Hello, I'm Ron Davis. I would like to talk to you about some recent research being done by Stanford University on ME/CFS. Some of this work has been supported by the Open Medicine Foundation, which for us has been absolutely critical.

DR. RONALD W. DAVIS
Stanford Genome Technology Center

Our approach for doing this research – which we call fast-tracking – is to find a cure for this disease. Our goal is to find a cure, not just find data that we can publish. So this whole thing is being driven by collecting lots of data, analysing that data, and moving on. It's not heavily controlled by hypothesis testing. We are not doing large numbers of patients to keep costs down, but we feel we can understand this disease by only using a small number of patients.

What we're looking for is a signature in this disease. Some of these are going to be biomarkers, and we really only focus on getting high quality data and following this up over the course of time. One of the things we are really good at is developing new diagnostic technology, and I am going to talk to you about that in this talk.

We are also going to develop a technology that will allow us to do drug screening. Currently we are restricted by testing drugs on patients, which is slow and costly and takes a long time. So, what I am going to show you is a technology that we think we can probably use for high-throughput screening. Also, it is likely that this technology will be applicable to other diseases like Lyme, fibromyalgia and so forth.

Now, the goal of the Stanford Genome Technology Centre is to develop innovative technologies to cut healthcare costs. We note this is not a very high priority for the NIH, which I am surprised at. However, private donations help us a lot in developing this.

One of our focuses is on developing engineered biosensors and devices. We also have a synthetic biology core that is used to develop new ways to do production of

drugs and test drugs. So we're trying to take on a whole systems approach for understanding disease and finding a treatment for those diseases.

For the Open Medicine Foundation, we're putting together an advisory board that will help us on this, because it is a complex problem involving medicine and other technologies, and we have experts in a variety of fields that will help us do this. And this is a pretty outstanding advisory board, and they have helped us a lot in coming up with ideas and approaches.
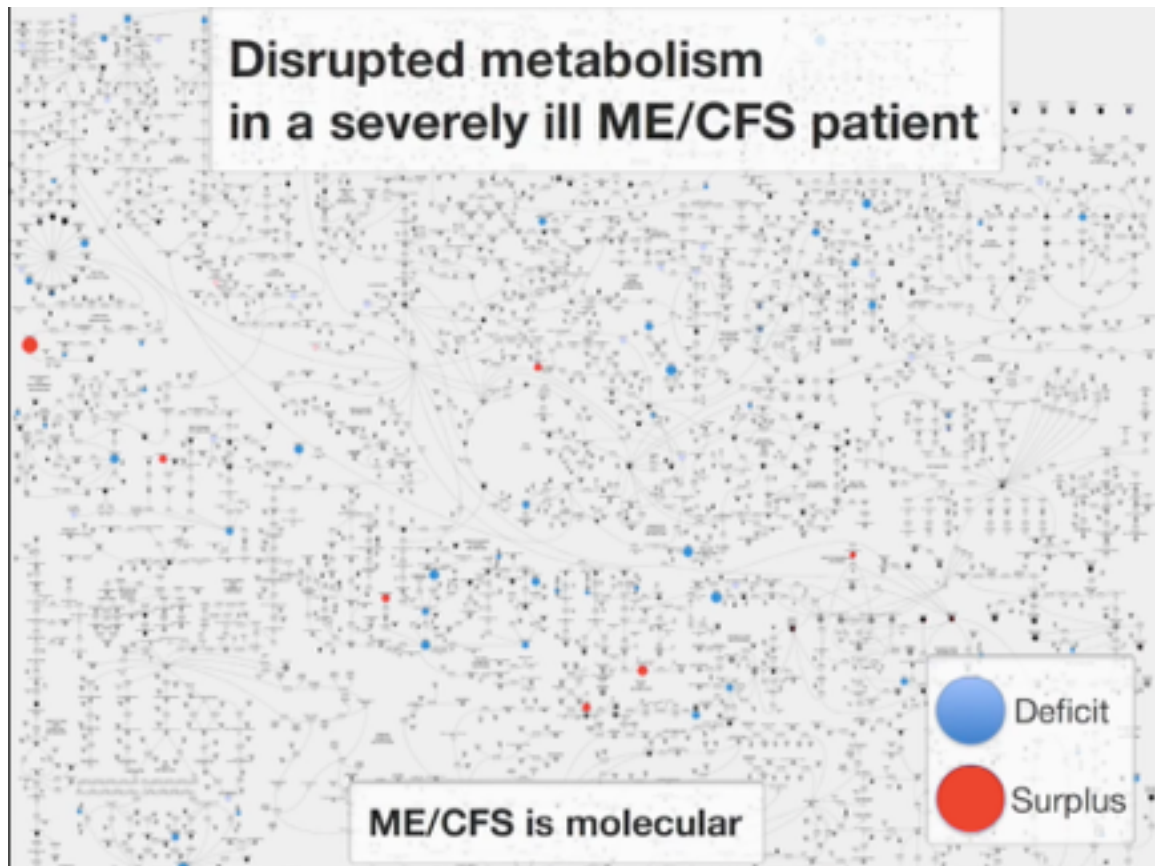
Now, let me digress a moment to talk about our big data study. That was launched some time ago, we're making progress on it. It is a hard problem because we could only take a small amount of blood from these patients and we only got enough blood to do each test once. We do not have backups. And we also promised them that we would not go back to them for new blood samples. It is not uncommon for technologies not to work, and you have to do backups. So we're being very careful to make sure everything works before we use the severely ill patients' samples.

We have a lot of data collected on them. We have a list of those we have done. We're going to continue working on this big data study. I'll mention one thing, is that to test out all this, we did one patient – that was my son – and in his case, he was okay with us going back and taking a second blood sample, and so we used his blood as the volunteer.

One of the problems that I faced is I wrote two grants to the NIH. They were both turned down because we were trying to do discovery, and they wanted us to only do hypothesis testing, and as I said to them: the scientific method is first observation, then hypothesis. And if you have virtually no observations you can't generate a good hypothesis. I think one of the big problems we have is that we do not know enough at the molecular level to generate hypotheses.

We analysed his blood and did a very large number of measurements. We did metabolomics, which was done by Metabolon. They analysed the data and sent it to me. It took me about 15 seconds of looking at their lists of metabolites, and I said "I understand this disease pretty well now", and that has set us on a direction that I think we're going to make progress with this disease. So that was a discovery, and that discovery was crucial for everything we're going to do now, and that came from Metabolon.

We're experts in a lot of different things, and those are mostly around DNA and RNA. We will be pursuing those in this big data study. But I want to show you something more specifically in the metabolism. So, here is a plot that was sent to us by Metabolon. It has all the metabolic pathways of the human being. You can't really see it, because there is so much data on there, but I have colour coded It and that should help. The colour coding is blue and red. Blue is a deficiency, red is a surplus of a metabolite. And these are what's called two standard deviations away from healthy controls, and are pretty seriously a problem. You can see an awful lot of blue and red. And that is very clear that there is metabolic problem going on.

Disrupted metabolism in a severely ill ME/CFS patient. Deficit (blue) / Surplus (red). ME/CFS is molecular.

You can also see an awful lot more blue than red, which suggests it is a hypo-metabolic disease. That is what I could see in the first 15 seconds. So you can look into all the metabolic cycles and learn a lot of detail about a particular patient, but a particular interest is looking at a citric acid cycle, which generates all of the energy for a person. And if you look at that you realise there is an awful lot of blue, that means the citric acid cycle intermediates are all very, very low. That suggests that this patient cannot generate energy or ATP very well.

If we look at a patient that has a mitochondrial defect, you see it all in red. That means that it accumulates the citric acid cycle, because it can't burn them. So that is a case in which a person cannot burn the glucose that they are getting. The fact that the citric acid cycle intermediates are somehow shutdown, and if we look at that we say that probably glycolysis is actually shutdown [in ME patients].

Fluge and Mella suggest pyruvate dehydrogenase is probably blocked. We have not investigated that, but it is consistent with glycolysis being shutdown. We also think that pyruvate kinase might be shutdown. Those are not inconsistent and it is possible there are blocks in both of them. This may be the heart of this disease.
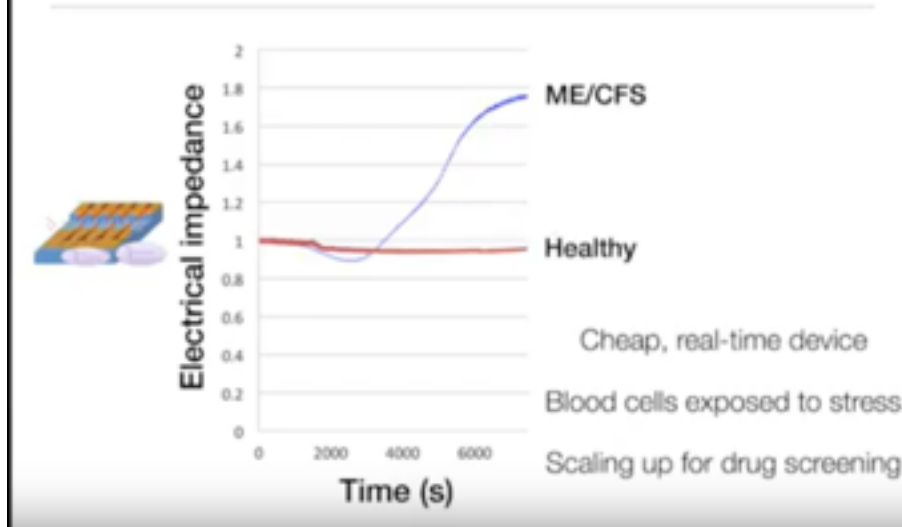
Now, we had developed a device and it is a nanofabricated device. In fact, I have one here in my pocket. This is our new instrument. Here's the instrument. It's basically like a computer chip. It has a small channel in it, that we can put about a tenth of a drop of blood in, and that is all we need for this assay. It has 2500 electrodes in it, and each electrode is sampled 100 times a second. So it generates a massive amount of data.
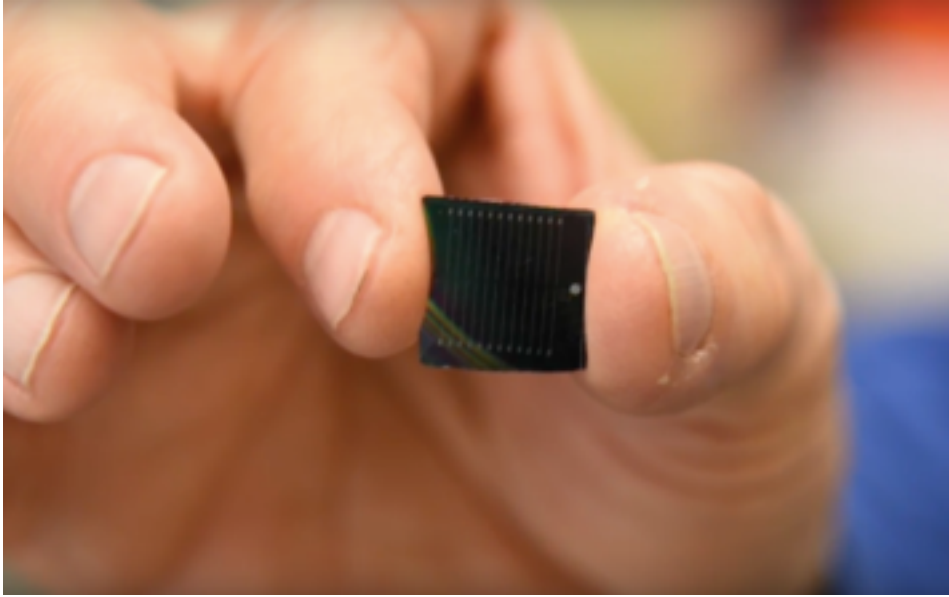
What we have noticed from this device is that if we put bacterial populations into this, we will get a certain electrical impedance signal. If we then add an antibiotic that kills the bacteria, the electrical impedance will rapidly increase. If the bacteria are resistant to the antibiotic, we see no change. So this is a metabolic testing device, we reasoned that maybe the cells from patients might respond similarly. So that is something we have currently been testing, and here is what it looks like.

So, if we put healthy cells and their serum into the device, it is pretty stable and does not change. If we put in ME/CFS cells and their serum, it doesn't change. However, if we put a demand on the cells, we require them to consume energy, and that demand is seen in this graph where there is a slight dip in the healthy controls – but they handle that demand quite well and don't change after that – however the cells from the ME/CFS patient, show a rapid increase in impedance. And that has been shown for every patient we have looked at, and also every healthy control is the same.

Towards a blood diagnostic for ME/CFS: nanoneedle biosensors

ME/CFS

Healthy

Cheap, real-time device

Blood cells exposed to stress

Scaling up for drug screening

Now, here's how we plan to use this. This can be a way to track down whatever's going on. The first experiment was to do a serum switch. If we take the serum from a healthy control and put it on CFS cells, they look healthy. If we take serum CFS patient and put it on healthy cells, they look like ME/CFS cells. In other words, the information that causes this rapid rise in impedance is in the serum, not the cell. That was quite surprising, but also good news, because there is something being released in the serum that is causing a lot of the effects. If it is in the serum, we probably can find it. And that is what we're trying to do now, which is find the component or components – most likely plural – that is causing this effect. And that is the intense effort that we are currently using. We have some good ideas. Now this is a good hypothesis, and we are now testing it.

But, more importantly, this provides us with an assay for a drug. In other words, if we take ME/CFS cells and serum, or just healthy cells and CFS serum, and we add a compound that blocks this process, we can test them. We can probably set this up into some form of high throughput assay.

Now, we've already found a couple of things that do this. Unfortunately, they're not going to be used for drugs. One of those is pyruvate. If we throw pyruvate into a situation that would show the increase in impedance, it doesn't happen – it would appear that pyruvate itself can cure the problem. Now that could be because the pyruvate enters into it after the block, or it could be that the pyruvate is actually inhibiting something - we don't know.

We've also found that if you add ATP the cells become normal. Well, that might not also be surprising because one of the problems is lack of ATP possibly. So this is kind of a good initial testing that indicates this may be a good approach, so the next stage is trying to find a number drugs that might help us in solving this problem.

Now we actually also plan to test all the things out there that people have found in the past, like Valcyte appears to help, which is an antiviral, but maybe does

something else. Rituximab has been shown to have an effect - maybe the antibody reacts to something. So those will be tested in this system.

Now this is very cheap, and it is a real-time assay. We're also looking to maybe even increase the throughput on this instrument, so that we can do massive drug screening.

Now, with this system, I don't think it's necessarily viable for patients at this stage to know what their answer is. Certainly not until we can put in a bit more work. What we're looking for is patients that have been clearly diagnosed with CFS, and then we look for the signal. I don't think at this stage it would be useful for patients to know what their response is. If it turns out that this is a good diagnostic test, and it could be, then we will try to figure out a way to disseminate that into the doctors' office. You can see that you could probably put this into a handheld device.

We have another device that we developed, that is called magnetic levitation. It separates out cells based on their magnetic properties. That is being used currently to count tumour cells in cancer patients, and also their profile. We have some signatures that we've seen in this device also from ME/CFS patients. And we're trying to combine both of these technologies.

So the magnetic levitation is a little device that fits onto an iPhone. It is very simple. It costs us around 5 cents per assay. We have another new technology that uses an inkjet printer to make the devices. Those devices can be made for about 1 cent. So we have a heavy focus on how to reduce cost of tests and simplicity of those tests and portability. So we're very optimistic that we can in fact find some inhibitor, and we're accumulating a list of things that we are trying with this device, and are hoping to find something that can block the signals we're seeing in the serum, and actually make patients feel better. It may also allow us to find a cure.

Thank you very much.

[Ron Davis then goes on to appeal for funding so they can hire more staff, which will get research moving more quickly. If you want to donate, you can do so at https://app.etapestry.com/onlineforms/OMF/newsletter.html]