

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 thrilled to welcome our guest, Dr. Christine Klein. Listeners, Christine is Director of the Institute of Neurogenetics and of the Section of Clinical and Molecular Neurogenetics at the Department of Neurology, as well as the Schilling Professor of Neurology at the University of Lübeck in Germany.

Today, we are excited to hear about her perspectives on advances and opportunities in genetics research, her role in the Global Parkinson's Genetics Program, as well as her research on genetic variants for Parkinson's disease. So, Christine, welcome to our show today. How are you?

Christine: I'm very well. Thank you. Good morning. Good afternoon, Marie. Over here, I'm in Germany. It's already four o'clock. I'm very happy to be your guest today. Thank you for having me.

Marie: Well, we are so excited to chat with you, and we are looking forward to learning more about you and all of the wonderful work that you've been doing. So, can you start off by telling us a little bit more about you? What is your background, and Christine, how did you find your way to your current position?

Christine: Yes, thank you. So, I am a neurologist by training. So, I first attended medical school, and I was very interested from the beginning in movement disorders. I thought that was very exciting. In fact, the brain really excited me, even when I did neuroanatomy. And then I was very lucky. After finishing medical school, I was able to go to the U.S. to Boston for two years for a post-doctoral fellowship with Xandra Breakefield at Harvard, and this is how I was introduced to neurogenetics.

And not only that, but we have a system here in Germany which is slightly different from the U.S. system, I suppose, where MDs also have to do a thesis. And so, I was very lucky again. So, I spent a year even before that during medical school and did one year of lab work. And that was also neurobiology.

And I was very lucky because my supervisor, Craig Garner, was also American, very rare at the time that Americans came to Germany. So, this was a wonderful opportunity.

And then I continued with my fellowship training, my clinical fellowship, and then finished my board certification as a neurologist, but always kept doing my research and built up my own team. And then I was trained also in clinical movement disorders by Tony Lang, where I went eleven times to Toronto to spend always the summers there, which was extremely educational. And since that time, and even before, we've been building up the Institute of Neurogenetics here in Lübeck, and we've been doing the genetics work. And for a number of years, we have been supported very generously by The Michael J. Fox Foundation. And I think this is a good opportunity. We didn't talk about this, but I want to say a big thank you. It's wonderful to have all this support, and not just the financial support, but also all the wonderful discussions we've been having, the scientific support as well. Thank you for that.

Marie: Absolutely. I think this is a wonderful collaborative environment here with The Michael J. Fox Foundation, and all of the scientists that work with this organization around the world. So, Christine, I'd love to talk about the field of genetics. You've been working in this neurogenetics field now for a number of years. Can you talk a little bit about how research in this field has maybe changed or evolved over the years as you've seen it happening in real time?

Christine: Indeed, I'm now old enough, which may be a good or a bad thing to remember at times when it was a lot more difficult to do genetics research. In fact, at the beginning, we had to call every single base with a special pen, using a big light box, I would say, and it was very cumbersome, and it took absolutely forever. Then some years later, when I was already in Boston, together with Xandra Breakefield and Laurie Ozelius, we had found an interesting region through linkage at the time, which is still being done sometimes. It can still be helpful, but it's no longer the way to find genes really. So, we had found this region of interest, and it was still relatively large.

So, I asked Laurie, well, Laurie, what do you think? Can we just simply not sequence that region? She looked at me and said, no, we cannot. There's no way to do this. So, when I think back, this was in the late '90s. It's been a while, but when I think back, this now seems almost funny, and it's really amazing how many things we're able to do now. And I find it also interesting how much technology driven our field really is. And really wonderful technologies, next generation sequencing, but also non-sequence-based technologies such as Bionano optical mapping and things like this have emerged. I think really giving us new opportunities, and as you know, this still results in great new discoveries. So, I'm very hopeful that there is still a lot more to be discovered.

Marie: Absolutely. And I know as the technology advances, as the methods sort of become more efficient, I think the amount of data becomes almost a benefit and a problem. So, how do you deal with the massive amounts of information that you're able to obtain relatively conveniently these days, compared to how it was even decades ago?

Christine: Absolutely true. And this is a big challenge. And I don't think I can claim that we're totally on top of this challenge. A colleague of mine sometimes likes to explain this particular relationship that you just described with two triangles, one flipped. And basically, at the beginning, we had a lot of wet lab effort and very little interpretation, because for example, we were only sequencing one gene and there's only so many bases to look at. Now, it's the other way around, just as you've implicated. So, we're doing several genomes maybe a day or something. And then we have all this data. And so, it's really a matter of very good bioinformatics to be able to really analyze this data well.

And this is something that we do here in-house, also in collaboration with colleagues on campus, but also really in collaboration with colleagues all over the world. And in fact, as you say, data analysis has become probably the bigger part of what we're doing these days. Another part, however, then is also, I think, important. And that is all the validation work that then follows. But again, even the validation work is closely connected to the bioinformatics work. So, this is really something that goes together and has to go together.

Marie: Certainly. And perhaps we can hone in specifically on Parkinson's disease as well. So, how has sort of studying the genetics of Parkinson's disease changed over time? What are some of these major advances or the accelerations that you've been seeing?

Christine: Yeah. So, thank you. That is obviously also a very exciting question. I have really witnessed myself, I think, from the very beginning. So, my very first Congress that I ever attended was in 1996 in Vienna. Beautiful venue there. And this was the Movement Disorders Congress. And this was the time when Larry Golbe had a talk and he showed mostly actually pictures, photos. Photos of a beautiful region in southern Italy.

And this is where the Contursi kindred originated. And so, he was showing these photos and he was showing videos of family members. And it turned out really it was clear that this was a familial form, genetic form, of Parkinson's. And then it took only, as you know, only one more year until 1997, until the first monogenic cause of Parkinson's was discovered. And that was the discovery of the alpha-synuclein gene. And this was really based on this large, large pedigree on linkage studies. So, all the traditional genetics we had at the time.

So, that was wonderful. And initially, people still even questioned that a little bit because this particular mutation or pathogenic variant, as we call it today, found in that kindred actually happened to be non-conserved in mouse. And so, it was a question, you know, how important this missense change really could be. But of course, today, we really know that this was a true finding. And we have learned so much about it from, although a very rare monogenic condition, we have learned so much about Parkinson's disease, and lewey bodies, and everything that's connected, and also risk factors that's connected to alpha-synuclein.

The next, I would say, very important gene, that's slightly different. That was discovered in Japan by Nobu Hattori. And this is a recessive form of Parkinson's, unlike alpha-synuclein, where it's dominantly inherited. So, it goes from generation to generation, whereas in the recessive forms, typically the parents are healthy, but you can have several siblings affected. And so, this is a different way. So, basically, you typically have several smaller families. And this is really also how in Japan, the PRKN gene was next then discovered.

And then, maybe I'll skip a few genes. I think the first gene that was then found by next-generation sequencing methods, where we already were now able to sequence long stretches of the genome, that was VPS35. So, this was for the first time really a new discovery based on next-generation sequencing technologies. And since that time, a few more genes and candidates have been proposed. And personally, I'm thinking that there could be more to be found in maybe non-coding regions of the genome. So, I'm thinking that could be another next step really, or a whole field that we might want to enter. And we are already entering.

Marie: Absolutely. And as you alluded to, I think part of the challenge of just sort of untangling this genetic story of Parkinson's disease is that it is a heterogeneous disease. There are a variety of different genes and things that could cause it. So, how has this played into just figuring out what that story is and how to tackle the next steps?

Christine: Yes. I'd like to thank you for that question. And in fact, it falls right into my lab. As we recently wrote a seminar for *The Lancet* on Parkinson's disease, and I was kind of invited by Bas Bloem to join him for that effort. And in fact, we wanted to name this article, although it's not only on genetics, but we wanted to name it "Parkinson's diseases," reflecting what you just said, but the journal was against it. But it's clear that there are different Parkinson's diseases, and I would even go so far that almost practically every patient has his or her own Parkinson's disease, because every single person with Parkinson's disease is different.

It's clear that there are different genetic forms, and I think that is important altogether when you also take into account pathogenic variants in GBA1, which is mostly considered a strong risk factor for Parkinson's. But if you take that all together, you will end up with a frequency of about 15%. And so, you may now say, okay, well, there's still 85% where we don't find anything, and that's a lot. And I agree with that. On the other hand, you can say, oh, well, I mean, 15%, that's not nothing. This is like every seventh patient or so, where we can find something. And hopefully, these findings in the future will be potentially actionable.

Marie: I agree, and I think an important step in understanding the genetics of Parkinson's disease is the Global Parkinson's Genetics Program, or GP2, a Program from Aligning Science Across Parkinson's (ASAP) with strong involvement and support from The Michael J. Fox Foundation. So, Christine, can you talk a little bit about how this project started and what your role has been?

Christine: This is a topic very close to my heart, and it's the most wonderful Parkinson's genetics program you may ever envision. It's really a dream come true project. It's large in scope. There are enough resources to do everything well, I would say, which is not always the case in research. And, most importantly I think, it's a huge and absolutely enjoyable privilege to work with all these colleagues really from all over the world. And I would like to acknowledge in particular the leadership, Andy Singleton and Cornelis Blauwendraat at NIH, who put this all together initially and who so generously share all of this now with us.

And yeah, how did it start? Well, for me, I heard about it for the first time in 2019. That's something really big, you know, might soon appear in the field of genetics of Parkinson's. So, I was very interested. And then there was an invitation to come to New York City in 2020. And this was actually at the end of January, probably the very last opportunity to come together in person before the pandemic started. And so we were there, probably around, I would say, 60 people or so, all, you know, experts in Parkinson's genetics, also very international from the very beginning. And so, we started to talk about, you know, how we could shape this program.

And so this is how it started. And already at the beginning, there were some ideas as to how one could perhaps best reflect the different genetic contributions to Parkinson's disease and investigate them further. And what I have not yet really talked about, also because not really my field so much, is the complex genetics of Parkinson's, which of course also plays a major, major role.

So, at the beginning, the idea was we definitely have to cover monogenic disease and also complex genetics. So, why don't we have these two, at the time they were called hubs, now they're called networks. So, this is how those two

networks were created at the beginning. And I'm fortunate enough to be co-leading, together with two colleagues, Niccolo Mencacci in Chicago and Joanne Trinh here in Lübeck, the monogenic network, whereas the complex network is represented by Huw Morris in London. This was my role from the very beginning.

Then, you know, there were a lot of things to be done. And luckily, we had a wonderful team. Another person I would like to mention here is Dr. Lara Lange. She is actually here in Lübeck, and she really from scratch also helped building up our monogenic network. So what did we have to do? We had to find first of all, you know, all these patients with a potential monogenic disease origin.

So, in other words, patients with an early age of onset, or family history, or both. Then we had to make sure that people could tell us about their families in a structured way. And so we had to set up a portal, again, which we did, you know, in collaboration with colleagues in Malaysia, Shen Lim and Enza Maria Valente in Italy. And we had to prioritize samples. This was led by Kishore Kumar in Australia.

So, lots of things to do. And we had to set up the sequencing pipeline, which is led by Zih-Hua Fang and initially also by Peter Heutink, who actually is no longer with GP2, unfortunately. So, this is how we put our teams together with these individual working groups. And I think by the end of the first year, we had at least, you know, a basic structure as to how we could start with this. And in fact, in order to try this out, we started with a 500 genome pilot. And so we started working with those groups that had already ethics in place that could share samples freely and data as well. And so, we did the first 500 genomes and tried to be also very inclusive and to try to include colleagues and patients, of course, from across the globe. And that was how it started. And then the pandemic came, but we continued with everything. A little slowed down, but not much. And now we had the second in-person meeting after Madrid last year, this year in Copenhagen, where we came all together, and more than 200 people this year. And it has grown to an amazing family.

Marie: Oh, that's wonderful. And you brought up an important point here, the necessity for inclusivity. I think there was an important gap that really needed to be filled when looking at the genetics of this disease. And that was that much of the research had been done on people who were white and of European ancestry from either North America or Europe. So, how did you go about kind of trying to fill that gap and recruit people from all over the world?

Christine: This is very, very true. And in fact, The Michael J. Fox Foundation, you know, prior to the GP2 program, they funded us to actually conduct a project on monogenic PD. And let me just take one step back. So, we had put together a

website which includes genetic information and clinical information on published patients with mutations in Parkinson's genes. And we had done this very carefully.

And then we said, okay, that's fine. So why don't we use this as the springboard? Everything, however, in the English language. So this, for example, excluded already all the reports in Spanish, or Chinese, or what have you, because for lack of better knowledge of the languages. So, this was the systematic review of the English language reports. And then we contacted every single corresponding author of every single one of these articles.

And we had a great response rate of over 50%. And were able to collect then the actual information beyond what was written in the papers. And then exactly what you just described happened. When we looked at the ethnicity and where people came from, it turned out that over three quarters of all of these patients where we had genetic information are of white European ethnic origin. And we kind of knew this, but I think it was worse than we expected. And obviously, this is a huge knowledge gap.

Marie: So then, Christine, once you identified this knowledge gap, what were some of the steps that you took to try to get that information to fill the gap?

Christine: Obviously, we had exhausted what we could do based on the literature. And of course, it's not surprising that those areas or those sites in the world that are underprivileged for, for example, research resources cannot publish, right?

Because they just don't have the means to do this. Although many of them are, you know, very well trained in movement disorders, for example, or even if they didn't have that opportunity, they're very interested in receiving training and contributing to the project. And this is what we did. And there's another colleague, Ignacio Mata, who is leading another big project within GP2 that is the underrepresented populations project. And he basically had already started, this was a natural extension of what he had already done, because being Spanish in origin himself, he had put together a large network in South America, or Latin America, called LARGE-PD.

And so he was the one systematically reaching out to others. And this was then enhanced by all these other people that came on board. For example, in Asia, we had Shen Lim and Ai Huey Tan, and they're in Malaysia. First of all, it was great to have them. But then they started reaching out to Vietnam, to Nepal, Indonesia, Bangladesh, to Sri Lanka. So, all of a sudden, you know, this was like a whole big avalanche. So, all of us went out, every single one of us went out to include, you know, everyone and anyone we knew that was not yet part of this network. And we were also contacted by some of these investigators ourselves. So, it was, I

would say, was not a systematic approach. Although we sometimes look at the map and say, okay, where's still uncharted territory? But we really used all these contacts. And this has led to the inclusion of a lot of countries and sites that were previously underrepresented or not represented in research.

Marie: I love this. I think this is the network effect in action.

Christine: Yes.

Marie: So, Christine, can you talk a little bit then about what are some of these key achievements or the things that this initiative has accomplished so far?

Christine: So, I mean, coming maybe from, you know, the very end. Obviously, we're interested in finding and understanding causes and genetic contributions of Parkinson's disease. I think we've already seen, and this is the big success that was published this year, you know, the African GWAS, which has shown that there is a specific influence of the GBA1 gene in the African population that we don't see in other populations.

So, we have already now demonstrated that it is absolutely worth the effort, for various reasons, particularly for scientific reasons, to include all other populations, because there will be very, very interesting insights. And it's possible that some diseases or some forms of Parkinsonism exist exclusively in certain areas, because of, for example, geographic, you know, isolation. And one such example that was already previously known, though, is X-linked dystonia-parkinsonism, which is a condition that is rare worldwide. But there are thousands affected in the Philippines, and this is actually the only country. So, all affected originate from the Philippines. And, in fact, all from the same island.

And so these types of conditions, I'm sure there are others out there that we don't know about, we are all missing so far. So this, I think, is one of the most important goals. At the same time, there's also, of course, a big cultural goal, if you will, to be, as you phrase it, inclusive. And to be able to, you know, really work with the whole world on this huge problem of Parkinson's and the genetics of Parkinson's, because this way, all together, we'll best be able to solve it, I think.

And this has proven very true. I think, you know, this interaction with so many colleagues whom probably otherwise I wouldn't have met has been extremely inspiring and really fruitful. And for me, one of the biggest perks of the whole project.

Marie: Oh, absolutely. I think this is an extremely large collaboration, just looking at the scale of it. So, Christine, can you talk a little bit about the importance of this team

science approach, really bringing people together, not only from all over the world, but from academia, industry, the clinical realm, and also just working together in a collaborative fashion to solve this big problem.

Christine: Yes, I think this is a very, very important point that you're raising. And I think you really named all the big stakeholders. Just as you said, I think all of them are absolutely vital to the eventual success. For example, industry, it's, you know, something that has to be done carefully. On the other hand, it can be extremely fruitful, because eventually we want all of this to result in better treatments as well. So, from the beginning, there has been exchange also with different companies. I personally think that this is very important. And they also have very interesting data. And so I think this exchange is very, very helpful as well.

Exchange with patients directly themselves has not yet taken place. And it's probably not necessarily, you know, at the heart of this particular research program. But what we are discussing and what we're working on very intensely at the moment is the question of return of results to patients. Just recognizing and being mindful of the fact that these are research results and not clinical diagnostic-quality results. So, this poses challenges also because obviously genetic counseling is a big issue, and not one size fits all, as you may imagine with all these different settings, and different cultures, and different also habits. And, you know, we have everything ranging from research results must not be shared to research results must be shared. So they're set at GP2. And this is fine because GP2 allows these different views that is quite broad and not easy to tailor to.

Marie: Certainly. Are there any other aspects of GP2 that you want listeners to know about or things that this initiative is working towards?

Christine: I think one really, really well-developed and really interesting aspect is the training network. And that is run by Alastair Noyce and his colleague Sumit Dey in London. And by now, a very, very impressive array of teaching opportunities online, but also in person, hackathons, meetings, opportunities to attend conferences, etc., have emerged and are really sought after by the trainees. Over 200 of them are part of the network at the moment.

And I should also say that GP2 is also running graduate programs. So, there are currently, I think, four PhD students. Another group of students will start very soon. We have just selected them. And this time, there were, I think, four or five times as many applications. So, the program catches on, and more and more people know about it and want to be part of it, which is great. So, I think the training aspect is fantastic and an amazing opportunity for everyone, really. What is also nice is that it is connected to the MDS, the Movement Disorder Society. So, there is an agreement, which is important also for the clinical

characterization, that we are able to use their rating scales, for example, and things like this. And also teaching activities that they offer. So, I think this is also a very nice partnership.

And then let me mention just one more beautiful aspect, which is also relatively new to the program. There are sabbaticals now. There have always been opportunities to visit other sites. But this has now been more formalized. And also there is now, you know, small funding available for this so that people can come, you know, for short term visits, typically up to six months at the most, and come to pretty much any GP2 site that can and would like to host another scientist.

And this is really, really nice. And I have to say, I think we've been hosting eleven people so far. And it's an absolute privilege to learn from them, and to interact with them, and have them here, and it's a cultural experience as well. So, this is really, really nice. And at this moment, actually, we're hosting four people, you know, from very different areas in the world, from South America to Thailand. And so this is fantastic.

Marie: Well, I appreciate you sharing a lot about this GP2 initiative. And I think it's making amazing strides in advancing our understanding of the genetics of Parkinson's disease. And you touched on just a little bit some of the issues surrounding genetic testing and delivery of results.

And I think this is a really important issue in the field. So can you talk about some of the work that you've done, just understanding the scene surrounding genetic testing, when it would be appropriate, and what the future looks like in terms of it being implemented in a routine setting?

Christine: So, within GP2, and I think this is very, very important to understand, GP2 is first and foremost a research project. So, when it was first conceived, returning results, and that is research results, was not really, you know, very high on the agenda. However, it then turned out, maybe not surprisingly, that returning results to patients is the major incentive for many of the centers, especially for those, of course, that have no easy access to genetic testing.

And it also turned out, through other studies, that patients and people with Parkinson's are very interested overall in genetics, and sometimes more interested than their doctors. And so, this is also something that we have to take into account. And then maybe the next question is what to return and who to return what. And so the first question is, do we return results only to patients (affected people), or do we return results also to controls (healthy people)?

Because in GP2, there is not only patients, but there is, of course, also many, many controls that need to be included, because we have to compare whatever

we find in patients to what we find or do not find in healthy people controls. And controls need to come from all these different ethnicities and sites, because certain things can obviously be meaningful in controls, let's say, of Asian origin, whereas in European controls, you wouldn't even see this. So this is something we also take very seriously, which poses a problem.

So, we have decided at the beginning to focus on the patients. The next big question is what to return? Do we return only definitely pathogenic variants? Or do we return also things that are a little less certain, or even variants of uncertain significance? And for now, we have decided to keep it simple to really focus on those with a known, or with a highly likely, at least, pathogenicity.

The next major question is, who returns and how do we return these results and who provides for the genetic counseling? And this is mostly unsolved, I have to say. There are good structures in place in some centers, but this is definitely not the rule. Another problem, and this is something that actually even some of the site investigators may not have realized, because it's really, you have to understand a bit better the techniques and the technology behind what we're doing in GP2, to appreciate what we can find with our methods and what we cannot find.

And let me just explain this briefly. So, for those where we suspect a monogenic cause, so a single variant or gene that explains the disease, there we perform whole genome sequencing. So, this is wonderful, because you really see every single base pair of your DNA sequence, of the genetic code. However, for those with complex PD, so where we don't suspect immediately a monogenic cause, they undergo a chip. And this chip is wonderful, and it has close to 2 million variants. It also covers the entire genome, but these are common variants, so not disease-causing. It also contains what we call a custom content. So, in other words, on this chip, in addition to the backbone, there is a large number of known pathogenic variants. But obviously, on the chip, you can only detect what's on the chip.

And the chip was designed and made a few years ago. So, first of all, it cannot be updated. Second of all, it will, and we discussed that at the beginning, it will only contain what's been described. So, it will miss, for example, variants that may be very important in certain populations, but we just don't know about them yet. So, this is bound to be something that will come with a lot of false negatives. And so when you then return results to a patient, it is very important to actually recognize and realize how the results were generated because with the whole genome, you have much better certainty than with this chip. It's a great research instrument, but it's not great for return of results, especially not in a diagnostic setting.

And then the next step is, although we take great care in everything we're doing, but we're not at the level of a genetic testing company that is CLIA-certified and that has their beautiful pipeline and barcodes. And even if we wanted to have that, we couldn't really quite or it's difficult because, especially when we work with more remote areas of the world, sometimes just shipping the samples and even sample mixups occur. So, all of these things may happen and again, may result in false results. So, this is another thing that we've been discussing. How can we validate, ideally using a fresh sample, making sure that there was no sample mixup and that we can really, by a Sanger sequencing, for example, confirm that this particular variant that was found, either on the chip or on whole genome sequencing, is actually there in that particular patient. So this is another concern. And that's also, of course, very costly and not something that has been built into the budget of GP2. So, lots of questions, but we're definitely not giving up. We're only at the beginning.

Marie: Absolutely. And you highlighted some of these issues surrounding sort of research-level genetic testing results. And I think the complications sort of balloon even further out when you start thinking about the implementation in a routine clinical setting. So, can you share your thoughts on what the future of that might look like?

Christine: I don't have the perfect answer to this, though, I have to admit. What is important, and this is actually how we started, and I didn't mention that yet. We first started out with a survey to all of the GP2 sites and we asked them a number of questions, how comfortable they feel, what kind of training they have, what their current practice is, and what their diagnostic and research setting looks like and things like that. So we got that data back, which is very interesting. Also, the majority feels that their patients want to know, which I think is very important.

And this really segues nicely really into your actual question. What would the future look like? And starting with the future, although it's always slightly difficult to predict the future, of course, but starting with the future, let's say, and this is my hope, all of our hope, that we will have gene-targeted treatments available one day. And as you know, there are now clinical trials ongoing. Then, of course, I think the genetic testing landscape would change because if we had something really actionable that we could offer, hopefully at reasonable cost, and with few side effects to patients with specific variants or specific genes that are mutated, then I would argue for trying to implement genetic testing as widely as possible.

We are not there yet, obviously. So the question is then, what is useful? Because also we don't want to harm the patient and because one of the most common scenarios and outcomes of genetic testing is, of course, variants of uncertain significance. So, we're sitting there not knowing what to do with this. And if anything, confusing the patient perhaps even a bit. So, I think this must not be

taken lightly and it is done at the discretion of the patient. That is very important and they need to understand what they're getting themselves into.

Because as you know, genetic testing often, if not always, somewhat affects all of the family, and it can really interfere with family planning and with career planning. And so this is a very, something that cannot be taken lightly. However, an important consideration, I think, is also that many patients, as I mentioned before, really want to know. So, what we do and most of us do, and there are no universal guidelines yet, but what is recommended to offer genetic testing in people with an early age of onset — somewhere between the age of 40 or 50, there's a bit of a debate — and those with a positive family history. Because sometimes we can actually find the genes and find the cause. And this is something as doctors, we're always trying to do, right? We try to find the cause of the disease. So there, I think it's clear.

But then there is a gray zone where you may or may not test. And maybe just one last word about this, as I mentioned at the beginning, it's about 15% who carry potentially actionable variants if gene-targeted treatment is becoming available, and some trials are already ongoing at the moment. So, then you may argue, well, it would be good if we had these patients available and we knew about their genetic status, because then we could actually include them in ongoing clinical trials. And in the clinical trials, we don't want just those with an age of onset at 30, but also the other ones.

So, from a clinical trial perspective, it would probably be good to have more clinical trial ready cohorts, which at this stage, we don't have for PD. And this is something that Mike Nalls is actually advocating. He's actually doing a lot of the modeling and the biostatistics or bioinformatics for GP2. But he's also very keen on translating GP2 into the practice and has started a program in a project within GP2 where we can start trying to build a clinical trial ready cohort.

Marie: Very interesting. And I think, Christine, you brought up some really interesting points in that response. And you're doing a lot of really cool research on these different genetic variants related to Parkinson's disease. So, is there a project that you're working on at the moment in the lab that you're the most excited about and want to share with all of us?

Christine: I'm really, really excited about a lot of things.

Marie: I was looking at your recent papers. I was like, there's a lot to choose from.

Christine: Yeah, we have great opportunities to work together with wonderful people. Interesting questions. So, what I'm personally extremely interested in, that is actually the phenomenon of reduced penetrance. And I think this is something

very positive, which is also one of the reasons why I like it. This means that not everyone who carries a pathogenic variant or mutation will develop the disease. Or if you say, okay, it's an age-dependent thing anyway, because typically nobody has it at the age of three, then it could also be, let's say, a very late age of onset as compared to others that have a much earlier age of onset.

So, understanding these factors that protect you, so from a really positive angle, protect you against or delay the onset of the disease, I find from a conceptual point of view, an extremely promising and important concept and really project. And this is something we're doing here. And maybe I can just highlight one recent paper that was published earlier this year, where we looked into modifiers of penetrance. Again, we found a few different things.

So, for example, mitochondrial mutational burden. Mitochondria, our little powerhouses of the cells, they obviously also have their genomes. When they get old, they need to be removed. And that doesn't always work very well. Sometimes when they're not removed at the right time, then they build up mutations. So, we looked into the mutational load in those mitochondria. And it turns out that people with Parkinson's have a higher mutational load. And this is significant, really, than those that have not developed the disease. Another very interesting finding, and a lot of this obviously will be somehow connected. So, genetics is not just done in isolation, I think, or will not explain everything in isolation.

There's also, of course, the environment. And one interesting study showed by Joanne Trinh that in patients of North African origin, all carrying the LRRK2 common pathogenic variant that's common there in this area in those patients. When she compared for environmental factors, people that are affected versus those that are unaffected. And she found that some of the protective environmental factors really also delayed the age of onset of the condition. So, for example, when you're a smoker, which protects against Parkinson's, which doesn't mean that I think we should all smoke, but it does protect against Parkinson's. And also black tea drinking. So, in those people that both smoked and drank black tea, the age of onset, when they carried a certain mutation, was much later than in those that did not do either of these things.

And so I'm thinking this is also a very interesting area that we are fully starting to really go into, which is this gene and the environmental factors and potentially later, even the gene-environmental interactions.

Marie: I think this is so cool. So, what have been some of the biggest surprises or maybe unexpected results, Christine, from the work that you've been doing?

Christine: Well, in a way, it's unexpected that we haven't yet found a new PD gene. And this is a little bit disappointing, I should say as well. But I should say that we're really only at the beginning.

It took a while really to set up all of GP2 and to bring so many centers on board. I could talk another hour about all the problems we have with getting all the ethics. And there's a wonderful compliance team led by J. Solle and Claire Wegel who deal with this. But this is a big deal. So, I'm thinking hopefully we'll find some actual new genes in the near future. And I think one answer will be also long-read sequencing that we are starting to do now as well. So, that's maybe one unexpected thing.

Then, what is maybe slightly unexpected, and we're really just at the beginning of analyzing the data that's now really coming in, but now at a really, really nice steady flow. What is interesting is that we're finding pathogenic variants or mutations and repeat expansions in genes previously really associated with other diseases. And sometimes there was the odd case where they wrote this can also be associated with Parkinson's, but we really now find a number of people with isolated Parkinson's. And you would never have thought of any of these genes.

So, that I think is also an interesting and somewhat surprising finding. But then all the really big studies are yet to be undertaken like burden analyses. The first GWAS will be done soon, other than the African one. And that I think was also, well, maybe not surprising, surprising, because we were hoping to find something special in other populations. But to find GBA1, which is again a gene that we have been studying so much in the context of PD, but then now to find it in that population in a different context, I think, was also somewhat surprising and very gratifying.

Marie: Absolutely. And I guess if you were to look at the big picture of the field here, what are some of the resources or tools or collaborations that you think are really having a big impact and helping to move the field forward towards answering these big questions related to the genetics of Parkinson's?

Christine: That's, as you already implicate, I think this is really a combination. And I think GP2 really checks all these boxes. And it's technology, as you mentioned, you have to have the cutting edge technology. Otherwise, you don't find things in intronic regions, for example. So, this is something you have and also the bioinformatics. And we're very fortunate to have the highest quality bioinformatics within GP2. So, that's one.

Second, you have to have the right patients, and people, and probands, and they need to be well-characterized. Because if that's not the case, then everything else that's following will not be relevant or may even be misleading. And just to

give you one example, in some areas, there is obviously at this stage, very little training still, for example, even in movement disorders. And at the beginning, there were some centers where it was very difficult for the clinicians there to really characterize the patients well.

And so, for example, initially, we found Huntington's disease, a mutation, so the repeat expansion of Huntington's disease in a seemingly Parkinson's family, which then turned out when we watched the videos, that they actually had a Huntington's disease-like picture. So again, this I think is very important to have the quality control for the genetic part. But that's relatively easy compared to the clinical part, where it's just as important. And for this, there are many great solutions. So, there's again, training, there are certificates from the Movement Disorder Society, and there's videos that help greatly. And this again, is also wonderful training for those that are part of GP2, because they can help watch these videos, see them, watch them together with their colleagues, and analyze them. So, this is another great opportunity. And then the third and last thing, which is probably the most important of all, is the amazing collaborative and open spirit of sharing and of doing this together, and of working together on the same goal. I think this is really the big hallmark, I suppose, of GP2. And I have to say, unparalleled.

Marie: Absolutely. And we've talked about some of the questions that remain to be answered throughout our conversations today. But I guess if you had to, again, take a step back and look at the big picture, Christine, what do you see as the biggest unanswered questions or maybe areas of opportunity for research in Parkinson's disease?

Christine: So, as much as I'm interested in genetics, I've been spending pretty much my professional life so far on genetics. And I mentioned this a little bit already. I think genetics will not be the answer to all and everything. And so I think taking into account the environment, which GP2 started doing, I think, is a really important step. I think what we're also starting to do, for example, there's one paper that just came out earlier this year. I mean, it's relatively simple when it comes to a monogenic cause and you have one gene, one mutation, and that explains everything. But that's, of course, the tip of the iceberg. So gene-gene interactions is another thing.

So really, I think we have to, and we're doing already polygenic scores and things like this, but I think we have to dig a lot deeper still to understand this network of things that causes or contributes to Parkinson's disease. And I will just reiterate what I said before. And I think we need to understand also the other side of the coin. And that is, what are the protective or the compensatory factors? Because first of all, we need to know about them. And second of all, they may also be actionable and exploitable. And so, I think this is another unmet need. And it

requires slightly different approaches because there, for this, to understand this really well, you have to study also healthy people and follow them longitudinally.

And this could be another huge unmet need that I would like to mention because we don't have that. We don't have that information. We barely have it for idiopathic Parkinson's. We don't have it practically, almost not at all, for genetic Parkinson's. We don't know really the natural histories and how this could be influenced. And so I think that's another huge unmet need that we will have to study. And why GP2 again, is a wonderful platform to do so.

Marie: Absolutely. And perhaps to wrap it up, Christine, could you share with us, I guess, how your work is really bringing us closer to that ultimate goal, finding a cure for Parkinson's disease or really contributing to improving the therapies for people with Parkinson's?

Christine: Well, first of all, I should acknowledge that my particular contribution or that of our group is a very small piece of the puzzle. So, that's important. And there are really fantastic outstanding scientists that have contributed already so much. And every single contributor to GP2, and also people that are not part of GP2 yet, I should say. They are also doing important work. So it's really a team science approach, a global team science approach.

And I'm thinking the biggest opportunity really lies in early sharing of everything that we are finding. Because that gives us an opportunity, not only to know more, many of us, but also to all start thinking about the next steps. And I think if there's one thing that we have learned is we're finding things, and that's great. And we're understanding some things. And that's also great. But also, honestly speaking, things have become more complicated, you know, then coming back to my early days in the '90s, we thought it would be a lot simpler. And that's not the case. And so sharing everything we have, and as a group, really starting to think about everything, and the best strategies to move forward, I think that's going to be key.

Marie: Well, Christine, we appreciate you being part of this big GP2 initiative. And we appreciate all the amazing work that you're doing in Parkinson's disease. So thank you so much for sharing more about yourself and your work with all of us today.

Christine: Thank you so much again for having me. This was such a privilege and great pleasure. Thank you.

Marie: Thank you so much, Christine, and the 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 <https://www.michaeljfox.org/researchresources>. Also visit the MJFF website for more information about the GP2 program that we

talked about today. And also, you can find new episodes of this show each month on the 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 out on our outstanding lineup of upcoming episodes. We look forward to connecting with you again in our next episode of *The Parkinson's Research Podcast*.