Robert Phair, PhD | Metabolic Traps: A new way to think about ME/CFS

https://www.youtube.com/watch?v=uej1LXzRbnY&index=16&list=PLl4AfLZNZEQPxjqF4ojAO3wdCFMeriNBK

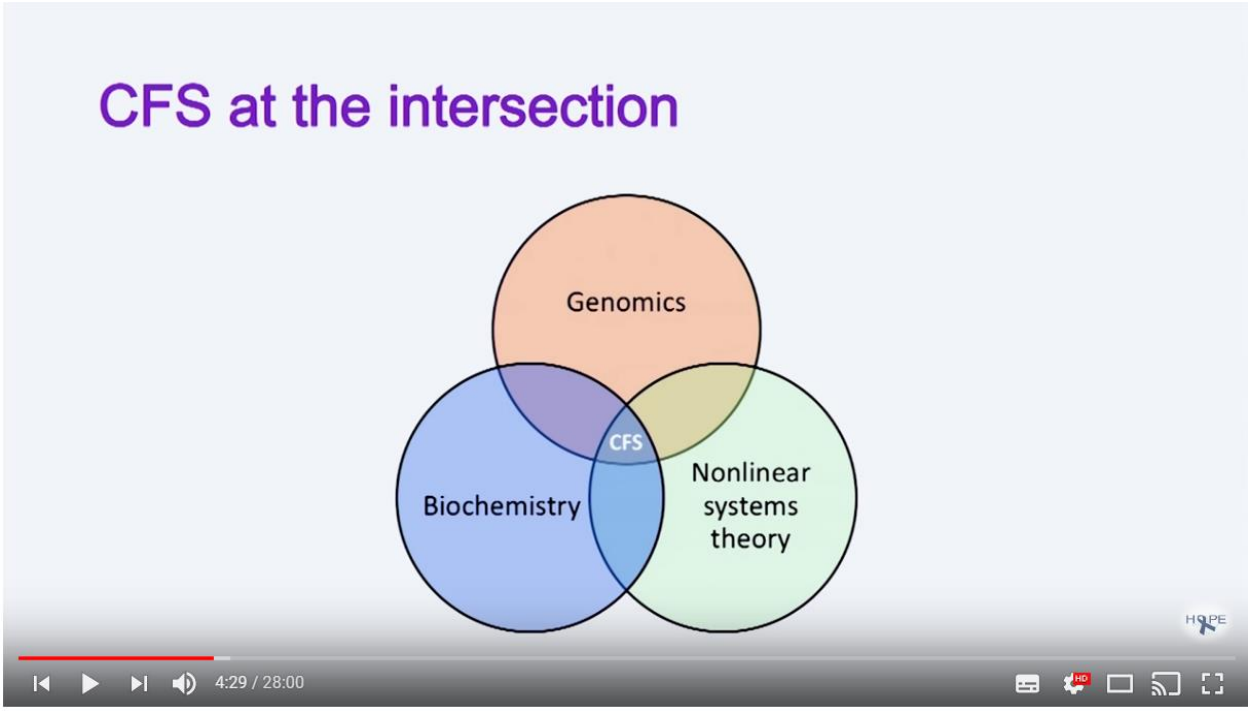
Dr. Raeka Aiyar: Our next speaker Rob Phair, who is with us here from Integrative Bioinformatics Incorporated, a local company. He's been collaborating with us, sort of out of interest and the goodness of his heart for a couple of years. He's about to present us with the theory for ME/CFS that I think a lot of you already know about judging from the questions that happened even before the symposium began and we're looking forward to hearing the latest about that.

Dr. Ron Davis: Rob came to us I think like three years ago and said he had read something in the Stanford Medical Magazine about Chronic Fatigue Syndrome and he said I want to try to help. He started a small company, Integrative Bioinformatics, here locally and said I'll donate one day a week of my time. So he started coming one day a week and getting involved, learning how to do sequence analysis and all sorts of things that he hadn't done before but he was very, very willing to do all of that. Now Robert is another nerd, that's not an insult it's a compliment, from MIT and as an engineer and then went and learned human physiology and so he knows both of those really well. His goal was to be able to make predictions and integrate data and in a kind of a systems engineering fashion. Now that wasn't very popular with NIH and review committees. So, he was at Johns Hopkins and he decided to quit and set up his own company and do it as a consulting and that has worked out pretty well but I'm glad you did that. Anyway, this was a perfect problem for him and he came to say he wanted to help and I said “Oh my gosh I've been really, really wanting a systems engineer that knows human physiology because I think that's where the answer is.” But there are almost none because NIH won't fund them and so it was fantastic that he came here and I really loved the idea. He will admit that it we don't know if it's right or not but we're testing it and he's going to tell you about the results of those tests. Thanks Rob.

[Applause]

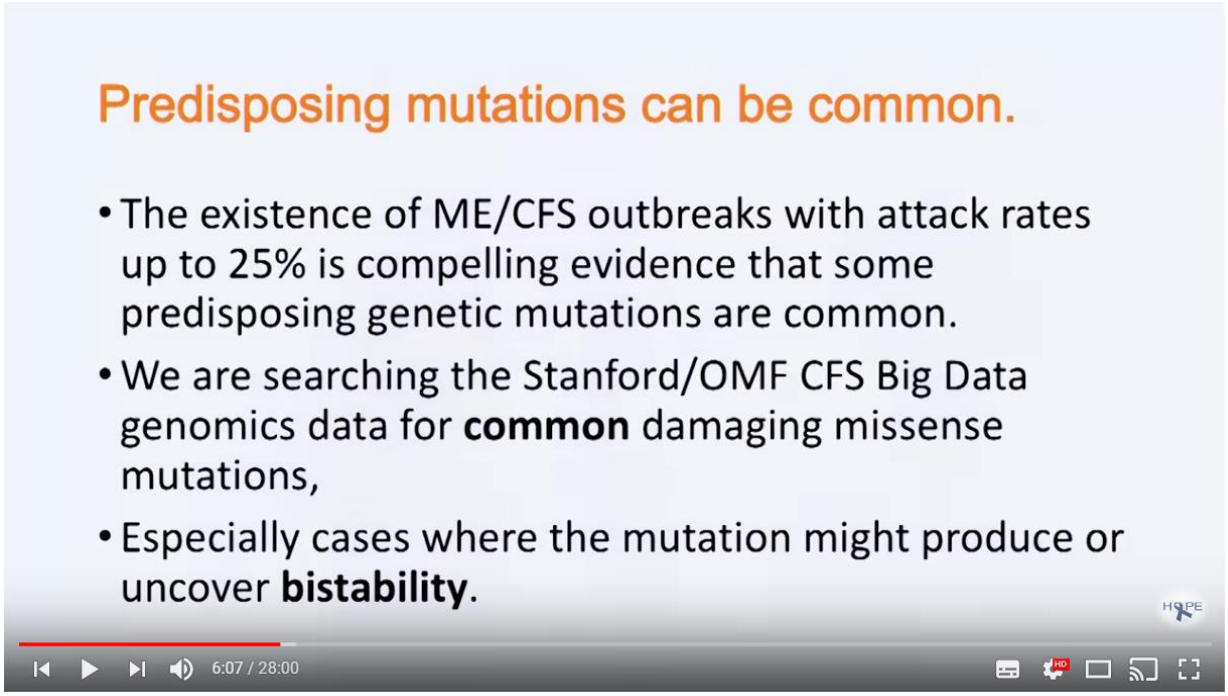
02:36 **Dr. Robert Phair:** Alright so I want to ask how many of you have read David Bell's book. This is called “The Doctor’s Guide to Chronic Fatigue Syndrome.” Now that's a good number of hands. This is the best book on the subject, it was published in 1994. I wish there was a supplement to this book to cover what's happened since. That supplement is being written by the scientists at this meeting. I've learned a heck of a lot from those people and I'm glad I'm here. So Chronic Fatigue Syndrome is a complex disease. The human body is a nonlinear system. David Bell says in this book that if you want to treat Chronic Fatigue Syndrome you have to know the symptoms and you have to know your physiology and your pharmacology. But if you want to cure Chronic Fatigue Syndrome you need to know its underlying mechanistic basis.

04:02 So I want to talk about today is a new way of thinking about Chronic Fatigue Syndrome, that has helped me at least pull a lot of pieces together, to see how the various symptoms and the various measurements we can make tell a systematic story. So to do that I'm going to talk about these three topics: genomics, biochemistry and nonlinear systems theory.

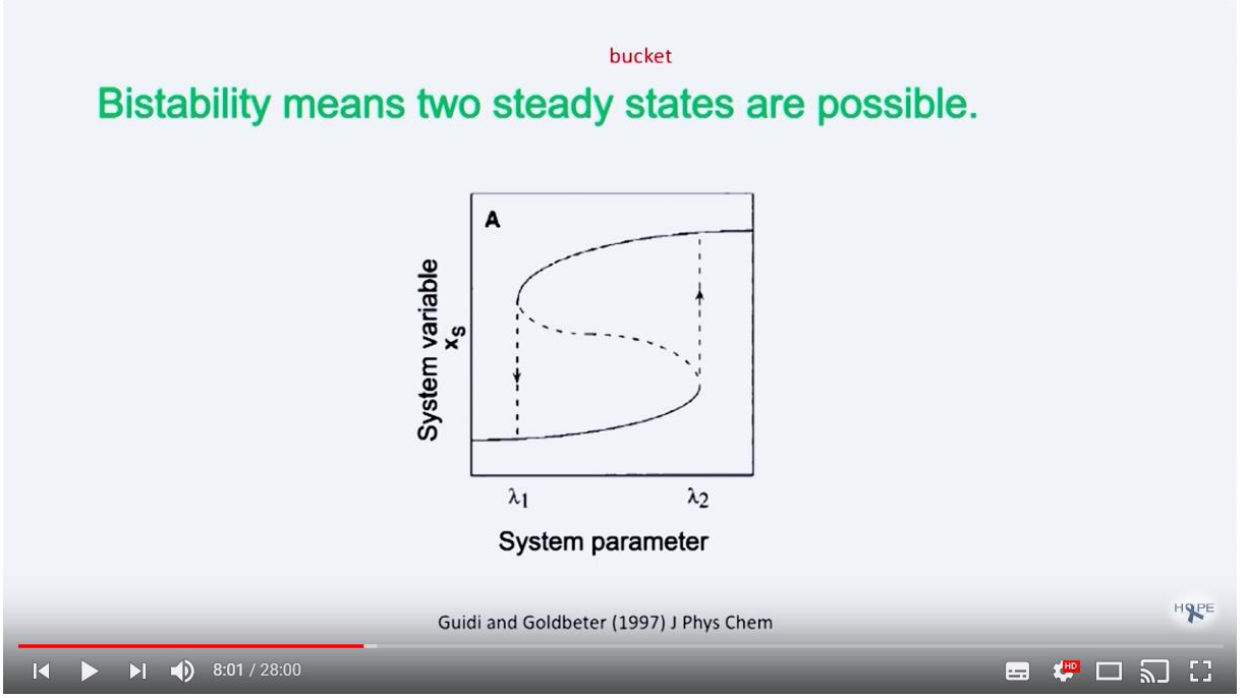


Now you might be saying what's nonlinear systems got to do with biology and I've been asked this question throughout my career as Ron alluded to. It's hard to get biologists to think about mathematics, it's also actually hard to get mathematicians to think about biology. But forty-five years ago I made a decision that my father never forgave me for. That decision was that I took my newly minted MIT electrical engineering degree and I went off to get a PhD in physiology. He thought this was a terrible idea. He's an engineer, my brother's an engineer, his dad was an engineer, I'm supposed to be an engineer too. So I want to convince you not only that nonlinear systems analysis belongs in the study of biological problems but that it can actually explain some of the things about CFS that have remained inexplicable.

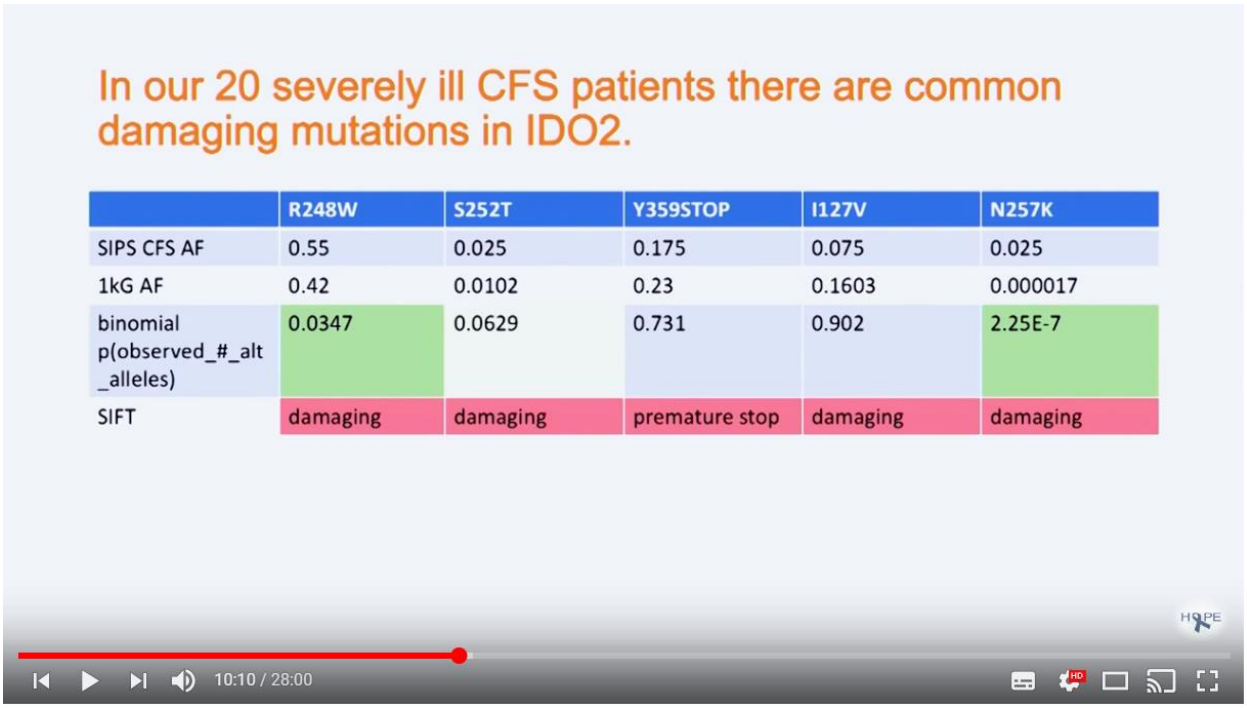
05:50 So let's start where I started. I started with David Bells book and with Osler's Web (https://www.oslersweb.com/the-book), Hillary Johnson's book. There were a lot of stories in those books about CFS epidemics. The existence of these epidemics is to me an important feature of the disease.



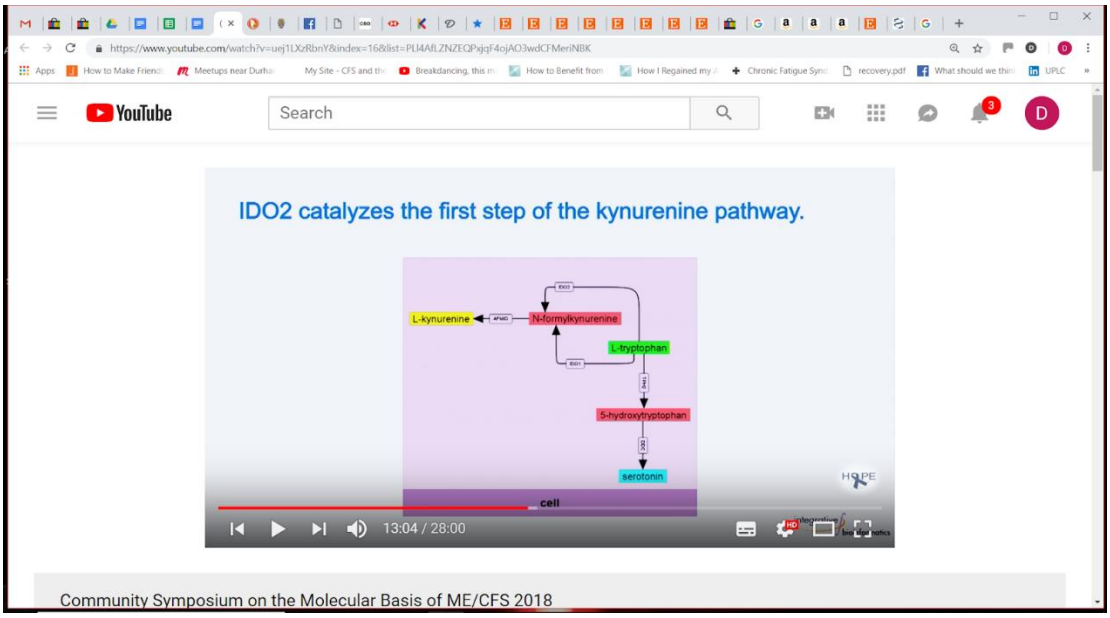
The fact that you can have attack rates, that means the fraction of the people who are exposed to whatever it was, that is upwards of ten or twenty five percent as it says in the slide, says to me that it's possible that any predisposing mutation is common. So as I arrived in the Stanford Genome Technology Center and started talking to Ron, it was just about the time that the Big Data study, the one funded by the OMF and run at Stanford, was coming to fruition. And so I had suddenly access to the genomic sequences, the genome sequence for twenty severely ill CFS patients and so I started searching not for rare mutations but for common ones because of this feature about the the presence of epidemics like the one at Incline Village and the one at Lyndonville, New York. And so I was especially interested in cases of mutations where the mutation might produce or uncover what's called a bistability. So I'm going to talk specifically about one such mutation or set of mutations today but at first I need to define for you or illustrate for you what a bistability is.



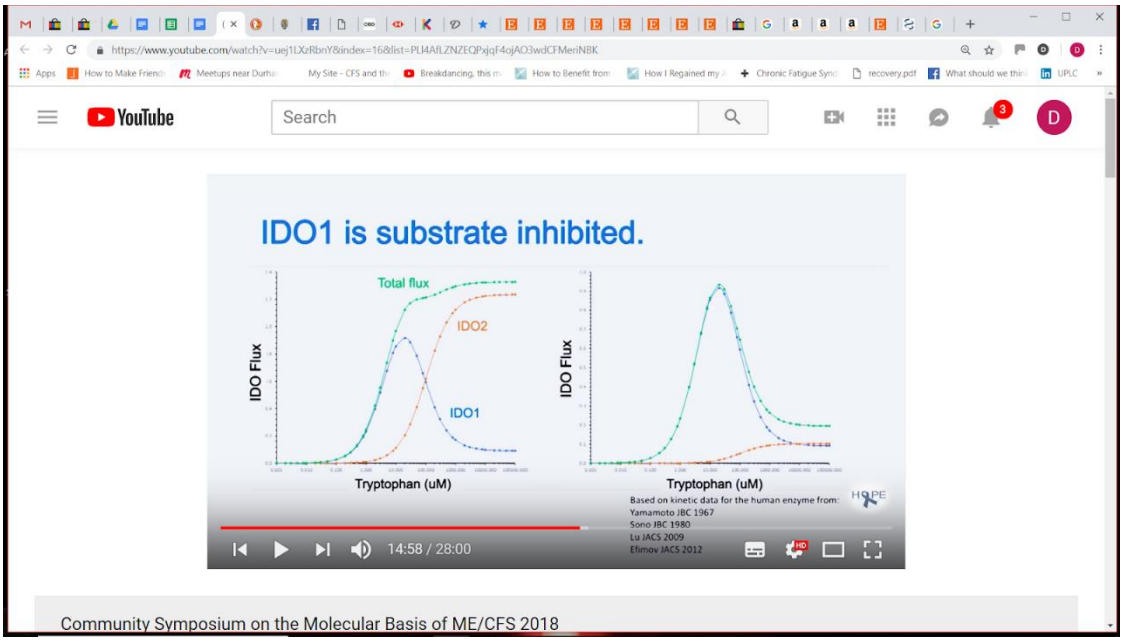
07:48 So here's a diagram. This diagram comes from a mathematicians papers from the Journal of Physical Chemistry and it describes what's known as a bistability on a nonlinear system. That means that this system, and it can be the human body, everyone should know that the human body is incredibly nonlinear system, can obtain two different steady states. One of them I'll just use as an example, the one at the top closest to the letter A in the diagram, is a solid line representing all the steady states that are healthy steady states. The solid line at the bottom as a curved line represents a whole set of unhealthy steady states. The points, they're labeled with mathematicians rigor, lambda 1 and lambda 2 are called the limit points. The hypothesis that I want to test here is that CFS represents a collision with one of those limit points. When you're on the healthy steady state, so in the upper left. I think I have a way to point to that. Right here at that point you fall off of the curve of healthy steady states and drop down onto this lower unhealthy diseased steady state. The axes (labels) of this graph are not very clear they are system parameter on the horizontal and system variable on the vertical. They represent features of the biological system that we're studying. This presence of two steady states is the thing that we're looking for in those genomic sequences because we want to see something, a message to us in the genome that tells us that a bistability is possible. So what was the first such genome mutation that we found.



10:16 That's here, in Chronic Fatigue Syndrome, in these twenty severely ill patients, we found several mutations in one gene called IDO2 (indoleamine 2,3-dioxygenase 2). I'll tell you in a minute what IDO2 is but you can see here that across the horizontal, in the blue bar, there are some amino acid (building blocks of proteins) symbols followed by a number, followed by another amino acid symbol. These represent mutations. It's an arginine (R) converted to a tryptophan (W) in the first column, for example, in the 248th amino acid of this protein and that mutation breaks the protein. The protein does not work if it has this mutation, nevertheless this mutation is very common. So if you look in the line that has the SIPS CFS allele frequency (AF) that says that our twenty patients have that 55% of the time. And the thousand genomes project (1kG AF), that's one of the standard reference genomes, has it 42 percent of the time so it's a very common mutation. 42 percent of the people in the room, maybe even more since we're studying CFS, have this mutation. It's significant (statistically) at the 0.03 level not the 10 to the minus ninth level (0.000000001) so I can't say it's perfect and I don't intend it to be. What's surprising is that even patients who don't have that very common mutation have another or even one or two or three other mutations in the very same protein IDO2. Although I don't know that all of those are broken biologically, I do know that all of the prediction software tools, one of them I've listed here is SIFT, that attempt to tell from the sequence whether or not it's broken or likely broken, predict that all of these are broken. Even the one in the middle here is a premature stop codon, so that's what we call a nonsense mutation, it doesn't even make the full protein. So these mutations provided a hint about what was going on in these severely ill patients that I wanted to follow up.



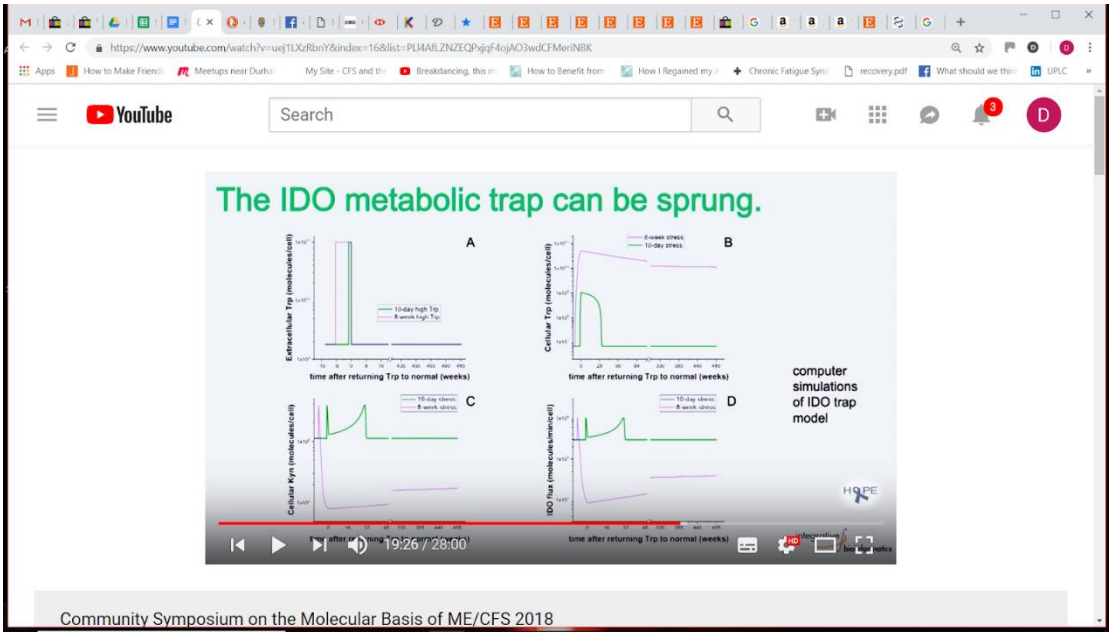
12:46 Okay so now what's IDO2. IDO2 is the first enzyme in a pathway known as the kynurenine pathway. This is the major degradation route for an amino acid called tryptophan. This is the least frequent amino acid in your proteins. Tryptophan is an essential amino acid, that means you cannot make it yourself* (see end of transcript for warning from Dr. Davis regarding self-experimentation). It is only available in the diet from organisms that can make it themselves. So tryptophan has two fates that we're going to talk about. There are other small or minor fates but big ones are the kynurenine pathway that goes from east to west in this diagram. East to west (right to left) and there are two enzymes that catalyze that first reaction the IDO2 enzyme I was just telling you about with its mutations, and IDO1 which does exactly the same thing. So you can say how can it even matter, this is what I was saying to myself, how can it even matter that IDO2 is broken when in fact IDO1 works just fine and does the same thing so it took me weeks, months maybe to figure this out. I don't know that I figured it out but I've got one way that it might work. Okay, just to make the system complete, there's a pathway going from north to south here that also is important for the fate of tryptophan and it is the production of serotonin, a molecule you've probably all heard of, it's involved in sleep regulation and many other things like controlling the immune system. So what could it be about the IDO system that actually matters since IDO1 works fine and it's only IDO2 that's broken. So that answer came from enzyme kinetics.



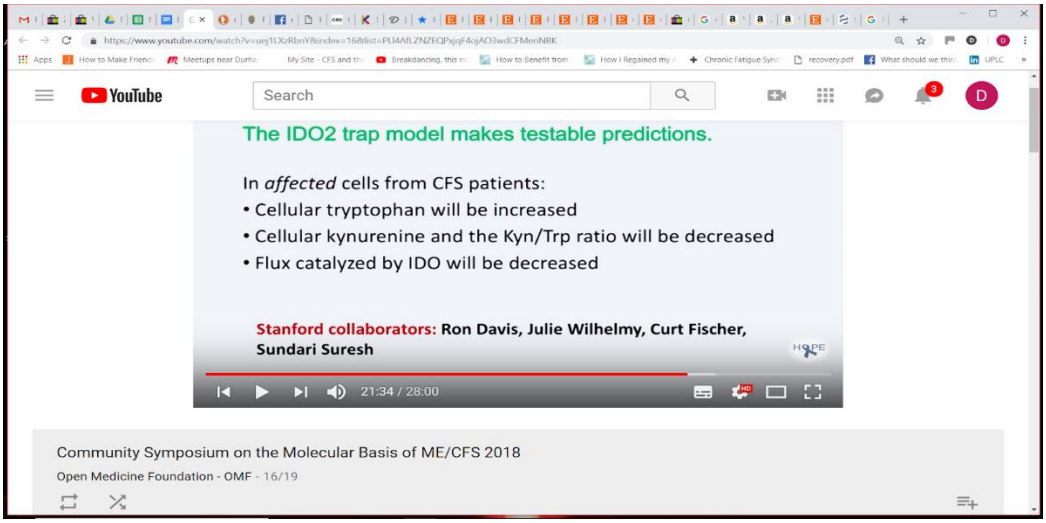
15:01 This is one of the more complex slides I have, but you know I've learned that the CFS patient community has read more papers on CFS than I have and so I'm not worried that these slides are over the top of your heads. I'm worried that you're going to ask a question I can't answer. Okay, so on the left hand side is a plot of tryptophan, that's the molecule that's the substrate for this enzyme. The molecule that's converted to other molecules. On the vertical axis is the flux through that enzyme, that means how many molecules are converted to product in a unit time, so maybe a thousand molecules per second, something like that. On the horizontal axis is the concentration of the tryptophan that's going into that reaction and you see for IDO2, the one that's broken, IDO2 has the classical shape. This is what we call a Michaelis Menten curve, named after the two women who discovered this feature of enzymes back in the 1800s, and it has the right shape for almost all enzymes. But for IDO1 the shape is not the same, it doesn't saturate up here and become a maximal velocity, instead it goes over a peak and comes back down again. So at very high tryptophan concentrations there's much less flux than there was before, much less production of kynurenine. So instead of having the sum of the total flux be something like an Michaelis Menten curve, if you get rid of IDO2 looks like what you have on the right. Right says IDO2 is in effect a backup system. It works at very high tryptophan concentrations to maintain a flux through the kynurenine pathway in the presence of a very high tryptophan concentration. So now you see what could go wrong, IDO2 doesn't work, it's broken. So now there's a much lesser flux and as soon as you go over this peak, as soon as somehow, and I'm thinking this is what happens in that initial stressor that you've encountered that puts you into the CFS disease, you were pushed over this peak. Once you get over the peak, now you're in trouble because now every time you increase the tryptophan concentration the flux through the enzyme goes down, and every time the flux through the enzyme goes down the tryptophan concentration goes up, and every time the tryptophan concentration goes up the flux through the enzyme goes down and so on and so on. You are pushed all the way down

the curve. You're stuck on the other side of this peak and as a result you're stuck in what I call the IDO metabolic trap.

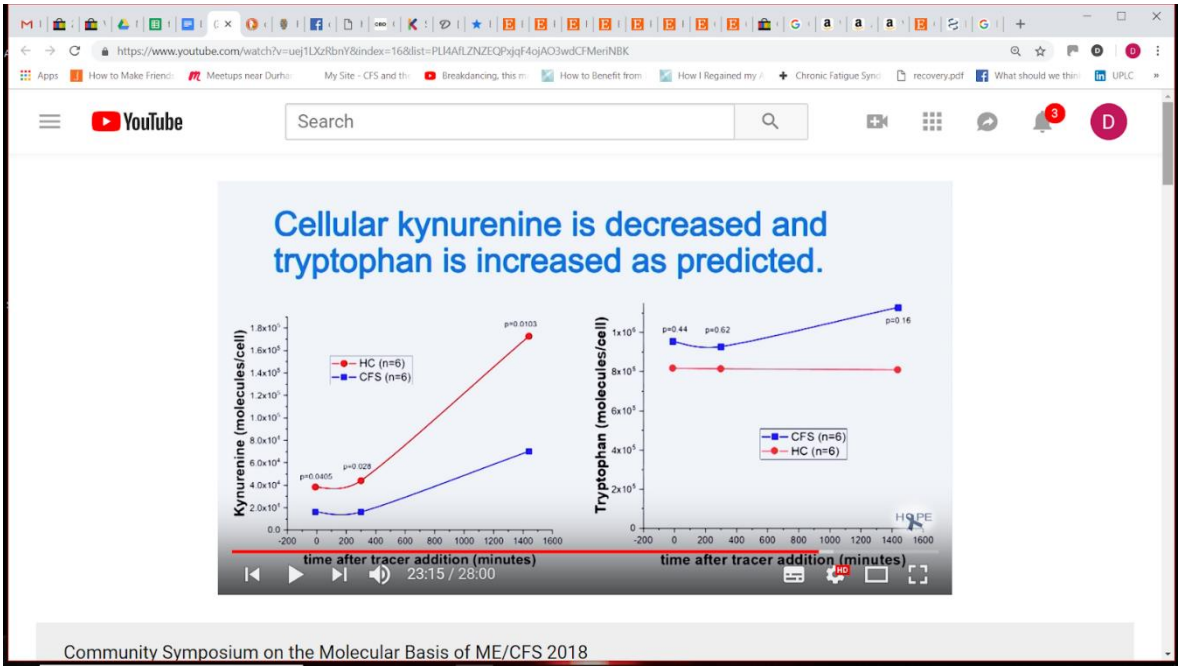
18:20 So the key points so far. Severely ill CFS patients have on average 1.7 broken copies of IDO2. There are only two possibilities. Yeah, 1.7 is a big number compared to two and, without IDO2 as a backup, at high tryptophan concentrations inhibition of IDO1 by its own substrate opens the door to a pathological steady state. This is an example of the bistability and nonlinear systems that I've been talking about since the beginning. So the next thing we did was show that that's not just hand waving. Yeah, I stood there, I talked about going over the peak in the IDO1 curve. Yeah, that sounds pretty good but we built a mathematical model.



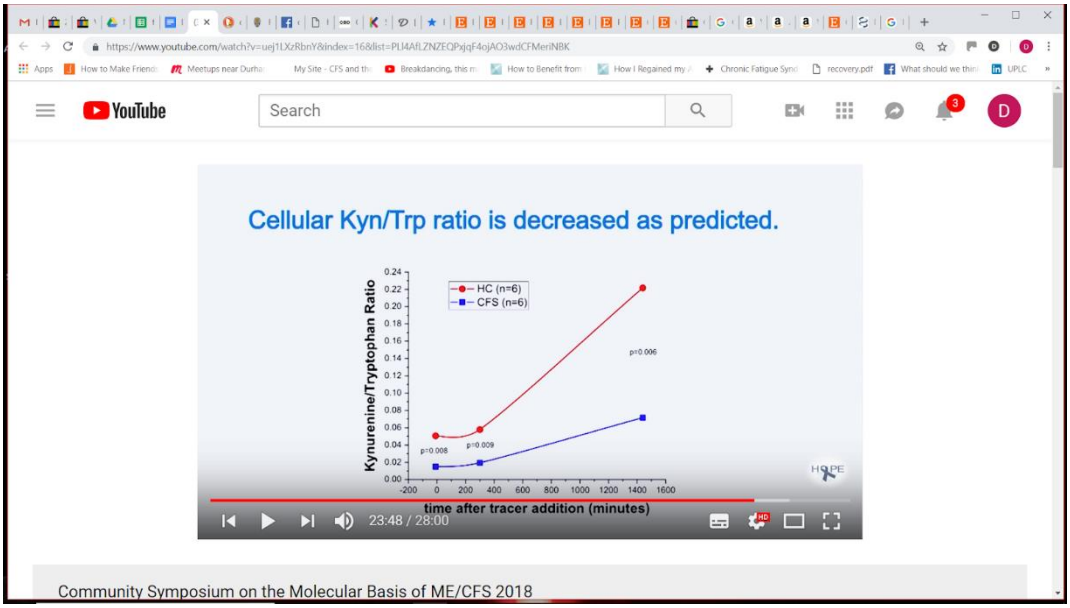
This is actually the part of the project that I actually do for a living so I'm happy with this. This is a model of that system that I drew, the tryptophan pathway through the kynurenine and the tryptophan pathway to serotonin. What the model shows is that a very large increase in tryptophan concentration, artificially produced in the model for only ten days, causes a change in tryptophan, as you might guess, inside the cell. When you remove that high tryptophan in the extracellular space, actually returns to normal but it takes 30 weeks, half a year. If you do that longer, if you have the high tryptophan for, I think this one is eight weeks, so two months worth of stressors then you never return even though you have removed the stressor. So, I take this tryptophan right back to where it was in control and everything in the cell stays where it was. So this is a very high level of tryptophan, this is a very low level of kyneurine, the downstream product, and this is the flux through that pathway, the IDO1 pathway, it's very depressed. So the model is consistent with the idea that, not only is it fun to look at, going over that peak it actually happens in theory, so now this model makes predictions that we can test.



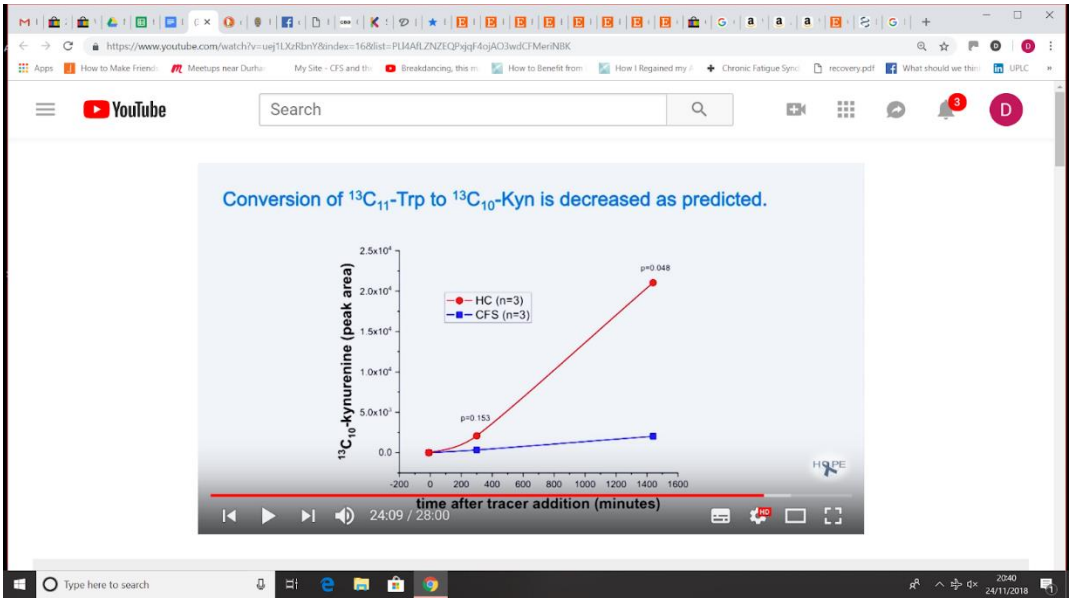
21:07 Now I couldn't test these things myself, a theorist. I did a lot of experiments by the Johns Hopkins but that was a long time ago and furthermore these experiments are a lot harder than the ones I used to do, so that meant I needed a team and so fortunately the OMF, when they found it they funded this grant that I'm working on and also funded a grant for Ron's (Dr. Ron Davis) team. So Julie Wilhelmy, Curt Fischer and Sundari Suresh have made it possible to make the measurements I'm going to show you. Curt Fischer is an expert in mass spectrometry and Julie Wilhelmy has the best experimental hands I have ever had the pleasure to work with, she is the best in the lab I have ever seen. So this team wanted to test these predictions; in affected cells cellular tryptophan will be increased. This is the tryptophan level inside the cells, so it's not going to be outside, it's inside the cells. Cellular kynurenine and the kynurenine/ tryptophan ratio will be decreased, that's another prediction, and finally the flux catalyzed by IDO should be lessened in CFS. So we undertook to do that.



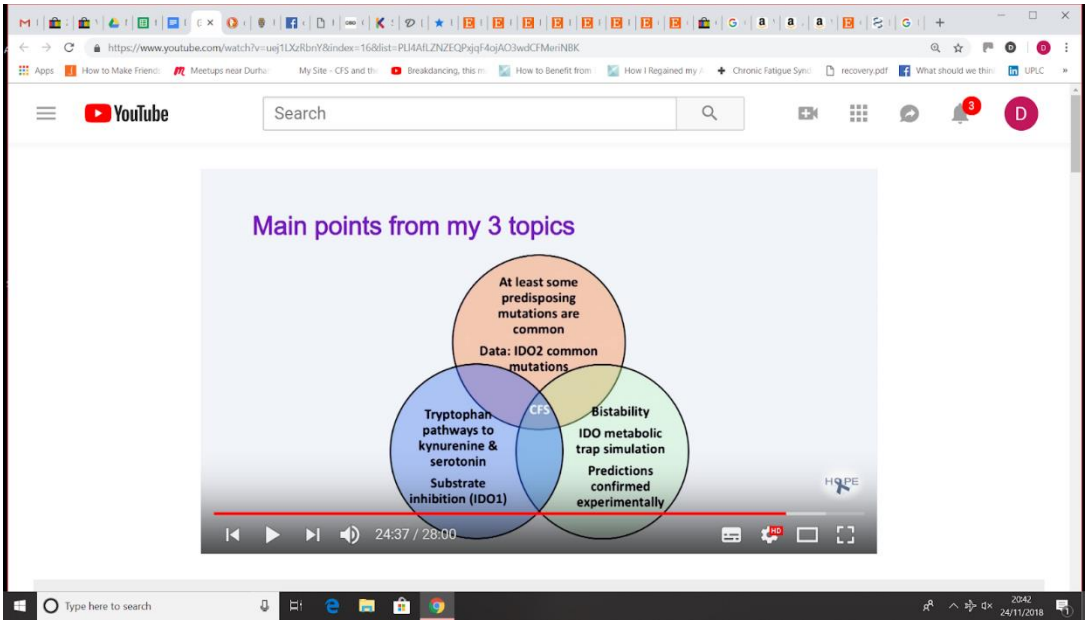
22:30 We've only been doing this for eight months, so this is only n=6 patients. On these two figures what we have is the kynurenine concentration in molecules per cell. The red data points are the healthy controls and the blue squares are the CFS patients. You can see that kynurenine is as predicted substantially lower than it is in the healthy controls. Time equals zero here is when we added a metabolic tracer, I didn't mention that I should have, in the design. Tracers are here used to follow the fluxes, so I'll show you in the next slide or two where the tracer goes. But this is the what's actually plotted on the horizontal axis, is the time after tracer addition. Over on the right-hand panel you see that the tryptophan in the cells is also high in the CFS patients as predicted. That's not statistically significant, I'm hoping it will be by the time we get to an n (sample number) much greater than 6.



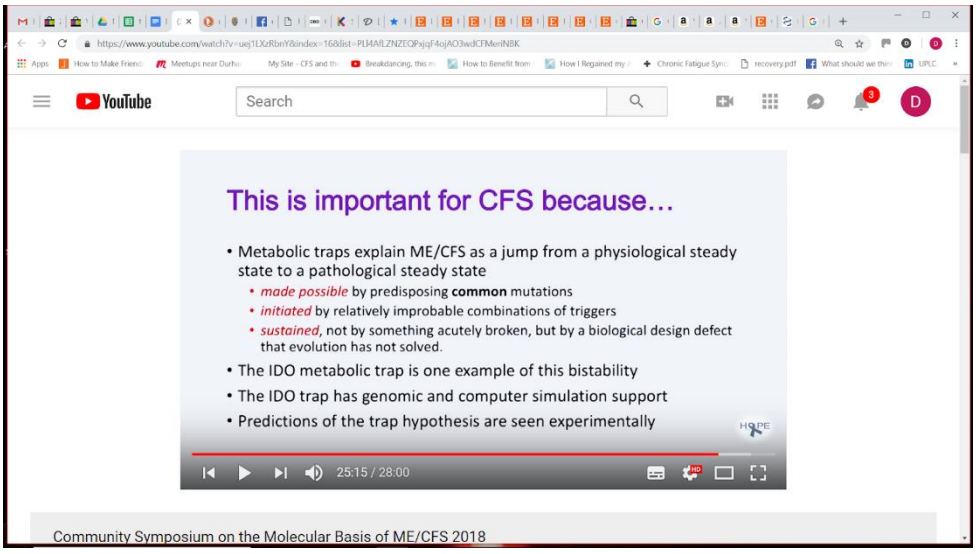
So the last one then is that the cellular kynurenine to tryptophan ratio is decreased, as predicted. This is highly significant in n=6 patients. So those are all consistent with the theory.



We also measured that flux that I told you about, using the carbon-13 labeled tryptophan. You can see that the blue curve here for CFS is again much depressed compared to the production of kynurenine in healthy controls.

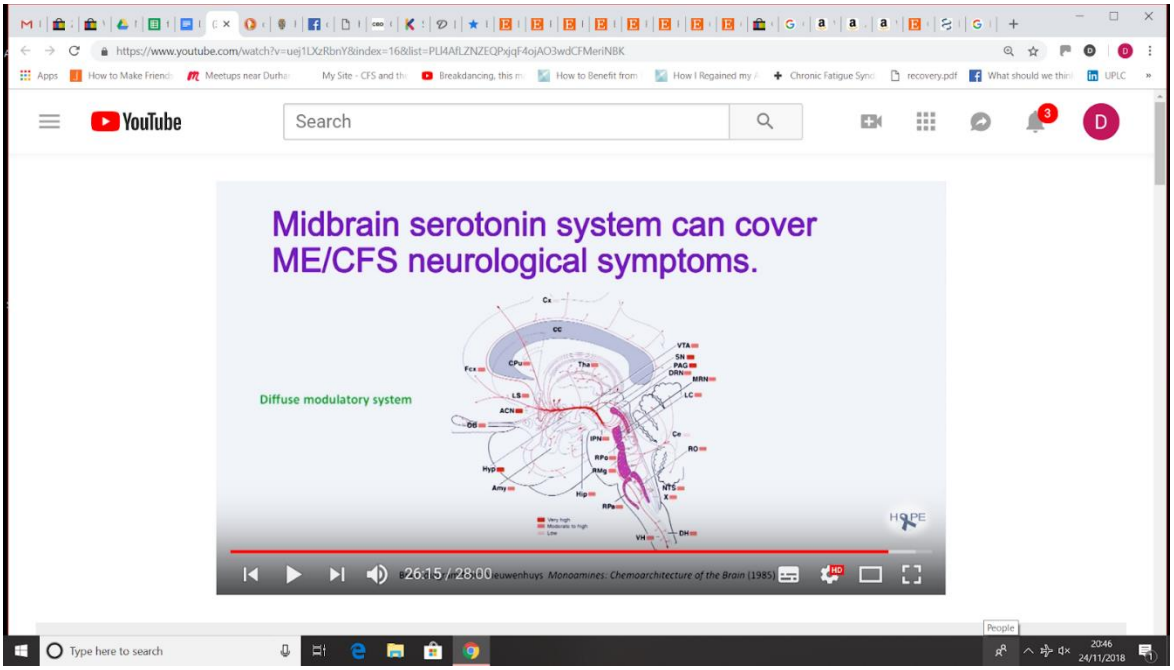


24:19 So my main points for each of those three topics that I discussed. There are at least some predisposing mutations are common. We have data on a particular set of mutations in IDO2 in the biochemistry part, the blue circle of my Venn diagram, there's tryptophan pathways to kyneurine into serotonin. I showed you what those were and then showed you about substrate inhibition, a particular feature of IDO1 that makes it relevant to our bistability, that might be the characteristic of chronic fatigue syndrome. Then we did the simulation to show that it works in theory and that some of the predictions of that model are confirmed experimentally.

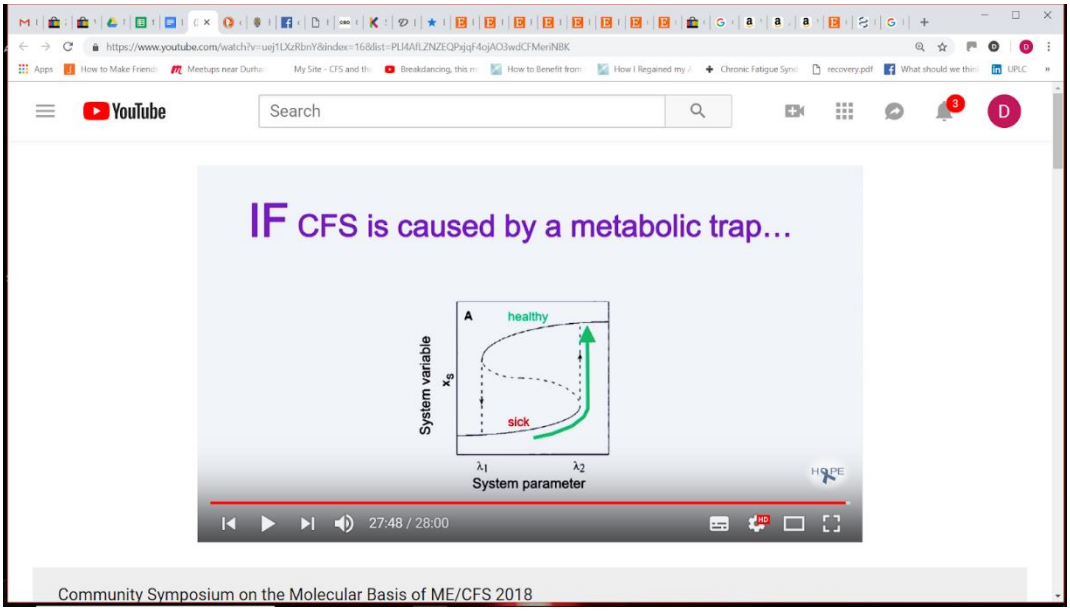


25:15 So this is important for CFS because metabolic traps explain ME/CFS as a jump, a jump from a physiological steady state to a pathological one. This jump is made possible by predisposing common mutations. It's initiated by a relatively improbable

combination of triggers. They're not always the same triggers, just any trigger that allows you to get into the bistable state, the low state of your bistability and finally is sustained not by something acutely broken, but by a biological defect that evolution hasn't solved yet. So the idea of metabolic trap is one example of this kind of bistability. It has some genomic and computer simulation support and it makes predictions that have been confirmed experimentally.



26:10 So I want to just spend a minute on why this might matter to you. This is a diagram of the brain in cross-section and in the purple you see six nuclei, these are called the raphe nuclei. They are the neurons of the serotonergic nervous system in the brain and those neurons are capable of controlling a vast number of portions of the brain that are relevant to CFS. I'm being shepherded off the stage,** (excerpt from panel discussion where Dr. Phair was asked to finish some thoughts at end of transcript) so let me tell you that cognitive function, movement, smell dysautonomia, motor neuron dysfunction, dopamine system, all of these headaches, air, hunger, POTS, parasympathetic dysfunction and pain, all of them have inputs from this serotonergic nervous system in the brain. I think that's relevant to the disease. How will we proceed? We're going to pursue the trap intensively. We're going to initiate collaborations, I must say in this week probably four new collaborations on the trap have emerged from our scientific discussions. We're going to design and test therapies based on the trap and see if we can make them work.



27:47 If, and that's a big if, CFS is caused by a metabolic trap then our job is to get the patient from the sick set of steady states back up through the limit point and onto the domain of healthy steady states. Thank you.

[Applause]

**Warning from Dr. Ron Davis, excerpt from his talk 'Ronald W. Davis, PhD | What's next?' https://www.youtube.com/watch?v=pFzOrknOylA&index=17&list=PLl4AfLZNZEQPxjqF4ojAO3wdCFMeriNBK*

11:02 ***Dr. Ron Davis:*** *Now the other thing I wanted to mention is the fact that it hasn't escaped our attention that we're talking about tryptophan and things that you can buy. What happens usually with patients if they see "Oh my gosh, that might make me well" and they start experimenting. This is a dangerous pathway to experiment on. We know for example if you take tryptophan you can actually cause autoimmunity. Auto-immunity is not necessarily curable by present technology. We were talking about some of this stuff a couple nights ago with with the people there. One point one person pointed out, yeah it's really dangerous when patients start experimenting because there was one possibility for a disease and they needed a particular drug but it wasn't available. So they contracted a company that synthesized it for them and they gave it to them. That is not tested in any animal models and they took the drug and it killed part of their brain and became totally paralyzed and then died. So it's really dangerous to self-experiment on yourself without really the people who know what's going on. That's why we're so strict about drugs and they have to be tested. If you make a new drug or a new company makes the same drug that's been made before it has to be tested because you can get yourself into big problems. So I'm urging people do not experiment with this pathway!! Give us some time to figure it out. Then you can see some immediate problems if this is all true the tryptophan levels can be very high, which will make your serotonin levels very*

high. The problem with that is that the body will self adapt to that and it reduces the level of receptors to make you less sensitive to serotonin. If you try to get yourself out of the trap, and this is what we have to be really careful about, your body's not used to the right level of serotonin and what is it gonna do to you? It may be awful, we don't know. It's certainly involved in the brain and we just don't know what would happen. The problem is by self-experimenting on these kind of critical pathways you can make yourself much much worse and that whatever you did to yourself might not be curable. So I'm just urging people please don't self-experiment! Give us the time to figure out the right way to do it and that's what we'll be trying, if we think it's right, that's what we will be trying to do. We're going to be working with the doctors and we're going to be working internationally with this thing. I'm hoping it's right because it would lead to an effective treatment and it might even lead to a cure but we have to test it and we have to build the resources to do that.

**Excerpt from 'Q&A Panel Discussion 2', https://www.youtube.com/watch?v=mSm8P5v8wCA , where Dr. Phair was allowed to finish some thoughts

2:50 **Dr. Raeka Aiyar:** So I'm happy to welcome our speakers from this afternoon backup here again. Since we were just talking about the the metabolic trap I want to start with that and address some questions, some of the common questions that came up. There are many, many questions but I'll get to as many as I can, again we had something like a hundred or so submitted so do bear with me as I try to address them. So Rob you had started to talk about how this would fit into some of the symptoms, into ME/CFS, at which point you accused me of shepherding you off the stage

Dr. Robert Phair: which of course you weren't doing

Dr. Raeka Aiyar: [laughs] so I wondered if there was anything left over there that you might want to address, because I think that might address some of the questions we got.

Dr. Robert Phair: Yeah, I didn't have much time to talk about serotonin and I talked more about the kynurenines. Ron ('Ronald W. Davis, PhD | What's next?' https://www.youtube.com/watch?v=pFzOrknOylA&index=17&list=PLl4AfLZNZEQPxjqF4ojAO3wdCFMeriNBK) added the two things that I should have said: one that serotonin might end up either being high or low depending on the cell type and so the consequences could be either a depression or an upregulation of the target neurons in the brain; and also the second that kynurenine has powerful effects in the cell, namely it's a regulator of the immune system. We think it could explain, we that means Ron and me, we talk all the time, and the immunologists aren't quite so sure, they of course know one heck of a lot more about immunology than we do, but there is a good evidence that IDO is required both for the clonal expansion of T-cells and for the function of NK (natural killer) cells. So symptoms you've heard about, a lot I'm sure, that can potentially be explained by the kynurenine being depressed.

Dr. Raeka Aiyar: That's good if that's the part that I prevented you from saying then that's great. So there were other questions about, sort of the you know, the therapeutic implications of this. Of course Ron addressed a lot of that in this follow-up remarks. I just, and I think it's important to sort of reiterate that caution towards the self experimentation, but if you might be able to take a look you know sort of further down the road. What are sort of the most important validations and where could that go for therapies? I think also related to this, and feel free to answer this in

any order, do you think that it's something that could explain the symptom progression or remission that happens in some patients, different types of severity and maybe even other diseases? Do you think that there might be implications there? I mean I guess it all sorts of fits into where do you see this going and how wide-ranging do you think the impact could potentially be.

Dr. Robert Phair: You know I sat with a group of patients at lunch and they were of course happy to hear my story but I told them that everyone who was on the stage in the morning had to be conservative, and I'm just a little crazier than them right. I want to cure this thing and so I have to find a balance between the conservatism of saying we have to test this again and again and again, and at the same time recognize there are a lot of people in pain. Now I want to do both things and I'm not sure how to do it but I wanted to balance the conservatism with some optimism. I'm an optimist by trade alright, so I can think of a lot of things to say about what we might be able to do in the future but I think it would be better right now to recognize that all the other scientists you heard speak might be the ones who are right. It's entirely possible that despite data showing consistency with a metabolic trap that they're the ones who are going to solve the problem. So I just want to tone down a little from the excitement I show when I present this. Science is a team effort made up of a whole bunch of entrepreneurs. Every scientist is working on his or her own to find a great idea, and then Ron brings all these people together, and they got all these different great ideas and they bounce off each other, and together we're going to figure this out. So in particular together, I wanted to make one more point about this together, every scientist who is up here talking today has a team of people behind him or her and I would like you to see some of those people. In the audience we have a bunch of those people who worked on all these projects. Now they didn't work on the projects that are presented by scientists from other states or other countries but they're representative. So I'd like everybody from the Stanford Genome Technology Center who works on these projects to stand up and show the patients and the caregivers that there are real people working really long hours on this project. Is there anybody here who fits that adept that description? [Several people stand in the audience.] So there's a team like that behind every other scientist.

Dr. Raeka Aiyar: I think they might be in the lab [laughter]

Dr. Robert Phair: All those grad students they've got a lot of grief. OK, that's enough on that.

Dr. Ron Davis: Speaking of pharmacology fix, something, now this is a cherry-picked support thing, this is not a hypothesis test right but what Robert is saying is the inhibition of IDO1 is what's causing the problem. So, it turns out that there is a drug that's been tested for cancer because they know that this pathway controls the immune system. So they have had a drug that inhibits IDO1, hoping to help to cure cancer. So far that drug hasn't worked the way they thought it would but it does inhibit IDO1 and one interesting fact, this is the cherry-picked one, is that when you inhibit IDO1 it causes extreme fatigue. That is very much fitting with the idea and it's the inhibition of IDO1 which may actually be causing your fatigue. Now that doesn't mean it doesn't involve the brain, of course it could, it's just that may be the initiating thing that causes the fatigue, it does something else, which does something else and it's in your brain. It tells you you're fatigued.

Dr. Robert Phair: Ron’s my biggest fan [laughter]

Dr. Ron Davis: Yep and what we, so what we really want to do is to activate IDO1, not suppress it, and we don't know how really to do that. Yeah so if there's anybody out there that wants to set up a pharmaceutical company that would be a good target. But you can see it happening in other

situations. So when you get a infection, not all infections, but many infections activate IDO1 and that is consistent with some of the patients saying, and we seen in our own son, you get better after an infection. In fact there are some cases that have been reported, that people actually get cured by an infection. They say I'm over it and you ask how'd you get over it, I had an infection and all the sudden I got better. Now don't go out there and eat dirt and try to find some microbe right, hoping to get a bad infection because it could kill you and it's not guaranteed to activate IDO1. But you know there are some things here that have substance that we can figure, we can explain, can explore.

Thank you to our wonderful team of volunteer transcribers for transcribing the Symposium.