**Jonas Bergquist, MD, PhD | The Neuroimmune Route in ME/CFS**

<https://www.youtube.com/watch?v=oXCEJxOmuAY&index=9&list=PLl4AfLZNZEQPxjqF4ojAO3wdCFMeriNBK&t=0s>

**Raeka Aiyar:** I want to welcome up our final speaker before the break, Jonas Bergquist from Uppsala University in Sweden.

**Ron Davis:** It's a pleasure to have Jonas here again. He gave us a talk last year. He's an expert in all sorts of things, including being able to handle virtually any instrument. He's also an MD and he can do all sorts of things with patients. One of the remarkable things—we were talking about making progress during our working group the last few days—I was lamenting that there was a chemical compound that we could not find and we didn't know how we could go forward with some of the things because it wasn't available. And he says, oh we just made that it Uppsala and it's been tested on humans. So I said, oh my gosh, why don't you do the experiments then? And we'll send you the protocols. And he agreed. So it’s things like that, just to illustrate why you want to be working together with people that are cooperative and that it moves things faster. Anyway—also he makes lots of discoveries and he announced one of those discoveries the last time he was here. He said he found a correlation between chocolate consumption and winning a Nobel Prize. So these are very important things!

**Jonas Bergquist:** Thank you very much, Ron, and thank you right now for the introduction also. It's a great pleasure to be back here. Yeah, I have to say that after these three days we had this time, I feel much more hopeful that we actually are migrating and moving ahead very, very rapidly into this field. And also to reach some more understanding which I think is extremely important in order to do anything.

So today I will focus a bit on the neuroimmune route of ME/CFS. And as you all understand this is very complex situation that we are looking at. But we have to take small steps at least and understand more of the biology that is behind the disease.

I will get you set into this complexity—and this is a natural phenomena that we all are dealing with on a constant basis. So the nervous system and the immune system communicates all over and all the time. And sometimes it's very important and positive and non-pathogenic route that we have in the body. The motor of development for instance and also maintenance of both the immune system and the central nervous system. But sometimes we have problems when the immune system for instance starts to attack the nervous system and also when the nerve system starts to down-regulate or maybe disturb the immune system’s positive action so then things can go wrong and become very complicated. We try to show you a bit of what we are going to start to understand some of these things in humans.

But I will start with something related to food since we're on this. Ron described what I told you about last year chocolate and Nobel awards are connected. If you eat enough chocolate you it will get a Nobel prize. Sweden or Switzerland do very well on the statistics there, on the correlation. But this year I want to tell you something else, maybe something related to maybe not being brilliant and smart but maybe to become stupid if you eat certain dishes. I will bring you into the Swedish tradition we have, I don't like it so much myself but I know there are people around that want to eat this stuff. I put it in the context of the complexity of biology we are going to look at where it's actually sort of small dishes of the analytes and molecules that we're going to address and we call it a Swedish smorgasbord which is the small collection of dishes we have on that tradition on this table. So the dish I will introduce is called surstromming, it's a very, very odd thing and it's something that I can’t recommend you to test it. but maybe see if this works a little movie here about it.

[Video on screen of a group of American young people sitting around a table as one of them opens a can of surstromming. The video’s title is: Americans Try Surstromming for the First Time. Dialogue: They say that when you first open a can of surstromming, it's one of the worst smells in the world. Let's not do it then! [Groaning] It's sewage in a can. Oh my god. [Music] I’ve got the worse gag reflex, I'm definitely gonna heave…]

[Bergquist] In order not to take too much time, I can recommend you to go and see this movie on YouTube. But it's a very funny way of describing traditional Swedish food. I think after watching the movie you want to go and buy this dish, I think.

But there are things in there that cause problems. So we have our things in our closed environment and also around us that disturbed new transmitters and immune reactive molecules that can of course cause this delicate balance between the immune system and the central nervous system to be offset. So poor diet, surstromming for instance, or toxic consumables that we approach sometimes, sensory overload, bowel dysfunction, genetics—of course, chronic stress, external stress, and then environmental toxins and things like that. So it all comes together and also makes a situation very complex when we study it. This busy slide, unfortunately, I would try just to tell you a bit what can what can be examples of things which can happen between the central nervous system and the immune system. So if you see in the top panel you see that we have the central stressors as those that affect the central nervous system directly. And it can be physical stress, it can be psychological stress, it can be immunological stress or infections, for instance. And you see that the brain will react on different ways. It will start to produce new transmitters that actually then can be transported and effect for instance a down regulation of the immune system or sometimes an upregulation of the immune system. And it can also go through the autonomic nervous system and control, for instance, our bowel function [such as] motility, secretion in our gut. And that, together with the peripheral stresses that could be an infection or inflammation, comes together with the immune cell regulation. And it all becomes a very, very complex situation where inflammation, motility, secretion, regulatory mechanism in the central nervous system, is affected.

Just to complicate things a bit—many years ago we discovered in our work that our classical neurotransmitters, catecholamines, they’re actually very important immune regulatory molecules also. So dopamine, for instance, can regulate the immune system. This little slide here just shows you what can happen. So if we have normal levels of catecholamines, immune cells—this is a t-cell. So they are very nicely regulated. They proliferate and they differentiate as they should. But if we have increased catecholamines in the region, actually we will affect the proliferation and differentiation and also induce apoptosis which is programmed cell death in the immune system. And it's not only external catecholamines, it's also internal. So the lymphocytes, they can produce their own catecholamines and they have receptors for these molecules. And they also have transporters so they can even transport these molecules into the cellular nuclei which is done a vail action.

So how can we do then to measure all these molecules to try to understand the system? Well it's not trivial. But by selecting different targets, if we go for genetic analysis [and] we approach groups like Ron's group which are skilled geneticists. If we do proteins, we do what we call proteomics. We measure with mass spectrometry which I will tell you a bit what it does—these complex protein mixtures that we have in a system. Or if we go for small metabolites, we call it metabolomics. We use in my lab a lot of instruments called mass spectrometers. That's very expensive balances as you can say that can monitor a delicate concentration of all different kind of molecules and tell exactly how the structure is, and how much of the molecules are in a complex molecular sample. Once you have the mass spectrometry, that doesn't solve everything. You have to also present your molecules in some delicate way to the instruments. So you have to get samples selected, for instance, body fluids that we can infuse into the instrument by technique called electrospray ionization. Or we can produce small droplets of the sample on a solid surface and then do laser ablation. Or we can present tissues directly into the instrument if we have a material tissue that we can measure. We are pretty sensitive when we do this analysis and that's the key. We want to measure things very accurately and at very high sensitivity.

I've made a little calculation for you just to understand what the sensitivity is when we talk about sensitivity. So let's say you take one piece of sugar and you put that in a cup of coffee. Mix it carefully and you will end up with the concentration of sugar in that cup of coffee, approximately 0.03 molar, mole per liter. And all of you remember Avogadro's number, I guess, from school. So if not this is it. So one molar is one mole per liter and that's six times ten to twenty three molecules per liter. Then we take that cup of coffee and then we bring it to our Olympic swimming pool next door and we spill that cup of coffee in that swimming pool. And then we mix that carefully. You can take a while to mix it out carefully in that big volume and then we will have one nano molar concentration in that pool. Then we take another cup of coffee and get some water from the first pool and put that in the second pool and mix it carefully. And then we draw a sample from that and then we get the concentration about ten femtoMolar. So ten times ten to minus fifteen mole per liter. And that is the sensitivity we can do. So we can measure how much sugar is in that second swimming pool. Unfortunately swimming pools are a bit problematic because you know it's not only water and sugar in a swimming pool. Kids pools—terrible! So we have problems that we have a matrix all the time. We have to fight background. So think about that next time you go to the swimming pool, or maybe not.

We do other things also. We don't only use mass spectrometers. We use targeted proteomics and targeted proteomics is when we know what we're looking for. So we're not screening, we are just measuring things with our precise methods. And we have used many different ways, but one of the ways that I will tell you shortly about this proximity extension assay. If you see at the lower panel here. What you do here—you have two binders that can detect your molecule of interest, a protein for instance. And whilst these two binders come close to each other they have single-stranded DNA on their … conjugated on them, as you see here. And those can ligate it and this ligation then can be amplified by PCR polymerase chain reaction. And this is a connector being developed in Uppsala by Olink which is a company and we use that. And we have used that in a number of studies where we also have included ME/CFS patients.

I mentioned this last year. I mean now we have concluded the data for the first Swedish population of ME/CFS. The unique thing here is that we have both cerebrospinal fluid which is my body fluid of interest and plasma from these patients. We also compare them with fibromyalgia patients. We have CFS and healthy matched controls. And we've been very, very careful with how we selected the material to make sure that we can draw conclusions. That's extremely important, especially when we have small populations. So we run selectively neuro inflammatory markers. 92 in the first set and just to compute what we found is in cerebrospinal fluid, we find significantly enriched molecules that tells us that patients with both Fibromyalgia and ME/CFS have a neuro inflammatory ongoing reaction that we can measure. Plasma samples, blood samples, did not reveal that much actually. A bit of disappointment to us. It would be much easier if we could measure this in blood but unfortunately that's not given so much yet.

We are now following up with a second study to validate our first findings but also to see what else we can do. And we have now included a small group of various highly selected patients. We have ME/CSF patients, we have our multiple sclerosis contrast patient group and then we have healthy matched controls. And we are focused only on cerebrospinal fluid. Seven of each is very small number but this is what we are going to do. We are going to set them into a totally new set of panels that have not been tested before for cerebrospinal fluid. And we have the luck to work together with Olink on this. So about 1,200 different selected markers will be selected for this. Based on the first two panels that we just got data from, actually already now we have 36 significantly differentiated cerebrospinal fluid related proteins that we can find in ME/CFS patients. And this of course has to be validated and in a larger set. We also see both similarities and differences between multiple sclerosis and ME which is, I think, of great importance to understand this. With the proteins that we found initially is also following what we found earlier which was very good. So we find neuro inflammation, cell damage and repair systems ongoing in these patients, which is of course great importance.

Secondly we do targeted steroid omics. We look at molecules that are of steroid family classes. Steroids are extremely important for controlling many different things in the body. For instance, reproduction and endocrinology, maturation of different cells, gene expression, neurological functions, etc. You know we use steroids for classic endocrine detection in many diseases but the trick is that in many studies there's only one or two markers measured at one point. But we have a possibility now to do a full screen, a steroidomics or steroid hormone analysis. We can measure the whole biochemical pathway with our techniques. What we’ve done in this first step—and this is also supported by the Open Medicine Foundation and the Swedish patient organization—is that we're selected 24 patients, 24 matched controls and then we have done in-depth screening, all of the steroids.

Now we'll just give you a brief idea of how it is done. We can do it in plasma now we don't need cerebrospinal fluid. We can do the derivatization and then a high-resolution separation using a separation technique called supercritical fluid chromatography and then high resolution mass spectrometry to measure what we have. What we found is, maybe not dramatic changes but we have changes that I think are relevant when we come to discussion of what's going on in the patient group. So if I have a general downregulation of many of the steroids, the only one that came out significant, as you see here, is pregnenolone. I will show you what that can mean. Pregnenolone is the key steroid in the biochemical pathway. So it's actually the precursor of the rest of the steroid profiles. I also put these blue arrows in the biochemical pathway here. All of these enzymatic ways that I marked here are located to a specific organelle. Is there anybody who can guess what organelle that is? Mitochondria. So pregnenolone is one of the neurotransmitters that are neurosteroids that we have in very high concentrations in the brain. It controls, together with the sulfate product, synaptic function, neuro protective functions and also enhances myelinization. It can also, together with its sulphate ester, improve cognitive and memory functions. So if we have a real neural reduction of pregnenolone that we can tie to some of the symptoms *and* it is produced in mitochondria. So things start to tie together in a nice way here.

Finally, how about autoimmunity? Autoimmunity is something that we've been working with now for a while. That is when the body reacts with immune system reactivity against something of your own, such as a protein or target of the body. Multiple sclerosis is one example of that autoimmunity disease. There are many different autoimmune diseases, around 80 chronic inflammatory diseases. Prevalence in the United States is up to 80 percent. It's more common in women than in men and for women it's actually the fourth largest cause of disease.

In ME/CFS patients there have been studies and there are ongoing studies also from other groups. I started approaching a group in Berlin run by Carmen Scheibenbogen. She had found in 2015 that she found in subsets of her patients with ME/CFS and upregulation of autoantibodies against muscarinic and Beta-2 adrenergic receptors which I found very interesting. So we asked for help and we also selected a bit broader panel of autoimmune reactivity for our studies and for our Swedish patients to look at adrenergic receptors or antibodies against them and also against the muscarinic receptors here. The biological function of adrenergic receptors and muscarinic receptors also tied to what we see in the patients as symptoms. The study design was that we took 48 patients with matched controls. We took both cerebrospinal fluid and plasma from these. We could verify and validate not only Carmen Scheibenbogen 's findings but also our own preliminary data. So we have a significant upregulation of autoantibodies against the adrenergic receptors, as you see here, and also some of the muscarinic receptors. This is a log scale plot here. So you see that in healthy controls and in patients we have differences but we have a big biological spread which is what we expect here. So everybody can carry some of these autoantibodies and still not have any symptoms but [they are] significantly enriched in the patients in this case, for some of them.

Moving ahead here. What we also found was in cerebrospinal fluid, no autoantibodies for the muscarinic and beta receptors which is something positive I would say. So we have validated the findings summarized here: significantly elevated in patients. We have observed now in two separate studies in Sweden and we also have no detectable levels of autoantibodies in our cerebrospinal fluid which is good.

Just something that is not totally related to ME/CFS but something that I think has given me at least some ideas of what to look for. We just published a study together with a lot of colleagues in Uppsala where we have looked at acute sleep loss in an experimental setup that I will tell you and the remarkable change that you can see often just 24 hours of sleep deprivation. So the setup of the study briefly is that we have young male healthy individuals, students, of course. We selected 15 of them and we asked them not to sleep for 12 hours when they were supposed to sleep. We take blood samples, we take adipose tissue, fat tissue, I'm going to take muscle biopsies and then we do screening of all different kinds of molecules. Just to summarize what we found is a dramatic change in genes, specifically those genes that are regulating our daily rhythm or circadian rhythms and we also find degradation of muscle tissue, we find an upregulation of adipose tissue. What is that meaning? Well, after only 12 hours of this deprived sleep, sleep disturbance, we have phenomena going on in the body. I know that patients have very [many] difficulties with their sleep so of course this is something I want to follow up on and see what kind of metabolic changes and proteomics changes and maybe gene changes we can see.

In conclusion: significantly elevated levels of inflammatory markers we have established especially in cerebrospinal fluid which is a target for me; disturbed stereoidogenesis in the patients that could be related to dysfunction in the mitochondria; significantly elevated levels of these autoantibodies; and sleep hygiene is very important.

That leads me into my final slide here. I had the chance to be on a tall ship race ship this summer for two weeks. We managed to sleep like three hours in a row for two weeks which is, I can tell you that you get tired and dizzy. I don't know if we didn't gain much fat. I think because food onboard wasn't the best maybe. But we had also very tough weather. We had eight nines before in this race so there was a fantastic expedition. Actually they came second in this race with the ship in the end of the race. With that I would like to state what Lord Kelvin said at one time, when you're face to face with the difficulty, you're up against the discovery. I think that's where we are now in this field. I really look forward to follow what's going on in all the other research groups around the world that are focusing on this. So a great thanks to Ron, Ron’s family, for arranging this, Linda with family and all their involvement in this research field. To the Open Medicine Foundation of course, to our Swedish patient organization’s support, colleagues all over the world and of course all patients and healthy controls that voluntarily gives [inaudible] for this research. So thank you very much. Tack så mycket.

[Applause]

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