Dr. Ron Davis Presents ME/CFS Research at the 2018 IIMEC Conference

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Dr. Ron Davis: Thank you and it's delightful to be here. I want to talk about some of the latest progress that we're making and I'd also like to give you some directions that we're moving forward with, whether they're right or not I don't know.

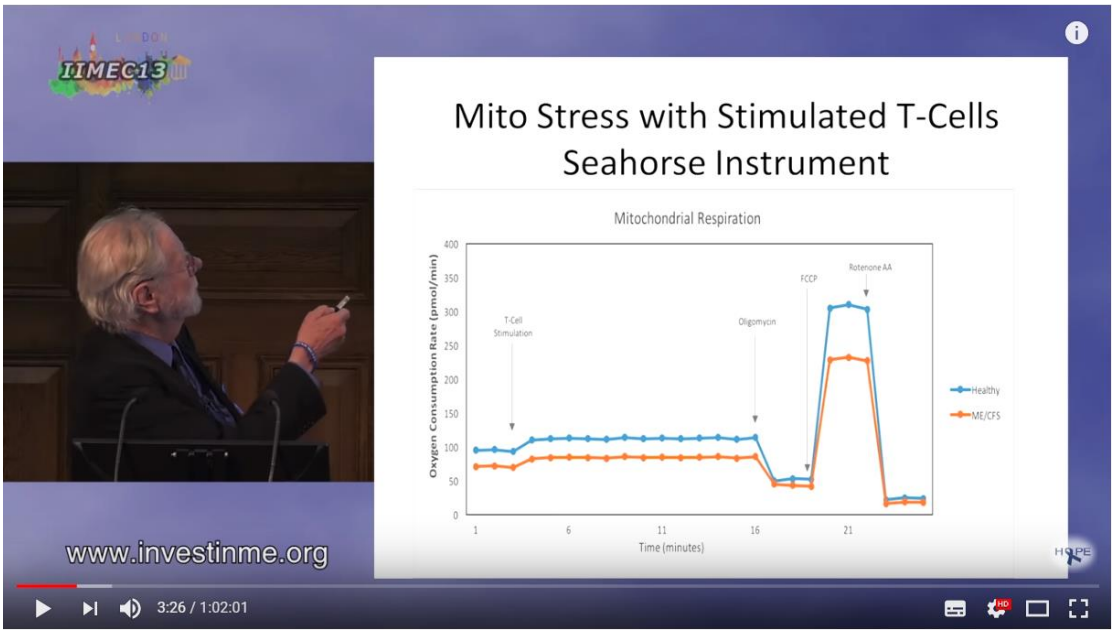
0:38 One thing I'd like to talk about is we have a scientific advisory board. This is our end ME/CFS project. It's supported by the Open Medicine Foundation. Everything I'm going to show you has been supported by the Open Medicine Foundation and we've had several of our advisors are here.

In fact, you've just heard Maureen Hanson. Jonas is one of our advisors. Mella and Fluge are on our advisory board. And also we have two people here, Ron Tompkins and Wenzhong Xiao, we just awarded a new cooperative center grant an award to start a collaboration. So we're trying to expand our efforts. They will be focusing a bit more on some of the clinical aspects that we need to do since I'm not a physician.

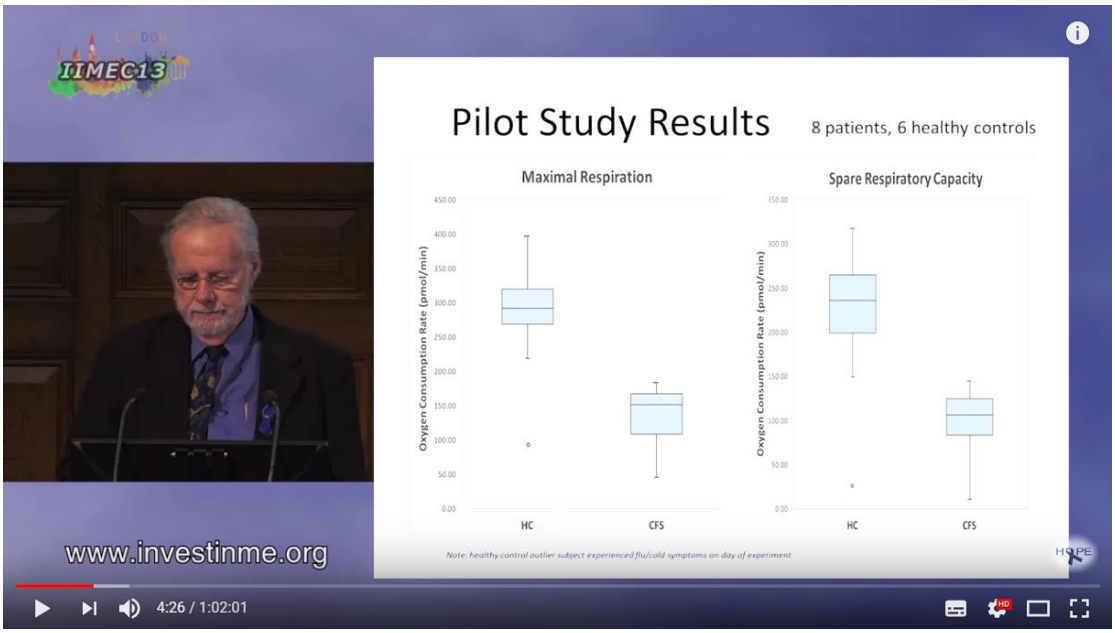
1:48 One of the things that was very clear for some time is that we need an assay. So many patients, including my son, were told that there was nothing wrong with them because the doctor carried out a bunch of tests and they all come back normal. Incidentally he's now severe, he's bed bound, he can't talk, he can't read and he's a very severe patient. I still take blood samples from him and rerun those same tests the doctors do and he's perfectly normal, there's nothing wrong with him. Those test are lousy for this test and that's the problem. And they often say it's in your head, or depression and we need something to say that's not true. Our focus right now is to say they're not healthy and all we're comparing them to are healthy controls and can we see a clear difference. That's pass number one. The next thing to figure out is what do we need to distinguish it from in terms of another disease. It's often very hard to get a biomarker that distinguishes it from all other diseases. For one thing you have to do all other diseases and I'm not sure that it's necessary. What we need to do is work with the physicians to know what do you

need to know in a diagnostic test of this type or can you use diagnose it from other diagnostic criteria, such as talking to the patient.

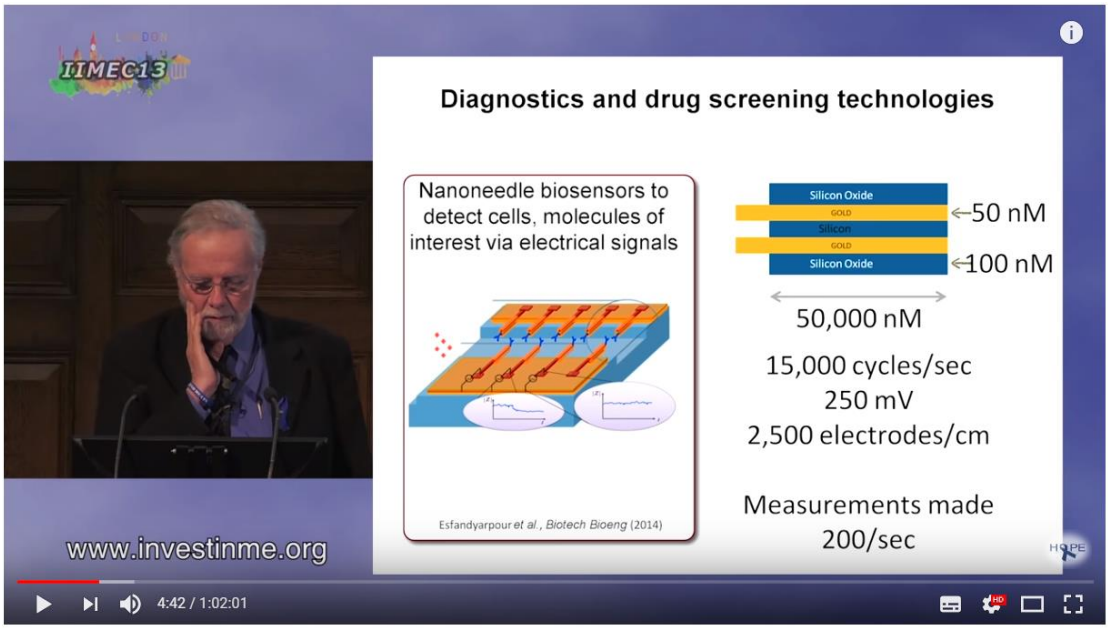
03:26 So our first pass is compare to healthy. We've done a standard instrument called the Seahorse. A number of people and scientists at this meeting will have used that instrument. And this just shows you an example and what we've done. What gives the best result is using isolated T-cells (blood cell that plays a role in immunity) from patients and then stimulated these T-cells and measuring their energy production. I'm not going to go in to how this test works I just want to show you this difference here.



And that's been pretty reproducible between the healthy controls and ME/CFS patients. if we don't use the stimulation in T-cells its more variable and its also dependent on when they've last eaten, and that's a really difficult thing for the patient to define and they can't eat before they come in. So this is something we're looking at as a possibility. As a test because its commercially available, the instrument costs $100,000 so I don't particularly like it.

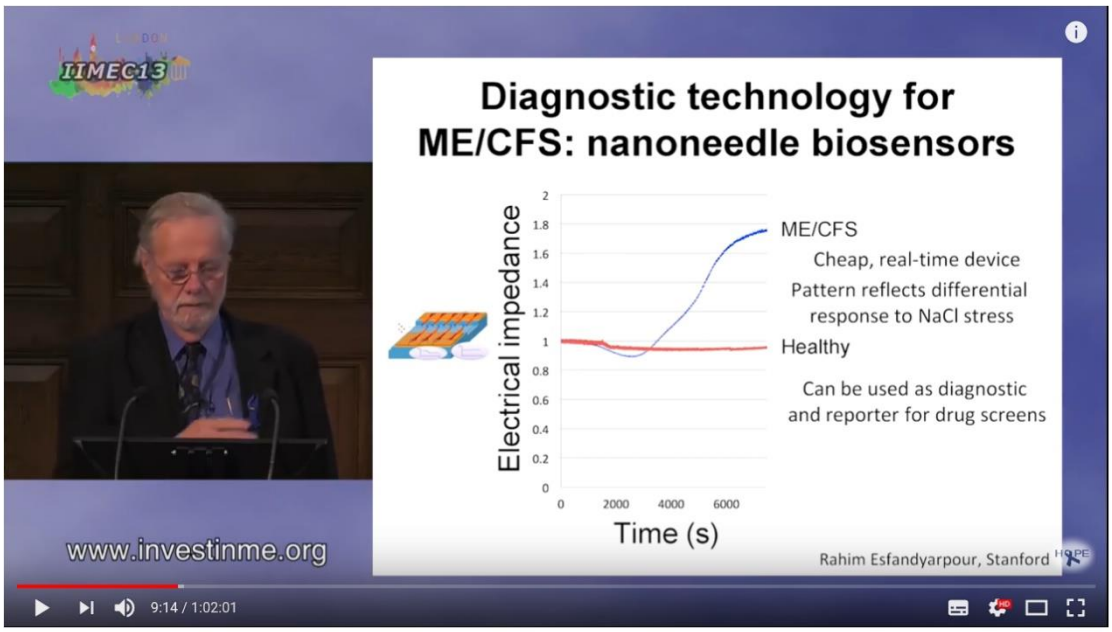


4:25 And this just shows our results from this in terms of comparing the differences, in terms of the pilot project.



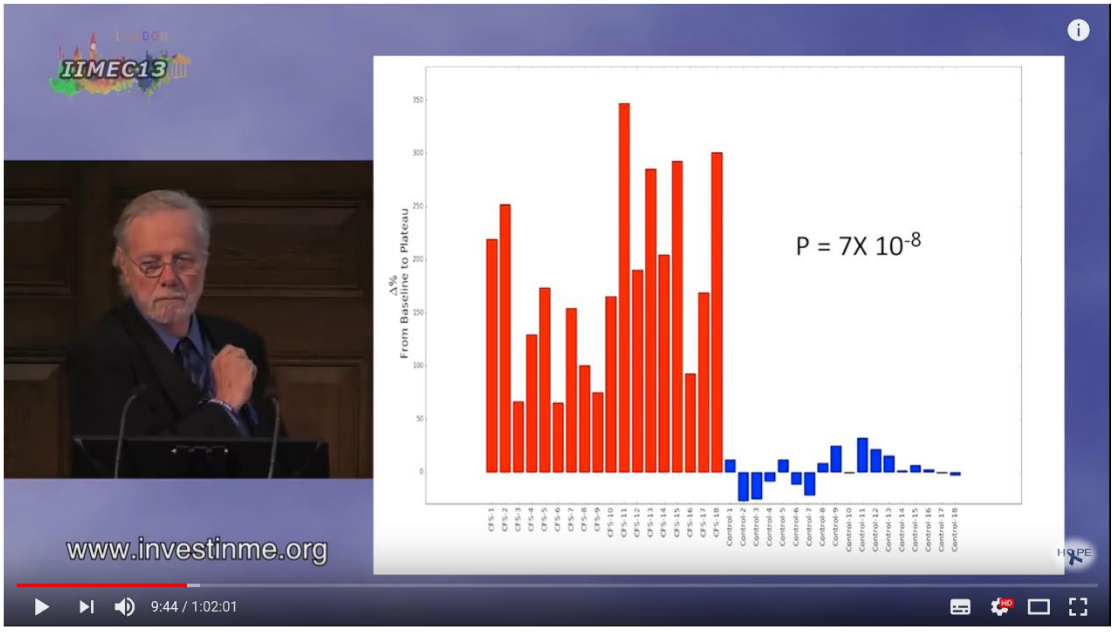
4:42 Another device we've developed and worked with extensively and we refer to as the Nanoneedle. And it's called the Nanoneedle because of the fabrication of it. It's a needle that's nano fabricated and we do it at Stanford. It has two electrodes in it, two gold electrodes, that conduct electricity, that are very tiny. They're 15 nanometers (nM) that's a very small distance. They're separated by an insulator and then its quite long. These needles stick into a little micro trough and we have 2500 of these in a centimeter. With these devices we measure what's called the electrical impedance. I'm not going to give the formula for electrical impedance, it's something engineers use a lot and it's very sensitive electrical measurement.

We make 400 measurements per second, so when we have done doing an experiment we've taken one billion measurements. That's much more useful than some medical devices that only make one measurement and you don't know what the variance is when you look at that. So we've worked out all the parameters for doing this that give us the best results. It is nano fabricated, that's a problem because most labs don't have a nanofabrication facility, but they can be made commercially. If we make them in large numbers commercially they won't be very expensive. We can probably make them for about $1 to maybe $5 and we've also figured out how to clean them, so we don't have to remake them/make new ones.

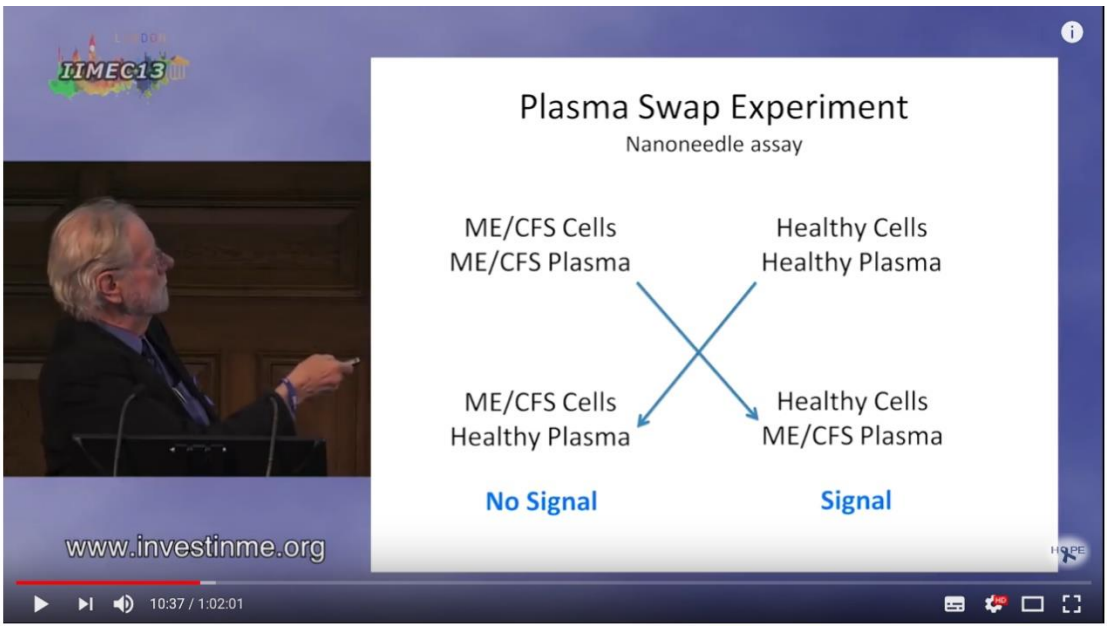


06:41 So here's what it looks like for almost all measurements. When we put a healthy sample in it's very flat. So we just take blood remove the red cells and it's basically one drop of blood and this is put on the device. And then, if we put on ME/CFS cells they're the same as healthy controls but what we reasoned is that because there is sort of an energy deficiency, and this is not necessarily correct, that if we made the cells work harder we would see a difference. And so what we do is we add sodium chloride, salt, and cells have to pump salt out of their cells. Salt goes into the cell it's got to get pumped out. The pump requires energy so by just simply putting salt, very simple, you make the cells work and what happens with them when they work is that their impedance increases. The reason we tried this experiment was if we did a culture of bacteria and looked at its impedance and then we add an antibiotic, if the antibiotic is going to kill the cell, the cell is still alive, but if it's going to kill the cell the impedance will change. If the antibiotic does not work it doesn't change so it's a very very fast way to tell what antibiotic to use and the same thing goes for tumors. We put an anti-tumor agent in, we can tell very quickly whether the tumor agents actually going to work on a patient or not and so we know a physiological change will change the impedance and that's what we're just looking for. And this has been very reproducible, if we take the same patient and do it a week later we get virtually an identical result. And it is dependent upon the patient so this is a very it's a very cheap way to do it. You can get it in real time it's not something that you have to wait weeks to get, so it's a response to sodium chloride and what might field use as a diagnostic tool. We've also tried it to treat the cells with some drugs. We've tried a few of these now and see what happens after the drug treatment and we have found a number of drugs that in fact seem to get

rid of this effect. We don't know if they're going to be useful or not but it could be used for drug screening and we'll explore it, we are exploring that now.

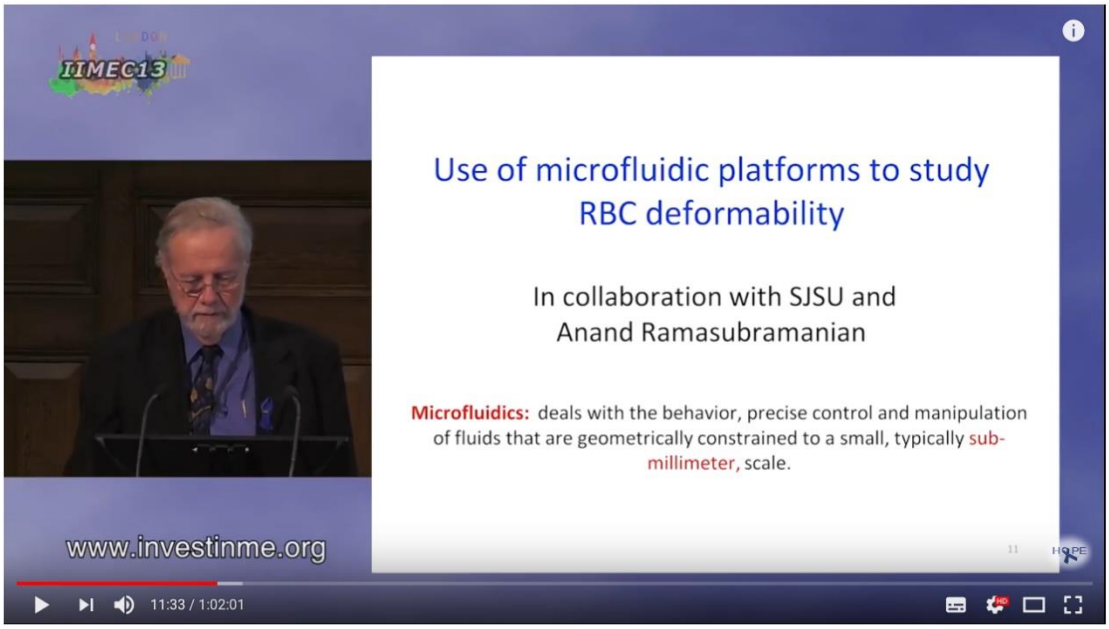


09:28 Let me just show you a few experiments. So what you show here is patients and healthy controls. So there's a very big difference and if you ask what is the probability that you could have gotten this by chance and the answer is seven times. It's about one chance in ten million and that's actually pretty good in terms of the diagnostic test.

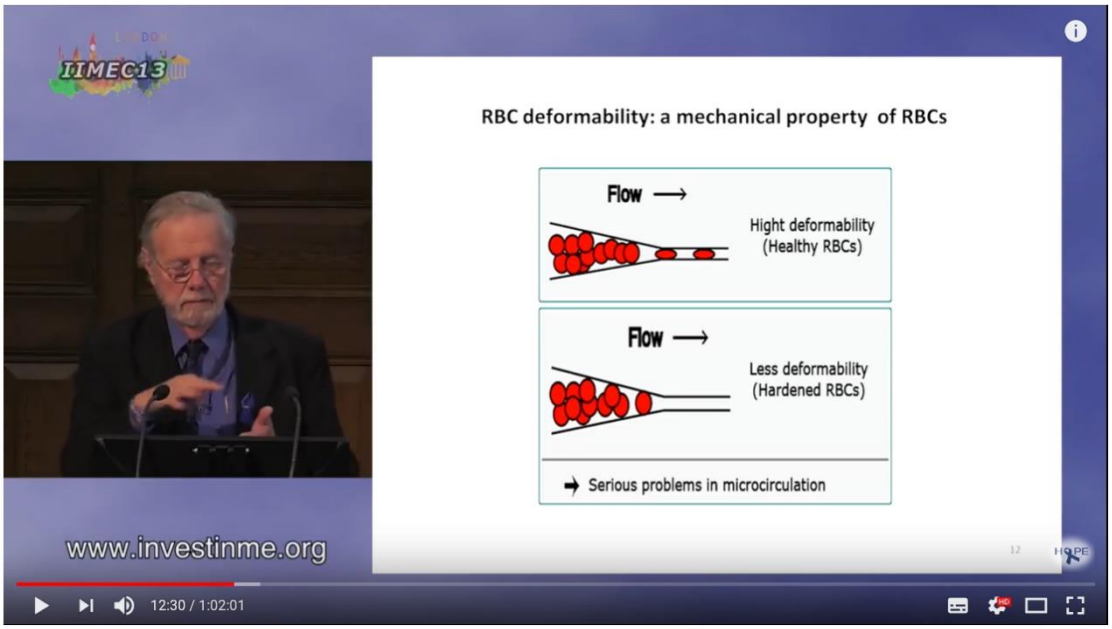


10:04 Now we have done a few experiments trying to understand what works in this assay and we call this the plasma swap experiment. So our initial thought is it's in the cell and so we decided okay well let's just test that out and we do a plasma swap where we take the plasma from ME/CFS cells and put it on healthy cells and vice versa. And what we find is that the signal that we get tracks with the plasma, it's

not the cell. That even suggests that the cells are actually pretty healthy but there's something in the plasma that's causing this effect. We don't know what it is yet but that's something that we need to figure out because it's going through the entire body. It may be causing some of the effects and if we can figure out what it is and what its size is, we might be able to find a way to remove it and that actually could be a treatment.

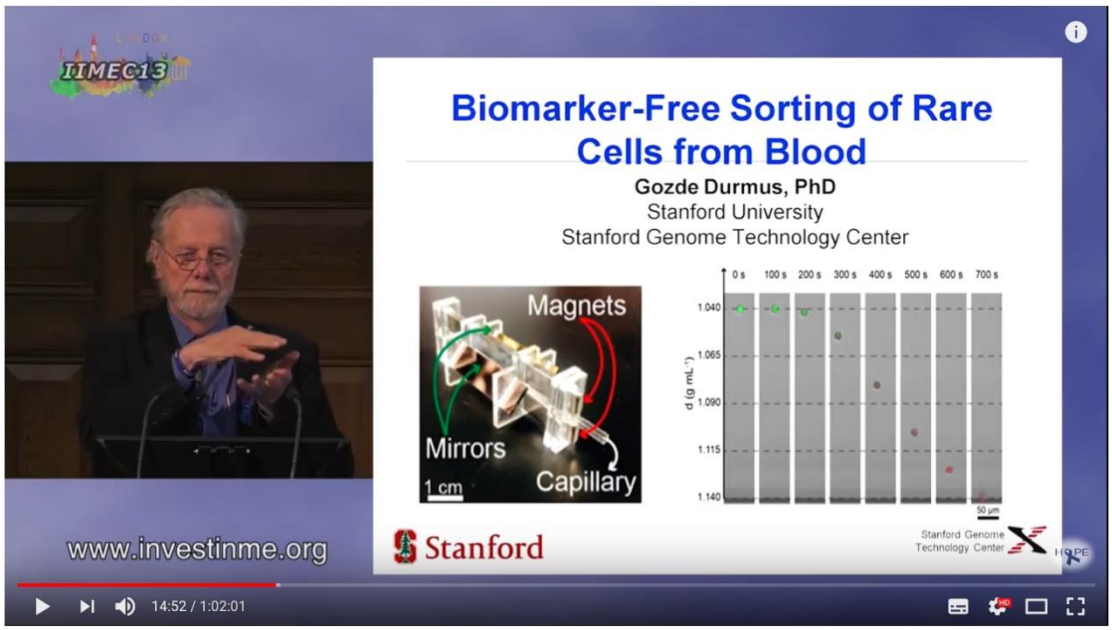


11:15 Now, I have another so I'm just going through a few devices because we have to figure out which device we should use. So we have another, this is much earlier. It's a collaboration. We've discovered a professor at San Jose State (SJSU) was developing this. We were going to develop it on our own and we realized he was already doing it so we decided we would collaborate.

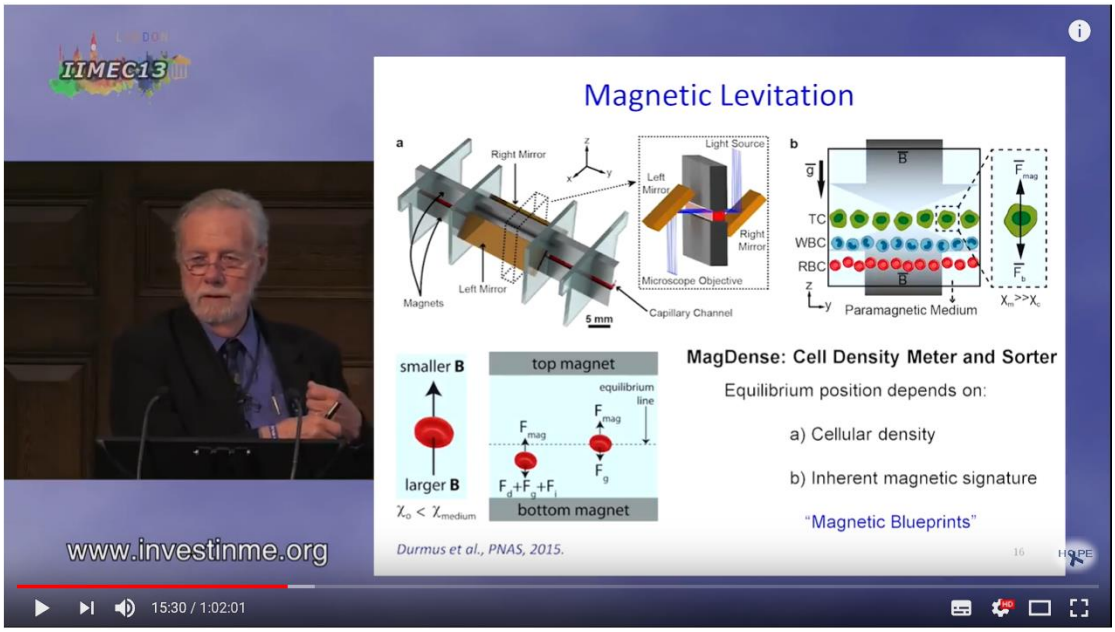


11:41 And it's a microfluidic device that measures deformability of red cells and the idea of this device is you have a larger channel than red cells. If the drop of blood again goes through and the red cells have to get squished normally when they go through your body. And this particular one channel is five microns which is a little bit larger than a lot of the capillaries. And then what happens is, if the cells are not as deformable, is that they'll get stopped just before going in because they have to deform and when they deform they may go slower through this. And then you can also measure how much elongation you have. So this is easy to do, you just image it and you do about a thousand cells and you average them, so this could be done relatively fast.

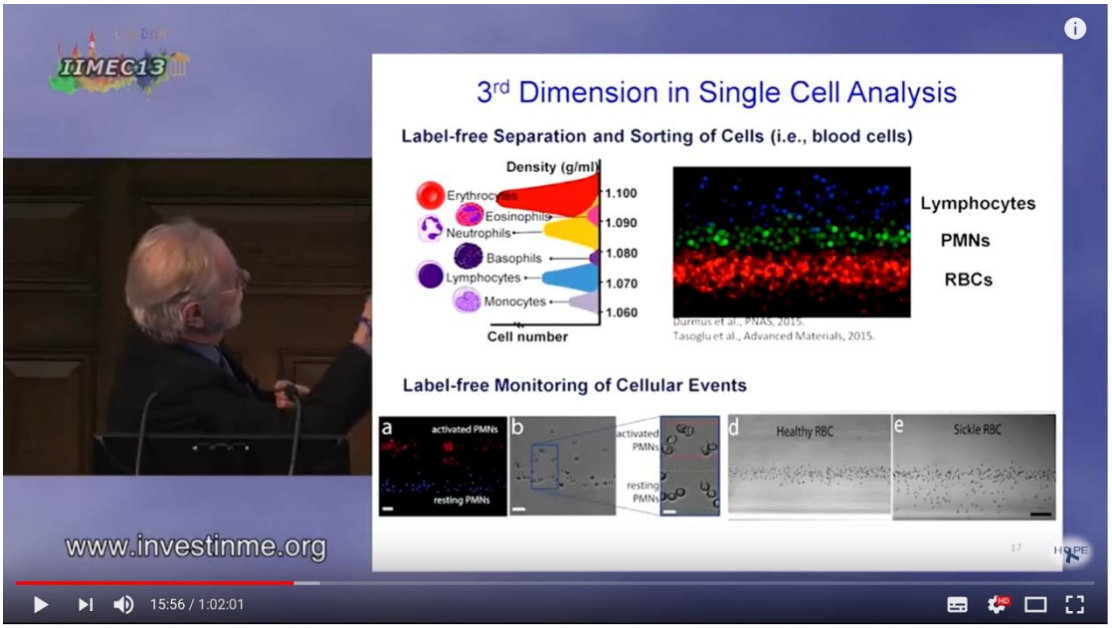
12:35 Here we go, you can see the red cells coming through this device. You can see that they're not being squished very much and so we have to redesign this instrument and go down to probably three to two microns (channels) and make it better. But in fact we do see a difference on bulk. So on bulk we're looking at we're looking at a thousand cells on average. They enter much slower, they have a slower transit velocity and they have less elongation. But right now that does not work for a diagnostic tool because there's a lot of variance. There's a lot of differences between different patients and and controls. So we have to have it so that the patient will always differ from a healthy control. I suspect it will work once we get down to a very small capillary. That involves a new fabrication of molds and so forth that we have to make and which is in the process. Again, this is a very inexpensive, very simple test. What physiological effect it has because of the lack of deformability I don't know, this is not a new idea, it's a little bit of a mystery we haven't figured it out yet but people have reported things of this type in the past and hasn't really been followed up on. Whether or not it actually changes the blood flow in a person isn't clear, but it could be used as a diagnostic. We will use an atomic force microscope to make much much cleaner measurements about the deformability. That's all set up at Stanford. It's very easy to do. We also have several chemical engineering professors that found this interesting and they'll also start doing some measurements on red cells, but much more detailed complex measurements Those could turn out to be a diagnostic as well.



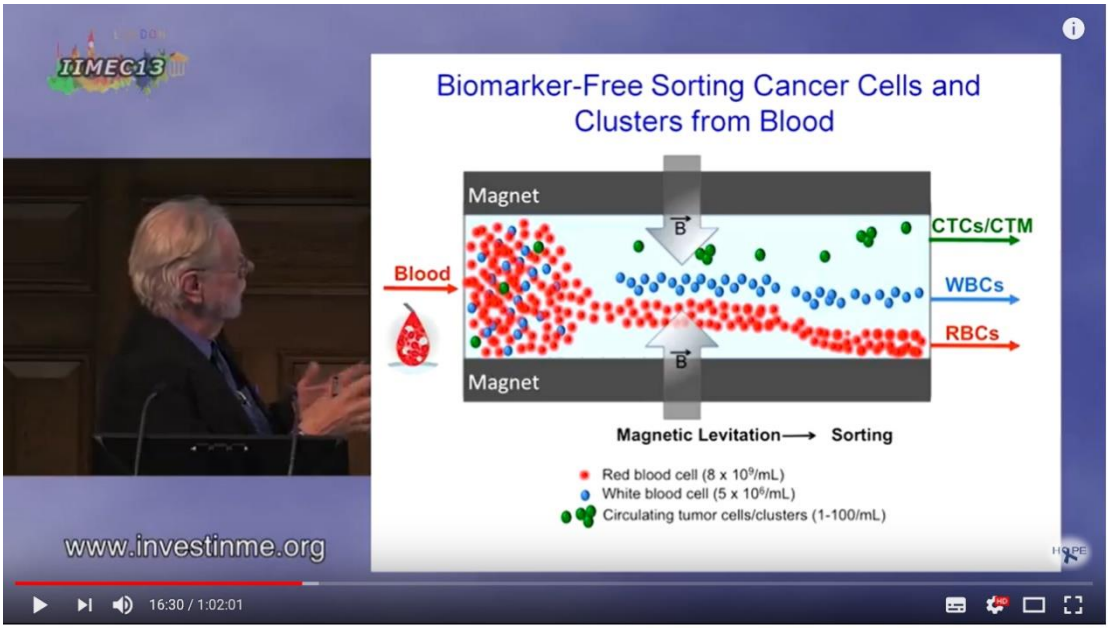
14:32 So here's another instrument that we have built and it's a device that measures magnetic levitation. And what that is, is we can suspend something in a magnetic fluid if we put a magnet on it and it'll create a density array. And in fact you can even suspend whole organisms in this, not this device because this is a micro device, but it allows us to suspend cells right quickly. So you put a blood sample in it and put a magnetic field on it and very quickly the cells will go to a density in the little tube.



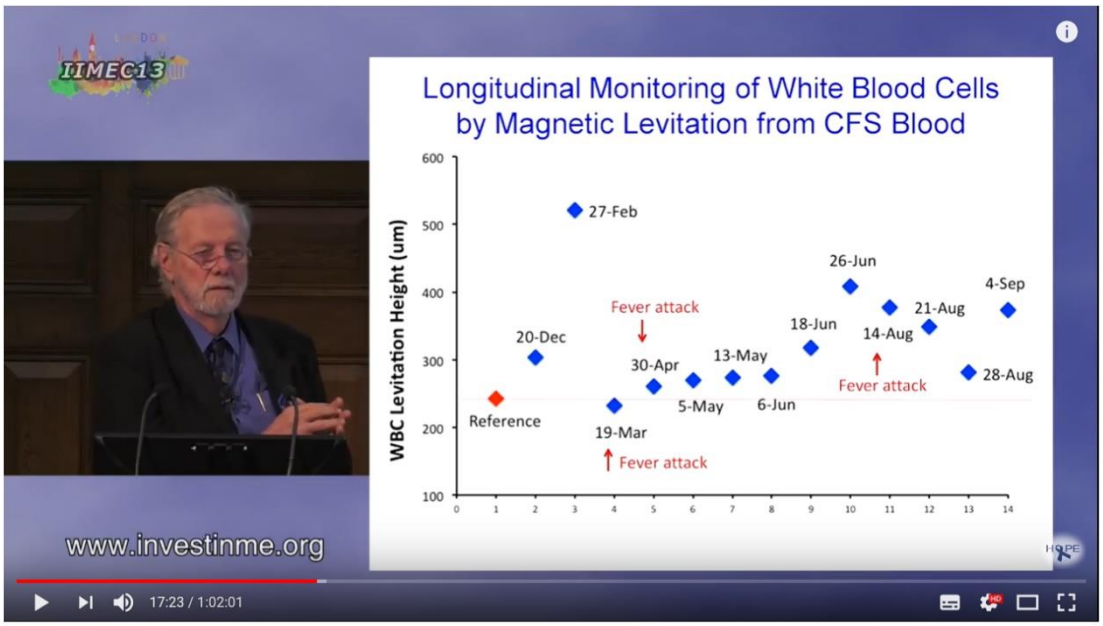
15:33 The little tube is here and what you can see and what this is developed for, we always have difficulty getting funding so you got to do something that's more fundable, we often have to do that, so cancer is more fundable. So this is developed to separate circulating tumor cells from the white cells and the red cells and it does work for that and that helped fund the development of this instrument.



16:03 And here you have some imaging of staining of the red cells, the white cells and the tumor cells. Well not this one the tumor cells are above this one, excuse me.

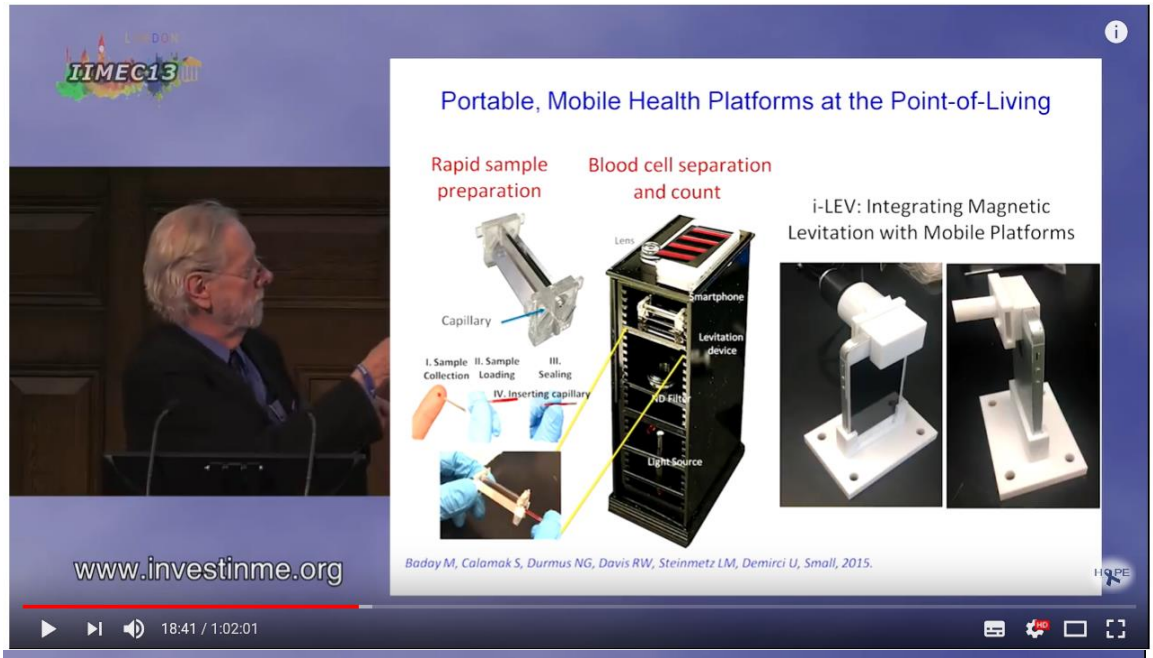


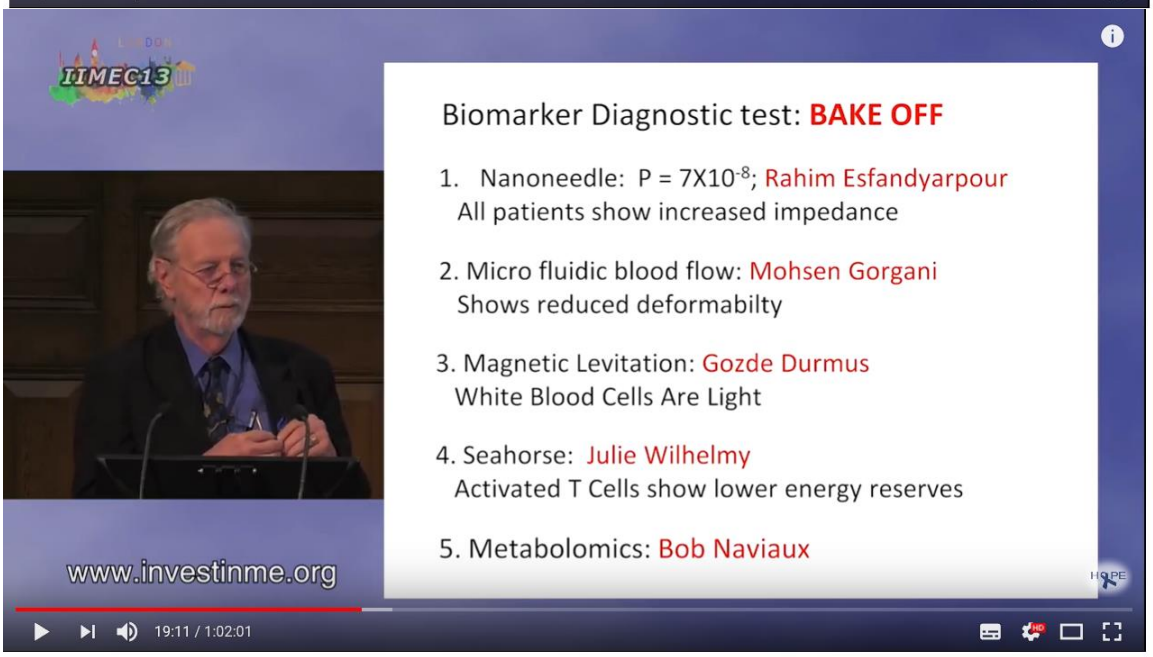
16:20 And we can in fact separate lots of different cell types as well with the device. But we've also then converted that into a property of the instrument where you separate the cells and they will separate by density. Here the circulating tumor cells are and then we have little ports that go out here and collect those cells. Now, what are the things you should know about this is that is the cost. The major cost, in fact the only cost of the running the instrument, is the capillary so each of these runs cost five cents.



16:50 And here's a longitudinal study just on one patient looking at the variation in density of the white cells and they vary quite a bit in density. There's one measurement which showed it was pretty close to the normal and that's relevant in terms of what happened. This measurement was made right after a bacterial infection in the blood and the significance of that is the patient became much much better after that. Now we made a mistake of saying it was because we put him on an antibiotic, so the antibiotic made the patient better. The answer's no because it was the fever. The bacterial infection made the patient better and there's other reports of that. Now, some people said it's a fever, I think it's the bacterial infection that made them better and we've seen a couple of examples of that but in general the cells are light. And the only problem would be this (higher point) but this would not be a diagnostic problem because this person was on antibiotics and you would not try to diagnose them under those circumstances. We are going to plan to use what goes on in a bacterial infection that makes them better and we're going to try to use that as a way to try to understand what's going on. I think people have already mentioned you want to look at people, what happens to them when they get better. One way to look at that is what happens in a bacterial infection.

18:40 And this is just a device. A lot of people are doing this to try to decrease cost of instrumentation and so the instrument is here and we're simply using the camera from the iPhone and then the phone is used as the computer for doing everything. So one of these smartphones is like the cheapest computer you can get. It makes it handheld and so you can make this whole instrument for less than a hundred thousand dollars and it's portable.

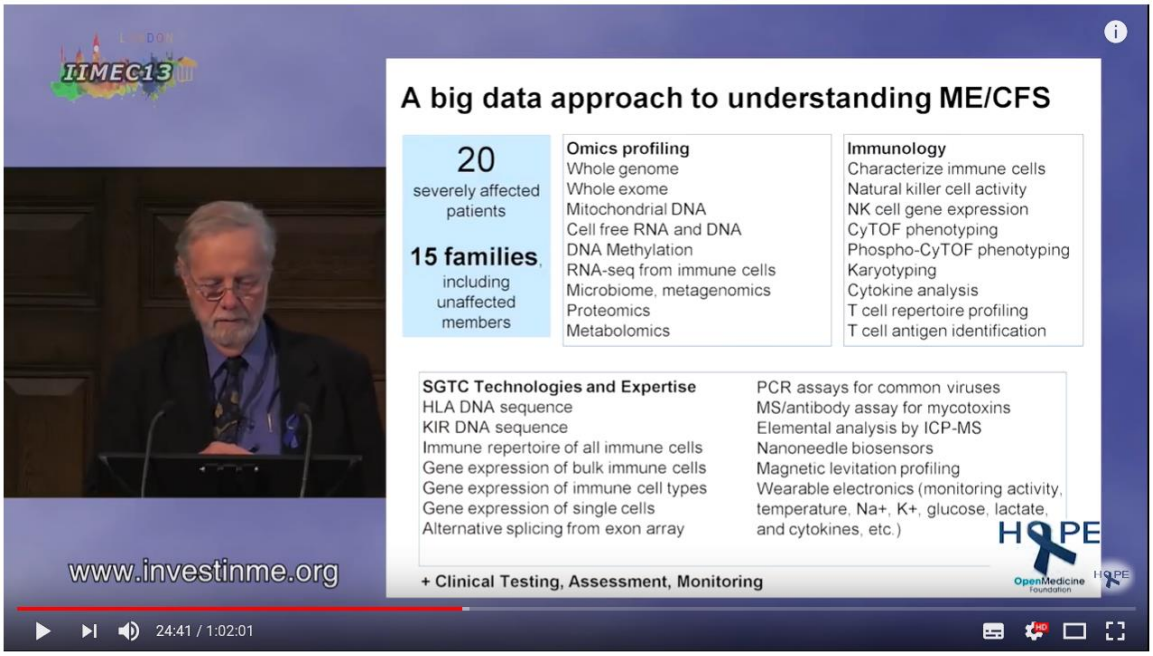




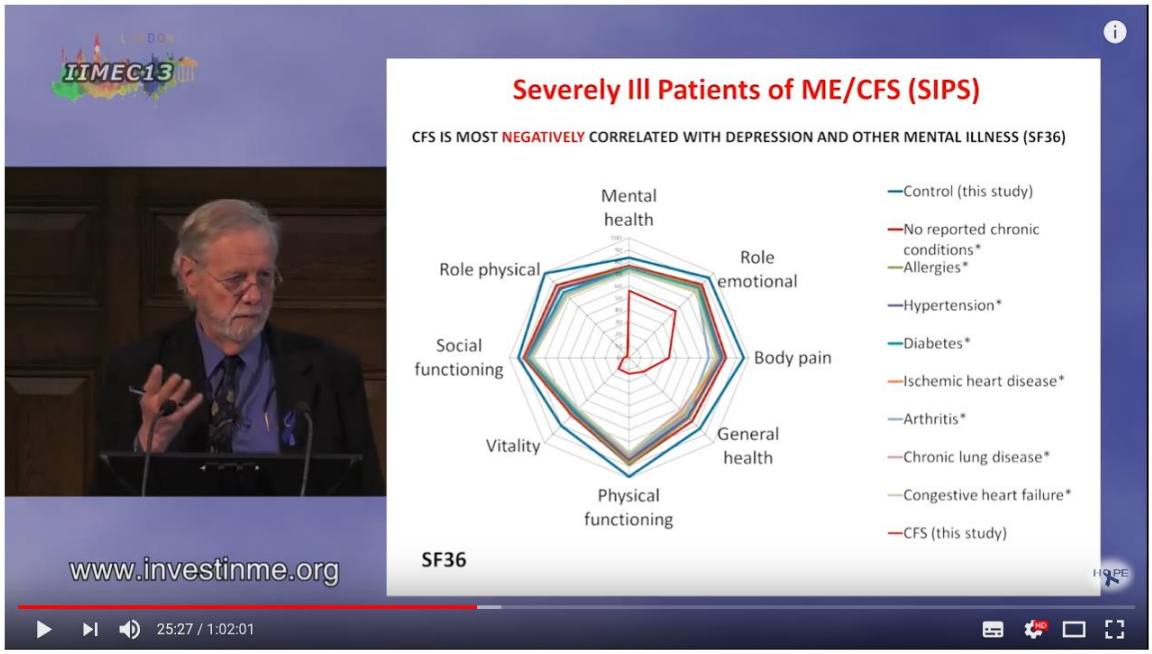
19:08 So I've just shown you a few examples of devices that we're trying to do and now we're going to hold what it's at least in the U.S. is called a bake-off. And it's done exactly like a bake-off so we'll take the next blood samples, that are now coming in, we run them with all instruments and we run them with what Bob Naviaux did with metabolomics. He found a signature that diagnosed the disease so we can compare it to that. So that will give us five different instruments to look at the consistency. Now one reason you do this is the fact you might find that there are some exceptions, that it doesn't work right but the other diagnostic tool did work right because it's measuring something different. So in fact you might have to use two different instruments and get much much better results. All we're looking for, we really don't want a lot of false positives, sorry false negatives, false positives are not such a problem. And the way I showed you this is that we have one instrument that can physically separate cells. One instrument uses red cells, the other instrument uses white cells so in fact we can combine all three instruments into one

and they all could use the same computer I think and probably the camera and so we could actually make all different measurements on the same device because they use different cell types. That's a possibility and that would allow us to get more information in a better test. The next thing to do is to look at other diseases that are closely related and how it performs. Now and that's the one that we have to talk to physicians about because it's really possible that other diseases will show the same behavior. Now we can always do something else like another biomarker to help try to separate and resolve those but our biggest goal is to show that the patients are clearly not healthy and there's something wrong with them.

21:31 Now I'd just like to turn to another bigger project that we have going on. This is all funded by the Open Medicine Foundation. It cost about two million dollars and it was to look at severe patients. Now what we really wanted to look at are bed-bound patients because people don't normally study them, because they don't come to clinics. And sometimes you'll hear severe patients in studies, those were the severest patients that came to the clinic, they're not necessarily severe patients that we would. So we should probably call these very severe patients or something because most of these are bed bound or at least housebound. And then in addition to that we're doing a new study on families. That's not done yet, I don't have data to show you. And that's looking at families where there's more than one affected and that is not uncommon unfortunately, and it's really horrible because one family having multiple affected is really hard and one reason for looking at these is that there's a reasonable chance that what's going on in those families is something genetic. And that will help us to get at the genetic basis, maybe, of the disease. So most people think that how you do research is you create a hypothesis and then you design a test to try to rule out that hypothesis. That's actually wrong, and Vicky's not here anymore (earlier speaker Dr Vicky Whittemore NIH) so I can say it, NIH has got it wrong (chuckles), and that is not the scientific method but that's what NIH requires if you write a proposal and that is incorrect. The scientific method is observation, hypothesis because if you don't have observations then you generate a random hypothesis based on nothing and will never get there. So what you want is observation and that observation creates an idea and that's the hypothesis you're testing it based on something you've observed and that's how all science has been done. Why they say that is, that when you do a grant and you test a hypothesis and evaluate it you'll make new observations and that's the basis for your next experiment and that can keep going on and on and on. What happens when you start a new disease, where you know almost nothing about at the molecular level? You have to make observations and then you can generate hypotheses. And in fact you heard Avi Nath's talk (earlier speaker Dr Avi Nath NIH) that's what he's doing, he's making lots and lots of observations, there's no hypothesis there. So the idea is to try to create as much data as we can. That's something we're very good at doing, I've done this for years.



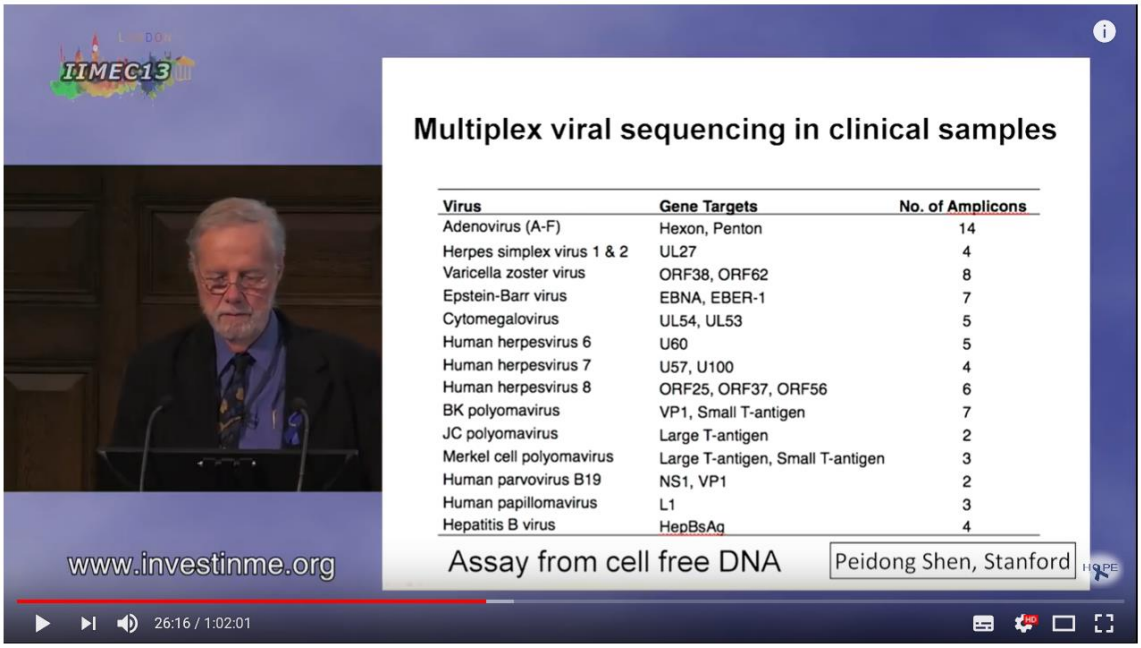
24:32 And so I'm just going to show you a few lists, you don't need to look through them, but these are the kinds of tests that we are we were planning to do. And that will generate an enormous amount of data, which is why it's taken so long to actually talk about it because it's a massive undertaking just to analyze it.



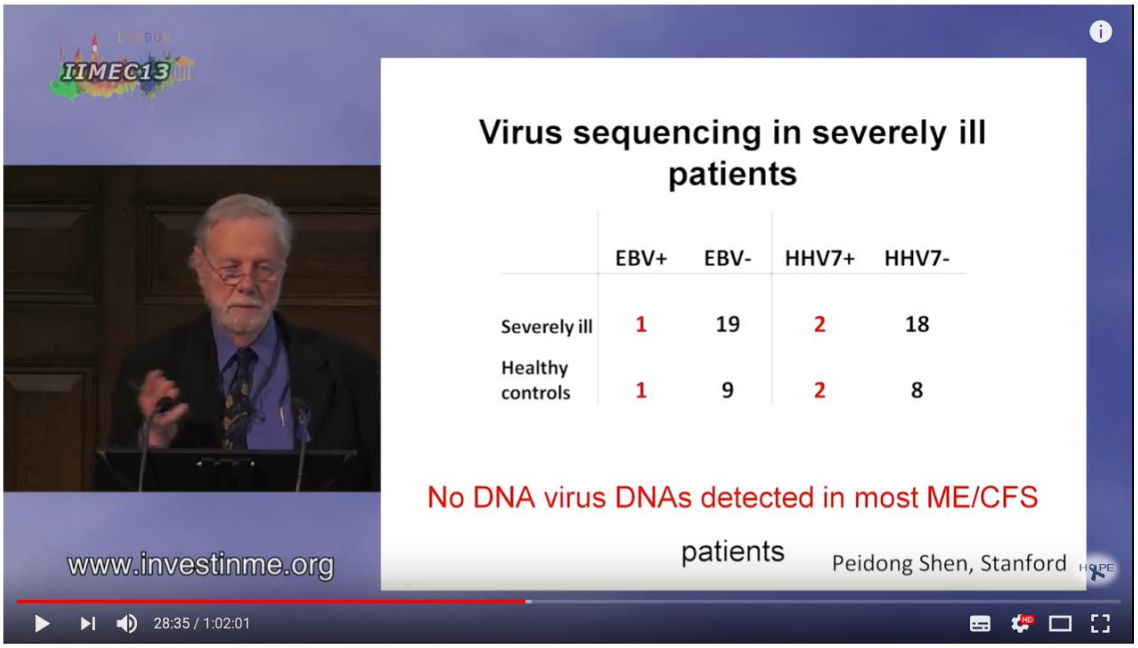
24:53 So let me just go through a few things that we found in that. One of the things is to just try to make an evaluation using standard tools. And this is the SF36 for these patients, how do they how do they rank for other diseases? We compared them to a number of diseases here and putting on different disabilities. And where the patients are really in here (most central on diagram) these patients are more severe than

almost any of these other diseases and I think that needs to be focused on for NIH to make them realize this is not being a little tired.

25:47 Now the other thing that we decided to do in this project is to test some of the ideas that patients have had, are they right or not. So I've heard a lot from patients that "Oh I keep getting viral infections . . .I get them all the time, it's really my real problem . . . I'm very susceptible to viral infections". And I ask them what virus do you think you're getting? "Oh I'm sure it's HHv7 or it's another herpes virus and that's what caused my illness in the first place". So we decided to actually test this and we had to develop a technology to really do it and to do what I thought was correct.



We decided to test this list of viruses. These are all DNA viruses, these are viruses that many of the patients say they think they have. And well what you do is what's called a PCR assay, polymerase chain reaction, because it's extremely sensitive and what you assay is cell-free DNA that's found in the blood. Now Ron Tompkins, who I work with, he always refers to the blood as the sewer of the body. It's also the nutrient carrier but it also carries away all the waste products that's not needed. So it's been found that if you have an infection anywhere in the body; brain, heart, anywhere in the body, some of those organisms will die being attacked by the immune system and DNA will get into the blood from that organism. So there's been a small startup company that's been looking at that and they always find DNA from an infecting organism in the blood. Some people say you don't want to look in the blood because what if it's not there. The organism may not be there but their DNA will be and so you can do a very, very sensitive test. But we didn't want to do a single test just because maybe we get a false positive or false negative. So we decided to test multiple regions of these viruses and these are the number of regions that we're testing, multiple regions of each of these viruses. Now that's an awful lot of tests and that's going to make it expensive. So what we decided to do is to rig it so that we can do all those simultaneously in one tube, so this is what we call a multiplexed assay which we're actually quite good at. We've actually made several of these types of tests for commercial diagnostics.

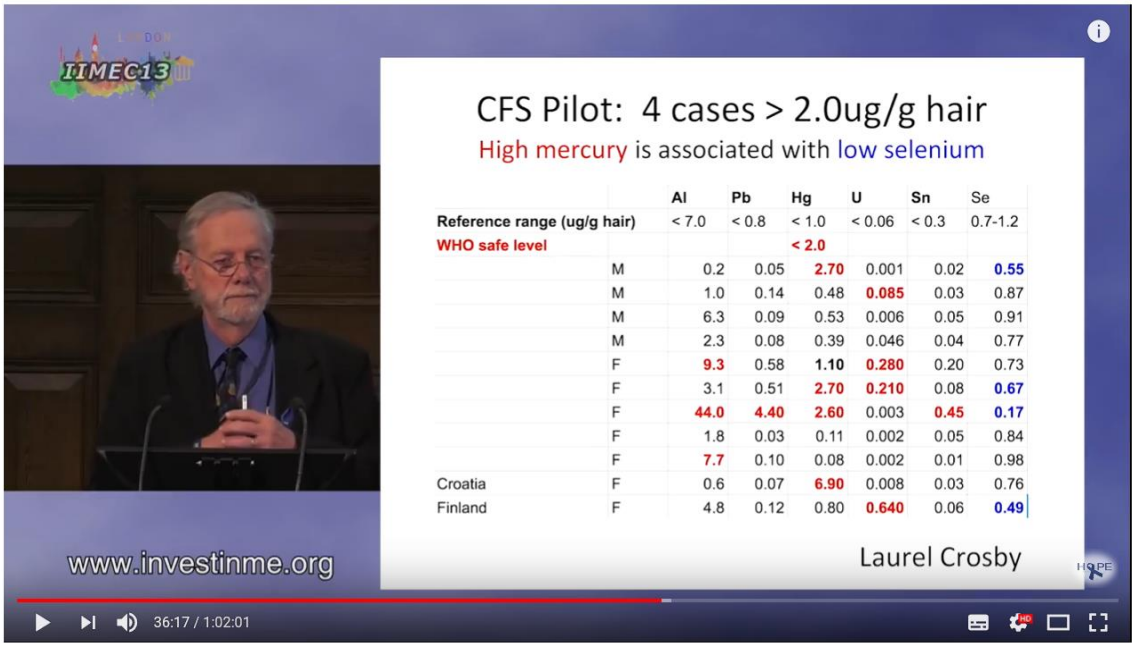


28:24 The results of that is basically there aren't virus infections that are different from healthy controls. A few people do have them but healthy controls have more in this small study, so it makes me suspicious that in fact they don't have viral infections. They have something else going on that feels like a virus infection and a lot of inflammation things will make you feel like that. Most of these viruses probably, by themselves, don't really do anything by themselves. It's not to their advantage to give a signal to the body that they're there. The body is the one that does the signaling that there's something wrong. And I think if you have that signal like inflammation it may feel like a viral infection. The only reason I'm stressing that point is that if it's most likely you don't have a viral infection you shouldn't be taking antivirals probably, because they're probably not that healthy for you. And the reason they're probably not that healthy is that the antivirals generally target the synthesis of the DNA from the virus and it works because it's a very primitive DNA polymerase, and in fact you can inhibit it without inhibiting your human polymerase. The problem is the mitochondrial polymerase is also a primitive one and some of these may actually inhibit the mitochondrial polymerase to some extent and given the fact that there seems to be mitochondrial lack of activity that's the probably not a good thing to do, if you don't need it. So I don't think patients should be taking anything that they don't really need to take, it's probably not a good thing to do. Now this just tests for the DNA viruses, we also have to test other things and I'll show you a couple of things. And that is that when, and I'm not showing you the data, we've done gene expression a fair amount of that. What Wenzhong has done is taken gene expression from the patients and compared it to every other gene expression that's ever been done. And there's a collection of that, it's 95 thousand other studies, and asked for the best match. The reason to do that is that it might give us a clue as to what may be going on. And the best match or close to the best match is a trypanosome infection, that's sleeping sickness found in Africa. How many people go to Africa regularly (chuckles)?! It doesn't seem likely it's a trypanosoma but anyway I looked up the symptoms of a trypanosome infection and was shocked to find out they look identical to Chronic Fatigue Syndrome. And they call it sleeping sickness because the sleep/wake cycle is inverted where people are awake all night and sleep during the day, and that's true for a lot of Chronic Fatigue Syndrome patients. So I come up with two possible conclusions from that and one is that this disease is actually caused by a trypanosoma. You would never necessarily know that, doctors would never figure it out because the diagnostic for trypanosome infection is to find

it in the blood. And it turns out if you look at the West African trypanosome, and I was in Africa looking at the AIDS epidemic there in 1983 and I met a lot of doctors then and so I called them up to talk to them about this, and they said it's very easy to identify the trypanosome in West Africa because it's very abundant in the blood but the East African one is very hard. You would do well if you find one, otherwise you treat it anyway because that's probably what it is. So it could happen that we could have a trypanosome in the rest of the world that was very very low level, it didn't kill you, which sleeping sickness does, and they don't spot it in the blood because it's rare. That is a real possibility. The other possibility is that the trypanosoma causes Chronic Fatigue Syndrome with high efficiency, that's why their symptoms are identical and that's why the transcription patterns match because it is Chronic Fatigue Syndrome. Now I don't know that but what we're doing is making probes for all the different trypanosomes, multiple probes, and while we're at it we're going to do all the parasites because they are also difficult to diagnose. And given the results that we've seen before, the DNA from these organisms should be in the blood and we'll look for it in the blood. Another thing that we need to do is try to figure out how to do the RNA viruses and that's not going to be easy because RNA is not very stable in the blood. We'll try to figure that out if we can make that work or some industrial group will do it and that that way we can look for an awful lot of infecting organisms.

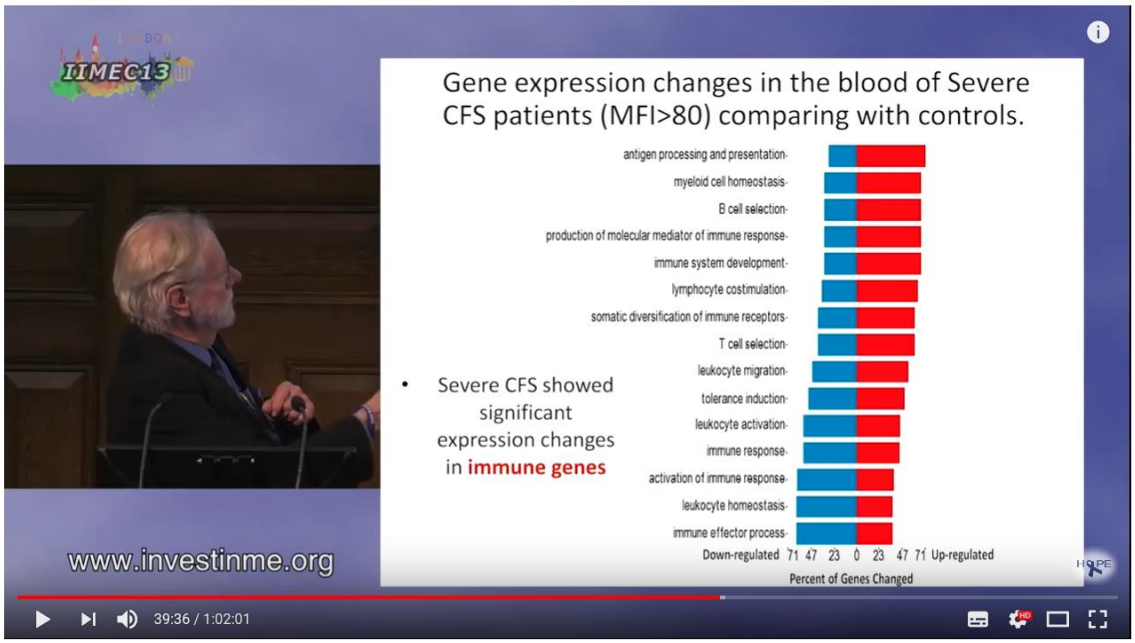
34:27 But there's other possibilities and in fact well maybe it's a new virus and we've had that excitement with XMRV, that's kind of discouraged people from looking for a new virus but it could be. So there's another way to do this and that is to isolate particles from the blood. And a particle would be a virus, a bacteria, a fungus, a parasite. You isolate the particles. There's a lot of DNA from human in the blood so you destroy all that DNA and then you extract the DNA from things that are left and you sequence massively. Then you compare that sequence to everything else has ever been done and you allow it to be highly mismatched and when you do that you can find new viruses and we've had a collaboration for that. Ian Lipkin does that as well, we're doing exactly the same thing he does and you can find new viruses. The person that was working with us on that said if we were to do that experiment in the early 80s, when the AIDS epidemic started, it would taken us about 24 to 48 hours to figure out HIV. That would be absolutely trivial. So these are very powerful methods for looking for new things and we have done that once and it comes up empty and it says that there probably isn't an infective organism with the caveats of these things. It's possible that if there's a parasite that it's not in the blood.

36:08 Another thing that the patients have told me is that they have heavy metal contamination and that they need to detox from their heavy metals and it's making them sick and the environment is exposing them to heavy metals. So we've done urine analysis on all the severe patients. They do not have any heavy metals, however what a lot of them have is they're low in essential metals. And we have a hypothesis of why that's true, of course it could be because they are detoxing all the time and they're removing the metals that are essential. What I mean by essential metals, are metals that are very important for your body to function. Things like copper a lot of people think copper is toxic. It's not, it's essential. If you have too much of it, it's a problem but very few people have too much. In the United States about 60% of the population is deficient in copper, very few people have copper toxicity. People who had a copper bracelet and used to wear them were told it's toxic, you've got to get rid of the copper bracelet. The amount of copper you'll get from your copper bracelet is about your daily requirement, by taking it off you now become copper deficient. So those patients didn't show us anything and the problem with that was it was a urine analysis and the way that that was done they don't do mercury. Mercury was one of the ones that we were the most worried about. So you have to do hair analysis and so we did.

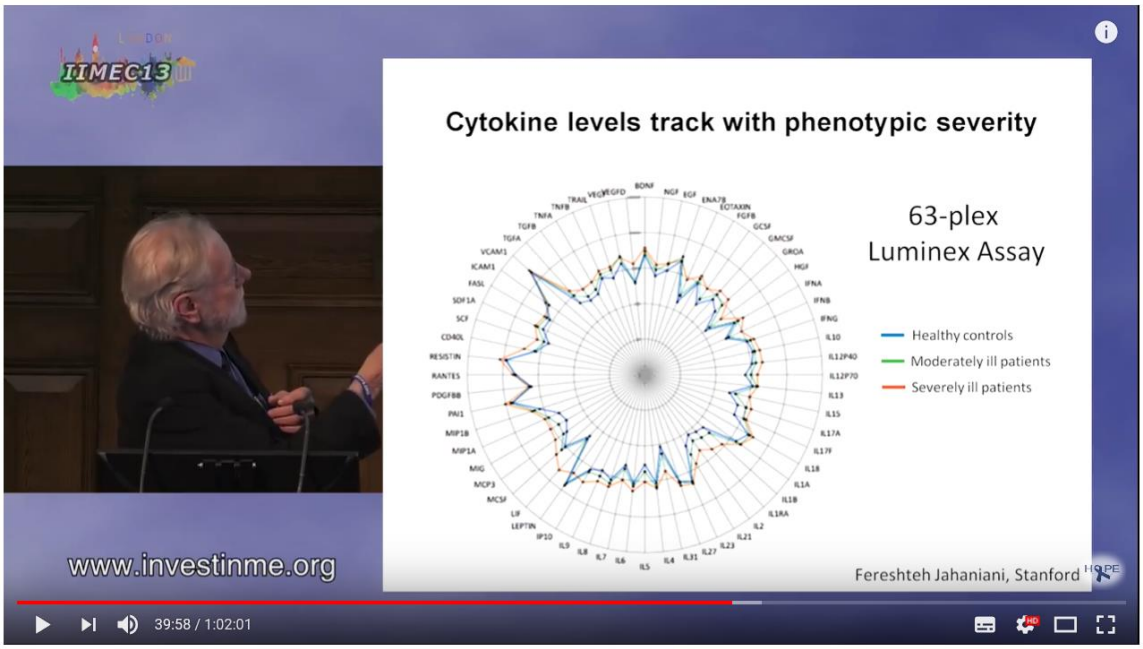


		Al	Pb	Hg	U	Sn	Se
Reference range (ug/g hair)		< 7.0	< 0.8	< 1.0	< 0.06	< 0.3	0.7-1.2
WHO safe level				< 2.0			
	M	0.2	0.05	2.70	0.001	0.02	0.55
	M	1.0	0.14	0.48	0.085	0.03	0.87
	M	6.3	0.09	0.53	0.006	0.05	0.91
	M	2.3	0.08	0.39	0.046	0.04	0.77
	F	9.3	0.58	1.10	0.280	0.20	0.73
	F	3.1	0.51	2.70	0.210	0.08	0.67
	F	44.0	4.40	2.60	0.003	0.45	0.17
	F	1.8	0.03	0.11	0.002	0.05	0.84
	F	7.7	0.10	0.08	0.002	0.01	0.98
Croatia	F	0.6	0.07	6.90	0.008	0.03	0.76
Finland	F	4.8	0.12	0.80	0.640	0.06	0.49

37:54 We took some new patients and did hair analysis on them to see about the mercury. So these are not the severe patients and what we found from that is that, this is mercury (pointing to Hg column) that's just a chemical symbol for mercury (Hg), and you can see in red that there were several patients that had a little over the limit on mercury and one patient had lead (Pb) toxicity. The other thing that's interesting about this if you have too much mercury you often have too little selenium (Se) and selenium is used as an antioxidant. We have all this oxidative damage, so you probably do not want to be low in selenium, so probably the low selenium is probably worse than the mercury being high. This is not terribly high, we've talked to each of the patients that were mercury high and it looks like they're all because they eat a lot of fish and especially things like salmon. It's okay to eat fish but don't overdo it because all these big fish have a fair amount of mercury in them. Then a little bit of a surprise is this one patient from Finland who has a fair amount of uranium. That's probably environmental, I don't know where that comes from and we don't know the medical consequences of the uranium.

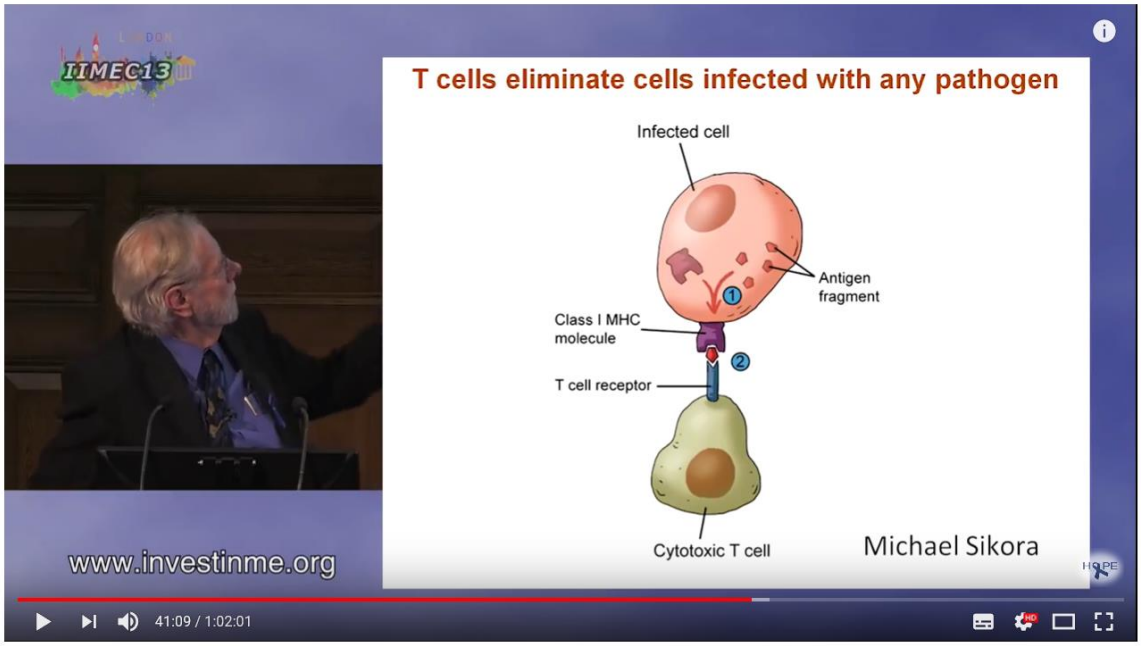


39:21 Now this just a collection not a whole gene expression study. I just wanted to show you that if it's in red it's increased in expression and if it's blue it's decreased, and these are just looking at the immune system. You see a lot of changes in gene expression in the immune system so that means that there's a lot of immunology going on and we're not surprised by that. This is just the data that shows there's a lot of immunological effects in these severe patients.

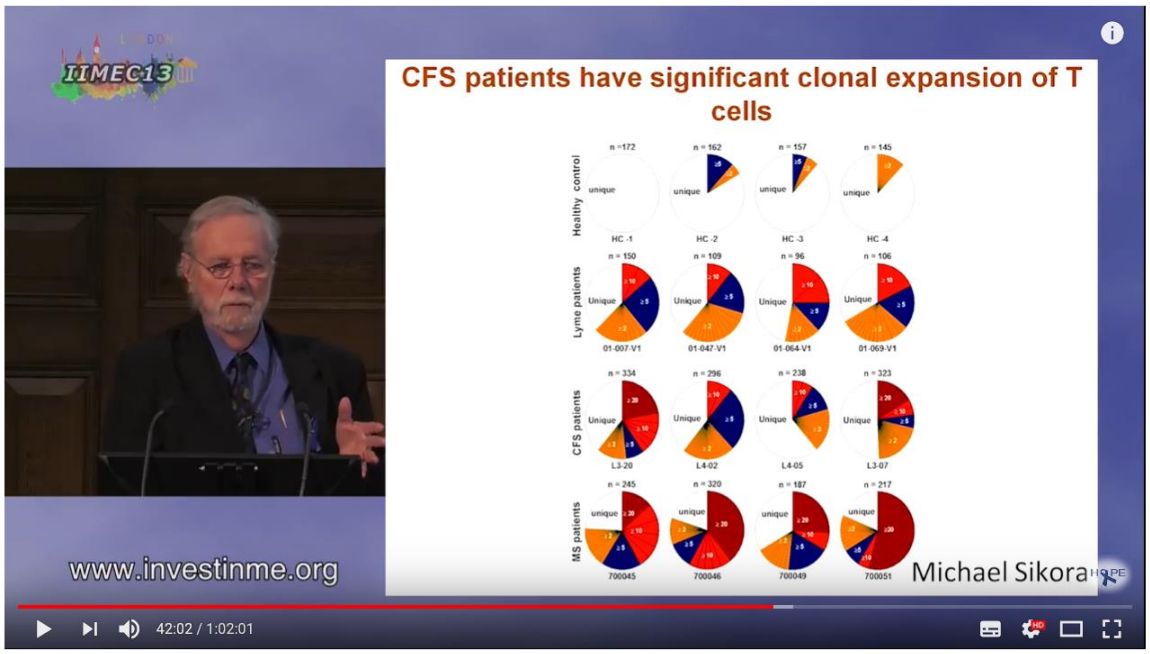


39:52 And then you can also do cytokines. This is a 63-plex. That's higher than what's been published so there's a few new ones that have been added to this. Here are the severe patients and what we can see in them is that they have even higher cytokines than had been seen before. We just put the circular plot

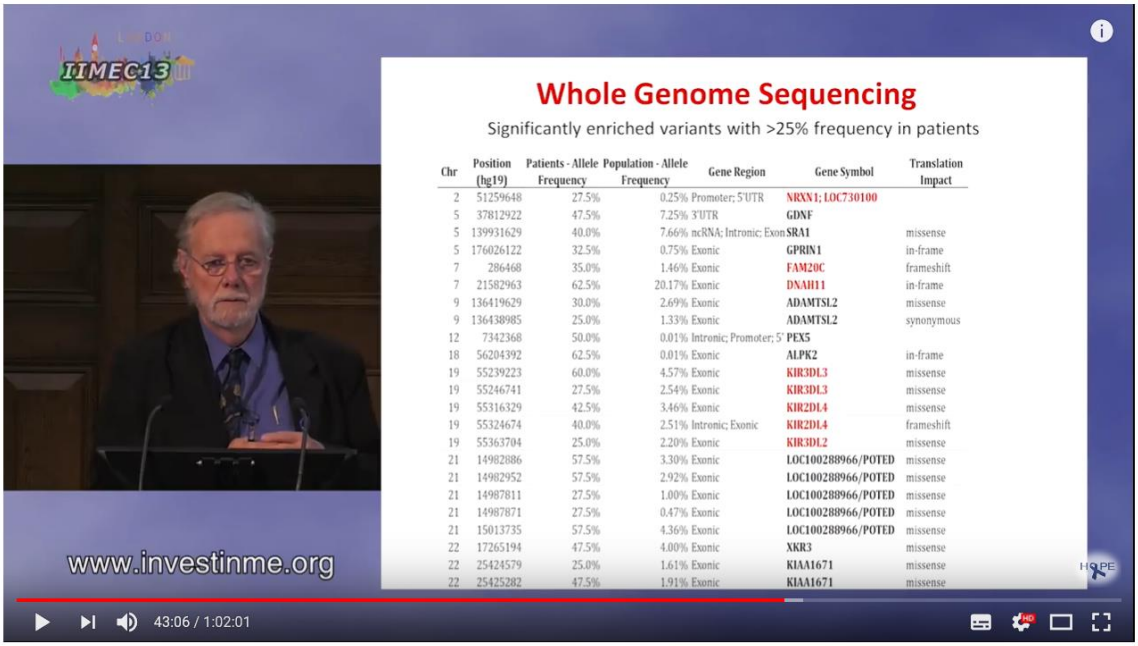
so we can put it all together. So there is a lot of immunology, that's not surprising but that's a big component of this disease. Then we want to look further in the immune system and what we'd like to do is understand what may be going on like in an autoimmune system situation. And so what we're looking for are T cell activation and that was found by Mark Davis in a small study and now the Open Medicine Foundation has funded that. That was part of our collaborative research center that got turned down but now we're going forward with that T-cell activation project and we're going to add to that doing the single cell expression analysis that Maureen Hanson mentioned.



41:03 T cells can recognize a foreign object through this T cell receptor and once they have identified it they can kill the foreign agent. So you have a whole bunch of these in your body and they're scanning your systems for something foreign. If they find something foreign then they amplify and make very large numbers of them and it's called the T-cell expansion.

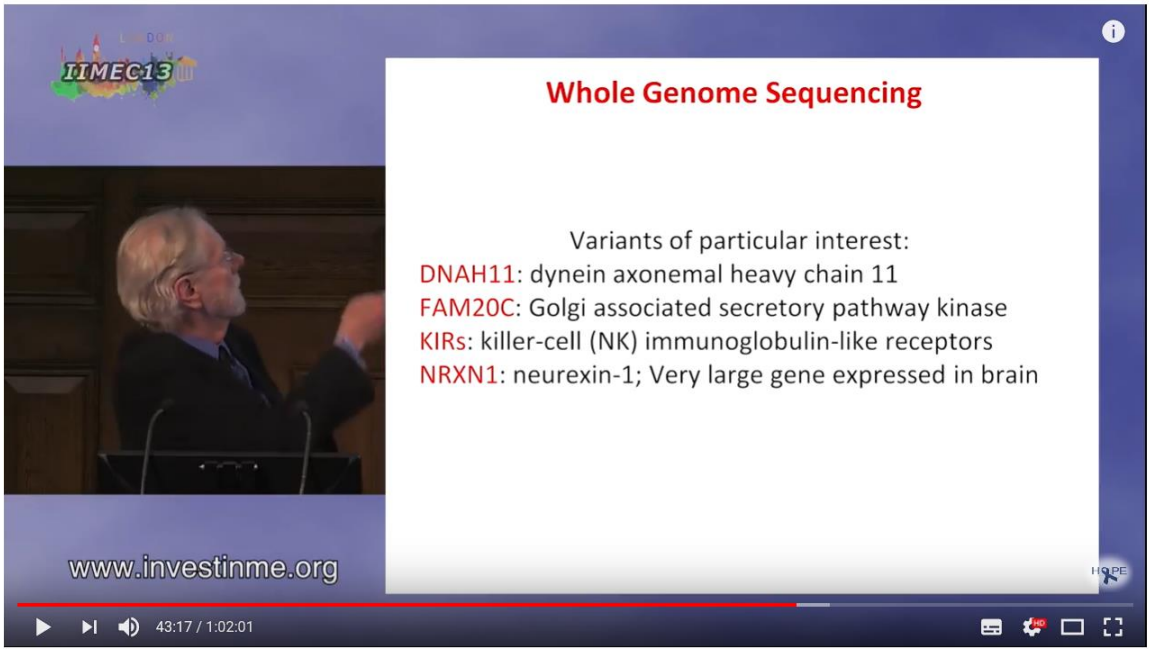


41:32 What we find in Chronic Fatigue Syndrome is that there is T cell expansion. I'm not going to go into the details of this plot but you can see it's filled in where healthy controls are not and Lyme disease looks very similar and MS (Multiple Sclerosis) looks very similar. So there is T cell expansion we need to explore that more. One of the reasons why this is really important is that it's saying it's recognizing something. We've looked for a foreign agent, we haven't found it but maybe it's still there and we've just failed to find it. We'd like to know what it's looking for, what it's seeing. Now it also could be seeing self so that's an autoimmune disease. Also a possibility there's something else that's triggering the immune system to be activated and it's not an autoimmune disease, so we'd like to find a result. That'll be very important.

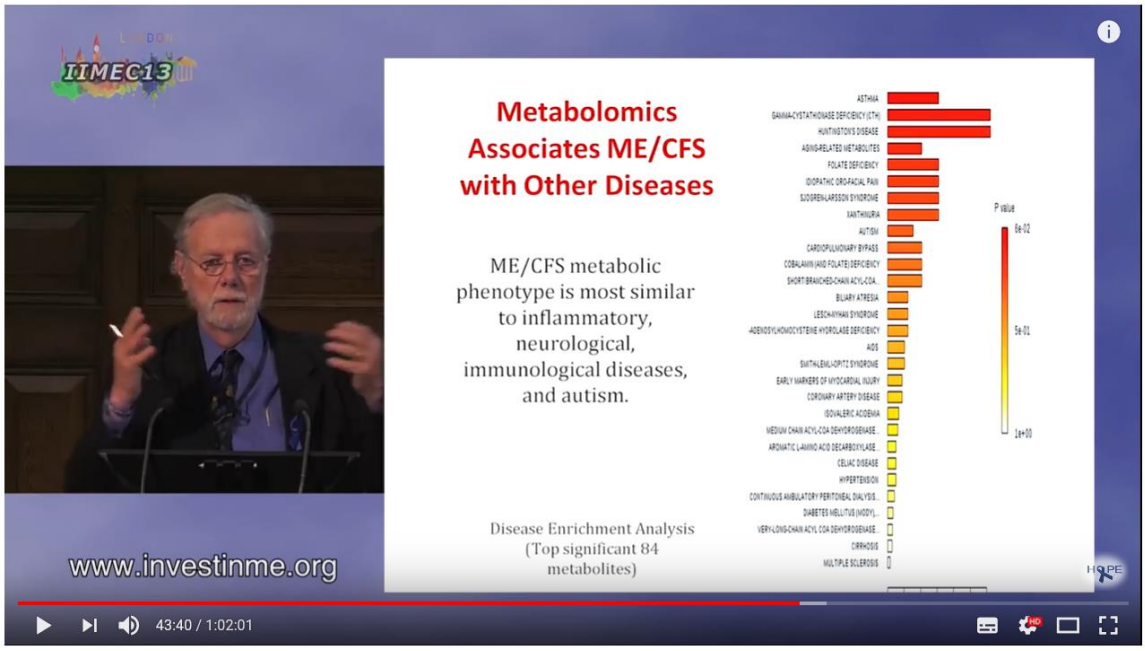


Chr	Position (hg19)	Patients - Allele Frequency	Population - Allele Frequency	Gene Region	Gene Symbol	Translation Impact
2	51259648	27.5%	0.25%	Promoter; 5'UTR	NRXN1; LOC730100	
5	37812922	47.5%	7.25%	3'UTR	GDNF	
5	139931629	40.0%	7.66%	ncRNA; Intronic; Exon	SRA1	missense
5	176026122	32.5%	0.75%	Exonic	GPRIN1	in-frame
7	286468	35.0%	1.46%	Exonic	FAM20C	frameshift
7	21582963	62.5%	20.17%	Exonic	DNAH11	in-frame
9	136419629	30.0%	2.69%	Exonic	ADAMTSL2	missense
9	136438985	25.0%	1.33%	Exonic	ADAMTSL2	synonymous
12	7342368	50.0%	0.01%	Intronic; Promoter; 5'	PEX5	
18	56204392	62.5%	0.01%	Exonic	ALPK2	in-frame
19	55239223	60.0%	4.57%	Exonic	KIR3DL3	missense
19	55246741	27.5%	2.54%	Exonic	KIR3DL3	missense
19	55316329	42.5%	3.46%	Exonic	KIR2DL4	missense
19	55324674	40.0%	2.51%	Intronic; Exonic	KIR2DL4	frameshift
19	55363704	25.0%	2.20%	Exonic	KIR3DL2	missense
21	14982886	57.5%	3.30%	Exonic	LOC100288966/POTED	missense
21	14982952	57.5%	2.92%	Exonic	LOC100288966/POTED	missense
21	14987811	27.5%	1.00%	Exonic	LOC100288966/POTED	missense
21	14987871	27.5%	0.47%	Exonic	LOC100288966/POTED	missense
21	15013735	57.5%	4.36%	Exonic	LOC100288966/POTED	missense
22	17265194	47.5%	4.00%	Exonic	XKR3	missense
22	25424579	25.0%	1.61%	Exonic	KIAA1671	missense
22	25425282	47.5%	1.91%	Exonic	KIAA1671	missense

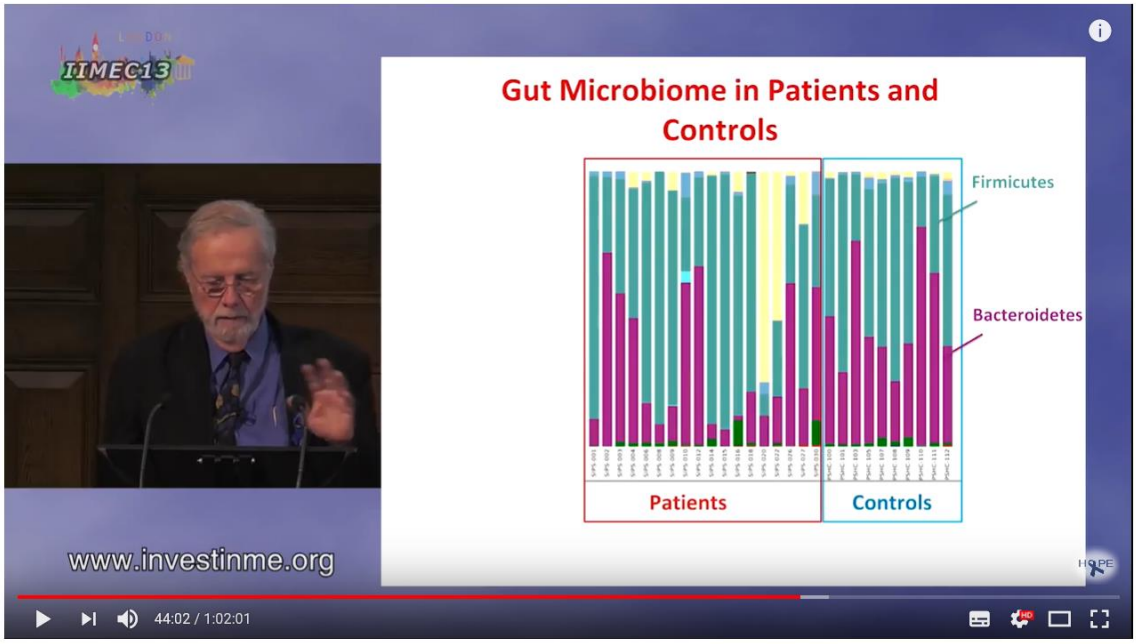
42:23 We've also done whole genome sequencing and I don't want to go into a lot of detail about that. All I've done here is list a few of the genes that appear to be very important that are different in ME/CFS patients and healthy controls. The highest rank is this gene and it's roughly a hundred times more prevalent as an alteration than in the healthy controls. But also are these genes and these genes are involved in the NK (natural killer) cells and there is the response of a NK cell recognition and they are different in patients versus healthy controls.



43:15 So we're now going to do a big, both HLA and KIR, DNA sequencing project and these are the ones that we'll continue to explore. This is an interesting one and it comes up first it's a very, very large gene and it's in the brain.



43:33 We also find a lot of metabolic changes. A lot of people have talked about that but you see a lot when you look at all the different small molecules in the blood. So when we take it from a broad point of view what we see is an activation of the immune system, a lot of genetic changes and a lot of metabolic changes. So that's what we're going to focus on, those two.



44:00 We've also done the microbiome and I don't think I'm going to talk about that, that's just for the record to have that as part of the database.

I wanted to get to, because I'm running out of time, a future direction. So what we're now doing is taking the metabolites that we see and the genetics that we see and we're combining them and that's something called the systems biology approach. The metabolites are affected possibly by the genetic changes. Then we're going to put it in the context of actually pathway analysis so make the whole flow chart of all the genetics, all the genes and all the metabolites. What we're now looking for is something we call a metabolic trap (Robert Phair) and I'm just trying to introduce that concept because that's a total different new concept. Because what we think is happening here, and this is our best guess at the moment, is because of the genetics and because of the inflammation that gets started, that you can get yourself into a situation where your pathways are normally supposed to work get altered because of the mutations and the metabolites. The enzymatic process gets trapped into a not very functional state and when I say trapped it means there is no easy way out. So what this would predict is that you would get something that would trigger it, and we think it is an infection that could change your metabolism to the point where it gets trapped. That would happen almost instantaneous, you'd go to sleep and you would wake up with the disease. That seems to fit and nothing you do would get you out of it that you can normally do. There will be no drugs that you could take to get you out of it. Now to some extent that's good news because it means we don't have to develop new drugs, that'll take 10 to 20 years. Also the good news is by understanding what it is, if this is all true, it will probably be very easy to fix but it won't be something that anybody's tried. We will have to manipulate the metabolism but it should be very inexpensive to do it and it should take a few days to get you out of it. So that's good news! So I'm telling you this simply because I'm so optimistic this is right, but unfortunately these things almost always come out wrong. So don't get too excited about it but just to give you an idea what researchers are trying to do here is that we're trying to figure out what's really going on and how to fix it. This is not about doing some research to get a publication, which seems to be the case for an awful lot of disease studies. This is trying to figure out what it is and what we need for that is everybody with all their expertise to be thinking this way and someone maybe, I hope, will come up with what's wrong. I don't think this is that complicated in the sense of something permanently wrong with your body. That's why I like the trap hypothesis because it just simply says you've fallen into a trap and you just don't know how to get out but by understanding all this we can get you out. That's why I'm really, I'm really hoping it's right. We'll know maybe within, I hope, several months to a year. It'll be more complicated than I've specified here, I know that, but I am optimistic. Thank you very much. (Applause)

Dr. Ian Gibson – Conference Chair: Thank you, thank you very much Ron. I'm sure that's given you a real flavor for research and how to do it along with the other talks you've heard today. I'm sorry I haven't given you time to have a lot of input yourself, you can ask Ron questions now of course but I hope you've had a day where you see there's lots of things going on and that's only part of it. We could have doubled this conference up in time with other people are going. It's because of you there are so many people now getting involved in this and thinking like Ron's and the others that you've heard today, are all going to be part of something. And there's going to be some moment when even the newspapers have to take an interest in it and admit that there's something going on and there's an explanation for it, we can do something about. And really it's because we have meetings like this. I want to thank the speakers of course. Thank you for all those who have helped get the conference going today and made sure it's been nice for us. It's exciting. It's nice to have exciting times in science and I think we started to get somewhere. And Ron thank you for your talk, a few questions now at the end. I don't want to keep you out because getting out of London is sometimes worse than getting in so yeah I know you need to get home. So you want to ask Ron a question. You've had a way of thinking about things which is perhaps new for you, and I know there's been a lot of technical details which is hard to take in. But I hope you're feeling the kind of excitement that's generated in a bunch of scientists and people who are sincerely interested in the

problems of ME and the effects it has on lives of people. The time has come, it's been around too long. But with this kind of work and effort something is going to happen, I feel sure.

Question from audience: There have been a number of reports that surprisingly cholesterol is high in many ME patients. Cholesterol is going to change the viscosity of the blood. Would that actually make a difference to your impedance measurements?
Dr. Davis: I missed a couple of your words because of the background noise.
Audience member: Cholesterol is high in many ME patients despite the fact that they are not obese. Cholesterol is going to change the viscosity of the blood especially as the blood changes from a temperature of 37 to 24 degrees. Could that change the impedance measurements in your instrument?
Dr. Davis: The disease affects a lot of lipid metabolism and that's I think why the white cells are light. That's why a lot of the metabolites are lipid containing so I don't know why that is. One thing we do see is a fair number of mutations in fat metabolism in the patients more so than in healthy controls, so there's something about that too. If you can't metabolize the fats well they'll accumulate and then you can get feedback systems that affect all sorts of other fats as well. You have to get rid of them and so you may not be able to oxidize them in the mitochondria and you then have to oxidize them in the ER (endoplasmic reticulum) and that just will foul your whole system up. So that's just speculation but I think we'll see it, we'll see a lot of alterations in things that involve lipids.
Audience question continued: Just wondering, whether basically a control group could be a number of healthy people with high cholesterol could maybe mimic what we're seeing in ME patients?
Dr. Davis: People high in cholesterol probably be genetically determined and it's probably very narrow in terms of the lipid problems that they have. I think this is very general there's something about metabolizing fat, and I don't necessarily understand it. It looks like also that we see a fair amount of the glucose being shunted over to the sorbitol pathway which then goes to fructose and then goes to fatty acid synthesis, so your body is even making fat. And so I don't understand it at the moment that there's something about lipids that is really off in the patients.

Question from audience: I had two thoughts that came to mind. One was your observation of improvement under fevered conditions and it turns out that there are both with fever itself but usually an association with the pyrogenic stimulus, which could be bacterial is an inducer of CRH (Corticotropin-releasing Hormone) and then of ACTH (Adrenocorticotropic hormone). And this phenomenon of improvement under fever conditions is also something that has been observed in individuals with autism who don't speak and then during a fever episode they do speak. So it might be could be something with the HPA (hypothalamic–pituitary–adrenal axis) axis and there's actually a clipped form of ACTH that had been used years ago in autism that had led to some improvements but then it went off patent and I never saw anything about it again. Something to think about. But the other thought was about the red cell deformability and the velocity, that even though you had some variability there that it is potentially an interesting phenomenon. But you know of course I happen to be doing work right now on sickle cell anemia and neuro-immune stroke risk in that population. But one of the things that happens with sickle cells, there are certain processes that are oxygen dependent and some that are not. So the deformability can be both oxygen-dependent and independent, depending on what level of plasma oxygen tension and so the question is in your system do you control for oxygen tension or can you control for oxygen tension? It might first of all reduce the variability and maybe allow you to see what the group specific differences might be.
Dr. Davis: Those are great suggestions and in fact there's quite a few of them there. One is that I think there's a close relationship between autism, the lack of speaking. The metabolites look very similar. There's obviously a difference in onset time and some of the differences you see in autism may just simply be because it starts at a very young age and it's affecting mental development and things of that type. But

one of the optimistic things you can also see is that Bob Naviaux has treated some autistic children with suramin, which blocks the purinergic receptor (receptors for both ATP and adenosine) and he got a great deal of improvement in them. I have been trying my darndest to get a hold of suramin. I can't and there's a big long story but it will be available. A new manufacturing plant is now being set up in the US and it may be available by the end of the year. So that's an optimism and the nice thing about that drug is its not very toxic. Also the curious thing is it's used for trypanosomes. That's what it was originally developed for and I don't understand that connection.

Dr. Ian Gibson: One last question please.

Question from audience: Following the results of the drugs on the impedance device have you any plans to trial those drugs on patients?
Dr. Davis: What we're trying are drugs that are FDA approved and we're not physicians so we can't. But we can do that with partnership with other physicians and that would be something I would be talking to Ron Tompkins about, we'll look at them. We did find one drug that seems to improve the ATP production and that's a drug called Ativan. I know that it helps because we've given it to my son and it really really helps him. But unfortunately it's only for a few hours and you habituate to it, so it's used for crisis management. So we give him Ativan if we have to take him to the hospital and he tolerates that so much better if he's on that drug. I don't know if it's GABA (Gamma-aminobutyric acid) receptors on the white cells and we've looked at the T-cell energy production and it's much higher.

Dr. Ian Gibson: Can I just bring it in by asking you how many people feel that we're moving forward in a very positive, determined way or who maybe think we're just just messing around? How many people think we are moving forward in a positive way?

(Murmured, unclear response from audience)

Dr. Ian Gibson: We will know how to get money, you know we'll get it. We'll get it you know, we'll get it.
Audience member: What is it you most need to move forward?
Dr. Davis: Probably money and the reason for that is what we really need are more people. But to get more people you need money and one of the problems with donations is the fact that you, if you want to use it, 70% of the cost of doing research is salaries and what you want to do is get really really good people and retain them. You have a hard time retaining people if they feel that you're going to run out of money because we're headhunted all the time and I lose people because they don't feel I will have the money to pay their salary. I just lost two fantastic people because they got really, really good job offers and they were willing to stay but I didn't have the money at that time to guarantee them a salary for the next year. So it's hard to find good people and what you really want is having two years out, that you have money for two years that will keep people. If you're going to run out in six months people will jump ship, so that's something people need to understand because an awful lot of research is done by donation unfortunately.

Dr. Ian Gibson: No, it's right obviously but if there's more than just money sometimes as well. You know, you need money but you need to get the right people who are enthusiastic and keen and talented. It's alright saying that the National Health Service needs more money but it's what it's used for. It's no use just taking it and paying for all the debts you've accrued over the last ten years. There's got to be real money that you can really invest in something: new technologies, new people, new education programs and so on and getting the public like you to get involved and to be able to contribute to what happens. And that's what happens here. You've ideas about what might go on because if somebody's out of order

and got it wrong you'll tell them. You know, now a lot of people don't like that, there's a professional sort of etiquette that some people think they know best. No they don't. They've gotta take the public with them. The public are the biggest political animals in the world. They know what's needed, know what they want, they see it and they've got to be listening to. The real trouble today is they're not being listened to, that's the thing. So it's not just money, it's getting them involved in it too to help you know and the money I think will follow. Remember when we couldn't do proton therapy for cancer, brain cancers, and a young man was taken by his mum and dad to Spain and got treatment there and suddenly seventy million pounds appeared the next day. I remember fighting for that for a proton therapy, the best method for brain cancer, and being told you can bugger off there's no money, you're not gonna get it. And that was a time when we were doubling the bloody budget you know. So I mean it appeared because they were pressured, different types of pressure. So there's going to be a political build-up, from the grassroots up as well, to make sure that the people at the top who've got the money, and there is a lot of it about, are listening. And if you look and watch, every week money appears for all sorts of things because there's agitation around it. I know there's a limit, I mean it's not unlimited, but at the same time they find it when they need it, you know. And I'm not going to talk about defense and bombs and all that stuff, that's the political message and it gets a bit boring sometimes but you know what I mean. They can find money for certain things when they need it. We have got to have the voice and make sure that we're doing it. I don't think we do enough in the press actually. I think it's very difficult. I mean sometimes it's an advantage not to have the press but they still don't believe in ME a lot of the journalists, the health journalists, in this country they just taken the message that it doesn't exist from the professionals, in the medical profession. That's what we've got to fight and you know that's for you guys to get involved in.
(Comment from audience about finding £60 million for brain cancer)

Dr. Ian Gibson: Yes absolutely, you know. That's because people have agitated for it. Certain types of people have agitated for it within the system, same with prostate cancer money suddenly appeared for that because the poor boys suddenly started admitting that they got cancer as well. Funny how the money suddenly appeared you know. So there's all sorts of difficult political decisions have to be made too but you're a major part of that. So get talking, get writing and get angry you know. And make sure that the kind of what we're hearing today and the speculations are really going to turn out to improve life for people.
(Applause)