Wenzhong Xiao, PhD | Results from the Severely Ill Patient Study (SIPS)

https://www.youtube.com/watch?v=_N1o2gbaCl4&index=8&list=PLl4AfLZNZEQPxjqF4ojAO3wdCFMeriNBK&t=1s

Raeka Aiyar: I'm now happy to welcome up our next speaker Wenzhong Xiao who is split between Stanford here at here in Ron's group at the Genome Technology Center and Massachusetts General Hospital as well as Harvard Medical School, so he's in a lot of places, and we're happy that he's in this place right now.

Ron Davis: Yeah, it's always a problem to know, "Where is Wen?" We call him Wen because I can pronounce that, and I've known him for a very long time, and he has some really unique capabilities, and one of them is he is an expert in physical chemistry, so he understands the details of how measurements are made and also one of the problems in those measurements. And he also is an expert in statistics so he can analyze the data. And he's been often asked to do a very large number of complex analysis. I know that the National Bureau of Standards called NIST now uses him a lot and he's recently told me last night that the FDA has given a lot of their data to help him out of course they don't have any money to pay him, because it is the federal government but he's greatly underrated, I think, and he's very humble. It's a light to continue to work with him over these years, so thank you Wen.

Wenzhong Xiao: First I'd like to thank everybody who have, especially the patients and the caregivers, who made the experts come here, and it's honor for me to stand in front of you and share with you some of the results that we produced in the past year. So as Dr. Fluge already mentioned, we in this room all know that ME/CFS is a serious disease affecting multiple body systems. Dr. Fluge mentioned the immune system, the metabolic system, and obviously the central nervous systems. So it has been known I think for a long time that it's likely the dysfunctions or dysregulations between all these systems. For example, the the so-called HPA axis, the immune cells, and the gut microbiome, the the muscle system of the patients that ought to work together and perhaps give rise to the symptoms that we see in the patients.

Therefore, the first study that was conducted by the Genome Technology Center under Ron's leadership and supported by Open Medicine Foundation is this Severely Ill Patient Study (SIPS) where we selected a few patients who had, you know, severe illness and compare that with the normal controls. And the idea there is to carefully look over all the possible measurements that are available today from the genes to proteins to metabolites to the function of themselves the gut microbiome and the functions of their organs and tissues and environmental exposure and their clinical records and try to identify perhaps a core set of features that can then be followed in a bigger study, and if these findings or subsets of these findings will be verified, they might lead to new discoveries and eventually better treatments.

So, a lot of people at Stanford as you can see there, as well as Open Medicine Institute and UCSF participated in this study. So, because of the time I wouldn't be able to go through all the findings, but in order to facilitate the collaboration, you know, with the research community, we

set up this website endMECFS.stanford.edu that has all the data and results that generated to date, and I think there are a few hundred researchers that have received access to this data set, and if anybody here or, you know, over the Internet would like to look at this data please contact us. It's just this registration process.

So, in terms of analysis there are three pieces that were considered. One is again comparing patients with controls to see whether there is a consistent difference which is shown in the middle, and on your left side we can also compare results from different measurements on the same patient to see whether, for example, at gene level there's a variant that could explain what you see in the metabolite level or, you know, functions of an immune cell for that particular person, so that's basically a cross-dimension analysis. And the third one which is shown on your right side because this is Silicon Valley, there's a large collection of machine learning statistical learning artificial intelligence tools that one can use to try to integrate all this data together and try to identify a few features that might be able to best explain what we see in patients as symptoms. Obviously the last one is still preliminary because we so far only looked at a few severely ill patients so if those predictions can be verified in a larger study then perhaps those can be used as either biomarkers or potential targets for therapy development

So as Fluge already mentioned, SF-36 is one of the ways to measure the physical impairments of the patient, and if we look at the score of these severely ill patients which are on the upper left side comparing to the controls which are on the lower right side. And there's, you know, a collection of different diseases where their SF-36 scores was recorded. You can see that patients and controls are clearly separate from each other, and, you know, also from other major diseases, and it's clearly different comparing to for example the scores of patients with depression.

So we then put the patient's, you know, on Fitbit and tried to measure their physical impairments. This is just one measurement which is a number of steps it takes for the patient versus the controls, and as expected these patients are mostly homebound and a lot of them are bedridden, so the number of steps they take obviously is very, very different than a healthy person. We also put those patients as well as controls under sleep monitoring, and as you can see here, for example, REM latency stage 3 latency between patients and controls, and the patients are in orange and controls in blue, you can see that typically it took much longer time for the patients to get into deep sleep, for example, and that might explain some of the, you know, problems that the patients feel.

So in terms of molecular mechanisms, the first thing we looked at was the infectious agents and exposure in these patients because that's the obvious place to look. And so I think Pei Dong, who is in the audience, he developed tools to sequence specifically 20 common viruses in humans, and we applied his tool to study patients and the controls and as you can see here, that's the number of positives in patients and in the controls. For example, for EBV there's one out of 20 patients that were tested positive and the same number of controls have tested positive so the bottom line is that we didn't see any significant enrichment in any of those 20 common viruses.

The second thing we did was in collaboration with UCSF. We tried to isolate the viral particles from blood and then conduct DNA sequencing, so that's a more shotgun approach, and the

most significant signal is what you can see in the down lower right corner, that's the anellovirus we know that another virus is perhaps the most dominant virus species in human, and you can see that for most of the patients and controls you do see that virus, but we don't see an increase in load of this virus or any other virus that we studied. And we also did antibody antigen tests of a few viruses as shown there and none of them were significantly different between patients and controls. In terms of bacterial infections, Lyme, Bartonella, and mycoplasma were tested, and again we didn't see a difference between patients and controls. In addition, heavy metals in urine were measured, and we couldn't see a difference either, so it seems like perhaps it's the human response to external stress that might contribute more to these disease, you know, at least from this particular data.

We then looked at the clinical tests. There were about 200 some clinical tests conducted for each patient and control. This is probably one of the most different results between patients and controls, and that's the morning cortisol level. We know that in in normal people you would have a high cortisol level in the morning, and that gradually goes down over the day, which is what you see in the blue line here, which are the controls for this study, but you see a much flatter response in the patients. We next looked at the cytokines. Since these are severely ill patients, so perhaps as expected, their cytokine response is much stronger comparing to some of the other published studies where their patients are not really limited to severe patients, so shown here are four cytokines that showed the biggest difference. The gm-csf, leptin, and the cxcl5 or ene78, these were reported in multiple publications before, and again the difference here is just the magnitude of change in severe patients comparing the controls is bigger than what was seen before.

One of the new findings that as far as I know hasn't been reported is this BDNF, which is brain derived neurotrophic factor, and it's very extensively studied in a number of neurological diseases such as Parkinson's, Alzheimer’s, etc. and it's a well-known marker for neurological diseases. As you can see there the patient level is about threefold less than controls. We know that BDNF is involved in the growth, the differentiation, and the maintenance of nerve cells, so this might indicate that there is a neurological component that we should look into. We actually also compared data from NASA and other space agencies where they tie people in bed for you know a few days to a month and measure, you know, different parameters in those, you know, normal people, and we did not see a decrease of this molecule in those studies, so this apparently is potentially unique to our study.

In terms of the metabolites in the plasma of the patients comparing to the controls, these are the three metabolites that showed the biggest difference between patients and controls. Indolepropionate, which is also called IPA, is also a known neuroprotective factor, and as you can see on the left side the patient level is much lower than the controls. Lysine and hydroxyproline were higher in patients than healthy controls. Dr. Fluge talked about some of the amino acid dysfunctions in patients, so we're trying to look into this further. Hydroxyproline obviously is related to proline which is a major component of collagen, for example. So because of time I’ll only talk about the indolepropionate because it's known that it's made in the gut by the gut microbiome so it's made by specific microbes in the gut from tryptophan to indolepropionate, and that goes through the gut barrier and gets to the brain, and it's neuroprotective, so in our patients of severely ill, the level of this particular molecule is much lower compared to the controls.

So that leads to us to look into the gut microbiome between patients and controls. So each, you know, orange or red dot there shows one patient and the blue dot there again shows the controls so you can see that the, you know, the general pattern of the microbiome in patients is much more diverse than the controls, which is more gathered together. So if we look at this specific species between the patients versus controls I think the biggest difference as you probably can see here in orange are those significant increased Verrucomicrobia in a subset of patients and these patients are mostly male. Comparing to any of those controls, we're still trying to figure out the biological, potential biological implication of this and why it only occurred in in male patients.

So we then did whole genome sequencing of these patients just to see whether there's a genetic component that might contribute to the symptoms in these patients, and this is just a very brief list of what we found that might be worthwhile in a bigger study to perhaps, you know, try to verify these findings. So top one in list is killer-cell immunoglobulin-like receptors and as it's well known that the NK cell functions in patients probably it's different than the controls, and it's interesting to see that a large number of NK cell immunoglobulin-like receptors of different in patients comparing to the general population in the U.S. Neurexin is another gene that's studied in a number of neurological diseases, OCD, you know, etc., and that's another one that we're trying to follow up.

The next two, dynein and FAM20C, which is Golgi-associated secretory pathway kinase, these two are known to be pathogenic or likely pathogenic so that's why we were trying to verify this in a bigger study. The last one, there's not much known about that gene, but there's a number of variants that look suspicious of the POTED, it's a membrane-associated protein and we're trying to do targeted studies on that one as well.

Since there was some suggestion of calcium channel involvement in this disease, we actually looked at those specific genes that were reported before, and unfortunately we couldn't see significance in that gene and associated genes. So, this is just a reminder, you know, just a sort of brief discussion of the killer-cell immunoglobulin-like receptors. They're important because they're on the surface of NK cells, and they work with the HLA genes to either activate or inhibit the NK cells, and the red boxes there show those genes that are different between patients and controls, and we're in collaboration with the Stanford Blood Center to do targeted sequencing of this group of genes together with HLA genes and try to see whether we can learn more about these genes.

And I just said in the beginning, since this is, you know, Silicon Valley so, you know, you do a bunch of, you know, machine learning and artificial intelligence you would try it. We will be able to identify a set of variants that would best explain a patient's symptoms, and, you know, again this is a preliminary. So because of time I'll just show you a blow-up version of part of this so you can see CFS is in the middle and the SF-36 parameters, Karnofsky score is another measurement of the physical function of the patient, and they, you know, they're expected to be strongly linked to the disease because they're used. And you see BDNF, the indolepropionate that are most connected to different aspects of SF-36. And you know tryptophan is down here. That's another molecule I think Dr. Phair will talk about, and the lysine and the hydrolysine over here. So again, this is only based on these severely ill patients, and is preliminary. We're trying

to incorporate other studies into this network, and I hope perhaps in the future I would be able to update you a more concrete result in terms of what we know.

So with that I'll just talk about the next steps. On the immune system side we are doing sequencing of KIR and HLA which are the ones that, you know, are significant from our study, and continue identification of the pathogen-associated molecules, basically the RNA viruses, for example, and see whether, you know, that's potentially different between patients and controls and the damage-associated molecules which are the host response between patients and controls. On the metabolism side we are doing a muscle biopsy of patients after exercise at Boston and the Dr. Tompkins will talk more about that I think later today. And together with molecular imaging looking into the central nervous system of these patients, and a number of speakers today will actually talk about more about either the metabolism side and the central nervous system side, and I think working together with all the people in this room and over the Internet, hopefully we can learn the big picture of this disease, and perhaps can identify a potential cure of this disease. And with that I'd like to thank you for your attention.

[Applause]

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