Post-exertion malaise in GWI: Brain, autonomic, and behavioral interactions

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GWI
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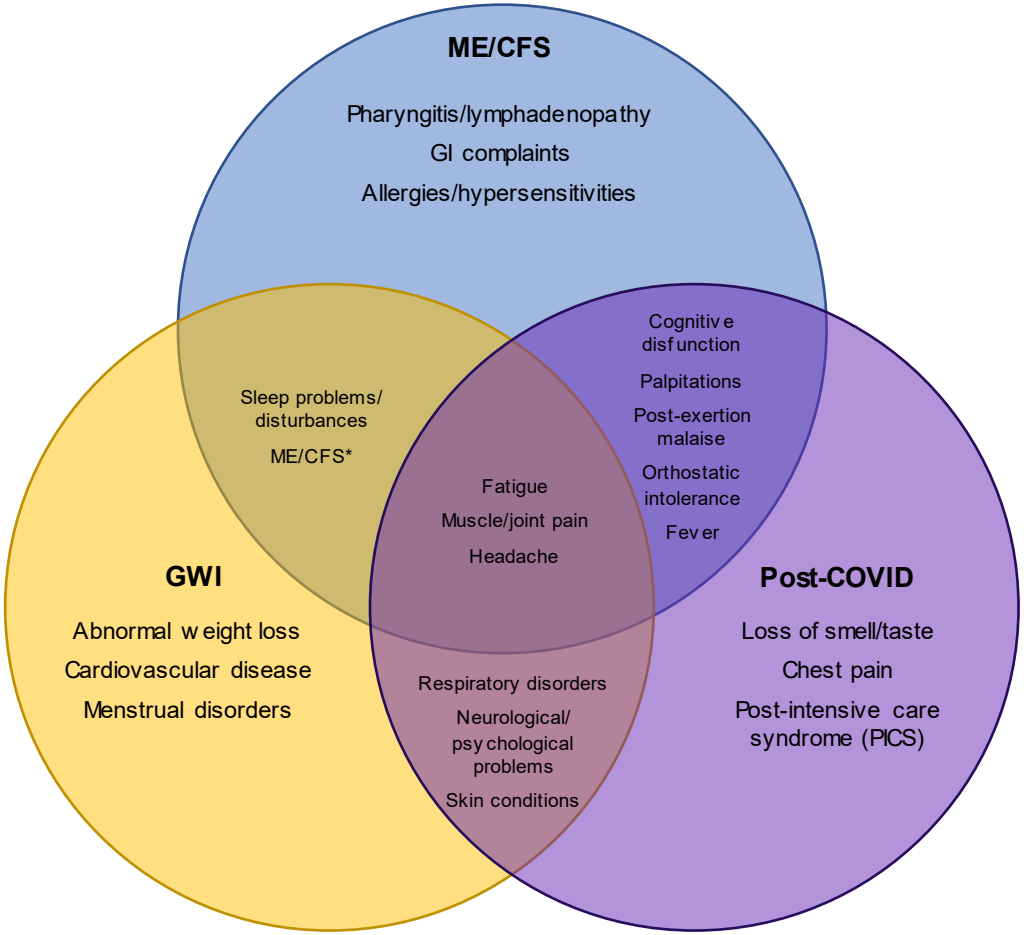
***Note: some of information contained herein is unpublished and preliminary data.**

Symptom Overlap in ME/CFS, GWI, and COVID

ME/CFS: symptoms persist for at least 6 months

GWI: symptoms persist for at least 6 months, appeared during active duty in Southeast Asia military operations by 12/31/21, and be at least 10% disabling. *Attributing illnesses include: Chronic Fatigue Syndrome, Fibromyalgia, functional gastrointestinal disorders, undiagnosed illnesses

Post-COVID: symptoms persist more than 4 weeks after initial infection and include asymptomatic cases



ME/CFS

80% of people with ME/CFS report a prodrome consistent with infection

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KEY FACTS · FEBRUARY 2015
Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)

What are the symptoms and other effects of ME/CFS?

- Reduction or impairment in ability to carry out normal daily activities, accompanied by profound fatigue
- Post-exertional malaise
- Unrefreshing sleep
- Cognitive impairment
- Orthostatic intolerance

Immunological Abnormalities

Metabolic Disturbances

Sex-specific Differences

CDC

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Prior Infections

Epstein-Barr virus
Ross River virus
Coxiella burnetti
→ 11% develop ME/CFS symptoms

SARS
MERS
→ 50% develop ME/CFS symptoms

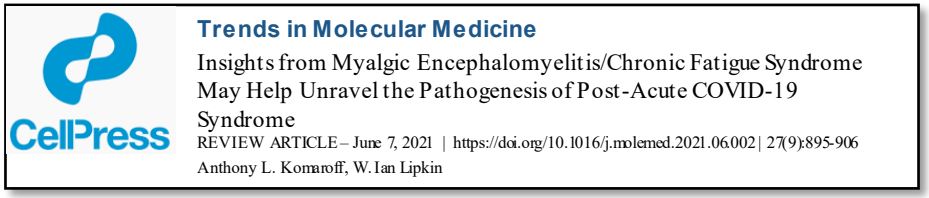
~~Human herpesvirus 6~~
~~Enterovirus~~
~~Rubella~~
~~*Candida albicans*~~
~~Bornaviruses~~
~~Mycoplasma~~
~~Human immunodeficiency~~
~~XMRV~~
→ Infections studied but not found to be a cause of ME/CFS

Post-COVID Sequelae

Acute disease:
- Neurologic signs and symptoms: 36% Wuhan, 82% Chicago
- Common: myalgia, headache, dysgeusia, anosmia, encephalopathy, psychiatric
- Less common: seizures, movement disorders, stroke, neuropathy
- Pathogenesis: indirect damage v. neuronal infection; infection of supporting cells, inflammation, vascular

Chronic disease:
- May occur with mild or no respiratory disease
- Cognitive dysfunction, headache, autonomic instability, sensory disturbances, anosmia, dysgeusia

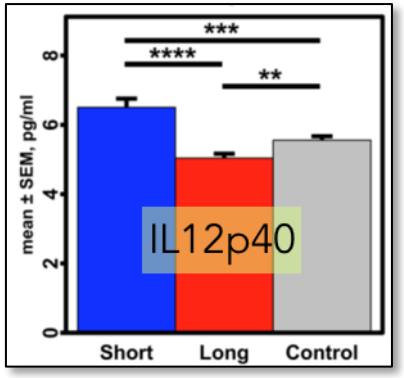
Similarities between ME/CFS and Long COVID:
- Viral prodrome
- Inflammation
- Cognitive dysfunction
- Autonomic instability

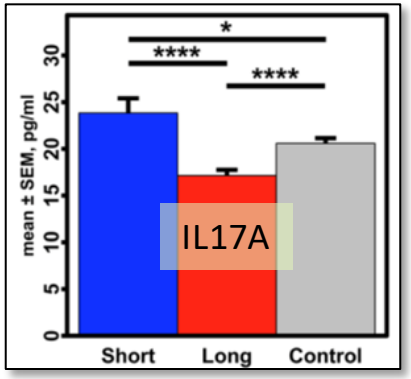


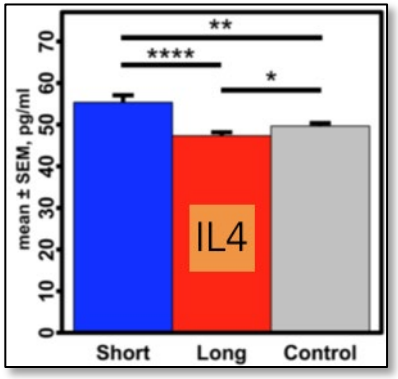
Pathogenesis:
- Autoantibodies to cytokines, chemokines, lymphocyte receptors, endothelial targets and multiple CNS targets including the orexin receptor (important in fatigue and sleep)

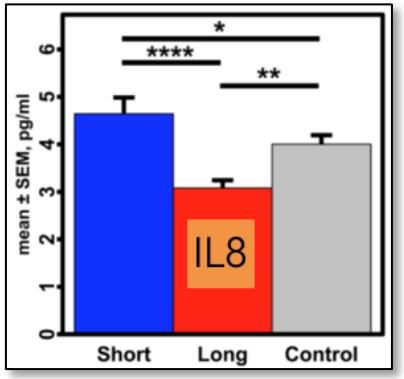
Research strategies:
- Dynamic analyses insights into energy metabolism, inflammation, and redox balance (mRNA, metabolomics)
- Stress testing to elicit and elucidate pathophysiology: in vivo (exercise and lean tests) and in vitro (TruCulture)

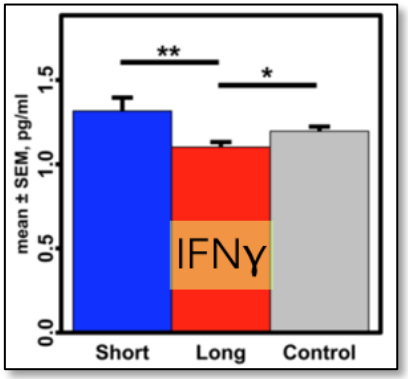
Cytokine Activation in Plasma in ME/CFS

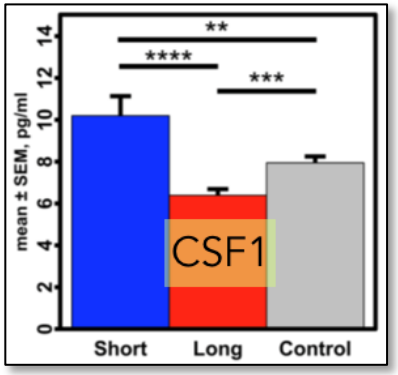












ScienceAdvances
RESEARCH ARTICLE | BIOMARKERS
27 Feb 2015 · Vol 1, Issue 1 · DOI: 10.1126/sciadv.1400121

Distinct plasma immune signatures in ME/CFS are present early in the course of illness

Hornig M, Montoya JG, Klimas NG, Levine S, Felsenstein D, Bateman L, Peterson DL, Gottschalk CG, Schultz AF, Che X, Eddy ML, Komaroff AL, and Lipkin WI

JCI
Research Article | Immunology | Metabolism
J Clin Invest. 2020;130(3):1491-1505. DOI: 10.1172/JCI132185

Myalgic encephalomyelitis/chronic fatigue syndrome patients exhibit altered T cell metabolism and cytokine associations

Mandarano AH, Maya J, Giloteaux L, Peterson DL, Maynard M, Gottschalk CG, and Hanson MR

PNAS
RESEARCH ARTICLE
PNAS August 22, 2017 114 (34) E7150-E7158; DOI: 10.1073/pnas.1710519114

Cytokine signature associated with disease severity in chronic fatigue syndrome patients

Montoya JG, Holmes TH, Anderson JN, Maecker HT, Rosenberg-Hasson Y, Valencia IJ, Chu L, Younger JW, Tato CM, and Davis MM

Unbiased Proteomic Analysis Provides Evidence of Persistent Immune Activation in ME/CFS

PLOS ONE

RESEARCH ARTICLE – July 21, 2020
https://doi.org/10.1371/journal.pone.0236148

Plasma proteomic profiling suggests an association between antigen driven clonal B cell expansion and ME/CFS

Milica Milivojevic, Xiaoyu Che, Lucinda Bateman, Aaron Cheng, Benjamin A. Garcia, Mady Hornig, Manuel Huber, Nancy G. Klimas, Bohyun Lee, Hyoungjoo Lee, Susan Levine, Jose G. Montoya, Daniel L. Peterson, Anthony L. Komaroff, W. Ian Lipkin

antigen-driven clonal B cell expansion

ME/CFS is associated with increased levels (>4x) in plasma levels of specific immunoglobulins

- IGHV3-23/30: OR = 4.439; p-value = 0.0182
- IGKV3(D)-11: OR = 4.527; p-value = 0.032
- IGHV3-23/30: OR = 4.545; p-value = 0.019

IGHV3-23/30

- Associations to lymphomas, anti-myelin associated glycoprotein neuropathy
- Induction: chronic stimulation from either microbial or auto-antigens
- Therapeutic implications: identify and remove stimulant, use kinase inhibitors
- ME/CFS patients are at an increased risk for lymphoma

BRAIN, BEHAVIOR, and IMMUNITY

Skewing of the B cell receptor repertoire in myalgic encephalomyelitis/chronic fatigue syndrome

RESEARCH ARTICLE – July 2021 | https://doi.org/10.1016/j.bbi.2021.03.023

Wakiro Sato, Hirohiko Ono, Takaji Matsutani, Masakazu Nakamura, Isu Shin, Keiko Amano, Ryuji Suzuki, Takashi Yamamura

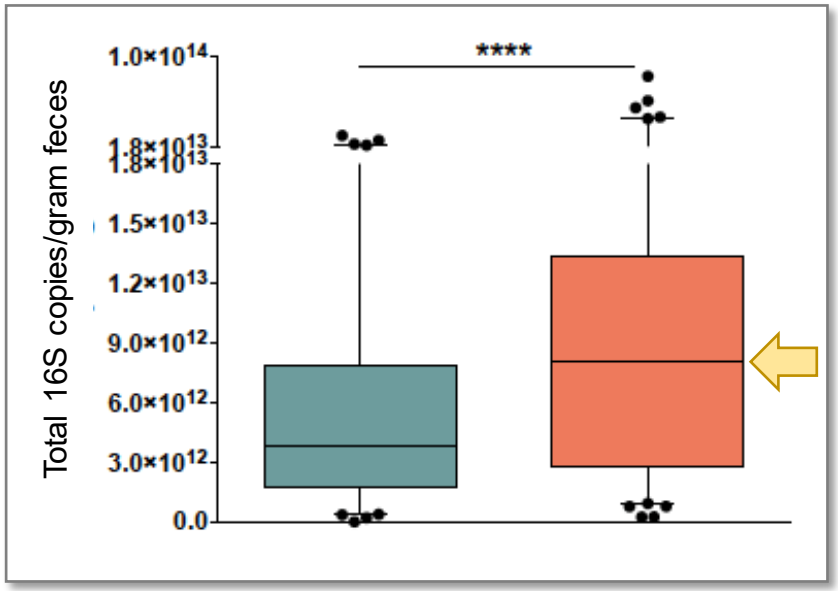
Independent confirmation of our findings

"Recently, an association between IGHV3-23/30 and ME/CFS has been shown using a plasma proteomic approach (Milivojevic et al., 2020). Despite differences in methodology, the fact that the expression of the same IGHV region was significantly increased in ME/CFS patients provides further evidence of the importance of IGHV3-30."

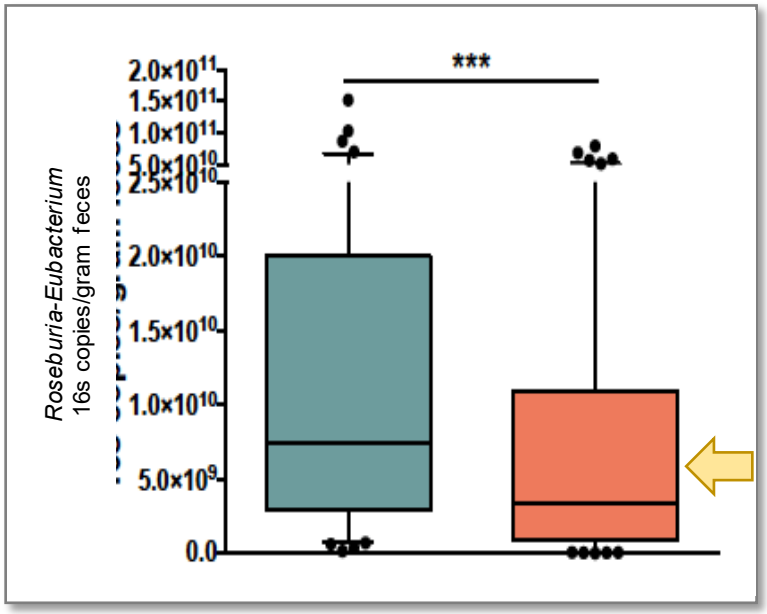
Implications: Finding and eliminating the trigger(s) may mitigate disease

Microbiology of ME/CFS

Total Fecal Bacteria *Increased*



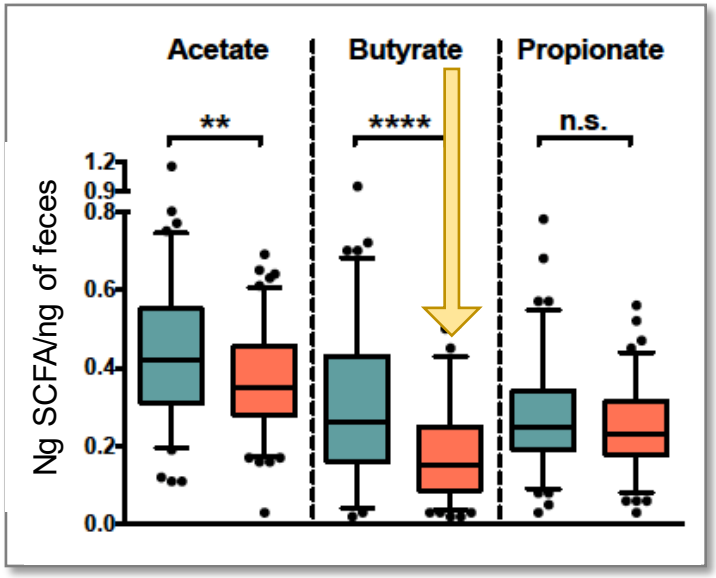
Total Butyrate-Producing Bacteria *Decreased*



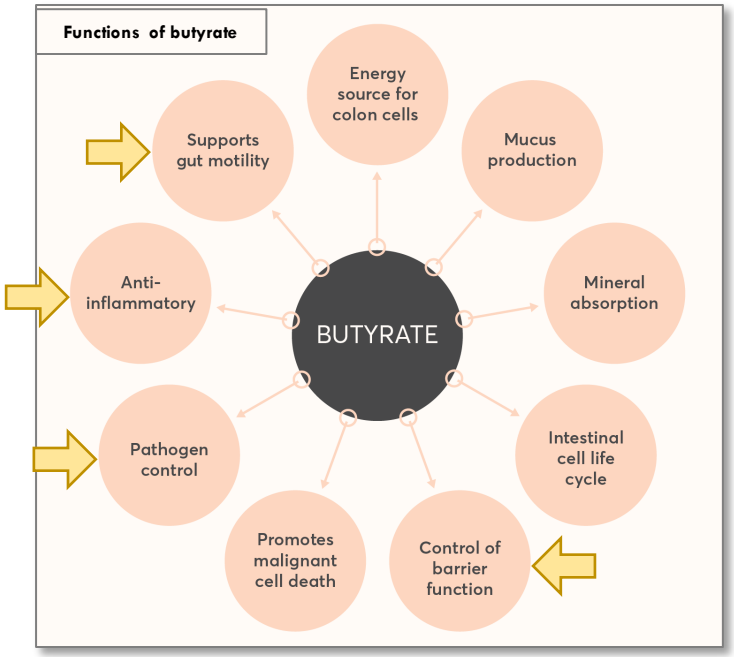
Controls (n=91)
ME/CFS (n=106)

Microbiology of ME/CFS

Confirmation of Decreased Butyrate



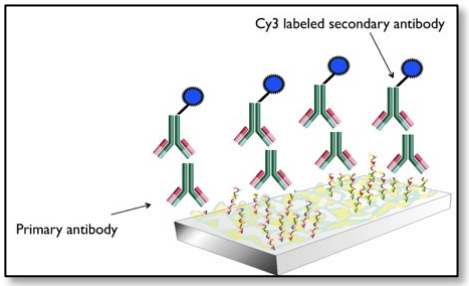
Implications of Decreased Butyrate



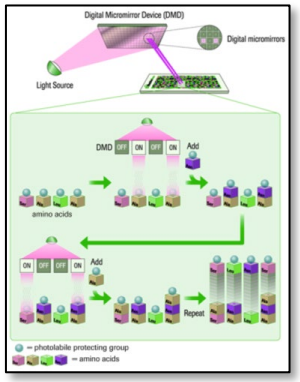
Implications: A rationale for clinical trial of prebiotics and probiotics?

Indirect Methods for Microbe Discovery and Implication

High-Throughput Serology Using Peptide Microarrays

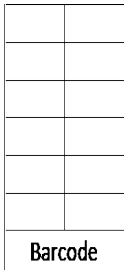


3M feature density
24M features needed to tile the vertebrate virus proteome

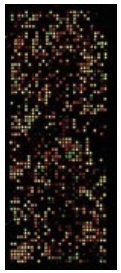


Synthesis in situ

Data Production



Virtual image of each array, peptides are printed randomly on each sub array, and co-ordinates known for each peptide.



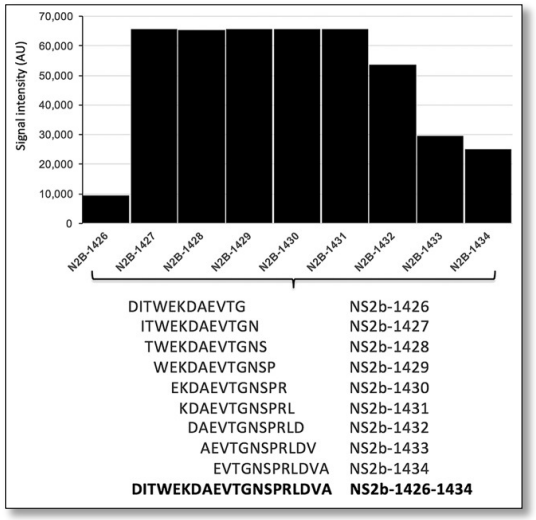
Scanned array image after secondary IgG and IgM Ab binding (Fluorescent signals)

Overlap images

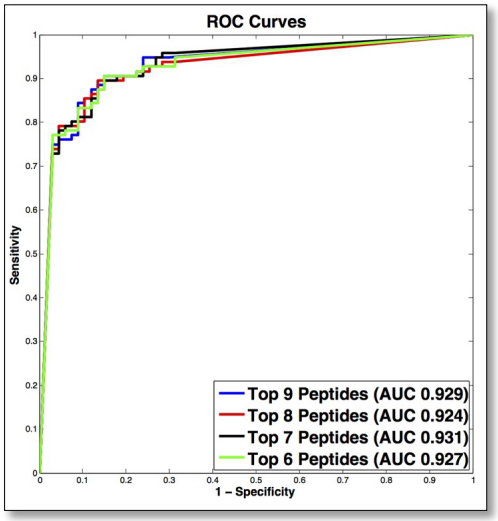
AA position	ZKV_AY632535	ZC1	ZC3	ZC4	ZC5	ZC7	ZC8	ZC10	ZC11	ZC12	ZC14	ZC15	CONTROL
1426	[illegible]	333.25	8182.3	413.29	12659.73	2017.06	6594.73	4150.09	9488.55	3633.04	840.34	548.96	1094.96
1427	[illegible]	5929.83	65535	40686.59	65524.72	65533.86	65502.77	62587.86	65533.94	65516.8	43074.78	29062.1	2326.87
1428	[illegible]	1167.64	65500.03	21902.73	65523.24	65127.44	64648.23	63418.59	65486.87	63716.99	31907.45	21128.54	1590.4
1429	[illegible]	17833.23	63912.63	15760.5	65535	65535	65442.69	65096.3	65534.21	65518.18	62389.82	49153.83	3619.51
1430	[illegible]	30032.74	65535	60224.11	65528.73	65240.54	65050.65	62511.76	65529.66	65484.65	58686.57	39390.5	3177.84
1431	[illegible]	44195.73	65506.73	64752.4	65517.85	65249.55	65164.64	65275.14	65532.21	65535	63452.59	61976.44	3653.07
1432	[illegible]	9107.15	54122.52	24994.3	63781.27	47079.49	32032.5	32277.86	53493.7	28045.73	19244.79	13894.04	773.36
1433	[illegible]	194.26	36612.42	1403.83	60530.99	26119.84	26386.36	13440.57	29573.1	17285.55	6787.69	9720.6	756.19
1434	[illegible]	193.6	25712.89	1453.79	41425.13	16979.84	15147.67	9767.21	24949.63	12211.56	4808.66	7190.39	916.53

Fluorescent signals converted to arbitrary units (data) and transferred to heat maps

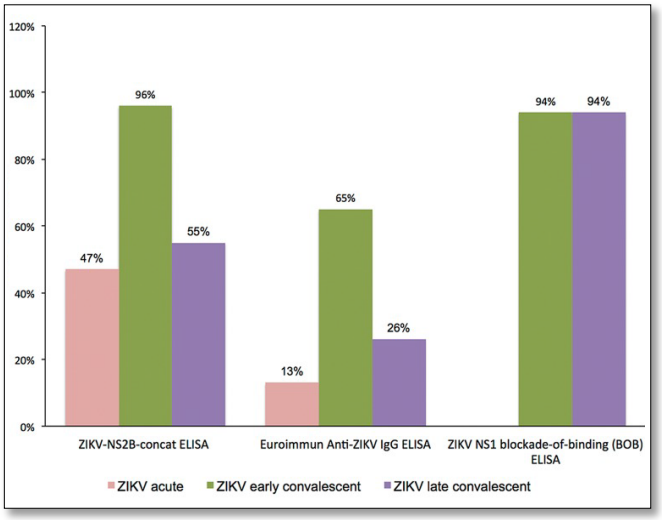
Diagnosis of Zika Virus Infection by Peptide Array and Enzyme-Linked Immunosorbent Assay



Identification of an immunoreactive 20-amino-acid ZIKV NS2B peptide

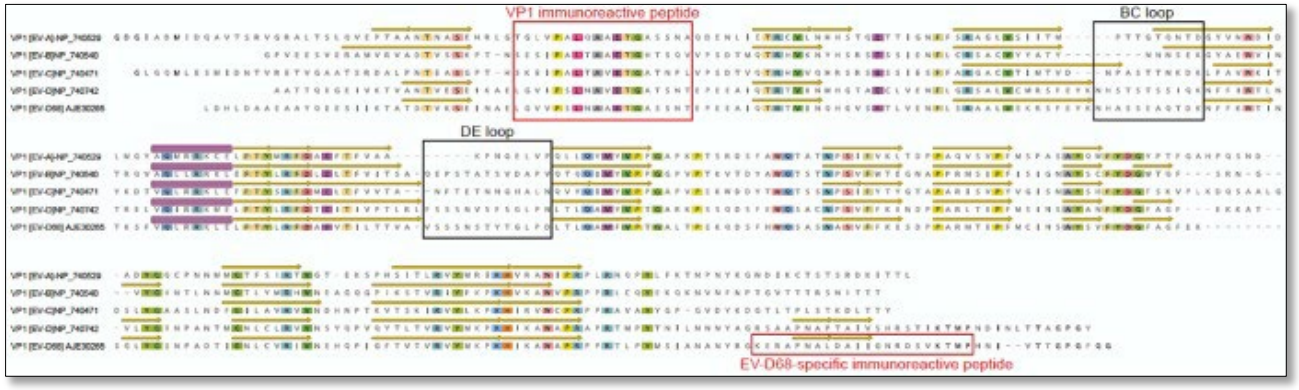


Average receiver operating characteristic (ROC) curves over 1,000 runs using the 9 overlapping peptides identified (comprising 20-aa ZIKV NS2B peptide), with an average area under the curve (AUC) of 0.931

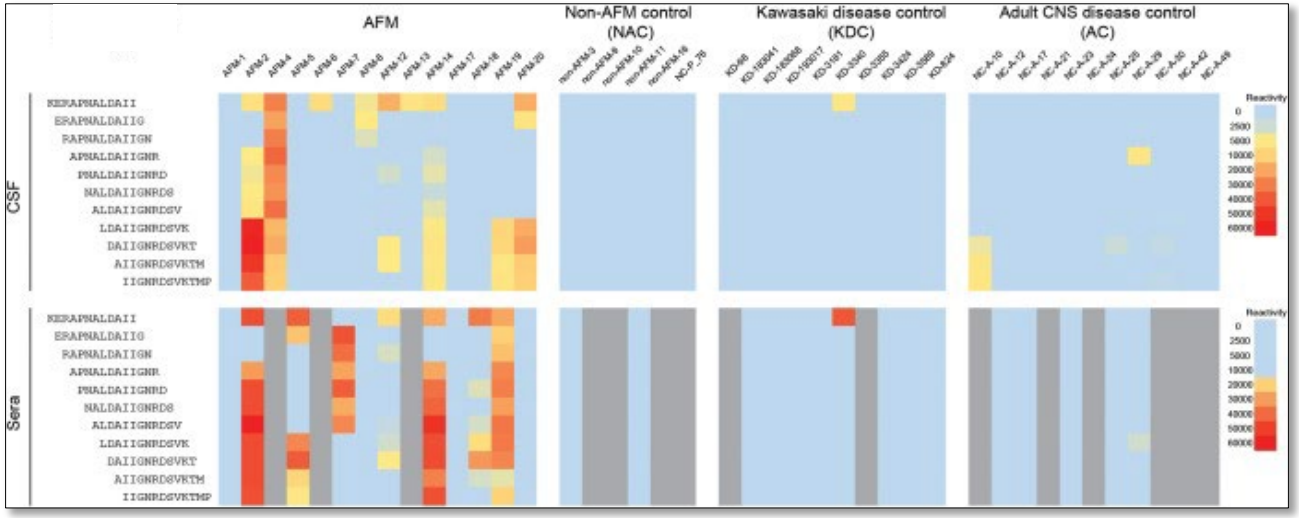


ZIKV-NS2B-concat ELISA sensitivity comparison with Euroimmun anti-ZIKV IgG ELISA and ZIKV-ELISA

Antibodies to Enteroviruses in Cerebrospinal Fluid of Patients with Acute Flaccid Myelitis



Identification of an immunoreactive peptide sequence region in VP1 protein of reference sequence entries for EV-A, EV-B, EV-C, and EV-D



Immunoreactivity against an EV-D68-specific 22-aa VP1 capsid peptide in patients with AFM, non-AFM controls (NAC), Kawasaki disease controls (KDC), and adult CNS disease controls (AC).

ME/CFS and Herpesviruses: Rationale and Previous Studies

Studies associating EBV and HHV6 with ME/CFS

Beyond Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: Redefining an Illness

Committee on the Diagnostic Criteria for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome; Board on the Health of Select Populations; Institute of Medicine

Washington (DC): National Academies Press (US); 2015 Feb 10.

Annals of Internal Medicine®

Reviews | 16 June 2015

Diagnostic Methods for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: A Systematic Review for a National Institutes of Health Pathways to Prevention Workshop

Haney E, Smith MEB, McDonagh M, Pappas M, Daeges M, Wasson N, Nelson H

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Reviews | 16 June 2015

Treatment of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: A Systematic Review for a National Institutes of Health Pathways to Prevention Workshop

Smith MEB, Haney E, McDonagh M, Pappas M, Daeges M, Wasson N, Fu R, Nelson H

Samples

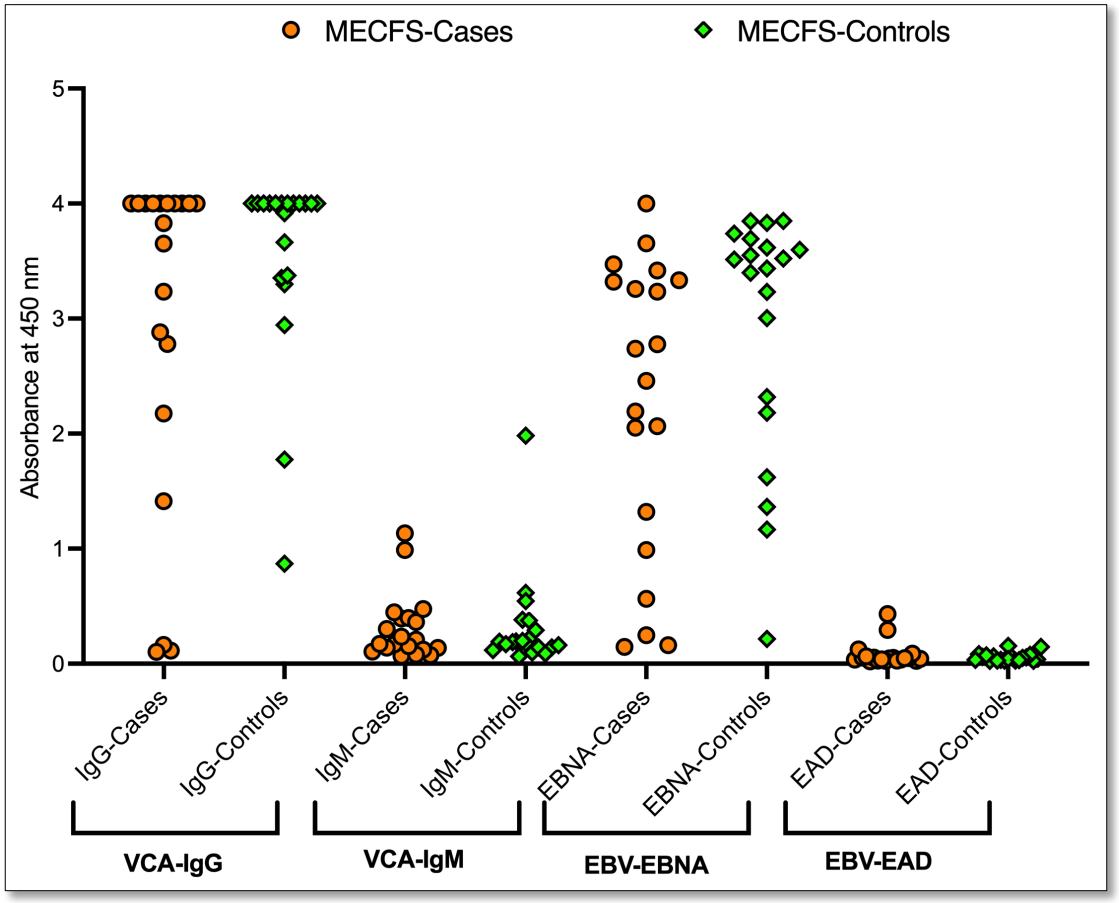
Forty samples tested on ELISAs and peptide array and incubated with anti human IgG and IgM antibodies.

- n= **20 ME/CFS subjects who had evidence of immune activation consistent with infection (levels of CD56bright/CD16dim NK cells higher than control)**
- n= **20 age and sex matched healthy controls.**

Derya Unutmaz in the ME/CFS Center at Jackson Laboratories

CII ID	JAX ID	Status	Year of Collection	Age at Collection	Sex	CD56hi CD16-
JPL-002	MECFS-018	case	2016	47	F	21.3
JPL-003	MECFS-037	case	2016	37	F	12.1
JPL-005	MECFS-073	case	2016	55	F	10.1
JPL-006	MECFS-104	case	2016	54	F	14.1
JPL-007	MECFS-120	case	2016	63	F	11
JPL-008	MECFS-133	case	2016	36	F	14.4
JPL-011	MECFS-172	case	2016	62	F	23.7
JPL-018	MECFS-285	case	2017	33	F	20.2
JPL-019	MECFS-296	case	2017	62	F	14.7
JPL-020	MECFS-302	case	2017	26	F	20.5
JPL-021	MECFS-121	Case	2016	56	F	19.9
JPL-022	MECFS-081	Case	2016	28	F	18
JPL-023	MECFS-170	Case	2016	50	M	15.4
JPL-024	MECFS-136	Case	2016	30	F	14
JPL-025	MECFS-132	Case	2016	37	F	12.2
JPL-026	MECFS-210	Case	2016	25	F	11.8
JPL-027	MECFS-021	Case	2016	22	F	11.4
JPL-028	MECFS-085	Case	2016	33	F	10.3
JPL-029	MECFS-100	Case	2016	30	F	10.3
JPL-030	MECFS-151	Case	2016	41	F	10.2
JPL-001	MECFS-016	control	2016	48	F	1.81
JPL-004	MECFS-049	control	2016	62	F	1.7
JPL-009	MECFS-157	control	2016	39	F	6.08
JPL-010	MECFS-158	control	2016	63	F	2.87
JPL-012	MECFS-178	control	2016	55	F	2.28
JPL-013	MECFS-197	control	2016	37	F	4.06
JPL-014	MECFS-214	control	2017	26	F	2.41
JPL-015	MECFS-215	control	2016	62	F	5.02
JPL-016	MECFS-243	control	2017	54	F	4.92
JPL-017	MECFS-270	control	2017	33	F	1.71
JPL-031	MECFS-HC-28	Control	2016	55	F	3.65
JPL-032	MECFS-HC-30	Control	2016	28	F	3.97
JPL-033	MECFS-204	Control	2016	52	M	1.87
JPL-034	MECFS-HC-42	Control	2016	31	F	7.33
JPL-035	MECFS-HC-191	Control	2016	38	F	7.55
JPL-036	MECFS-HC-41	Control	2016	25	F	6.9
JPL-037	MECFS-HC-27	Control	2016	22	F	3.65
JPL-038	MECFS-HC-161	Control	2016	33	F	3.13
JPL-039	MECFS-HC-165	Control	2016	32	F	0.8
JPL-040	MECFS-HC-65	Control	2016	41	F	4.89

Recombinant Protein EBV ELISAs Revealed No Differences Between ME/CFS and Healthy Controls



Multi-dimensional Analysis Plots of IgG Reactivity in Peptide Chips Discriminated ME/CFS Cases and Healthy Controls

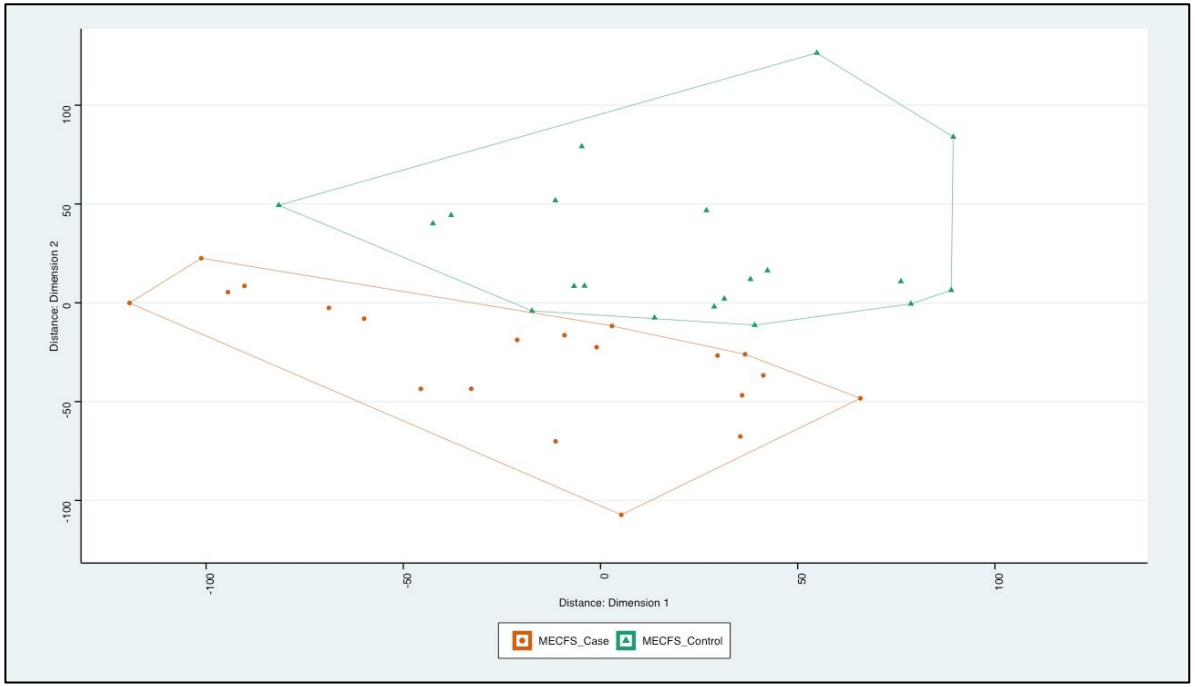
A MDS plot was created for IgG analysis using IgG signal data for each peptide. Signal data points were filtered such that only peptides that showed signal > threshold in any sample were retained. Threshold was calculated by calculating mean+2*SD of random peptides.

This step reduced the initial number of peptides from 376,388 to 108,107

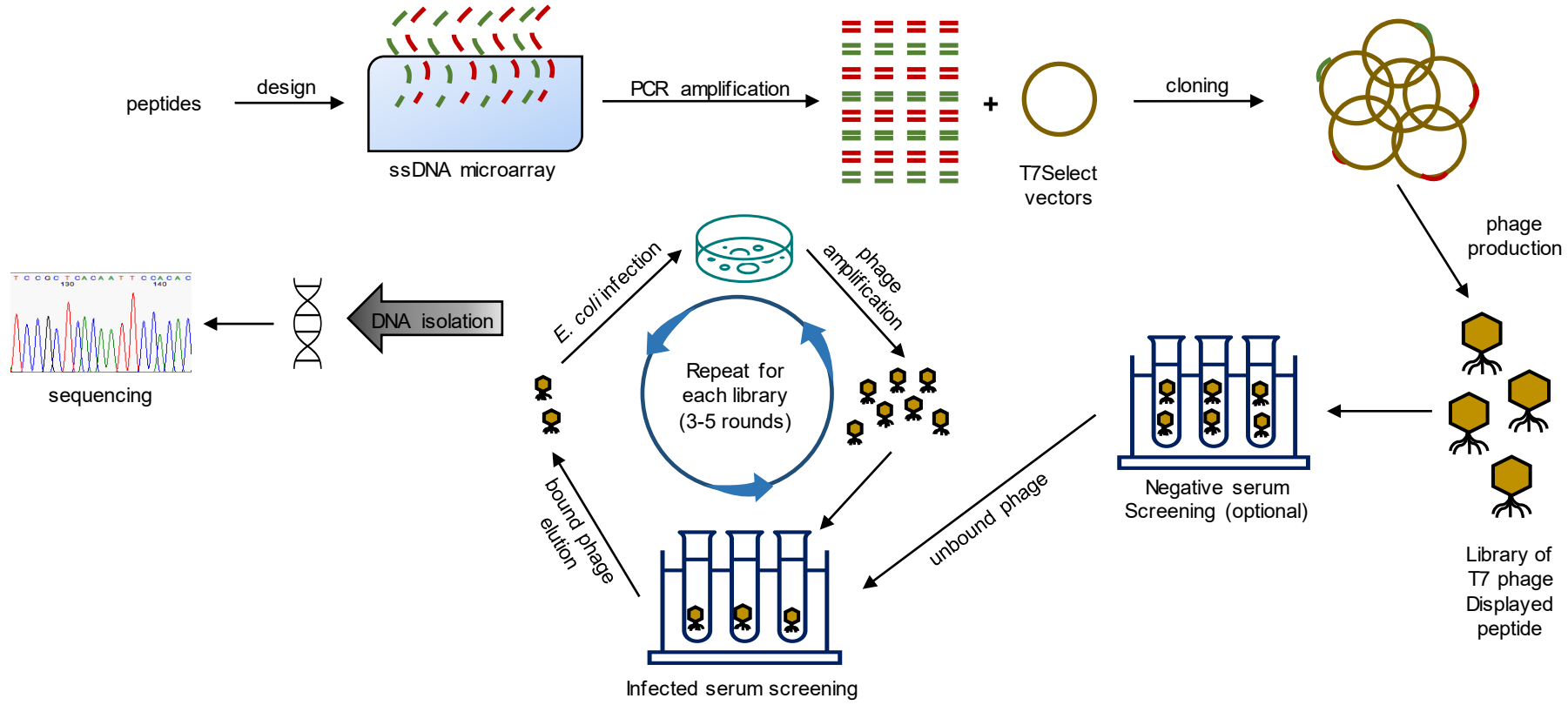
Stats for IgG
Total number of peptides on chip: 376,388
Number of random peptides: 500
Number of filtered peptides at threshold 10K: 108,107

ME-CFS-Case vs ME-CFS-Controls
Number of peptides significant for MECFS_Case: 22,023
Number of peptides significant for MECFS_Controls: 29,415

Number of epitopes: IgG MDS: 1745



Multiplex Serology Using Phage Display



Concordance Between Peptide Array and Granular Phage Display

Opportunities to Discriminate Between Infections With Related Viruses and Find Evidence of Reactivation

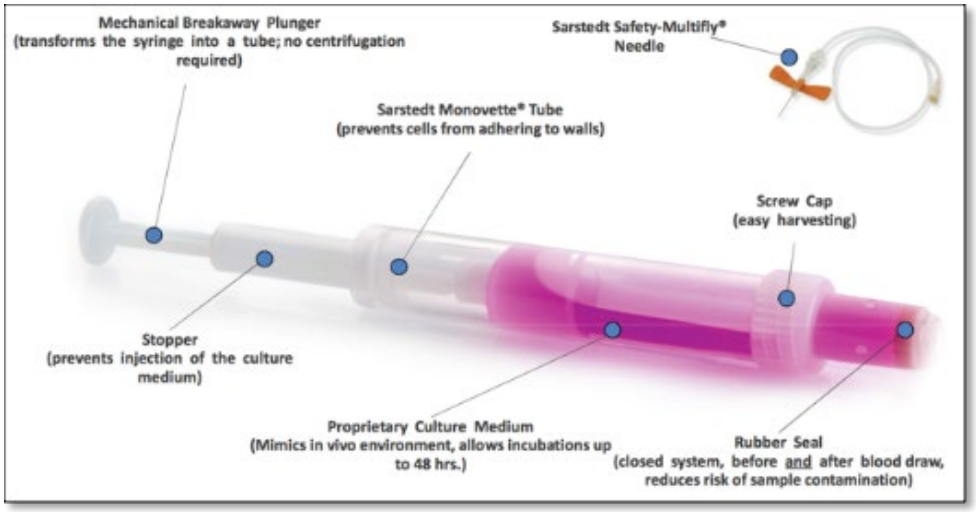
Reaction of Herpesvirus in ME/CFS Patients

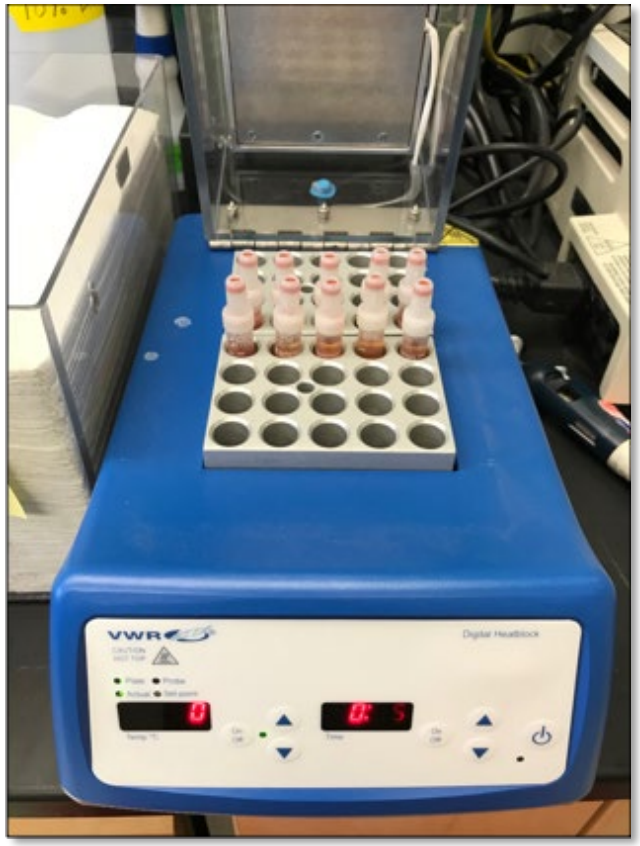
Virus	Protein	CASE1	CASE2	CASE3	CASE4	CASE5	CASE6	CASE7	CASE8
HHV4	BZLF1	●			●				
HHV4	C M protease					●		●	●●
HSV1	E- Protein	●	●●	●●	●●			●●	
HHV-6A	Helicase	●	●●		●●		●●		
HHV3	Large Tegument Protein	●●	●●	●●	○	●●	●●	○	○
HHV3	UL32			●	●		●		○

Convalescent COVID-19 Patients

Virus	Protein	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6
Coronavirus	NP	○	●○	●○	●○	●	
		●○	●○	●○	●○	●○	●
	GP	●	●○	●	●	●	●○
				●○	●○	●	●
		●○	●○	●○	●○	●○	○
		○	●○	○	○		●
		○	●○	○	○	○	
			●			●	

TruCulture System

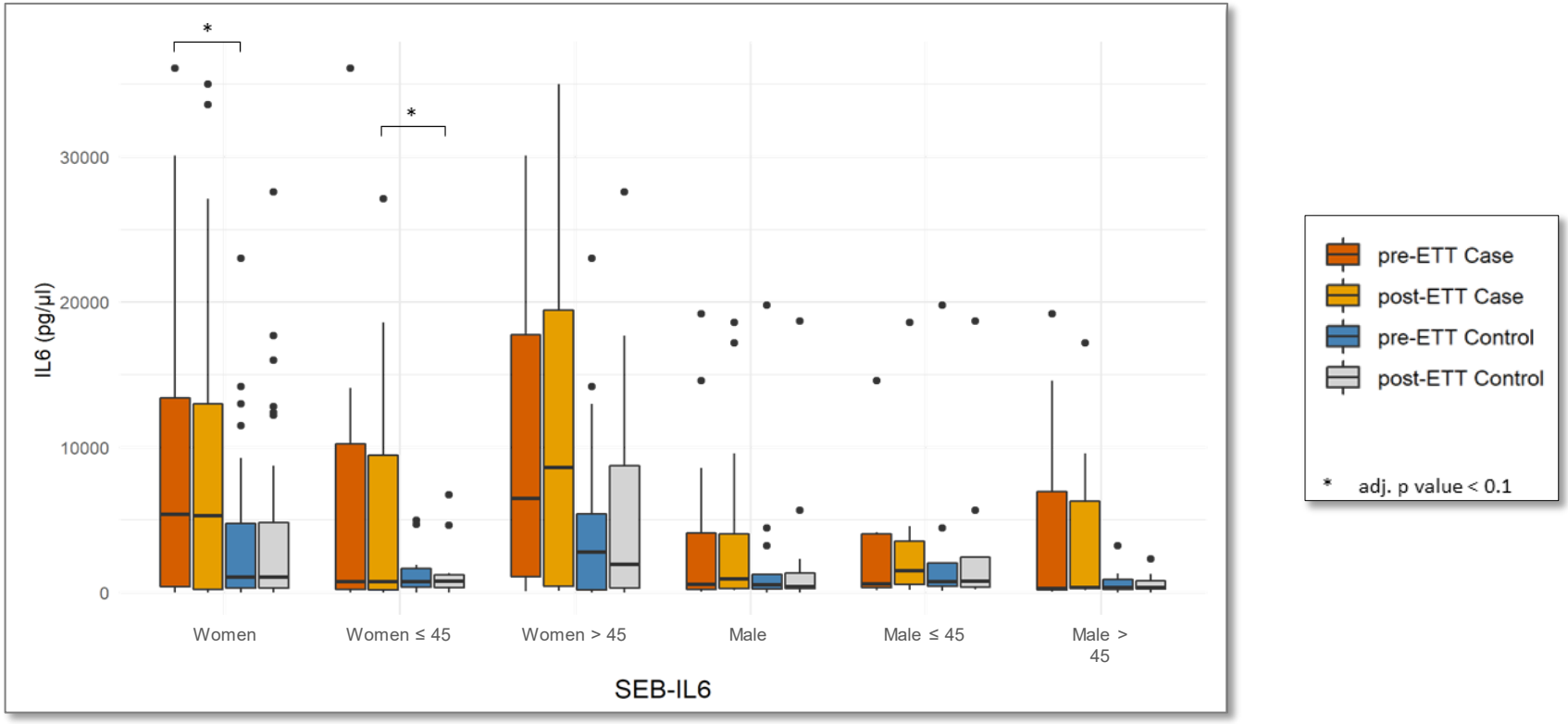




Exercise Tolerance Testing (ETT)
Blood draw before and 24 hours after ETT
PBMC incubated with SEB, poly I:C, LPS, HKCA, control buffer
Supernatant assayed for cytokines, metabolites

Higher IL6 Responses to the Superantigen SEB in Women with ME/CFS

Highest response in women >45Y



Cytokine Analyses

- **ME/CFS: 1.5x higher concentrations ($p<0.05$) of T-cell cytokines (GM-CSF, IL-17) after *Staphylococcus* enterotoxin B stimulation**
- **Cytokine levels higher in ME/CFS females having lower plasma levels of 17β-estradiol** (notably higher in females>45y)

Bulk RNASeq Analyses

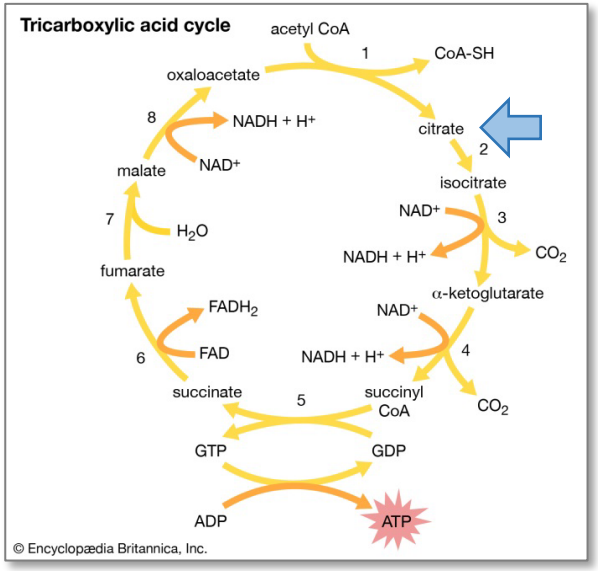
- **ME/CFS: 1.25x ($p<0.05$) higher levels of T-cell surface receptor beta variable 14 (TRBV14) mRNA**
- **ME/CFS: 1.3x ($p<0.05$) lower levels of the programmed cell death 1 ligand 1 (PD-L1) mRNA**

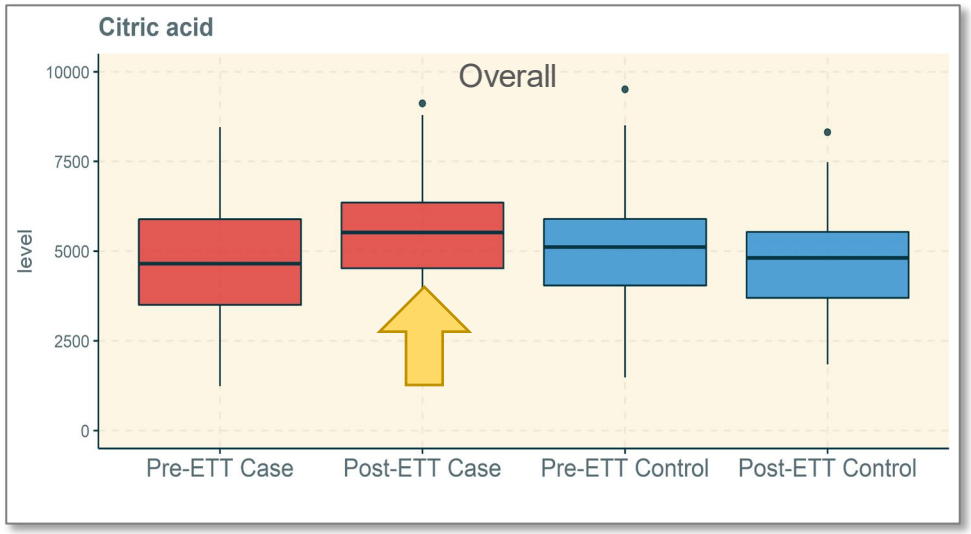
Interpretation

- Sex- and age-associated differences in ME/CFS are consistent with the function of 17β-estradiol as a regulator of inflammation
- T-cells in ME/CFS subjects are more sensitive to superantigens
- Potential mechanisms for enhanced sensitivity to superantigens may include increased density of TRBV14 receptors and reduced levels of PD-L1

Mitochondria in ME/CFS Patients are Slow to Recover After Exercise

TCA cycle impairment
Mitochondrial damage
Oxidative stress
Inflammation





Accumulation of citric acid indicates that the cycle is blocked

GWI Proteomic Subject Demographics

Demographics		Group 1 (n=27)	Group 2 (n=36)	p-value
Sex n (%)	M	25 (92.6%)	32 (88.9%)	0.620
	F	2 (7.4%)	4 (11.1%)	
Site n (%)	NJ	9 (33.3%)	18 (50.0%)	0.186
	WI	18 (66.7%)	18 (50.0%)	
Age mean (SD)		52.8 (5.7)	52.8 (4.2)	0.976

Methods

Plasma collected **before**, **immediately after**, and **24 hours after exercise in GWI subjects and matched controls**

Somalogic panel established to detect **1,512 protein analytes**, implicated in **inflammation**, **metabolic disorders**, and **neurological diseases**

Levels of individual proteins log-transformed and compared between GWI cases and controls using generalized linear mixed models adjusted for age, sex, and site. (*Similar analyses performed with ME/CFS subjects and matched controls before and 24 hours after exercise.)*

Differences in protein levels between case and control groups at the three time points

Trajectories of protein levels in case and control groups between each of the three time points

Results

No *individual* proteins were significantly associated with the outcome (case vs control, *FDR* > 0.1) at any of the three time points

Ingenuity Pathway Analysis (IPA) revealed significant group-specific difference in biological pathways (*FDR* < 0.1)

Baseline Before Exercise

GWI v. Control	ME/CFS v. Control
Metabolic Pathways	
Mitochondrial function	Ceramide signaling
Glucocorticoid receptor signaling	Circadian rhythm signaling
Inflammation Signaling	
HIF1α signaling	***HIF1α signaling***
STAT3 pathway	***STAT3 pathway***
PI3K/AKT signaling	***PI3K/AKT signaling***
p38 MAPK signaling	***p38 MAPK signaling***
Pathogen-induced cytokine signaling pathway	***Pathogen-induced cytokine signaling pathway***
FXR/RXR activation	LXR/RXR activation
Complement system	IL-17 signaling
	GM-CSF signaling
	LI-8 signaling
	HMGB1 signaling
	TGF-β signaling
Neuronal Signaling	
Axonal guidance signaling	***Axonal guidance signaling***
Neuregulin signaling	***Neuregulin signaling***
Neuroinflammation signaling	

24 Hours After Exercise

GWI v. Control	ME/CFS v. Control
Metabolic Pathways	
Glucocorticoid receptor signaling	***Glucocorticoid receptor signaling****
Mitochondrial function	
Oxidative phosphorylation	
Glutathione redox	
Inflammation Signaling	
HIF1α signaling	***HIF1α signaling***
STAT3 pathway	***STAT3 pathway***
Pathogen-induced cytokine signaling pathway	***Pathogen-induced cytokine signaling pathway***
FXR/RXR activation	LXR/RXR activation
	IL-17 signaling
	GM-CSF signaling
	LI-8 signaling
	HMGB1 signaling
	TGF-β signaling
	PI3K/AKT signaling
	p38 MAPK signaling
Neuronal Signaling	
	Neuroinflammation signaling

**Meyer JD, …, Stegner A, Cook D. Fatigue: Biomedicine, Health & Behavior, 2013*

Trajectories: Before → Immediately After Exercise

GWI Cases* – Increased	Controls – Increased
Cellular communication network factor 1 (O00622)	***Cellular communication network factor 1 (O00622)***
Lymphocyte-specific protein 1 (P33241)	***Lymphocyte-specific protein 1 (P33241)***
Transaldolase (P37837)	***Transaldolase (P37837)***
T-cell surface antigen CD2 (P06729)	***T-cell surface antigen CD2 (P06729)***
Neutrophil cytosol factor 1 (P14598)	***Neutrophil cytosol factor 1 (P14598)***
Protein S100-A12 (P80511)	***Protein S100-A12 (P80511)***
	Aldo-keto reductase family 1 member C3 (P42330)
	NAD(P)H dehydrogenase [quinone] 1 (P15559)
	Hexokinase-2 (P52789)
	Myeloblastin (P24158)
GWI Cases* – Decreased	**Controls – Decreased**
Pancreatic triacylglycerol lipase (P16233)	None

**No comparable ME/CFS data*

Trajectories: Before → 24 Hours After Exercise

GWI Cases – Increased	ME/CFS Cases – Increased	Controls – Increased
Triggering receptor expressed on myeloid cells 1 (Q9NP99)	No significant findings	No significant findings
GWI Cases – Decreased	**ME/CFS Cases – Decreased**	**Controls – Decreased**
Succinate dehydrogenase assembly factor 1, mitochondrial (A6NFY7) Peroxisome proliferator-activated receptor gamma (P37231) Glutathione S-transferase theta-2 (P0CG29)	No significant findings	No significant findings

Summary

There are significant similarities in plasma proteomic profiles between GWI and ME/CFS subjects at baseline and 24 hours after an exercise challenge

Abnormalities are found in pathways associated with inflammation, mitochondria, peroxisomes, and neural signaling

Pending Deliverables

Metabolomic analyses
Biogenic amines
Complex Lipids
Oxylipins

Transcriptomics
Pre-, immediately post, 24 hours post exercise

Enterovirus serology
Other serology?