Biomarker and Pathogen Discovery in Chronic Illness

W. Ian Lipkin
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Mailman School of Public Health
College of Physicians and Surgeons
Columbia University

EPIDEMIOLOGY CIRCA 1854

GENES

ENVIRONMENT

microbes
xenobiotics
toxins
stressors
learning
diet

TIMING

Three Years

Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 4184–4188, June 1990
Neurobiology

Isolation and characterization of Borna disease agent cDNA clones

(limbic system/behavioral disorders/central nervous system infection)

W. Ian Lipkin*†, Gabriel H. Travis‡, Kathryn M. Carbone§, and Michael C. Wilson*

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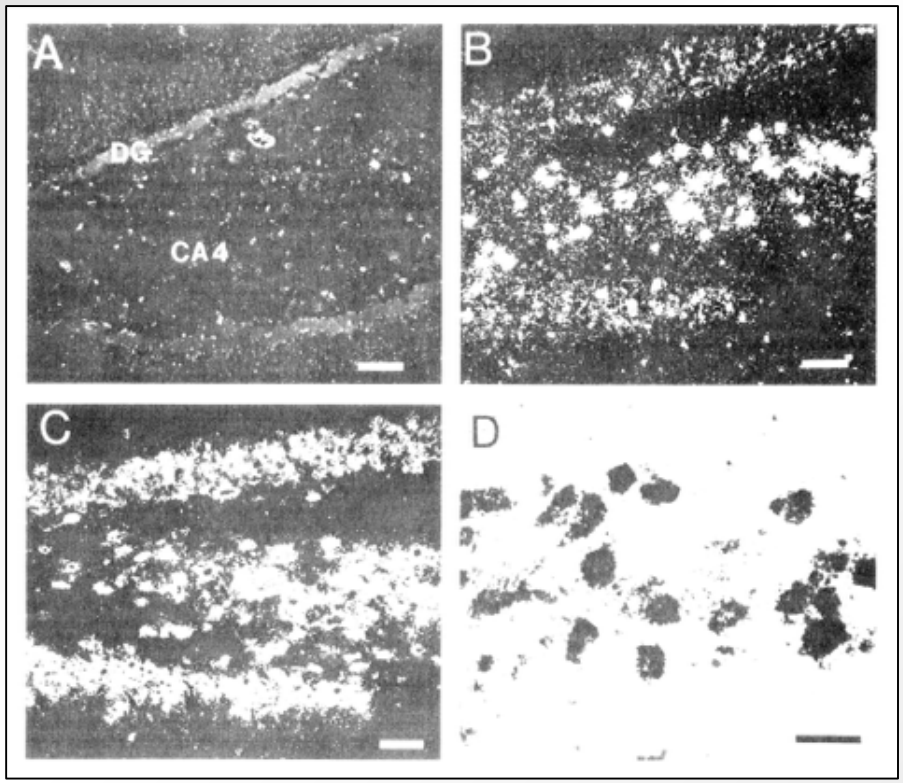
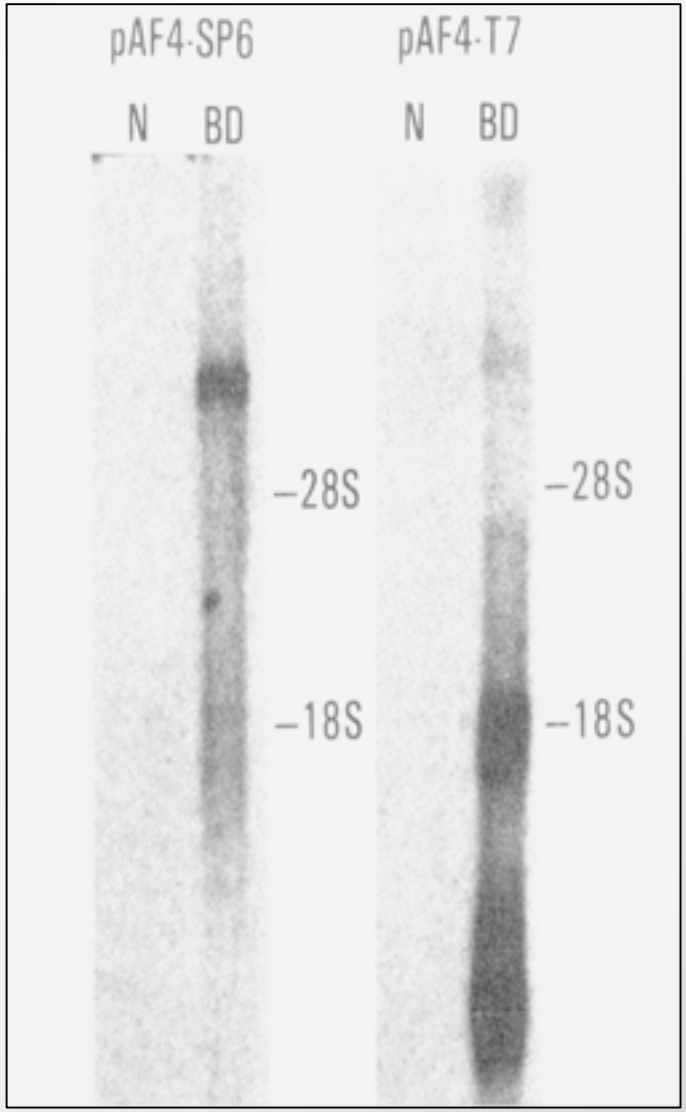
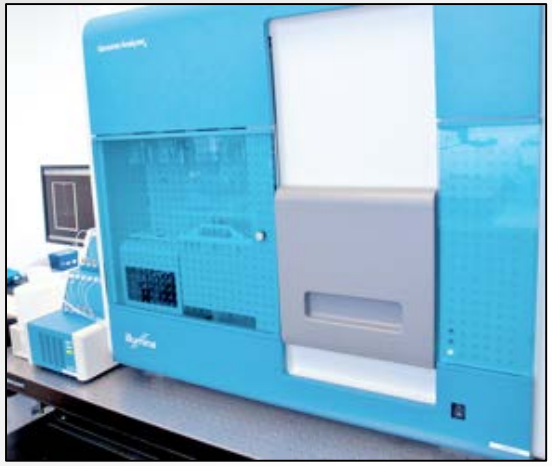


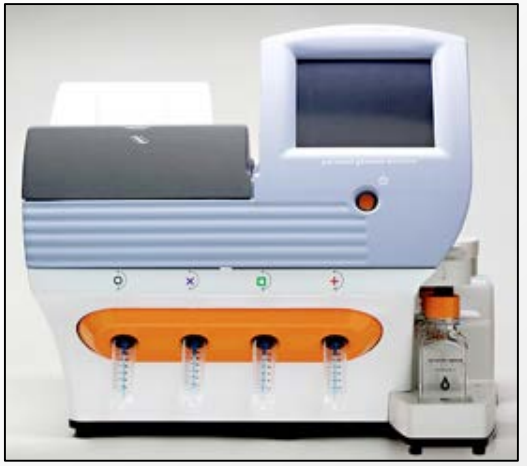


Evolution of High-Throughput Sequencing

Roche 454



Illumina



Ion Torrent

Illumina HiSeq X Ten

Oxford Nanopore MinION

A Selection of >1,200 Viruses Discovered/Characterized at CII (2009-17)

Humans

Adenoviruses (1 species)
Astroviruses (4 species)
Bocaviruses (4 species)
Cosaviruses (4 species)
Enteroviruses (9 EVA, 15EVB, 8EVC, 1EVD (EV68),1 untyped)
Dengue Virus
LuJo Virus
Orthobunyaviruses
Parvoviruses (3 species)
Phlebovirus (7 species)
Rotaviruses (1 species)
Rhabdobirus (2 species)
Rhinovirus A, C
Polyomaviruses

Other Mammals

Bat Adenoviruses
Bat Astroviruses
Bat Bocaviruses
Bat Coronaviruses (35+ species)
Bat Hepaciviruses and Pegiviruses
Bat Filovirus (distant relation to Ebola and Marburg)
Bat Herpesviruses
Bat Paramyxoviruses
Bat Parvoviruses
Bat Polyomaviruses
Canine Hepacivirus
Canine Kobuvirus
Cattle Orbivirus (6 species)

Cattle Orthomyxovirus (2 species)
Cetacean Influenza Virus
Cetacean Polyomavirus
Gorilla Parvovirus
Gorilla Metapneumovirus
Hedgehog Rhabdovirus
Horse Pegiviruses
Minke Whale Astrovirus
Porcine Astrovirus
Porcine Circovirus
Porcine Picobirnavirus
Rodent Hepaciviruses and Pegiviruses
Sea Lion Reovirus

Avians

Avian Bornavirus (2 genotypes)
Avian Farmington Virus
Turkey Hepatitis Virus (4 genotypes)

Insects

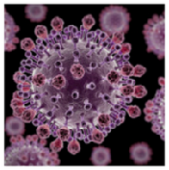
Mosquito Rhabdovirus (8 species)
Mosquito Orbivirus
Mosquito Alphavirus
Mosquito Nidovirus
Mite Rhabdovirus
Insect Phlebovirus (19 species)
Insect Negevirus
Metagenomic studies of *Apis mellifera*

Fish/Reptiles/Other

Piscine reovirus (Salmon)
Clam retrovirus
Snake nidovirus
Tilapia Lake virus

Licensing Agreements
Roche (2017); Grifol (2018)

2015 WORLD CHANGING IDEAS



H1N1 INFLUENZA is one of many viruses snared by a single new test.

Trawling for Viruses

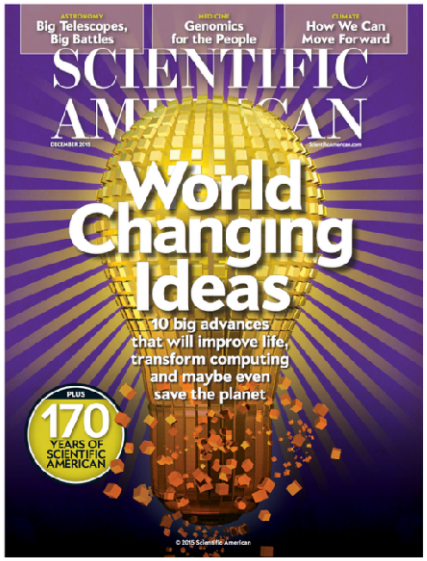
A new method identifies every virus in a given sample with near-perfect accuracy

When doctors want to identify the virus behind an infection, they usually turn to the polymerase chain reaction (PCR), a method for "amplifying" scattered bits of DNA into a sample large enough to study. To use PCR, however, a physician must know what kind of virus to look for, and that involves guesswork.

This past September a team of Columbia University researchers described a new method that could eliminate that guesswork. The technique, which has the unfortunate name of "virome capture sequencing platform for vertebrate viruses," or VirCapSeq-VERT, can find every virus in a given drop of saliva, tissue or spinal fluid with near-perfect accuracy. The method makes it possible to simultaneously analyze 21 samples in less than 48 hours at an estimated cost of just $200 per sample. It can also detect novel or mutated viruses, so long as they are at least 40 percent identical to known ones. "When someone goes into an emergency room and winds up having all kinds of tests run, it costs thousands of dollars," says W. Ian Lipkin, John Snow Professor of Epidemiology at Columbia University's Mailman School of Public Health. "This method is very inexpensive and allows us to personalize medicine by telling you exactly what you have."

To develop the technique, Lipkin and his colleagues first created a database of more than 1,000 vertebrate viruses. Then they synthesized genetic probes to match every strain of every virus—two million of them, each a strand of DNA 25 to 50 nanometers long. When a probe encounters a matching virus, it binds to it. To extract those viruses, laboratory workers add magnetic beads measuring one to three microns in diameter to the mix; a chemical linker binds the beads to the genetic probes and the viruses they have captured. Researchers then insert a tube containing the mixture into a magnet stand, which pulls the probes to the tube's walls. After researchers isolate and wash the probe-bead-virus combos, they genetically sequence the viruses, eliminating the risk of false positives. Lipkin and his colleagues are now looking to team up with a commercial provider that can distribute the technology to hospitals and clinics around the world. They are also planning on adding probes for all known infectious bacteria and fungi.

—R.N.



The Atlantic

SUBSCRIBE SEARCH MENU

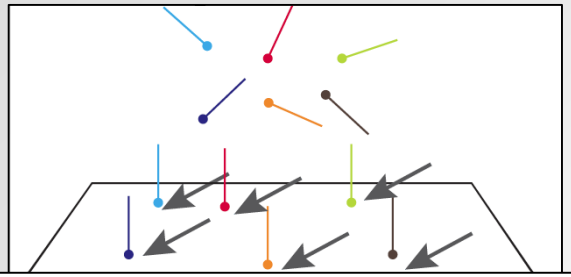
The New Technique That Finds All Known Human Viruses in Your Blood

And some unknown ones, too

ED YONG | SEP 22, 2015 | SCIENCE

Ian Lipkin, a virus hunter from Columbia University, recently received a blood sample from colleagues at the National Institutes of Health. They came from a man who had received a bone-marrow transplant and had fallen mysteriously ill, with evidence of severely inflamed blood vessels. In analyzing a similar case a few years back, Lipkin had discovered a new polyomavirus, part of a family that can cause disease in people with compromised immune systems. Perhaps this new case would yield another new virus.

PROBE LIBRARY



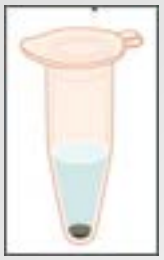
Biotinylated oligonucleotides synthesized on slide

Released from slide to generate soluble probe pool

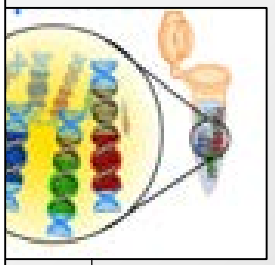
Thomas Briese

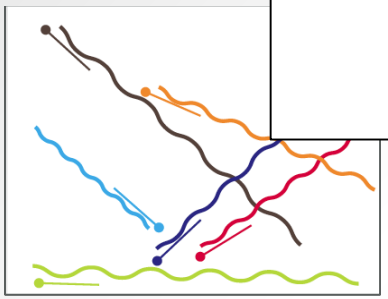
TARGET EN

Sample

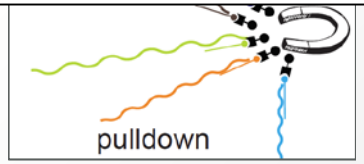


Pre-capture amplification





Hybridization



Streptavidin capture and pulldown

Post capture amplification

Sequencing

BacCapSeq: Method for Pan-Bacterial Diagnosis, Surveillance, and Discovery

Objective: identify known and potential human bacterial pathogens as well as antimicrobial resistance (AMR) genes

Probe Selection:

- 1.2M protein coding sequences from PATRIC database
- 30,178 virulence factors from VFDB database
- 2,169 AMR genes from CARD database

Probe Set Design:

- 4.2M total probes
- 75 bp each
- 152 bp of inter-probe space

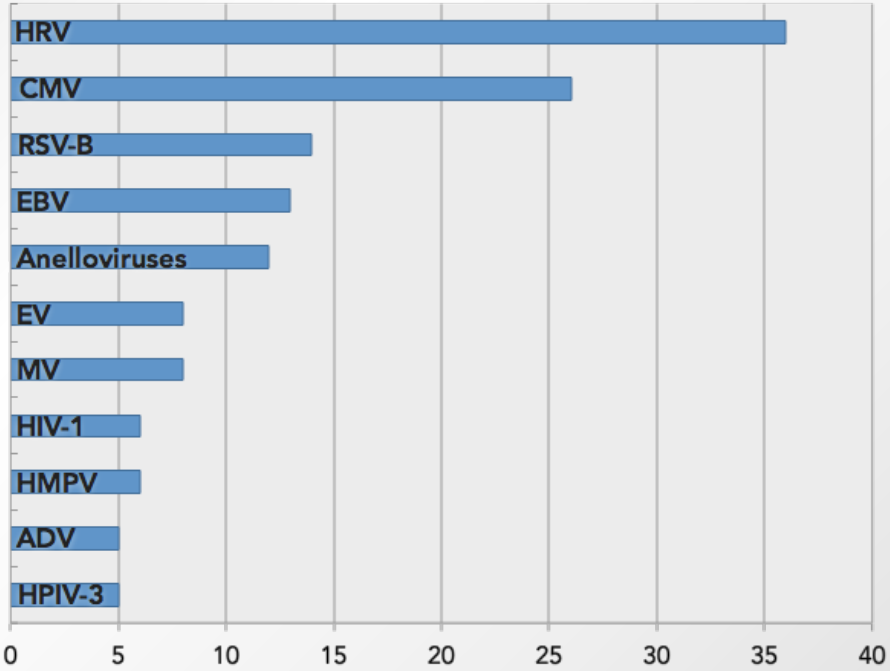
Partnership with the Bill and Melinda Gates Foundation

VirCapSeq-VERT Analysis of Severe Acute Respiratory Infection in Africa

VIRUSES DETECTED*	#
Human rhinovirus	36
Cytomegalovirus	26
Respiratory syncycial virus b	14
Epstein-Barr virus	13
Anellovirus	12
Measles virus	8
Enterovirus	8
Metapneumovirus	6
Human immunodeficiency virus 1	6
Human parainfluenza virus 3	5
Adenovirus	5
Respiratory syncycial virus A	4
Coronavirus	4
Picobirnavirus	3
Human rotavirus	3
Human polyomavirus	3
Human parainfluenza virus 1	3
Human herpes virus 6	3
Rubella virus	2
Human parainfluenza virus 2	2
Human herpes virus 7	2
Hepatitis B virus	2
Hepatitis A virus	2
Salivirus A virus	1
Porcine circovirus-2	1
Parovirus b19	1
Norovirus	1
Influenza A virus (H1N1)	1
Human parainfluenza virus 1	1
Human bocavirus	1

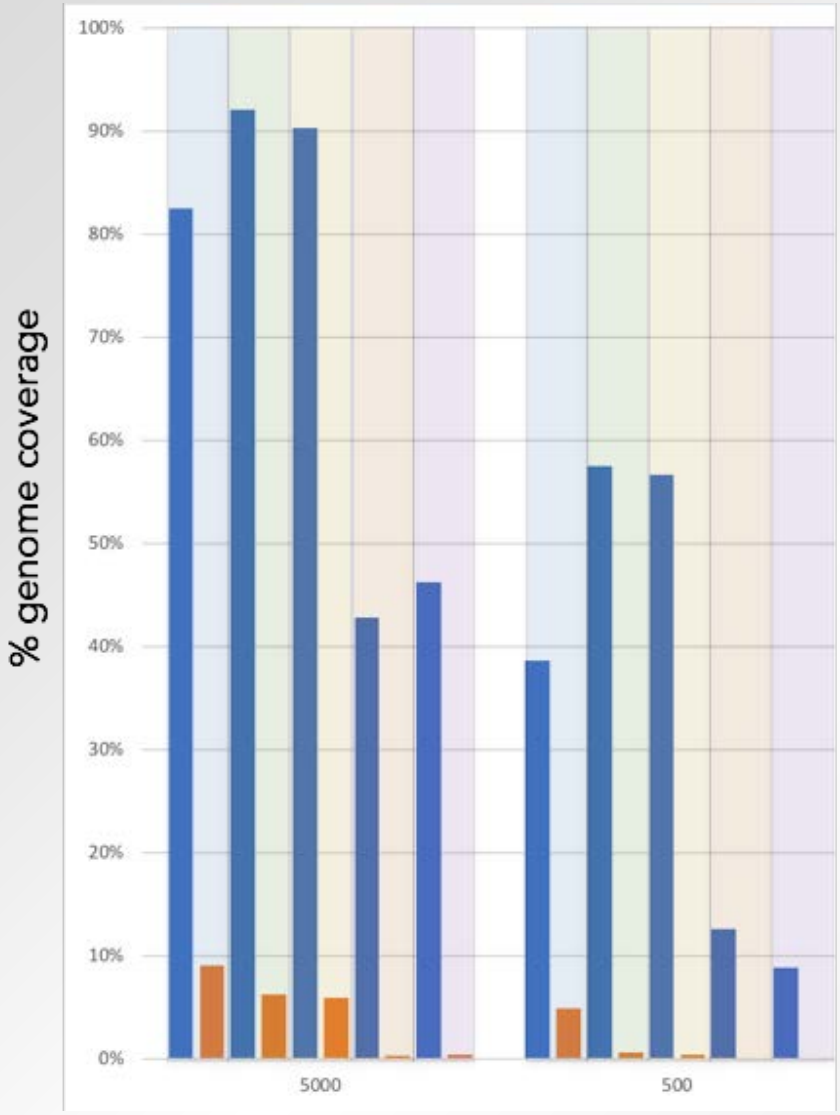
**** Respiratory viruses are in bold***

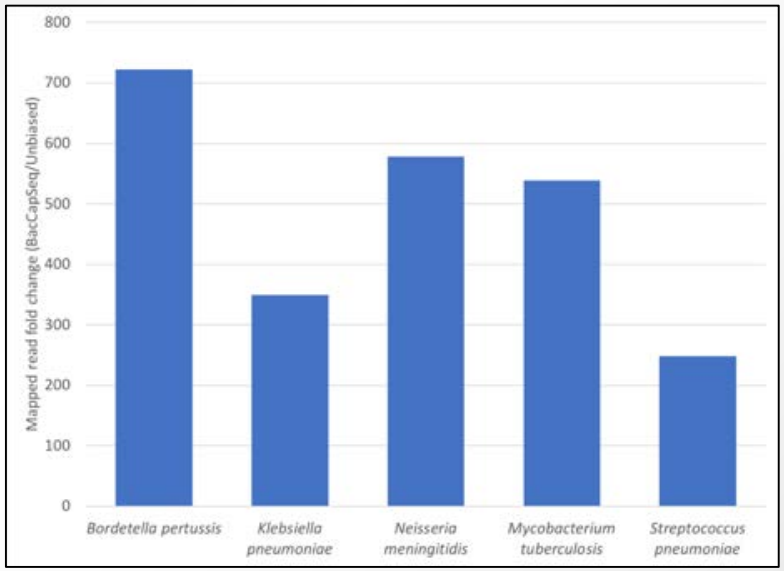
113 influenza-negative nasal swabs from the Uganda Virus Research Institute



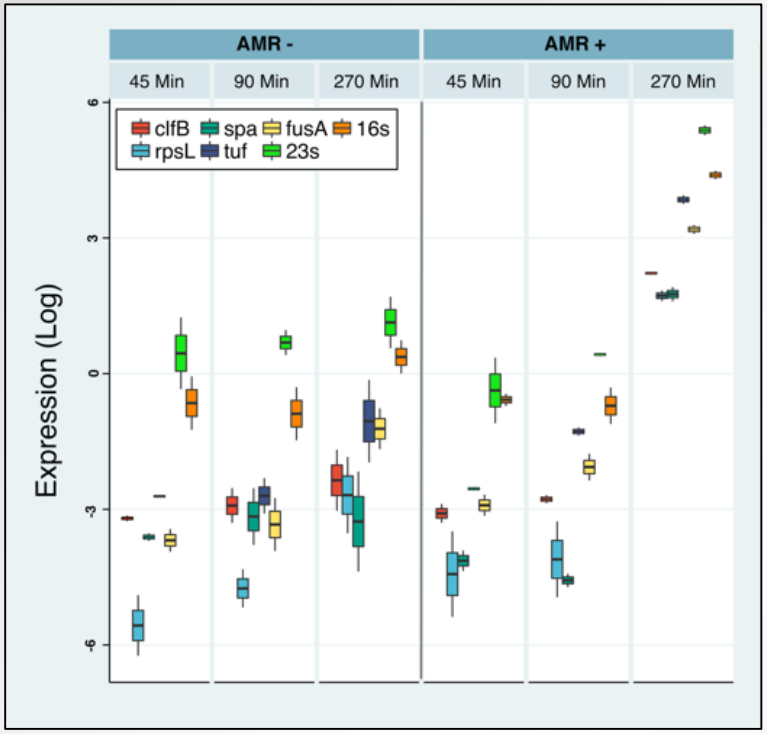
VirCapSeq-VERT detected an influenza virus that was not detected by PCR

BacCapSeq v. Unbiased Sequencing





BacCapSeq: High Abundance Transcripts, Material Growth, and AMR Resistance



Increases in levels of transcripts expressed by beta-lactamase positive *S. aureus* 45, 90, and 270 minutes after exposure to ampicillin (0.06 ug/ml)

tuf: promotes GTP-dependent binding of aminoacyl-tRNA to the A-site of ribosomes during protein biosynthesis

fusA: promotes GTP-dependent translocation of the ribosome during translation

spa: Protein A is a 42 kDa surface protein originally found int he cell wall of the bacteria Staphylococcus aureus

clfB: clumping factor B, a fibrinogen-binding MSCRAMM (microbial surface components recognizing adhesive matrix molecules) adhesion of Staphylococcus aureus, also binds to the tail region of type I cytokeratin 10

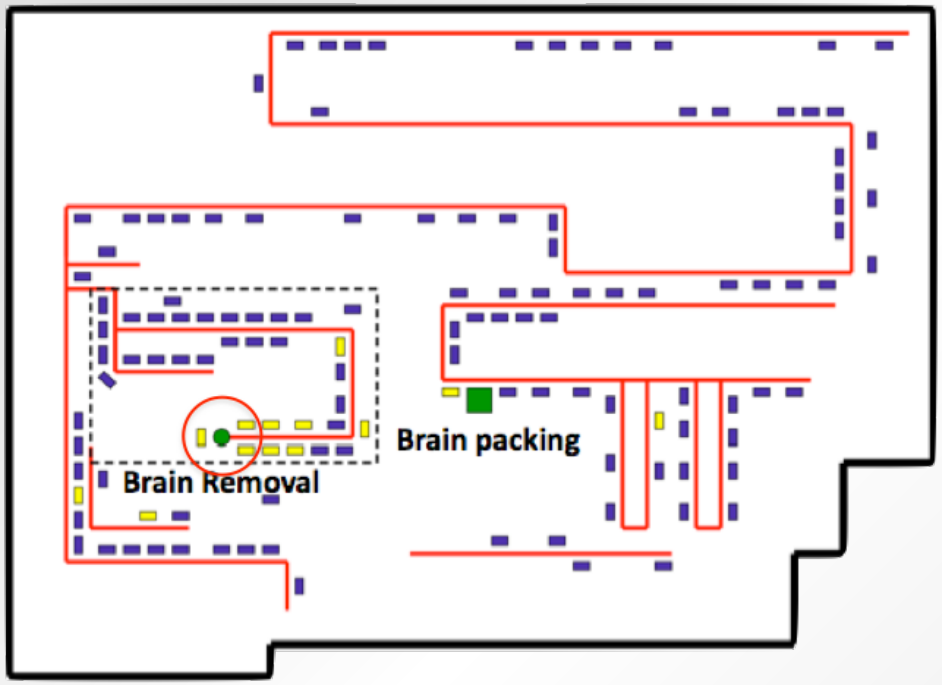
rpsL: interacts with and stabilizes bases of the 16S rRNA involved in tRNA selection in the A site and with the mRNA backbone

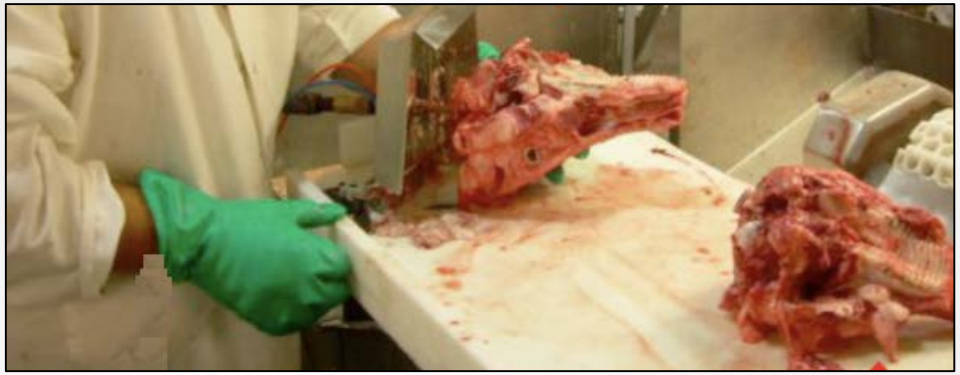
Date: Tue, 29 Jan 2008 03:12:14 -0500 (EST)
From: ProMED-mail <promed@promed.isid.harvard.edu>
Subject: PRO/AH/EDR> Undiagnosed neuro. synd.,
porcine plant workers - USA (02): (IN,MN)

UNDIAGNOSED NEUROLOGIC SYNDROME, PORCINE PLANT WORKERS
****

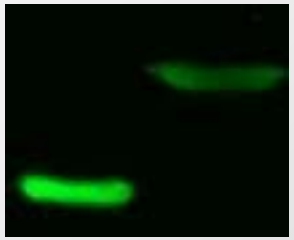
All of the subjects worked in a pork processing plant in Austin, Minnesota, in an area of the facility where the pigs' heads are processed. In mid-January of 2008, there were reports of an additional cluster of patients with similar symptoms among individuals working in a pig processing plant in Indiana.

MN Department of Health, Mayo Clinic, CDC, Columbia University



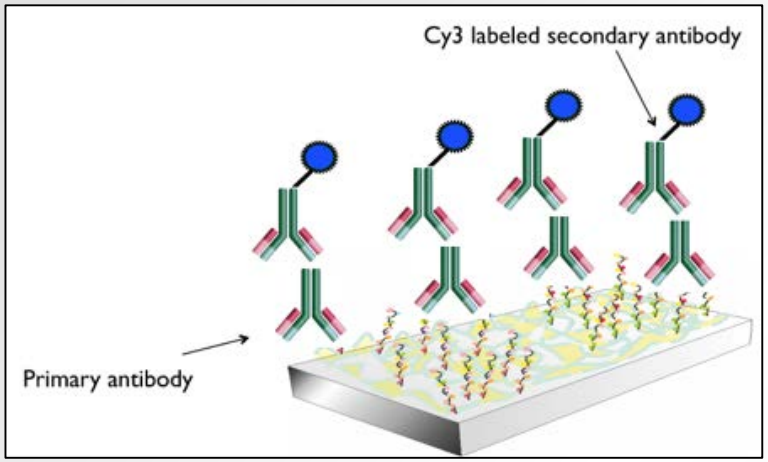


cytokines
Abs to CNS/PNS

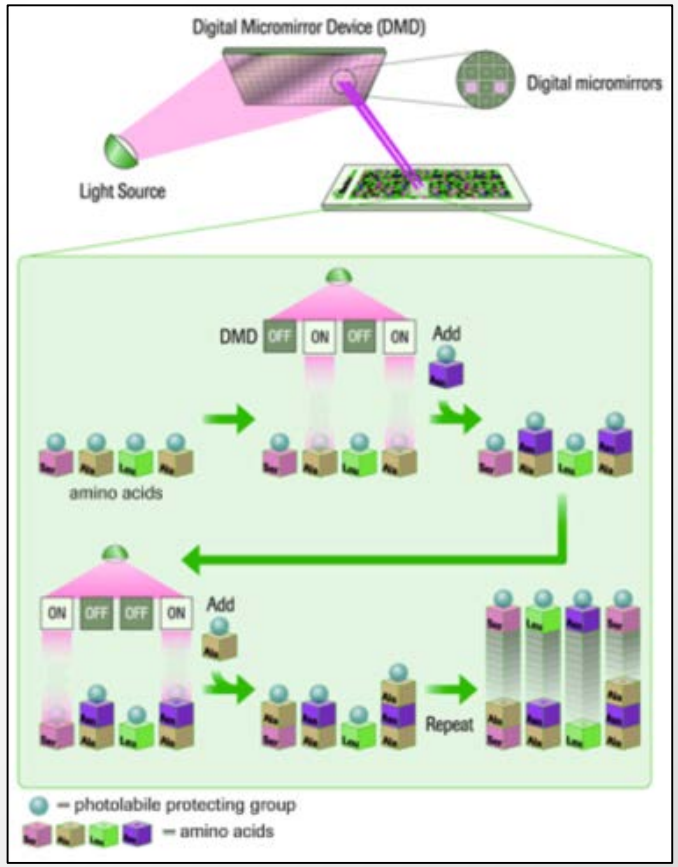


Historical Infection:
High Throughput Serology Using Peptide Microarrays

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



Synthesis in situ



Serochip for Tick-Borne Diseases (TBD-CHIP)

3M feature platform for multiplex serological detection of tick-borne agents

Antigens

B. burgdorferi
OspA, OspB, OspC, OspD, VlsE, DbpA, DbpB, BmpA, P100, OppA2; FlaA, FlaB, FliL, FlgE, DnaK, RevA, P66, LA7, BBK07, BBK32, BBK50, OspE, OspF, Erp (A,B,C,D,G,H,K,L,M,O,P,Q,X,Y), Mlp (A, H I, J); Bdr (M, K, P, Q, R, S, T, U), BBA04, BBA36, BBA57, BBA64, BBA65, BBA66, BBA68, BBA69, BBA73, BBA74

B. miyamotoi
GltQ, FhbA, ipA, P66, OppA2, FlgG, FlaB, FliL, VLP (1, A1, A2, C1, C2, C3, D1, D2, D3, D4, D5, D5S, D6S, D6, D7S, D8, D9, D10, 3S, A2S, 4S 15/16, 18), VSP (1,2, 3, 4, 6)

B. microti
BMN1 (-2, -3, -4 -5 -6, -7, -8, -9, 10, 11, 12 13, 17, 20), GPI 12, AMA1

A. phagocytophilum
MSP2, MSP4, MSP5, P55, P62, Omp1N

E. chaffeensis
P156, P120, P28/omp-1, Gp47, VLPT, SP-related protein

R. rickettsii
OmpA, OmpB, OmpW, Porin 4, adr1, adr2

Powassan virus
Polyprotein

Heartland virus
N, Gn, Gc, L

Long Island tick rhabdovirus
N, P, M, G, L

Agent	Number of designed 12-mer peptides
Borrelia burgdorferi	91,338
Borrelia miyamotoi	23,946
Anaplasma phagocytophilum	16,787
Babesia microti	11,333
Powassan virus	7,688
Rickettsia rickettsii	5,855
Ehrlichia chaffeensis	4,156
Heartland virus	4,153
Long Island tick rhabdovirus	3,949
Total peptides	**169,205**


Rafal Tokarz


Nischay Mishra

Tokarz, et al. Scientific Reports, 2018

TBD-CHIP Detects Previously Unknown Babesia Co-Infections

SAMPLE	Standard assays			TBD-Chip antigen signal intensity							
	ELISA	Western Blot IgM	Western Blot IgG	BBK12	DbpA	P66	FlaB	VlsE (C6)	OspC	oppA	p100
Neg Ctrl 1	NEG	NEG	IND								
Neg Ctrl 2	NEG	NEG	IND								
Neg Ctrl 3	NEG	NEG	NEG								
EA Lyme 1	POS	POS	NEG	+			+	++			
EA Lyme 2	N.A.	POS	IND				++	++	+++		+++
EA Lyme 3	NEG	POS	IND						+	+++	
EA Lyme 4	IND	POS	IND						++		
EA Lyme 5	N.A.	POS	IND						+++		
EA Lyme 6	POS	POS	IND					+	++	+	
EA Lyme 7	POS	POS	IND				+++	+	+++		
EA Lyme 8	IND	POS	IND	+++	++			+++			
EA Lyme 9	N.A.	POS	IND	+			+++	+++	+++	+++	+
EA Lyme 10	POS	POS	NEG		+		++	+++	+	++	
AD Lyme 1	POS	IND	POS				+++	+++			
AD Lyme 2	POS	NEG	N.A.		++	+++	+++	+++			
AD Lyme 3	POS	POS	POS	+++	+++	+++	+++	+++			
AD Lyme 4	POS	POS	POS	+++	+	+++	+	+++			
AD Lyme 5	POS	POS	POS	+			+++	+++			
AD Lyme 6	POS	N.A.	POS	++			+++	+++	+++		+
AD Lyme 7	POS	POS	POS	++	+++	++	+++	+++	+		
AD Lyme 8	POS	POS	POS	+		+++	+++	+++	+++		
AD Lyme 9	POS	NEG	POS	+++	+++	++	+++	+++			
AD Lyme 10	POS	POS	POS	+++	+++	+++	+	+++			

Negative controls

Early acute Lyme disease

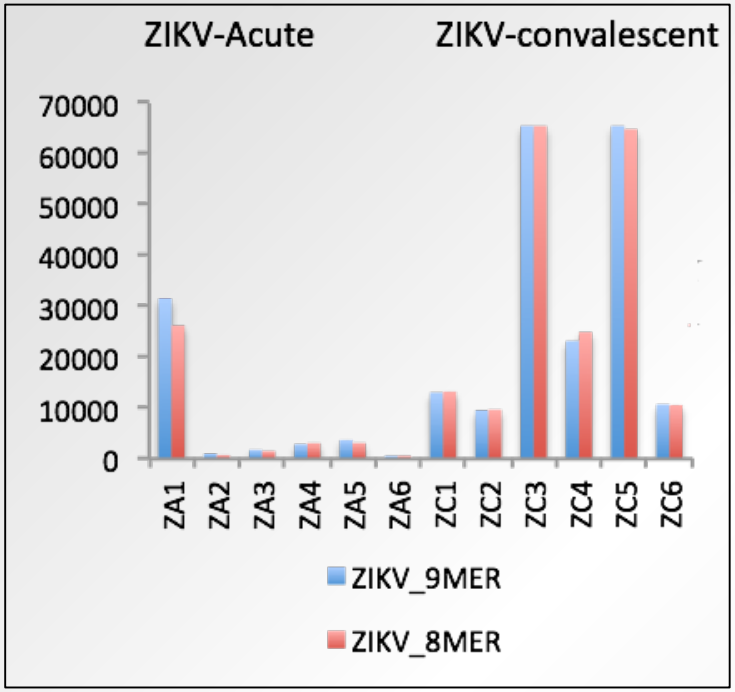
Acute disseminated Lyme disease

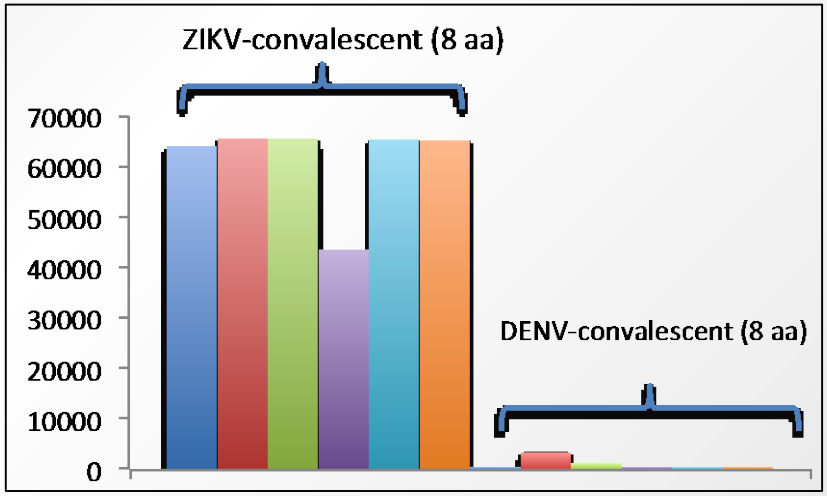
N.A. - data not available; POS - positive; NEG - negative; IND- indeterminate; + signal intensity

Positive for Babesia

Identification of a Zika-Specific Peptide

Tiled 8- and 9-mer peptides in the Zika NS2B region are specific in peptide chip





Nischay Mishra

Williams, et al. mBio, 2018

CAPRISA

Vaginal co-infection, inflammation, and HIV transmission

Background: Cohort of South African women treated with anti-retroviral Tenofovir gel to test effectiveness for prevention of transmission of HIV

Transmission was reduced but there were still some women that had acquired HIV

High cytokine concentration in vaginal lavage in this group of women

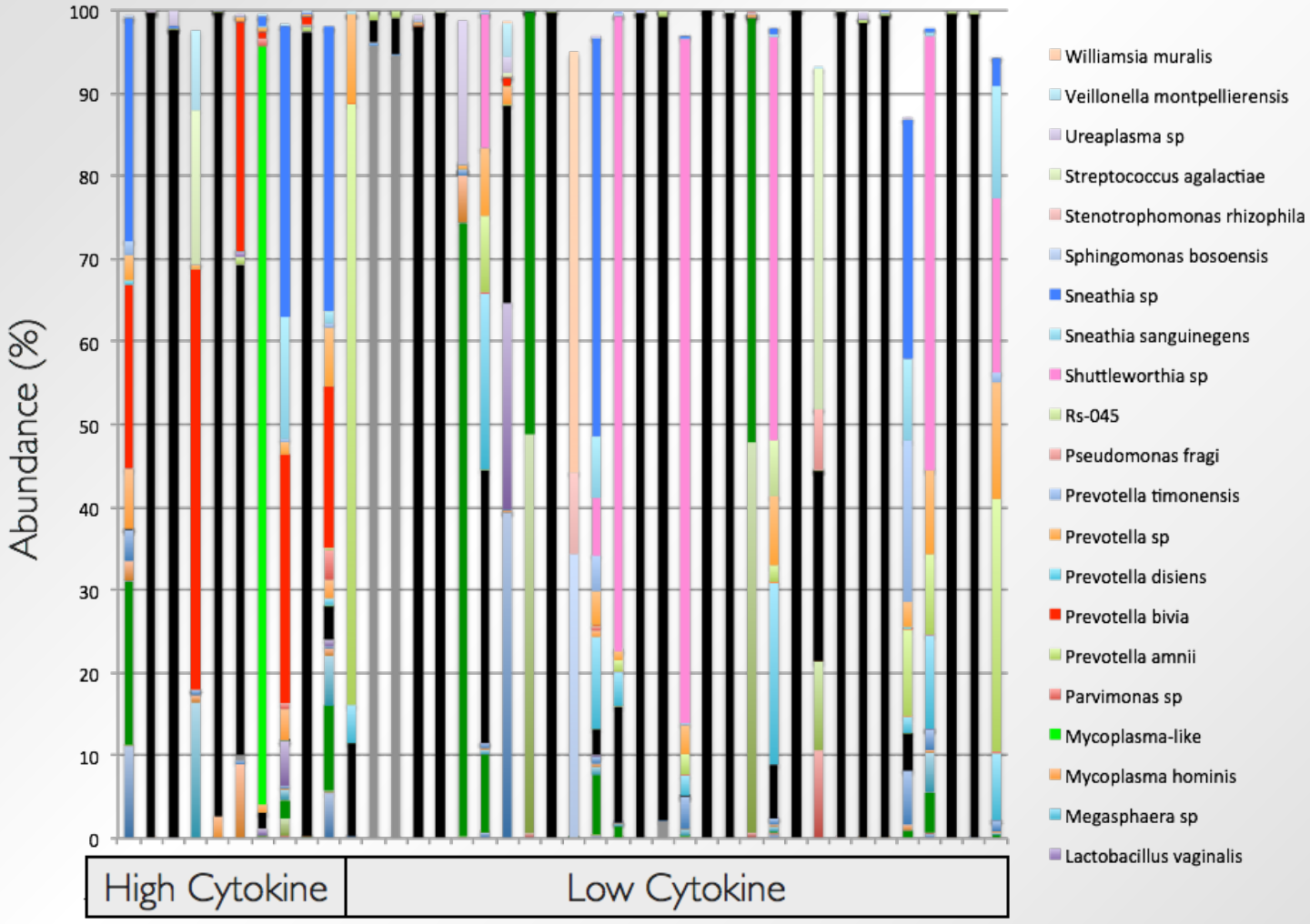
Hypothesis: high cytokine concentration associated with an altered vaginal bacterial, fungal, viral flora/infection


Brent Williams


Salim Abdool Karim


Quarraisha Abdool Karim

Top 10 Most Abundant Bacteria Per Subject (16S Sequencing)



P. bivia Associated with Genital Inflammation and Enhanced HIV Acquisition

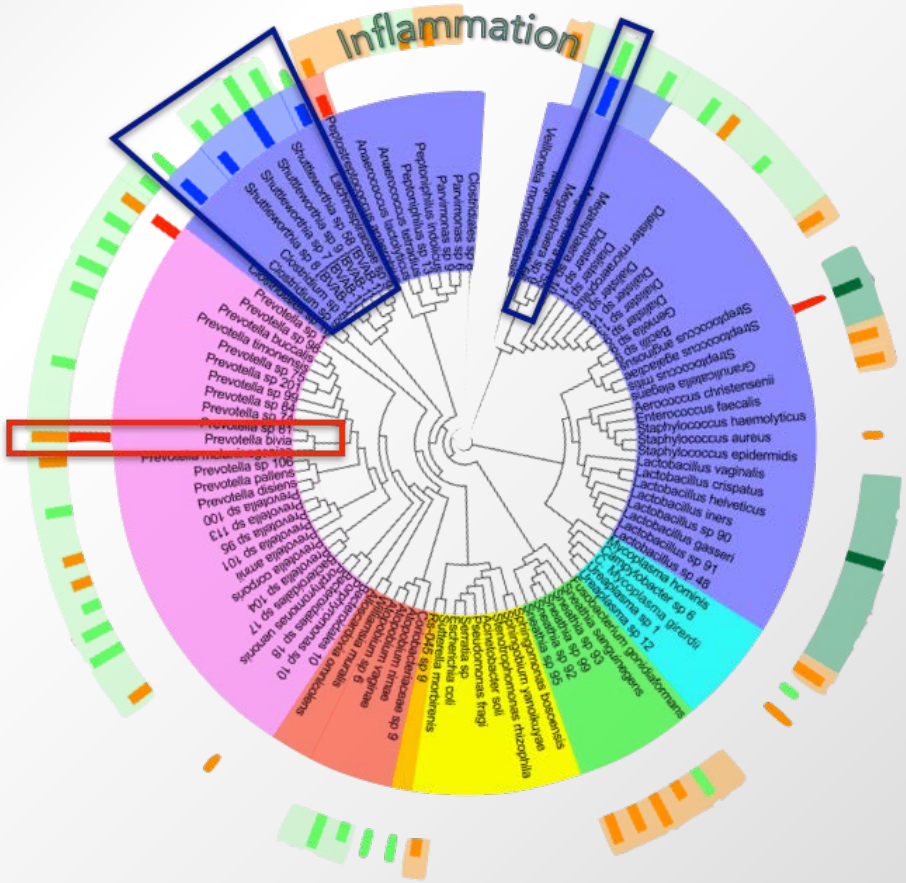
n=119 women
cervicovaginal lavage cytokine and microbiome analysis

Phylum:

- Firmicutes
- Tenericutes
- Fusobacteria
- Proteobacteria
- TM7
- Actinobacteria
- Bacteroidetes

log_{10}LDA score (≥2):

- HIV-positive
- HIV-negative
- HC (High cytokine)
- LC-1 (Low cytokine-1)
- LC-2 (Low cytokine-2)



Bar length indicates LDA score

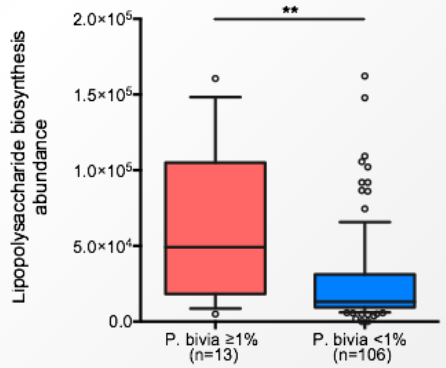
P. bivia is Associated with Genital Inflammation and Enhanced HIV Acquisition

Women with *P. bivia*
19x more likely to have genital inflammation
13x more likely to acquire HIV

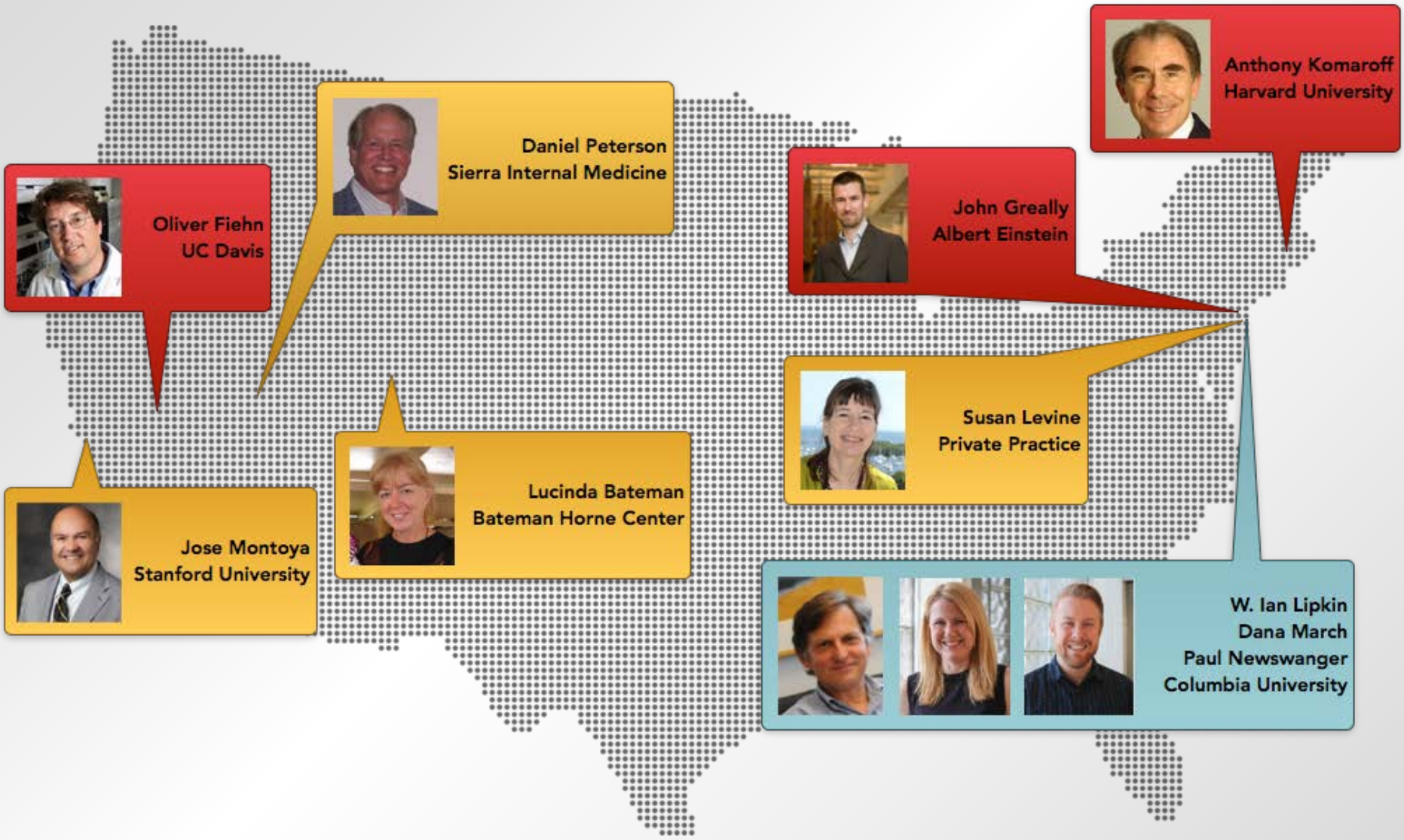
	***P. bivia* OR**
High cytokine	19.2 (95% CI: 4.0-92.4) p<0.001
HIV positive	12.7 (95% CI: 2.1-77.8) p=0.006

Potential mechanism

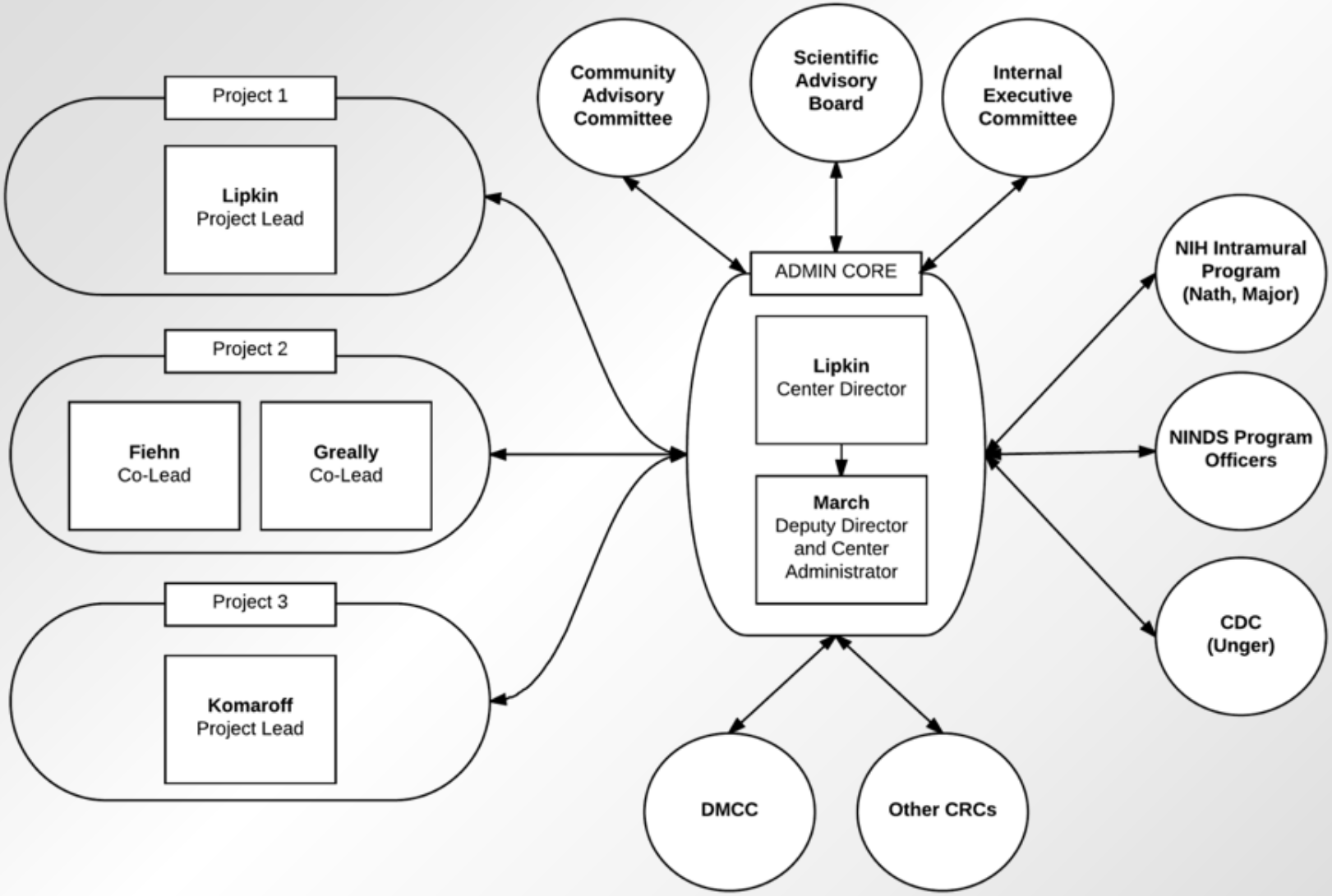
↑ *P. bivia* = ↑ Lipopolysaccharide



Center for Solutions for ME/CFS



CfS for ME/CFS Organizational Structure



Highlights in the History of the Center

Journal of NeuroVirology (1999) 5, 495–499
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http://www.jneurovirol.com

Absence of evidence of Borna disease virus infection in Swedish patients with Chronic Fatigue Syndrome

Birgitte Evengård*,[1], Thomas Briese[2], Gudrun Lindh[1], Shaun Lee[2] and W Ian Lipkin*,[2]

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differential exposure to infectious agents. Although serum immunoreactivity to BDV proteins observed in Swedish CFS patients by ELISA may reflect infection with related microbial agents that induce cross-reactivity with conformational determinants on BDV proteins (Kliche *et al*, 1996) and β-galactosidase, the serologic findings are also consistent with nonspecific polyclonal B-cell activation. Indeed, increased levels of antibodies against different microbial agents and other viruses, such as EBV, have previously been shown in sera from CFS patients (Jones *et al*, 1985; Straus *et al*, 1985) and interpreted as evidence of polyclonal activation.

A Multicenter Blinded Analysis Indicates No Association between Chronic Fatigue Syndrome/Myalgic Encephalomyelitis and either Xenotropic Murine Leukemia Virus-Related Virus or Polytropic Murine Leukemia Virus

Harvey J. Alter[a], Judy A. Mikovits[b], William M. Switzer[c], Francis W. Ruscetti[d], Shyh-Ching Lo[e], Nancy Klimas[f,g], Anthony L. Komaroff[h], Jose G. Montoya[i], Lucinda Bateman[j], Susan Levine[k], Daniel Peterson[l], Bruce Levin[m], Maureen R. Hanson[n], Afia Genfi[o], Meera Bhat[o], HaoQiang Zheng[c], Richard Wang[a], Bingjie Li[e], Guo-Chiuan Hung[e], Li Ling Lee[n], Stephen Sameroff[o], Walid Heneine[c], John Coffin[p], Mady Hornig[o], and W. Ian Lipkin[o]

Nagy-Szakal *et al. Microbiome* (2017) 5:44
DOI 10.1186/s40168-017-0261-y

Microbiome

RESEARCH Open Access

Fecal metagenomic profiles in subgroups of patients with myalgic encephalomyelitis/ chronic fatigue syndrome

Dorottya Nagy-Szakal[1†], Brent L. Williams[1†], Nischay Mishra[1], Xiaoyu Che[1], Bohyun Lee[1], Lucinda Bateman[2], Nancy G. Klimas[3,9], Anthony L. Komaroff[4], Susan Levine[5], Jose G. Montoya[6], Daniel L. Peterson[7], Devi Ramanan[8], Komal Jain[1], Meredith L. Eddy[1], Mady Hornig[1] and W. Ian Lipkin[1*]

RESEARCH ARTICLE

BIOMARKERS

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

Mady Hornig,[1,2*] José G. Montoya,[3] Nancy G. Klimas,[4] Susan Levine,[5] Donna Felsenstein,[6] Lucinda Bateman,[7] Daniel L. Peterson,[8] C. Gunnar Gottschalk,[8] Andrew F. Schultz,[1] Xiaoyu Che,[1] Meredith L. Eddy,[1] Anthony L. Komaroff,[9] W. Ian Lipkin[1,2,10]

Aims: Project 1

Aim 1: Determine between-group differences in the ME/CFS and control bacteriome, mycobiome, and virome.

- **Aim 1.1.** Profile bacterial communities of oral & fecal samples from ME/CFS cases & controls using shotgun metagenomic sequencing.
- **Aim 1.2.** Profile fungal communities in oral & fecal samples from ME/CFS cases & controls using internal transcribed spacer (ITS) sequencing.
- **Aim 1.3.** Profile virome composition and dynamics in oral and fecal samples and peripheral blood mononuclear cells (PBMC) from ME/CFS cases and controls using virome capture sequencing for vertebrate viruses (VirCapSeq-VERT).
- **Aim 1.4.** Quantitate the burden of potential pathogens identified in Aims 1.1-1.3 using quantitative polymerase chain reaction (qPCR).

Aim 2: Investigate between-group differences in prevalence of antibodies to microbes associated with ME/CFS in Aim 1 and in the prevalence of autoantibodies.

Aim 3: Profile plasma immune signatures in ME/CFS cases and controls surveyed in Aims 1 and 2.

Aim 4: Integrate microbial exposure (Project 1), plasma cytokine, metabolomic, plasma and PBMC RNA-seq data (Projects 2 and 3) to find relationships that may provide insights into ME/CFS sub-types, risk factors, and biomarkers with implications for pathogenesis, diagnosis and treatment**.**

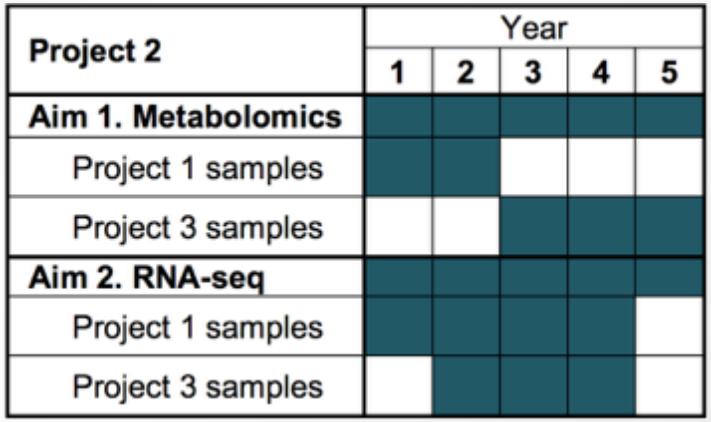
Project 1	Year				
	1	**2**	**3**	**4**	**5**
Aim 1.1. Metagenomics	■				
Aim 1.2. ITS		■			
Aim 1.3. VirCapSeq-VERT		■			
Aim 1.4. qPCR	■	■	■	■	■
Aim 2. Serology			■	■	■
Aim 3. Immune signatures	■				
Aim 4. Topological data analysis				■	■

Aims: Project 2

Aim 1: Profile metabolic changes in peripheral blood plasma of ME/CFS patients and controls at rest and after Lean Test and Exercise Tolerance Test (ETT) challenge.

Aim 2: Profile cellular repertoire and gene expression changes in PBMC of ME/CFS patients and controls at rest and after Lean Test and ETT challenge.

Aim 3: Test how molecular markers of metabolites and gene expression are associated with sub-types of ME/CFS.



Aims: Project 3

Aim 1: Establish the ME/CFS Practice Network as a hub for state-of-the-art translational research.

Aim 1.1. Extend and coordinate the already existing practice network.

Aim 1.2. Extend an existing integrated database to incorporate additional patients and data points.

Aim 1.3. Create the foundation for dynamic, longitudinal data collection using mobile devices through a participatory process that engages the ME/CFS community, clinicians, and researchers.

Aim 2: Mine existing databases to identify distinct features of ME/CFS and to identify subtypes that differ in pathogenesis, biomarkers, or clinical tests required for diagnosis, prognosis, or response to interventions.

Aim 2.1. Identify significant risk factors for illness through case-control comparisons.

Aim 2.2. Identify sub-types of ME/CFS.

Aim 3: Profile metabolomic, plasma immune signature, and gene expression responses to orthostatic and exercise stress tests.

Aim 3.1. Assess the impact and clinical utility of the Lean Test (orthostatic stressor).

Aim 3.2. Assess the impact of an ETT.

Project 3	Year				
	1	2	3	4	5
Aim 1.1. Practice network	■	■	■	■	■
Aim 1.2. Database integration	■	■			
Aim 1.3. Mobile app	■	■	■		
Aim 2.1. Case/control differences	■				
Aim 2.2. Identify sub-types	■				
Aim 3.1. Lean Test	■	■			
Aim 3.2. ETT		■	■		

ME/CFS Analyses

Fecal metagenomics

- CFS Exension: 50 cases/50 controls; 4 sites
- *Nagy-Szakal, et al*. Microbiome 2017

Plasma metabolomics

- CFI Extension: 50 cases/50 controls; 4 sites
- *Nagy-Szakal, et al.* Scientific Reports 2018

Cerebrospinal fluid metabolomics

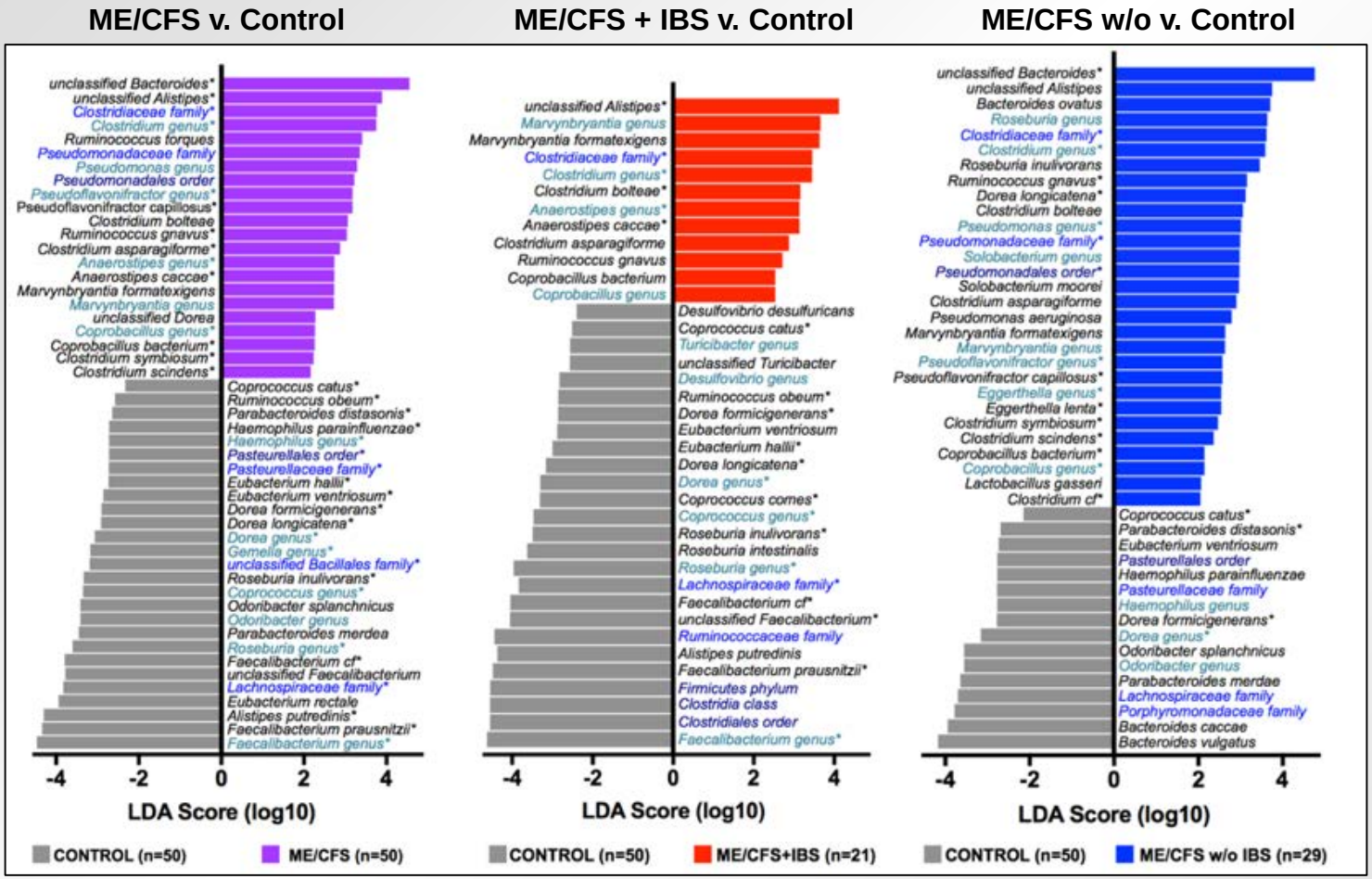
- Independent study: 60 cases/62 controls
- Additional samples (Natelson/Marques)
  - 4 cases/32 controls: 12 healthy, 20 post treatment (Lyme disease)
- *Lee, et al.* Unpublished

Plasma proteomics

- CFI Extension: 50 cases/50 controls; 4 sites
- *Analysis in progress*

Chronic Fatigue Initiative, Hutchins Family Foundation
Edward P. Evans Foundation
Simmaron Research
Solve ME/CFS

Fecal Metagenomic Profiles in ME/CFS

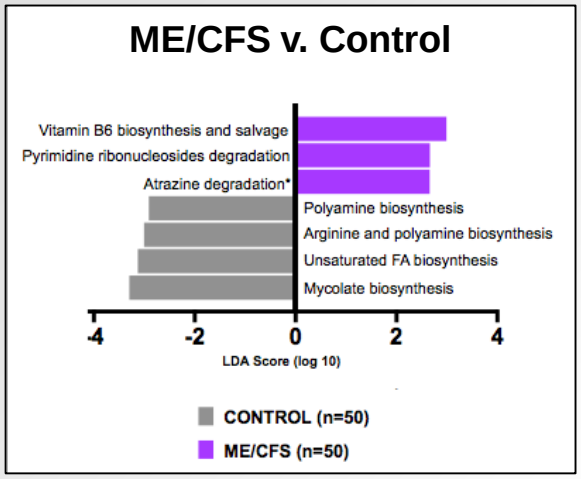


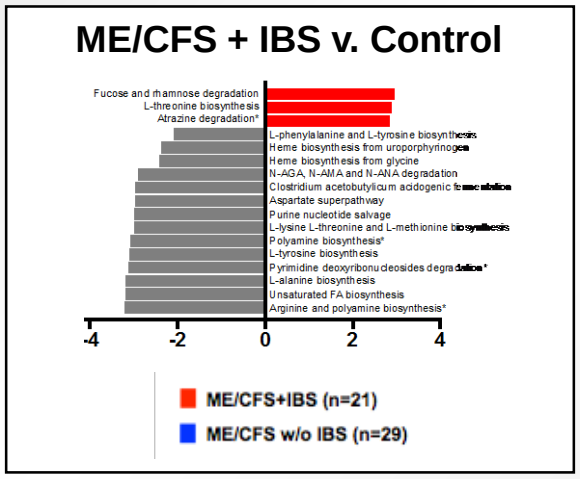
Linear discriminant effect size (LEfSe)

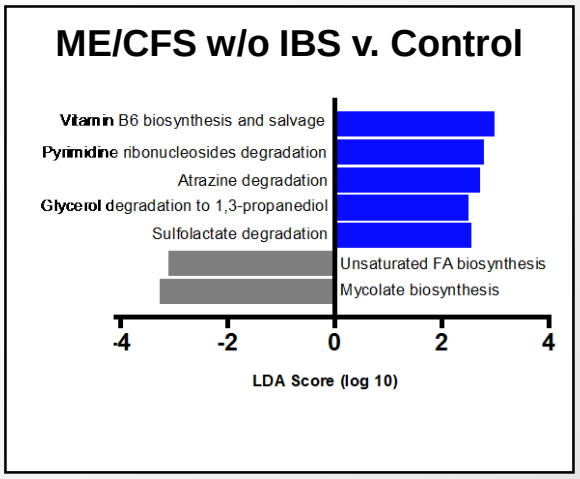
Nagy-Szakal, et al. Microbiome 2017

Dora Nagy-Szakal

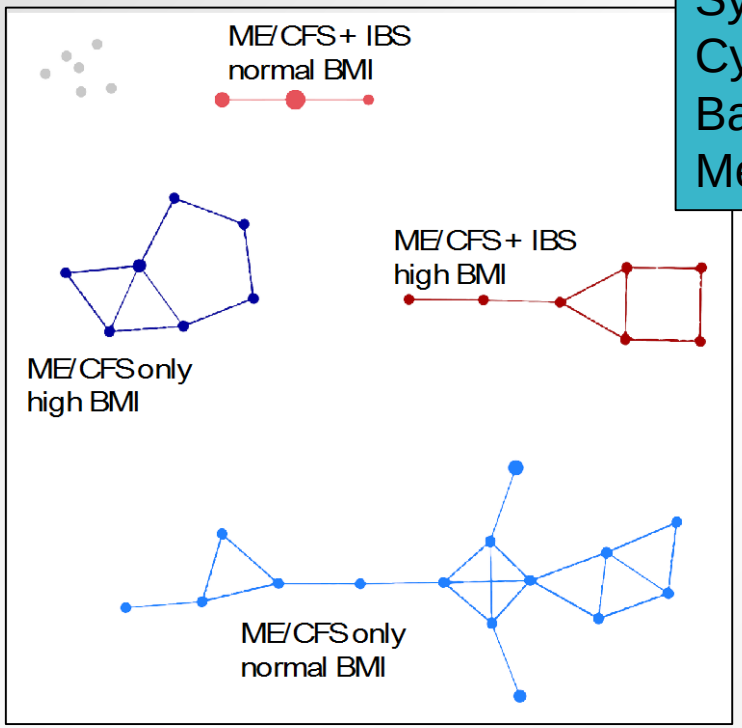
Fecal Bacterial Metabolic Pathways in ME/CFS with and without IBS







AYASDI Topological Analysis of ME/CFS



Symptoms
Cytokines
Bacteria
Metabolic Pathways

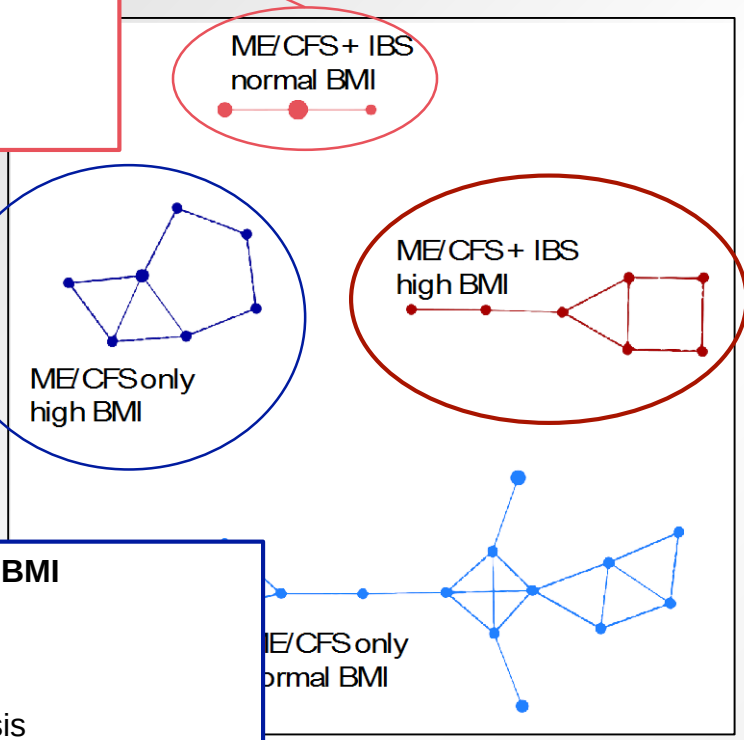
Metric: normalized correlation
Lenses: IBS status and BMI

Dora Nagy-Szakal

AYASDI Topological Analysis of ME/CFS

ME/CFS+IBS and normal BMI
↓ Coprococcus species
↓ *Collinsella aerofaciens*

General health
Physical function



ME/CFS only and high BMI
↑ *Clostridium leptum*
↑ IL-4, IL-5, IL-15

↑ Propanediol biosynthesis
↑ Fatty acid ß-oxidation
↓ L-glutamine and L-histidine biosynthesis

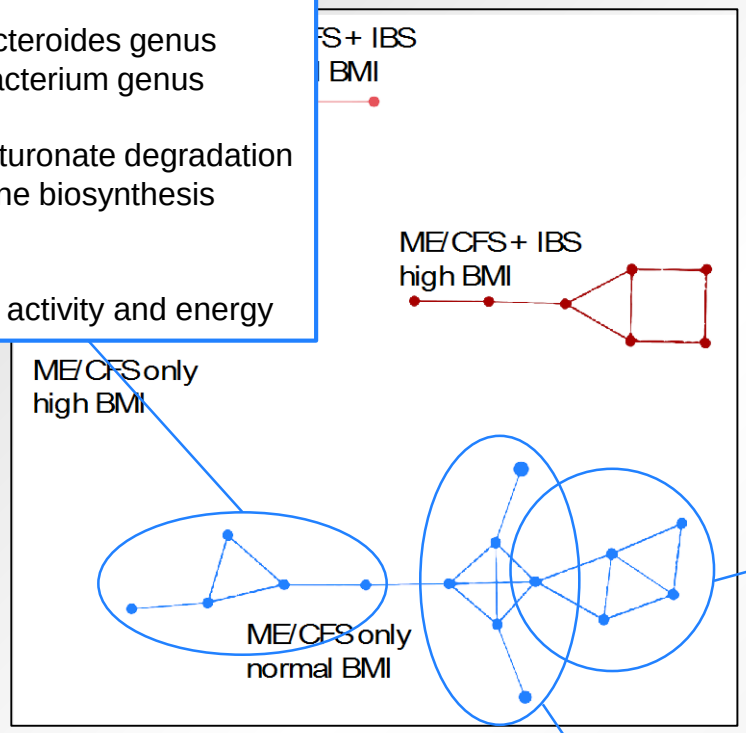
Physical and social function
General, mental, and physical fatigue

ME/CFS+IBS and high BMI
↓ Faecalibacterium species
↑ Leptin

↓ L-arginine biosynthesis
↓ Purine and pyrimidine degradation
↓ Biofidobacterium shunt (CH degradation)
↓ L-isoleucine degradation

Pain and physical function
General, mental, and physical fatigue
Reduced motivation, energy, and activity

AYASDI Topological Analysis of ME/CFS



Group 1
- ↓ Parabacteroides genus
- ↓ Bifidobacterium genus

- ↑ D-galacturonate degradation
- ↓ L-arginine biosynthesis

Pain
Reduced activity and energy

Group 3
- ↓ Roseburia genus
- ↓ VEGF-A
- ↓ IL-1RA, IL-12p40, IL-12p70, IL-15, IL-22, ß-NGF, LIF

- ↑ Peptidoglycan biosynthesis
- ↑ Pyrimidine degradation
- ↑ Allantoin degradation

Group 2
- ↑ *Eubacterium siraeum*
- ↓ IL-27, BDNF, IFN-γ

- ↓ Fatty acid biosynthesis
- ↑ Mannan (CH) degradation

ME/CFS Plasma Metabolomics

Targeted and untargeted analysis of >600 metabolites

50 ME/CFS cases and 50 control plasma (CFI Extension samples); 4 sites

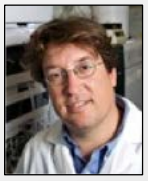
Primary metabolites (115)
- Tryptophan metabolism, sugars, hydroxyl acids, ketone bodies, and other energy-metabolism compounds

Positive electrospray ionization complex lipids (207)

Negative electrospray ionization complex lipids (96)
- Mono- and diacylglycerides, fatty acids, ceramides, sphingomyelins and phospholipids

Biogenic amines (109)
- Branched and unbranced acylcamitines, TMAO, choline, and amino acids

Oxilipins (46)
- Bioactive oxilipins, steroids, and bile acids


Oliver Fiehn


Dinesh Bharupal


Dora Nagy-Szakal


Bohyun Lee

Top 10 Metabolites that Distinguish ME/CFS from Controls

ME/CFS vs. Control					
Compound name	Direction in ME/CFS	Chemical Pathway	Mann-Whitney U-test p-value	Logistic Regression	
				Odds ratio	p-value
LPC 18:2	Decreased	PC	0.004	0.512	0.013
Betaine	Decreased	carnitine-choline	0.006	0.551	0.021
TG 53:5	Increased	TG	0.001	1.699	0.028
α N-phenylacetyl-L-glutamine	Increased	amino acid	0.004	2.457	0.015
PC 30:0	Decreased	PC	0.017	0.330	0.001
SM d32:1	Decreased	SM	0.017	0.513	0.009
PC 33:0	Decreased	PC	0.002	0.397	0.000
Urobilin	Increased	bilirubin	0.010	2.086	0.023
ε Caprolactam	Increased	amino acid	0.033	1.925	0.041
TG 54:8	Increased	TG	0.003	1.751	0.018

LPC: lysophosphatidylcholine
PC: phosphatidylcholine
SM: sphingomyelin
TG: triglyceride

Top 10 Metabolites that Distinguish ME/CFS IBS Subgroups from Controls

ME/CFS+IBS vs. Control					
Compound name	Direction in ME/CFS+IBS	Chemical Pathway	Mann-Whitney U-test p-value	Logistic Regression	
				Odds ratio	p-value
LPC 18:2	Decreased	PC	0.000	0.305	0.004
Ceramide d36:1	Increased	ROS, gut permeability	0.002	2.825	0.015
γ Butyrobetaine	Decreased	mitochondrial TCA cycle	0.000	0.470	0.003
LPC 18:1	Decreased	PC	0.001	0.396	0.011
5-Methylthioadenosine	Increased	one-carbon / nicotinate	0.001	3.715	0.005
TG 49:2	Increased	TG	0.006	1.844	0.043
Ceramide d40:0	Increased	ROS, gut permeability	0.002	2.340	0.024
TG 51:3	Increased	TG	0.001	2.459	0.020
Betaine	Decreased	mitochondrial TCA cycle	0.016	0.555	0.028
Ceramide d42:0	Increased	ROS, gut permeability	0.016	2.014	0.028

ME/CFS w/o IBS vs. Control					
Compound name	Direction in ME/CFS w/o IBS	Chemical Pathway	Mann-Whitney U-test p-value	Logistic Regression	
				Odds ratio	p-value
PC 33:0	Decreased	PC	0.000	0.386	0.000
PC 38:2	Decreased	PC	0.000	0.487	0.007
PC 30:0	Decreased	PC	0.000	0.323	0.001
Tyrosine	Decreased	neurotransmitter	0.008	0.333	0.001
PC 36:6	Decreased	PC	0.028	0.545	0.015
TG 54:6 A	Increased	TG	0.001	2.267	0.009
TG 53:5	Increased	TG	0.004	1.736	0.030
TG 54:8	Increased	TG	0.003	1.674	0.030
Tyr Met Lys	Increased	neurotransmitter	0.011	2.893	0.015
PC 32:2	Decreased	PC	0.001	0.483	0.006

LPC: lysophosphatidylcholine **PC:** phosphatidylcholine **TG:** triglyceride

Ceramides Linked to ROS, Gut Permeability

ME/CFS+IBS vs. Control					
Compound name	Direction in ME/CFS+IBS	Chemical Pathway	Mann-Whitney U-test p-value	Logistic Regression	
				Odds ratio	p-value
LPC 18:2	Decreased	PC	0.000	0.305	0.004
Ceramide d36:1	Increased	ROS, gut permeability	0.002	2.825	0.015
γ Butyrobetaine	Decreased	mitochondrial TCA cycle	0.000	0.470	0.003
LPC 18:1	Decreased	PC	0.001	0.396	0.011
5-Methylthioadenosine	Increased	one-carbon / nicotinate	0.001	3.715	0.005
TG 49:2	Increased	TG	0.006	1.844	0.043
Ceramide d40:0	Increased	ROS, gut permeability	0.002	2.340	0.024
TG 51:3	Increased	TG	0.001	2.459	0.020
Betaine	Decreased	mitochondrial TCA cycle	0.016	0.555	0.028
Ceramide d42:0	Increased	ROS, gut permeability	0.016	2.014	0.028

ME/CFS w/o IBS vs. Control					
Compound name	Direction in ME/CFS w/o IBS	Chemical Pathway	Mann-Whitney U-test p-value	Logistic Regression	
				Odds ratio	p-value
PC 33:0	Decreased	PC	0.000	0.386	0.000
PC 38:2	Decreased	PC	0.000	0.487	0.007
PC 30:0	Decreased	PC	0.000	0.323	0.001
Tyrosine	Decreased	neurotransmitter	0.008	0.333	0.001
PC 36:6	Decreased	PC	0.028	0.545	0.015
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TG 53:5	Increased	TG	0.004	1.736	0.030
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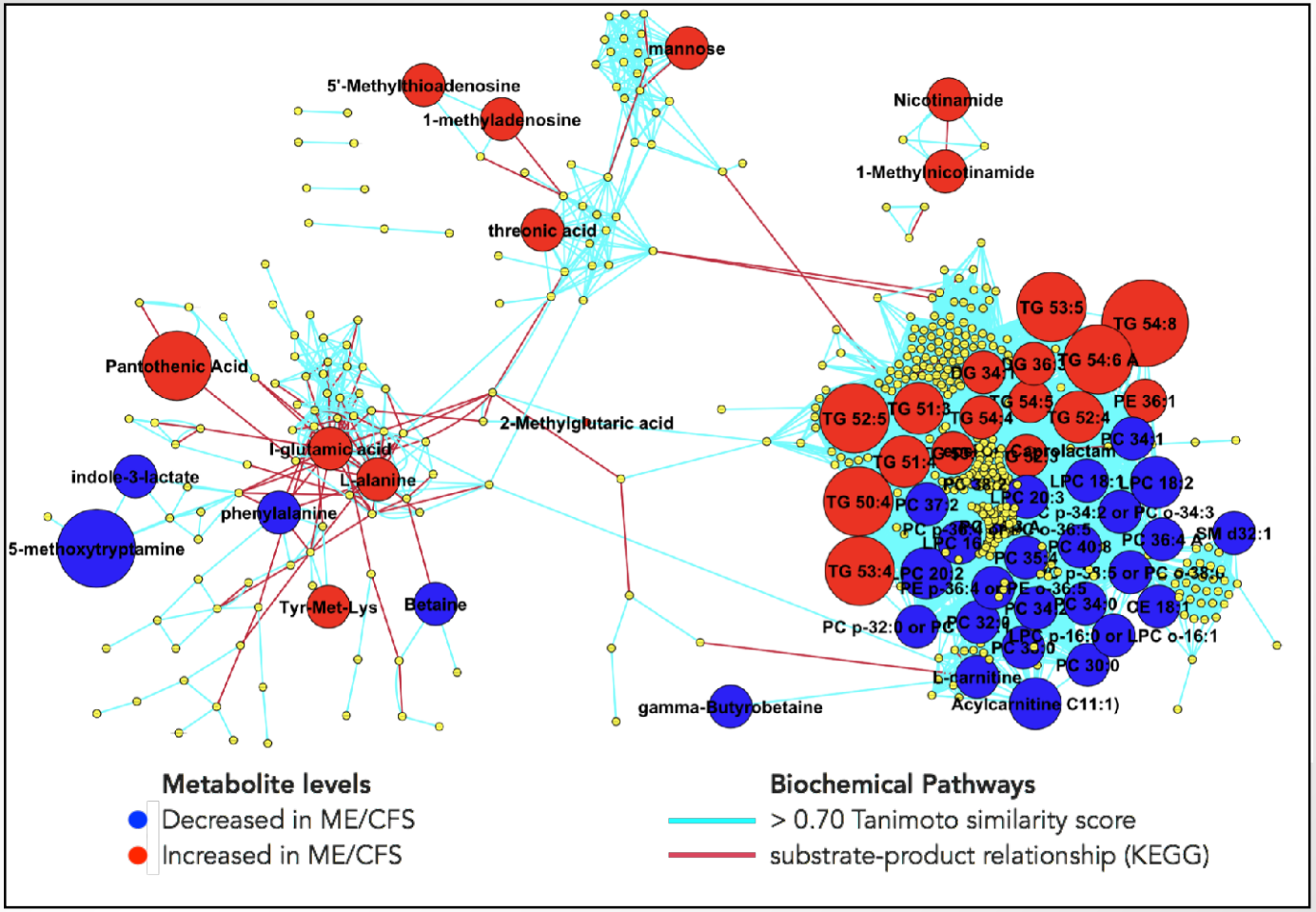
Tyr Met Lys	Increased	neurotransmitter	0.011	2.893	0.015
PC 32:2	Decreased	PC	0.001	0.483	0.006

LPC: lysophosphatidylcholine **PC:** phosphatidylcholine **TG:** triglyceride

Ceramide Levels Differ in ME/CFS with and without IBS

Compound name	Direction	Mann-Whitney U-test p-value	Logistic Regression		Random Forest	
			Odds ratio	p-value	Importance	RF ranking
ME/CFS+IBS vs. Control						
Ceramide d36:1	Increased	0.002	2.825	0.015	0.971	2
Ceramide d40:0	Increased	0.002	2.340	0.024	0.565	8
Ceramide d42:0	Increased	0.016	2.014	0.028	0.444	9
Ceramide d34:1	Increased	0.005	2.257	0.025	0.252	14
Ceramide d38:1	Increased	0.004	2.961	0.005	0.051	26
Ceramide d40:1	Increased	0.048	2.490	0.018	-0.004	35
ME/CFS w/o IBS vs. Control						
Ceramide d43:1	Decreased	0.007	0.469	0.008	0.061	37
Ceramide d42:1	Decreased	0.035	0.600	0.050	0.025	45

Altered Plasma Metabolite Levels in ME/CFS



Barupal and Fiehn, MetaMapp

Metabolomics and Metagenomics in ME/CFS

ME/CFS cases (n=50)

- ↓ Betaine correlates with ↓ [Firmicutes] *Anaerotruncus colihominis*
- ↓ PC 30:0 correlates with ↓ [Bacteroidetes] *Alistipes putredinis*

ME/CFS + IBS cases (n=24)

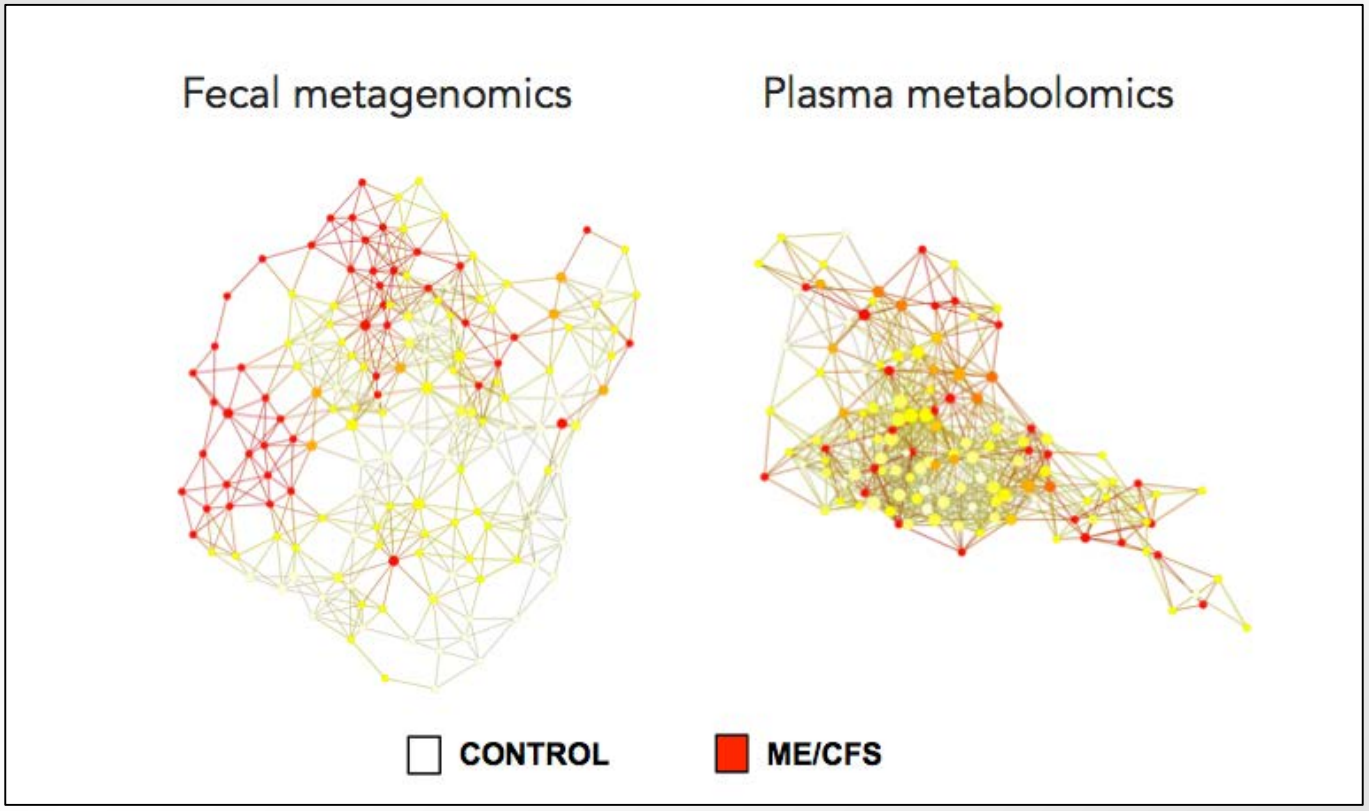
- ↓♂ Butyrobetaine correlates with ↑ [Firmicutes] *Faecalibacterium cf*
- ↑ 5-Methylthio-adenosine correlates with worse general health
- ↑ Ceramide d42:0 correlates with more severe physical fatigue

ME/CFS without IBS cases (n=26)

- ↓ Tyrosine is correlated with ↓ [Bateroidetes] *Parabacteroides distasonis*
- ↑ TG 54:6A and TG 54:8 associated with ↓ [Bacteroidetes] *Bacteroides caccae* and [Firmicutes] *Dorea formicigenerans*

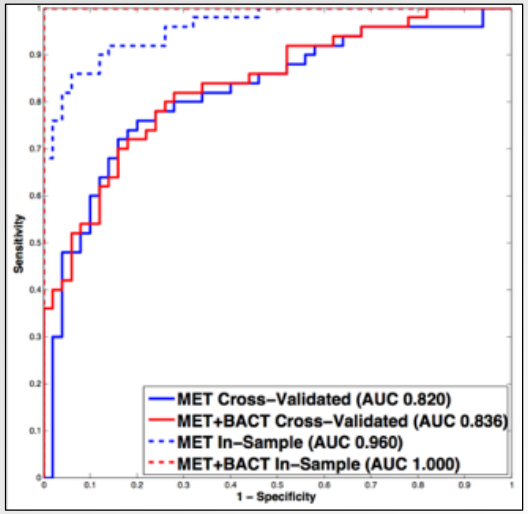
Topological Integration with Clinical and Laboratory Data

Fecal metagenomic features were stronger drivers of the network distinction than plasma metabolomics features



Improved Diagnostic Performance with Metabolomic and Bacterial Biomarkers of Plasma

All ME/CFS v. Controls

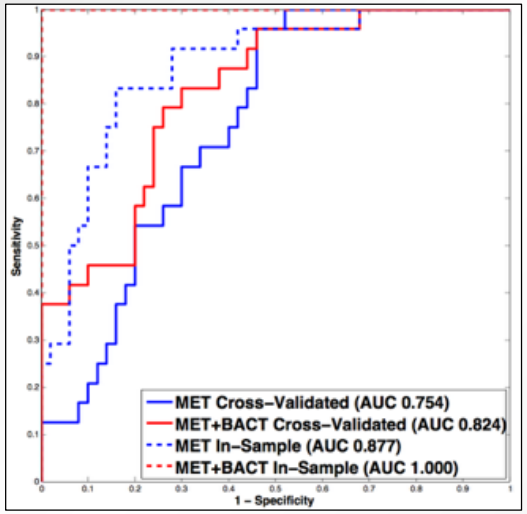


Bacteria alone:
cross-validated AUC=0.745

Metabolites alone:
cross-validated AUC=0.820

Bacteria and metabolites:
cross-validated AUC=0.836

ME/CFS with IBS v. Controls

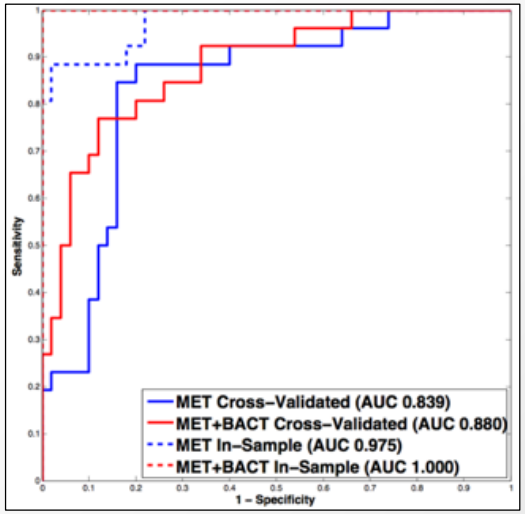


Bacteria alone:
cross-validated AUC=0.791

Metabolites alone:
cross-validated AUC=0.754

Bacteria and metabolites:
cross-validated AUC=0.824

ME/CFS w/o IBS v. Controls



Bacteria alone:
cross-validated AUC=0.754

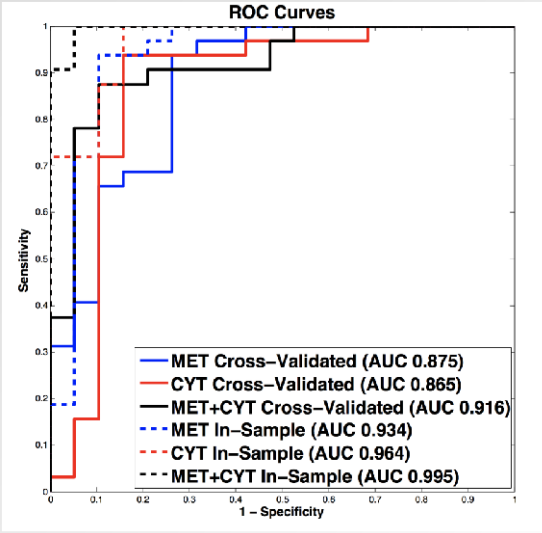
Metabolites alone:
cross-validated AUC=0.839

Bacteria and metabolites:
cross-validated AUC=0.880

Nagy-Szakal, et al. Scientific Reports 2018

Improved Diagnostic Performance with Metabolomic and Cytokine Biomarkers of Cerebrospinal Fluid

A. ME/CFS vs. ND control

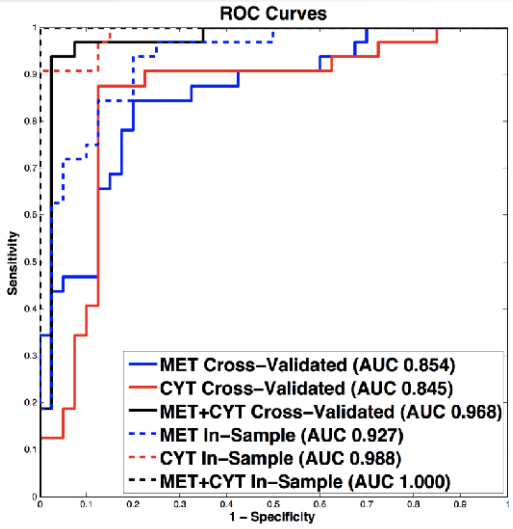


Metabolites alone:
cross-validated AUC=0.875

Cytokines alone:
cross-validated AUC=0.865

Metabolites and cytokines:
cross-validated AUC=0.916

B. ME/CFS vs. MS comparator

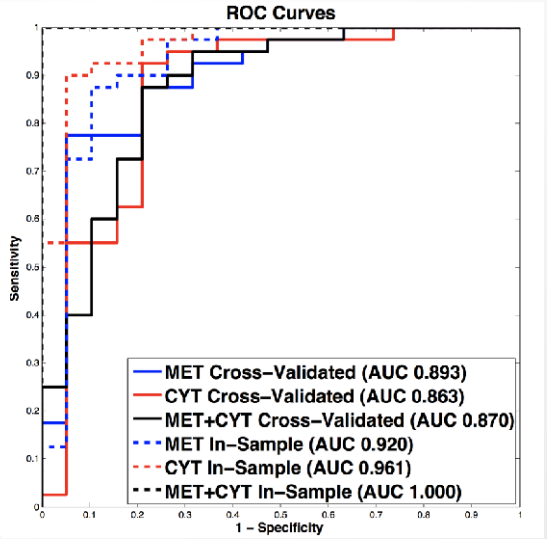


Metabolites alone:
cross-validated AUC=0.854

Cytokines alone:
cross-validated AUC=0.845

Metabolites and cytokines:
cross-validated AUC=0.968

C. MS comparator vs. ND control



Metabolites alone:
cross-validated AUC=0.893

Cytokines alone:
cross-validated AUC=0.863

Metabolites and cytokines:
cross-validated AUC=0.870

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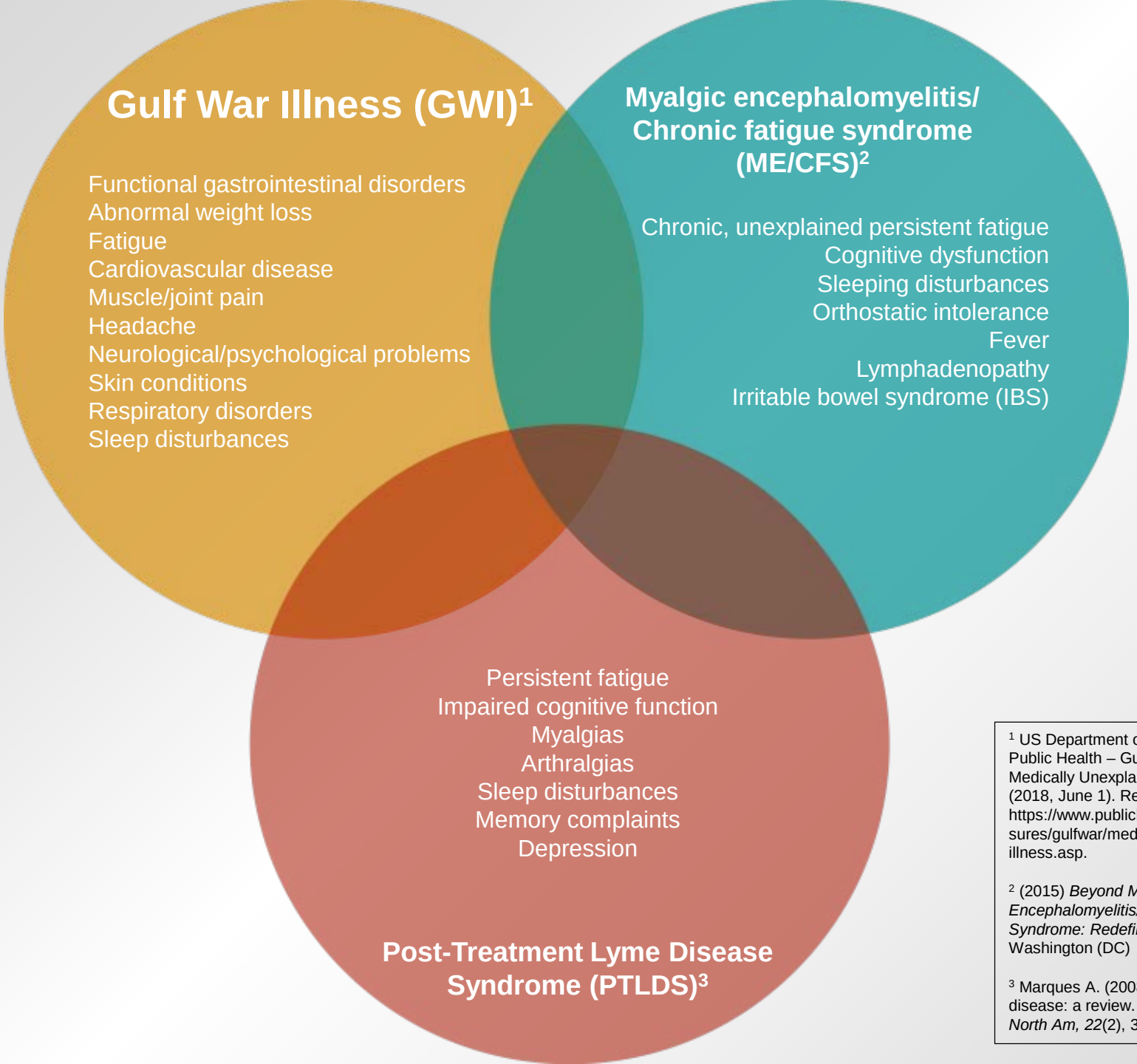

[1] US Department of Veterans Affairs: Public Health – Gulf War Veterans' Medically Unexplained Illnesses. (2018, June 1). Retrieved from https://www.publichealth.va.gov/exposures/gulfwar/medically-unexplained-illness.asp.

[2] (2015) *Beyond Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: Redefining an Illness.* Washington (DC)

[3] Marques A. (2008). Chronic Lyme disease: a review. *Infect Dis Clin North Am, 22*(2), 341-360, vii-viii.