Whither to the Creeping Paralysis? Progress on the road to curing motor neuron disease

Transcript

Date: Thursday, 23 February 2012 - 6:00PM
Location: Barnard's Inn Hall
23 February 2012

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Progress on the Road to
Curing Motor Neuron Disease

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I am a neurologist. I see people with motor neuron disease, and I have been working in this field for nearly 20 years. It is a field that has changed dramatically and, hopefully, this evening, I can persuade you that we are making progress and are in a better position to discover a cure for this dreadful disease.

I thought I would begin by explaining what the disease is. I am going to talk about how we, as clinicians, make the diagnosis, what the symptoms and signs are and the treatments we can offer.

I am also going to talk about the disease process itself: the pathology, what actually happens in the brains and spinal cords of our patients. I want to focus in particular on the proteins that accumulate there.

I am then going to talk about the genetics. When I started out, I was working in the Genetics Department and the Neurology Department as a sort of hybrid. My geneticist colleagues did not consider the disease to be genetic, but we have subsequently discovered a number of genes related to it. I am going to talk about two genes in particular, both of which were discovered in our laboratory: TDP-43 and fused in sarcoma (FUS).

We can obviously advise patients who come from families in which motor neuron disease runs as a trait, and we can help them in terms of being tested. However, we can also use these gene defects to study the disease process in cells and animals, and we can then, hopefully, target our therapies more effectively and also screen them to see what might work in our patients.

Jean-Martin Charcot was the first person to really identify this as being a disease based on the degeneration of motor neurons. He gave it an admittedly terrible name: Amyotrophic Lateral Sclerosis. Lord Brain (a very appropriate name for a neurologist!) decided that motor neuron disease would be a much more sensible name, but of course this never really caught on. In America, it is named after a famous baseball player called Lou Gehrig, who died of the disease. However, the most evocative name for it is probably “the creeping paralysis”, a colloquial term written on death certificates throughout the Middle Ages and right up until the 18th and 19th centuries.

Lou Gehrig was a very wonderful baseball player. He almost never missed a game. The year before he was diagnosed, however, his batting average dropped, which indicated that there were some subtle motor problems. He was dead within eighteen months of diagnosis.

Don Revie, a footballer, also died of this condition. There is a theory amongst many of my colleagues that occupations involving a high degree of exertion predispose people to getting motor neuron disease, which is possible.

David Niven died of this disease and his ‘thumbs-up’ symbol has been used by the Motor Neuron Disease Association ever since.

We also believe that Mao Tse-Tung died of this disease. Of course, I was not his physician and information regarding his death is not clear, but certainly there were features suggesting that he had motor neuron disease near the end of his life.

Where does it occur? In fact, it is a global problem. There are no communities that are free of motor neuron disease, but it has a very high incidence in two areas: one in the Kii Peninsula in Japan and one in Guam. The Guamanian one is slightly atypical in the sense that it is also associated with a form of Parkinson’s disease and dementia: sometimes they come in combination and sometimes they are separate. Interestingly, it peaked in the 1950s and has been decreasing ever since.

More men are affected than women, the ratio roughly 1.7 to 1. It is a disease that comes on in later life, but in fact, it does affect people in their twenties and I have seen several teenagers with this condition.

The number of people in the UK who have this condition at any point in time is approximately 5,000, and about 300,000 people worldwide, so it is not uncommon. The incidence is much higher, but people do not live very long with this condition, so the prevalence is lower.

What do we hear from our patients when they come to see us? Firstly, they say that they have developed a disability. It may be weakness of grip or tripping on the pavement or slurred speech, if it affects the throat muscles first, but the really difficult thing about this condition is that it progresses. It progresses relentlessly, and people are then unable to use their arms properly to hold objects, to cut their food, to feed themselves, to go to the toilet, to wash themselves, and eventually they are unable to speak properly or speak at all. They have difficulty swallowing their own saliva and eventually they have difficulty breathing, and that is what limits life.
For a long time people said that one positive aspect of motor neuron disease was that the mind remained unaffected. However, it does actually affect the mind, to a very subtle degree, in about 30-50% of people, but this is something I will talk about a bit later.

The really challenging part of this condition is that it is not relapsing or remitting; there is no really effective treatment that can arrest it or even give you some relief from it. It is progressive, with disability always increasing. You know that what you have learnt to come to terms with and accommodate this month will change; you will face new disabilities and new challenges in the months to come. Although this is a relatively uncommon condition, it is the most common reason that people seek euthanasia. I will not go into the rights and wrongs of that debate - it is a completely interesting and separate debate - but it does suggest the personal challenge that people who have this condition face.

What is the biological and anatomical basis for the condition? There are a series of neurons that can be affected, but the main neurons affected are those that control movement (hence its name). There is a group of neurons that controls the planning of movement in the frontal lobes, but then the execution of movement is elicited by the upper motor neuron and the lower motor neuron. We see different deficits based on whether it is predominantly an upper motor neuron problem or a lower motor neuron problem. The lower motor neuron problem causes major weakness: the muscles become very thin and fasciculate or flicker. The upper motor neuron, on the other hand, does two things: it activates the lower motor neuron but it also holds it in a state of readiness and reduces its automatic excitability. As a result, when the upper motor neuron is failing to work, we see stiffness and spasticity, exaggerated reflexes. There is the extensor plantar response, the test that neurologists love to do, where they scratch the bottom of your foot. In this instance, the toe goes up; in most adults, the toe would go down.

Interestingly, sensation is rarely affected, and eye movements are retained. So, some of my patients who are no longer able to communicate with their voices actually have an ‘eye gaze’ mechanism whereby they can select letters and communicate thought through a computer, by picking out letters and making sentences. Bladder and bowel functions are usually spared in this context.

What tests do we do? We do not have a test for this disease. There is not a single blood or electrical scan test that can tell us, without a doubt, that a person has motor neuron disease. Instead, we do a variety of tests that exclude other conditions. For example, we test the nerves in the body to see whether there is a neuropathy that might be mimicking motor neuron disease.

In particular, we scan the spine. There are sometimes problems involving compression of the spinal cord due to bone overgrowth or discs and compression of the nerve roots, and that can look like motor neuron disease.

We also take blood tests because there are some autoimmune conditions that can attack the motor nerves in a very select fashion. These conditions are important to exclude because they are actually treatable. We can administer drugs that suppress the immune reaction and reverse the motor deficits.

So, the diagnosis can be difficult to make, and often takes 12-18 months. Of course, survival is only three years from symptom onset, on average, so people can really face a very short time between diagnosis and death.

Well, what can we offer in terms of treatment? There is one drug, Riluzole, that works for motor neuron disease, but it has a modest effect. The drug became licensed in the UK and in most parts of the world, and it has been validated by the National Institute of Clinical Excellence, NICE, as being an effective and cost-effective therapy for this disease.

We looked at our patient population to see if what we were doing was making a difference. We looked at our dietary regime, our ventilatory support, the general impact of our care, and we were a bit disappointed to see that the only thing that had really made a difference in terms of the survival curves of our clinic population was Riluzole. So, we really do believe it works, but if you have a three month increase over an eighteen month period in a drug trial, that is not really a substantial change. We need to do better than that.

We also have something called non-invasive ventilation. When you are no longer able to take in enough oxygen at night and release carbon dioxide, we can give positive pressure ventilation to help the respiratory muscles while you are asleep, and it really does make a very big difference.

It not only increases the length of your life, but also improves its quality. If you are retaining carbon dioxide and not getting enough oxygen, you wake frequently at night, and the one thing to make anybody mad is to be woken every few hours by breathlessness. Sufferers retain carbon dioxide, and wake feeling drunk and drowsy and they often fall asleep during the day. Patients who are given this treatment get a good night’s sleep, they feel refreshed in the morning, and they do not tend to fall asleep during the day.

I am now going to talk about what happens in the brain and the nervous system that leads to motor neuron disease.

The upper motor nerve cells, which sit in the motor cortex in the precentral gyrus (particularly layer five), degenerate. They are the cells that provide input to the lower motor neurons. We talked about the kinds of
symptoms and signs that they generate. It is the population of motor nerve cells, which sits in the spinal cord, that makes the connection out to the muscles – and which are also lost. It is a degenerative process, which usually starts at one part of the body and then spreads to others. There are new theories about why that might be, how the proteins that accumulate are perhaps being transmitted across synapses, infecting local neurons. I am not sure that there is convincing evidence of that, but what ultimately happens is that almost all of the motor neurons are affected and people develop paralysis throughout their body.

In 2006, we identified the protein known as TDP-43 (the TAR DNA-binding protein), and its molecular weight is 43 kilodaltons. This was a huge advance for us. Before, we knew there were proteins that were accumulating, but we did not know what they were. 95% of all people with motor neuron disease have this particular protein accumulating. It normally sits in the nucleus and it is involved in regulating gene editing and transcription, but in cases of motor neuron disease, it leaves the nucleus and aggregates in the cytoplasm. It could be toxic because it is not in the right place doing the right thing, or it could be toxic because it is doing the wrong thing in the wrong place in the cytoplasm.

It was also exciting to discover that this protein does not just accumulate in motor neuron disease. It also accumulates in a condition called fronto-temporal dementia. Previously, dementia and MND were thought to be very separate things, but in fact, this protein is found in about 60% of patients with fronto-temporal dementia. We have subsequently identified that dementia and motor neuron disease can run through the same family. Just recently, we have identified a gene that accounts for both of these.

Anyway, what is TDP-43? It is a crucial protein that is involved in stitching together genes before they go and make proteins. However, it needs to be kept in the right place, doing the right thing, otherwise it can aggregate. We can use what is known as a ‘western blot’ to analyse proteins in the brain and prove that they can aggregate.

This is not unique. Motor neuron disease, fronto-temporal dementia - they are both part of a family of degenerative diseases, of late onset, that relate to protein accumulation.

In the case of Alzheimer’s Disease, we see two proteins: one is beta amyloid, accumulating outside the cell (so-called Amyloid plaques); and we also see these tangles of the microtubule associated protein Tau, which forms inside the neurons, which is very similar to the shape of inclusions we see with motor neuron disease.

In Parkinson’s Disease, in fact, we see these Lewy bodies in the cell, and they are made up of a different protein called alpha-synuclein.

So, there is a whole family of conditions. I have just mentioned two here, but there are about fifteen in which different proteins are accumulating. These proteins become sticky, they go in the wrong places, and they start to cause disease.

Now I am going to talk about my favourite subject, which is genetics. Fifteen years ago, before we actually had much in the way of genetic evidence, I argued that, “Genetic research will provide the strongest clues to the causes of MND and give us powerful tools to model it... Only when we understand the mechanisms of disease will we find drugs capable of curing MND”.

I say “causes” because, although we call it one disorder, it has multiple causes. Not only do we discover why it is happening, we can also use these gene defects to model the disease and discover how it is happening. Only when we actually understand the mechanisms of disease can we really make smart therapeutic targets and try and treat this effectively.

Let me give you a very brief bit of cell biology. A chromosome is like a ball of very tightly bound wool, with DNA in it. DNA gets read by polymerases and they make mRNA. This is a messenger RNA that is going to go on and tell the cell which amino acids to put together to make the protein. When we have mutation, possibly just a single base change in the DNA, it leads to a mutation within the mRNA and that gets turned into a mutation within the protein itself. As a result, those proteins no longer behave the way they should and they can become sticky, such as the case of TDP-43 and FUS. It is these proteins that are abnormal in this particular condition; interestingly, these two proteins are actually involved in stitching together the mRNA when it is being read from the DNA, which shows you how important they are.

What are we doing in the way of technologies? We have been using ‘Sanger sequencing’, which reads every single base in a tiny fragment. We can read about 500 base pairs at a time. It might take us a week to do that sort of experiment. Indeed, the first draft of the Human Genome was published in 2000 and took fifteen years – it was just one person’s genome and cost about £200 million. Things are different now. With these sorts of machines, we can do that in about a week, for about £2,000. I am confident that we will identify all the major genes that are responsible for familial motor neuron disease, and a large number that will be responsible for sporadic disease as well.
This graph charts gene discovery. When I entered the field, we had only discovered the gene called SOD1, superoxide dismutase (“SOD all so far”!). But then, as you can see, there was an almost exponential increase in the number of genes discovered. Not all of these are strongly associated with ALS, but some of them are.

The vast majority (95%) of our patients have no family history of motor neuron disease at all, but we do find gene defects. C9orf72 is the ‘latest’ defect, published about three or four months ago. We find mutations in our patients of about 4% overall. In some populations, this is as high as 10% or 20%, particularly in Northern Europe.

Some people (about 5%) have a family history of motor neuron disease, and in those individuals we very frequently find mutations – more than 50%. The most common mutation is C9orf72. In the UK, it is about 24%, but I recently screened a sample set from Belgium and it was 86% of their undiagnosed genetic cases of familial disease. This figure is also very high in Scandinavia and parts of Germany.

SOD1 accounts for about 20%, and these two new genes - although relatively rare - have given us insight into the disease mechanism. You have to remember that although all these genes are implicated, it is this particular protein, TDP-43, which is present and accumulates in 95% of people. This means that the other genes responsible for this condition are somehow mostly feeding into the TDP-43 pathway.

So, how does this help us? Firstly, when people come to us with a family history of motor neuron disease, we can identify the gene defect. We can tell them why this has happened to them. We can also give them advice about other members of the family, so that people can be tested for the same gene defect. Obviously, it is a very challenging to find out whether you carry such a fatal gene defect, but I do a lot of counselling with families in that regard.

Secondly, everybody knows about genetics now, and even people who have got no family history at all realise that there are genetic risks. As a result, there are people who want to know whether they carry a gene defect.

Lastly, we can make a difference for the next generation. Obviously, for a number of years, we have been able to screen prenatally for SOD1, and now other genes, but not everybody could face the termination of a pregnancy and that is fully understandable. Therefore we now offer something called pre-implantation genetic diagnosis, which is in vitro fertilisation - fertilising the eggs in the laboratory. When they reach the ten-cell stage, we can take a cell out and genotype it. We can see whether that particular embryo carries the gene defect, and
those embryos that do not carry it can be put back in the womb and hopefully the mothers will go on to deliver happy, healthy babies. We are now doing this for SOD-1 in this country - an important step forward.

So, although the population effect of familial motor neuron disease is small, the biological impact of these gene discoveries has been really huge. I could tell you about the number of times these papers have been cited and the kind of impact they have made, but it is a bit like a stone being thrown into a very still pool. The ripples are spreading out through the research community, and it really is changing all that we do.

When the TDP-43 protein was identified in motor neuron disease patients, we immediately thought that this was a good candidate gene. We screened 156 different families and we found only one mutation in one individual; I find variants all the time and most of them are not significant. It took me a year to track down, in different laboratories around the world, these samples here. I have shown that the other person who was affected also carried the mutation (signified by M), whereas the others had the healthy copy of the genes, and they did not get this disease.

I was then contacted by one of the samples, who invited me to a family wedding (not something that happens very often to a researcher). His family worked all over the world, in the military service, and they were coming together for his wedding. His wife was not so pleased, but I turned up on the morning of the wedding with a lot of tubes and needles and we bled most of the family. We showed that the two brothers who were affected carried the mutation, while the other siblings did not. Lastly, a family from Australia turned out to have the mutation as well.

I could see that the mutant variant was segregating this disease: basically, those people that had the disease also had this. This made me think that it was more likely to be real. We also identified, in patients who had no family history at all, two neighbouring mutations, which gave us a bit more genetic evidence. However, it was not until we actually did some experiments in tissues and cells that we saw real evidence for disease.

One of the experiments we do is to take an egg and look at the developing embryo. One of my colleagues, Vineeta Tripathi, cannulates the central canal of the spinal cord in the egg, only a few days post-fertilisation. After this, she can inject DNA and drive it into one side. When we injected SOD-1, we saw that it was toxic and that we had a model that worked.

We then carried this out for TDP-43. We injected the healthy copy and did not see cell death; when we injected the mutant copy, we did see cell death. We were very pleased to have our findings published in *Science* in 2008.

Subsequently, my colleague Corinne Houart is modelling the disease effect in zebrafish. Frank Hirth is doing this in flies, and Don Cleveland and I are generating mouse models with these gene constructs.

I am now going to talk about stem cells. The first paper on the subject to really catch my attention was published in 2003 by Tom Jessell and Hynek Wichterle. They took embryonic stem cells, cells taken from the embryo in its earliest stages and which can turn into any cells in your body. They used two chemicals, retinoic acid and ‘sonic hedgehog’, which is a growth factor (it is really called that!). Using these chemical signals, which they had identified in the spinal cord of patients, they could turn them into motor neurons. When they put these motor neurons into the spinal cord of a chick, they went to the right place where motor neurons live, they put their processes out on the right part of the nerve roots, and they made contact with muscles. Subsequently, they showed that these mouse motor neurons were activating the chick muscle and were fully integrated.
We wanted to do something similar. We planned to make motor neurons in a dish – not an easy aim by any means, but one which we have recently achieved.

Our first idea was to use cloning technology. You will have heard of Somatic Cell Nuclear Transfer. The idea is that you take a donor egg cell, take its nucleus out, you insert a whole cell (not just the nucleus) and then stimulate it to reprogram it, to make it think that it is an embryonic stem cell. As it started to form an embryo, we would take out the inner cell mass, we would grow these pluripotent stem cells, drive them to become motor neurons, and discover the drugs that could cure motor neuron disease. It sounded fantastic. Of course, like most of these things, it does not always work out like that.

It was Ian Wilmut (of ‘Dolly the Sheep’ fame) who approached me to work on this. We actually got a licence to use human eggs to do this.

Around that time, Woo-Suk Hwang claimed that he had managed to do this: successfully use nuclear transfer to make an embryonic stem cell from a human individual. He did actually manage to clone this dog, and he is a very sincere scientist, but of course his work was discredited. Somebody in his lab was making up the results and he and his team were horribly humiliated. He very nearly went to jail for this.

Also around this time, in Shanghai, Huizhang Sheng said that she could achieve this, not by using human eggs, but by using rabbit and cow eggs. So, following our disastrous encounter with Woo-Suk Hwang, we held a press conference and I made the fatal comment: “Of course we can now use rabbit and cow eggs”. It is interesting to say this was warmly received by the press, who came up with headlines like “Franken-bunny” and “Moo-tant”; you may remember the furore surrounding the animal-human hybrid embryo debate. There was very nearly a law passed that would ban this work altogether, and we spent about nine months trying to persuade the politicians that that was not a good idea, and the public that we were not cruel and devious scientists trying to create some sort of mutant; we were actually working on an important disease with the aim of helping people. In the end, we did not need to use this method because new technology came along.

We were rescued from those sorts of disastrous and difficult experiments by an extraordinary man called Shinya Yamanaka. He is actually an orthopaedic surgeon who wanted to make cartilage for people that were having problems with their joints. He developed a technique whereby he could insert what we call early stem cell genes. We thought we would adapt this to make motor neurons from our patients. Basically, we take a skin cell from a person who has a gene defect which is responsible the motor neuron disease, we put early stem cell genes in, and they then become induced pluripotent stem cells – cartilage, gut tissue, but also brain tissue and therefore motor neurons. So the idea is not to make motor neurons to replace the motor neurons in the patient, but to actually be able to look at these cells and study the disease mechanism in a more normal fashion. We cannot look inside the spinal cord and brain of our patients, but we can study the cells that come from them. When I was writing applications for the grants to do this, it sounded reminiscent of science fiction and we did not know whether we could make it. In fact, we have evidence that this is a promising technique.

The second gene I want to talk to you about is Fused in Sarcoma, otherwise known as FUS.

The discovery of the gene is a very sad story. I met a lady who had seen all five of her children die of motor neuron disease, in their thirties, and she lived long enough to see five of her grandchildren die of the disease as well. They survived, on average, for thirteen months following their first symptom. Usually, they died within six months of diagnosis, and the weakness in this family always started in the arms. It is a tragedy that is very hard to fathom.

However, it did allow us to identify a region on chromosome 16 which carried a defective gene. We did not know which one. In fact, there are 400 genes there and we were sequencing them. It was a very arduous task and we worked very closely with some excellent colleagues in the US, in particularly Bob Brown from Boston. One of the genes happens to be Fused in Sarcoma. Together we identified mutations in this particular gene, and found that it happens to be a ‘cousin’ of TDP-43 - another RNA processing gene.

We started inserting the gene into cells. Healthy copies of FUS went into the nucleus, as it should do. However, when we put in a mutant gene from one of our patients, instead of being in the nucleus, it was outside the nucleus in the cytoplasm. We believe that it is interfering with something called a nuclear localising signal. When we chopped that bit off (essentially a truncation mutation), it sticks outside in the cytoplasm as well, again suggesting that whatever is in there that has been disrupted by this mutation is responsible for taking it into the nucleus. When we took the bit we chopped off, the NLS, and put it back onto the mutant protein, it goes back into the nucleus, essentially rescuing the phenotype. So, we now know that the mutation is acting by taking it outside the nucleus and putting the protein into the cytoplasm.

I am going to give a quick summary of the biology that we have reviewed. TDP-43 and FUS, when functioning normally, stitch together the gene. The whole gene is read across the DNA, but only parts of it are put together, and these proteins are involved in the splicing machinery to do that. They also traffic genes around the place, the mRNA, they take it out to the places where it needs to be delivered to be translated into protein. But what we have seen in disease is that they mis-localise. They go outside the nucleus, where they should be doing most of their function, and they start to aggregate in an unprotected fashion. They then need to be degraded by the proteasome, but there is something wrong with this process, or degraded by the autophagy process, but there
is a failure of that. So, anything that increases the tendency for it to sit out in the cytoplasm or leads to a
decrease in the clearance by these two mechanisms, will lead to the aggregation of these proteins, and that is
what we believe to be the toxic mechanism behind this. It is interesting that they are both RNA processing
proteins.

In 2006, where were we? For fronto-temporal dementia, we had mapped Tau. We had nothing else. We did not
know anything else, in terms of pathology. And for MND we had SOD1 - we did not know anything else. But in
2012, we now have TDP-43, which accounts for a large proportion of patients with fronto-temporal dementia.
95% of people, we know, have got TDP-43 deposited, and a small number have FUS. Also, interestingly, FUS
appears in fronto-temporal dementia as well.

Where are we with the genes? In FTD, we have this very brand new gene which affects both C9orf72, and many
other genes account for it too, but you can see that we still have a long way to go in terms of identifying the
genes that feed into the deposition of these proteins - I am not out of a job just yet! When we have a new gene
discovery, it is very exciting, because we can immediately start putting them into cells and discover why the gene
mutation might be misbehaving, causing toxicity. We can then model the disease on very simple organisms (the
fruit fly, the zebrafish, the mouse) but also on human brain tissue, which is essentially the perfect model. We are
very grateful to our patients and their families for donating tissues over time, so that we are able to validate the
things we are seeing in these very simple animal models.

But that is not all. We really want to discover drugs that are effective and having these cell lines helps us to
screen hundreds of thousands of compounds and take the very best of those to our fruit flies and zebrafish
and mice to see whether we can inhibit or reverse the disease process.

There is nothing about motor neuron disease that makes me think that it is going to be incurable. A lot of the
clues will come from genetics, but we really need to work this into cellular animal models and undertake complex
drug screening before we can really make a difference.

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