Drug discovery for genetic forms of Parkinson's in clinical trials:

Marc Van Grieken (Dundee Research Interest Group) interviews Dario Alessi, Miratul Muquit and Esther Sammler

Key terms

Heredity – the genetic passing of characteristics from one generation to another.

Gene – the physical and functional unit of heredity. Genes are made up of DNA and can act as instructions to make molecules called proteins**.**

DNA - a self-replicating material present in living organisms, that is the carrier of genetic information.

Amino acid - small molecules that are the building blocks of proteins.

Protein - a naturally occurring substance present in all living organisms that consists of amino acids joined together. Proteins make up many essential biological compounds such as enzymes, hormones, and antibodies. The information to produce a protein is encoded by genes in the cell's DNA.

Enzyme - a protein that acts as a catalyst in living organisms, they regulate the rate of chemical reactions in the body.

Mitochondria - membrane-bound cellular machinery or 'organelles' that generate most of the chemical energy needed to power the biochemical reactions within cells. Chemical energy produced by the mitochondria is stored in a small molecule called adenosine triphosphate (ATP).

Phosphorylation - A biochemical process that involves the addition of phosphate (a charged particle that contains the mineral phosphorus) to an organic compound. The structural change induced by phosphorylation can regulate the activity of proteins, making them either activated or inactivated. Phosphorylation is carried out through the action of enzymes known as phosphotransferases or kinases.

Kinase - A type of enzyme that adds chemicals called phosphates to other molecules, such as sugars or proteins. This may cause other molecules in the cell to become either active or inactive. Kinases are a part of many cellular processes.

Ubiquitylation - also referred to as ubiquitination, is the process of attaching ubiquitin, a small protein found in almost all human tissues, to another targeted protein. Ubiquitylation can change the activity or role of the protein within the cell, it is commonly associated with the degradation process of marking specific proteins to be broken down.

Ligase - an enzyme that can cause and accelerate the joining or 'ligation' of two molecules by forming a new chemical bond.

Mutation – the changing of the structure of a gene, resulting in a variant form. Gene mutations may make you more likely to develop certain conditions, such as Parkinson's.

LRRK2 – leucine-rich repeat kinase 2 (also known as dardarin), is a kinase enzyme that in humans is encoded by the LRRK2 gene. LRRK2 is thought to be involved in numerous cellular functions but overactivity of LRRK2 and mutations in the LRRK2 gene have been associated with both early- and late-onset Parkinson's.

Rab proteins - Ras-associated binding proteins are small guanosine triphosphatases (GTPases), which are enzymes that control the activation state of proteins. Rab proteins are involved in the regulation of protein transport into and out of cells. Overactivity of LRRK2 can result in too much phosphorylation of Rab proteins and this over the course of many years leads to defects in cells.

PINK1 - PTEN induced kinase 1 is a kinase enzyme encoded by the PINK1 gene. It is thought to protect cells from stress-induced dysfunction of mitochondria. PINK1 activity causes the parkin protein to bind to mitochondria to induce the removal or destruction of those mitochondria. Mutations in PINK1 are associated with early-onset Parkinson's.

Parkin – a protein encoded by the Parkin RBR E3 Ubiquitin Protein Ligase (PRKN) gene. Parkin is involved in the breaking down or 'degradation' of unneeded proteins through the tagging of damaged proteins with molecules called ubiquitin. Ubiquitin acts as a signal for the unneeded proteins to be moved into specialized cell structures known as proteasomes, where the proteins are broken down or 'degraded'. Inactivation and mutations causing Parkin to become inactive are associated with early-onset Parkinson's.

FBXW7 – F-box/WD repeat-containing protein 7 (FBXW7 or FBW7) is a protein that in humans is encoded by the FBXW7 gene. FBXW7 is part of the ubiquitin-proteasome system and is affected in many human cancers. Parkin ubiquitylates FBXW7I and the reduced ubiquitylation resulting from Parkin inactivity has been implicated in Parkinson's.

Recessive – when two copies of an abnormal gene must be present in order for the condition to develop.

Dominant – when a single copy of an abnormal gene is enough for the condition to develop.

Autophagy - the removal or destruction of damaged or redundant cellular components.

Mitophagy – mitochondria specific autophagy, the removal or destruction of damaged mitochondria.

ASAP- Alliance of Science Across Parkinson's

White blood cells - part of the body's immune system that help to fight infection and other diseases. Types of white blood cells include monocytes, lymphocytes (T cells and B cells) and granulocytes (neutrophils, eosinophils, and basophils).

Homogeneous – being the same or alike. It may be used to describe entities showing alike features.

Idiopathic – the term used to describe any disease or condition which arises spontaneously or for when the cause is unknown.

Paraquat – a toxic chemical that is widely used as weed killerl. Paraquat exposure has been associated with Parkinson's.

Pathogenesis – the chain of events leading to the development of a disease.

Alpha synuclein – a protein that, in humans, is encoded by the SNCA gene. It is a neuronal protein that is thought to be involved in the regulation of neurotransmitter release. In Parkinson's, alpha synuclein folds into the wrong shape or 'misfolds' and can bind together into protein clumps or 'aggregates' known as Lewy bodies.

PET ligand – Positron emission tomography (PET) scans are used to produce detailed 3D images of the inside of the body, including within the brain. A ligand is a molecule that binds to another molecule known as a 'receptor'. PET scans can use radioactively labelled ligands to image whether a particular receptor has been bound to.

Biosample – A biological material (for example a tissue biopsy or samples of blood, cerebral spinal fluid, urine) that contains information from which experimental data can be extracted.

Bioassay – an experiment to measure the potency of a substance by testing its effect biological samples, cells, or tissues.

Biomarker – a naturally occurring molecule, gene, or characteristic by which a particular disease or physiological process can be identified.

Transcript

Marc

Good afternoon everyone, my name is Marc Van Grieken and I was diagnosed with Parkinson's some 16 years ago. I'm a landscape architect and I am fellow of the Landscape Institute. I recently also became a fellow of EUPATI, EUPATI stands for the European Patient Academy for Therapeutic Innovation. Basically, this course trained me to potentially act as a patient advocate in medicine development as part of patient and public involvement, which is an issue we will briefly touch on a bit later.

I am absolutely delighted to be here this afternoon and would like to introduce you to, on my left, your right, Dr Esther Sammler who is a clinical scientist and splits her time between research and acting as a clinician at Ninewells Hospital in Dundee.

Next over, further to my left, further to your right, is Dr Professor Miratul Muqit who is also a clinical neurologist as well as a researcher at this institute.

I will ask both of them in a minute to give an introduction to what they do at this institution. But before that, I would like to introduce you to the last speaker, certainly not the last or least, Professor Dr Dario Alessi.

He has been instrumental in the science associated with genetics and how this may be a cause of people developing Parkinson's.

If I may go back to you Esther, would you be ready to say a very brief few words about what a typical day might entail.

Esther

Ok. Thank you very much for the introduction, Marc. So, I am a clinician scientist, I spend my time partly in the hospital, seeing and treating people with Parkinson's and other neurological conditions, I run clinical trials in Parkinson's disease. But the majority of my time of the week is spent here in the MRC protein phosphorylation and ubiquitylation unit of the University of Dundee, in the building where we are meeting today.

And at the research group, that is basically performing research from bench to bedside and back and forth. So basically, like trying to take discoveries from basic science into the human system and see how far we can get. So that's what I do.

Marc

Thank you, a bit later I will ask you what phosphorylation means but we'll get back to that.

Miratul, I understand that you also work as a clinician in the hospital seeing people with Parkinson's or other neurological conditions and you work in the lab. You may be involved in one of the ASAP grants? A-S-A-P, as soon as possible as far as I am concerned but it stands for the Alliance of Science Across Parkinson's, is that correct? Can you just say a tiny bit about what your day looks like?

Miratul

Thanks very much Marc and it's a pleasure to be here with you today. So, I'm also a clinician scientist, I have two roles here, one is, as you mentioned Marc, seeing patients with Parkinson's and managing their Parkinson's. But I also have a research lab here and we've been mainly studying how two genes, that are been linked to Parkinson's, affect the batteries within our cells. These are the batteries that produce all the energy in our cells, and we think this is a very important cause of Parkinson's. And you've mentioned the ASAP work that we do and one of our roles in the ASAP work is to really define better how different mutations in Parkinson's affect these batteries or 'mitochondria' as its also known scientifically and how when they go wrong it leads to Parkinson's.

Marc

Quickly Miratul, what are mitochondria?

Miratul

These are very small structures in virtually every cell of our body. They have several functions but what they are mostly known for is to produce energy, so the energy of the cell is in the form of a chemical called ATP and this is where we produce energy. But it turns out they also have many other functions and there's been a very longstanding link between these mitochondria being abnormal and the development of Parkinson's. But it's only, I would say, in the last sort of decade that we are starting to get a much better understanding of precisely what is going wrong in these mitochondria which is specific to the Parkinson's link.

Marc

Thanks. Dario, not only are you… I looked on the web and the sheer number of citations and papers you have been involved in is incredible. You are obviously very affluent in the science of it, but you are also the director of this institute. Would you be willing to say a little bit about your day and how all these different responsibilities are matched and what challenges they pose?

Dario

Yes, so a typical day would be for me to come into the lab in the morning and spend time looking at people's data and discussing ideas and future experiments and what it means. And you know we get very excited when an experiment works and if it doesn't work then we try and learn why it didn't work and try to come up with a new idea of how to make it work. Then we have a lot of collaborations with pharmaceutical companies and also this ASAP collaboration and other international collaborations. I would say, every day I would have 3 or 4 zoom calls to discuss how international collaborations and the data that emerges from this. Then I also deal with the administration that is less exciting but really important to deal with. So, typically that would be my day, which starts at half past 8 until 8 o'clock at night would be a typical working day.

Marc

Thank you. One of the things we have to get over is the very long, wordy name of the organisation, the University of Dundee's, Medical Research Council's, Protein

Phosphorylation and Ubiquitylation unit. I haven't a clue what phosphorylation means and what ubiquitylation means. It took me as you probably remember, 3 months at least to try and practice it before I could pronounce it.

Dario, what is phosphorylation and what is ubiquitylation?

Dario

Yes, everyone says we should change the name of our unit because it is too complicated, you know phosphorylation and ubiquitylation, but one of the reasons we are keeping this is because it is unique, there is no other place in the world that has such a name. But maybe more importantly, these are the highways of communication that a cell uses to do everything it does, to control its wellbeing, its response to nutrients, its survival and essentially these are chemical processes that regulate you know biology. Every aspect of biology is controlled by phosphorylation and ubiquitylation and that's what we study, how it controls biology.

Marc

So, you have the cell, and the cell might be subject to phosphorylation, so what happens?

Dario

So, at a molecular level it is extremely simple, you have an enzyme, and your enzyme has to be, sometimes it needs to be low activity and sometimes it needs to be high activity, and you know phosphorylation is the easiest method that biology has to switch something from a low activity to a high activity. So, what happens is a phosphate group, the chemical phosphate, gets attached to the enzyme, at a region of the enzyme, and this causes the shape of the enzyme to change so it can become more active, it can become less active, it can make the enzyme move to a different part of the cell, like the mitochondria, it's a very global, important process. And in this process, most diseases are caused by disruptions in this process. So, this is why we are really interested to study it in Parkinson's disease as it is caused by disruptions both in phosphorylation like in LRRK2, disrupts the phosphorylation part of it. And then Miratul's work with PINK1 is also an example of phosphorylation and Parkin is ubiquitylation.

Marc

May I stop you for a minute, LRRK2, PINK – what are they?

Dario

So, these are really important enzymes that are in all cells of the body but especially in cells in the brain and immune cells. And these are the enzymes that are often mutated in people with an inherited mutation in the part that causes Parkinson's disease.

Marc

Thank you. I now understand a lot more about what phosphorylation is but what is ubiquitylation?

Dario

Let Miratul discuss this, yes.

Miratul

In a similar way to phosphorylation, ubiquitylation is also a chemical alteration in mainly proteins or the machines within the cell, that can affect their function. Unlike phosphorylation, which is quite a small chemical modification, just a few sort of elements, the ubiquitin itself is a larger moiety, it is actually a protein within itself. But in some ways and in the way that the ubiquitin is added to proteins is a little bit more elaborate involving three enzymes, as opposed to one enzyme, to attached this to the machines or proteins. But the effect is very similar, if a protein receives a ubiquitin addition, if it is an enzyme its activity will change from high to low or the opposite. Or it can change the location of that protein or machine within the cell. And the regulation of this is very important in human health because similar to phosphorylation, there are many human diseases in which there are mutations in the enzymes that participate in this ubiquitylation. And so disruption of ubiquitylation is linked to many diseases, including Parkinson's and there are a number of proteins, so for example my own group has studied one, Parkin, which is an enzyme that is important in ubiquitylation. There's also another protein that is linked to Parkinson's, FBXO7 which Dario has also studied, that is also involved in ubiquitylation. So, its another very important system in the cell for communication and function.

Marc

Good, Esther – how does this relate to your work?

Esther

So, I think Dario might like to talk a bit more about his efforts to really understand what LRRK2 does on a molecular level because he's spent years making huge discoveries and moving the field and Parkinson's research forward. And then I come in because I basically, like you know taken the basic science discoveries to then say ok, but this is in a mouse, this is in a cell dish but is this of any relevance, can we make it relevant to the human being as a subject. So, I spent the last 2 years really trying to develop methodologies and assays to test for better pathways. There are two pathways which are switched on and off in patient samples. But I think it would make more sense for Dario to explain a little more about the basic science discoveries.

Marc

I think that would be great because I think from a previous conversation, I think you said it took you years rowing against the stream before you got the result.

Dario

Yes, science is a long-term approach, so I think the story starts in about 2004, when I was really working on the enzymes really involved in cancer and high blood pressure at the time and I came across these papers showing that mutations in the LRRK2 gene caused Parkinson's disease. And LRRK2 is a type of enzyme called a kinase

which puts phosphorylation on proteins so I had a lot of expertise in this area, although I had no expertise of Parkinson's I was just fascinated by how mutations in a gene LRRK2 could be linked to Parkinson's disease. So we started working on this in 2004 and normally when you work on a kinase enzyme, it takes 2 or 3 years to work out what it does and all the previous ones I had worked on it never took us more than 3 years to understand the biology. But with LRRK2 it was very difficult, by 2007 we hadn't even made any advance at all. And then my theory has also been that if something doesn't work, you don't give up until the job is done and you know, if possible, try to invest more effort into this project. So, in 2007, rather than just having one person working on this we put two people working on this and hoped that by 2010 we would get there but by 2010 we hadn't got there and then we had to basically throw the kitchen sink at the problem. The key was to do collaborative science because one lab by themselves can't really have enough expertise to solve the problem. So, we got 5 academic labs, 2 companies – GSK and MERCK, and we got the Michael J Fox Foundation, so we built a huge team of researchers to tackle this problem. And it really took us until 2016 when we discovered that the key thing that LRRK2 does is that it puts these phosphorylations on a new set of enzymes called Rab proteins. And this is the central biology that is disrupted in people who have mutations in LRRK2. This has led to a lot of essential, fundamental information that other researchers could then build on to study the pathways. In a way it provides the framework of knowledge that can then be exploited by further work. But what we now know is that mutations in LRRK2 actually switch on the enzyme, so they make it work more, they make it hyperactive. Its like if something went wrong with your car and you pressed on the accelerator, and it went twice as fast than what you would expect. That is basically what is happening in people who have mutations in LRRK2. This acceleration of the biology leads to these Rab proteins having too much phosphorylation on them. And this over the course of many years leads to defects in cell biology that probably results in loss of dopaminergic neurons, that results in Parkinson's disease. And you know, because of this what is exciting in terms of the knowledge, is that companies are now able to develop new drugs that can target LRRK2, in a way to make the accelerator not be as efficient as it was before, so when you press it down the power doesn't go as fast as it was before and that would hopefully lead to some benefits, may be actually be possible we hope to slow or even stop the progression of the disease. And even with the knowledge that this pathway is too active in people with this mutation, may be with other Parkinson's patients it might be possible to do preventative medicine and treat people with LRRK2 inhibitors before they develop the onset of most of the symptoms Parkinson's disease. And that's sort of an important question that people are thinking about.

Marc

That sounds very promising if I understand that rightly So some people have a genetic mutation that may cause Parkinson's, your now looking at other options to stop that operation of that faulty gene.

Dario

Yes, in the future of LRRK2. Our working hypothesis is that we were sure that in these people the disease is caused by the hyperactivation of this LRRK2 pathway and

therefore it would make sense that if you could dampen this pathway down before the people get the disease, so long as it is safe to do that and you can get drugs that safely do this then it is possible that this could stop the onset of the disease and that would be fantastic. There are issues with, regulatory issues about giving healthy people a drug, you know normally you get a drug once you are sick, but here it would be rethinking the rule book and giving healthy people the a drug to stop them becoming sick and that's another discussion for another day but you know I'm a firm believer that in the future this is what we need to try and aim for. But obviously now the key focus is to see whether the therapies would slow the progression of a patient's disease and that would be a first step and the would be a landmark if we could achieve that.

Marc

You referred to a few times a team of scientists or a collaboration. Is it generally the case that scientists really share all their findings across the globe and work collectively?

Esther

I've been working with Dario for a long time, first as a PhD student and then towards independence and I think there are huge differences when you talk to other researchers but I think that one of the fascinating benefits of working here in our team and in our unit is really that there is the firm belief that we will really only move forward by collaborating with others, by sharing and by talking openly about our science. I can't say that this is true for every other research group or every other institution, but I think like that's really something that's a hallmark of the success of our unit.

Dario

I think it's becoming more; I think science and scientists in general now, not everybody, are getting more collaborative and more open. I think there's still a long pathway to go, a lot of scientists keep their stories a secret until they are published but we want to try and reverse this and make all the information available at a very early stage well before the publication to talk about our results to make all our reagents that are funded by charity funding and tax payers money, available as soon as we make them and discuss our very early findings. And you get a lot of feedback as well if you discuss some early results, that are slightly preliminary even, by talking about them people can give you some very good ideas and feedback, they can suggest some important experiments, collaborators can come and work with you because you present that data so progress is dramatically accelerated by being open and having this open, sharing culture and working in a collaborative way. You know, Parkinson's disease is so complicated, the only way we are going to solve it is by having all the researchers working together, almost in a network and this is what this ASAP collaboration is trying to achieve, it's trying to set up a network of maybe 3 or 400 researchers working in Parkinson's disease. You know, together you can achieve much more than the same number of scientists working independently.

Marc

Yes indeed. At a very basic understanding of genetic research, Esther I want to come to you and follow up with Miratul, I understand that you are looking at translational aspects of genetic research and Miratul is looking at looking at further genes and other genes please could you tell us a bit about the work that you are personally involved in.

Esther

So, Dario mentioned all the basic science discoveries in the LRRK2 field which took very many years. I had a little bit of an advantage because obviously I was part of the lab and knew what was going on and I was fascinated the progress in the LRRK2 field, and my question was whether we could possibly look at this switching on and off the pathway and whether we could measure that in a human bio-sample. The first thoughts that went to my mind was what kind of bio-sample or biomatrix should we use, you know, spinal fluid which is probably difficult to get, especially if you want to experiment whether something is even working, then there is peripheral blood which is relatively easy to obtain, or even human tissue samples. I really spent quite a lot of time with other people here in the unit to somehow look at different populations of blood cells, so white blood cells – neutrophils and monocytes because they are quite a homogeneous group of cells that express high levels of LRRK2 but also its substrate Rab10 and other substrates of LRRK2 that are phosphorylated by the LRRK2 kinase. So, we spent quite a lot of time to then really like show that you see in blood cells, from healthy individuals in the first instance, that you can actually see the phosphorylation event of LRRK2, that a phosphate group is attached to Rab10 and if you block the pathway with an inhibitor *ex vivo* (which means after you have collected the blood) that this pathway is switched off. So, that was the first translational research output in this field that we used here. Then obviously the next question was, is this in anyway relevant for interrogating samples derived from people with Parkinson's. And there we fully like, you know, had quite a lot of success working again with like international collaborators in the States and in Vienna in Austria, in Spain and also in Germany, where we then took blood from people affected by Parkinson's disease, with and without genetic changes in LRRK2 and actually in some other genes. And we actually discovered that certain genetic changes associated with Parkinson's disease really like, significantly activate this pathway. You can really see this in a blood sample of these individuals and when you treat those cells (after they have been collected from the blood, after it has been collected from the individuals) with LRRK2 kinase inhibitor the signal completely disappears which proves that this event, this activation is mediated by LRRK2. And, you know, that's been really, really fascinating research to be involved in.

Marc

How are you going to prove that? How are you going to prove that - are there clinical trials?

Esther

Clinical trials are hugely regulated, like any kind of research that involves human, like healthy individuals or patients, but clinical trials would usually be with like a medicinal product, where they look at like how efficient something is. So, obviously,

in my research I do not administer LRRK2 kinase inhibitors (yet), I just work on this as a tool, that would be like in the hands of pharmaceutical companies who are the sponsor for research into for example LRRK2 kinase inhibitors. These clinical trials are in early stages, in humans, are underway at the moment, so that's hugely exciting for us to follow this and hopefully to also be involved.

Marc

That's fantastic, so we have heard about Dario's base, foundation laying research, and how Esther's building on that particularly. Miratul, how does your research relate to this and build on it?

Miratul

So, our research has also been more foundational at the moment, rather than translational. Parkinson's disease is likely to be due to several causes that including genetics, several different genetic causes, and our laboratory has been particularly interested in two genes, one we call PINK1 and one called PARKIN. Just a little brief background is that we've known since the early 1980s from studying people who have passed away from Parkinson's, that there is an accumulation or excess of damage to the mitochondria in patients with Parkinson's but we didn't understand what that was due to and in some ways studying these two proteins, so PINK1 like LRRK2 is a protein kinase and Parkin is this other enzyme in the ubiquitin pathway, it's called a ubiquitin ligase, trying to understand, in my lab we are trying to understand how do mutations in these two enzymes lead to Parkinson's, whilst asking an essential question, our work, together with other labs in the world, has actually found a function for them that explains how mitochondrial damage is controlled in the cell. And particularly this is important in brain cells because brain cells don't renew, you know, you're stuck with your brain cells for most of your life.

Marc

They will die off?

Miratul

They will die off and so, cells are actually exposed to lots of different types of damage in their lifetime. Cells are exposed to damage of their DNA but they've developed many protective or repair pathways to mitigate against that and similarly mitochondrial are being constantly exposed to damage and it turns out these two proteins Parkin and PINK1 work together to first of all sense whether there's mitochondrial damage and once they sense that there is they sort of active, the PINK1 becomes activated and that then activates the Parkin, together they actually signal and some ways create a signal in the cell that this mitochondria is damaged and that then leads to the elimination or removal of that mitochondria. And what our work has been particularly interested in has been the nuts and bolts of that process, how it senses the damage, how it then labels the damaged mitochondria, and others have shown how that labelled mitochondria is then removed. And it turns out this is actually quite important in Parkinson's, we've actually be able, we're lucky enough to actually, some of the key regulatory steps of this process, we've been able to find rare patients who have particular gene variants, in a particular

regulatory step, and shown that that has been sufficient to cause Parkinson's. So that in some ways has really been able to validate that this is a very important cause of Parkinson's as well as elaborates on some biology which is important for understanding how brain cells work and how they maintain themselves to survive.

Marc

Well, thank you for talking about that personal effect, what would this mean for a family if someone found out that he or she has a genetic cause of Parkinson's. Is this carried forward to his or her children? Is there anything known about that? Are there issues associated with that you want to discuss?

Dario

Yes, I think either one of those two would be able to tell you the answer to that question.

Esther

I can, I can start, I think like you know, I think there are different levels of complexity here. So, first of all you need to see what questions that you want to answer, and I think also, like you know, it is very depends on what kind of gene we are talking about. So, for example, if you come to me and say, like you know, does it matter to me today whether or not I have a genetic mutation in any of the genes associated with Parkinson's, with regards to my treatment, so basically like just for you at the moment, the answer is probably at this point in time, if you are not participating in a clinical trial, so just for your clinical care of your self – probably not. So then, like you know, so then the question can be like, so you know, in what instances do you do genetic testing? I think when you look, you know, in worldwide, genetic testing is not always part of the clinical care of Parkinson's patients. I think, like you know, in the future, I believe that will become, you know, an important part to us but at the moment it is not in most instances. So, if you know, one important question can be why does someone have Parkinson's, so usually Parkinson's is considered as a condition that affects people who are older, so if you have somebody who looks like he or she has Parkinson's but is maybe like 20 years old or 25 years old, then like your genetic testing can be really, really helpful because then it can explain a clear cut diagnosis. Or if you have someone who looks like Parkinson's but has additional features, again genetic testing can help with formalising, or you like really giving a diagnosis and a label to someone. Then obviously that's on the basis of an individual but then obviously when you then look at family that's a completely different question again. We have different modes of inheritance, so usually, like you know, most genes you have like two copies – one from your mother and one from your father, and you have two different modes of inheritance. So sometimes, so you know, you can have what's known as recessive conditions where you need both copies of the same genes, so the one from your father and one from your mother to be altered to develop the condition. So, Miratul can talk more about this with regards to, for example Parkin. Or we can take the example of LRRK2, you really only need one of the altered copy of the gene to predispose you for Parkinson's. An important other aspect is that often the penetrance, so the likelihood of a genetic predisposition to develop Parkinson's disease to then actually cause Parkinson's

disease in an individual is incomplete and age dependent. This means that for other family members, if there is known mutation of, for example LRRK2 in that family, so other family members who then may test, get tested and also find to have the gene mutation but not have Parkinson's, first of all they, it is difficult to predict at this point in time whether throughout their lifetime, living to their 60s, 70s, 80s, whether they will actually develop Parkinson's disease and if they develop Parkinson's disease – at what time. So with regards to, you know, family counselling its kind of difficult, so for family counselling it would be, its more of, you have an increased risk compared to the general population for developing Parkinson's disease but in the case of LRRK2, even if you are a gene carrier of an abnormal copy, it does not automatically mean that you will develop Parkinson's disease throughout your lifetime. But again, that is a very, very, very individual conversation that one needs to have and also very dependent on the gene that we are talking about, or even the particular mutation. So that's why, like you know, I think doing genetic testing and identifying something is easy enough but then really having that conversation about what that means for the individual affected by Parkinson's disease for their treatment, for their condition, and for the wider family, that's on a different page.

Marc

Miratul, I better ask you a left field question on this issue. We're in Scotland at the moment and there's 12,000 people with Parkinson's disease in Scotland, 10,800 (90%) have idiopathic Parkinson's disease – we don't know what causes it. You are heading an enormous amount of effort to get to grips with the genetic, potential genetic link, how does this research also benefit the other 90%? Or should I be arguing that the money spent on the 10% could be money better spent on the 90%? This is a bit of a theoretical question.

Miratul

I am very convinced that it would, so there are 10,800 idiopathic, and I'm very convinced that that group it's not one disease mechanism, and whilst they may not have any genetic evidence for a disturbance in a particular pathway, I think it's highly likely that they'll have an imbalance in that pathway for maybe a non-genetic reason but nevertheless its effect is the same as having a genetic mutation. Certainly, if you look at the patients that I look after with idiopathic, I'm always struck by the variability, some people you know, I'm always now very much more vague about what the future lies because my experience tells me I really, sometimes it's hard to know if somebody's going to have a very slow course, very, very slow – you know, sometimes not needing treatments for the first few years and some that are getting treatment and escalation pretty quickly. And then, some have the more average passage. So clearly very varied in the idiopathic group, very varied, but why is that? We haven't, obviously without, we don't have the tools and the clinic yet to really go beyond the genetics to classify the type.

Marc

Am I right to say there are also environmental factors that may influence the development (or not) of Parkinson's? That they could link to the variants that you call them or rather the mutations in the genetics?

Miratul

Absolutely, so I think the environmental, where that fits in is that that might be the trigger for then the alteration of a genetic pathway. These environmental insults like be in some ways disrupting or activating

Dario

Some of them could be activating the LRRK2 pathway, maybe they are exposed to pesticides or toxins, you know, then that would active the LRRK2 pathway in people who are exposed to these, and this would then cause or lead to Parkinson's disease.

Marc

I think there is increasing evidence that paraquat is in that category, particularly in Ohio of the United States

Miratul

I think that's where Esther's work is very important because her studies are actually looking at these pathways at the level of the protein. And so, this is where you may in the future be able to take those 10,800 and be able to classify which pathways are disturbed and therefore the genetic research that's been done here, on the nuts and bolts of it, will be able to benefit the 10,800 because we will then be able to identify which of them share the defect of the disruption of that pathway.

Dario

But, regarding your funding question, I think that's a very interesting question there and you know, for example, we have a clinical trial now that's on the most advanced LRRK2 inhibitors being done by Denali and Biogen. They are actually doing two separate clinical trials, my understanding is – one in people with LRRK2 mutations, and then they are also doing a parallel trial with people with idiopathic disease, to see whether they would work in that group as well. So it could be that the knowledge from the LRRK2 field may treat well the people with the mutations but if it is also found to treat some of the people with idiopathic disease, that's an example of where the benefit, you know, moves forward onto the others. And its very hard to work on a disease if you don't know the cause of the disease. It's like if you have your car that doesn't work, if you don't understand your car and you don't know how it works, you can't fix it. You know, so the advantages of the genetics is that it tells you what the cause of the problem is and then that enables you to then study that cause, the biology and come up with ideas to better diagnose, treat and prevent. And, you know, that's why I think it's really valuable to support, you know, the work that you really know what the causes is. If you want to work in what paraquat's doing, paraquat's probably targeting hundreds of proteins in a cell and, you know, just the feasibility to work on all those hundreds of effects is virtually impossible to do that well, with good science so I think the money is well spent, focusing it on the known causes and then trying to exploit that information as much as possible.

Marc

Thank you, Dario. There's at least one more subject, oh Esther, did you want to comment?

Esther

Yes, and I think when you look at the really exciting developments that clinical trials are now moving into the direction of the area of disease modification. So not symptomatic treatment to alleviate symptoms but only looking at the root cause of the condition, most of these clinical trials are either underway in early clinical trials (phase 1 and phase 2), and most of them really like, you know, target genetic pathways that have evolved from genetic Parkinson's where we know that a single genetic change can cause the disease. So, the hope is that, really like that would have overspill, including benefit for others. And also, like, what Miratul really pointed out that, you know, if I see someone with Parkinson's in my clinic, that person is really like, you know, different from the next person. And so, like to get a little bit more the grasp of like to stratify people on a clinical basis, on a genetic basis, and a mechanistic basis, imaging and so on, I think that is really like, it will help us to, you know, once these treatments come available, to match the individual person with Parkinson's to the best available intervention and treatment.

Miratul

I was just going to add, not to wish to diminish enthusiasm in clinical trials, but I think the biology research we do is extremely challenging, and I also think that the clinical trials of Parkinson's, compared to other disease areas, is also equally as challenging. Particularly as we don't have yet the kind of tools that are useful to kind of classify patients so that's why in summary sticking to the primarily genetically well understood groups of patients remains, I think, still the kind of, the best route. I think that whilst it would be fantastic to know that the group of idiopathics could benefit from our gene targeted therapy, I think the bar to see effects in a disease which we all know is not as rapidly progressive, does vary significantly. You know, because of the lack of tools to monitor the disease you need to do lots of assessments, you have to have very large numbers in that group, which is very expensive as well, I still think that focusing on the genetically well worked out groups is key. And that sort of in some ways in the Alzheimer's trials, in some ways that's where there has been glimmers of success. It's come from the sort of well -worked genetically understood groups.

Marc

I think that that's good because it's given me the last aspect that I want to go onto next and that's people with Parkinson's involvement in research, public and patient engagement. It is increasingly obvious to me that more and more public and patient involvement in research or involvement in medicine development is vital. I think it has become clear that you need to be able to explain some of the things at a very scientific level of activity is required, how can you involve complete lay people in the context of your work, in a meaningful way? Have you got any views on that, and how important is that to you?

Miratul

What I can say about it is, so in our work I think we already here at the University of Dundee and obviously you've been very sort of key to that, Marc, is being first of all getting patients to have some sort of access to the researchers, to at least have a chance to meet them, hear what's being done, maybe not fully understand on the initial sort of exposure but to have that sort of access to ongoing contact – I think, I think that has been very helpful for us to basically allow us to sort of distil our work in a way that can first of all can be understandable. I think we have to do; we obviously need people to be interested but we also have to be able to distil that in a way to keep them on the message of what we are doing. But in terms of the more wider question of how does it benefit research, I think that one of the real advantages of our sort of research for involving patients is ultimately we started off working on our foundational work, from discoveries made in patients – the genetic discoveries are in patients. But you know this has been shown in biology many times, that you can find interesting things in a cell if you study it enough, but the question is – is what you've discovered really fundamental to the disease process? Clinicians use the word pathogenesis, is actually the pathway that you've found, is it in the pathogenesis of the disease? Because it might be that the protein that you work on does 10 things and 9 of the things are really important for the cell but it's not what is wrong in Parkinson's. So how do you prove that? Well you prove it if you find the discoveries in the cell which you can then go to the patients again and show that what you've found actually is linked to the development of the disease, of the progression of the disease. Or you disrupt it using a drug showing that you can arrest the disease. So for us, I think that we need patients for that last step. We've been very fortunate in our own work to at least show that some of the discoveries that we've made, we've been able to find rare patients that we can go back to and confirm that actually this regulation step we found is actually involved in the pathogenesis, its not just a property of what we found in the cell. So, I think it is very important.

Marc

Is that linking data, sorry wrong word, is that linking a patient to a discovery or to identifying a direct cause?

Miratul

Yes, it is having I guess the luck to find a patient with a very specific genetic mutation in a particular gene that is involved in the pathway and a particular maybe amino acid that you think is involved in a particular function, so that you have a chance of to find that and then you also have to be also you know rigorous enough to exclude other potential confounding effects. So, if you can have that very, very, and in some ways, you have a patient that can help your research.

Marc

I understand that. May we go back to you Dario? You posted a hypothesis essentially back in 2006 if I recall properly before you started research into LRRK2, with posing such a question, how would you potentially involve public and patients.

Dario

I think, so in 2004 we started and I was just a biochemist, still I'm a biochemist, and we did probably wrong in that time is that we had never met a person with Parkinson's disease at that time. You know, and I just worked you know in the lab probably by myself and that's how it was in those days. And you know then because of that we probably didn't you know think deeply enough and you know we probably missed a lot of tricks at the time that would have maybe revolutionised the way we did things now. But nowadays I think we've learnt our lessons and in fact I get all my inspiration from talking to people like you and other people impacted by Parkinson's and Esther often meets her patients in the next office to mine to explain them their genetic diagnosis and after she's talked to them she brings them to my lab or to my office and we talk to the patient and it's just such an inspiring thing. You know, to have that opportunity to talk to someone impacted with the condition. I think, Marc, you've helped me write abstracts for grant applications or re-write them I should say to make them really accessible. Our students in my lab, now come from different countries to gain research experience and one of the first things I get them to do is to meet Marc and your colleagues to discuss, and you know talk to them and this really inspires them, and they come out of those meetings, you know, really motivated to do research and to study. So, I think it's really critical that we talk to each other and we work out what's going on and you know it's a really important partnership. That's how I see it.

Marc

It is certainly extremely important from people with Parkinson's point of view, and I can vouch for the fact that the Dundee research interest group, set up and established by Parkinson's UK, has always been felt extremely welcome here.

Trying to round up a bit, the thousand million dollar question, Esther where are we going to be in 10 years? What's your opinion, what's your hope? What's your expectation, because I know you all work more than the time you said, you said you work from 8am to 8pm and I see emails from all of you at all sort of times.

Dario

We are here form 8am to 8pm but then we go home and we continue working probably.

Marc

So where will we be in 10 years time?

Esther

Ok, so stepping back a little bit, obviously my main focus is Parkinson's but obviously I am a neurologist and I look after people with other conditions as well. And I think that like the reason for me to go into neurology and neurological research and neurodegenerative conditions is that I think that in my lifetime, as a practicing clinician, I will treat people, with Parkinson's, with other conditions, successfully with disease modifying medications. So, to really like change the trajectory of these conditions, so that's really what gives me the motivation to basically work in all these different roles. So, I really want to somehow see us jumping forward and you know like change the way we see people in clinic, you know like how we develop

partnerships with the patient to develop an individual treatment plan and also having the tools available to us to say, ok there's this particular medication or this particular trial or intervention and this will change the underlying disease progression. So, that's kind of, you know, where I think we need to be. You know, there is urgency but this is what we ultimately want to do, so that's what to somehow when I look back at my career, what I want to be able to say – we started off and there was a lot of optimism and a lot of knowledge and this over the years translated to effectively treating people with neurological conditions like Parkinson's disease with disease modifying treatments.

Marc

Fantastic

Miratul

I think the same, I think in 10 years we'll be a lot busier in the clinic, I think we **(will)** be busier, and it will be more intensive as well. There will be urgency for people to be referred early and to be seen early because we'll have these treatments. There will be an urgency and intensity to monitor them, these treatments are not going to be, you know at any new medicine exposed to man, it's very unlikely that they will be completely side effect free or free of adverse effects. We're going to have to have more time spent on patients on the drugs we are offering them, and they are going to be required to be monitored. But maybe different types of monitoring, you know certain, there for example there are already a couple of Parkinson's drugs that, the current way we do neurology, there was one drug in particular that was quite effective but it caused liver problems and so in general it's available but we don't really use it because it's felt that the monitoring required for the liver, with the current infrastructure that we have it's probably safer for patients to not to be given it. I think that will change, I think we'll have an infrastructure in the clinics and the hospitals which is much more akin to what some of our more intensive colleagues, may be in like oncology have, where there are lot more people, nurses, young doctors, and patients and just busier and it's going to be great because they'll be patients getting drugs that we know are slowing down the progression of the disease, we'll have to deal with situations where somebody's got a side effect, but that will be, you know, we'll just deal with it. I hope also myself that not just the drugs but that we have better bioassays as well. It is something that is really crying out for in Parkinson's but brain imaging – still no brain imaging that we can offer that are actually tracking disease in a way that links to the severity. It would be nice to think that with the knowledge that we are accumulating with say synuclein aggregation, somebody develops a PET ligand that we can use to track disease progression carefully. But also other types of biomarkers as well. But I think it is probably going to be, you know currently I do one day in the clinic, in the hospital, I suspect in 10 years if I'm still, you know, able to work I will probably be doing more because I'll have to. It's going to be more intensive, more work required.

Marc

Fascinating.

Dario

In the 10 year time scale I'm very optimistic but you know that we'll have something that will for the LRRK2 inhibitors, we'll have answered the question decisively, you know whether they work or not and in which patients will benefit from those. Hopefully that benefit will be wide not very narrow. And I think once you can start treating better in slowing the progression of the Parkinson's down, you can build on that, you know you can make better inhibitors, you can consider other pathway components, you can combine it with boosters of the PINK system and mitophagy that Miratul and my colleagues are working on that we haven't discussed. And there's going to be lots of other ideas that are being tested now, so I think in the 10 year time period I'm optimistic that you know we'll have some treatments that will work for well for some people. And then we'll have so much more knowledge that we'll be able to rapidly improve from that starting point.

Marc

It's been fascinating being about to listen to you and ask you questions. Miratul, many thanks for giving the time and explaining your work so clearly. Esther, many thanks to you too and Dario, of course making us welcome again. I hope that you all have a little bit more insight in terms of the very fundamental research taking place here in Dundee and that you shared their optimism. Thank you.

All

Thank you