UCSF
University of California
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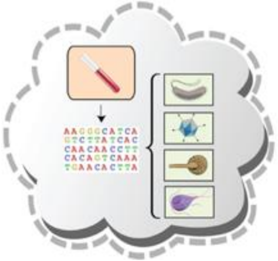

Clinical Metagenomic and Host Response RNA Sequencing for Diagnosis of Infection-Associated Chronic Illnesses

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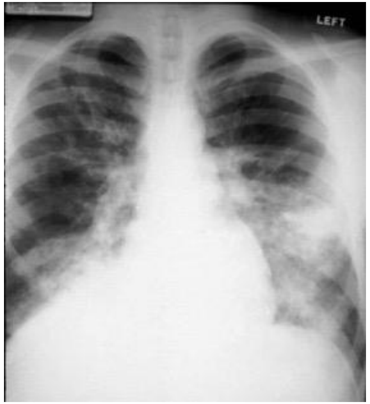
Chan-Zuckerberg Biohub Investigator
University of California, San Francisco

Disclosures

- SURPI+ software, “Pathogen Detection using Next Generation Sequencing” (PCT/US/16/52912), filed by University of California, San Francisco

- Scientific Advisory Board for Mammoth Biosciences, BiomeSense, Poppy Health, and Delve Bio and Co-Founder of Delve Bio

Major Diagnostic Challenges in Acute Infectious Diseases



Meningitis / Encephalitis

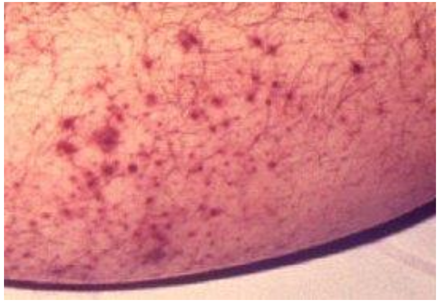
40 – 60% unknown cause

- Glaser, et al., (2006) *CID* 43:1565-1577
- Vora, et al., (2010) *Neurology* 82:443-451

Pneumonia

15 – 62% unknown cause

- Van Gageldonk-Lafeber, (2005) *CID* 41:490-497
- Louie, et al., (2005) *CID* 41:822-828
- Ewig, et al. (2002) *Eur Respir J* 20:1254-1262
- Jain, et al., (2015) *NEJM* 373(5)



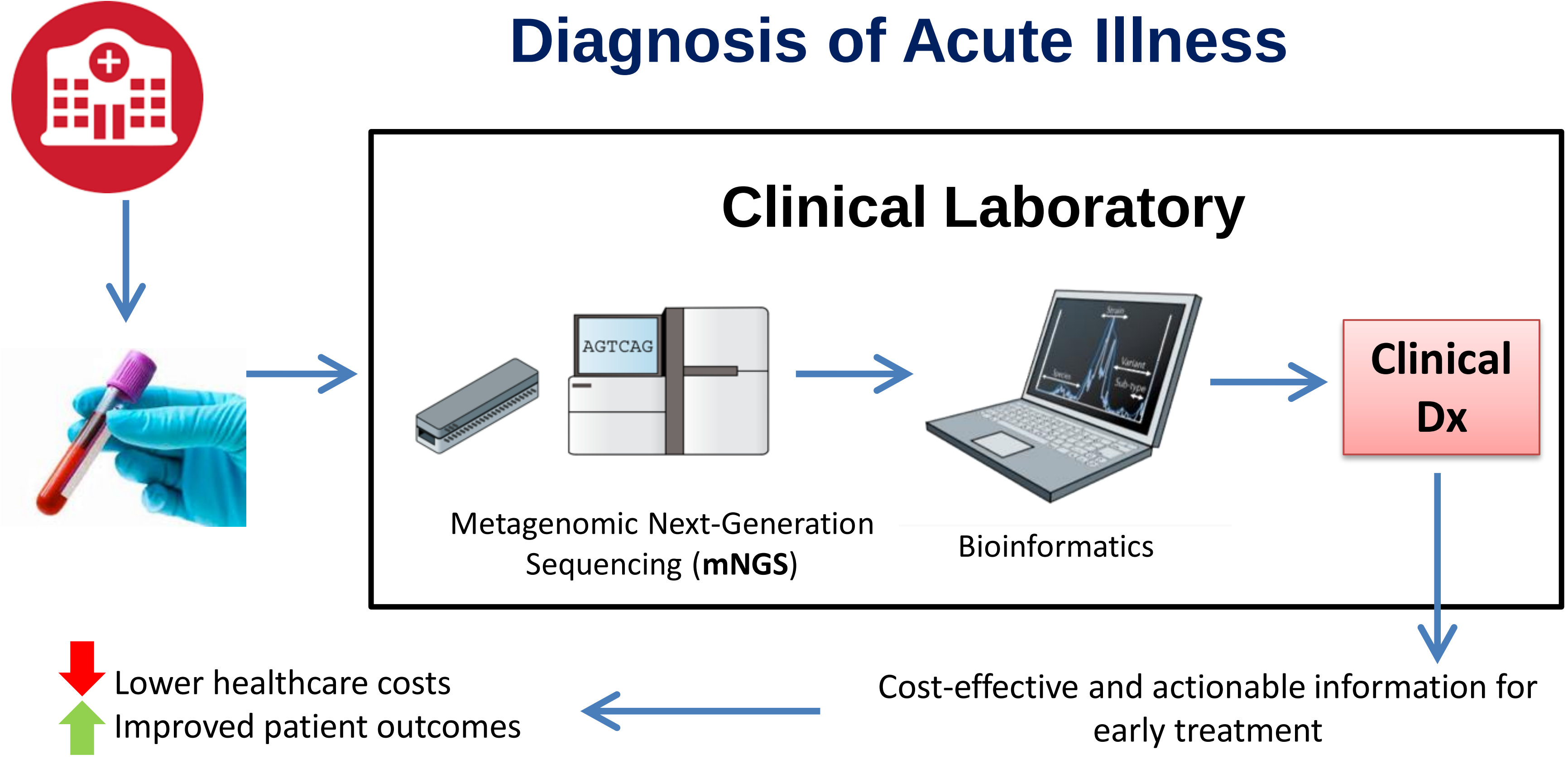
Fever / Sepsis

~20% unknown cause

- Eber, et al. (2010) *Arch Intern Med* 170:347-353
- Vincent, et al. (2015) *Critical Care Med* 43(11).

Failure to obtain a timely diagnosis leads to delayed / inappropriate therapy, increased mortality, and excess healthcare costs

Clinical Metagenomic Sequencing for Diagnosis of Acute Illness



Clinical Laboratory

Metagenomic Next-Generation Sequencing (**mNGS**)

Bioinformatics

Clinical Dx

Cost-effective and actionable information for early treatment

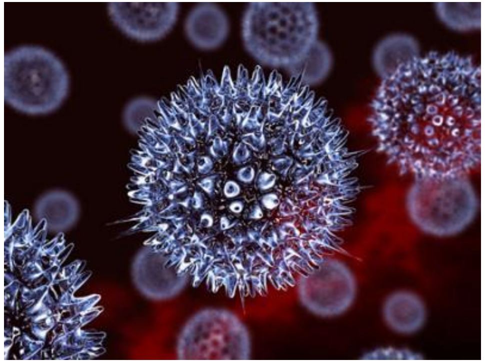
⬇ Lower healthcare costs
⬆ Improved patient outcomes

Turnaround time: hours – days (versus days – weeks)

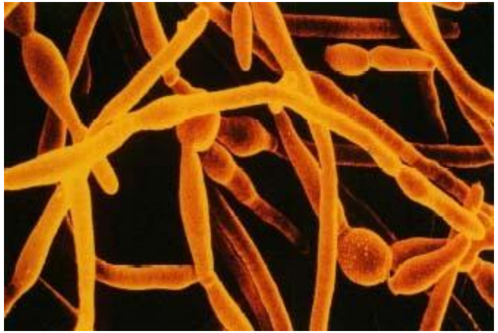
Nearly all Pathogens can be Identified by mNGS

Bacteria

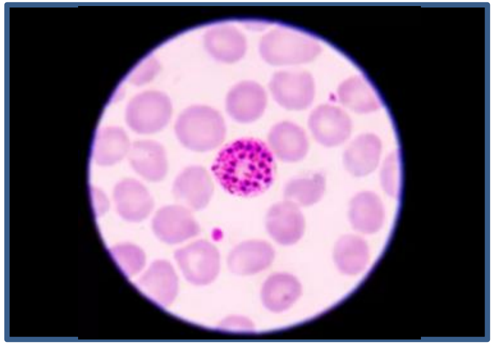
Viruses

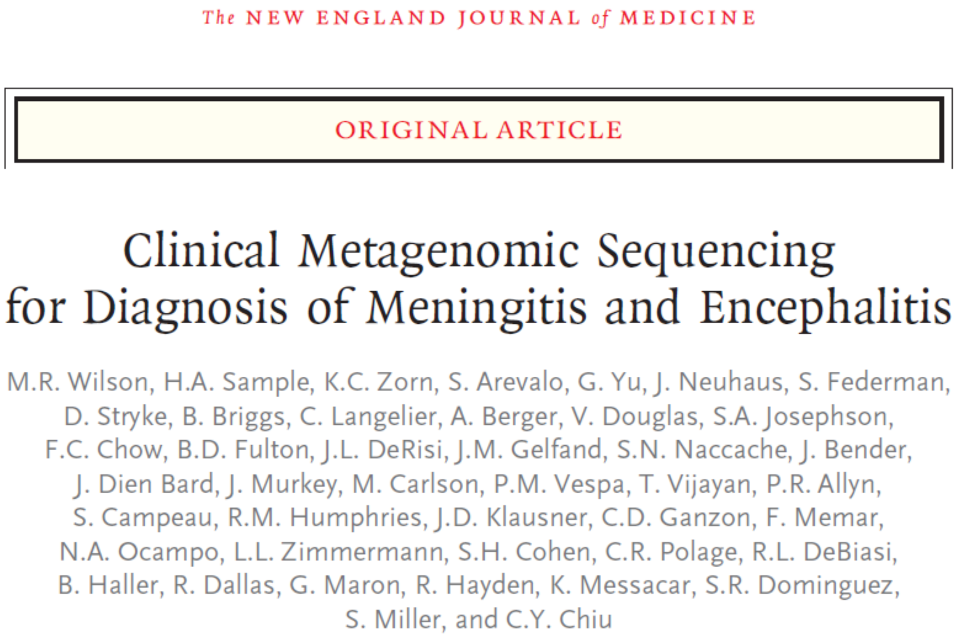


Fungi



Parasites



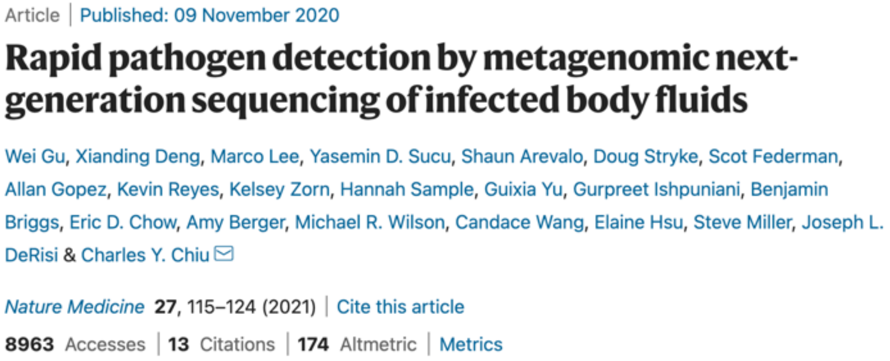


The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis

M.R. Wilson, H.A. Sample, K.C. Zorn, S. Arevalo, G. Yu, J. Neuhaus, S. Federman, D. Stryke, B. Briggs, C. Langelier, A. Berger, V. Douglas, S.A. Josephson, F.C. Chow, B.D. Fulton, J.L. DeRisi, J.M. Gelfand, S.N. Naccache, J. Bender, J. Dien Bard, J. Murkey, M. Carlson, P.M. Vespa, T. Vijayan, P.R. Allyn, S. Campeau, R.M. Humphries, J.D. Klausner, C.D. Ganzon, F. Memar, N.A. Ocampo, L.L. Zimmermann, S.H. Cohen, C.R. Polage, R.L. DeBiasi, B. Haller, R. Dallas, G. Maron, R. Hayden, K. Messacar, S.R. Dominguez, S. Miller, and C.Y. Chiu



Article | Published: 09 November 2020

Rapid pathogen detection by metagenomic next-generation sequencing of infected body fluids

Wei Gu, Xianding Deng, Marco Lee, Yasemin D. Sucu, Shaun Arevalo, Doug Stryke, Scot Federman, Allan Gopez, Kevin Reyes, Kelsey Zorn, Hannah Sample, Guixia Yu, Gurpreet Ishpuniani, Benjamin Briggs, Eric D. Chow, Amy Berger, Michael R. Wilson, Candace Wang, Elaine Hsu, Steve Miller, Joseph L. DeRisi & Charles Y. Chiu ✉

Nature Medicine **27**, 115–124 (2021) | Cite this article

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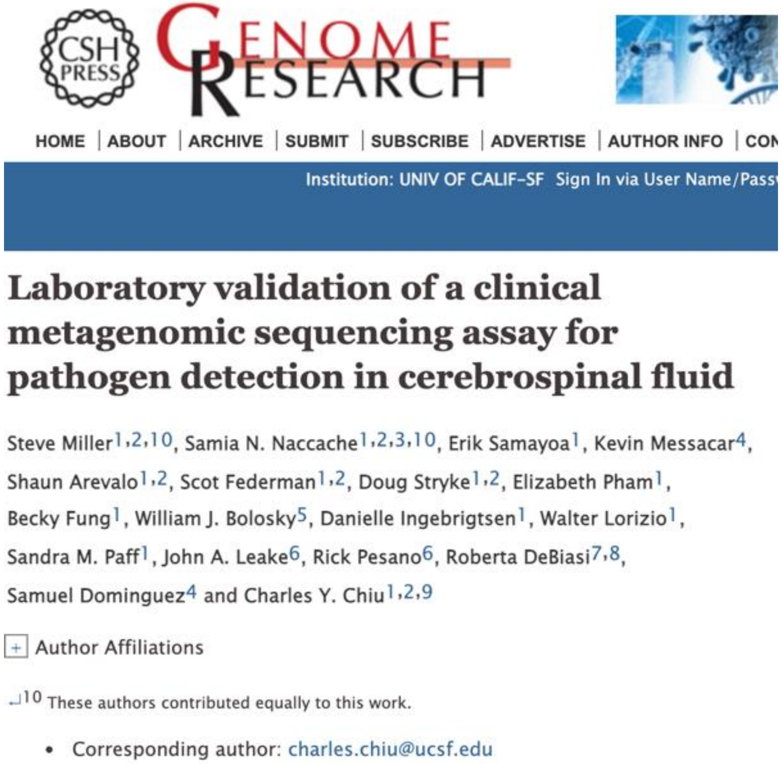


Laboratory validation of a clinical metagenomic sequencing assay for pathogen detection in cerebrospinal fluid

Steve Miller[1,2,10], Samia N. Naccache[1,2,3,10], Erik Samayoa[1], Kevin Messacar[4], Shaun Arevalo[1,2], Scot Federman[1,2], Doug Stryke[1,2], Elizabeth Pham[1], Becky Fung[1], William J. Bolosky[5], Danielle Ingebrigtsen[1], Walter Lorizio[1], Sandra M. Paff[1], John A. Leake[6], Rick Pesano[6], Roberta DeBiasi[7,8], Samuel Dominguez[4] and Charles Y. Chiu[1,2,9]

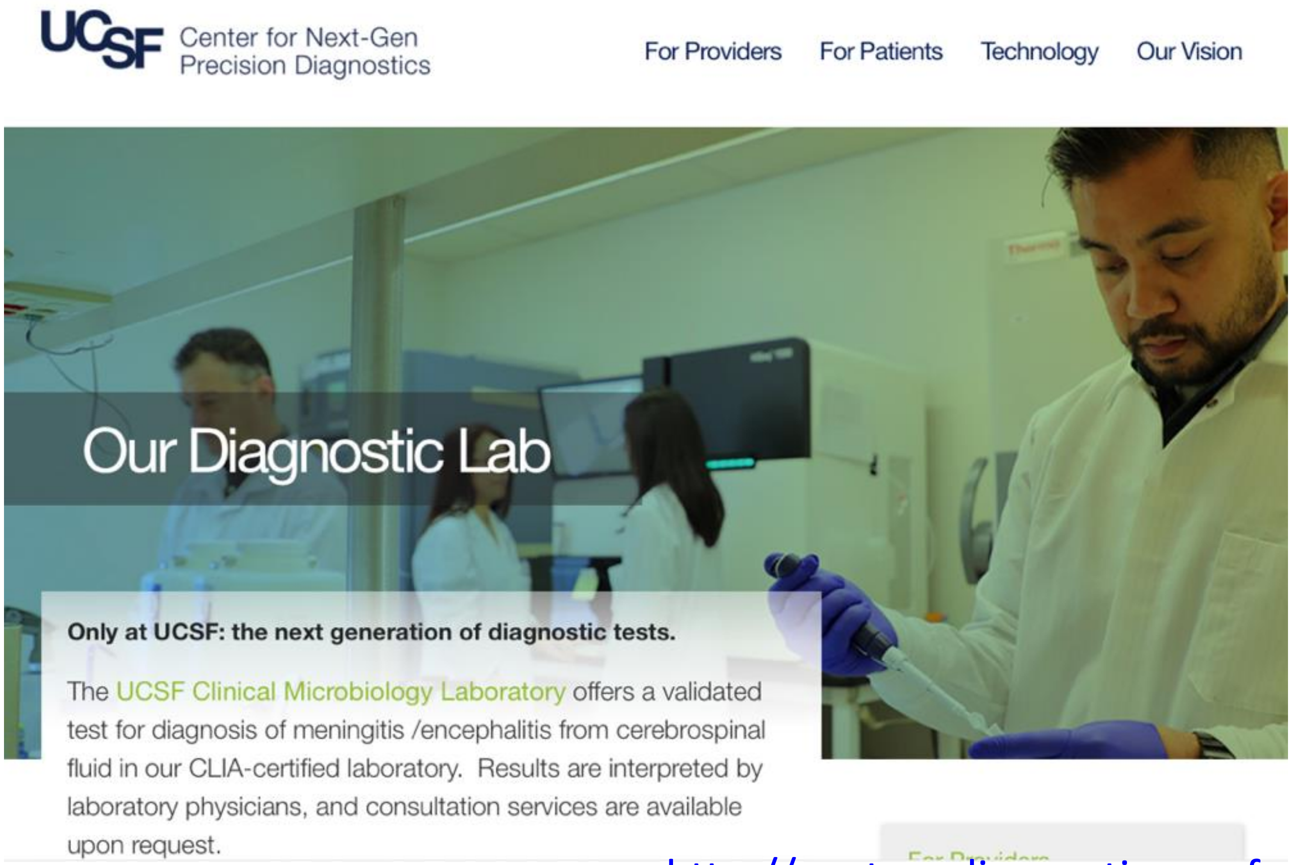
⊞ Author Affiliations

[10] These authors contributed equally to this work.

- Corresponding author: charles.chiu@ucsf.edu

CLIA-Validated Clinical mNGS Assays at UCSF

- CSF
- Plasma
- Body Fluids



Our Diagnostic Lab

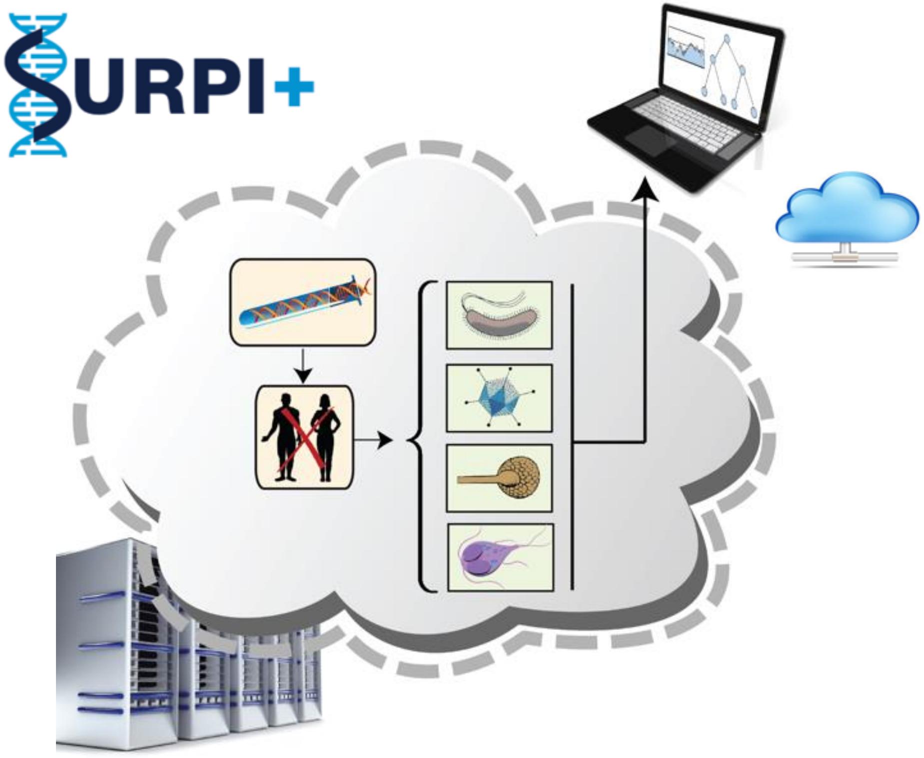
Only at UCSF: the next generation of diagnostic tests.

The UCSF Clinical Microbiology Laboratory offers a validated test for diagnosis of meningitis /encephalitis from cerebrospinal fluid in our CLIA-certified laboratory. Results are interpreted by laboratory physicians, and consultation services are available upon request.

http://nextgendiagnostics.ucsf.edu

The SURPI Bioinformatics Pipeline

“Sequence-based ultra-rapid pathogen identification” (minutes – hours)



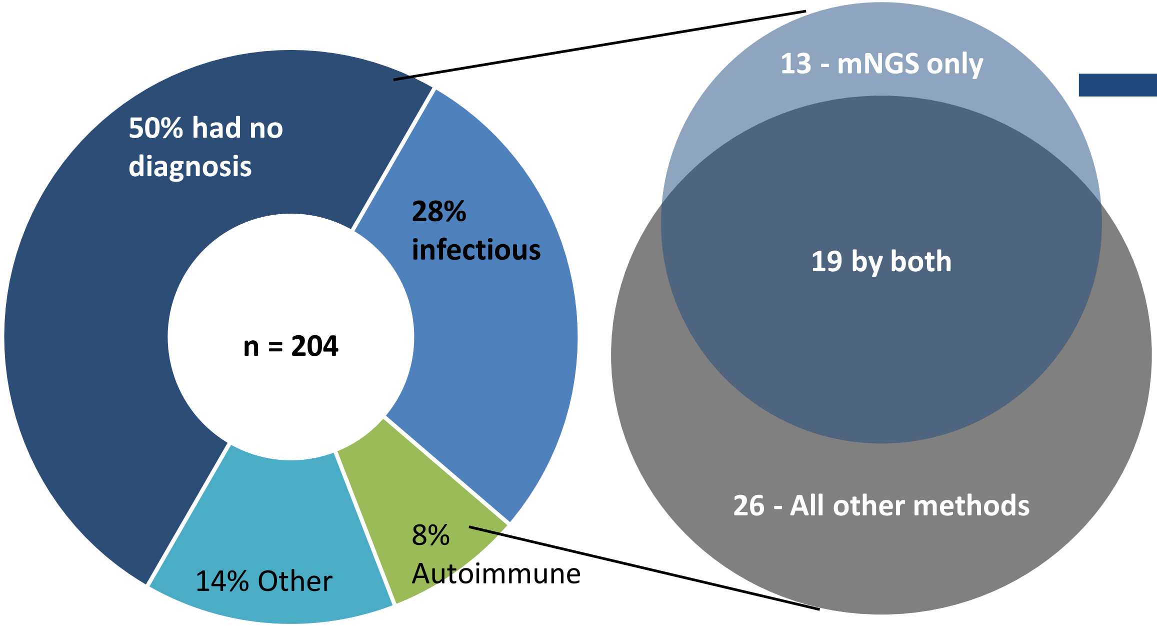
- ***Directly addresses computational analysis bottleneck***
- ***SURPI+ (clinical version) – automated analysis***

Naccache, *et al.*, 2014, *Genome Research 24(7):*1180-1192

Clinically Actionable Diagnosis of Neurological Infections by Metagenomic Next-Generation Sequencing (mNGS)

Clinical Trial Summary

Diagnoses (n=58)



Clinical Utility

9 cases had direct clinical impact

- *Neisseria sp.* – changed antibiotics
- *Nocardia farcinica* – changed antibiotics
- *Candida tropicalis* – changed antibiotics, discontinued antifungals
- **Hepatitis E virus – antiviral, stopped liver transplant**
- *Enterobacter aerogenes* – changed antibiotics
- *Enterococcus faecalis* – changed antibiotics
- *Streptococcus mitis* – changed *antibiotics*
- *Streptococcus agalactiae* – changed antibiotics
- *Streptococcus agalactiae* – changed antibiotics

Note: Conventional diagnostics include culture, PCR, serology (antibody), and antigen testing

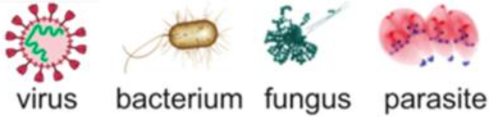
Wilson, et al., 2019, *NEJM*, 380:2327-2324

Roughly ¼ of all diagnoses were made using mNGS alone

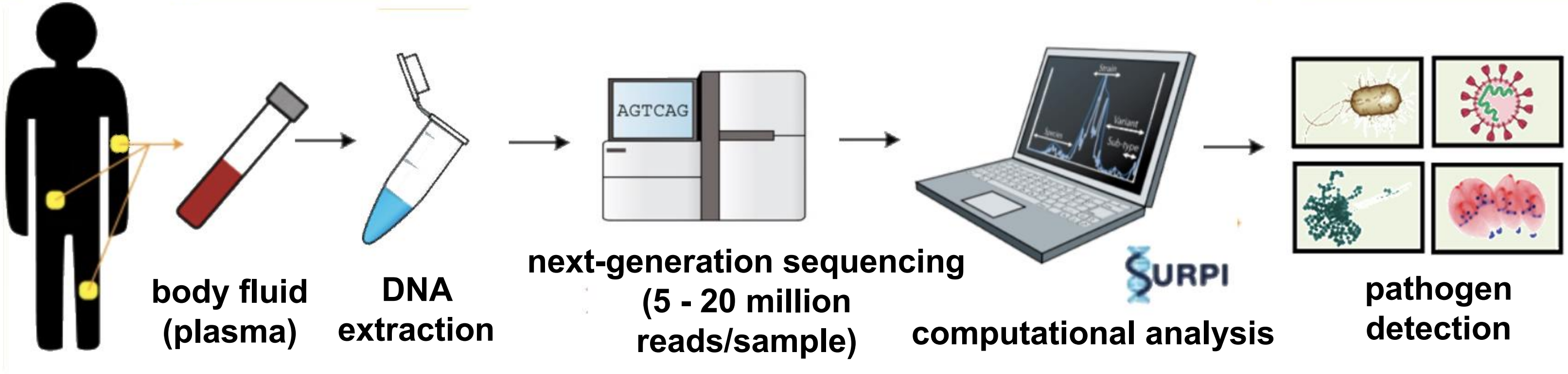
Representative Set of mNGS Assay Positives (2016-2023)

Streptococcus agalactiae (CSF)
Enterobacter aerogenes (CSF)
Human herpesvirus 1-8 (CSF, plasma)
Cryptococcus neoformans (CSF)
enterovirus D68 (CSF)
JC polyomavirus (CSF)
SARS-CoV-2 (respiratory fluid)*
Candida sp. (CSF)
Aspergillus sp. (CSF)
Scedosporium sp. (CSF)
St. Louis encephalitis virus (CSF)
hepatitis E virus (CSF)
Neisseria meningitidis (CSF)
MW polyomavirus (CSF)
Capnocytophaga canimorsus (plasma)
Trypanosoma cruzi (CSF)
Angiostrongylus cantonensis (CSF)
rubella virus[#] (CSF)
yellow fever virus[#] (CSF)
Potosi virus** (CSF)
astroviruses*** (CSF)
Colorado tick fever virus (CSF)
Balamuthia mandrillaris (CSF)
Naegleria fowleri (CSF)
Acanthamoeba sp. (CSF, plasma)
Chlamydia psitacci (CSF)
Brucella meletensis (CSF)
Mycoplasma pneumoniae (plasma)
respiratory viruses (respiratory fluid)[&]
Cache Valley bunyavirus (CSF)
bufavirus (plasma)
Tropheryma whipplei (CSF)
Powassan virus (CSF)
human bocavirus 1 (plasma)
Legionella anisa (plasma)
Candida sp. (CSF and plasma)
Gardnerella vaginalis (plasma)
Scedosporium sp. (CSF and plasma)
Coxiella burnetti (plasma)
Fusarium sp. (CSF and plasma)
parvovirus B19 (plasma)
Leptospira santarosai (CSF)
Mycobacterium sp. (CSF, plasma, respiratory fluid)
Kingella kingae (plasma)
BK polyomavirus (plasma)
Bartonella quintana (plasma)
Borrelia burgdorferi (CSF)

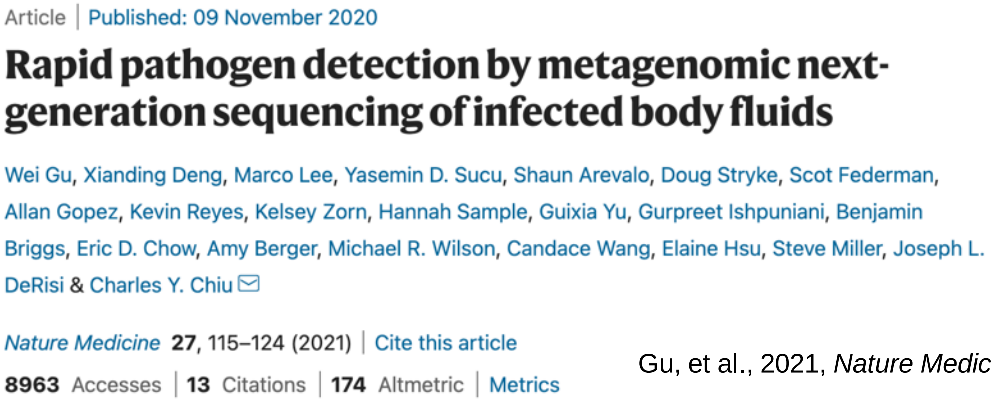
*reads align to bat coronaviruses (using 2019 SURPI+ database)
**arbovirus that has never been described before in human infection in a fatal immunocompromised patient
***neuroinvasive species such as VA-1/HMO-C
[&] RSV, influenza A/B, parainfluenza viruses 1-4, seasonal coronaviruses, adenovirus
[#] vaccine-associated case or outbreak



Metagenomic Sequencing of Body Fluids from Infected Patients



body fluid (plasma) → DNA extraction → next-generation sequencing (5 - 20 million reads/sample) → computational analysis → pathogen detection



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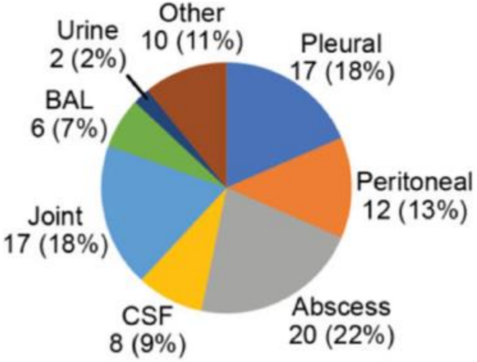
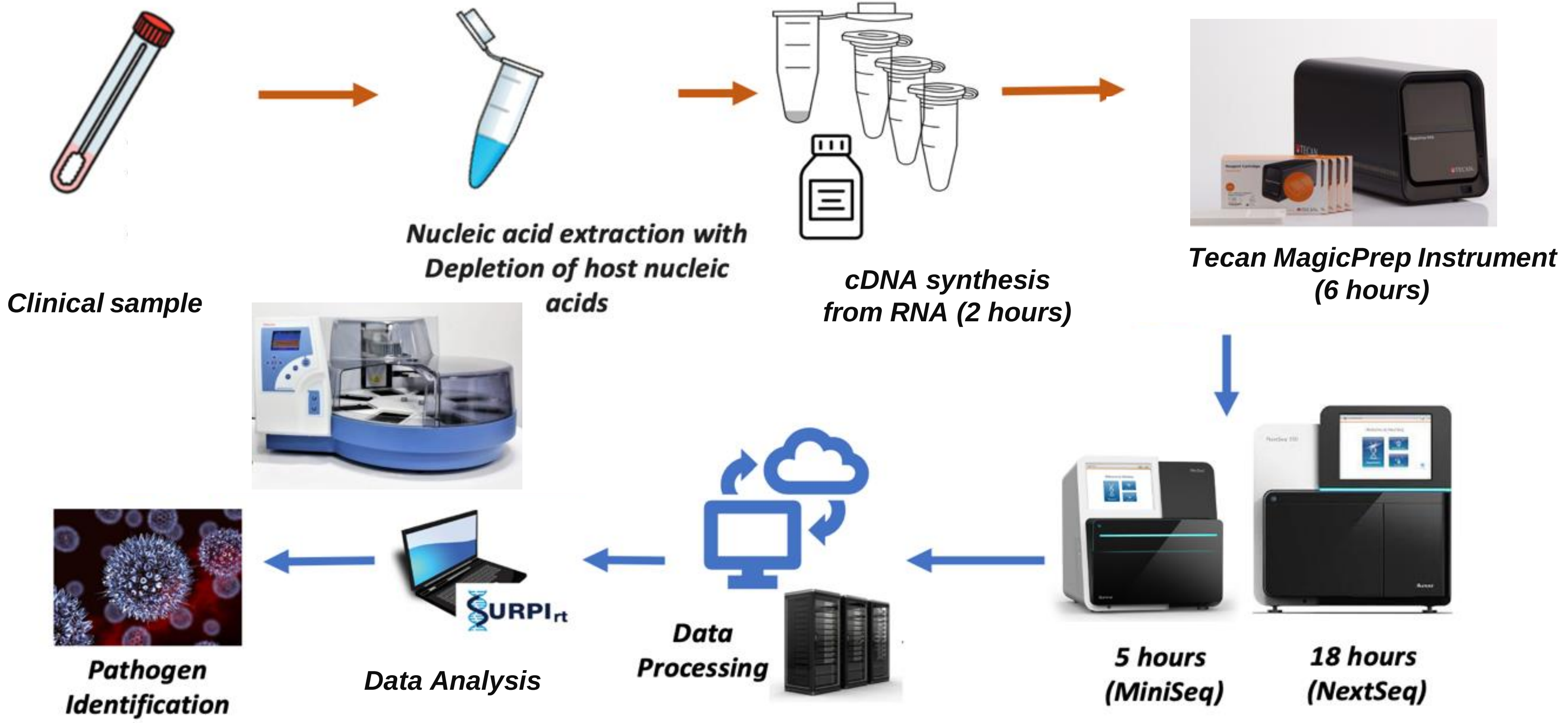


Gu, et al., 2021, *Nature Medicine*, 25:115-124.

Clinical Workflow for mNGS Based Pathogen Detection



Clinical sample

Nucleic acid extraction with Depletion of host nucleic acids

cDNA synthesis from RNA (2 hours)

Tecan MagicPrep Instrument (6 hours)

5 hours (MiniSeq)

18 hours (NextSeq)

Data Processing

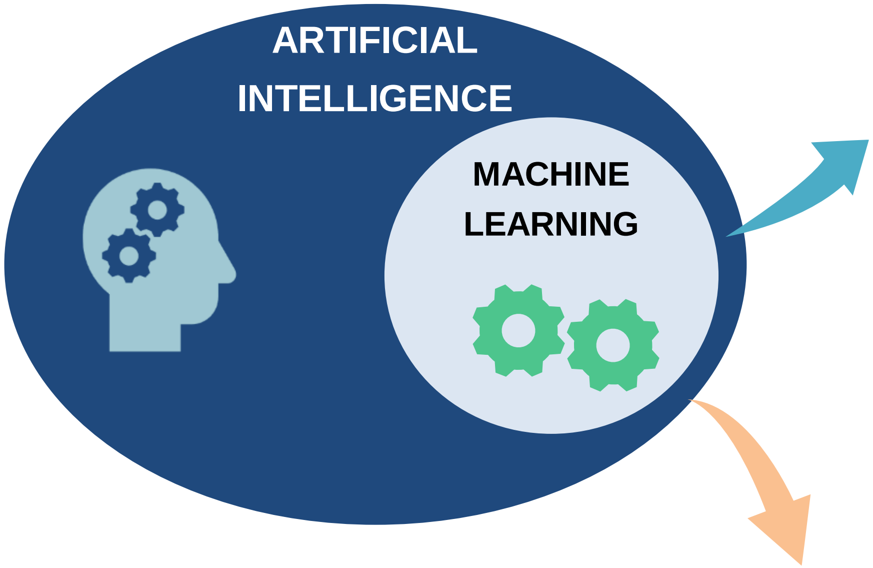
Data Analysis

Pathogen Identification

- *12 – 24 hours turnaround time → are same day results feasible?*
- *Goal for mNGS to be performed routinely by clinical and public health labs*

Host Response Based Diagnostics for Acute and Chronic Illnesses

PRECISION MEDICINE



ARTIFICIAL INTELLIGENCE

MACHINE LEARNING

DIAGNOSTICS

- CANCER
- RARE DISEASE GENETICS
- HOST IMMUNE RESPONSE TO INFECTION

THERAPEUTICS

chronic

LYME DISEASE

- ❖ 476,000 cases/year (CDC Data and Surveillance)
- ❖ difficult to diagnose
- ❖ lack of accurate diagnostic assays early Lyme disease

ME/CFS AND LONG COVID

- ❖ Diagnostic tests not available

acute

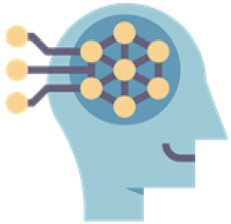
Neurologic, bloodborne, and respiratory infections

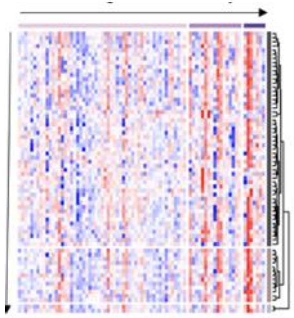
Gene Expression (Transcriptome) Profiling by RNA-Seq

Sample and Clinical Metadata Collection

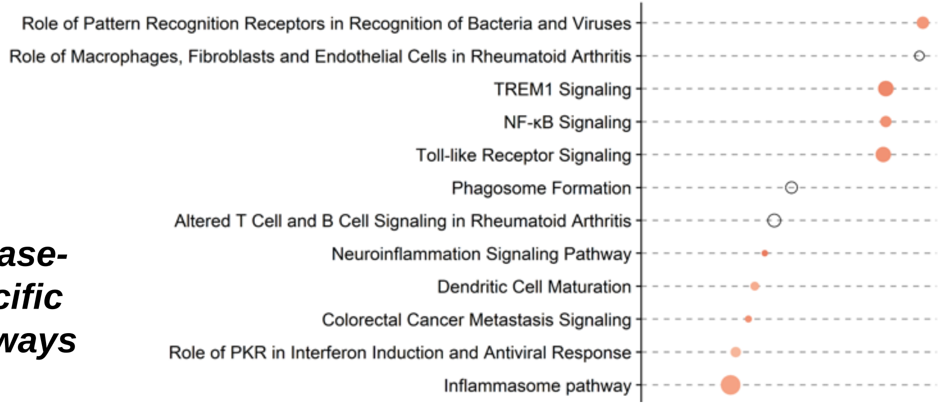
Nucleic Acid Extraction and NGS Library Preparation

DEG Analysis and Machine Learning-Based Modeling

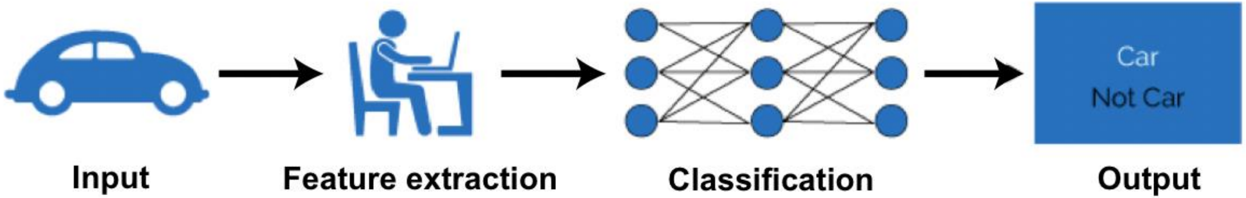




Disease-Specific Pathways



Machine Learning Based Analysis Workflow



Car

Not Car

Input → Feature extraction → Classification → Output

Train

Image features → **Training** → **Honda Accord**
Tesla Model 3
Toyota Camry

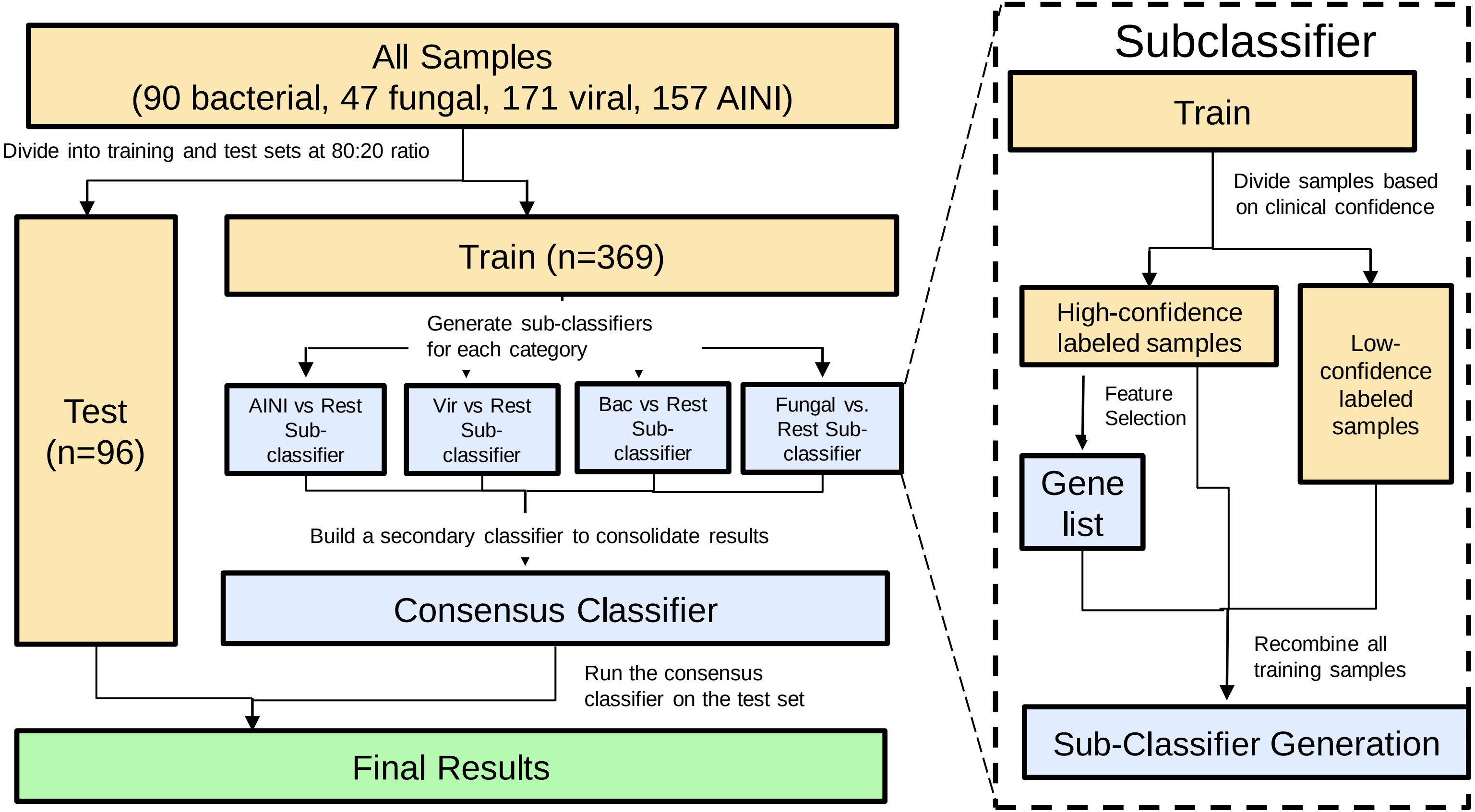
~80% of data

Deploy

Image features → **Learned model** → **Prediction: Tesla (Model S)** ✓

Test classifier model performance with ~20% of data

Design of a CSF Host Response Classifier for Differential Diagnosis of Neurological Illness (Two-Stage Binary Classification Approach)



All Samples
(90 bacterial, 47 fungal, 171 viral, 157 AINI)

Divide into training and test sets at 80:20 ratio

Test
(n=96)

Train (n=369)

Generate sub-classifiers for each category

AINI vs Rest Sub-classifier

Vir vs Rest Sub-classifier

Bac vs Rest Sub-classifier

Fungal vs. Rest Sub-classifier

Build a secondary classifier to consolidate results

Consensus Classifier

Run the consensus classifier on the test set

Final Results

Subclassifier

Train

Divide samples based on clinical confidence

High-confidence labeled samples

Low-confidence labeled samples

Feature Selection

Gene list

Recombine all training samples

Sub-Classifier Generation

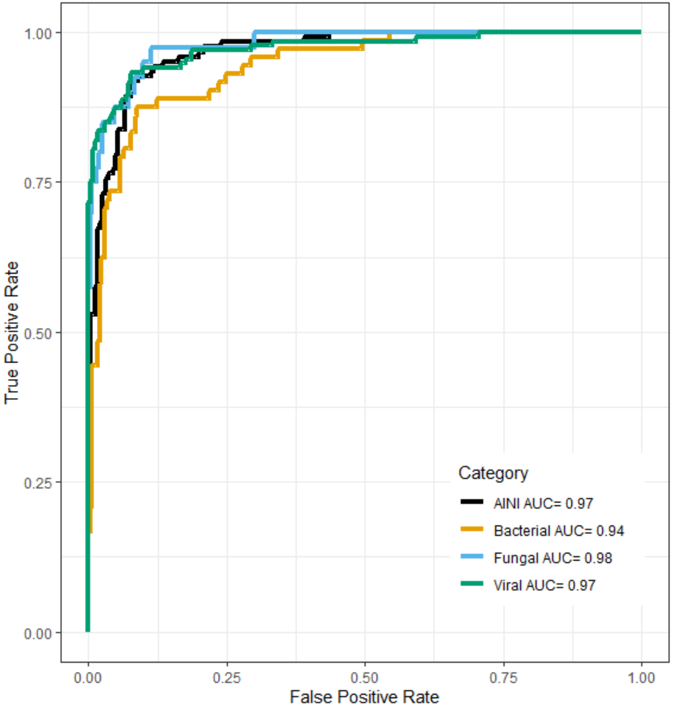
- Independent gene lists
- Independent sub-classifiers
- Better at finding agent-specific markers

Omura and Chiu, et al., 2023, in preparation

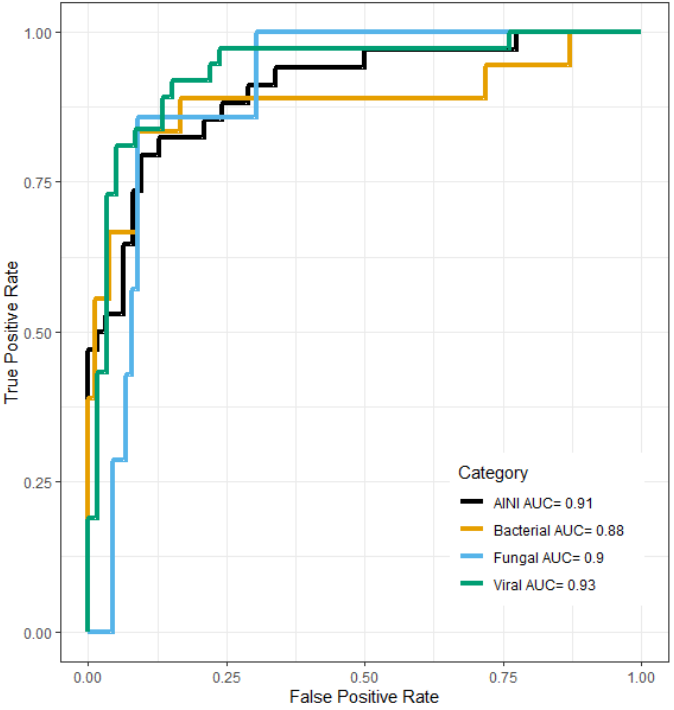
Host Response Classifier Performance

(Two-Stage Binary Classification Approach)

Training Set



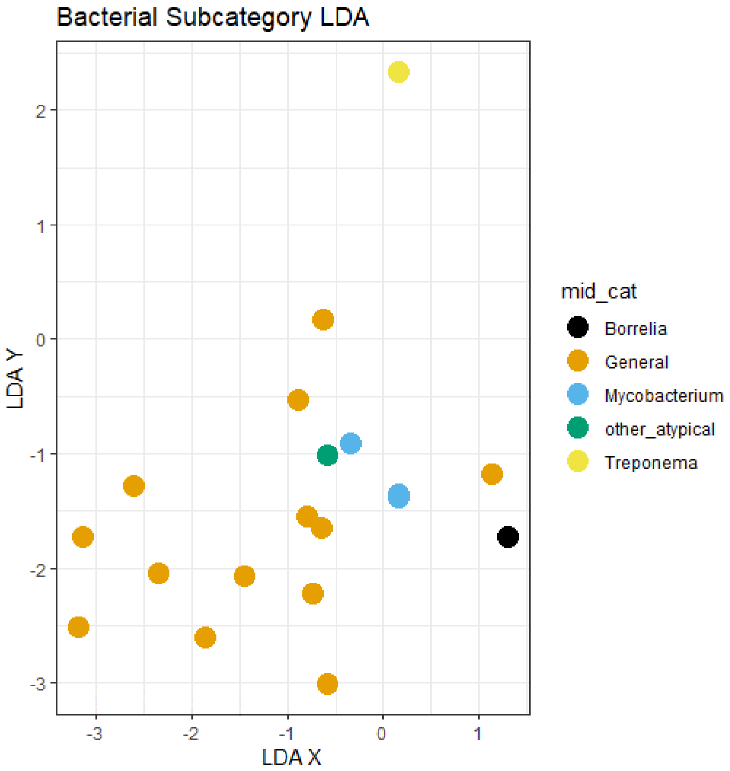
Test Set

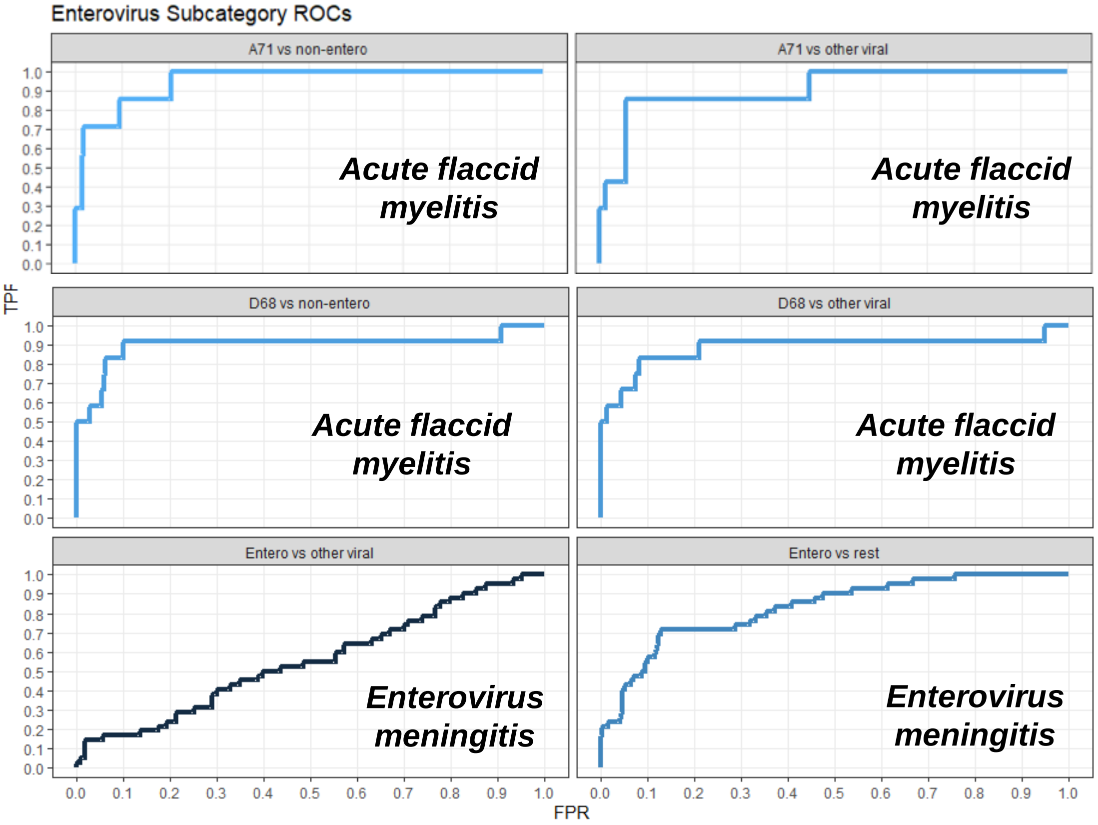


Omura and Chiu, et al., 2023, in preparation

>90% accuracy for all 4 comparisons

Subcategory Classification


Bacterial Subcategory LDA


Enterovirus Subcategory ROCs

A71 vs non-entero

Acute flaccid myelitis

A71 vs other viral

Acute flaccid myelitis

D68 vs non-entero

Acute flaccid myelitis

D68 vs other viral

Acute flaccid myelitis

Entero vs other viral

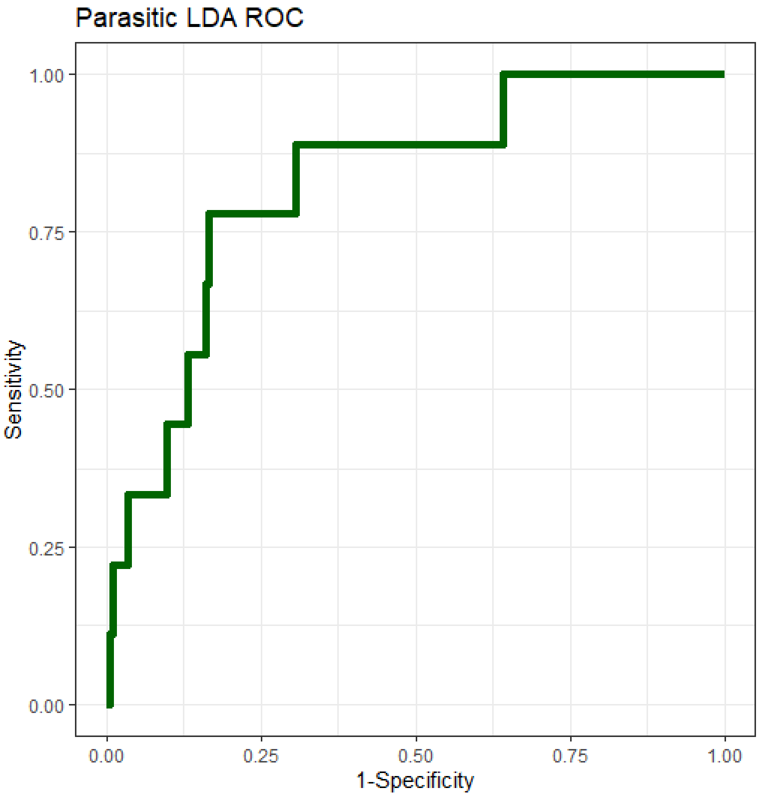
Enterovirus meningitis

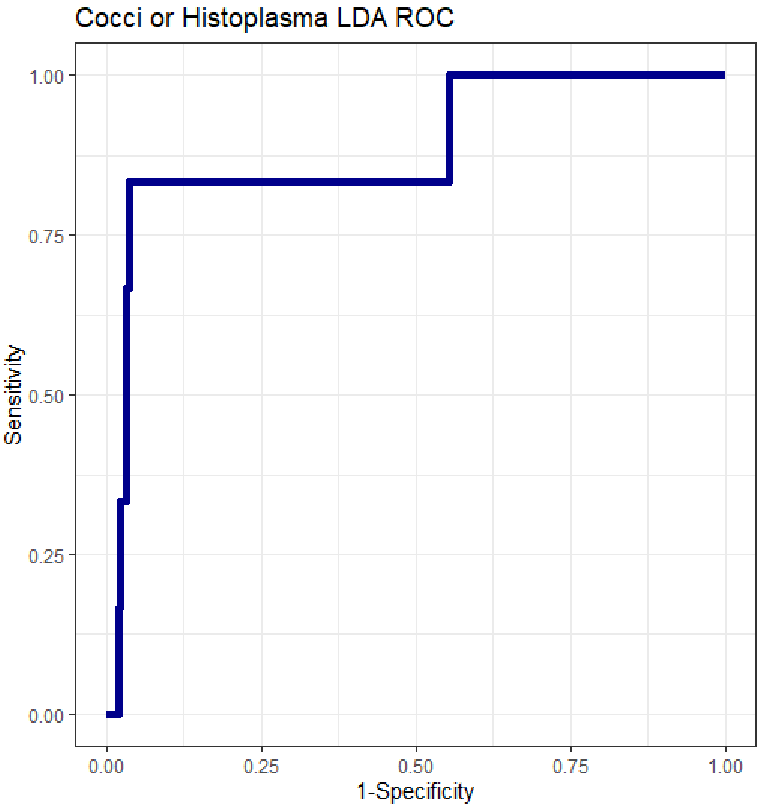
Entero vs rest

Enterovirus meningitis

Omura and Chiu, et al., 2023, in preparation

Subcategory Classification



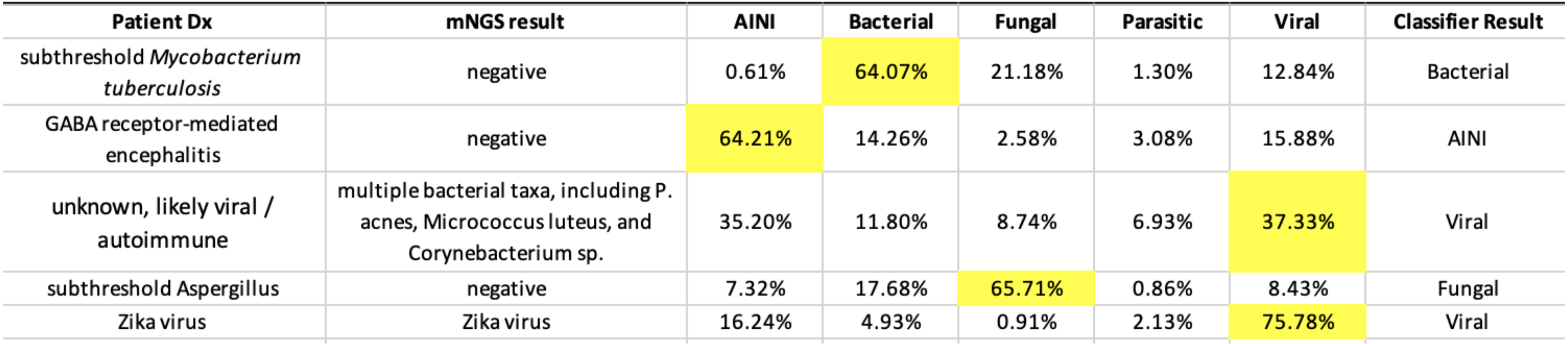


AUC = .83

Omura, Wilson, Chiu, et al., 2023, in preparation

AUC = .88

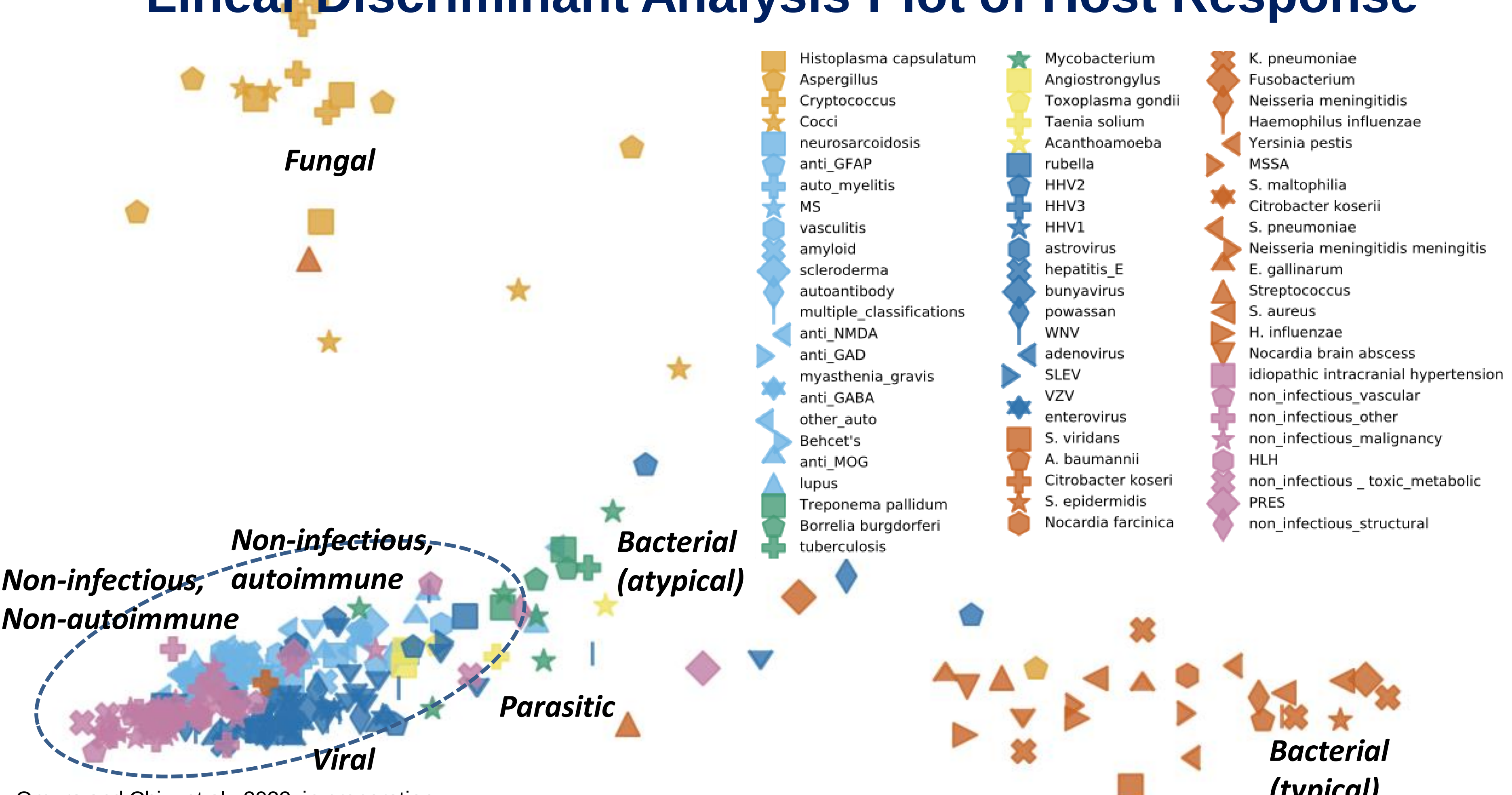
Examples of Host Response Classifier Calls



Patient Dx	mNGS result	AINI	Bacterial	Fungal	Parasitic	Viral	Classifier Result
subthreshold *Mycobacterium tuberculosis*	negative	0.61%	64.07%	21.18%	1.30%	12.84%	Bacterial
GABA receptor-mediated encephalitis	negative	64.21%	14.26%	2.58%	3.08%	15.88%	AINI
unknown, likely viral / autoimmune	multiple bacterial taxa, including P. acnes, Micrococcus luteus, and Corynebacterium sp.	35.20%	11.80%	8.74%	6.93%	37.33%	Viral
subthreshold Aspergillus	negative	7.32%	17.68%	65.71%	0.86%	8.43%	Fungal
Zika virus	Zika virus	16.24%	4.93%	0.91%	2.13%	75.78%	Viral

Omura, Wilson, Chiu, et al., 2023, in preparation

Linear Discriminant Analysis Plot of Host Response



Omura and Chiu, et al., 2022, in preparation

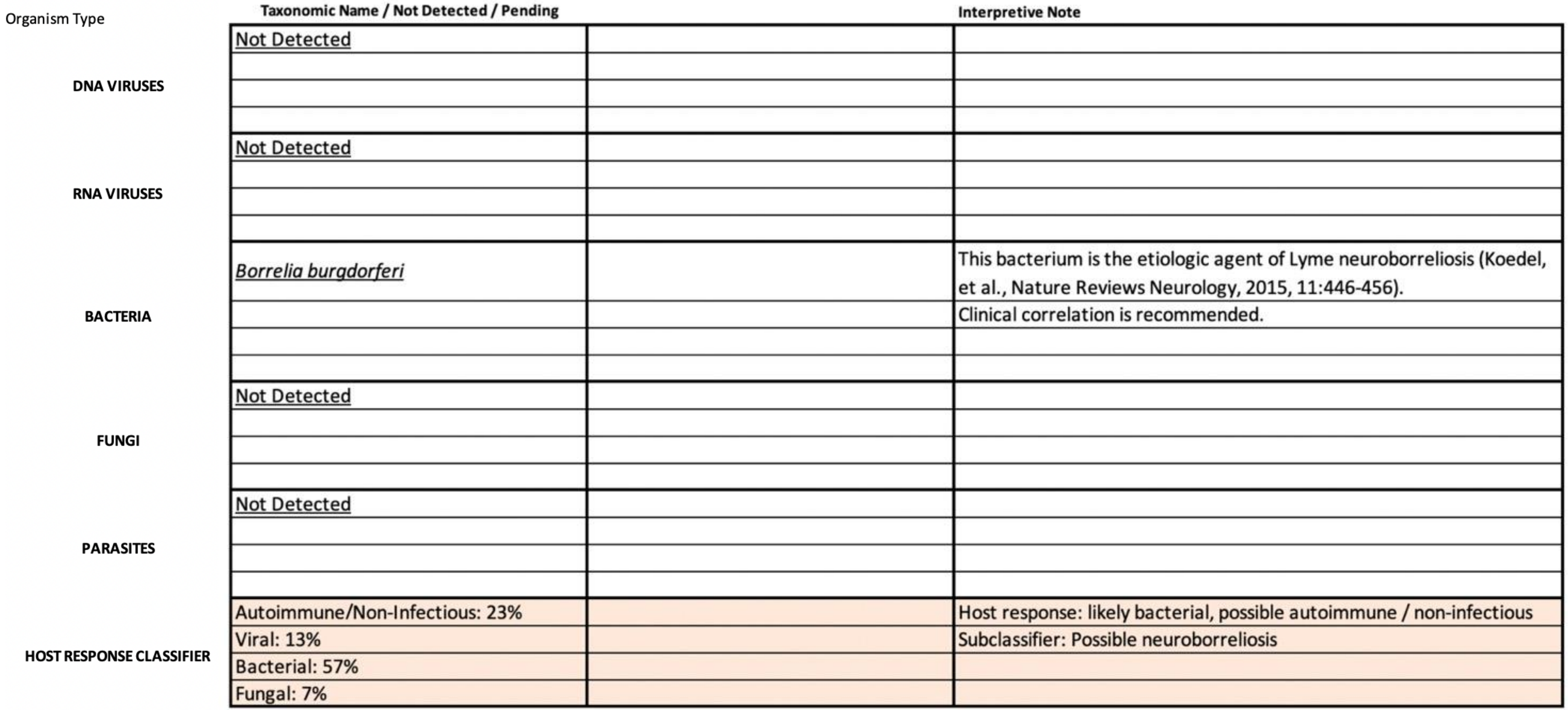
CSF Subclassifier Analysis:
Acute / Chronic Neurologic Syndromes

Subcategory	AUC	Classification Strategy*
amyloid	0.811321	SVM
cancer	0.717778	SVM
lupus	0.754717	SVM
multiple sclerosis	0.896226	LDA
paraneoplastic	0.796296	SVM
structural	0.777778	SVM
vascular	0.886792	LDA
vasculitis	0.839623	SVM

**SVM, support vector machines; LDA, linear discriminant analysis*

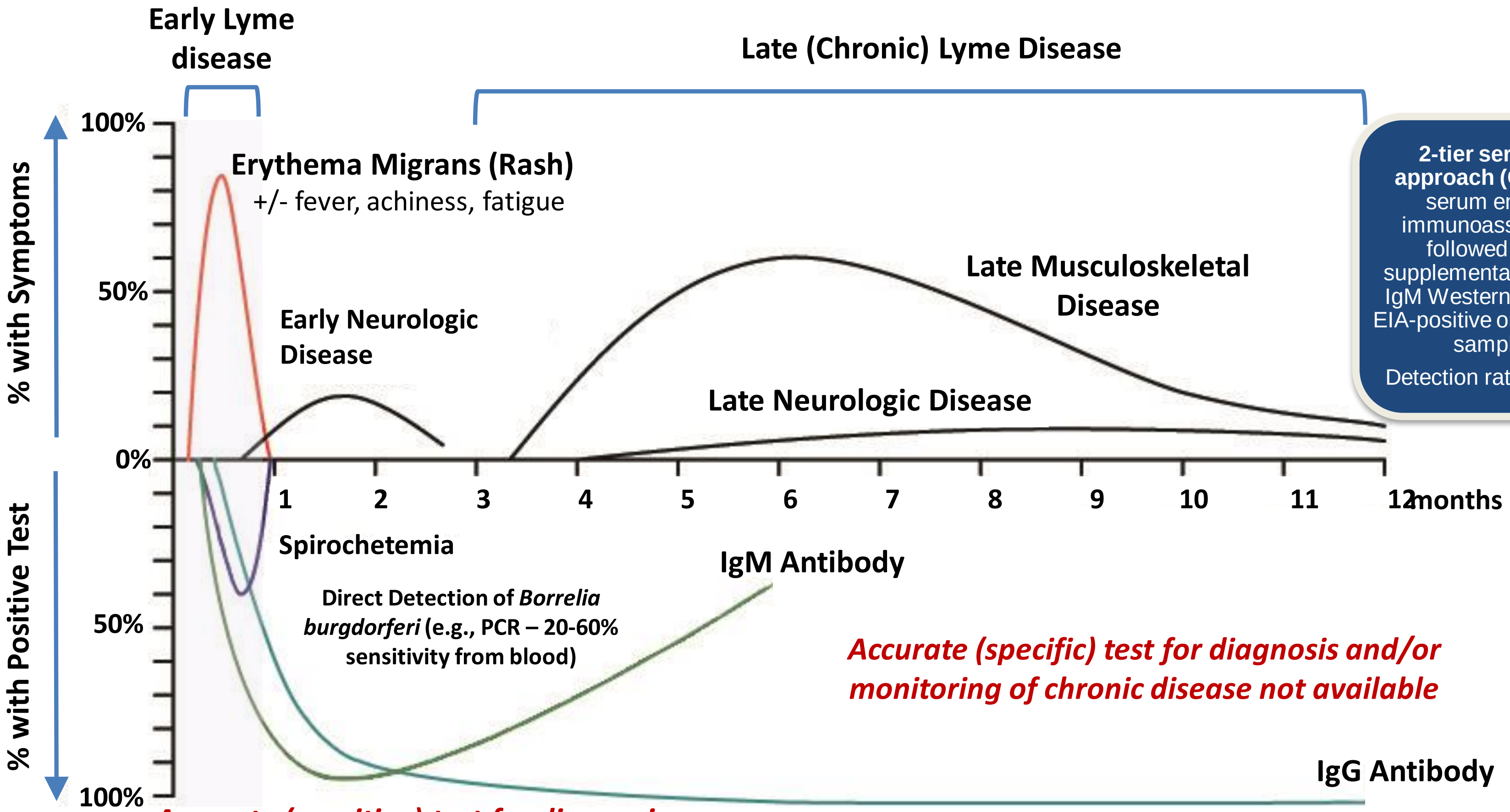
Omura, Wilson, Chiu, et al., 2023, in preparation

CSF mNGS: Clinical Results Report



Organism Type	Taxonomic Name / Not Detected / Pending		Interpretive Note
DNA VIRUSES	Not Detected		
RNA VIRUSES	Not Detected		
BACTERIA	*Borrelia burgdorferi*		This bacterium is the etiologic agent of Lyme neuroborreliosis (Koedel, et al., Nature Reviews Neurology, 2015, 11:446-456).
			Clinical correlation is recommended.
FUNGI	Not Detected		
PARASITES	Not Detected		
HOST RESPONSE CLASSIFIER	Autoimmune/Non-Infectious: 23%		Host response: likely bacterial, possible autoimmune / non-infectious
	Viral: 13%		Subclassifier: Possible neuroborreliosis
	Bacterial: 57%		
	Fungal: 7%		

Challenges in Diagnosis of Lyme Disease



2-tier serologic approach (CDC): (1) serum enzyme immunoassay (EIA) followed by (2) supplementary IgG and IgM Western blotting of EIA-positive or -equivocal samples.

Detection rate: 29-40%

Accurate (specific) test for diagnosis and/or monitoring of chronic disease not available

Accurate (sensitive) test for diagnosis of acute infection not available

What is the Diagnosis?

40 y/o patient from California, with chronic weakness, diffuse muscle pain, and lower-extremity numbness

- Lyme disease – Two-tiered antibody testing NEGATIVE
- Borrelia burgdorferi PCR, Anaplasma PCR, Babesia PCR NEGATIVE
- Borrelia burgdorferi IgG C6 peptide, p83-93 **POSITIVE**
- Borrelia burgdorferi IgM C6 peptide **POSITIVE**
- Borrelia afzelli IgG OspA **POSITIVE**
- Borrelia bavariensis VIsE1 **POSITIVE**
- Babesia microti WCS (whole-cell sonicate) IgG **POSITIVE**
- Bartonella henselae SucB IgG **POSITIVE**
- Anaplasma phagcytophilum Msp5/Msp2 (p44) IgG **POSITIVE**

Classifier for Early Lyme Diagnosis from PBMCs

PBMCs (31-gene panel)

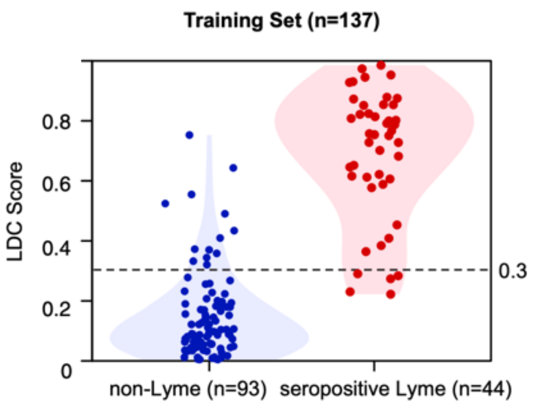
90% sensitivity

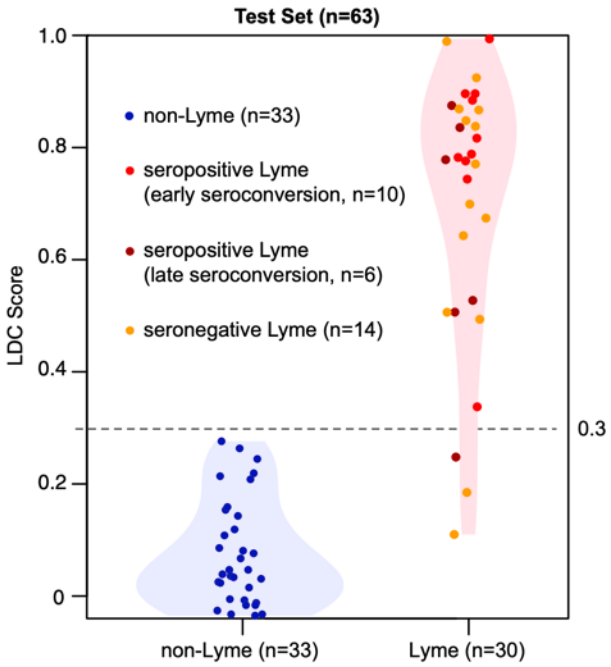
100% specificity

95% accuracy

<u>Limitations</u>:

- no controls from other tickborne infections
- PBMC isolation is not routinely done in the clinical lab





Performance (Seropositive Lyme)

	Lyme	non-Lyme
LDC +	15	0
LDC−	1	33

sensitivity = 93.7%
specificity = 100%
accuracy = 98.0%

Performance (Seronegative Lyme)

	Lyme	non-Lyme
LDC +	12	0
LDC−	2	33

sensitivity = 85.7%
specificity = 100%
accuracy = 95.7%

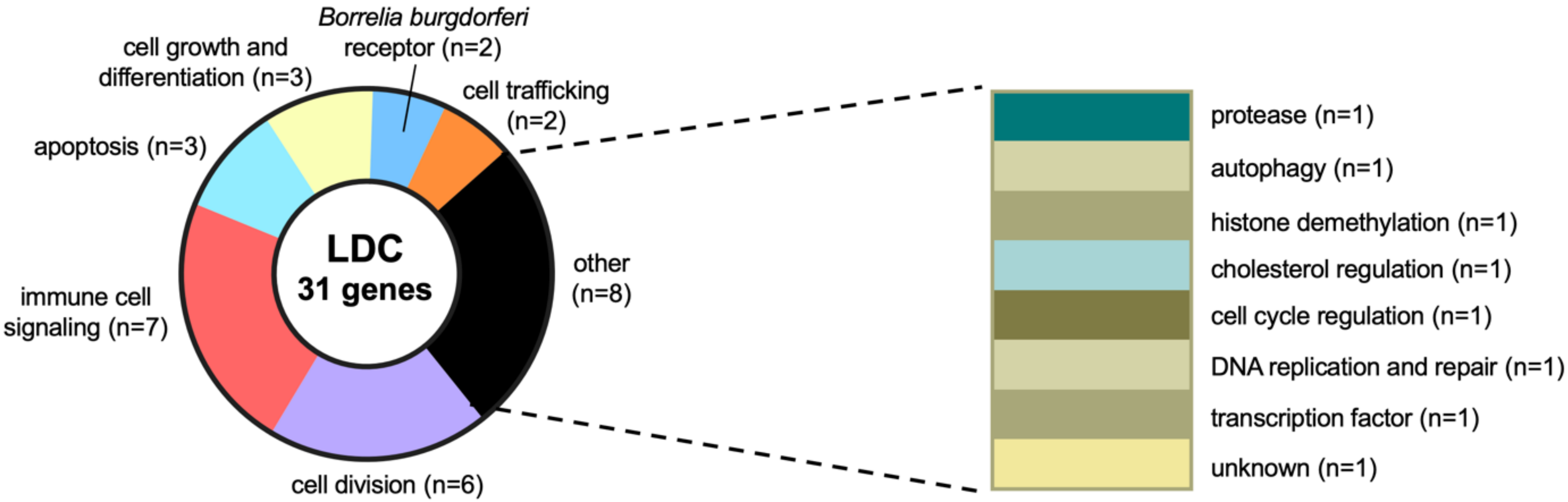
Performance (Overall)

	Lyme	non-Lyme
LDC +	27	0
LDC−	3	33

sensitivity = 90%
specificity = 100%
accuracy = 95.2%

Servellita V, Bouquet J,.. Chiu CY. *Communications Medicine*, 2022. doi:10.1038/s43856-022-00127-2

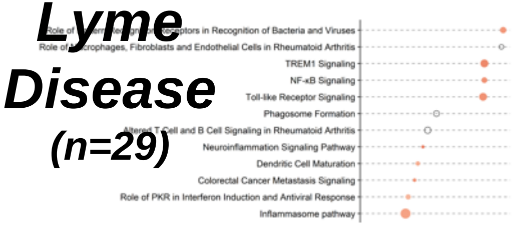
Classifier for Early Lyme Diagnosis from PBMCs



Servellita V, Bouquet J,.. Chiu CY. *Communications Medicine*, 2022. doi:10.1038/s43856-022-00127-2

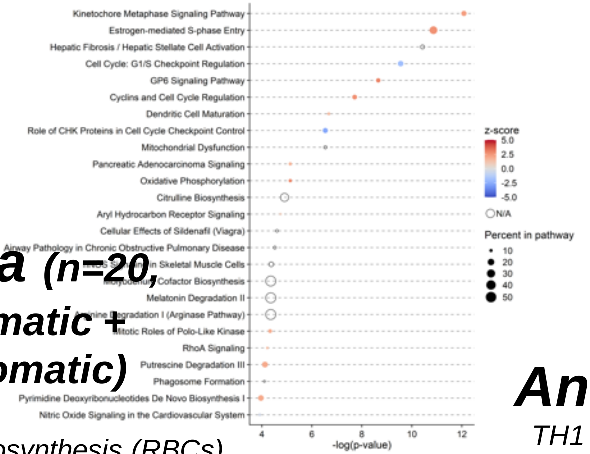
RNA Profiles of Tickborne Infections from Blood

Lyme Disease (n=29)



Inflammasome pathway
TREM1 signaling
TLR signaling
NF-kB signaling
Immune Cells in Rheumatoid Arthritis
IL-6 signaling

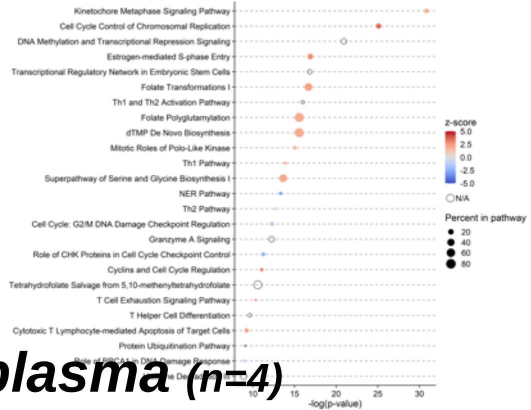
Babesia (n=20, symptomatic + asymptomatic)



Citrulline Biosynthesis (RBCs)
Nitric Oxide Pathways (Sildenafil)
GP6 Signaling (RBCs)
Dendritic Cell Maturation
*Phagosome Formation**

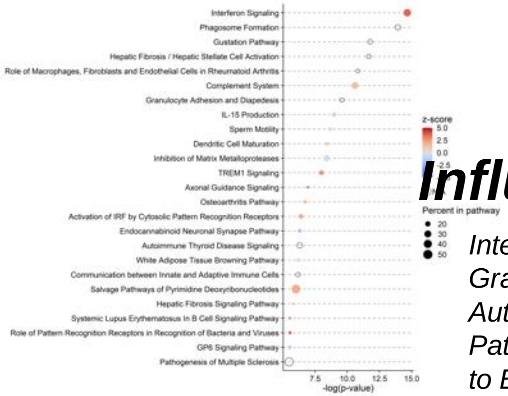
**involvement in canine babesiosis*

Anaplasma (n=4)



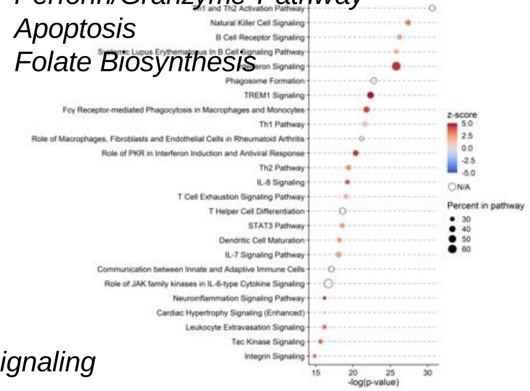
TH1 and TH2 Activation Pathways
Perforin/Granzyme Pathway
Apoptosis
Folate Biosynthesis

Influenza (n=18)



Interferon Signaling
Granulocyte Adhesion
Autoimmune Signaling
Pattern Recognition Receptors to Bacteria and Viruses

Bacterial sepsis (n=5)



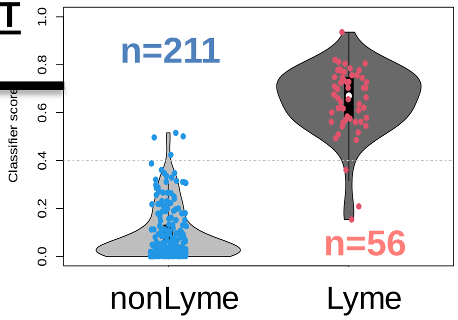
Phagocytosis
TREM1 Signaling
Interferon and NK Cell Signaling
IL-6 and IL-7 Signaling

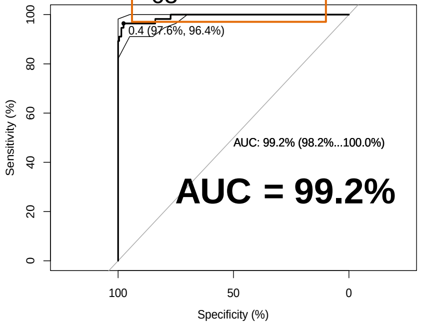
Unpublished data, collaboration with Lyme Disease Biobank, American Red Cross, Columbia University

Gene Expression Classifier for Early Lyme from Whole Blood

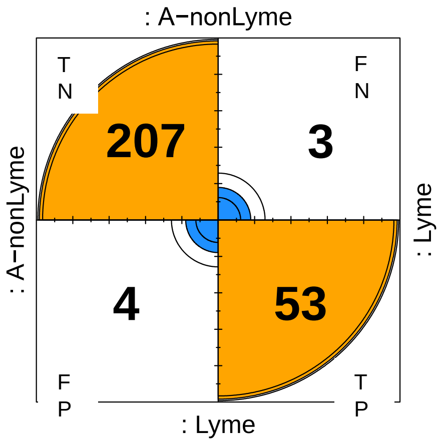
TRAINING SET

Lyme: 56
sepsis: 23
influenza: 17
covid: 25
asympBab: 67
sympBab: 5
anaplasma: 4
ill_ctrls: 7
donor_ctrls: 63





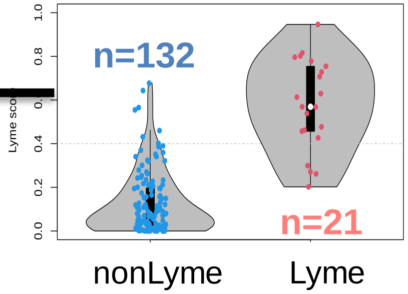
AUC = 99.2%

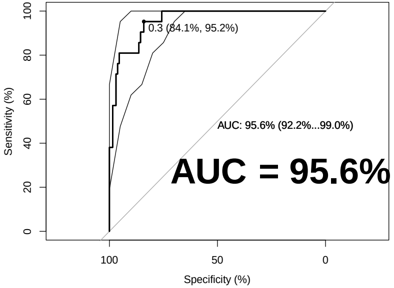


Accuracy : 0.9738
95% CI : (0.9467, 0.9894)
Sensitivity : 0.9464
Specificity : 0.9810

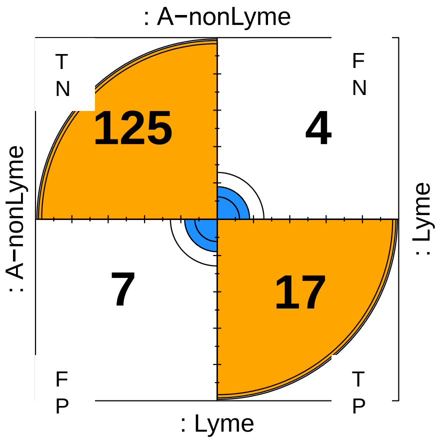
TEST SET

Lyme: 21
sepsis: 12
influenza: 6
covid: 11
asympBab: 26
sympBab: 2
anaplasma: 4
ill_ctrls: 7
donor_ctrls: 71





AUC = 95.6%



Accuracy : 0.9281
95% CI : (0.875, 0.9636)
Sensitivity : 0.8095
Specificity : 0.9470

Unpublished data, collaboration with Lyme Disease Biobank, American Red Cross, Columbia University

Key Lyme-Associated Genes Identified using the SHAP (Shapley Additive Explanations) Algorithm

ADGRE4P (Adhesion G Protein-Coupled Receptor E4)

- leukocyte adhesion and migration

GPR15 (G-protein coupled receptor 15)

- chemokine receptor
- up-regulated in patients with persistent Lyme symptoms in PBMC (Bouquet, et al. 2016)
- differentially expressed in early Lyme patients in PBMC (Servellita, et al. 2022)

DUSP5 (Dual specificity phosphatase 5)

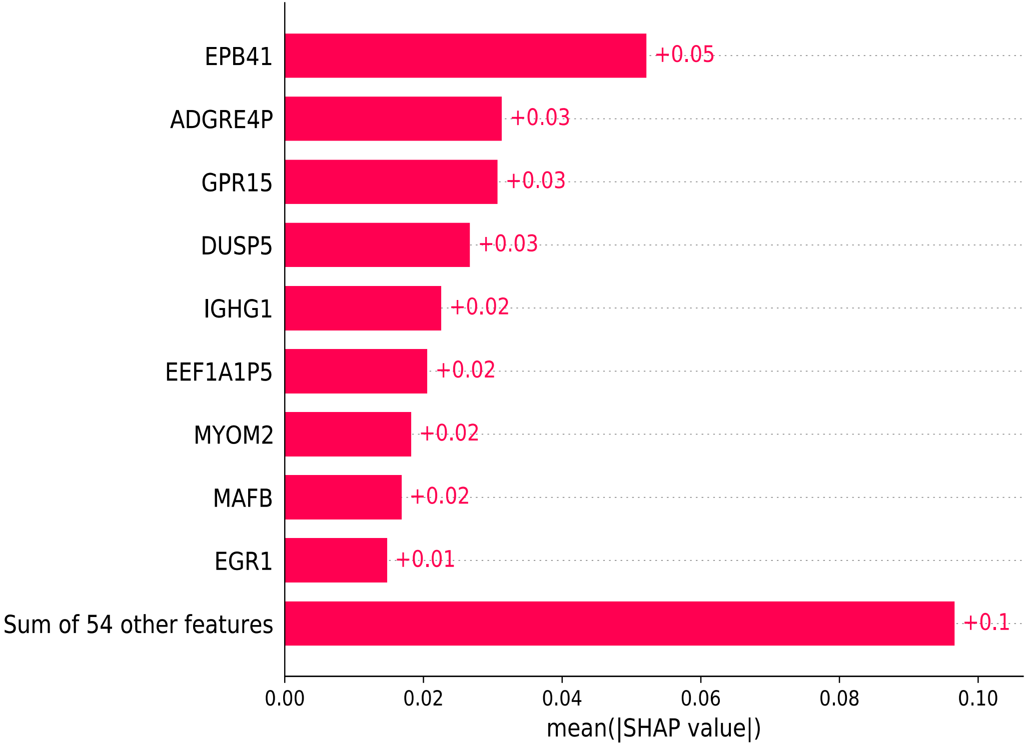
- cellular proliferation and differentiation
- up-regulated in PBMCs stimulated with live *Borrelia burgdorferi* (Salazar, et al. 2009)

IGHG1 (Immunoglobulin heavy constant gamma 1)

- activation of immune response; defense response to other organism; and phagocytosis
- up-regulated after *B. burgdorferi* stimulation (Scholl, et al. 2016)

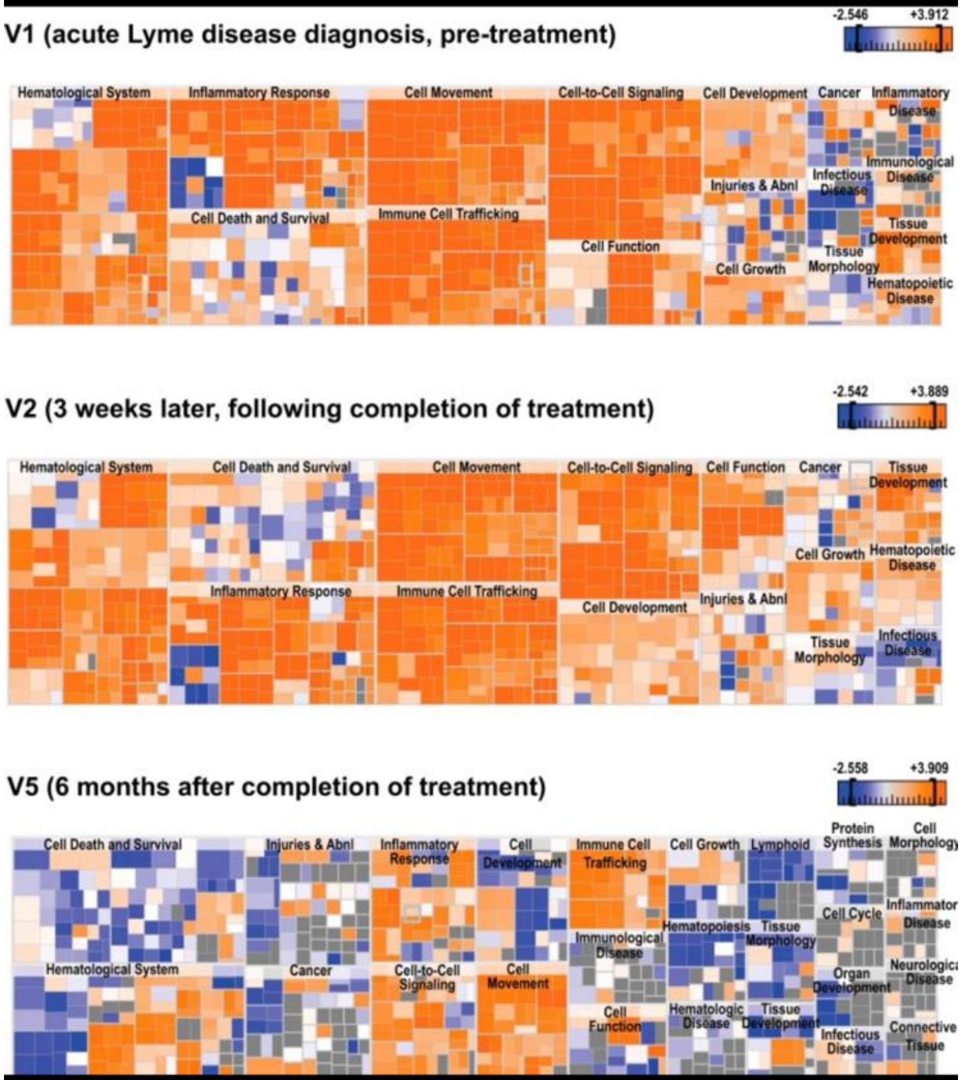
EGR1 (Early growth response 1)

- one of the top genes with greatest fold changes in LD subjects relative to healthy donors in PBMC (Petzke, et al. 2020)
- down-regulated in PBMC stimulated with *B. burgdorferi* (Krupna-Gaylord, et al. 2014)



Unpublished data, collaboration with Lyme Disease Biobank

Persistent Gene Expression Signature in Lyme Disease


V1 (acute Lyme disease diagnosis, pre-treatment)

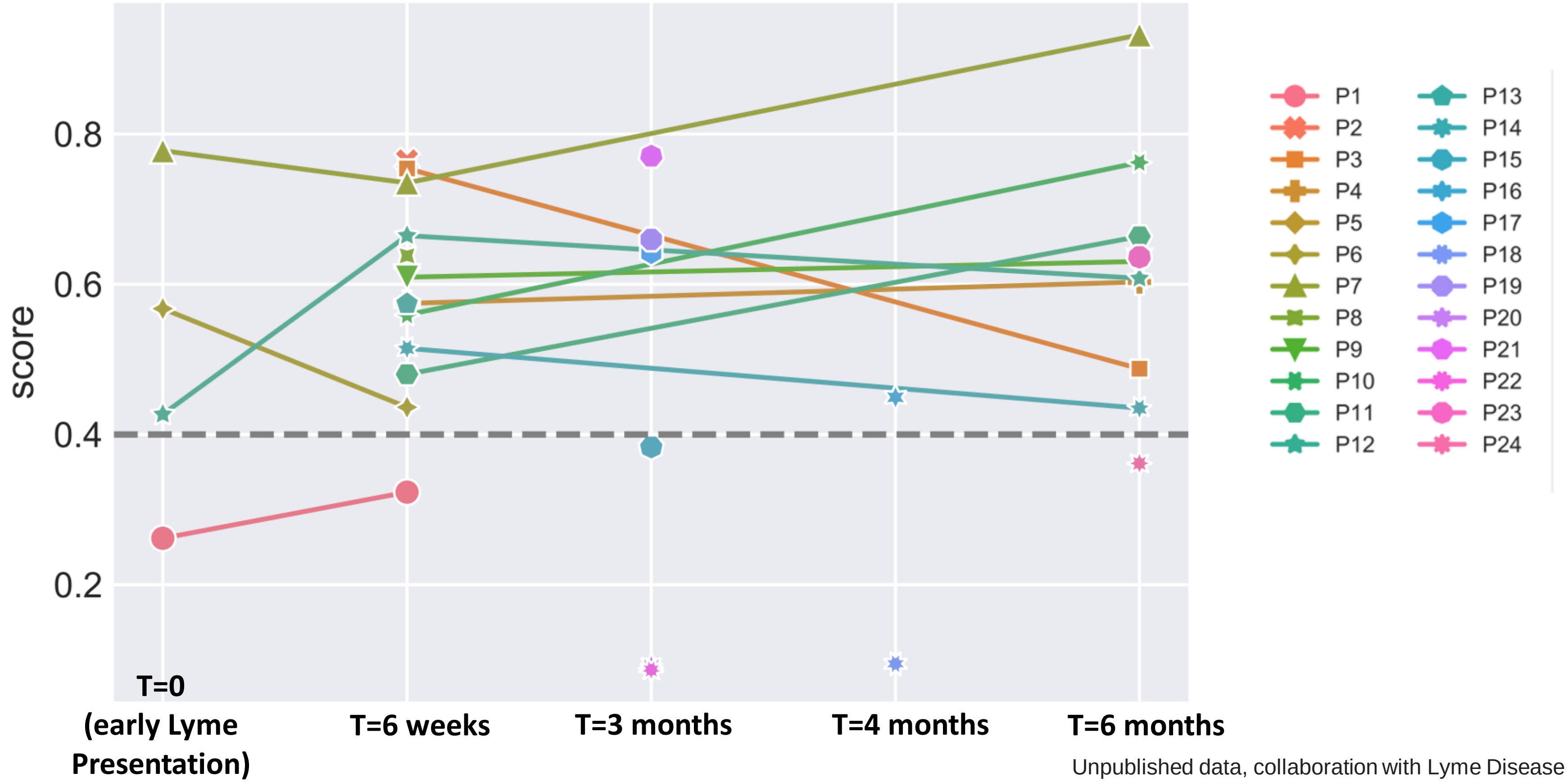
V2 (3 weeks later, following completion of treatment)

V5 (6 months after completion of treatment)

Bouquet, et al., 2016, *mBio,* 12(7):e00100-16

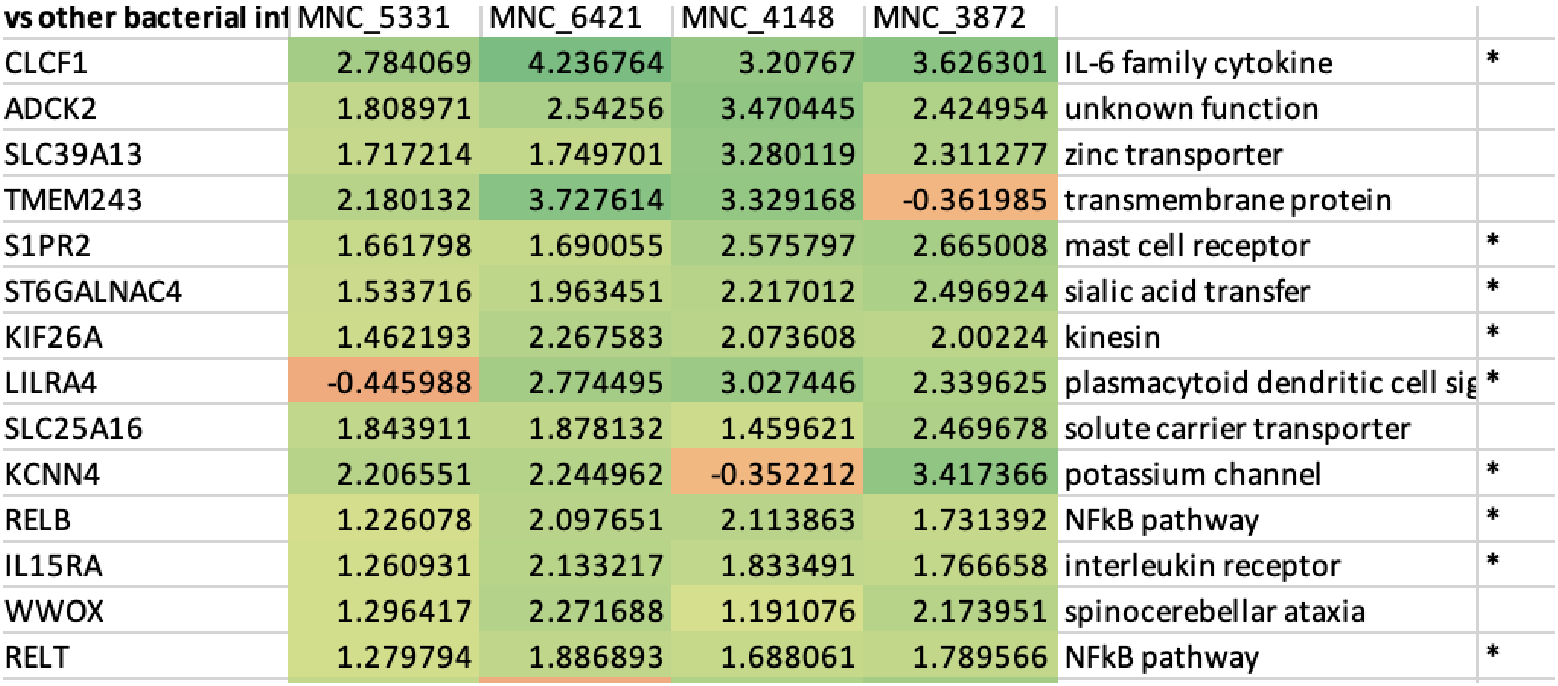
Longitudinal Host Response Analyses

(n=24 patients with confirmed Lyme)



Unpublished data, collaboration with Lyme Disease Biobank

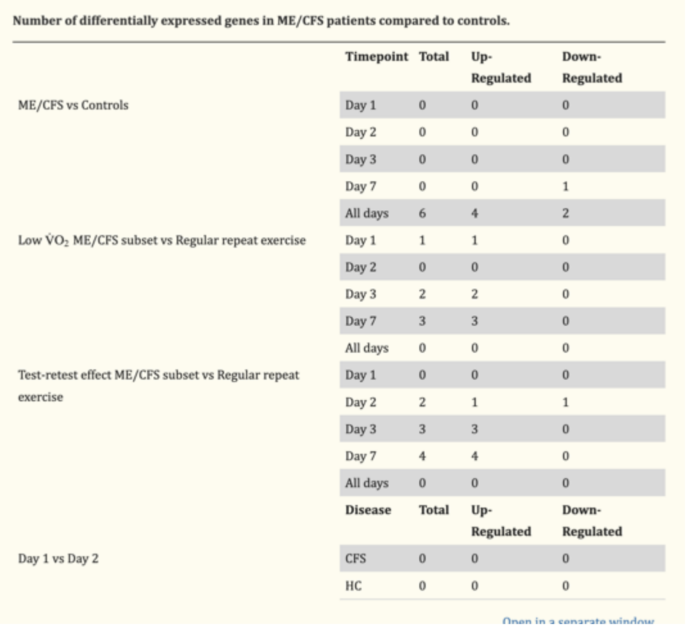
CSF Subclassifier Analysis:
Lyme Disease (Neuroborreliosis)



vs other bacterial inf	MNC_5331	MNC_6421	MNC_4148	MNC_3872		
CLCF1	2.784069	4.236764	3.20767	3.626301	IL-6 family cytokine	*
ADCK2	1.808971	2.54256	3.470445	2.424954	unknown function	
SLC39A13	1.717214	1.749701	3.280119	2.311277	zinc transporter	
TMEM243	2.180132	3.727614	3.329168	-0.361985	transmembrane protein	
S1PR2	1.661798	1.690055	2.575797	2.665008	mast cell receptor	*
ST6GALNAC4	1.533716	1.963451	2.217012	2.496924	sialic acid transfer	*
KIF26A	1.462193	2.267583	2.073608	2.00224	kinesin	*
LILRA4	-0.445988	2.774495	3.027446	2.339625	plasmacytoid dendritic cell sig	*
SLC25A16	1.843911	1.878132	1.459621	2.469678	solute carrier transporter	
KCNN4	2.206551	2.244962	-0.352212	3.417366	potassium channel	*
RELB	1.226078	2.097651	2.113863	1.731392	NFkB pathway	*
IL15RA	1.260931	2.133217	1.833491	1.766658	interleukin receptor	*
WWOX	1.296417	2.271688	1.191076	2.173951	spinocerebellar ataxia	
RELT	1.279794	1.886893	1.688061	1.789566	NFkB pathway	*

Omura, Wilson, Chiu, et al., 2023, in preparation

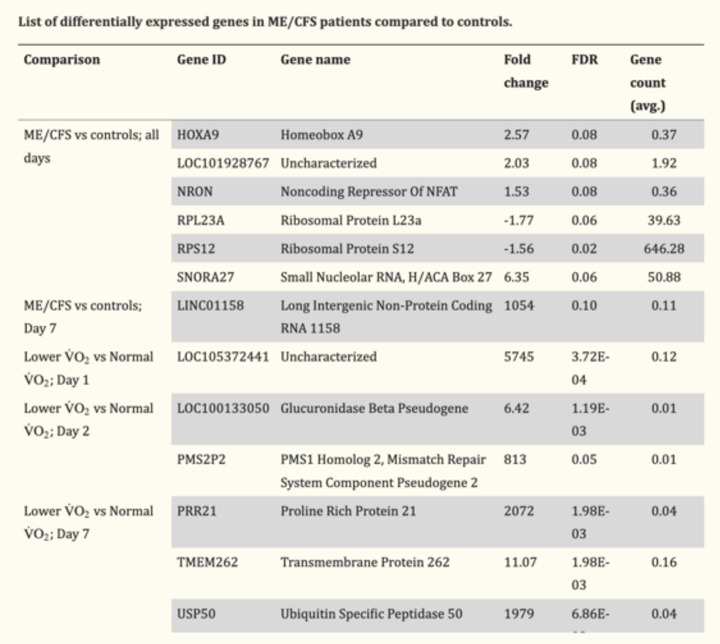
Chronic Fatigue Syndrome and RNA Gene Profiling



Number of differentially expressed genes in ME/CFS patients compared to controls.

	Timepoint	Total	Up-Regulated	Down-Regulated
ME/CFS vs Controls	Day 1	0	0	0
	Day 2	0	0	0
	Day 3	0	0	0
	Day 7	0	0	1
	All days	6	4	2
Low $\dot{V}O_2$ ME/CFS subset vs Regular repeat exercise	Day 1	1	1	0
	Day 2	0	0	0
	Day 3	2	2	0
	Day 7	3	3	0
	All days	0	0	0
Test-retest effect ME/CFS subset vs Regular repeat exercise	Day 1	0	0	0
	Day 2	2	1	1
	Day 3	3	3	0
	Day 7	4	4	0
	All days	0	0	0
	Disease	**Total**	**Up-Regulated**	**Down-Regulated**
Day 1 vs Day 2	CFS	0	0	0
	HC	0	0	0

Open in a separate window



List of differentially expressed genes in ME/CFS patients compared to controls.

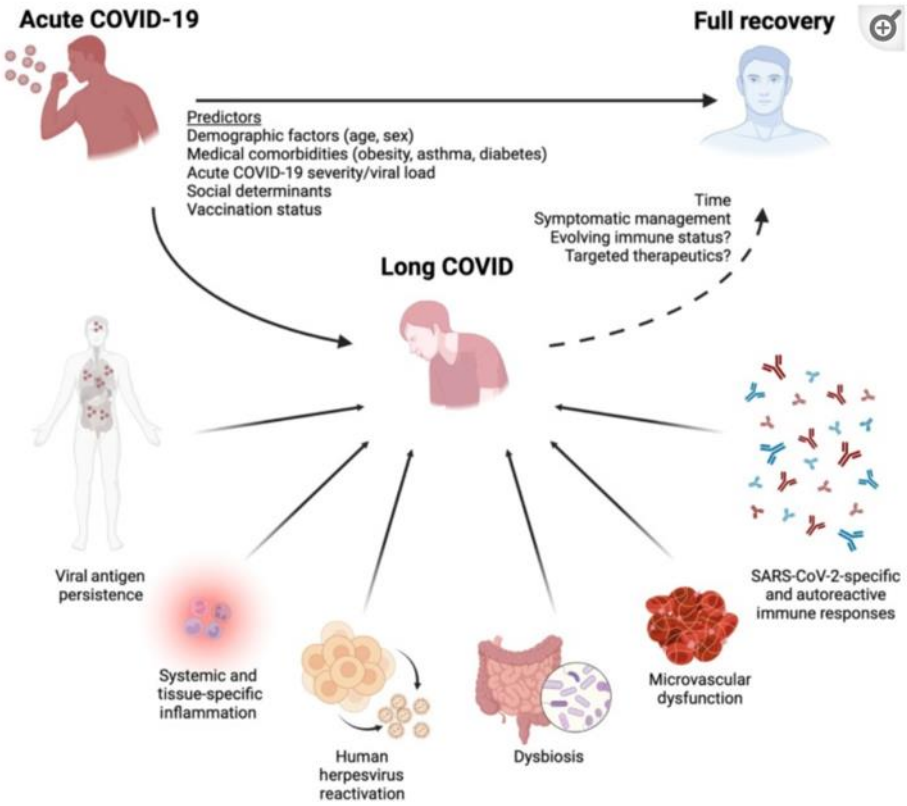
Comparison	Gene ID	Gene name	Fold change	FDR	Gene count (avg.)
ME/CFS vs controls; all days	HOXA9	Homeobox A9	2.57	0.08	0.37
	LOC101928767	Uncharacterized	2.03	0.08	1.92
	NRON	Noncoding Repressor Of NFAT	1.53	0.08	0.36
	RPL23A	Ribosomal Protein L23a	-1.77	0.06	39.63
	RPS12	Ribosomal Protein S12	-1.56	0.02	646.28
	SNORA27	Small Nucleolar RNA, H/ACA Box 27	6.35	0.06	50.88
ME/CFS vs controls; Day 7	LINC01158	Long Intergenic Non-Protein Coding RNA 1158	1054	0.10	0.11
Lower $\dot{V}O_2$ vs Normal $\dot{V}O_2$; Day 1	LOC105372441	Uncharacterized	5745	3.72E-04	0.12
Lower $\dot{V}O_2$ vs Normal $\dot{V}O_2$; Day 2	LOC100133050	Glucuronidase Beta Pseudogene	6.42	1.19E-03	0.01
	PMS2P2	PMS1 Homolog 2, Mismatch Repair System Component Pseudogene 2	813	0.05	0.01
Lower $\dot{V}O_2$ vs Normal $\dot{V}O_2$; Day 7	PRR21	Proline Rich Protein 21	2072	1.98E-03	0.04
	TMEM262	Transmembrane Protein 262	11.07	1.98E-03	0.16
	USP50	Ubiquitin Specific Peptidase 50	1979	6.86E-	0.04

Only 6 DEGs between CFS patients and controls

(application pending for samples from the UK National ME Biobank)

Bouquet and Chiu, et al., PloS ONE, 2019; 14(3): e0212193.

Long COVID



RNA Host Response Profiling of Whole Blood from Long COVID Patients

(collaboration with Drs. Michael Peluso and Steven Deeks)

Peluso and Deeks, Trends Immunol, 2022, 43(4): 268-270.

Take-Home Messages

- Metagenomic next-generation sequencing (mNGS) is promising for broad-based, agnostic diagnosis of infections
- RNA mNGS data can be leveraged to simultaneously interrogate the patient’s host response
- Host response profiling using RNA gene expression (“transcriptomics”) can enable identification of biomarkers that can lead to tests that will not only diagnose diseases but to monitor the chronic disease course longitudinally
- The signal from cell-free RNA (dead or dying cells) in addition to cell-associated RNA (immune response cells) should be considered when developing diagnostic assays for infection-associated chronic illnesses
- The development of host response assays needs close coordination with regulatory bodies (CLIA, CLSI, FDA, etc.) regarding the development of reference materials and requirements for clinical validation

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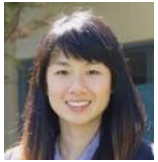
Charles Omura

Alicia Sotomayor-Gonzalez



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