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Highlights of Professor Anthony Komaroff's webinar "HOT AREAS IN ME/CFS RESEARCH" on

24th May 2018 for The Solve ME/CFS Initiative

Margaret Williams **4th June 2018**

https://www.youtube.com/watch?v=VprqU9knS4Y

Note: For the sake of clarity, a few words/phrases (eg. repetitions) have been omitted from actual quotations in Professor Komaroff's webinar. Any errors in this text are the fault of the author but the emphases are Professor Komaroff's own.

INTRODUCTION

Anthony Komaroff MD is Professor of Medicine at Harvard Medical School, Boston, Massachusetts, and Senior Physician at the world-renowned Brigham and Womens' Hospital (located adjacent to Harvard Medical School and one of the top US hospitals). For 35 years he has cared for and studied patients with ME/CFS; he has served as an advisor on the illness to the NIH, CDC, the US Surgeon General, and to the IOM/National Academy of Sciences.

His 2018 webinar is divided into sections:

1. CONTROVERSY ABOUT ME/CFS
2. STUDIES OF THE BRAIN
3. STUDIES OF THE IMMUNE SYSTEM
4. STUDIES OF ENERGY METABOLISM/MITOCHONDRIA
5. STUDIES OF THE STRUCTURE & EXPRESSION OF GENES (EPIGENETICS)
6. STUDIES OF THE MICROBIOME
7. QUESTIONS & ANSWERS

Professor Komaroff's take-home message is his conviction that, whilst there is still no accurate diagnostic test for ME/CFS, there are now many biomarkers and that those biomarkers provide objective, measurable evidence of underlying biological abnormalities in ME/CFS.

At the end of his webinar, Professor Komaroff said: "I think we can say now, on the basis of 35 years of research, that the illness is not simply the expression of physical, somatic symptoms by people with a primary psychological disorder; in fact,

thousands of studies show that there are robust underlying biological abnormalities in the brain and autonomic nervous system, in the reaction to exercise, in the immune system, in energy metabolism, gene expression and gene structure, and differences in the microbiome".

He continued: "Why am I optimistic? I think back to 30 plus years ago when many of us began to care for patients with ME/CFS and to study the disease. It was a wasteland then. Practically nobody had heard of the disease, including most doctors, and those doctors who had were generally very sceptical that it was a disease; frankly, those of us who did think it was a real disease caused by underlying biological abnormalities really didn't have a lot of evidence for that then. Today we do".

SECTION 1: CONTROVERSY

Professor Komaroff began by saying:

"Hello everybody. About a year and a half ago the Solve ME/CFS Initiative asked me to do a webinar on hot areas of ME/CFS research. A lot has happened since and I want to focus on a few of the many recent studies that seem to me to have the most promise for understanding this illness and for getting us to our ultimate goals, which are a highly accurate diagnostic test and a cure".

SLIDE: CONTROVERSY: "IS ME/CFS REAL?"

- In an illness defined exclusively by subjective symptoms, is there evidence of objective underlying biological abnormalities?
- Could those biological abnormalities theoretically explain the symptoms?
- Do the abnormalities in fact correlate with the symptoms?

"Why has this illness been controversial?"

"I think it was controversial because it was at least initially defined exclusively by subjective symptoms, and so any doctor or scientist is going to ask is there evidence of objective, measureable underlying biological abnormalities in the people with these symptoms? Fair question. And if there are, could those biological abnormalities theoretically explain the symptoms, and if they could, do the results of the studies show that in fact the abnormalities correlate with the symptoms? Those fundamental questions have been pursued in a lot of different ways".

"Doctors often say they did all the usual standard laboratory tests and nothing turned up. That is true, but there are a few standard laboratory tests that in fact

dramatically distinguish patients with this illness from healthy subjects of the same age and sex".

SLIDE: **COMMON LAB ABNORMALITIES IN ME/CFS**

A Case Control Study Involving over 20,000 Laboratory Tests in Over 700 Patients in Two Geographic Areas Over 10 Years

	Odds Ratios	95% C.I.	P-value
• Immune Complexes	26.5	3.4 – 206	= 0.002
• Immunoglobulin G	8.5	2.0 – 37	= 0.004
• Atypical lymphocyte count above 2%	11.4	1.4 – 94	= 0.03

Reference: Bates DW, Komaroff AL et al. Arch Intern Med 1995:155:97

"This is a case-controlled study we did over 20 years ago. It was a big study – it involved 20,000 laboratory tests from over 700 patients in two different geographical areas over ten years. We wanted to wait until we had enough data to say something definitive. Three standard laboratory tests clearly discriminated -- distinguished – between the patients with the illness and healthy subjects. They were effectively biomarkers".

"But today we are going to be talking about – in contrast to such standard laboratory tests – more esoteric potential biomarkers that are turning up in a variety of research studies".

"Those involve studies of the brain; the effects of exercise and post-exertional malaise -- important because the post-exertional malaise is a symptom that is at the core of this illness; studies of the immune system; studies of energy metabolism and the mitochondria; studies of the structure and expression of genes, and then studies of the microbiome".

"Those are the hot areas that I want to focus on today".

SECTION 2: STUDIES OF THE BRAIN

SLIDE: **CNS INVOLVEMENT IN ME/CFS**

- Neuroendocrine dysfunction: impairment of multiple limbic-hypothalamic-pituitary axes (involving cortisol, prolactin and growth hormone) and serotonin (5-HT) system
- Cognition: impairments in information processing speed, memory and attention, not explained by concomitant psychiatric disorders
- Autonomic dysfunction: impaired sympathetic and parasympathetic function in 30-80%
- MRI: multiple abnormalities
- SPECT: areas of reduced signal
- PET: immune cell activation (neuroinflammation)
- EEG abnormalities: sharp spike waves, distinctive spectral coherence pattern, impaired connectivity

"First let's start with studies of the brain. The brain and nervous system in general have been studied in a wide variety of ways, with well over 1,000 published studies. The hormones in the brain, the neuroendocrine system of the brain, has been shown in a variety of ways to be different in patients with this illness. Thinking is affected in this illness, particularly impairments in information processing speed, memory and attention. The autonomic nervous system which controls the vital functions of the body like heart rate and blood pressure is impaired in the majority of studies – both arms of it. MRI studies show multiple abnormalities – MRI now can look at the brain in a variety of different ways and all of them that have been reported find abnormalities. SPECT scanning is another way of looking at the brain, primarily at blood flow in the brain, and the metabolism of brain cells. PET scanning similarly – in some ways better than SPECT – can look at a really critically important issue, which is whether the immune system of the brain is activated in patients with this illness and I'll show some of the data about that. Finally, studies of brain waves – EEG – also have been shown to clearly distinguish patients with this illness from healthy individuals and from individuals with major depression".

SLIDE: ION CHANNEL ABNORMALITIES IN ME/CFS

- 115 cases v 90 non-fatigued controls
- 240 polymorphisms (SNPs) for 21 ion channels of the transient receptor potential (TRP) type
- Results find 13 polymorphisms significantly different in CFS cases, most of them in the TRPM3 ion channel

Reference: Marshall-Gradisnik S et al. Immunol & Immunogen Insights 2015:7:1

"Recently there have been a series of studies from a laboratory in Australia that have found abnormalities in ion channels. These are very important in how cells function, particularly in cells of the brain. They are the most interesting brain-related studies I'm aware of recently, but they need to be replicated in other laboratories".

SLIDE: FATIGUE & PAIN-SENSING MOLECULES: NORMALS v ME/CFS, Post-Exercise

Sensory: Ion channel receptors / Adrenergic / Immune
Reference: Light AR et al. J Pain 2009:10:1099
White AT et al. Psychosom Med 2012:74:46

"This is a study that's not new. It's one way of looking at what happens with exercise in patients with ME/CFS. They measured in white blood cells a bunch of different molecules, some involving ion channels, some involving the autonomic nervous system, and some involving the immune system. They asked patients with ME/CFS and healthy people of the same age and sex to exercise on the standard exercise test. When the healthy individuals did that and all these molecules were measured at baseline before the exercise began and then at 30 minutes, 8 hours, 24 hours and 48 hours after exercise, the levels of a few of these molecules bumped up after the exercise, but there wasn't a really big difference: exercise didn't seem to change things very much from baseline. And then they did the same tests in patients with ME/CFS. What they saw was obviously a dramatic difference. Many of their molecules shot way up following exercise and remained up two days after the exercise had been completed. What does it mean? Why did that happen? I don't think we know, but is it an objective, measureable difference between the patients and their healthy counterparts? I think it's pretty clear".

SLIDE: PET EVIDENCE OF BRAIN INFLAMMATION DISTINGUISHES CFS FROM HEALTHY

Reference: Nakatomi Y et al J Nucl Med 2014:55:945-950

"This is a PET study conducted in Japan that was one of the first times that a technology allowed us to look at whether the immune system, particularly the innate immune system in the brain, was activated. It's very hard to study the chemistry that's going on inside the brain of a living human being, but the PET technology allows you to begin to do that. Specifically, they used a marker, or a tracer, that attaches to a ligand and what they found was that the levels of this tracer were much higher in the brains of patients with ME/CFS than in healthy individuals of the same age and sex, so – a dramatic difference, a clear difference. On the other hand, (the study used) a relatively small number of patients and healthy people and it hasn't yet to my knowledge been repeated by another laboratory and needs to be, but it is important because it's consistent with one of the theories as to why the symptoms of this illness are caused".

EFFECTS OF EXERCISE / CAUSES OF POST-EXERTIONAL MALAISE IN ME/CFS

SLIDE: ABNORMALITIES OF MUSCLE IN ME/CFS

- Central sensitisation: Decreased pain threshold, generalised hyperalgesia
- Increased reactive oxygen & nitrogen species: eg. ↑ TBARS (products of lipid peroxidation)
- Mitochondrial dysfunction: Reduced levels of succinate reductase, cytochrome-C oxidase & Co-enzyme Q10
- Bioenergetic dysfunction: ↓ proton efflux after exercise; ↑ intramuscular acidosis with exercise

  Reference: adapted from Rutherford G et al. J Aging Res 2016

"Now what about the effects of exercise and the basis for the post-exertional malaise? There are many different studies that have been done on muscle in people with ME/CFS, both the muscle at rest as well as muscle following exercise".

"There is now pretty strong evidence for different kinds of abnormalities that involve muscle in the nervous system's sensing of what's happening in muscle. There is a central sensitisation of pain in people with this illness, that is, people with the illness feel pain more easily".

"There are increased reactive oxygen and nitrogen species in the muscle of brain and measureable also in the blood: mitochondria – the parts of the cells that generate energy – are functioning adversely as reflected by a number of different ways of studying them, and the energy production itself is reduced, as reflected in several different ways of studying them".

SLIDE: **CENTRAL SENSITISATION IN ME/CFS:**
PRESSURE PAIN THRESHOLD PRE-AND POST EXERCISE
Reference: Malfliet A et al. Pain Physician 2018:21:E13-E24

"This is one study, the latest study this year, and it corroborates what several previous studies have shown about central sensitisation. These are both patients with ME/CFS (blue bars on slide) and healthy individuals of the same age and sex (orange bars on slide) both before they exercise and after they exercise. The height of these bars is how much pain they experience to a certain degree of pressure. The higher the bar, the more the pain. At baseline, the patients are more pain-sensitive than their healthy matched controls. Following exercise, whereas pain thresholds go down in healthy people, they go up in the patients with ME/CFS and the difference in pain sensitivity in the patients and the healthy controls becomes much greater. Several studies have shown that; this is just the latest".

SLIDE: **MUSCLE ACIDOSIS DURING EXERCISE:**
Determined by MR Spectroscopy
Reduced anaerobic threshold causes ↑ lactic acid
Reference: Jones DEJ et al. Eur J Clin Invest 2012:42:186

"This is a study of what happens to the acid level in muscle following exercise. pH is a way of measuring acid: the lower the pH, the more the acid. At rest, there's no real difference in the amount of acid in muscle of healthy control subjects and patients with CFS but following exercise, the pH plummets, which means acid levels go up in the muscle; the reason is that the patients are making more lactic acid in their muscle because they have shifted to anaerobic metabolism from aerobic metabolism at a much earlier stage in exercise than healthy people".

SLIDE: **EFFECT OF EXERCISE ON SYMPTOMS**
Change in sum severity of 10 symptoms post-exercise
Reference: Cook DB et al. Brain, Behaviour and Immunity 2017:62:87

"It's not just pain that's different following exercise in patients with ME/CFS. This study looked at ten different symptoms of ME/C FS including pain but not limited to pain, summed them all into a single score and measured the change pre-exercise versus post-exercise. (In) the healthy controls, this score of symptoms went down a little bit following exercise in the people who were healthy, but in patients with ME/CFS, following the exercise, that score of symptoms went way up".

SLIDE: **EFFECT OF EXERCISE ON COGNITION**
Reference: Cook DB et al. Brain, Behaviour and Immunity 2017:62:87

"Here's a way of studying thinking, cognition itself, with exercise. This study used a test call PASAT, which is a test primarily of information processing speed, and they did the test three times, both before the exercise and three times after the exercise, both in patients with ME/CFS and in healthy control subjects".

"At the first test, the results were not very different between the patients with ME/CFS and the healthy individuals. (When) they tested pre-exercise, the number of errors started to go up in the patients and with the third time it went up even more, whereas in the healthy people, the number of errors was actually going down. Then, following exercise, the number of errors in the patients went up even higher, whereas it remained lower in people who were healthy. So – a clear difference, both before exercise, but especially after exercise, between patients with ME/CFS and healthy people of the same age and gender".

SECTION 3: STUDIES OF THE IMMUNE SYSTEM

SLIDE: PLASMA CYTOKINE LEVELS CORRELATE WITH SEVERITY OF SYMPTOMS

51 cytokines, 192 cases and 392 healthy controls

Reference: Montoya JG et al. PNAS 2017:114:E7150-E7158
Komaroff AL et al. PNAS 2017:114:8914-8916

"What about studies of the immune system?"

"This is a very important study from Stanford, Dr Jose Montoya and Dr Mark Davis, one of the world's most eminent immunologists, published in the Proceedings of the National Academy of Sciences – a very large study, nearly 200 patients matched to nearly 400 healthy individuals of the same age and sex. In each of these people, 51 different cytokines were mentioned. The cytokines are chemicals that the immune system uses to orchestrate its attack when the immune system thinks there is something foreign in the body that it needs to wipe out. Cytokines have been of great interest to people studying ME/CFS for two reasons: we know that cytokines, particularly cytokines in the brain, when they are manufactured, cause many of the symptoms that people with ME/CFS have, including fatigue and many others, cognitive, mood changes, pain, so measuring cytokine levels has been very important because of the evidence that the immune system is activated in this illness, and that would mean that cytokine levels would be higher, and that could mean that those higher cytokine levels were causing some of the symptoms of the illness. So what they did in this study from Stanford was to measure cytokine levels in three different groups of patients: those who were the least severely symptomatic, those in the middle, and those most symptomatic, and they found that for 17 of the 51 cytokines that they measured, there was a clear correlation between the level of the cytokines and the intensity of the suffering. The higher the level of the cytokine, the more the suffering".

"You can see the same pattern generally throughout this whole spectrum of 17 different cytokines".

"This is important because if these cytokines are not just a marker for the disease but might actually be explaining some of the symptoms of the disease, you would expect their levels to correlate with the symptoms, and they appear to (do so) in this study".

SLIDE: CHANGES IN CYTOKINE LEVELS AFTER EXERCISE

- study of 24 ME/CFS patients and 24 healthy controls
- measurements of 51 cytokines/immune-related molecules pre- and post-submaximal exercise test
- statistically significant differnces in cytokine levels both at baseline and, particularly, after exercise

- the molecules that best distinguished ME/CFS from healthy subjects were IL-1β, IF-α, CD40L, CXCL1 and platelet activation inhibitor (PAI)

Reference: Mongehetti KJ et al. Sci Rep 2018:8:2779

"Since we know that exercise can produce a flare of symptoms in people with this illness, you'd like to think that exercise triggers a different cytokine response in patients with the illness that might explain the symptoms of post-exertional malaise".

"This is another study from Stanford, a smaller study, and they report that there is in fact an immune signature that is characteristic of ME/CFS patients following exercise compared with healthy people following exercise, and the cytokines or immune-related molecules that are most important in that signature are shown (on the slide)".

SLIDE: **CYTOKINES IN SPINAL FLUID IN ME/CFS**

Reference: Hornig M et al. Molecular Psychiatry 2016:21:261-269

- 32 ME/CFS v 40 MS v 19 age & sex-matched healthy controls
- 51 different cytokines measured in spinal fluid
- Highly significant differences between ME/CFS & healthy controls for most cytokines: ME/CFS often like MS
- "Consistent with immune activation in the CNS, and a shift towards a T-helper type-2 pattern"

"All those cytokines are cytokines in the blood, but if the cytokines are the cause of the symptoms of the illness, since it's the brain that experiences the symptoms, you would want to know what the cytokine levels in the brain were: that's hard to measure. The closest you can come is to measure cytokines in the spinal fluid (which) reflects – though not exactly – what's happening in the brain itself".

"In Columbia, the Centre for Infection and Immunity did a study of spinal fluid in these patients – the same 51 cytokines that the Stanford study employed, almost the same, not quite -- and they found that in the spinal fluid cytokines, just as had been seen in the blood, there were highly significant differences between the patients with ME/CFS and the healthy individuals".

"Indeed, the cytokine levels in the brain of the ME/CFS patients looked very similar to those they had measured in the spinal fluid of patients with MS".

SLIDE: **Antibodies to dUTPases in ME/CFS**

- dUTPases are proteins produced both by virus and human cells that activate innate immunity (TLR2 → NFkB)
- Antibodies to human dUTPase & EBV dUTPase are significantly higher in ME/CFS than healthy control subjects

Reference: Halpin P et al. J Med Virol 2017:89:1636

"Anther possibly related recent report that I found very interesting is this one: in the innate immune system, when it's activated, it can cause some of the symptoms of this illness when its activated in the brain".

"What activates the innate immune system? There are proteins called dUTPases that are made by some viruses and also made by human cells, and they activate the innate immune system. A team from Dr Klimas' group in Miami and from Ohio State – Marshall Williams' group – measured antibody levels to the human dUTPase and that of the EBV and found that they were significantly higher in patients with this illness than in healthy control subjects".

"When these dUTPases are given to animals, it provokes innate immunity and also provokes a lethargic behaviour that looks as if the animal is experiencing a state of chronic fatigue".

SLIDE: **Telomere Length & Implied Aging**

Reference: Rajeevan MS et al. J Transl Med 2018:16:44

- The length of the telomeres – the ends of the chromosomes in cells – is a sign of how rapidly the cells are aging
- CDC Report: in ME/CFS, telomeres are shorter

"Chronic activation of the immune system or chronic inflammation in the body also affects how rapidly the body ages and one measure of how rapidly a body is aging – or more accurately, how rapidly the cells of a body are aging – is in measuring the length of the telomeres. The telomeres are the DNA at the end of the chromosomes in the cells and in all of us. As we grow older, as our cells grow older, the telomeres shrink a little bit and keep shrinking with age, and so the length of a telomere is a measure of how rapidly cells are aging. Here is this report from the CDC of the length of telomeres in healthy individuals and in individuals with ME/CFS. A clear, statistically significant difference says that the cells of patients with this illness are aging more rapidly than healthy individuals of the same age. How much more rapidly? To the extent you can estimate this from human lifespan implications from cellular aging implications, the additional years of aging in the patients with ME/CFS compared with healthy people of the same age is 15 to 30 years".

SLIDE: **SUMMARY: THE IMMUNE SYSTEM IN ME/CFS**

- Something has activated several different parts of the immune system
- What has activated the immune system is unclear, but infectious agents are a plausible possibility in some cases
- Immune system activation in or near the brain and the nerves that come from it could explain many of the symptoms of CFS

"The immune system in ME/CFS has been activated – several different parts of the system have been activated. This has been shown by hundreds of studies going back 25 years. What has activated the immune system is unclear: infectious agents could be the trigger in some cases; it's also possible that activation in or near the brain could explain some of the symptoms of the illness".

SECTION 4: ENERGY METABOLISM, OXIDATIVE STRESS AND NITROSATIVE STRESS

SLIDE: THE ENERGY METABOLISM HYPOTHESIS

If the organism experiences a lack of energy, perhaps there is a defective energy metabolism at the cellular level

"About twenty years ago someone presented at a conference the theory that if human beings said they just lacked the energy they used to have, maybe the problem was that their cells were not making as much energy as the cells used to. My first reaction when I heard that was that it was pretty simplistic and unlikely to be true, but I was wrong. I think there's now abundant evidence that energy metabolism is adversely affected in patients with ME/CFS. One way of looking at that is the emerging science called metabolomics".

SLIDE: EARLY LESSONS FROM METABOLOMICS

- Studies employing optimal techniques (eg. mass spectrometry) for identifying levels of hundreds of metabolites in patients with ME/CFS compared to matched controls
- Levels of most metabolites are lower, as occurs in hibernation
- Abnormalities incriminate impairment of cellular energy (regulated by the availability of NAPDH)
- Possibly a defect in one critical enzyme – pyruvate dehydrogenase
- Defects in pathways converting sugars, lipids and amino acids into energy

References: Naviaux RK et al. PNAS 2016:113:E5472-80
Fluge O et al. JCI Insight 2016:1:e89376
Yamano E et al. Sci Rep 2016:6:34990
Germain A et al. Mol Bio Syst 2017:13:371-379

"We now have the ability to simultaneously in a blood sample or sample of other parts of the body measure hundreds and hundreds of different chemicals made in the body. If you did that in enough people with enough different chemicals or molecules, you can begin to look for patterns of metabolism in the person's body. You could imagine doing it twenty years ago but you never thought you'd be able to, but in fact we are".

"One of those studies – there are now five or ten of them published and many more under way – one of the interesting things found in several studies is that many of the natural chemicals in our body are being manufactured at lower levels than in healthy people, similar to what you see in animals who hibernate. Many of these metabolites that are lower are involved in energy metabolism. One study has fingered one particularly potentially crucial enzyme" (ie. pyruvate dehydrogenase – see slide above).

"Defects in converting all four of our major sources of energy have been reported, the four being oxygen, sugars, lipids and amino acids, so -- lots of emerging evidence that energy metabolism in the cells of people with ME/CFS is adversely affected".

SECTION 5: GENE STRUCTURE & GENE EXPRESSION (EPIGENETIC) STUDIES

"Another hot area: gene structure and gene expression studies. There are two ways that a gene can cause disease: if a gene is built wrong -- if the gene structure is wrong – that can cause disease. But even if the structure is perfectly normal, if the gene is not turned on and off appropriately – that's called gene expression – that also can cause disease".

SLIDE: **GENES (DNA) → mRNA → Protein**
Normal gene makes normal protein

"Let's just refresh your biology. Genes, made of DNA, when they are turned on, when they are expressed, make a messenger RNA that goes on to make a protein. When a gene is defective in the way it is built -- when there is a defect – it produces a different, defective, messenger RNA that produces a defective protein".

SLIDE: **GENE STRUCTURE STUDIES**

SNPs in genes involved in neurotransmitter regulation

Reference: Fukuda S et al. Life Sci 2013:92:183
Narita M et al. Biochem Biophys Res Comm 2003:31:264
Smith AK et al. Psychoneuroendocrinology 2008:33:188
Smith AK et al. Neuropsychobiology 2011:64:183
Sommerfeldt L et al. Acta Paediatrica 2011:100:293

SNPs involved in HPA axis regulation

Reference: Rajeevan MS et al. Genes Brain Behav 2006:6:167

SNPS involved in inflammation/immune response

Reference: Carlo-Stella N et al. Int J Immunopathol Pharmacol 2009:22:745

Smith J et al. J Clin Pathol 2005:58:860

Carlo-Stella N et al. Clin & Experimental Rheumatol 2006:24:179

Rajeevan MS et al. Human Immunol 2015:76:553

"Are there such defects that have been reported in the genes of patients with ME/CFS? Yes, there have been a number".

"They involve genes involved in the regulation of neurotransmitters, the chemicals in the brain that the brain uses to function. (There are) changes in genes that are involved in the HPA axis – this is one of the most important brain hormone axes that affects human functioning and any defect in genes that involve inflammation or the immune response".

SLIDE: EPIGENETIC (GENE EXPRESSION) STUDIES

- Disease is caused not just by mutated genes
- It is also caused by perfectly normal, non-mutated genes, when those genes are not "expressed" (turned on or off) appropriately
- Gene expression is controlled by many "epigenetic" forces
- Epigenetic studies are increasingly being done in ME/CFS v healthy controls

"Increasingly there are also studies showing that even when the genes are built perfectly fine, they are being turned on or off incorrectly in people with this illness – they are being expressed incorrectly".

SLIDE: GENE EXPRESSION STUDIES IN ME/CFS

Many studies have found abnormal expression of genes, particularly those that

make proteins involved in immune activation, energy metabolism and neurohormones

involved in the stress response, to occur more often in people with ME/CFS

Reference: Whistler T et al. J Transl Med 2003:1:1-8

Powell R et al. Clin Exp Allergy 2003:33:1450-6

Kaushik N et al. J Clin Pathol 2005:58:826-832

Kerr J et al. J Clin Pathol 2008:61:730-739

"For instance, many studies have found abnormal expression of genes involved in immune activation, in energy metabolism and in the brain hormones – neurohormones – that are involved in the stress response in people with ME/CFS".

"There are a variety of ways in which genes are turned on and off (by the cells). Four of the major ways are the methylation of genes (the DNA methylome), the expression of microRNAs, which control genes being turned on and off, the elaboration of transcription factors which are proteins that simultaneously turn on multiple genes, and HDAC expression – (this is) like a blanket draped over a gene that shuts it off from expressing itself, and all of the four have been shown to be abnormal in patients with ME/CFS compared with healthy controls".

SLIDE: EPIGENETIC CHANGES IN ME/CFS

Comparing ME/CFS to healthy controls:

- The DNA methylome is different, particularly with regard to glucocorticoid
  sensitivity genes and in genes important in cellular metabolism
- Expression of microRNA is different, particularly in NK cells
- Difference in transcription factor levels: increased levels of NFkB
- Increased HDAC expression, leading to decreased gene expression

References: de Vega WC et al. PLoS One 2014:9:e104757
de Vega WC et al. BMC Med Genomics 2017:doi:10.1186/s12920-017-0248-3
Petty RD. PLoS One 2016: doi:101371/journal.pone0150904
Maes M et al. NeuroEndocrinology Letters 2007:28:456
Jason L et al. Brain Behav Immun 2011:25:1544

"Abnormalities in all four of these central mechanisms by which genes are turned on and off have been found in people with ME/CFS".

SECTION 6: THE MICROBIOME AND ME/CFS

"Finally, the microbiome".

SLIDE: HOW THE MICROBIOME MAY AFFECT THE BRAIN

- The human microbiome: 10 times as many bacterial cells as human cells,
  containing 5- 8 million genes, compared to our 20,000+ genes
- Microbes in our gut:
  -- Synthesise hormones & neurotransmitters (eg. norepinephrine, serotonin,

dopamine, ACh, GABA)
-- Synthesise molecules of inflammation (cytokines, prostglandins) & elicit the
production of those molecules by the gut immune system
-- Through inflammation, create a "leaky gut": the tight junctions that bind
gut epithelial cells together become loose, allowing bacteria & toxins to
enter the blood
Reference: From Navaneetharaja N et al. J Clin Med 2016:5:55

"You probably have heard a lot about the microbiome, so what are we talking about?"

"Each of us human beings lives all their lives with a lot of other microbes that are on us and inside us. In fact there are ten times as many of their cells as there are our own human cells in each of us. Much more important than that, there are more than a hundred times as many of these microbial genes in and on each of us than there are our own genes. And more important than that, these microbial genes make molecules like human-related hormones, neurotransmitters, molecules that affect inflammation, or the immune system, and all of these chemicals made by the bacteria in the microbiome can get into our blood stream and affect our metabolism and our human health".

"One of the things that inflammation caused by the microbiome and the bacteria in our gut can do is cause the gut wall to become leaky, called leaky gut".

SLIDE: **GUT BARRIER DAMAGED MAY TRIGGER INNATE IMMUNITY**

Breach in gut barrier → LPS translocate to blood → LPS binding protein (LBP) up
+ sCD14 (LPS-LBP receptor) up: triggering innate immunity
Reference: From Giloteaux L et al. Microbiome 2016:4:30

"In a healthy individual, the lining of our gut is a wall of cells that are tightly attached to each other – it's an impenetrable wall and that's good, because inside our guts are bacteria and toxic substances being made by those bacteria and you wouldn't want (them) to get into your body through the blood stream. The wall in a healthy gut keeps them out, but when the microbiome of the gut is causing inflammation in the gut, that wall becomes leaky and one of the things that happen is shown in this slide".

"This is a bacterial toxin called LPS or lipopolysaccharide; the levels of this toxin in the blood stream of ME/CFS patients, according to a good study from Cornell, are significantly higher than in healthy individuals. The same was true of two other molecules".

"More provocatively, a recent study from Cornell found that following exercise in patients with ME/CFS, actual live bacteria get into the blood: levels that were not measureable before exercise now become measureable following exercise, whereas you do not see that in healthy individuals".

SLIDE: EXERCISE CAUSES GUT BACTERIA TO ENTER THE BLOOD IN PATIENTS WITH ME/CFS

FERMICUTES/CLOSTRIDIA/----/LACHNO X1Va

Reference: From Shukla SK et al. PLoS One 2015:10(2):e0145453, doi:10.1371/journal.pone.0145453

SLIDE: METAGENOMIC GUT MICROBIOME STUDY

- 50 ME/CFS and 50 matched healthy controls
- Relative abundance of several genera were significantly associated with ME/CFS:
  Faecalibacterium, Roseburia, Dorea, Coprococcus, Ruminococcus, Coprobacillus
- Bacterial metabolic pathways also distinguished cases v controls
  Pathways associated with:
  -- unsaturated fatty acid biosynthesis ↓
  -- Vit B6 biosynthesis salvage ↑
  -- Pyrimidine ribonucleoside degradation ↑
  -- Atrazine degradation ↑

Severity of pain, fatigue and motivation correlated with the abundance of bacterial taxa and bacterial metabolic pathways

Reference: From Nagy-Szakal D….Lipkin WI. Microbiome 2017:5:44

"A study from Columbia from the Centre for Infection and immunity has looked at the genes and the bacteria that you infer from those genes in 50 patients with ME/CFS and matched healthy control subjects".

"There appears to be a microbiome signature that is characteristic of ME/CFS – different kinds of bacteria are present in the gut, and the metabolic pathways that reflect those bacteria are also different in important ways from what you see in healthy individuals".

"More to the point, the levels of these different metabolic pathways seem to correlate with how severe the pain and fatigue are in patients with ME/CFS".

"This is a single study and people are in the process of trying to repeat it to see if they can find the same thing".

"So – we've covered a lot of ground in a short time: studies of the brain, of the immune system, energy metabolism, gene structure, gene expression, the microbiome. Let me try to summarise:

SLIDE: **ME/CFS: In Summary…**

- The pathogenesis is still obscure, and the causes are probably multiple
- The case definition of CFS likely encompasses several illnesses with similar symptoms, but different triggers
- No tests yet have adequate sensitivity and specificity for diagnosis
- No proven treatments

But...

Professor Komaroff went on to show other slides and to speak about a much more positive situation.

"I've shown you lots of things that are wrong, that are measurable, but what's causing all of them? We don't know that yet".

"The case definition of this illness likely encompasses several different illnesses that have similar symptoms but are triggered by different things in different people with the illness. We don't yet have diagnostic tests that are adequately sensitive and specific – adequately accurate – to make a diagnosis but (we have) many tests, many biomarkers, that clearly are different in the patients from healthy subjects of the same age and sex".

"But I think we can say now, on the basis of 35 years of research, that the illness is not simply the expression of physical, somatic symptoms by people with a primary psychological disorder, in fact, thousands of studies show that there are robust underlying biological abnormalities in the brain and autonomic nervous system, in the reaction to exercise, in the immune system, energy metabolism, gene expression and gene structure, and differences in the microbiome".

"I said in the previous webinar, but I didn't repeat it today, that it's a very attractive hypothesis to believe – and (there is) considerable evidence consistent with it – that the same physiology that causes sickness behaviour -- what makes any of us or any animal feel sick when we get an infection of have inflammation in our body -- that chronic fatigue syndrome may share underlying mechanisms with sickness behaviour, and that the underlying long-held hypothesis that an activated immune system in the brain may be responsible for the symptoms of the illness I think is increasingly plausible, meaning that there is increasing evidence to support that".

"And I think that the recent suggestion that the gut microbiome may be one trigger of this illness in some patients is also plausible".

"So since the last webinar, I would just finish by saying there have been hundreds of important new articles published, many scientific meetings around the world; the NIH has launched a major research project on the NIH campus, has funded three large five-year research programmes and has announced publicly its desire to substantially increase its investment in worthy ME/CFS research. CDC has continued its very active research programme and is working with NIH to develop better research tools for ME/CFS going forwards. To my way of thinking, this is all good news".

"No, we haven't yet got an accurate diagnostic test or a cure, but I'm convinced that every step forward brings us closer to those goals".

"Why am I optimistic? I think back to 30 + years ago when many of us began to care for patients with ME/CFS and to study the disease: it was a wasteland then, it was a wasteland. Practically nobody had heard of the disease, including most doctors, and those doctors who had were generally very sceptical that it was a disease, and frankly, those of us who did think it was a real disease caused by underlying biological abnormalities really didn't have a lot of evidence for that then. Today we do, thanks to research, and with continued support from the government and from private foundations, we're going to be better able to diagnose and treat this illness".

"That's my conviction. Thank you very much".

SECTION 8: QUESTIONS & ANSWERS

Several important topics were addressed in the Q&A session:

(1) asked about treatment, including the efficacy of antivirals , Professor Komaroff said: "You can't conclude that any treatment is beneficial until you conduct a random controlled trial; encouraging experience needs to be followed up with random controlled trials before we can draw conclusions".

(2) asked about the effect of short telomere length, he said: "We can't draw inferences about life expectancy – it's a way of saying there is a very substantial shortening of the telomeres in the study in people with ME/CFS. What that says about life expectancy, we just don't know".

(3) asked about the need for continuing medical education programmes (CME credits) about ME/CFS, Professor Komaroff said: "I know CDC has conducted a number of CME programmes; Medscape, a website for doctors, has done a bunch of CME activities on ME/CFS, and online, on the CDC and NIH websites, there is health information both for general public and for health professionals. There are many medical educational conferences like medical grand rounds that have been dedicated to ME/CFS – not as many as many of us would like, but still, a lot. It was invisible 35 years ago; today there's much more information available".

(4) asked about biomarkers, he said: “What a biomarker means to me is an objective measurement that can distinguish people with an illness from healthy people or from people with other diseases”. Referring to his own study from 1995 in which three biomarkers had been identified, he continued: “That’s what these three tests in this very large study did – they distinguished patients with ME/CFS from healthy individuals. But none of them was so accurate that you would want to use them as a diagnostic test, that is, there are people who had normal results but who really had the illness, and there were people who had abnormal results who did not have the illness. There were a few, in each case, but it’s just not good enough to call it a diagnostic test. The main point here is that patients with this illness were 27 times more likely to have an abnormality in this test than healthy people. That’s not a subtle difference; it’s not a good enough diagnostic test but it is a biomarker”.

(5) asked about the rituximab trial, Professor Komaroff said: “I’ve been waiting for (Fluge & Mella) to publish the results of this phase three study and I really can’t comment beyond that, except to reflect (on) subsets. Every clinical trial of any treatment for any disease that’s ever been done -- you’re studying a large group of people and if that large group of people is not homogeneous – if they don’t have exactly the same disease, then there may well be subsets within the large group that will benefit, but that gets lost – you don’t see it in the clinical trial because there weren’t enough of the people in that subset to affect the average result of the whole group. So – a negative clinical trial does not mean that there are not subsets that might benefit from the treatment. I’m just waiting to see the published results of the larger phase three study before knowing what to make of it”.

Professor Komaroff was warmly thanked by Carol Head from The Solve ME/CFS Initiative.