Biomarker and Pathogen Discovery in Chronic Illness

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EPIDEMIOLOGY CIRCA 1854
Isolation and characterization of Borna disease agent cDNA clones

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

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Proc. Natl. Acad. Sci. USA
Neurobiology

Three Years
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)
- Rhabdovirus (2 species)
- Rhinovirus A, C
- Polyomaviruses

**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*

**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)
PROBE LIBRARY

Biotinylated oligonucleotides synthesized on slide

 Released from slide to generate soluble probe pool

TARGET ENRICHMENT

Pre-capture amplification
End-repair, linker barcode ligation, size selection
Fragmented DNA/cDNA

Sample

Hybridization

Streptavidin capture and pulldown

Fold Increase

Lung

Blood

10,000
1,000
100
10
1

FLUAV
EV-D68
MERS-CoV
DENV
EBOV
WNV
CVV
HHV-1

Sequencing

Post-capture amplification
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

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

*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

**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 in the 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

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)
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.
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*

*B. miyamotoi*
GilQ, 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

Tokarz, et al. Scientific Reports, 2018
TBD-CHIP Detects Previously Unknown Babesia Co-Infections

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>Standard assays</th>
<th>TBD-Chip antigen signal intensity</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ELISA</td>
<td>Western Blot IgM</td>
</tr>
<tr>
<td>Neg Ctrl 1</td>
<td>NEG</td>
<td>NEG</td>
</tr>
<tr>
<td>Neg Ctrl 2</td>
<td>NEG</td>
<td>NEG</td>
</tr>
<tr>
<td>Neg Ctrl 3</td>
<td>NEG</td>
<td>NEG</td>
</tr>
<tr>
<td>EA Lyme 1</td>
<td>POS</td>
<td>POS</td>
</tr>
<tr>
<td>EA Lyme 2</td>
<td>N/A</td>
<td>POS</td>
</tr>
<tr>
<td>EA Lyme 3</td>
<td>NEG</td>
<td>POS</td>
</tr>
<tr>
<td>EA Lyme 4</td>
<td>IND</td>
<td>POS</td>
</tr>
<tr>
<td>EA Lyme 5</td>
<td>N/A</td>
<td>POS</td>
</tr>
<tr>
<td>EA Lyme 6</td>
<td>POS</td>
<td>POS</td>
</tr>
<tr>
<td>EA Lyme 7</td>
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<td>POS</td>
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<tr>
<td>EA Lyme 8</td>
<td>IND</td>
<td>POS</td>
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<tr>
<td>EA Lyme 9</td>
<td>N/A</td>
<td>POS</td>
</tr>
<tr>
<td>EA Lyme 10</td>
<td>POS</td>
<td>POS</td>
</tr>
</tbody>
</table>

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
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

<table>
<thead>
<tr>
<th></th>
<th><strong>P. bivia OR</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>High cytokine</td>
<td>19.2 (95% CI: 4.0-92.4) p&lt;0.001</td>
</tr>
<tr>
<td>HIV positive</td>
<td>12.7 (95% CI: 2.1-77.8) p=0.006</td>
</tr>
</tbody>
</table>

**Potential mechanism**

\[ P. bivia = \text{Lipopolysaccharide} \]
Center for Solutions for ME/CFS

- Anthony Komaroff
  Harvard University
- Daniel Peterson
  Sierra Internal Medicine
- John Greally
  Albert Einstein
- Susan Levine
  Private Practice
- Oliver Fiehn
  UC Davis
- Jose Montoya
  Stanford University
- Lucinda Bateman
  Bateman Horne Center
- W. Ian Lipkin
  Dana March
  Paul Newswanger
  Columbia University
CfS for ME/CFS Organizational Structure
Absence of evidence of Borna disease virus infection in Swedish patients with Chronic Fatigue Syndrome

Birgitté Övergård, Thomas Iriess, Gudrun Lindh, Shaun Lee and W Ian Lipkin

Department of Immunology, Microbiology, Pathology and Infectious Diseases, Clinic for Infectious Diseases 173, Karolinska Institutet at Huddinge University Hospital, S-141 86 Huddinge, Sweden; Laboratory for the Study of Emerging Diseases, 3101 Gillette Neuroscience Facility, University of California, Irvine, California, CA 92697-4292, USA

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; Strauss 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


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

Dorothy Nagy-Szokal, Brent Williams, Nischay Mohai, Xiaoyu Che, Bohyun Lee, Lucinda Bateman, Nancy G. Klimas, Anthony L. Komaroff, Susan Levine, Jose G. Montoya, Daniel L. Peterson, Devi Ramanan, Komal Jain, Meredith L. Eddy, Mady Hornig, and W. Ian Lipkin

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

Mady Hornig, José G. Montoya, Nancy G. Klimas, Susan Levine, Donna Felsenstein, Lucinda Bateman, Daniel L. Peterson, C. Gunnar Gottschalk, Andrew F. Schultz, Xiaoyu Che, Meredith L. Eddy, Anthony L. Komaroff, W. Ian Lipkin
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.
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 subtypes 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.
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)

Plasma proteomics
- CFI Extension: 50 cases/50 controls; 4 sites
- Analysis in progress
Fecal Metagenomic Profiles in ME/CFS

Linear discriminant effect size (LEfSe)

Nagy-Szakal, et al. Microbiome 2017
Fecal Bacterial Metabolic Pathways in ME/CFS with and without IBS

**ME/CFS v. Control**
- Vitamin B6 biosynthesis and salvage
- Pyrimidine ribonucleotides degradation
- Atrazine degradation*
- Polyamine biosynthesis
- Arginine and polyamine biosynthesis
- Unsaturated FA biosynthesis
- Mycotoxin biosynthesis

**ME/CFS + IBS v. Control**
- Furano and flavone degradation
- L-tryptophan biosynthesis
- L-tryptophan degradation
- L-tryptophan biosynthesis
- L-tryptophan degradation
- L-arginine biosynthesis
- L-arginine degradation
- Unsaturated FA biosynthesis

**ME/CFS w/o IBS v. Control**
- Vitamin B6 biosynthesis and salvage
- Pyrimidine ribonucleotides degradation
- Atrazine degradation
- Glycol degradation to 1,3-propanediol
- Sulfobaculate degradation
- Unsaturated FA biosynthesis

Legend:
- CONTROL (n=50)
- ME/CFS (n=50)
AYASDI Topological Analysis of ME/CFS

Metric: normalized correlation
Lenses: IBS status and BMI
AYASDI Topological Analysis of ME/CFS

**ME/CFS+IBS and normal BMI**
- **↓** Coprococcus species
- **↓** *Collinsella aerofaciens*
  - General health
  - Physical function

**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

**ME/CFS only and high BMI**
- **↑** *Clostridium leptum*
- **↑** IL-4, IL-5, IL-15
- **↑** Propanediol biosynthesis
- **↑** Fatty acid β-oxidation
- **↓** L-glutamine and L-histidine biosynthesis
  - Physical and social function
  - General, mental, and physical fatigue
AYASDI Topological Analysis of ME/CFS

**Group 1**
- ↓ Parabacteroides genus
- ↓ Bifidobacterium genus
- ↑ D-galacturonate degradation
- ↓ L-arginine biosynthesis

Pain
Reduced activity and energy

**Group 2**
- ↑ Eubacterium siraeum
- ↓ IL-27, BDNF, IFN-γ
- ↓ Fatty acid biosynthesis
- ↑ Mannan (CH) degradation

**Group 3**
- ↓ Roseburia genus
- ↓ VEGF-A
- ↓ IL-1RA, IL-12p40, IL-12p70, IL-15, IL-22, β-NGF, LIF
- ↑ Peptidoglycan biosynthesis
- ↑ Pyrimidine degradation
- ↑ Allantoin 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 unbranched acylcamitines, TMAO, choline, and amino acids

Oxilipins (46)
  Bioactive oxilipins, steroids, and bile acids
Top 10 Metabolites that Distinguish ME/CFS from Controls

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

**LPC**: lysophosphatidylcholine  
**PC**: phosphatidylcholine  
**SM**: sphingomyelin  
**TG**: triglyceride
Top 10 Metabolites that Distinguish ME/CFS IBS Subgroups from Controls

### ME/CFS+IBS vs. Control

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Direction in ME/CFS+IBS</th>
<th>Chemical Pathway</th>
<th>Mann-Whitney U-test p-value</th>
<th>Logistic Regression p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPC 18:2</td>
<td>Decreased</td>
<td>PC</td>
<td>0.000</td>
<td>0.305 0.004</td>
</tr>
<tr>
<td>Ceramide d36:1</td>
<td>Increased</td>
<td>ROS, gut permeability</td>
<td>0.002</td>
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</tr>
<tr>
<td>γ-Butyrobetaine</td>
<td>Decreased</td>
<td>mitochondrial TCA cycle</td>
<td>0.000</td>
<td>0.470 0.003</td>
</tr>
<tr>
<td>LPC 18:1</td>
<td>Decreased</td>
<td>PC</td>
<td>0.001</td>
<td>0.396 0.011</td>
</tr>
<tr>
<td>5-Methythioadenosine</td>
<td>Increased</td>
<td>one-carbon / nicotinate</td>
<td>0.001</td>
<td>3.715 0.005</td>
</tr>
<tr>
<td>TG 49:2</td>
<td>Increased</td>
<td>TG</td>
<td>0.006</td>
<td>1.844 0.043</td>
</tr>
<tr>
<td>Ceramide d40:0</td>
<td>Increased</td>
<td>ROS, gut permeability</td>
<td>0.002</td>
<td>2.340 0.024</td>
</tr>
<tr>
<td>TG 51:3</td>
<td>Increased</td>
<td>TG</td>
<td>0.001</td>
<td>2.459 0.020</td>
</tr>
<tr>
<td>Betaine</td>
<td>Decreased</td>
<td>mitochondrial TCA cycle</td>
<td>0.016</td>
<td>0.555 0.028</td>
</tr>
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</tr>
</tbody>
</table>

### ME/CFS w/o IBS vs. Control

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Direction in ME/CFS w/o IBS</th>
<th>Chemical Pathway</th>
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<tr>
<td>PC 33:0</td>
<td>Decreased</td>
<td>PC</td>
<td>0.000</td>
<td>0.386 0.000</td>
</tr>
<tr>
<td>PC 38:2</td>
<td>Decreased</td>
<td>PC</td>
<td>0.000</td>
<td>0.487 0.007</td>
</tr>
<tr>
<td>PC 30:0</td>
<td>Decreased</td>
<td>PC</td>
<td>0.000</td>
<td>0.323 0.001</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Decreased</td>
<td>neurotransmitter</td>
<td>0.008</td>
<td>0.333 0.001</td>
</tr>
<tr>
<td>PC 36:6</td>
<td>Decreased</td>
<td>PC</td>
<td>0.028</td>
<td>0.545 0.015</td>
</tr>
<tr>
<td>TG 54:6 A</td>
<td>Increased</td>
<td>TG</td>
<td>0.001</td>
<td>2.267 0.009</td>
</tr>
<tr>
<td>TG 53:5</td>
<td>Increased</td>
<td>TG</td>
<td>0.004</td>
<td>1.736 0.030</td>
</tr>
<tr>
<td>TG 54:8</td>
<td>Increased</td>
<td>TG</td>
<td>0.003</td>
<td>1.674 0.030</td>
</tr>
<tr>
<td>Tyr Met Lys</td>
<td>Increased</td>
<td>neurotransmitter</td>
<td>0.011</td>
<td>2.893 0.015</td>
</tr>
<tr>
<td>PC 32:2</td>
<td>Decreased</td>
<td>PC</td>
<td>0.001</td>
<td>0.483 0.006</td>
</tr>
</tbody>
</table>

**LPC**: lysophosphatidylcholine  **PC**: phosphatidylcholine  **TG**: triglyceride
Ceramides Linked to ROS, Gut Permeability

### ME/CFS+IBS vs. Control

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**LPC**: lysophosphatidylcholine  
**PC**: phosphatidylcholine  
**TG**: triglyceride
Ceramide Levels Differ in ME/CFS with and without IBS

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<tr>
<th>Compound name</th>
<th>Direction</th>
<th>Mann-Whitney U-test p-value</th>
<th>Logistic Regression</th>
<th>Random Forest</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>U-test p-value</td>
<td>Odds ratio</td>
<td>p-value</td>
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<td>Increased</td>
<td>0.016</td>
<td>2.014</td>
<td>0.028</td>
</tr>
<tr>
<td>Ceramide d34:1</td>
<td>Increased</td>
<td>0.005</td>
<td>2.257</td>
<td>0.025</td>
</tr>
<tr>
<td>Ceramide d38:1</td>
<td>Increased</td>
<td>0.004</td>
<td>2.961</td>
<td>0.005</td>
</tr>
<tr>
<td>Ceramide d40:1</td>
<td>Increased</td>
<td>0.048</td>
<td>2.490</td>
<td>0.018</td>
</tr>
</tbody>
</table>

| 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 ↓ [Bacteroidetes] Parabacteroides distasonis
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

Lee, et al. Submitted 2018
Gulf War Illness (GWI)¹
- Functional gastrointestinal disorders
- Abnormal weight loss
- Fatigue
- Cardiovascular disease
- Muscle/joint pain
- Headache
- Neurological/psychological problems
- Skin conditions
- Respiratory disorders
- Sleep disturbances

Myalgic encephalomyelitis/Chronic fatigue syndrome (ME/CFS)²
- Chronic, unexplained persistent fatigue
- Cognitive dysfunction
- Sleeping disturbances
- Orthostatic intolerance
- Fever
- Lymphadenopathy
- Irritable bowel syndrome (IBS)

Post-Treatment Lyme Disease Syndrome (PTLDS)³
- Persistent fatigue
- Impaired cognitive function
- Myalgias
- Arthralgias
- Sleep disturbances
- Memory complaints
- Depression

