, it turns out that it's both regulated by Rabs, and it's also then LRRK2 also regulates Rabs itself. It's quite a complicated cycle.

The biology of this is still being unraveled, but it looks like the signals in the cell that might result from dysfunction in certain organelles that lead to the activation of a Rab on the surface of these organelles. And then these then recruit LRRK2 to that surface of the organelle where LRRK2 will then phosphorylate more Rabs at this surface and this will switch on biology to maybe counteract this dysfunction.

Marie: This is very interesting. I think unraveling the mysteries of the biology, the mechanisms that are contributing to this process is really exciting. And we've mentioned, of course, some of the really exciting accomplishments that you've had in the work that you've been doing. And among them, you were very recently awarded the 2023 Robert A. Pritzker Prize for Leadership in Parkinson's Research. So, Dario, what did it mean to you to be selected for this award?

Dario: I mean, being able to contribute to the research that The Michael J. Fox Foundation does for Parkinson's disease, this has been the privilege of my scientific career. And for The Michael J. Fox Foundation then to select me for this year's recipient for this prize was extremely special. And I still think there's obviously lots of work to do, and I'm enormously appreciative of the opportunity that I've been given by being able to interact with The Michael J. Fox Foundation and then this wonderful staff who work there.

Marie: Certainly. And I know this particular award is presented to scientists who excel both in the science and the research side of things, but also in mentorship. So, Dario, can you comment on the importance of mentorship and your role?

Dario: I think I might have mentioned at the beginning, in 2004, when I was wanting to work on Parkinson's disease, there weren't any researchers in my university or probably anywhere in Scotland that were doing this type of research. You might be pleased to know that through the efforts of The Michael J. Fox Foundation, and other opportunities that have come from our research, that we've been able to build Parkinson's disease research quite significantly in my university. And we now have around 30 researchers working in this area, including two clinical scientists, Miratul Muqit and Esther Sammler, who we work very closely with. We built lots of ties with the pharmaceutical industry, and we built huge numbers of international collaborations with colleagues all over the world.

So, I'm very pleased that we've been able to build something quite special from scratch. And we provided opportunities to train other researchers who I hope will spend the rest of their careers working to understand and treat Parkinson's disease. And as a special mention for my wonderful close collaborator, Suzanne Pfeffer, I think we've also encouraged her to work on Parkinson's. She was doing amazing work in other areas, and her work is making huge contributions to the field. And I think we've helped encourage her to come into this area.

I hope that our way of working together, maybe as a team, and sharing our ideas and reagents immediately with everybody else without restriction is becoming the norm. I think now that virtually everyone working with The Michael J. Fox Foundation follows these practices. So, I hope we played a small role in helping reveal the value of such policies and working in this way. And for me, the key thing is that this will hopefully accelerate progress and then bring major benefit to patients sooner than it would otherwise if we worked just by ourselves and didn't share ideas and reagents.

Marie: Certainly. And I think it's wonderful, Dario, that you're really helping to bring more investigators into this Parkinson's research field. And I know that, in addition to growing the field with current investigators, you're also helping contribute to developing the next generation of investigators. So, working with trainees, students, things like that in the lab. Can you talk a little bit more about this side of the mentorship that you do?

Dario: When all is said and done, probably one of the most important things that a scientist will do is training young scientists and giving them that opportunity. And it's quite hard to mentor a young scientist who's never had much experience of doing research. They can come from different backgrounds. At the University of Dundee, we're a small university, and it's quite a low income area here. So, we get a lot of students from all parts of the world, maybe not the top universities they come from, but they're very brilliant, nonetheless.

And we give them this opportunity. We train them well. We teach them when you do an experiment — my student this morning was showing me some data, and I often say this, where's your positive control? Where's your negative control? And to think about that before you do the experiment to make sure the data is rigorous, and the replicates are good, and you use the most rigorous methodologies, and we're not cutting any corners. You're making sure you use the state-of-the-art technologies, and thinking about the experiments deeply, and also analyzing the data you get, and then thinking about what it means and what will be your next experiment.

And there's always like 100 experiments you can think to do, and you only have two pairs of hands and so much budget. Some of the times the trick is to work out of those 100 experiments, what is the best one for me to do, and why? And you don't get this right all the time. So, you get it wrong most of the time, but that's the experience. And then you have to instill the discipline of working reasonably hard, and reading the literature, going to talks, communicating your data effectively, enjoying it. You have to enjoy doing science, and it's fun. And you've got to go to meetings and all those things. So, it's a great privilege to be able to do this and it's a very important part of my job.

Marie: Absolutely. And as we mentioned previously, in your career you've developed some valuable assays also that many others can now benefit from, so your work is touching a lot of different people in the field. Are there other tools or resources or maybe collaborations, Dario, that you see that are really moving the field forward?

Dario: Yes, I think that from The Michael J. Fox Foundation's work, there's this new organization called Aligning Sciences Across Parkinson's Disease that The Michael J. Fox Foundation is also very heavily involved in. In this collaboration, they actually have around 70 groups now working on understanding, and treating, and preventing Parkinson's disease. Each one of these groups is made up of four or five different teams, so there's something like 300 teams involved in this project from all over the world. And they use what they call a CRN, so a collaborative research network. This is run by Ekemini Riley and Randy Schekman, and it's an amazing collaboration where they have so many researchers all working together.

The data has to be shared almost immediately, and all reagents and ideas are shared. And I think this is the biggest experiment that's ever been done, certainly in the history of Parkinson's disease research. We have one of the teams involved in this project, one of the 70 teams, and it's just amazing the amount of progress and work that's been taking place in all aspects of understanding Parkinson's.

And I think where we were in 2004 where you couldn't even get any funding to do one project, and now you have much more opportunities, and there's so much research ongoing. I think this is fantastic. And to the communities that support Parkinson's research, we're obviously extremely grateful to all the donors who work so hard to place funds to support Parkinson's research, which as I said before, there's no disease-modifying therapies, and it affects so many people. And the numbers of people who are having Parkinson's disease are going up. So, this is a really important challenge of medical research — is to try and treat Parkinson's disease.

Marie: As you mentioned, this is a really important time to be working in Parkinson's and also a really exciting time to be working in the area. And you mentioned this massive collaboration, but I know you also have a unique collaborative environment there in the University of Dundee in the Division of Signal Transduction Therapy. So, can you tell us a little bit more about what makes this so unique?

Dario: We like, as I said before, working in a really collaborative manner. Most of our research comes from government money and obviously The Fox Foundation and other foundation money. I believe that it's essential that we make available all our knowledge and tools, to not just other researchers, but also to pharmaceutical companies because they will be the ones who will do the preclinical work, and develop the drugs, and test them. So, we have a structure that we call the Division of Signal Transduction Therapy Unit, in which we enable pharmaceutical companies to basically collaborate with us on projects where we have expertise and a mutual interest to work with them.

So, we typically have the students or postdocs who work on projects that are interesting for the companies. And the companies often suggest these projects, and then we host the students or postdoc to work on this project using our expertise and knowledge. And it's fantastic because we get lots of good ideas from the companies. We get access to lots of reagents, and we can access their technologies. And this enables us to start working in the new areas that we could never get funded on because we're not an expert in those areas often.

And it also enables the students and the postdocs to get experience with working with companies, and they can actually go to the company to do some research. And vice versa, the companies can also send researchers to our place to get training to do certain projects. And because of that, many of our trainees end up working with pharmaceutical companies, which is great. They're the ones who are going to make the drugs. And having that experience, they kind of have a head start in their interviews for working with the company. And then they get good contacts from their time here. So, I think that's another important benefit of this.

Marie: Well, Dario, I think that is really cool and a wonderful collaborative environment that you've developed there over the years. And as you mentioned, there are still many important questions that the research community is working on in Parkinson's disease. I guess, if you're thinking about the future here, Dario, what do you see as maybe the most important questions that remain to be answered, or maybe the biggest areas of opportunity, in Parkinson's disease research?

Dario: Yeah, we don't know how much of the biology we don't understand. Sometimes, in the middle of the night, I wake up and say we only understand one percent of the biology. And sometimes you even think 0.1% if I'm being particularly in a dark mood. It could be 10%. I think there's incredible amounts still to understand.

I think we have a sketch of what might be going on, but I'm sure there's at least 10 to 100 times more things to understand than we currently know. I think for the LRRK2 area, where people are really excited about in the field as a whole now, is that the LRRK2 seems to be involved in regulating an organelle in the cell called the lysosome. This encompasses about 1% of the cell, but this is sort of a recycling plant in the cell that goes around recycling a lot of damaged components and then dissolving them up to their constitutive ingredients that regenerates new nutrients the cell can then use to build new things again.

And it seems that disruption of this pathway — the lysosome, it comes from the endosome, so it's often called the endolysosomal pathway — lies at really the heart of Parkinson's disease. There's about 20 genes that are known to cause Parkinson's disease, and I think more than 10 of these genes are involved in regulating this endolysosomal pathway. So, it's going to be incredibly exciting to really understand in much better detail how these components work, how they're controlled, how mutations that cause Parkinson's disease affect their biology, and then to harness this information to develop new assays to better treat and prevent, and also better diagnose Parkinson's disease.

Also, I think there's not just one type of Parkinson's disease. I think Parkinson's disease can be caused in many different ways. So, I think one will need to stratify — at the moment, your patient has Parkinson's disease or doesn't have Parkinson's disease — but I think in the future, with this increased knowledge, we'll be able to say this patient has LRRK2-driven Parkinson's, this patient doesn't have LRRK2 (it might be alpha-synuclein-driven), this patient might have PINK1 or Parkin, and these are different components of the system. So, understanding the cause of the Parkinson's disease in different people impacted by the condition will enable them to get the best therapies that might work best at halting the disease, or even reversing some of the symptoms.

So, that's really important. What's even more important, all evidence suggests that people with Parkinson's disease may have a disease 10 to 20 years before the onset of major symptoms. So, this is called the prodromal phase of the disease. So, if you are able to diagnose people in this phase of the disease, and this is something that I know The Michael J. Fox Foundation is doing amazing work on with this alpha synuclein test that they're developing, then you might be able to tell people, okay, you're in this prodromal phase of Parkinson's disease. And you can work out what pathways are affected in that patient and administer them with a drug that would pause the disease in its tracks at that stage, and they wouldn't go on to develop any of the symptoms of Parkinson's, or it would be massively delayed. This is why research is so important. And I think all the things I've said are possible, but it requires lots more very detailed and vigorous research.

Marie: You've got work to do Dario.

Dario: Definitely, definitely. We don't know how high the mountain that we need to climb is to achieve what I've just said, but I can assure the listeners that the researchers, like myself and The Michael J. Fox Foundation and other funders of this area, won't stop working until the job is done.

Marie: Definitely, and we really look forward to continuing to improve our understanding of that underlying biology to improve not only the diagnosis and treatment, but also as you're alluding to, the prevention, ultimately, of Parkinson's disease. So, maybe our last question here, Dario, can you comment on how your work specifically is bringing us closer to this big goal of finding a cure or contributing to these improved therapies and diagnoses?

Dario: I think the finding of the Rabs is really important because it provides a way to assess the LRRK2 pathoactivity in people and assess the efficacy of drugs that target the LRRK2 pathway, but then we're also working on other components of the system. So, for example, it turns out when the Rabs are phosphorylated by LRRK2, there's also an enzyme that counteracts that biology — a protein phosphatase. And it's a specific enzyme called PPM1H that regulates this reaction. So, we're exploring ideas whether it's possible to boost the activity of PPM1H in cells as an alternative method to counteract increases in LRRK2 activity. I mentioned before as well that our companies have made these type one kinase inhibitors that target LRRK2, but we and others in this field have shown that it's also possible to target LRRK2 with another class of inhibitors called a type II kinase inhibitor. And these are potentially interesting because they actually have properties of trapping the LRRK2 in the inactive conformation, so it doesn't become activated.

There's a lot of interest to develop improved type II kinase inhibitors so we can test whether they maybe have less toxicity in the lung and the kidney than type I compounds. And then, as I told you as well before, Rabs also activate the LRRK2 pathway by recruiting it to membranes by binding it to this end terminal region. So, if we can understand that biology better, we could provide drugs that would block the activation of these Rabs that lead to the inappropriate activation of the LRRK2, either by blocking the activation of the Rabs, or by blocking the Rabs from recruiting the LRRK2. This is something I think is particularly interesting. Everyone else I've talked to thinks this is a completely crazy idea.

Marie: Ambitious, we'll call it.

Dario: Most of the ideas will be completely crazy, but nevertheless, as Marco Baptista said, we shouldn't leave any stone unturned. And then further downstream, we've just discovered there's a new component that we call TMEM55B, and this component's on the lysosome. This is one of the target organelles for LRRK2, and we think that this component seems to bind to many genes involved in Parkinson's disease we've found. And we think that this might function as another type of enzyme called an E3 ligase. So, this is very technical, but this might be involved in regulating the stability and degradation of other proteins. So, we're very excited at the moment to see whether or not this protein TMEM55B is indeed a E3 ligase, and whether this can be explored further.

So, in a way, we're walking down the pathway, step by step. Each step seems to take four or five years of work, but each step reveals new ideas, new knowledge, new reagents to better study the disease, and potential new drug targets. And there's many people now working in this field doing great work. The lab of Shawn Ferguson at Yale have just discovered a new signaling pathway, known as the STING pathway, that seems to control the LRRK2 activity. And that pathway has been already targeted for other disease areas. So, it's possible that that could also result in new exciting treatments in the future. So, I think there's going to be lots of opportunities to develop new approaches to treat Parkinson's.

Marie: Absolutely. And Dario, for you, what has been the most satisfying, or perhaps the most rewarding, part of working in this LRRK2 research area?

Dario: Yeah. We've been working at this for over 19 years now, and by far the most rewarding thing that I've been able to witness is, in April this year, I was able to see the first patients in the UK participate in the Biogen-Denali trial at our local hospital here in Dundee. And after so many years' work, seeing a patient take three tablets — we don't know, obviously, if it was the inhibitor or the placebo — but to me, it felt like a sort of a landmark in our path for this work. And obviously, the next stage will be to know whether these compounds actually offer benefits to

the patients, but I think it offers so much hope that they will. And that was, for me, the most special moment.

Marie: Well, Dario, we truly appreciate all the outstanding research that you've done over the years and that you are continuing to do in the area of Parkinson's research. And we truly appreciate you joining us for this conversation today, so thank you so much for your time.

Dario: Thank you, Marie. It was a great pleasure talking to you and lots of fun. Thank you.

Marie: Well, Dario, it's been wonderful to speak with you, and listeners, it's been great to have you 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. 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*.