Symposium for Promoting the Advancement of Research Knowledge in ME/CFS Early Career Researchers

December 11, 2023

Clinical Center (Building 10)

FAES Classrooms 6 & 7 and Terrace

NIH Campus

Bethesda, MD

SPARK ME | The Future of ME/CFS Research

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Clinical Center (Building 10), FAES Classrooms 6 & 7 and Terrace, NIH Campus, Bethesda, MD

9:00 – 9:05 am	Welcome – Vicky Whittemore, PhD, NINDS
9:05 – 9:15 am	Introduction and Welcome – Walter Koroshetz, MD – Director, NINDS
9:15 – 10:00 am	Building Your ME/CFS Research Career Moderator: Jessica Maya, PhD – Cornell University • Perspective from Brent Williams, PhD – Columbia University • Perspective from Xiang Xu, PhD – Mt. Sinai
10:00 – 10:30 am	Overview of Grant Writing for Young/Early Career Investigators Moderator: Brent Williams, PhD • Vicky Whittemore, PhD – NIH/NINDS • Joe Breen, PhD – NIH/NIAID
10:30 – 10:45 am	Break
10:45 – 11:30 am	Panel of ME/CFS Non- Profit Partners Moderator: Agostina Casamento-Moran, PhD – Johns Hopkins University • H. Timothy Hsiao, PhD – Solve ME/CFS Initiative • Jamie Seltzer – #MEAction • Richard Simpson – Invest in ME Research • Linda Tannenbaum – Open Medicine Foundation
11:30 – 12:15 pm	Networking Breakout Groups
12:15 – 1:15 pm	Lunch (Sponsored by Nova Southeastern University) and Poster Session
1:15 – 3:00 pm	Short Research Presentations by Young/Early Career Investigator Participants Moderator: Chloe Jones, University of Alabama, Birmingham

1:15 – 1:30 pm	Developing a mouse model of myalgic encephalomyelitis Madeleine Uys – North-West University (Presenting via Zoom)
1:30 – 1:45 pm	Antibody Reactivity to the Intestinal Microbiome in Severe Myalgic Encephalomyelitis Patients Katharine Seton, PhD – Quadrum Research Institute (Presenting via Zoom)
1:45 – 2:00 pm	Identification of biomarkers for ME/CFS from metabolites and proteins in blood Katie Glass, PhD – Cornell University
2:00 – 2:15 pm	Effect of Physical Exertion on CNS Oxidative Stress and Metabolism in ME/CFS Nicholas Hampilos, MD – Weill Cornell Medicine
2: 15 – 2:30 pm	Epigenetic Reprogramming of CD8+ T cell Populations Drives Exhaustion in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) David Iu – Cornell University
2:30 – 2:45 pm	UpTime: A Digital Biomarker for ME/CFS Bella Rond – Bateman Horne Center
2:45 – 3:00 pm	ME/CFS Pathophysiology Investigated by Invasive Cardiopulmonary Exercise Test (iCPET) and Autonomic Function Testing Johanna Woodward Squires – Brigham & Women's Hospital
3:00 – 3:15 pm	Break
3:15 – 4:30 pm	Short Research Presentations by Young/Early Career Investigator Participants Moderator: Katie Glass, PhD – Cornell University
3:15 – 3:30 pm	Augmentation of Anaerobic Pentose Phosphate Pathway Triggers Tetrahydrobiopterin Biosynthesis in Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome (ME/CFS) patients with Orthostatic Intolerance: A Pilot Study Sarojini Bulbule – Simmaron Research Institute
3:30 – 3:45 pm	Tracking Peripheral Immune Cell Infiltration of the Brain in ME/CFS Chloe Jones – University of Alabama, Birmingham
3:45 – 4:00 pm	Evidence for the contribution of hemolysis to post-exertional malaise pathophysiology in myalgic encephalomyelitis Atefah Moezzi – University of Montreal (Presenting via Zoom)
4:00 – 4:15 pm	Exercise capacity in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) treated with long-term pyridostigmine Sarra Maan Al-Zayer – Brigham & Women's Hospital
4:15 – 4:30 pm	Single-cell transcriptomics of ME/CFS circulating immune system before and after symptom provocation Tien Luyen Vu – Cornell University

4:30 – 5:00 pm	Young/Early Career Investigator Network – discussion of ways the young investigators can network, collaborate across labs, countries and continents Co-Moderators: Agostina Casamento-Moran, PhD – Johns Hopkins University and Karen Karen Giménez-Orenga, Universidad Católica de Valencia San Vicente Mártir/EMERG
5:00 pm	Adjourn

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Speaker Abstracts

Developing a Mouse Model of Myalgic Encephalomyelitis

Madeleine M Uys, Brian H Harvey, Stephan F Steyn, Corniel van Rooyen

Centre of Excellence for Pharmaceutical Sciences, Translational Neuroscience, North-West University, Potchefstroom, South Africa

Background: Myalgic encephalomyelitis (ME) and chronic fatigue syndrome (CFS) are persistent debilitating neuroimmune disorders leaving 25% of patients housebound with severe fatigue, myalgia, and cognitive impairment. There are no widely accepted animal models for ME/CFS, which hampers the discovery of treatment options. Importantly, purported animal models for CFS lack validity in that acute (<7 days), but not long-term persistence of aberrant behavior and biochemical measures are reported.

Method: Polyinosinic:polycytidylic acid (Poly I:C) is a synthetic double-stranded RNA which induces a viral immune response in humans and mice. To model a chronic state of viral-induced immune activation, we will administer Poly I:C (n=24; Exposure Group) or vehicle (n=24; Control Group) once weekly intraperitoneally for 6 weeks to male and female C57BL6/J mice, starting from age 8 weeks. Additionally, the effect of physical and psychological stress/exhaustion in the acute aftermath of a viral infection will be modeled to investigate its possible contribution to immune persistence, by subjecting a subgroup each of the Exposure Group and Control Group (n=12 each) to exhaustive swim exercise within 48 hours after weekly Poly I:C injections. During the 6-week exposure paradigm and for 4 weeks thereafter, grip strength and endurance as well as locomotor activity will be measured weekly as parameters of fatigue. Impairment of short-term working memory will be tested at week 10 in the novel object recognition test and Y-maze. Hereafter mice will be sacrificed and blood and brain harvested to measure multiple cytokine levels and parameters of oxidative stress and DNA damage.

Email: Madeleine.Uys@nwu.ac.za

Antibody Reactivity to the Intestinal Microbiome in Severe Myalgic Encephalomyelitis Patients

Katharine A. Seton[1], Marianne Defernez[1], Andrea Telatin[1], Sumeet K. Tiwari[1], George M. Savva[1], Antonietta Hayhoe[1], Alistair Noble[3], Ana Carvalho[4], Steve James[1], Amolak Bansal[5], Thomas Wileman[1,2], Simon R. Carding[1,2]

[1]Quadram Institute Bioscience, Norwich Research Park, Norwich, UK
[2]Norwich Medical School, University of East Anglia, Norwich, UK
[3]The Pirbright Institute, Woking, UK
[4]Experimental Arthritis Treatment Centre for Children, University of Liverpool, Liverpool, UK
[5]Spire St Anthony's Hospital, Surrey, UK

Background: The pathogenesis of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is multisystemic involving the immune and gastrointestinal (GI) systems. Patients often exhibit GI microbiome alterations, discomfort and pain, and elevated blood biomarkers of intestinal permeability. To further explore the role of the GI microbiome in disease pathology we conducted a feasibility study in severely affected ME/CFS patients to test the hypothesis that intestinal permeability and consequential microbial translocation results in the breakdown in immune tolerance leading to the development of systemic antibodies against gut microbes. We also assessed the feasibility of, and barriers to, including severe ME/CFS patients in research.

Methods: Five pairs of severe ME/CFS patients and matched same-household healthy controls were recruited. Serum IgG and faecal IgA levels and reactivity to intestinal microbes were assessed using enzyme linked immunosorbent assays (ELISA) and flow cytometry. Serum IgG reactivity to autologous and heterologous intestinal microbes were assessed using flow cytometry and ELISA. IgG-Seq, a method which combines flow-cytometry based bacterial cell sorting and metagenomics, was used to profile the systemic anti-microbiota IgG repertoire. Finally, correlations between the systemic anti-microbiota IgG repertoire and the blood microbiome were assessed.

Results: We identified immune dysfunction in severe ME/CFS patients, characterised by reduced capacity and reactivity of serum IgG to stool microbes, regardless of their origin.

Conclusion: This study provides the rationale for further investigations involving larger cohorts of ME/CFS patients to further explore immune-microbiome interactions. It also highlights considerations for including severe ME/CFS patients in research.

Email: Katharine.seton@quadram.ac.uk

Identification of Biomarkers for ME/CFS from Metabolites and Proteins in Blood

Katherine A. Glass, Maureen R. Hanson

Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA

ME/CFS lacks a clinically available diagnostic test. One impediment in evaluating treatments for ME/CFS is the absence of molecular biomarkers to establish an objective effect of therapies. Therefore, our goal was to determine circulating biomarkers that accurately distinguish ME/CFS patients from healthy controls.

We measured 10,201 compounds including metabolites (Metabolon HD4 global metabolites and complex lipids platforms) and proteins (SomaLogic and Olink platforms) in the blood of 103 subjects: 60 ME/CFS patients and 43 healthy controls. Cohorts were age- and BMI-matched and included both female and male subjects.

We selected 544 compounds via significance in a linear model or a fold change cutoff. The subjects were partitioned into 80% training and 20% validation cohorts with even splits of patients and controls (with 250 bootstrapped resamples). We compared the ability of six machine learning algorithms to correctly classify subjects in the validation cohort using the 544 features. For each algorithm, 10-fold cross validation was used on the training cohort to reduce overfitting. The two best performing algorithms, neural networks and elastic net, were further optimized and the top 50 features for each algorithm were identified. All area under the receiver operating characteristic (AUROC) curves of 250 resamples for the top 50 compounds were >0.9 and medians for both algorithms were 1 (perfect classification). 26 compounds were in the top 50 features for both algorithms, including 23 SomaLogic proteins and 3 Olink proteins.

These candidate biomarkers can be further tested in additional subjects and possibly developed as a diagnostic test for ME/CFS.

Emails:
kg432@cornell.edu
mrh5@cornell.edu

Effect of Physical Exertion on CNS Oxidative Stress and Metabolism in ME/CFS

Nicholas H. Hampilos[1 *], Xiangling Mao[1], Jihyun Lee [1]., Charlene Thomas[2], Maureen R. Hanson[3], Andrew Grimson[3], Susan M. Levine[3], Geoffrey E. Moore[3], Arindam RoyChoudhury[2], Betsy A. Keller[4], Dikoma C. Shungu[1]

[1] Department of Radiology, Weill Cornell Medicine, New York, NY, USA
[2] Division of Biostatistics, Department of Population Health Sciences, Weill Cornell Medicine, New York, NY, USA
[3] Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA
[4] Department of Exercise Science & Athletic Training, Ithaca College, Ithaca, NY, USA
*Corresponding author: nhh4001@med.cornell.edu

Introduction: The exacerbation of ME/CFS symptoms following exertion, known as post-exertional malaise (PEM), is a cardinal yet poorly understood feature of ME/CFS. The aim of this study was to investigate whether physical exertion has a measurable effect on central oxidative stress and glycolytic metabolism in ME/CFS using proton magnetic resonance spectroscopy (MRS).

Methods:
Study sample: The study enrolled 31 subjects with ME/CFS and 21 healthy controls (HC).
Exercise challenge: Subjects underwent maximal-effort cardiopulmonary exercise testing (CPET) on a cycle ergometer.
MRS: Occipital cortex (OCC) and left striatum glutathione (GSH), a critical antioxidant and oxidative stress marker, and ventricular lactate, a glycolytic metabolism marker, were measured with MRS pre and 24 hours post CPET.

Results:
Baseline: Levels of OCC GSH were 10.3% lower in ME/CFS than in HC ($p=0.02$), while striatal GSH did not differ between groups ($p=0.2$). Ventricular lactate was 17.1% higher in ME/CFS versus HC ($p=0.003$).
Post CPET: Neither GSH nor lactate levels changed significantly with CPET in either group. However, as at baseline, GSH and lactate levels remained lower and higher, respectively, in ME/CFS than HC.

Conclusion: The observed OCC GSH deficit and ventricular lactate elevation in ME/CFS are consistent with our prior studies. Surprisingly, CPET had no effect on brain GSH or lactate, suggesting no exacerbation of central oxidative stress or glycolytic metabolism, despite the known disabling effects of physical exertion in ME/CFS patients. Alternatively, changes in GSH and lactate could have been missed if they normalized before or occurred after the second MRS.

Epigenetic Reprogramming of CD8+ T cell Populations Drives Exhaustion in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)

David S. Iu[1]*, Jessica Maya[1]*, Luyen T. Vu[1], Elizabeth A. Fogarty[1], Paul R. Munn[2], Jennifer K. Grenier[2], Maureen R. Hanson[1], Andrew Grimson[1]

[1]Cornell University, Department of Molecular Biology and Genetics, Ithaca, NY
[2]Cornell University, Genomics Innovation Hub and TREx Facility, Ithaca, NY
*These authors contributed equally to this work.

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a severe, debilitating disease with striking similarities to long-COVID/PASC, but its etiology remains unclear. Multiple lines of evidence point towards immune dysfunction as a potential cause for ME/CFS, and we previously reported metabolic changes such as increased fatty acid oxidation, decreased glycolysis, and decreased mitochondrial membrane potential in ME/CFS CD8+ T cells, features commonly found on exhausted T cells. To comprehensively characterize the gene regulatory state underlying potential transcriptional dysregulation, we first re-analyzed a single cell transcriptomic atlas of ME/CFS by reclassifying CD8+ T cells, where we found that various cell clusters upregulated exhaustion-associated genes. We then generated high resolution profiles of gene expression and chromatin accessibility in effector memory CD8+ T cells (T_{EM}) and naïve CD8+ T cells by RNA-seq and ATAC-seq at baseline and further analyzed different CD8+ subsets following symptom provocation. We observed in ME/CFS T_{EM} upregulation of key transcription factors associated with exhaustion and downregulation of genes controlling key metabolic activities, as well as an altered chromatin landscape consistent with exhaustion programs, while ME/CFS T_N displayed a potential loss of naïve identity. Flow cytometry of exhaustion markers detected a higher frequency of exhaustion-associated transcription factors, validating our bioinformatic analyses. Our dataset serves as an important resource for understanding ME/CFS pathology and supports a novel state of immune cell dysregulation in ME/CFS which may offer new treatment options.

First author contact: dl649@cornell.edu
Corresponding author contact: agrimson@cornell.edu

UpTime: A Digital Biomarker for ME/CFS

Bella Rond[1], Yifei Sun[2], Candace Rond[1], Sarah Scheiving[1], Lucinda Bateman[1], Shad Roundy[2], Suzanne D. Vernon[1]

[1]The Bateman Horne Center of Excellence, 24 South 1100 East, Suite 205, Salt Lake City, UT, USA
[2] Department of Mechanical Engineering, University of Utah, Salt Lake City, UT, USA

OBJECTIVE: Hours of upright activity is a clinically meaningful measure of ME/CFS disease severity. Our goal was to develop an objective measure of hours of upright activity that could be used as a clinically meaningful endpoint and outcome measure in ME/CFS clinical trials.

METHODS: 51 subjects were enrolled including 30 ME/CFS participants, 15 Long COVID participants, and 6 healthy controls. Subjects reported HUA (defined as hours per day spent with feet on the floor) and completed questionnaires to assess the impact of orthostatic intolerance on daily activities and symptoms over the course of 7 days. Each participant wore a wearable device equipped with a sensor that continuously measured upright activity, or UpTime, over 7 days. Data analysis used one-way ANOVA.

RESULTS: ME/CFS participants had significantly lower UpTime compared to healthy controls ($p < 0.00004$). UpTime was able to distinguish between ME/CFS participants, Long COVID participants, and Health Controls ($p < 0.022$). While self-report orthostatic symptoms and hours of upright activity and steps per day were different between patients and controls, there was greater variance in these measures resulting in overlap and lack of statistical difference between the groups.

CONCLUSIONS: ME/CFS UpTime was significantly better at predicting ME/CFS and Long COVID disease severity compared to self-reported hours of upright activity or passively measured steps per day. Next steps are being taken to qualify UpTime as a digital biomarker for ME/CFS.

ME/CFS Pathophysiology Investigated by Invasive Cardiopulmonary Exercise Test (iCPET) and Autonomic Function Testing

J. Squires[1], S. Al-Zayer[1], W. Xiao[2], P. Novak[3], D. Systrom[1]

[1]Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
[2]Department of Surgery, Science, and Bioengineering, Massachusetts General Hospital, Boston, MA, USA
[3]Department of Neurology, Brigham and Women's Faulkner Hospital, Harvard Medical School, Boston, MA, USA

Introduction: Mechanisms underlying exercise and orthostatic intolerance in ME/CFS have been uncovered by invasive cardiopulmonary exercise testing (iCPET) and autonomic function testing (AFT), but the relationships between the two are not known. This study aims to determine if there is overlap of cardiovascular and respiratory pathophysiology in patients who have undergone both tests.

Methods: Between January 2017 and April 2022, 62 patients were identified with a contemporary iCPET and AFT. Key variables from the iCPET included peak oxygen uptake (pVO2), cardiac output (pQc), right atrial pressure (pRAP), and systemic oxygen extraction (Ca-vO2). Key variables from the autonomic testing included epidermal and sweat gland small fiber neurite density, electrochemical skin conductance, change in heart rate (ΔHR), and end tidal carbon dioxide (ΔETCO2) from supine to upright during the tilt table test (TTT).

Results: All 62 patients demonstrated preload failure (pRAP < 6.5mmHg). Of this group, 54 patients (87.1%) fulfilled NAM criteria for ME/CFS, with 32 testing positive (59.3%) for small fiber neuropathy (SFN) using either morphological and/or functional testing. Significant correlations were found between pVO2 and ΔHR ($r=-0.439$, $P<0.05$) and ΔETCO2 ($r=0.474$, $P<0.05$) during TTT. The same tilt table variables were found to be significantly correlated with pQc ($r=-0.365$, $P<0.05$ and $r=0.351$, $P<0.05$) from the iCPET.

Augmentation of Anaerobic Pentose Phosphate Pathway Triggers Tetrahydrobiopterin Biosynthesis in Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome (ME/CFS) patients with Orthostatic Intolerance: A Pilot Study

Sarojini Bulbule[1], Carl Gunnar Gottschalk[1,2], Molly E Drosen[1], Daniel Peterson[3], Leggy A Arnold[4], and Avik Roy[1,2,4]

[1]Research and Development Laboratory, Chemistry Building, 3210 N Cramer Street, Suite # 214; Milwaukee, WI
[2]Simmaron Research Institute, 948 Incline Way, Incline Village, NV
[3]Sierra Internal Medicine, Incline Village, NV 89451;[4] Department of Chemistry and Biochemistry, University of Wisconsin-Milwaukee, 3210 N Cramer Street, Milwaukee, WI

Tetrahydrobiopterin (BH4), an essential cofactor of amino acid metabolism, was found to be strongly elevated in ME/CFS patients with Orthostatic intolerance (ME+OI). However, the molecular mechanism of BH4 upregulation is poorly understood in ME+OI patients. Here, we report that the activation of non-oxidative pentose phosphate pathway (PPP) plays a critical role for the biosynthesis of BH4 in ME+OI patients. Microarray-based gene screening followed by realtime PCR-based validation, ELISA assay, and finally enzyme kinetic studies of various enzymes of PPP such as glucose-6-phosphate dehydrogenase (G6PDH), transaldolase (TALDO), and transketolase (TK) enzymes revealed that the augmentation of PPP is critical in the pathogenesis of ME+OI. While exploring the molecular mechanism of BH4 upregulation, implementation of non-oxidative PPP by treating human microglial cells with ribose-5-phosphate under a hypoxic condition of 85%N_2/10%CO_2/5%O_2 followed by the analysis of BH4 upregulation via ELISA, immunoblot and dual immunocytochemical analyses confirmed that the activation of non-oxidative PPP is indeed required for the upregulation of BH4. Interestingly, application of ME+OI plasma samples in hypoxia-acclimatized human microglial cells followed by the analyses of BH4 expression reiterated that the non-oxidative PPP is essential for the upregulation of BH4 in ME+OI patients. Moreover, the siRNA knocking down of *taldo1* gene not only abrogated the BH4 upregulating effect of ribose-5-phosphate or ME+OI plasma, but also inhibited the upregulation of iNOS and iNOS-derived NO in human microglial cells indicating that the non-oxidative PPP-induced BH4 could stimulate inflammatory response in ME+OI patients.

To whom correspondence should be addressed:
Avik Roy, Ph.D.
Simmaron Research R &D Laboratories.
3210 N Cramer Street # 214
Chemistry Building
Milwaukee, WI 53211
Email: aroy@simmaron.com
Ph: (773) 263 8182

Tracking Peripheral Immune Cell Infiltration of the Brain in ME/CFS

Chloe L. Jones[1], Suzanne E. Lapi[2], Denise Jeffers[2], Jennifer L. Bartels[2], Hailey H. Houson[2], Jonathan McConathy[2], Sharon L. White[2], and Jarred Younger[1]

[1]Department of Psychology, University of Alabama at Birmingham, Birmingham, AL, USA
[2]Department of Radiology, University of Alabama at Birmingham, Birmingham, AL, USA

Introduction: ME/CFS may be caused by infiltration of peripheral immune cells into the brain, resulting in neuroinflammation. This study tests the first positron emission tomography (PET) brain imaging of autologous leukocytes radiolabeled with [^{89}Zr]oxine. The long half-life of Zirconium 89 (3.2 days) allows for cell tracking over multiple days, which is essential for capturing peripheral cell migration to the brain.

Methods: To assess if [^{89}Zr]oxine labeled leukocytes could be detected three days after reinjection, four healthy individuals completed the protocol. Leukocytes were isolated from blood and incubated with [^{89}Zr]oxine. Labeled cells were washed to remove traces of unbound [^{89}Zr]oxine and re-suspended for injection into participants. Whole-body computed tomography (CT) and PET brain scans were conducted immediately following injection, and at 5-, 24-, 48-, and 72-hours post-injection.

Results: There were no reported side effects or adverse events. Images obtained 72-hours after injection established that [^{89}Zr]oxine labeled leukocytes remained detectable. Whole-body CT imaging showed labeled leukocytes accumulated in the liver, spleen, and bone marrow, as expected. PET brain imaging revealed the highest uptake in the blood, bone marrow, and muscle tissue of the head as measured by regions of interest in the superior sagittal sinus, C2 and C3 vertebrae, and temporalis muscle. Healthy participants did not exhibit leukocyte infiltration into brain tissue.

Conclusion: Results confirm that tracking peripheral leukocytes on PET imaging over multiple days with [^{89}Zr]oxine is feasible. Future scans will be performed on ten ME/CFS patients to test if the illness involves peripheral immune cell infiltration of the brain.

First author email: chloej@uab.edu
Corresponding author email: younger@uab.edu

Evidence for the Contribution of Hemolysis to Post-exertional Malaise Pathophysiology in Myalgic Encephalomyelitis

Atefeh Moezzi,[1,2] Jonas Bergquist,[3] Wenzhong Xiao [4,5], Peng Li [5], Anita Franco,[1] and Alain Moreau [1,2,6]

[1]Laboratoire Viscogliosi de génétique moléculaire des maladies musculo-squelettiques, Centre de recherche du CHU Sainte-Justine
[2]Département de biochimie et médecine moléculaire, Faculté de médecine, Université de Montréal
[3]Department of Analytical Chemistry and Neurochemistry, Biomedical Centre, Uppsala University, Uppsala, Sweden
[4]Stanford Genome Technology Center, Stanford University School of Medicine, Palo Alto, CA 94304, USA
[5]ME/CFS Collaborative Research Center at Harvard, Massachusetts General Hospital, Boston, MA 02114, USA
[6]Département de stomatologie, Faculté de médecine dentaire, Université de Montréal

Background: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a complex disease with unknown etiology. Post-exertional malaise (PEM) is the cardinal symptom of ME/CFS, and results in a severe exacerbation of many symptoms including an aberrant inflammatory response associated with physical exertion. Furthermore, fatigue and muscle pain have been linked to tissue oxygenation disruptions due to abnormalities in red blood cells (RBC) of ME/CFS patients.

Hypothesis: We hypothesized that induction of PEM through a standardized provocation maneuver will reveal more specific biomarkers that can illuminate ME/CFS pathophysiology. Specifically, this study aimed to characterize the molecular drivers of PEM onset and severity by looking at the proteomic alterations caused by PEM induction.

Methodology: A total of 61 ME/CFS patients and 20 age-matched controls were recruited for a global plasma proteome analysis. To reproduce PEM safely, we introduced a post-exertional challenge using a therapeutic massager device and blood samples were taken from participants at baseline (T0) and after PEM induction through a 90 min stimulation (T90).

Results and conclusion: Preliminary mass spectroscopy measurements revealed that plasma haptoglobin (Hp) was significantly down-regulated by almost 2-fold at T90 vs T0, in ME/CFS patient and such decrease was much more pronounced in severely affected cases comparing to the moderate group. Due to the predominant role of Hp in detoxifying free hemoglobin in hemolytic conditions, reduction in its circulating levels after the stress challenge could be suggestive of hemolysis triggered by PEM induction likely due to abnormalities in RBCs that remain to be characterized.

Project funded by Open Medicine Foundation USA and Open Medicine Foundation Canada

Exercise Capacity in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) Treated with Long-term Pyridostigmine

S. Al-Zayer[1], J. Squires[1], D. Systrom[1]

[1]Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Background: The pathophysiology underlying exertional intolerance in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) remains poorly understood. Previously, a single dose of 60 mg pyridostigmine, a reversible acetylcholinesterase inhibitor, was found to acutely improve aerobic capacity in patients (Joseph, P. et al. Chest 2022; 162:1116–26).

Aim: To build upon these prior findings, this study aimed to evaluate the long-term effect (>1 month) of pyridostigmine treatment on exercise intolerance in ME/CFS.

Method: Between 2017-2022, patients who met the National Academy of Medicine criteria for ME/CFS and had a minimum of two submaximal exercise tests (Shape Medical System, MN) were evaluated. Patients who began pyridostigmine after their baseline test were considered the treatment group. Patients who were not treated with pyridostigmine were considered the control group. Measurements were taken at baseline (T0) and most recent follow-up (T1).

Results: Thirty-seven patients in the treatment group and sixteen control patients were selected for analysis. At the follow-up evaluation (T1; 690 ± 547 days), the treatment group (dose range: 24-360mg/d) demonstrated a significant increase in the Oxygen Uptake Efficiency Slope (OUES) (T0: 1.82 ± 0.56, T1: 1.98 ± 0.53; P=0.044) and 1-minute Heart Rate Recovery (HRR) (T0= 27.79 ± 12.43 bpm, T1= 42.69 ± 16.63 bpm, P<0.0001). These differences were not observed in the control group OUES (T0: 1.62 ± 0.40, T1: 1.77 ± 0.47; P=0.258) and HRR (T0 = 24.69 ± 13.19 bpm, T1= 36.20 ± 14.62 bpm, P=0.101).

Conclusion: Long-term treatment with pyridostigmine improved aerobic capacity in ME/CFS as demonstrated by an increase in OUES and HRR.

Author Contact Information:

S. Al-Zayer sal-zayer@bwh.harvard.edu

J. Squires MSc jsquires1@bwh.harvard.edu

D. Systrom MD dsystrom@bwh.harvard.edu)

Single-cell Transcriptomics of ME/CFS Circulating Immune System Before and After Symptom Provocation

Tien Luyen Vu[1]; Faraz Ahmed[2]; Hongya Zhu[1]; David Shing Huk Iu[1]; Elizabeth A. Fogarty[1]; Yeonui Kwak[1]; Weizhong Chen[1]; Carl J. Franconi[1]; Paul R. Munn[2]; Ann E. Tate[2]; Susan M. Levine[3]; Jared Stevens[4]; Xiangling Mao[5]; Dikoma C. Shungu[5]; Geoffrey E. Moore[6]; Betsy A. Keller[6]; Maureen R. Hanson[1]; Jennifer K. Grenier[2]; Andrew Grimson[1]

[1]Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA
[2]Genomics Innovation Hub and TREx Facility, Institute of Biotechnology, Cornell University, Ithaca, NY, USA
[3]Levine Clinic, Private Practice, New York, NY, USA
[4]Workwell Foundation, Ripon, CA, USA
[5]Department of Radiology, Weill Cornell Medicine, New York, NY, USA
[6]Department of Exercise Science and Athletic Training, Ithaca College, Ithaca, NY, USA

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a long-term disease affecting various body systems with undefined cause. The immune system is key to disease an symptom development. However, most gene expression studies in ME/CFS are limited to main immune cell lineages. Here, we aim to provide a detailed description of the circulating immune system of ME/CFS at a single cell level at baseline and 24h after symptom provocation. Peripheral blood mononuclear cells collected from cohort of healthy controls and ME/CFS cases before and 24h after challenge were subjected to single cell RNA sequencing. After quality control and data integration, there were no major changes in cell composition across different cell types. Notably, classical monocytes in ME/CFS patients exhibited the highest number of differentially expressed genes at both time points. Naïve and effector memory CD4 T cells, and NK cells also display significant alterations in gene expression. Genes involved in inflammation, migration, and tissue recruitment were upregulated in ME/CFS for classical monocytes, indicating a potential increase in tissue resident macrophages. Moreover, we were able to detect dysregulated and normal monocytes within patients by applying machine learning approaches. Surprisingly, we detected improper alteration of platelet activities in patients upon analyzing the transcriptomes at baseline and post-exercise challenge. Taken together, we provide, for the first time, a high throughput characterization of the circulating immune system in ME/CFS at baseline and after symptom provocation. This study also serves as a valuable resource to investigate alterations in ME/CFS from a molecular and cell biology standpoint.

First author contact: louisvu@cornell.edu

Corresponding author contact: agrimson@cornell.edu

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Poster Abstracts

Exercise Capacity in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) Treated with Long-term Pyridostigmine

S. Al-Zayer[1], J. Squires[1], D. Systrom[1]

[1]Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Background: The pathophysiology underlying exertional intolerance in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) remains poorly understood. Previously, a single dose of 60 mg pyridostigmine, a reversible acetylcholinesterase inhibitor, was found to acutely improve aerobic capacity in patients (Joseph, P. et al. Chest 2022; 162:1116–26).

Aim: To build upon these prior findings, this study aimed to evaluate the long-term effect (>1 month) of pyridostigmine treatment on exercise intolerance in ME/CFS.

Method: Between 2017-2022, patients who met the National Academy of Medicine criteria for ME/CFS and had a minimum of two submaximal exercise tests (Shape Medical System, MN) were evaluated. Patients who began pyridostigmine after their baseline test were considered the treatment group. Patients who were not treated with pyridostigmine were considered the control group. Measurements were taken at baseline (T0) and most recent follow-up (T1).

Results: Thirty-seven patients in the treatment group and sixteen control patients were selected for analysis. At the follow-up evaluation (T1; 690 ± 547 days), the treatment group (dose range: 24-360mg/d) demonstrated a significant increase in the Oxygen Uptake Efficiency Slope (OUES) (T0: 1.82 ± 0.56, T1: 1.98 ± 0.53; P=0.044) and 1-minute Heart Rate Recovery (HRR) (T0= 27.79 ± 12.43 bpm, T1= 42.69 ± 16.63 bpm, P<0.0001). These differences were not observed in the control group OUES (T0: 1.62 ± 0.40, T1: 1.77 ± 0.47; P=0.258) and HRR (T0 = 24.69 ± 13.19 bpm, T1= 36.20 ± 14.62 bpm, P=0.101).

Conclusion: Long-term treatment with pyridostigmine improved aerobic capacity in ME/CFS as demonstrated by an increase in OUES and HRR.

Author Contact Information:

S. Al-Zayer sal-zayer@bwh.harvard.edu

J. Squires MSc jsquires1@bwh.harvard.edu

D. Systrom MD dsystrom@bwh.harvard.edu)

Circulating FGF-21 Levels are Associated with Myalgic Encephalomyelitis Disease Severity

Ghazaleh Azimi [1,2,3,4], Wesam Elremaly [2,3,4], Anita Franco [2,3,4], Alain Moreau [1,2,3,4,5]

[1] Department of Biochemistry and Molecular Medicine, Faculty of Medicine, Université de Montréal, 2900 Edouard Montpetit Blvd, Montreal, QC, H3T 1J4, Canada
[2]Viscogliosi Laboratory in Molecular Genetics of Musculoskeletal Diseases, Sainte-Justine University Hospital Research Center, 3175 Cote-Ste-Catherine Road, Montreal, QC, H3T 1C5, Canada
[3] Open Medicine Foundation ME/CFS Collaborative Center at CHU Sainte-Justine/Université de Montréal, 3175 Cote-Ste-Catherine Road, Montreal, QC, H3T 1C5, Canada
[4]ICanCME Research Network, Sainte-Justine University Hospital Research Center, 3175 Cote-Ste-Catherine Road, Montreal, QC, H3T 1C5, Canada
[5] Department of Stomatology, Faculty of Dentistry, Université de Montréal, 2900 Edouard Montpetit Blvd, Montreal, QC, H3T 1J4, Canada

Background: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS), is a heterogenous disease with uncertain etiology, and associated with a vast array of symptoms.

Purpose: This prospective study aims to investigate the contribution of circulating Fibroblast Growth Factor 21 (FGF-21) in ME/CFS pathogenesis.

Methods: 236 participants were recruited, including ME/CFS patients (n=178) and matched sedentary health controls (HC, n=58). All participants completed standard questionnaires (SF-36, MFI-20 and DSQ) to assess their health status. Blood samples were collected and circulating FGF-21 levels were measured by ELISA and correlated with disease severity. Data were compared by T-test and ANOVA, while effects were considered significant if P-value was less than 0.05.

Results: Plasma FGF-21 levels in individuals with ME/CFS were significantly higher (216 ± 18 pg/mL) compared to HC group (147 ± 17 pg/mL). Furthermore, we classified the ME/CFS patients into three groups based on their plasma FGF-21 levels: low (<50pg/ml), normal (50-200 pg/ml), and high (>200 pg/ml). Subsequently, we analyzed the correlation between these FGF-21 groups and the severity of the disease. Our results revealed that individuals with higher FGF-21 levels demonstrated more disease severity scores, encompassing physical, mental, and sleep problems, than the low FGF-21 group.

Conclusion: Our results suggest a potential link between elevated plasma FGF-21 levels and ME/CFS symptom severity, highlighting the significance of FGF-21 as a possible biomarker and actionable therapeutic target. Further investigation into the exact mechanisms underlying this association could shed light on potential therapeutic strategies for managing ME/CFS.

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Author Contact Information:

ghazaleh.azimi@umontreal.ca wesam.elremaly.hsj@ssss.gouv.qc.ca

anita.franco.hsj@ssss.gouv.qc.ca alain.moreau.hsj@ssss.gouv.qc.ca

Role of Soluble Sphingomyelin Phosphodiesterase Acid-like 3B (SMPDL3B) in Myalgic Encephalomyelitis

Rostami-Afshari Bita[1,2,3,4], Akoume Marie-Yvonne[2,4,5,6], Mella Olav[7], Fluge Øystein[7], Moreau Alain[1,2,3,4,8]

[1]Département de biochimie et médecine moléculaire, Faculté de médecine, Université de Montréal
[2]Laboratoire Viscogliosi de génétique moléculaire des maladies musculo-squelettiques, Centre de recherche du CHU Sainte-Justine
[3]Open Medicine Foundation ME/CFS Collaborative Center at CHU Sainte-Justine/Université de Montréal, 3175, chemin de la Côte-Sainte-Catherine, Montréal, QC, H3T 1C5, Canada
[4]Réseau de recherche ICanCME, Centre de recherche du Centre hospitalier universitaire Sainte-Justine, 3175, chemin de la Côte-Sainte-Catherine, Montréal, QC, H3T 1C5, Canada
[5]IRBK, Université Internationale de Libreville, Gabon
[6]Département de Biologie Cellulaire et Moléculaire et Génétique, Université des Sciences de la Santé de Libreville, Gabon
[7]Department of Oncology and Medical Physics at the University of Bergen, Haukeland University Hospital, Bergen, Norway
[8]Département de Stomatologie, Faculté de Médecine Dentaire, Université de Montréal, 2900, boulevard Édouard-Montpetit, Montréal, QC, H3T 1J4, Canada

Background: Sphingomyelin phosphodiesterase acid-like 3B (SMPDL3B), is a multifunctional protein bounds at the cell membrane by a glycophosphatidylinositol (GPI) anchor and prompted our interest in the context of myalgic encephalomyelitis (ME) given its role in the regulation of innate immunity and lipid metabolism.

Hypothesis: We propose that reduction of membrane bound SMPDL3B proteins and the elevation of its soluble form increases the severity of several ME symptoms through the perturbation of C-C chemokine receptor type 3 (CCR3) signaling.

Method: Two independent ME cohorts were studied: A Canadian discovery cohort composed of 147 ME patients and 62 age- and sex-matched controls; and an independent replication Norwegian cohort composed of 141 ME patients. All participants completed self-reported health questionnaires (SF-36, MFI-20 and DSQ). Plasma SMPDL3B levels were measured by an ELISA method. The potential mechanism of action of soluble SMPDL3B proteins was examined by cellular dielectric spectroscopy (CDS), by exposing human lymphocytes (Jurkat cells) to CCR3 agonist in presence of human plasma-rich or immuno-depleted in SMPDL3B proteins as well by using purified recombinant SMPDL3B proteins.

Results: The analysis of both ME cohorts revealed a significant association between soluble SMPDL3B protein levels and the severity of the symptoms. Higher plasma levels of SMPDL3B were observed in ME patients with orthostatic intolerance (OI) compared to ME patients without vascular abnormalities. Cellular response to soluble SMPDL3B was inhibited by CCR3 specific antagonist.

Conclusion: Collectively, our findings led to the identification of soluble SMPDL3B as a potential prognostic biomarker and therapeutic target for ME.

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Using [^{11}C]PBR28 Positron Emission Tomography to Assess Neuroinflammation in ME/CFS and PASC

Hannah Bues[1,2], Ludovica Brusaferri[1,3], Minhae Kim[1,3], Paula Lara-Mejia[1,2], Sydney Brumfield[1,2], Deena Saadi[1,2], Darin Dougherty[1,2], Marco Loggia[1,3], Michael VanElzakker[1,2]

[1]Athinoula A. Martinos Center for Biomedical Imaging
[2]Department of Psychiatry, Massachusetts General Hospital/Harvard Medical School
[3]Department of Radiology, Massachusetts General Hospital/Harvard Medical School

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a diagnostic label given to those experiencing symptoms such as post-exertional malaise, unusually strong fatigue, cognitive problems, widespread pain, and autonomic dysfunction, often following the acute phase of an infection. People with post-acute sequelae of COVID-19 (PASC) or "Long-COVID" experience symptoms that overlap with ME/CFS, to varying degrees depending on PASC phenotype and severity. While the symptom presentations of PASC and ME/CFS are similar, very little is known about the extent to which the pathophysiology of these conditions overlap. Evidence of a neuroinflammatory process in ME/CFS has been shown using methods such as magnetic resonance spectroscopy and first-generation radioligand positron emission tomography (PET) scanning. However, the present study is the first to use the second-generation radioligand [^{11}C]PBR28 to study neuroinflammation in both ME/CFS and PASC, and compare the two conditions. [^{11}C]PBR28 binds to the translocator protein, an outer mitochondrial membrane protein expressed by glial cells. Increased signal can reflect glial activation, glial cell density, or infiltration of peripheral immune cells. [^{11}C]PBR28 PET scanning is considered to be a specific and sensitive method to study neuroinflammation, and an improvement on previous radioligands. Here we present [^{11}C]PBR28 PET imaging data from ME/CFS, PASC, and healthy control participants. Highlighted regions show increased PET signal in cases versus controls. Future studies should attempt to delineate among the potential non-mutually exclusive reasons for neuroinflammation, including immune dysregulation, injury, or presence of leftover viral protein or virion.

Email: Hannah Bues, hbues@mgh.harvard.edu

Augmentation of Anaerobic Pentose Phosphate Pathway Triggers Tetrahydrobiopterin Biosynthesis in Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome (ME/CFS) patients with Orthostatic Intolerance: A Pilot Study

Sarojini Bulbule[1], Carl Gunnar Gottschalk[1,2], Molly E Drosen[1], Daniel Peterson[3], Leggy A Arnold[4], and Avik Roy[1,2,4]

[1]Research and Development Laboratory, Chemistry Building, 3210 N Cramer Street, Suite # 214; Milwaukee, WI
[2]Simmaron Research Institute, 948 Incline Way, Incline Village, NV
[3]Sierra Internal Medicine, Incline Village, NV 89451;[4] Department of Chemistry and Biochemistry, University of Wisconsin-Milwaukee, 3210 N Cramer Street, Milwaukee, WI

Tetrahydrobiopterin (BH4), an essential cofactor of amino acid metabolism, was found to be strongly elevated in ME/CFS patients with Orthostatic intolerance (ME+OI). However, the molecular mechanism of BH4 upregulation is poorly understood in ME+OI patients. Here, we report that the activation of non-oxidative pentose phosphate pathway (PPP) plays a critical role for the biosynthesis of BH4 in ME+OI patients. Microarray-based gene screening followed by realtime PCR-based validation, ELISA assay, and finally enzyme kinetic studies of various enzymes of PPP such as glucose-6-phosphate dehydrogenase (G6PDH), transaldolase (TALDO), and transketolase (TK) enzymes revealed that the augmentation of PPP is critical in the pathogenesis of ME+OI. While exploring the molecular mechanism of BH4 upregulation, implementation of non-oxidative PPP by treating human microglial cells with ribose-5-phosphate under a hypoxic condition of 85%N_2/10%CO_2/5%O_2 followed by the analysis of BH4 upregulation via ELISA, immunoblot and dual immunocytochemical analyses confirmed that the activation of non-oxidative PPP is indeed required for the upregulation of BH4. Interestingly, application of ME+OI plasma samples in hypoxia-acclimatized human microglial cells followed by the analyses of BH4 expression reiterated that the non-oxidative PPP is essential for the upregulation of BH4 in ME+OI patients. Moreover, the siRNA knocking down of *taldo1* gene not only abrogated the BH4 upregulating effect of ribose-5-phosphate or ME+OI plasma, but also inhibited the upregulation of iNOS and iNOS-derived NO in human microglial cells indicating that the non-oxidative PPP-induced BH4 could stimulate inflammatory response in ME+OI patients.

To whom correspondence should be addressed:
Avik Roy, Ph.D.
Simmaron Research R &D Laboratories.
3210 N Cramer Street # 214
Chemistry Building
Milwaukee, WI 53211
Email: aroy@simmaron.com
Ph: (773) 263 8182

Understanding the Behavioral Features of Fatigue in Long COVID

Agostina Casamento-Moran[1,2 ρ], Joonhee Leo Lee[1,2], Alba Azola[3,5], Laura Malone[2,4,5], Vikram S. Chib[1,2,6*]

[1] Department of Biomedical Engineering, Johns Hopkins School of Medicine; Baltimore, MD, 5 USA
[2] Kennedy Krieger Institute; Baltimore, MD, USA
[3] Department of Pediatrics, Johns Hopkins School of Medicine; Baltimore, MD, USA
[4] Department of Neurology, Johns Hopkins School of Medicine; Baltimore, MD, USA
[5] Department of Physical Medicine and Rehabilitation, Johns Hopkins School of Medicine; 10 Baltimore, MD, USA
[6] Kavli Neuroscience Discovery Institute, Johns Hopkins University; Baltimore, MD, USA

Fatigue is one of the most common and debilitating symptoms of Long COVID; however, it remains poorly understood and undertreated. Critical hurdles to understanding and treating fatigue include its multidimensional nature and the lack of quantitative methods for characterization. Here, we behaviorally characterized features of fatigue in individuals with Long COVID and healthy controls (HC): fatigue ratings (how tired one feels), perceived effort (how one perceives a previously exerted force), and decisions to exert effort (an individual's decision to engage in prospective effortful actions). We found that individuals with Long COVID reported higher fatigue ratings than HC and that fatigue ratings were correlated with clinical measures of perceived physical status and depression. Causal modeling showed that perceived physical status mediated the relationship between fatigue ratings and depression. We found that motor control was similar between individuals with Long COVID and HC. However, individuals with Long COVID overestimated the perceived amount of exerted effort due to an altered activation of the agonist and antagonist muscles. Finally, we found that individuals with Long COVID and HC make similar decisions to engage in prospective effortful tasks. Together, these results illustrate how a suite of behavioral assays is necessary to begin to understand the multidimensional nature of fatigue in Long COVID.

[ρ]First author's email: acasame1@jhu.edu

[*]Corresponding author's email: vchib@jhu.edu

Comprehensive Plasma Metabolomic Analysis in ME/CFS with Exercise Tolerance Test

Xiaoyu Che[1,2], Amit Ranjan[1], W. Ian Lipkin[1,3,4,5]

[1]Center for Infection and Immunity, Mailman School of Public Health, Columbia University; New York, NY, USA
[2]Department of Biostatistics, Mailman School of Public Health, Columbia University; New York, NY, USA
[3]Department of Epidemiology, Mailman School of Public Health, Columbia University; New York, NY, USA
[4]Department of Neurology, Vagelos College of Physicians and Surgeons, Columbia University; New York, NY, USA
[5]Department of Pathology, Vagelos College of Physicians and Surgeons, Columbia University; New York, NY, USA

We conducted a comprehensive metabolomic analysis of plasma from 56 ME/CFS subjects and 52 matched healthy controls before and 24hrs after exercise tolerance testing (ETT). Levels of metabolites were compared using both frequentist and Bayesian frameworks. Levels of citrate increased from before to after ETT in ME/CFS subjects, but not in controls—a finding consistent with dysregulation in the TCA cycle in ME/CFS. In ME/CFS subjects, the ratios of arginine/citrulline and ornithine/citrulline were higher at baseline but decreased after ETT—a finding consistent with dysregulation in the urea cycle. The ratios of kynurenine/tryptophan, a biomarker of the tryptophan metabolism, were lower than controls at baseline but elevated after ETT. Large differences in lipid clusters and metabolites were observed. In ME/CFS, carnitines were downregulated after ETT, and levels of various forms of phosphatidylcholines (PCs) decreased from before to after ETT. Whereas triglycerides and diglycerides were upregulated in ME/CFS at both time points, levels of ceramides were reduced from before to after ETT in controls, but not in ME/CFS. We observed distinct metabolic profiles in different subgroups of ME/CFS. Patients with short illness duration (<3 years) and male patients both exhibited disruption of ether/non-ether phospholipid (PL) balance in response to ETT, where the non-ether PL levels decreased, and the ether PL levels increased. Female ME/CFS younger than 45 had the most prominent downregulations of oxylipins at baseline. These findings suggest metabolic and physiological disturbances related to energy metabolism, urea cycle, lipid modeling/ remodeling, and inflammation and provide comprehensive insights into the pathophysiology of PEM in ME/CFS.

Emails:

xc2273@cumc.columbia.edu (XC)

ar4111@cumc.columbia.edu (AR)

ofiehn@ucdavis.edu (OF)

wil2001@cumc.columbia.edu (WIL)

Participant Recruitment for ME/CFS Research

Luke Collings, Sarah Scheiving, Candace Rond, Lucinda Bateman, Suzanne D. Vernon

The Bateman Horne Center of Excellence, 24 South 1100 East, Suite 205, Salt Lake City, UT, USA

Most clinical research studies do not enroll enough participants. This causes delays or failures in research efforts to develop diagnostic tests, discovery biomarkers, and new treatments. Participant recruitment for ME/CFS research is particularly challenging for many reasons including <10% of people with the disease have been diagnosed, various diagnostic criteria, high rates of comorbid diseases and conditions, and unrepresentative patient samples. This has resulted in small sample sizes for many ME/CFS research studies intended to identify potential biomarkers that cannot be replicated and clinical trials that cannot demonstrate the effectiveness of a given intervention.

We have implemented a multipronged approach to overcome recruitment challenges and improve participation in clinical research and trials conducted at the Bateman Horne Center. We have focused on realistic recruitment estimates, eligibility criteria that are not too broad or too narrow, team members that are trained and engaged in recruitment, budgeting for recruitment, and minimizing the pre-screening burden for research and trial participants.

This presentation will give examples of some of the screening tests we use to assess whether potential participants are appropriate candidates for inclusion in the research and provide a guide to improve representative participant recruitment in future ME/CFS research and clinical trials.

Comparison of T-cell Receptor Diversity of People with Myalgic Encephalomyelitis versus Controls

Joshua J Dibble†[1,2], Ben Ferneyhough†[3], Matthew Roddis†[3], Sam Millington[3], Michael D Fischer*[3], Nick J Parkinson*[3], Chris P Ponting*[1]
† Authors contributed equally. * Authors contributed equally.

[1] MRC Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, Crewe Road South, Edinburgh EH4 2XU, UK
[2]Massachusetts General Hospital & Harvard Medical School, Boston, MA, USA
[3]Systems Biology Laboratory UK, Abingdon, Oxfordshire OX14 4SA, UK

Myalgic Encephalomyelitis (ME; sometimes Chronic Fatigue Syndrome or CFS) is a chronic disease without laboratory test, detailed aetiological understanding or effective therapy. Its frequent onset after infection might indicate that it is an autoimmune disease or that it arises from abnormal T-cell activation.

To test this hypothesis, we sequenced the genomic loci of α/δ, β and γ T-cell receptors (TCR) from 40 human blood samples from each of four groups: severely affected people with ME/CFS; mildly or moderately affected people with ME/CFS; people diagnosed with Multiple Sclerosis, as disease controls; and healthy controls. Seeking to automatically classify these individuals' samples by their TCR repertoires, we applied a machine learning method. However, despite working well on a simulated data set, this approach did not partition samples into the four subgroups, beyond what was expected by chance alone. Our findings do not support the hypothesis that blood samples from people with ME/CFS frequently contain altered T-cell receptor diversity.

Joint-first author: Joshua Dibble (jjdibble@mgh.harvard.edu)
Correspondence to: Nick Parkinson (nickp@sbl-uk.org)
Michael Fischer (mikefischer219@gmail.com)
Chris Ponting (chris.ponting@ed.ac.uk)

Cell-free RNA Signatures of Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome

Anne Gardella, Conor Loy, Daniel Eweis-LaBolle, Joan Sesing Lenz, Iwijn De Vlaminck

Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY, USA

ME/CFS affects approximately 3 million individuals in the United States alone, subjecting patients to debilitating symptoms of extreme exhaustion and widespread pain that can persist for years. Diagnosing ME/CFS is particularly challenging and often relies on symptom monitoring as well as ruling out several other similarly presenting diseases, frequently resulting in missed or incorrect diagnoses. Circulating cell-free RNA (cfRNA) offers a potential avenue for biomarker discovery and new diagnostic tools. Recent advancements in cfRNA from blood plasma have demonstrated potential to diagnose diseases, including cancer and preeclampsia, as well as distinguish between infectious diseases like COVID-19 and multisystem inflammatory syndrome in children. In collaboration with the Center for Enervating NeuroImmune Disease at Cornell, we have sequenced cfRNA from the plasma of 30 patients and 30 control participants who were age and BMI matched. The plasma samples were collected prior to a cardiopulmonary exercise test. Our preliminary results have identified hundreds of differentially abundant genes in ME/CFS patients compared to controls. Our research will demonstrate that machine learning and modeling techniques applied to this data can identify potential disease-specific biomarkers, leading to advancements in ME/CFS diagnostics. These findings promise to enrich our understanding of the intricate biology underlying ME/CFS and underscore the efficacy of cfRNA as an innovative diagnostic tool for this debilitating illness.

Emails:
aeg267@cornell.edu
cjl332@cornell.edu
de256@cornell.edu
jsl26@cornell.edu
vlaminck@cornell.edu

Proteomic Adjustments Following Induction of Post-exertional Malaise

Arnaud Germain, Melissa A. Eckert, Ludovic Giloteaux, Katherine A. Glass, Maureen R. Hanson
Cornell University, Ithaca, NY

The overarching symptom of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is post-exertional malaise (PEM), worsening the slew of symptoms ME/CFS sufferers are experiencing with no anticipated reprieve from rest or sleep. Although exercise is widely recognized as a central pillar to both physical and mental health in healthy people – proven to prevent inflammation and many chronic diseases – PEM rapidly emerges in ME/CFS patients when low, patient-specific physical, mental and/or emotional effort limits are reached, which severely constrains their lives.

In this context, we acquired a high-throughput plasma protein profiling dataset over the course of a two-day cardiopulmonary exercise test (CPET) protocol, and scrutinized proteomic adjustments in response to exercise in ME/CFS subjects compared to sedentary controls. Our proteomics dataset spans a 10-log range (from fM [10^{-15}] to μM [10^{-6}] concentrations), with a total of over 3.5 million protein measurements for 6,361 unique proteins.

The analysis of our extensive dataset reveals stark differences between the 96 females and 36 males selected in our 132-subject cohort. Also, our analysis took advantage of our longitudinal sample collection, by using a linear mixed model, c-means time series clustering and t-sne classification. We found significant disturbances in ME/CFS patients compared to controls, with many abnormalities pertaining to exercise. Enrichment analysis revealed disruptions of the immune and nervous system as well as energy-related pathways, while correlation analysis with symptom severity exposed strong and significant associations.

Deciphering the proteomic changes occurring during PEM onset provides fundamental information about underlying physiological abnormalities in the disorder.

Emails:
Presenting Author: ag297@cornell.edu
Corresponding Author: mrh5@cornell.edu

Extracellular Vesicle Protein Cargo in ME/CFS Cases and Controls Following Maximal Exercise

Ludovic Giloteaux[1], Katherine A. Glass, Arnaud Germain, Carl J. Franconi and Maureen R. Hanson*

Cornell University, Ithaca, NY

Previously, 18 ME/CFS and 17 healthy sedentary females completed a maximal cardio-pulmonary exercise test and provided blood samples. We used quantitative untargeted proteomics to examine protein cargo of plasma-derived extracellular vesicles (EVs) before, 15 minutes and 24 hours post-exercise. We found large-scale, transient increases in the abundance of EV proteins in response to maximal exercise in healthy females. We also demonstrated that EV signaling post-exercise is highly disrupted and reduced in females with ME/CFS. Through enrichment analyses, we showed that the dysregulated proteins are involved in many pathways and systems, including platelet functions, the immune system, muscle contraction, the central nervous system, and endothelial signaling. We found many strong and significant correlations between the change in EV protein abundance post-exercise in ME/CFS and key symptoms including myalgia, arthralgia, and fatigue.

Recently, the same experiment and analyses were performed on a male cohort of 12 ME/CFS and 12 sedentary controls. We found fewer proteins in male ME/CFS patients with increased abundance due to exercise and that the differentially abundant proteins in patients vs. controls were predominantly lower in ME/CFS cases. EV proteins from the male cohort were strongly enriched in apoptosis-related pathways, neutrophil degranulation, biological oxidations, and muscle contraction. Similar to the female cohort, many strong and significant correlations were found between the change in EV protein abundance post-exercise and key symptoms.

Overall, these findings suggest that EV signaling dysregulation post-exercise may be contributing to ME/CFS pathophysiology in both sexes, particularly to the inability to recover from exertion.

[1]Presenting author: Ludovic Giloteaux, lg349@cornell.edu,
Co-authors: Katherine A. Glass, Arnaud Germain and Carl J. Franconi
*Corresponding author: Maureen R. Hanson, mrh5@cornell.edu

HERV activation segregates ME/CFS from fibromyalgia and patients fulfilling both clinical criteria

Karen Giménez-Orenga[1], Eva Martín-Martínez[2], Lubov Nathanson[3], Elisa Oltra[4]*

[1] Escuela de Doctorado, Universidad Católica de Valencia San Vicente Mártir, Valencia, Spain.
[2] National Health Service, Manises Hospital, Valencia, Spain
[3] Institute for Neuro-Immune Medicine, Dr. Kiran C. Patel College of Osteopathic Medicine, Nova Southeastern University, Fort Lauderdale, FL, USA
[4]Department of Pathology, School of Health Sciences, Universidad Católica de Valencia San Vicente Mártir, Valencia, Spain

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) and fibromyalgia (FM) are chronic diseases with poorly understood pathophysiology and diagnosis based on clinical assessment of unspecific symptoms. The recent post-COVID-19 condition, which shares similarities with ME/CFS and FM, has raised concerns about viral-induced transcriptome changes in post-viral syndromes. Viral infections, and other types of stress, are known to unleash human endogenous retroviruses (HERV) repression that if maintained could lead to symptom chronicity. This study evaluated this possibility for ME/CFS and FM on a selected cohort of female patients complying with diagnosis criteria for ME/CFS, FM, or both, and matched healthy controls (n=43). The results show specific HERV fingerprints for each disease, confirming biological differences between ME/CFS and FM. Unexpectedly, HERV profiles segregated patients that met both ME/CFS and FM clinical criteria from patients complying only with ME or FM criteria, while clearly differentiating patients from healthy subjects, supporting that the highly prevalent comorbidity condition must constitute a different nosological entity. Moreover, HERV profiles exposed significant quantitative differences within the ME/CFS group that correlated with differences in immune gene expression and patient symptomatology, supporting ME/CFS patient subtyping and confirming immunological disturbances in this disease. Pending issues include validation of HERV profiles as disease biomarkers of post-viral syndromes and understanding the role of HERV during infection and beyond.

Email:

Presenting Author: karen.gimenez@ucv.es
*Corresponding author. Email: elisa.oltra@ucv.es

Identification of Four Clinical Subgroups of ME/CFS Patients Using Model-based Clustering

Katherine Glass, Geoffrey Moore, Paul Munn, Carl Franconi, Maureen Hanson
Cornell University, Ithaca, NY

Identifying clinically relevant subgroups of people with ME/CFS may allow for tailored treatment strategies. Our objective was to uncover latent subgroups of ME/CFS patients with differing clinical profiles by performing model-based clustering of specific symptom severity (SSS) scores and metrics of overall severity.

91 ME/CFS subjects completed comprehensive medical history surveys (ClinicalTrials.gov NCT04026425). Model-based clustering was performed using several survey variables (*Mclust* R package). Features that maximized the overall certainty of cluster assignment and the model information criterion were selected. The final model included the SF-36 general health score, the SF-36 physical component summary, the percentage of waking time spent reclined, and all nine domains of the SSS scores. A four-cluster model was optimal. Clusters were compared using Welch's ANOVA with Games-Howell posthoc. Levels of 6,361 proteins were measured in plasma (n = 79; SomaScan Assay).

The four clusters have significantly different overall severity and severity of key symptoms (recurrent sore throat, lymph node tenderness, muscle pain, and joint pain): 1) LOWER overall severity and LOWER symptom severity (n=37); 2) HIGHER overall severity and HIGHER symptom severity (n= 35); 3) LOWER overall severity and HIGHER symptom severity (n=9); and 4) HIGHER overall severity and LOWER symptom severity (n=10). We also found significant differences in the plasma proteomes of the four clusters (linear mixed model, $q < 0.1$).

We identified four subgroups of ME/CFS patients with significant differences in symptomology and overall disease severity. Characterizing molecular signatures of clinical subgroups may elucidate pathophysiologic mechanisms contributing to ME/CFS heterogeneity.

Epigenetic Reprogramming of CD8+ T cell Populations Drives Exhaustion in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)

David S. Iu[1]*, Jessica Maya[1]*, Luyen T. Vu[1], Elizabeth A. Fogarty[1], Paul R. Munn[2], Jennifer K. Grenier[2], Maureen R. Hanson[1], Andrew Grimson[1]

[1]Cornell University, Department of Molecular Biology and Genetics, Ithaca, NY
[2]Cornell University, Genomics Innovation Hub and TREx Facility, Ithaca, NY
*These authors contributed equally to this work.

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a severe, debilitating disease with striking similarities to long-COVID/PASC, but its etiology remains unclear. Multiple lines of evidence point towards immune dysfunction as a potential cause for ME/CFS, and we previously reported metabolic changes such as increased fatty acid oxidation, decreased glycolysis, and decreased mitochondrial membrane potential in ME/CFS CD8+ T cells, features commonly found on exhausted T cells. To comprehensively characterize the gene regulatory state underlying potential transcriptional dysregulation, we first re-analyzed a single cell transcriptomic atlas of ME/CFS by reclassifying CD8+ T cells, where we found that various cell clusters upregulated exhaustion-associated genes. We then generated high resolution profiles of gene expression and chromatin accessibility in effector memory CD8+ T cells (T_{EM}) and naïve CD8+ T cells by RNA-seq and ATAC-seq at baseline and further analyzed different CD8+ subsets following symptom provocation. We observed in ME/CFS T_{EM} upregulation of key transcription factors associated with exhaustion and downregulation of genes controlling key metabolic activities, as well as an altered chromatin landscape consistent with exhaustion programs, while ME/CFS T_N displayed a potential loss of naïve identity. Flow cytometry of exhaustion markers detected a higher frequency of exhaustion-associated transcription factors, validating our bioinformatic analyses. Our dataset serves as an important resource for understanding ME/CFS pathology and supports a novel state of immune cell dysregulation in ME/CFS which may offer new treatment options.

First author contact: dl649@cornell.edu
Corresponding author contact: agrimson@cornell.edu

Tracking Peripheral Immune Cell Infiltration of the Brain in ME/CFS

Chloe L. Jones[1], Suzanne E. Lapi[2], Denise Jeffers[2], Jennifer L. Bartels[2], Hailey H. Houson[2], Jonathan McConathy[2], Sharon L. White[2], and Jarred Younger[1]

[1]Department of Psychology, University of Alabama at Birmingham, Birmingham, AL, USA
[2]Department of Radiology, University of Alabama at Birmingham, Birmingham, AL, USA

Introduction: ME/CFS may be caused by infiltration of peripheral immune cells into the brain, resulting in neuroinflammation. This study tests the first positron emission tomography (PET) brain imaging of autologous leukocytes radiolabeled with [^{89}Zr]oxine. The long half-life of Zirconium 89 (3.2 days) allows for cell tracking over multiple days, which is essential for capturing peripheral cell migration to the brain.

Methods: To assess if [^{89}Zr]oxine labeled leukocytes could be detected three days after reinjection, four healthy individuals completed the protocol. Leukocytes were isolated from blood and incubated with [^{89}Zr]oxine. Labeled cells were washed to remove traces of unbound [^{89}Zr]oxine and re-suspended for injection into participants. Whole-body computed tomography (CT) and PET brain scans were conducted immediately following injection, and at 5-, 24-, 48-, and 72-hours post-injection.

Results: There were no reported side effects or adverse events. Images obtained 72-hours after injection established that [^{89}Zr]oxine labeled leukocytes remained detectable. Whole-body CT imaging showed labeled leukocytes accumulated in the liver, spleen, and bone marrow, as expected. PET brain imaging revealed the highest uptake in the blood, bone marrow, and muscle tissue of the head as measured by regions of interest in the superior sagittal sinus, C2 and C3 vertebrae, and temporalis muscle. Healthy participants did not exhibit leukocyte infiltration into brain tissue.

Conclusion: Results confirm that tracking peripheral leukocytes on PET imaging over multiple days with [^{89}Zr]oxine is feasible. Future scans will be performed on ten ME/CFS patients to test if the illness involves peripheral immune cell infiltration of the brain.

First author email: chloej@uab.edu
Corresponding author email: younger@uab.edu

Post-Exertional Malaise Provocation Induces Different Profiles of Cognitive Impairment in ME/CFS

Corinne Leveau[1,2,3,4], Iurie Caraus[1,3,4], Evguenia Nepotchatykh[1,3,4], Ghazeleh Azimi[1,2,3,4], Wesam Elremaly[1,3,4], Anita Franco[1,3,4], Alain Moreau[1,2,3,4,5]

[1]Viscogliosi Laboratory in Molecular Genetics of Musculoskeletal Diseases, Sainte-Justine University Hospital Research Center, Montreal, Qc, Canada
[2]Department of Biochemistry and Molecular Medicine, Faculty of Medicine, Université de Montréal, Montreal, Qc, Canada
[3]Open Medicine Foundation ME/CFS Collaborative Center at CHU Sainte-Justine/Université de Montréal
[4]ICanCME Research Network
[5]Department of Stomatology, Faculty of Dentistry, Université de Montréal, Montreal, Qc, Canada

Problematic. Myalgic encephalomyelitis (ME) is associated with a wide range of symptoms including post-exertional malaise (PEM), which often results in cognitive impairment. Symptoms are known to vary in frequency and severity between individuals.

We hypothesized that ME patients could be stratified in different clusters according to their cognitive impairment levels triggered by PEM induction in response to a standardized provocation maneuver.

Methods. A prospective cohort was designed to enroll persons with ME (n=53) and matched sedentary controls (n=16). All participants were subjected to PEM induction through a 90-minutes mechanical arm stimulation. Six cognitive domains were evaluated with BrainCheck test (BrainCheck, Inc., USA) at baseline (T0) and after stimulation (T90). Each participant received a score and a population percentile based on their task performance.

Results. Performance between the groups was significantly different at T90 in four out of six cognitive tasks ($p < 0.05$). We then stratified the ME group in three clusters by k-means method based on their Δ percentile rank (T90-T0) for each task. We also investigated whether changes in the expression level of circulating miRNAs previously associated with ME and other molecules identified in diseases with a cognitive impairment component were consistent with the clusters. Conclusion. These results showed the impact of PEM on cognition in the context of ME as well as the variability of cognitive domains affected. The new proposed clustering approach using the BrainCheck test results will allow a better understanding of the contribution of miRNA and epigenetic mechanisms underlying neurocognitive impairment triggered by PEM.

Blood Brain Barrier Permeability is Increased in ME/CFS: An MRI Study

Sheryl Liu[1,2], Dengrong Jiang[3], Rodolphe Leforestier[2], Hanzhang Lu[3], Benjamin Natelson[4], Xiang Xu[2]

[1]Livingston High School, Livingston, NJ, USA
[2]BioMedical Engineering and Imaging Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[3]Department of Radiology, Johns Hopkins University, Baltimore, MD, USA
[4]Department of Neurology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

The etiology of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) remains elusive. It has been hypothesized that increases in blood-brain barrier (BBB) permeability in patients with ME/CFS lead to many of the symptoms experienced by these patients. A dysfunctional BBB is associated with chronic neuroinflammation and can lead to a sustained illness with chronic relapse recovery cycles. Post-Acute Sequelae of SARS-CoV-2 infection (PASC) patients, many of whom also meet ME/CFS diagnostic criteria, differ primarily in the shorter duration of their illness compared to those with classic ME/CFS. Assessing BBB permeability across PASC ME/CFS, classic ME/CFS, and healthy individuals could validate this hypothesis and deepen our understanding of ME/CFS pathophysiology. Traditional method for measuring the BBB permeability involves dynamic contrast enhanced magnetic resonance imaging using Gadolinium contrast agents. Since these contrast agents are large molecules, they are sensitive to major leakage of BBB, but may not detect subtle changes of BBB permeability. In this study, we applied a recently developed, non-contrast MRI technique to measure the BBB permeability to water. This method calculates the water extraction fraction and the BBB's permeability-surface-area product by tracking arterially labeled blood spins flowing into cerebral veins. The study was conducted on PASC ME/CFS patients (N=7), classic ME/CFS patients (N=9) and healthy controls (N=8). It was found that there was no significant difference in water extraction fraction between the three groups, but the permeability surface area product was significantly higher in the classic ME/CFS group than the PASC or healthy control groups.

Sheryl Liu: sherylliu100@gmail.com

Corresponds to:
Xiang Xu
xiang.xu@mssm.edu
1 Gustave Levy Place, Box 1234
New York, NY, 10029

Investigating T Cell Populations for Immune Cell Dysfunction in ME/CFS

Jessica Maya, Maureen R Hanson
Dept. of Molecular Biology and Genetics, Biotechnology Building, Cornell University, Ithaca, NY

Emerging research suggests significant immune system and metabolic dysregulation in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). Our prior investigations revealed notable alterations in ME/CFS T cells, including reduced glycolysis, decreased mitochondrial membrane potential, and increased fatty acid oxidation, resembling characteristics typically observed in exhausted T cells – a state commonly associated with chronic viral infections and cancer. This reversible exhausted state can lead to diminished cell proliferation, reduced cell survival, and decreased cytokine production. To ascertain whether T cell exhaustion is a contributing factor in ME/CFS, we conducted an in-depth analysis of CD4+ and CD8+ T cells, as well as their respective subsets, with a focus on inhibitory receptors and transcription factors associated with exhaustion. We collected blood samples from 15 ME/CFS patients and 14 healthy controls. Using magnetic beads, we isolated CD4+ and CD8+ T cells and examined approximately 15-17 markers using the FACSymphony analyzer, allowing us to identify distinct T cell populations (naïve, effector, memory, regulatory, and helper T cells). The inclusion of inhibitory receptors like PD-1 and transcription factors like Tox allowed us to identify stages of T cell exhaustion within circulating cells. The results from our flow cytometry panels revealed pronounced exhaustion profiles within multiple T cell populations, indicating higher levels of dysregulated T cells in ME/CFS patients when compared to healthy controls. These compelling findings provide valuable insights into the presence of exhausted and dysregulated phenotypes within ME/CFS T cells, potentially serving as a guide for the development of future therapeutic approaches for this complex, challenging illness.

Emails:

Jm2629@cornell.edu

Mrh5@cornell.edu

Investigating the role of iNOS in endothelial dysfunction in ME/CFS

Claire F. McNally, Maureen R. Hanson
Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA

The vascular endothelium regulates vascular tone, thrombosis, and cellular adhesion and permeability. ME/CFS patients exhibit impaired blood perfusion to the periphery and subsets of patients have significantly lower median flow mediated dilation and post-occlusive reactive hyperemia, two standard measures of endothelial function, than healthy controls. Impaired ability of the vasculature to supply oxygen to cells could explain many of the energetic symptoms of ME/CFS. ME/CFS plasma has been reported to cause a decrease in production of nitric oxide, the molecule which regulates vascular homeostasis, in human umbilical vein endothelial cells (HUVECs). The primary source of endothelial nitric oxide (NO) is the endothelial isoform of nitric oxide synthase (eNOS), but the inducible NOS isoform (iNOS) is also present. iNOS produces NO continuously and in a large burst once activated by infection or chronic inflammation inducing a hyperinflammatory response. If iNOS becomes uncoupled, it produces large quantities of reactive oxygen species (ROS), perpetuating inflammation. The effect of ME/CFS plasma on iNOS induction in the endothelium has not been explored.

We will report on the effect of ME/CFS plasma from 10 ME/CFS and 10 healthy controls on iNOS induced NO and ROS production in human endothelial cells (HUVECs) using a flow cytometry-based assay. The contribution of iNOS is measured by incubating plasma treated HUVECs with DAF-FM and CellRox Deep Red in the presence or absence of the iNOS specific inhibitor, 1400W dihydrochloride.

This study can help elucidate the molecular basis of endothelial dysfunction and inflammation in ME/CFS.

Emails:

Presenting Author: cfm58@cornell.edu

Corresponding Author: maureen.hanson@cornell.edu

Predicting Trajectories and Long-Term Sequelae in Long COVID: An Exploratory Pilot Study

Diana Petre[1,2,3,4], Evguenia Nepotchatykh[1,3,4,5], Iurie Caraus[1,3,4], Wesam Elremaly[1,3,4], Anita Franco[1,3,4], Alain Moreau[1,2,3,4,5,6]

[1]Viscogliosi Laboratory in Molecular Genetics of Musculoskeletal Diseases, Sainte-Justine University Hospital Research Center, Montreal, Qc, Canada
[2]Department of Biochemistry and Molecular Medicine, Faculty of Medicine, Université de Montréal, Montreal, Qc, Canada
[3]Open Medicine Foundation ME/CFS Collaborative Center at CHU Sainte-Justine/Université de Montréal
[4]ICanCME Research Network, Sainte-Justine University Hospital Research Center, Montreal, QC, Canada
[5]Molecular Biology PhD Program, Faculty of Medicine, Université de Montréal, Montreal, QC, Canada
[6]Department of Tomatology, Faculty of Dentistry, Université de Montréal, Montreal, QC, Canada

An increasing number of patients are presenting with "Long COVID" (LC), also known as post- acute sequelae of SARS-CoV-2 (PASC), a constellation of symptoms that the World Health Organization (WHO) is defining as post-COVID syndrome. Among known long-term sequelae, LC patients present a high risk of developing myalgic encephalomyelitis (ME). ME epidemics have historically followed viral outbreaks, while most ME patients describe viral infections as a trigger. We propose that LC onset and development of specific clinical trajectories like ME are caused by epigenetic alterations, notably changes in specific circulating microRNA expression.

In this prospective study, fifty LC patients (18-55 years old), who were non-hospitalized following their SARS-CoV-2 infection and presenting LC symptoms persisting over six months were recruited and compared against post-COVID and pre-pandemic healthy participants. All participants completed three health questionnaires (SF-36, MFI-20, DSQ). The expression levels of 11 circulating microRNAs were measured by RT-qPCR on plasma samples. By combining principal component analysis and Random Forest Models, LC patients classify into six distinct groups corresponding to specific clinical outcomes.

Our results showed that 55% of LC patients exhibited a molecular profile and symptoms corresponding to ME with or without fibromyalgia (ME+FM) as a comorbidity, while 10% of them were classified in the FM group and the others were distributed into three distinct groups: neurological diseases, respiratory illnesses, and severe allergies (MCAS).

It is anticipated that this diagnostic/prognostic panel of 11 microRNAs could pave the way for early and more selective medical interventions to prevent long-term sequelae associated with LC.

Emails:
diana.petre@umontreal.ca
alain.moreau.hsj@ssss.gouv.qc.ca

Impact of Age and Sex on Microbial Susceptibility and Immune Activation in ME/CFS

Amit Ranjan[1], Xiaoyu Che[1,2], W. Ian Lipkin[1,3,4,5]

[1]Center for Infection and Immunity, Mailman School of Public Health, Columbia University; New York, NY, USA
[2]Department of Biostatistics, Mailman School of Public Health, Columbia University; New York, NY, USA
[3]Department of Epidemiology, Mailman School of Public Health, Columbia University; New York, NY, USA
[4]Department of Neurology, Vagelos College of Physicians and Surgeons, Columbia University; New York, NY, USA
[5]Department of Pathology, Vagelos College of Physicians and Surgeons, Columbia University; New York, NY, USA

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a disabling and complex disorder often associated with immune dysfunction. We analyzed supernatant cytokine profiles and peripheral blood mononuclear cell transcript profiles in individuals with ME/CFS and controls after ex vivo stimulation with immunostimulatory agents before and 24hrs after exercise. Cytokine measurements included 56 ME/CFS subjects and 52 matched controls. Transcript profile analyses were performed on a subset of 31 ME/CFS subjects and 31 controls matched on age and sex. The levels of cytokines (IL-β, IL-2, IL-6, IL-23, TNF-α and IL-8) and a chemokine (CXCL5) were higher in ME/CFS subjects than controls after stimulation with *Staphylococcus* enterotoxin B (SEB). Exercise did not significantly impact levels of cytokines. The elevated immune responses were more prominent in female patients, particularly in those over 45 years of age who had lower plasma levels of 17-β estradiol than their younger counterparts. At baseline (prior to stimulation), the older (>45Y) and younger (≤45Y) female ME/CFS subjects differed in RNA profiles to their controls. The older females had higher levels of transcripts encoding mast cell specific proteases *TPSB2* and *TSAB1*; T cell-receptors *TRAV23DV6*, *TRBV-29*, and *TRBV-12*; ribosomal proteins; and regulatory long non-coding RNAs *IL21-AS1, SNHG-8, PRKCQ-AS1.* The younger females had lower levels of transcripts encoding human leukocyte antigen genes *HLA-DQA, HLA-DQB* and *HLA-DRB4*. These findings may provide insight into hypersensitivity to microbial stimuli and immune activation in ME/CFS and its sub-groups.

Emails: ar4111@cumc.columbia.edu (AR)

xc2273@cumc.columbia.edu (XC)

wil2001@cumc.columbia.edu (WIL)

UpTime: A Digital Biomarker for ME/CFS

Bella Rond[1], Yifei Sun[2], Candace Rond[1], Sarah Scheiving[1], Lucinda Bateman[1], Shad Roundy[2], Suzanne D. Vernon[1]

[1]The Bateman Horne Center of Excellence, 24 South 1100 East, Suite 205, Salt Lake City, UT, USA
[2] Department of Mechanical Engineering, University of Utah, Salt Lake City, UT, USA

OBJECTIVE: Hours of upright activity is a clinically meaningful measure of ME/CFS disease severity. Our goal was to develop an objective measure of hours of upright activity that could be used as a clinically meaningful endpoint and outcome measure in ME/CFS clinical trials.

METHODS: 51 subjects were enrolled including 30 ME/CFS participants, 15 Long COVID participants, and 6 healthy controls. Subjects reported HUA (defined as hours per day spent with feet on the floor) and completed questionnaires to assess the impact of orthostatic intolerance on daily activities and symptoms over the course of 7 days. Each participant wore a wearable device equipped with a sensor that continuously measured upright activity, or UpTime, over 7 days. Data analysis used one-way ANOVA.

RESULTS: ME/CFS participants had significantly lower UpTime compared to healthy controls ($p < 0.00004$). UpTime was able to distinguish between ME/CFS participants, Long COVID participants, and Health Controls ($p < 0.022$). While self-report orthostatic symptoms and hours of upright activity and steps per day were different between patients and controls, there was greater variance in these measures resulting in overlap and lack of statistical difference between the groups.

CONCLUSIONS: ME/CFS UpTime was significantly better at predicting ME/CFS and Long COVID disease severity compared to self-reported hours of upright activity or passively measured steps per day. Next steps are being taken to qualify UpTime as a digital biomarker for ME/CFS.

Objective Sleep and Pupillometry Measurements in Participants with ME/CFS and Controls

Deena Saadi, Hannah Bues, Michael VanElzakker

Massachusetts General Hospital/Harvard Medical School

We present preliminary objective data relating to sleep and sensory sensitivity in individuals with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and controls using quantitative dynamic pupillometry and an at-home wearable sleep device.

Markers of abnormal sleep such as sleep instability – measured via polysomnography and multiple sleep latency testing – have been found to be common in those with ME/CFS. Visual processing – assessed via in-lab contrast sensitivity testing – has also been found to be abnormal in the retina and subcortical visual pathways in patients with ME/CFS. Although sleep and sensory problems are part of ME/CFS diagnosis criteria, their measurement is often only possible in lab settings with specialized instruments. We aim to replicate and add to these findings using more accessible and portable instruments.

Data presented here are part of larger ongoing ME/CFS studies that measure sleep and pupillary responses separately or together. We conducted an at-home sleep test using a wearable *SleepImage* CheckMe O_2 Recorder that quantifies sleep duration, sleep apnea and overall sleep quality via a sleep quality index (SQI) score, alongside heart rate and oxygen saturation. Pupillary responses – a measure of autonomic function and sensory sensitivity – was measured using the PLR-3000 NeurOptics pupillometer, a portable hand-held device that records and analyzes how an individual's pupil constricts and dilates in response to a brief light stimulus.

We present preliminary analysis from these two objective measures of autonomic dysregulation- sleep and pupillary response- as part of a larger mosaic of dysautonomia in participants with ME/CFS.

The Role of Irisin in the Pathogenesis of Myalgic Encephalomyelitis

Souma B[1,2,3,4], Elremaly W[1,3,4], Elbakry M[1,3,4,5], Akoume M-Y[1,3,4,6], and Moreau A[1,2,3,4,7]

[1]Viscogliosi Laboratory in Molecular Genetics of Musculoskeletal Diseases, Sainte-Justine University Hospital Research Center, Montreal, Qc, Canada
[2]Department of Biochemistry and Molecular Medicine, Faculty of Medicine, Université de Montréal, Montreal, Qc, Canada
[3]Open Medicine Foundation ME/CFS Collaborative Center at CHU Sainte-Justine/Université de Montréal
[4]ICanCME Research Network, Sainte-Justine University Hospital Research Center, Montreal, QC, Canada
[5]Biochemistry section, Chemistry Department, Faculty of Science, Tanta University, Tanta, Egypt
[6]Department of Pharmacology and Toxicology, Faculty of Medicine, Université des Sciences de la Santé (USS) de Libreville, Libreville, Gabon
[7]Department of Stomatology, Faculty of Dentistry, Université de Montréal, Montreal, QC, Canada

Background: Myalgic encephalomyelitis (ME) is a heterogeneous disorder with uncertain pathogenesis. Post-exertional malaise (PEM) is the hallmark of ME. Irisin is a myokine synthesised by the muscles and it has been suggested that elevation of irisin mediates the beneficial effects of exercise.

Objective: To explore the role of irisin in the pathogenesis of ME, notably in the context of PEM.

Methods: This prospective study involved 92 ME patients and 40 matched sedentary healthy controls (HC). Each participant completed different health questionnaires (SF-36, MFI-20, DSQ) to evaluate their health status. Blood samples were collected at two time points, at the baseline (T0) and after applying a stress test (T90) to induce PEM. Circulating irisin levels were measured using an ELISA method. Western blot was used to detect possible distinct irisin forms. T-tests and ANOVA were used to determine statistical significance ($P < 0.05$).

Results: The plasma irisin levels in the ME group were lower (15 ± 0.5 µg/mL) compared to HC group (17 ± 0.7 µg/mL) at baseline. Following PEM induction, an increase in irisin levels was only observed in HC group. Males with ME had significantly lower circulating irisin levels compared to females. Elevation of plasma irisin levels in male patients exhibited significant correlations with the worsening of physical fatigue and PEM severity. Our Western blot results indicated that the observed difference in irisin levels between the two sexes could not be attributed to post-translational modifications.

Conclusion: The findings of this study may be of interest in ME management and suggest a new role for irisin as a potential biomarker for ME severity.

ME/CFS Pathophysiology Investigated by Invasive Cardiopulmonary Exercise Test (iCPET) and Autonomic Function Testing

J. Squires[1], S. Al-Zayer[1], W. Xiao[2], P. Novak[3], D. Systrom[1]

[1]Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
[2]Department of Surgery, Science, and Bioengineering, Massachusetts General Hospital, Boston, MA, USA
[3]Department of Neurology, Brigham and Women's Faulkner Hospital, Harvard Medical School, Boston, MA, USA

Introduction: Mechanisms underlying exercise and orthostatic intolerance in ME/CFS have been uncovered by invasive cardiopulmonary exercise testing (iCPET) and autonomic function testing (AFT), but the relationships between the two are not known. This study aims to determine if there is overlap of cardiovascular and respiratory pathophysiology in patients who have undergone both tests.

Methods: Between January 2017 and April 2022, 62 patients were identified with a contemporary iCPET and AFT. Key variables from the iCPET included peak oxygen uptake (pVO2), cardiac output (pQc), right atrial pressure (pRAP), and systemic oxygen extraction (Ca-vO2). Key variables from the autonomic testing included epidermal and sweat gland small fiber neurite density, electrochemical skin conductance, change in heart rate (ΔHR), and end tidal carbon dioxide (ΔETCO2) from supine to upright during the tilt table test (TTT).

Results: All 62 patients demonstrated preload failure (pRAP < 6.5mmHg). Of this group, 54 patients (87.1%) fulfilled NAM criteria for ME/CFS, with 32 testing positive (59.3%) for small fiber neuropathy (SFN) using either morphological and/or functional testing. Significant correlations were found between pVO2 and ΔHR ($r=-0.439$, $P<0.05$) and ΔETCO2 ($r=0.474$, $P<0.05$) during TTT. The same tilt table variables were found to be significantly correlated with pQc ($r=-0.365$, $P<0.05$ and $r=0.351$, $P<0.05$) from the iCPET.

High Circulating miR-29a-3p and miR-150-5p Levels are Associated with Vascular Instabilities in Myalgic Encephalomyelitis

Yasaman Vahdani [1,2,3,4], Evguenia Nepotchatykh [2,3,4,5], Wesam Elremaly [2,3,4], Iurie Caraus [2,3,4], Mohamed Elbakry [2,3,4] Anita Franco [2,3,4], Moreau Alain[1,2,3,4,5,6]

[1]Department of Biochemistry and Molecular Medicine, Faculty of Medicine, Université de Montréal, 2900 Edouard Montpetit Blvd, Montreal, QC, H3T 1J4, Canada
[2]Viscogliosi Laboratory in Molecular Genetics of Musculoskeletal Diseases, Sainte-Justine University Hospital Research Center, 3175 Cote-Ste-Catherine Road, Montreal, QC, H3T 1C5, Canada
[3]Open Medicine Foundation ME/CFS Collaborative Center at CHU Sainte-Justine/Université de Montréal, 3175 Cote-Ste-Catherine Road, Montreal, QC, H3T 1C5, Canada
[4]ICanCME Research Network, Sainte-Justine University Hospital Research Center, 3175 Cote-Ste-Catherine Road, Montreal, QC, H3T 1C5, Canada
[5]Molecular Biology PhD Program, Faculty of Medicine, Université de Montréal, 2900 Edouard Montpetit Blvd, Montreal, QC, H3T 1J4, Canada
[6]Department of Stomatology, Faculty of Dentistry, Université de Montréal, 2900 Edouard Montpetit Blvd, Montreal, QC, H3T 1J4, Canada

Background: Myalgic Encephalomyelitis (ME) is a complex disorder characterized by lasting fatigue and post-exertional malaise (PEM), associated with vascular abnormalities, including orthostatic intolerance (OI) and postural orthostatic tachycardia syndrome (POTS). However, the causes of POTS remain uncertain, previously, we identified eleven specific miRNAs associated with ME, PEM, and disease severity.

Hypothesis: We propose that alterations in circulating microRNAs could potentially contribute to vascular instabilities in ME and exacerbate the disease severity.

Objectives: To investigate the expression profiles of a panel of 11 circulating miRNAs previously associated with ME pathogenesis, explicitly focusing on their potential involvement in vascular instabilities.

Methods: 114 ME patients and 43 matched healthy controls (HC) were enrolled in this prospective study. Within the ME group, two subgroups were identified: ME (n=101) and ME with OI/POTS (n=13). All participants completed standard questionnaires to assess their health status. Blood samples were collected at two time points: baseline (T0) and after stress test to induce PEM over 90 minutes of stimulation (T90). The expression levels of 11 miRNAs were measured using qPCR. ANOVA was performed to compare the data among the different groups.

Results: The severity scores of physical fatigue, general fatigue, and reduced motivation were higher in ME+OI/POTS compared to ME and HC. Furthermore, the expression profiles miR-29a-3p and miR-150-5p at T90 were significantly elevated in ME+OI/POTS compared to other groups.

Conclusion: These findings suggest a potential association between miR-29a-3p and miR-150-5p and vascular abnormalities, offering valuable insights into the disease and may pave the way for new therapeutic approaches.

Emails:

Yasaman.vahdani@umontreal.ca
evguenia.nepotchatykh@umontreal.ca
wesam.elremaly.hsj@ssss.gouv.qc.ca
iurie.caraus.hsj@ssss.gouv.qc.ca

mohamed.elbakry.hsj@ssss.gouv.qc.ca
anita.franco.hsj@ssss.gouv.qc.ca
alain.moreau.hsj@ssss.gouv.qc.ca

Single-cell Transcriptomics of ME/CFS Circulating Immune System Before and After Symptom Provocation

Tien Luyen Vu[1]; Faraz Ahmed[2]; Hongya Zhu[1]; David Shing Huk Iu[1]; Elizabeth A. Fogarty[1]; Yeonui Kwak[1]; Weizhong Chen[1]; Carl J. Franconi[1]; Paul R. Munn[2]; Ann E. Tate[2]; Susan M. Levine[3]; Jared Stevens[4]; Xiangling Mao[5]; Dikoma C. Shungu[5]; Geoffrey E. Moore[6]; Betsy A. Keller[6]; Maureen R. Hanson[1]; Jennifer K. Grenier[2]; Andrew Grimson[1]

[1]Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA
[2]Genomics Innovation Hub and TREx Facility, Institute of Biotechnology, Cornell University, Ithaca, NY, USA
[3]Levine Clinic, Private Practice, New York, NY, USA
[4]Workwell Foundation, Ripon, CA, USA
[5]Department of Radiology, Weill Cornell Medicine, New York, NY, USA
[6]Department of Exercise Science and Athletic Training, Ithaca College, Ithaca, NY, USA

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a long-term disease affecting various body systems with undefined cause. The immune system is key to disease an symptom development. However, most gene expression studies in ME/CFS are limited to main immune cell lineages. Here, we aim to provide a detailed description of the circulating immune system of ME/CFS at a single cell level at baseline and 24h after symptom provocation. Peripheral blood mononuclear cells collected from cohort of healthy controls and ME/CFS cases before and 24h after challenge were subjected to single cell RNA sequencing. After quality control and data integration, there were no major changes in cell composition across different cell types. Notably, classical monocytes in ME/CFS patients exhibited the highest number of differentially expressed genes at both time points. Naïve and effector memory CD4 T cells, and NK cells also display significant alterations in gene expression. Genes involved in inflammation, migration, and tissue recruitment were upregulated in ME/CFS for classical monocytes, indicating a potential increase in tissue resident macrophages. Moreover, we were able to detect dysregulated and normal monocytes within patients by applying machine learning approaches. Surprisingly, we detected improper alteration of platelet activities in patients upon analyzing the transcriptomes at baseline and post-exercise challenge. Taken together, we provide, for the first time, a high throughput characterization of the circulating immune system in ME/CFS at baseline and after symptom provocation. This study also serves as a valuable resource to investigate alterations in ME/CFS from a molecular and cell biology standpoint.

First author contact: louisvu@cornell.edu

Corresponding author contact: agrimson@cornell.edu

7T MRI Showed Hippocampal Subfield Volume Differences in ME/CFS and Long COVID

Kiran Thapaliya[1*], Sonya Marshall-Gradisnik[1], Natalie Eaton-Fitch[1], Markus Barth[1,2], Maira Inderyas[1], Leighton Barnden[1]

Introduction: Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and long COVID patients show overlapping symptoms of fatigue, neurocognitive impairment, unrefreshing sleep, physical function, and pain. Studies show that 13-58% of long COVID patients met ME/CFS criteria and exhibited symptoms of fatigue, neurocognitive impairment, disability, and autonomic dysfunction. Neurocognitive impairment is one of the most frequently reported symptoms in both cohorts. Neurocognitive impairment in ME/CFS and long COVID patients includes memory loss, difficulty in decision-making, word finding and reasoning, and attention deficit. The neurocognitive impairment in ME/CFS and long COVID patients could be due to the changes in the hippocampus structure because the hippocampus and its subfields play an important role in cognitive function and are also involved in memory function.

Methods and Results:
Therefore, we used ultra-high field 7T MRI to investigate the hippocampal structural changes in 30 ME/CFS, 15 long COVID, and 15 healthy controls. We found left: subiculum head (ME/CFS vs. HC: p=0.002; long COVID vs HC, p=0.01), and pre-subiculum head (ME/CFS vs. HC: p=0.005; long COVID vs HC, p=0.004) volumes were significantly larger in ME/CFS and long COVID patients compared to healthy controls after multiple comparison correction. Interestingly, we did not observe a significant difference in the hippocampal subfield volumes between ME/CFS and long COVID.

Conclusion: This study showed structural changes in the hippocampus that may contribute to neurocognitive impairment in both ME/CFS and long COVID patients. Therefore, existing brain research findings in ME/CFS patients may help to better understand the neurological dysfunction in long COVID patients.

Email: k.thapaliya@griffith.edu.au

A Software for Integrating Multiple Methylation Studies: Feature Selection and Classification

Tao Xu[1], Dawei Li[1]

[1]Department of Immunology and Molecular Microbiology, Texas Tech University Health Sciences Center, Lubbock, TX, USA

Discoveries from high-throughput experiments are often hard to replicate because of study-level random effects and high dimensionality. Methods ignoring this, even with cross-validation, typically lead to perfect classification in training set but poor prediction in independent testing data. We developed a comprehensive R tool to identify reliable predictors that can classify cases and controls more consistently among different datasets through integrative analysis. Our approach first calculates a score for each genetic locus by combining differential analysis *P* values of the same locus from each study adjusted by sample size, and then selects the loci with the least scores (i.e., the most significant loci); then it uses these selected loci to conduct multivariate integrative partial-least-square discriminant analysis to determine linear combinations of loci as potential predictor. We applied the software into all the existing ME/CFS DNA methylation array datasets, including four PBMC datasets and three saliva datasets from different labs. Our integrative analysis found 662 DNA methylation probes that yielded 80% accuracies when predicting ME/CFS diagnosis in an independent dataset. Because our identified probes are independent of effects of studies/labs, our result appeared to be more likely to be replicated independently. We are currently applying this method also into RNA-seq datasets.

Email: taoxu@ttuhsc.edu

dawei.li@ttuhsc.edu

Structural and Oxygen Metabolic Magnetic Resonance Imaging of long-COVID and ME/CFS

Xiang Xu[1]*, Sera Saju[1], Puneet Belani[2], Michelle Blate[3], Tiffany Soto[3], Benjamin Natelson[3]

[1]BioMedical Engineering and Imaging Institute, Icahn School of Medicine at Mount Sinai, NY
[2]Department of Radiology, Icahn School of Medicine at Mount Sinai, NY
[3]Department of Neurology, Icahn School of Medicine at Mount Sinai, NY

Background: Many individuals who had COVID continue to experience a constellation of symptoms long after recovery from the initial illness (post-acute sequelae of SARS-CoV-2, PASC). The most frequently reported symptoms were fatigue, post exertional malaise and cognitive dysfunction, which are the main symptoms of ME/CFS. Clinically, many of the PASC patients fulfill diagnostic criteria for ME/CFS. In this project, we are conducting comprehensive magnetic resonance imaging (MRI) to determine similarities or differences in brain anatomy and metabolism between PASC and non-PASC ME/CFS patients, as well as healthy controls (HCs).

Methods: Multiparametric MRI: (1) high resolution comprehensive brain structural imaging; (2) in vivo measurement of venous oxygenation in the draining vessels of brain and global cerebral blood flow. Behavioral: Fatigue level was evaluated using the Multidimensional Fatigue Inventory and the physical functioning scale (PFS) of the Short Form 36.

Results: White matter hyperintensities were identified in a few participants. A Significant difference in venous oxygen saturation level, Yv, was observed among the three groups (p = 0.0074). Pair-wise comparison showed that the PASC group had significant lower Yv than both the HC and the non-PASC CFS groups (p = 0.004 and 0.0173, respectively). The Yv of the non-PASC CFS group did not differ significantly from HCs. A strong correlation (r=0.61) was found between the PFS and Yv.

Conclusion: The structural changes are likely non-specific to ME/CFS with or without PASC. Yv is correlated with physical function and is significantly lower for PASC-ME/CFS patients as compared to non-PASC-ME/CFS or HCs.

Correspond to:

Xiang Xu, PhD
1 Gustave Levy Place, Box 1234
Icahn School of Medicine at Mount Sinai
New York, NY, 10029

Organizing Committee

Agostina Casamento-Moran, PhD – Johns Hopkins University

Katharine (Katie) Glass, PhD – Cornell University

Chloe Jones – University of Alabama, Birmingham

Jessica Maya, PhD – Cornell University

Katharine Seton, PhD – Quadrum Institute

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