Alain Moreau, PhD - Deciphering MicroRNAs code in ME/CFS Pathogenesis

https://www.youtube.com/watch?v=RzlKezyJVQc

Ron Davis: Well it's a great honor to have Alain come here. I've known him not too long but we knew it was really important to look at these small RNA molecules that regulate everything. We didn't really have an expert in it and then I learned that Alain was focusing on that as well as other things. And I said, oh that's great now we don't have to do it. So that's just as an illustration of how to make things move more quickly. You find in some fields people – here, somebody's doing something and they said well it must be a good idea we better do it too. There's just a lot of duplication of effort. We need validation but not necessarily a lot of duplication. So that's how I got connected with him and we've shared a lot of stuff together. It's a great pleasure to have Alain here.

Alain Moreau: Thank you, Ron. It's a great pleasure to be here. I would like to thank Ron's team and Linda and the Open Medicine Foundation for the opportunity to share with you new results about the role of microRNA in the pathogenesis of ME/CFS. So here are my disclosures.

I would like to present to you today and do some review of the current challenges that we are facing not only as a researcher, but of course, the patients and the clinicians. [Here's a] brief description of the Quebec Cohort which I started from scratch.

As of today it is probably one of the largest cohorts of ME/CFS in Canada. I'll share with you those results. We're developing some interest [stuff] about the role of circulating microRNA and what can we do with that? So we give you some example of potential clinical utility of using these small non-coding RNA as biomarkers and potentially as a potential therapeutic target and where are the next steps.

As you may know, and this is a graph that probably summarizes quite well what is happening in ME/CFS and also in maybe other severe chronic diseases.
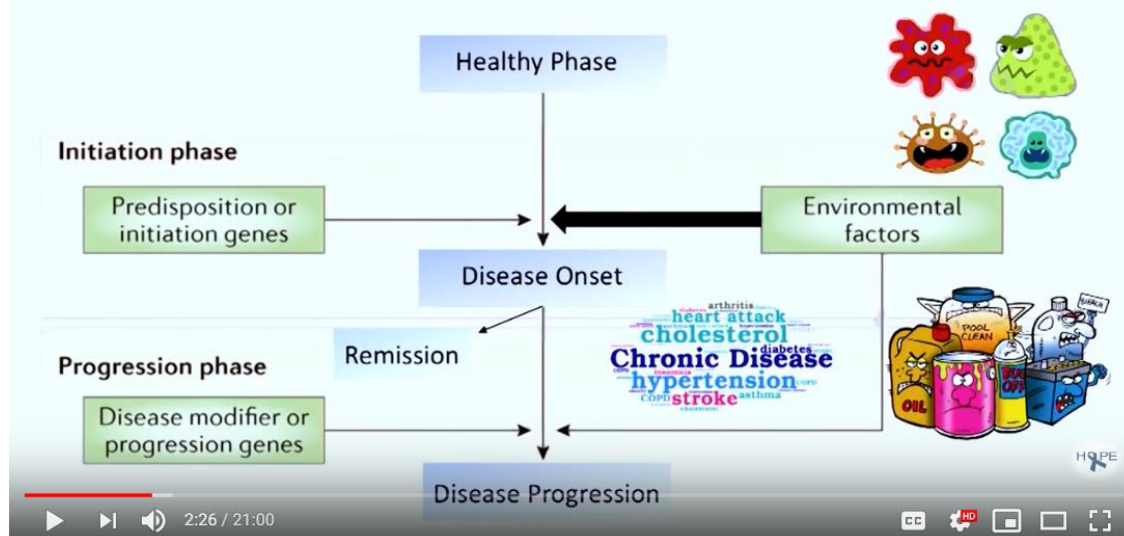
So the patients have a healthy phase in his life or her life and there is some initial triggers that often involve viral infections, could be also bacterial infection. So these are the little bugs on the top corner [referring to the slide] and those factors have a huge impact. We suspect that there are

some pre-disposition factors that will further react to these primary infections somehow. Why? You have an infection and this infection is often prolonged and beyond the infection you have also some of the factor chemicals, and different … environmental factors, heavy metals…, can act as well as the triggers. So that brings you to the disease onset. A few of the patients can have a full remission while others will [or] may continue to progress either because they are continuously exposed to some environmental factors but they might have also [the] presence of disease modifiers that will further induce a progression of the disease in the chronicity. So we don't know much about that but we need to understand that. So the relationship between environmental factors and the genes that may predispose to have this type of response. I think this is something very important.

So last year I show you different type of elephants. Now I switch for apples. How can we separate you?

Jonas [Berquist] talked about food and we never communicated but we'll stay in the field of food. So how can we face or address the clinical heterogeneity of ME/CFS? How to select and compare? It's clear that we are facing a spectrum as part of the disease. There is not a single entity and that starts with some difficulties in the clinical definition issues of ME/CFS. How we selected patients in the past, as [compared to how] we select patient today, and maybe how we will select patient in the future using novel tools. We are working often on small cohorts for different reasons, often due to a lack of significant funding. They have some maybe issue about different ethnic background and also the toys! As a researcher we are using this multiplicity of omics methods which are very expensive and sophisticated methods but again there's not a single lab in the world that can allow to use all your mix in the same labs. So that's why it's very important that we can collaborate.

To add to the difficulty, to further define the patient, patients come to us at different disease stages. They use different drugs, different supplements. It's very hard even with very sophisticated [?] to further understand that. But this is part of the reality of the patient. We have to understand that and plus, ME/CFS [is] occurring in ageing populations. You also [have the]

additional challenge of comorbidities because there is no such a thing as a pure ME/CFS. You have ME/CFS and maybe also arthritis and ME/CFS with some form of cancers, ME/CFS with rheumatoid arthritis, autoimmune disorder. So we need to introduce and understand that. Plus in the past whatever were your disease triggers. So we need to understand that but we can hardly follow the source of those triggers in a new path. We can have a reflection about what is happening by looking at different factors.

So our hypothesis is [that] ME/CFS is caused by a disturbance in the expression of small non-coding RNA, called microRNA which modulate immune function, energy metabolism and physiological stress response. And indeed we believe that microRNA could be link between environmental factors, genetic predisposition, and phenotypic difference—why some patients have those symptoms and others have different symptoms. So you are not like the same, so you are different types of apples. Some of your even disguise yourselves as some oranges! So we need to understand that. Why it's so important is because if we were good at understanding [and] maybe using microRNA, it could be a good link to establish the link between these stages, the disability across cohort, and eventually help us to have a better understanding of the etiology and to get our mind not only the molecular epigenetic and genetic mechanism but eventually follow in identify for the first time therapeutic targets.

7 PROMISING RESEARCH AVENUE
Role of circulating microRNAs in ME/CFS : small but powerful!

Ref: Van Rooij E. «The Art of microRNA Research» Review Circulation Research, 2011
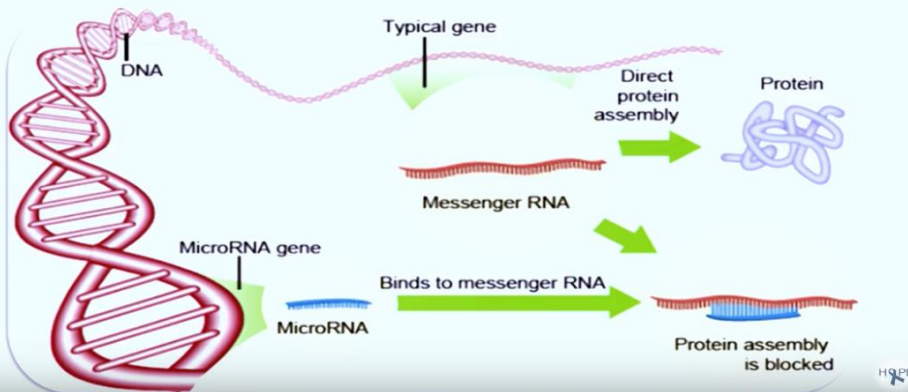
So this is a brief description about why micro RNA are very important. MicroRNA are part of your genomes. They are expressed and they have different molecules. They have different stressors. Only stress by itself can trigger [or] down regulate microRNA. MicroRNA will attach to messenger RNA and will do two things. The first thing, it will introduce a [??] so the messenger will disappear which mean that it won't be translated into protein. So you will have less of the protein. Or it can also block the translation with the ribosomal machinery and you won't have any protein anymore. Why this is very important is because one single microRNA can target up to 200 different genes and many genes can be targeted by different micro RNA. A very small molecule but very, very powerful and can harm you by many ways.

Here is a demographic and clean that off our cohort.

8 STUDY POPULATION (1)
Demographic and clinical data of Quebec cohort

ME/CFS Patients*	Women	Men
N	88	24
Age at diagnosis	36 ± 11 (13-59)	35 ± 10 (18-52)
Age at time of test	50 ± 10 (24-78)	48 ± 13 (13-67)
Daily activity (hrs)	4 ± 3 (1-13)	7 ± 4 (1-15)

Healthy Subjects	Women	Men
N	24	34
Age at time of test	47 ± 11 (28-65)	50 ± 10 (30-65)

* According to the Canadian consensus criteria

As of today we are around more than two hundred patients. 85% of them are homebound, so we have to send clinical nurses to test them. And of course we need to establish some reference values so we need to have healthy volunteers that are willing to participate in the test.


Because the patients are so different we need to try a way to regulate such differences. We developed what we call a stress test. Thanks to the work of one patient that I know is watching today—this patient I will just call him Christian. So Christian stressed me a lot about if you have to develop a test, you should develop a test that can mimic the post exertional malaise and this is what we have done with the help of Christian. We apply a cuff, an inflated cuff from a massage machine. So this machine technique is offering a therapeutic massage. [It is a] very gentle massage but this gentle massage after 90 minutes create a stress that mimic the symptom of post-exertional malaise. Trust me—ok it sounds a bit weird! We never push anyone to the emergency room, thank God, but it's really working.

We have a value at baseline and every 30 minutes up to 90 minutes. So each patient becomes their own control which is the beauty of this test. Whatever you are taking, medication, whatever, you're suffering the disease from 3 years or 20 years, you become your own control and we are doing the same with healthy controls.

So this is the workflow of the analysis:
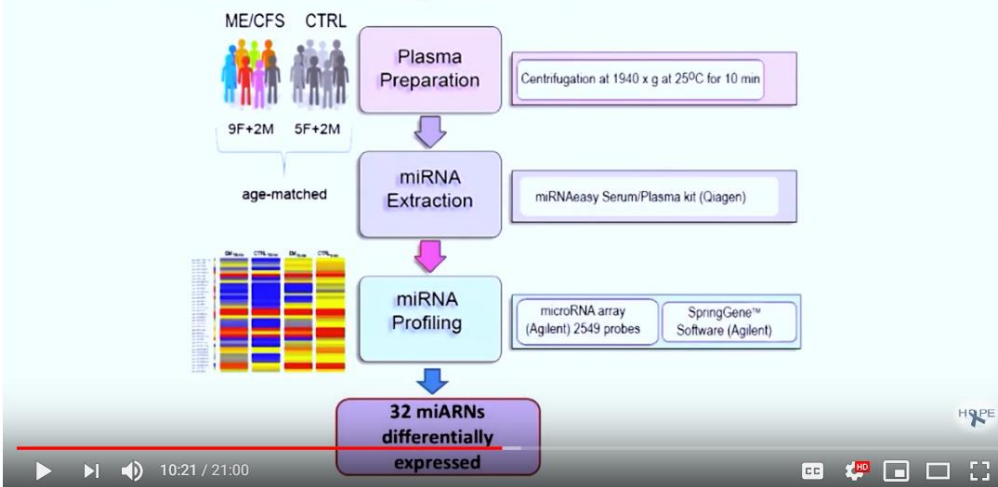
We prepare the plasma of the patient, we extract the microRNA, we establish a profile and from a potential soup of 2500 micro RNA we end up with 32 micro RNA that seem to be highly associated with ME/CFS. So this is a big reduction. I'm not saying that we capture all of them because you can use different [Agilent?] technology but from 2500 and then with 32 that we started to validate.

So what do we learn so far?
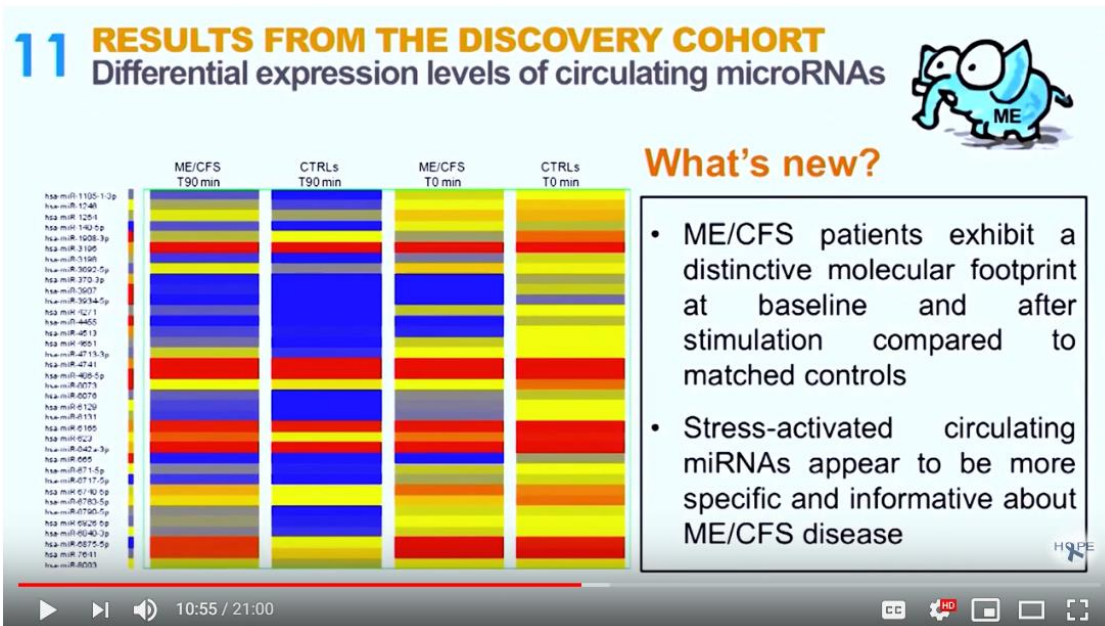
You have different code of colors that show you the difference between the stimulation and at baseline. So ME/CFS patient exhibit of a distinct molecular footprint at baseline and also at stimulation. And what we saw so far, we have more information after the stimulation of the

microRNA that seems to be more informative about ME/CFS disease. And we are doing the relative value after stimulation and at baseline for each participant.

I will give you a few examples of what we have found so far.
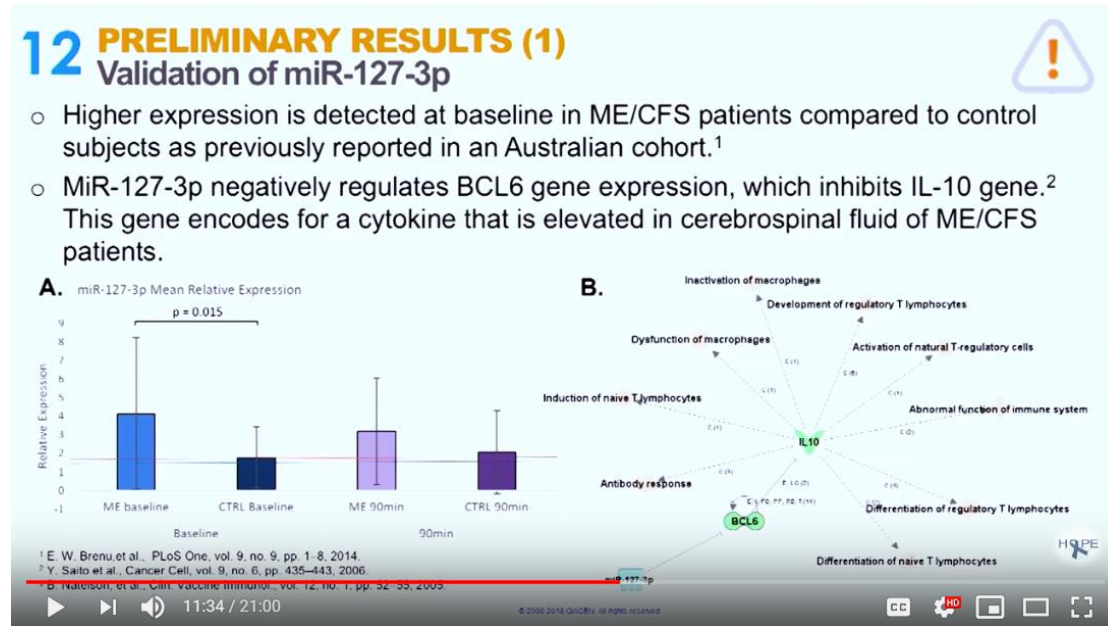
So we find a microRNA and they have weird names. They always start with miR then you have some number. 127-3p, this miR has been previously discovered in an Australian cohort of ME/CFS patients. For us that was very encouraging that Canadians and Australians look alike even though we are very far apart. What is very interesting in terms of pathways is this miR. You see the value at baseline is elevated. This is the control and the same after stimulation. But what is very important, we have access to a software called EPA that allowed us to predict or link the microRNA to validated targets. In that case this miR is targeting BCL6 which is a negative regulator of interleukin 10 which is highly important in the regulation of different functions involving lymphocyte T for instance. And the elevation of interleukin 10 has been previously report in cerebrospinal fluid of ME/CFS. So again we replicate results from others for the very first time. Good news! This means that this miR may really [be] associated with ME/CFS and we can give to this miR a role, a significance in the metabolism. So that could impact lymphocytic developmental function.

Another miR is the miR 145 P.
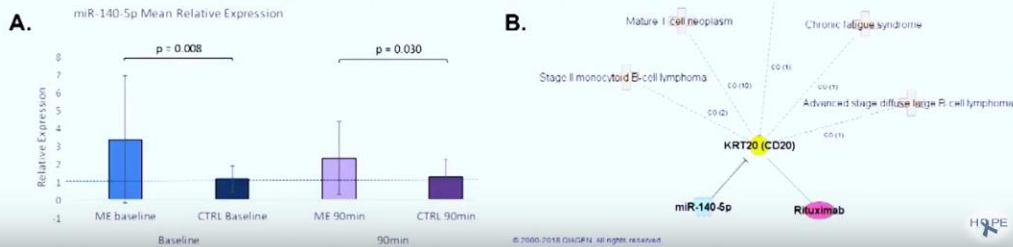
So this miR is elevated at baseline in many patients, not all of them, and after stimulation. But this miR is targeting membranous receptor called CD20 which is the receptor targeted by Rituximab. So when I saw that result I thought that probably Dr. Fluge would be interested and we need to talk. This week we agreed that it would be worth it to assess his patients in a clinical trial, to see which patient have a low level of this miR that could be good candidates versus the ones that have high levels of this miR that could not be good candidates for Rituximab. If you remember the presentation of that, one third of the patients [are] non-responders. I don't know, I don't know his patients but this is part of this meeting. We are engaged now in a possible collaboration to further examine each patient to understand why the non-responders are possibly the ones that produced that.

So just to show you the link. This is the value of individual patients.
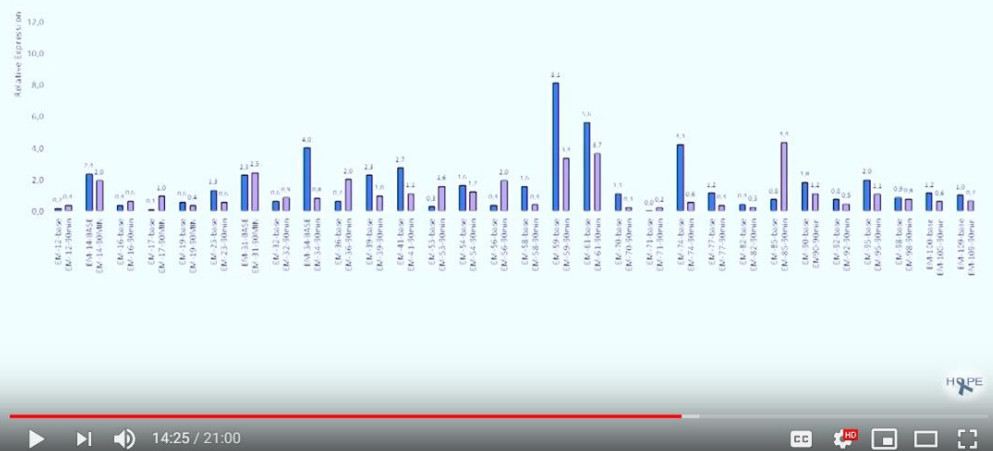
So you have in blue at baseline and in purple after stimulation. You can see that, not easily but if you count them, 27% at baseline show at least a two fold over expression of this microRNA. So I don't know yet if two fold is enough to disqualify the patient but that with a group of patients, I wouldn't consider for a Rituximab. But the other ones—you see the patients with very low level, that could be potentially someone that could respond better to Rituximab treatment. So this is something that we need to explore and through collaboration. It's the only way to assess that.

Another miR, miR-150-5-P:
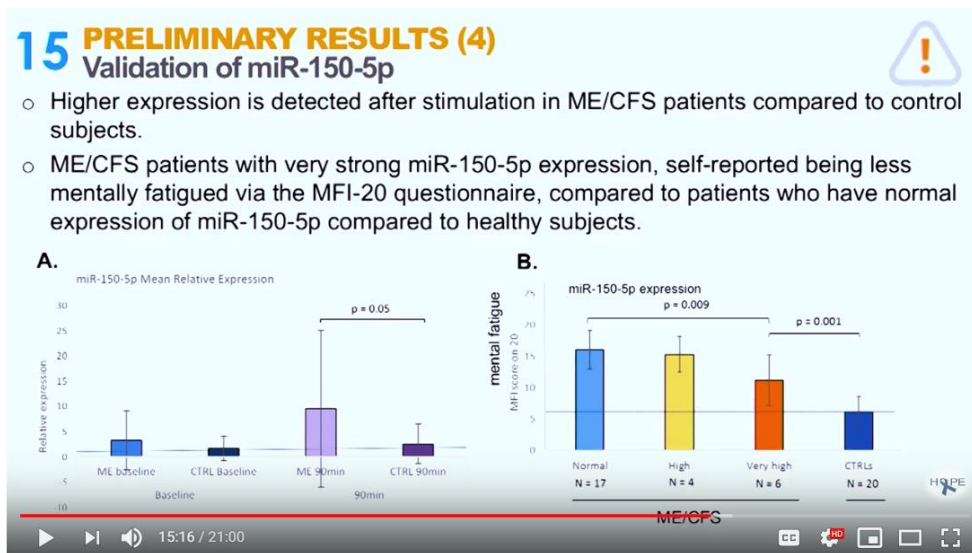
Again, we saw a very, very strong over expression only after stimulation. And we try to assess or qualify the patient. What is special to the patient having this high level? And we saw that patients having very high levels of this microRNA are the one that have the better mental fatigue score. So is going against the concept of stressing the patient. Those stressed by the stimulation at least for this microRNA, seem to have a better improvement of the mental fatigue. Very interesting.
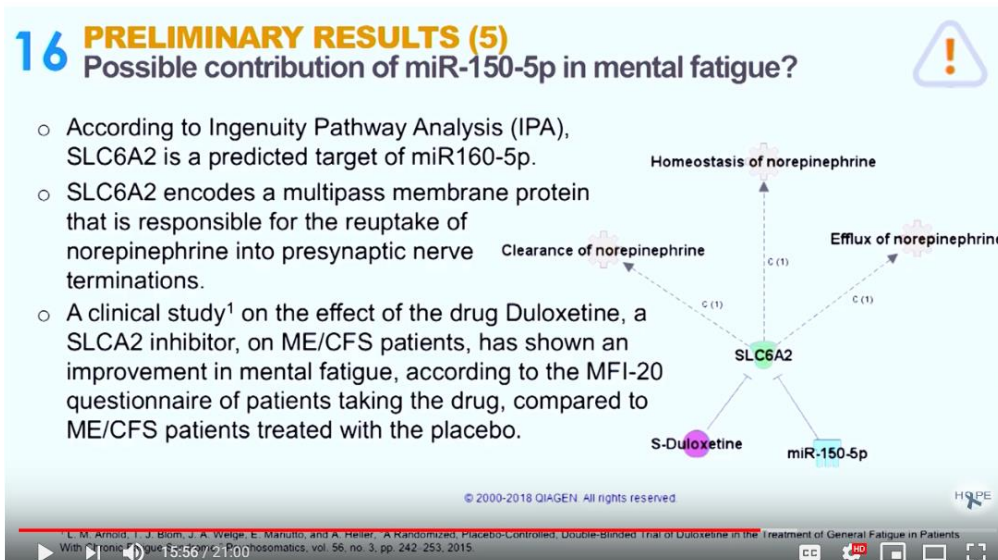
It becomes even more interesting because this miR is targeting a gene called SLCA2 which encodes a multipass membrane protein that is involved in the reuptake of norepinephrine.

The EPA pathway automatically gave us also other molecules that do the same thing. Duloxetine was a drug that's been tested in ME/CFS patients before I knew what was happening and they end up with the same conclusion, the duloxetine is not improving fatigue but is improving mental fatigue as well. Different cohort, different strategy, same conclusion. So again that further strengthened the value of what we are doing.
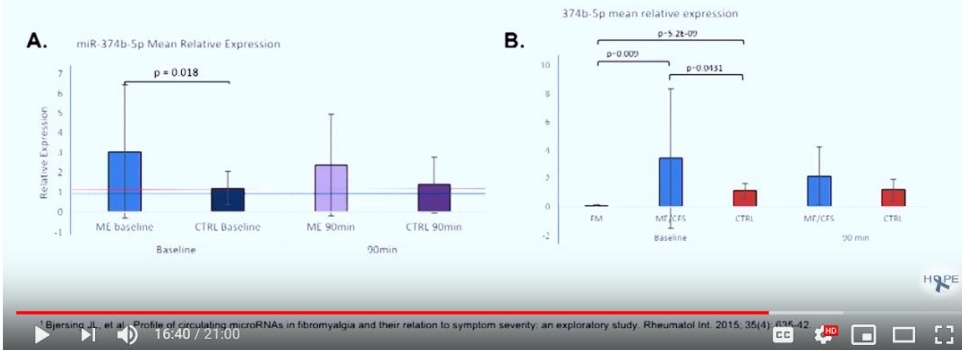
Another miR that is this one is interesting is this miR-374b-5p. It is very low in fibromyalgia patients and it is going in the opposite direction in ME/CFS patients. So possibly this is a way to clearly differentiate at the molecular level between ME/CFS and fibromyalgia. So very interesting molecules.

Now this is where the kind of fun things happen.

18 MOLECULAR PROFILING OF ME/CFS
Stratification of ME/CFS patients using circulating miRNAs?

ME/CFS subgroup 1

29a-3p	127-3p	140-5p	150-5p	181b-5p	374b-5p	486-5p
-4,13	-20,4	-4,1	40,6	-13,8	-22,1	-65,6
-40,90	-73,5	-49,0	54,9	-19,3	-111,1	-68,1
-18,91	-15,4	-25,2	74,2	-1,8	-28,7	-39,2
-13,09	-32,5	-19,1	50,4	1,9	-42,1	-16,2
-25,32	-28,9	-37,1	72,3	-3,9	-71,3	-4,6
-11,15	-33,5	-15,3	3,5	-12,8	-9,5	-25,0
-2,20	-27,5	-18,0	7,9	-28,3	0,0	-10,8
-12,71	-40,6	-26,7	10,9	-21,1	-16,2	-63,8

ME/CFS subgroup 2

29a-3p	127-3p	140-5p	150-5p	181b-5p	374b-5p	486-5p
5,66	-12,4	-11,4	3,6	17,3	-12,3	-38,7
7,52	-9,0	-6,8	33,4	14,8	-4,5	-41,4
8,59	-10,1	-1,6	49,8	18,8	-3,8	-18,9
6,86	-9,3	-7,4	40,8	1,9	-2,0	-23,0
12,73	-19,0	-12,3	55,9	16,3	-16,1	-68,3
17,24	-20,6	-9,4	54,4	19,6	-8,6	-31,3
5,32	-12,6	-11,2	41,4	-10,1	9,8	-50,0
32,82	-23,4	-5,5	83,0	-28,7	9,6	-96,7

ME/CFS subgroup 3

29a-3p	127-3p	140-5p	150-5p	181b-5p	374b-5p	486-5p
16,98	10,4	23,5	37,7	14,1	7,7	7,3
35,09	25,6	29,9	53,4	28,5	32,2	58,3
42,16	12,3	24,7	103,6	25,9	23,9	26,3
0,63	0,8	1,6	51,4	-1,3	4,9	20,8
30,28	22,2	18,8	73,7	23,0	10,9	-42,6

ME/CFS subgroup 4

29a-3p	127-3p	140-5p	150-5p	181b-5p	374b-5p	486-5p
-1,95	-1,7	9,1	-10,1	2,3	8,2	8,4
-1,26	10,7	7,7	-53,6	12,0	7,6	-1,8
15,48	17,1	24,7	-94,1	6,3	24,5	2,0
5,54	3,9	4,8	-20,5	10,8	-3,2	-67,0

17:01 / 21:00

Each line, so you have seven microRNA that we are considering. Each line represents a patient. Forget about the number and the number was not really important. But the yellow is a diminution when it's stimulated, in the blue it's a positive effect, so the stimulation increased this microRNA. You can see the very specific patterns that allowed us to classify patients in four specific subtypes. I'm not telling you that this is the final version of the whole thing but this is the beginning of something.

Now why doing this molecular statification that's the key question.
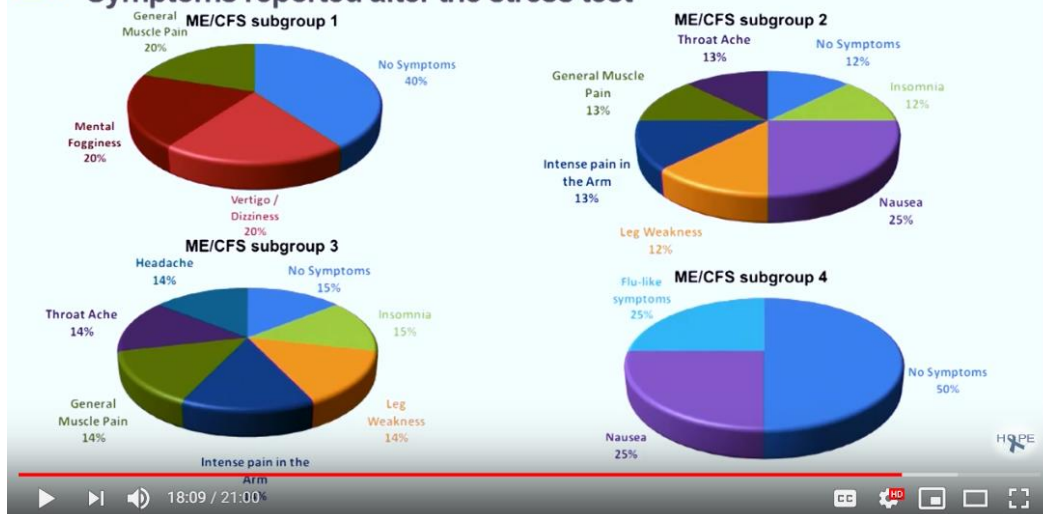

19 MOLECULAR PROFILING OF ME/CFS
Level of post-exertional malaise, post-test

Legend:
- Tired the day of the test but fine the next day
- Fatigued
- Tired the day of the test and the next day
- Fatigued and in pain
- In shape after the test and day after but tired the following day
- Relapse following the test
- No symptoms

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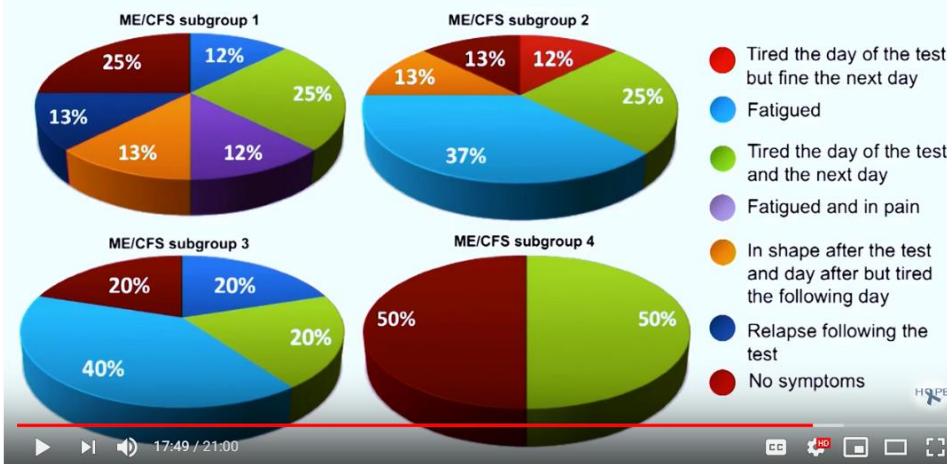
Why? Because the subgroup one when we ask the patients following the test what are the symptoms of post exertional malaise, that's the one that report the worst symptoms at the level of post exertional malaise, as opposed to the subtype number four which show at most, half of them no symptoms at all.
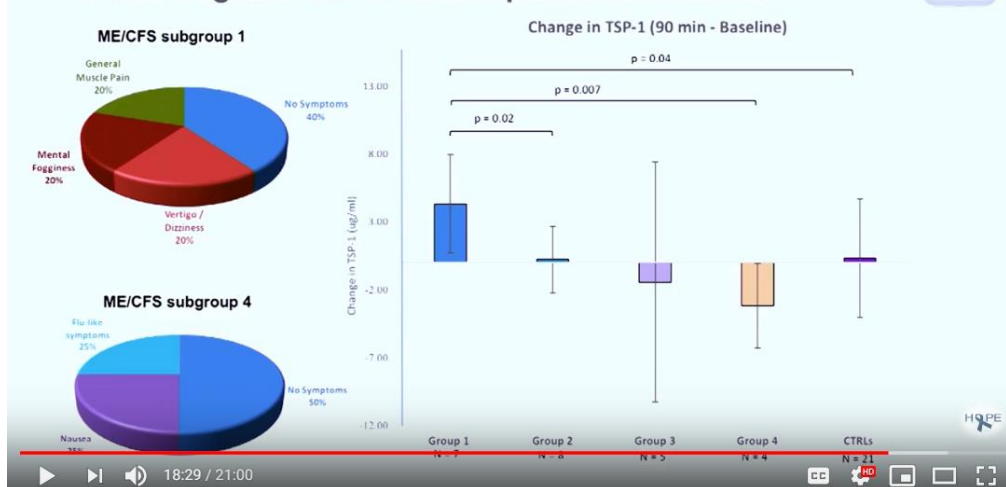
If we ask for other symptoms, the group one shows mental fogginess and vertigo dizziness. Very interesting, as opposed to again the number four which seems to have less symptoms or no symptoms at all—very interesting.

So doing that we look about to revisit other biochemical factor that we have identified in the past. I put up the first pie chart is the group one, the last one is group 4. And you see looking about a factor called thrombospondin-1 which is an anti-angiogenic factor. So TSP-1 is more elevated after the stimulation in group one. And it could contribute to a kind of vasoconstriction in those patients which explained why they feel a mental fogginess because you have some effect on the brain blood flow. As opposed to the group 4 which have less symptoms, is going in the opposite direction. What is very interesting is thrombospondin-1 is known to act through two receptors. Now we could actually start in vitro experiments to validate. If I block one receptor or the other one with an agonist or an antagonist I can either mimic or prevent these effects. Don't

start to look at the literature and try to have those drug and experiment on yourself. We don't know yet to what extent, but this is the beginning of something. That's why I put a warning on my slide. So where we are going. We need to further validate the remaining microRNA in a large ME/CFS cohort because right now we can split them in four subtypes.

We need also to validate in other cohorts. The key word here is replicate, replicate, and replicate. But I think we are on to something and the only way that we can bring a clinical value of what we're doing is through collaboration. We need to work and this week was an incredible week because now we establish some collaboration and we can start to assess the validity and utility of this new molecular statification.

Finally I would like to acknowledge special thanks to all the patient participants and healthy volunteers that also are participants to our test because the test is a bit demanding. It's at least two hours. And also thanks to the support to the many advocacy groups that support our work. Similar is the foundation for their incredible funding and a big thanks to my staff and graduate students and collaborators, that without them we, I, would not be here today. So many thanks for your support. Thank you.

Thank you to the volunteer transcriber for this transcript.