

UCSF
University of California
San Francisco

Solving Neurologic Mysteries: Next-Generation Approaches to Diagnosis

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Disclosure

Solving Medical Mysteries
Providing the next generation of clinical testing to diagnose unexplained diseases

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Defining Our Terms

- Meningitis: inflammation of the meninges
- Encephalitis: inflammation of the brain
- Myelitis: inflammation of the spinal cord

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Meningitis

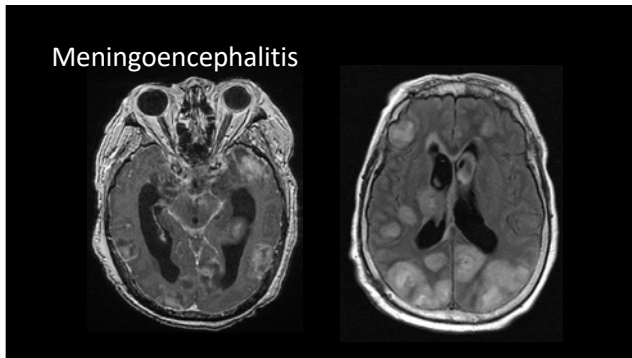
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Encephalitis

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Myelitis

6



Meningoencephalitis

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Encephalitis: Morbidity and Mortality

- ~20,000 cases/year in the United States
- \$2 billion in inpatient costs
- ~10% mortality
- Survivors are frequently disabled – speech, memory, mobility

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Encephalitis of Unknown Etiology

- > 50% unknown cases in 26 of 41 studies in recent meta-analysis
 - Not explained by # of pathogens tested for
- California Encephalitis Project
 - 7 years (1,570 cases): 63% of cases unknown

Granerod J, et al. Neurology 2010;75:924-932
Glaser CA, et al. CID 2006;43:1565-1577

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Diagnostic Challenges

- > 100 pathogens cause encephalitis
- Wide variation
 - Geography
 - Season
 - Patient demographic
 - Epidemics

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Emerging Neurologic Infections

- West Nile virus
- Periodic measles, mumps outbreaks
- Chikungunya virus
- Zika virus
- Powassan virus
- Nipah and Hendra viruses
- Enterovirus A71
- Chandipura virus
- Monkeypox virus
- Ebola virus
- PML in HIV and other immunosuppressed patients
- Arenavirus (LCMV-like) in solid organ transplant pts
- Dengue virus in the Florida Keys
- ? SARS-Coronavirus-2 ?

- Rate of severe neurological symptoms caused by emerging viruses
 - 39% commonly do so
 - 10% rarely or occasionally do so

Liu W, et al. BMC Med 2015;13(1):22-30
Tyler KL. Ann Neurol 2005;58(5):701-709

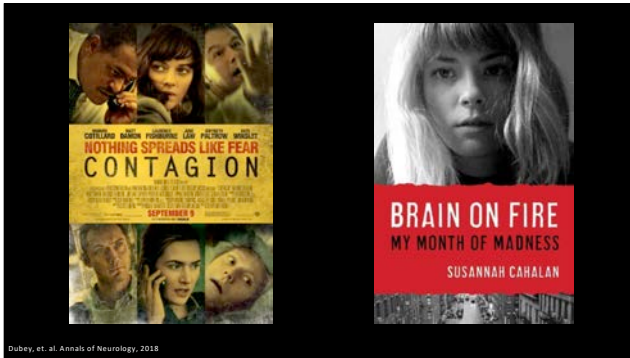
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Zika Virus

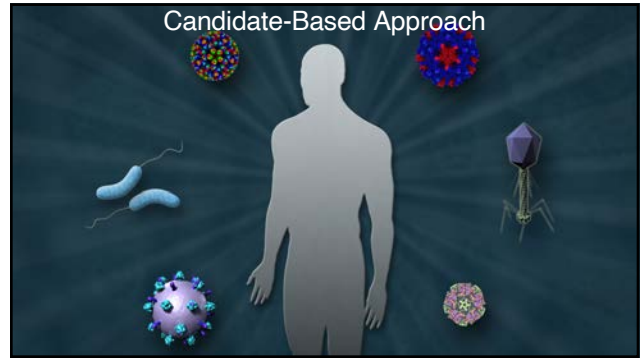
- Phylogenetic analyses indicate Zika was introduced to Brazil in 2013
- 18 months before it was detected
- Nearly 2 years before recognized as a cause of microcephaly, meningoencephalitis and Guillain-Barré syndrome

Faria NR, et al. Science 2016

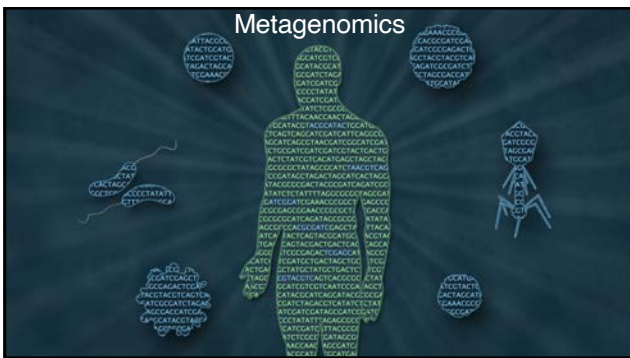
12



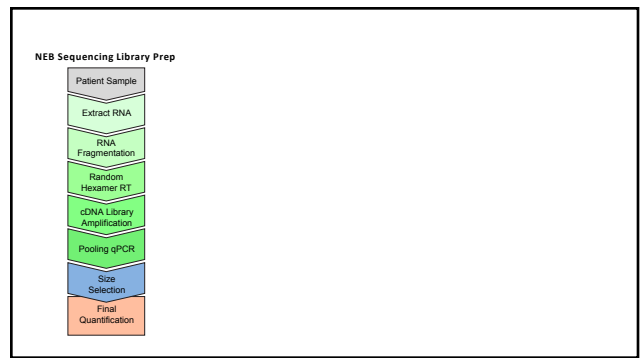
13



14



15



16

NEB Sequencing Library Prep

	Read-Pairs Retained	Time (min)	Component	Target	
Raw Sequence (fastq files)	19,574,979	100%	0.0	NA	NA
Removal of Human Reads	1,632,130	8.3%	5.2	STAR v2.4.2	Hg38/RefSeq/RepBase
Quality Filter	816,065	4.1%	5.8	PriceSeqFilter v1.1.2	read-pairs
Compression of Redundant Reads	96,913	0.49%	5.9	cd-hit-dup v4.6.4.2015	read-pairs
LZW Complexity Filter	92,074	0.47%	6.3	low-filter (script)	read-pairs
Paired-End Human Removal	2,232	0.01%	6.9	bowtie2 v2.2.4	Hg38/RepBase
Alignment to nt database	1,683	<0.01%	7.4	gmap/gsnapl v2015-12	NCBI nt 2015
Alignment to rr database	1,467	<0.01%	9.3	rapsearch2 v2.23	NCBI rr 2015
Taxonomic Stats/Reporting			9.4	MySQL v5.5.53	NCBI Taxonomy

Abbreviations: NT, nucleotide; NR, non-redundant protein.

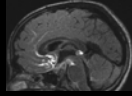
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Four Niches to Fill

- Novel, highly divergent pathogens
- Pathogens not linked with a clinical phenotype
- Rare pathogen not on the differential
- "Rule out" infection in suspected autoimmune cases

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Early Success / Proof Of Concept



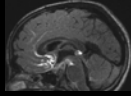

Neuroleptospirosis

- Unanticipated bacterial infection
- 48-hour turnaround
- Dramatic clinical improvement

• Wilson MR, et al. NEJM 2014

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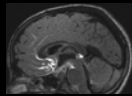

Balamuthia mandrillaris

- Unanticipated amoebic infection
- New cause of endophthalmitis

• Wilson MR, et al. NEJM 2014
• Wilson MR, et al. Ann Neurol 2015

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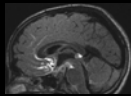

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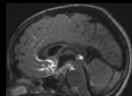


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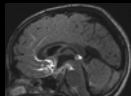


Cache Valley virus

- New cause of chronic encephalitis
- Undetected spread of an arbovirus to a new continent

• Wilson MR, et al. NEJM 2014
• Wilson MR, et al. Ann Neurol 2015
• Wilson MR, Swan D, et al. Ann of Neurol 2017

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Early Success / Proof Of Concept

Neuroleptospirosis

- Unanticipated bacterial infection
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• Wilson MR, et al. NEJM 2014
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Early Success / Proof Of Concept

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 - Unanticipated bacterial infection
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• Wilson MR, et al. NEJM 2014
• Wilson MR, et al. Ann Neurol 2015
• Wilson MR, Sloan D, et al. Ann of Neurol 2017

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 - Unanticipated bacterial infection
 - 48 hour turnaround
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- Balamuthia mandrillaris**
 - Unanticipated amoebic infection
 - New cause of endophthalmitis
- Candida dubliniensis**
 - Unanticipated fungal infection
 - IV drug use not disclosed

• Wilson MR, et al. NEJM 2014
• Wilson MR, et al. Ann Neurol 2015
• Wilson MR, Sloan D, et al. Herit Neurol 2017
• Wilson MR, O'Donovan B, Gelfand J, et al. JAMA Neurol 2018

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Pitfalls I

- Low input nucleic acid from cerebrospinal fluid (picogram quantities)
- Human background

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Pitfalls I

- Low input nucleic acid from cerebrospinal fluid (picogram quantities)
- Human background
- NGS library kit benchmarking
- Depletion of Abundant Sequences by Hybridization (DASH)

• Gu W, Crawford ED, O'Donovan B, Wilson MR, et al. Genome Biol 2016
• Ramani V, Shendure J. Genome Biol 2016

28

Pitfalls II

- Environmental contamination

29

Pitfalls II

- Environmental contamination
- Weighted z score-based scoring algorithm to separate bona fide pathogen sequences for spurious environmental sequences

• Wilson MR, O'Donovan B, Gelfand J, et al. JAMA Neurol 2018

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Case 1

- 40 year-old physician with 15 years of relapsing myelitis and arachnoiditis
- Evaluated and treated at top academic medical centers

Beck E. et al. Ann Neurol 2019

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Case 1

- 40 year-old physician with 15 years of relapsing myelitis and arachnoiditis
- Evaluated and treated at top academic centers
- Immigrated to US from India at age 22; lived in AZ, NY and MD
- No history of intravenous drug use
- No known animal, mosquito or tick exposures

Beck E. et al. Ann Neurol 2019

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Case 1

- 2002: Subacute onset of back pain and malaise -> night sweats, fever, anorexia, severe headache and neck stiffness
- CSF
 - WBC 454 (25% lymphocytes, 15% neutrophils, 60% monocytes)
 - Protein 96
 - Glucose 23

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Case 1



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Case 1

- Treated for tuberculous meningitis for 3 months (had to stop due to medication side effects)

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Case 1

- Treated for tuberculous meningitis for 3 months (had to stop due to medication side effects)
- 2006: epidural anesthesia for childbirth -> Temp 102F, headache, neck stiffness, back pain, night sweats -> got antibiotics
- ...but low-grade symptoms for months before acute onset bilateral gluteal and left leg pain

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Case 1

- CSF
 - WBC 130 (83% lymphocytes, 2% neutrophils, 13% monocytes)
 - Glucose 10
 - Protein 132
- Laminectomy at L5-S1 for biopsy -> non-specific inflammation
- 1 more year of anti-tuberculosis therapy

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Case 1

- Significant improvement but intermittent low back pain and chills

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Case 1

- Significant improvement but intermittent low back pain and chills
- 2015: days after thyroid surgery developed low back and leg pain and numbness with chills, headache, fever and neck stiffness
- Given valacyclovir + prednisone with dramatic improvement
- Recurrent symptoms when steroids tapered

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Case 1

- Since May 2015 has been on >35 mg of daily prednisone plus...
 - Mycophenolate mofetil (up to 3000mg daily) for 8 months
 - Methotrexate (Jan 2017 to present)
- Chronic, dull aching pain in back and buttocks that worsens with sitting, bending or twisting or moving her head
- Monthly gets general malaise, Temp 101F and more severe throbbing pain for 1-2 weeks

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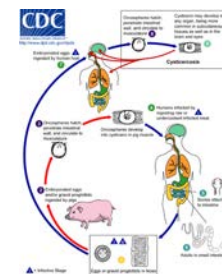
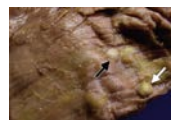
Case 1

- CSF
 - RBC 156
 - WBC 30 (92% lymphocytes, 4% neutrophils, 4% monocytes)
 - Protein 41
 - Glucose 47
- IgG index 2.41

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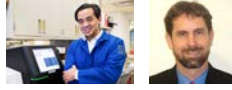
Cysticercosis

- *Taenia solium* tapeworm
- Two intermediate hosts
 - Pig
 - Human
- Definitive host: human



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CLIA Validation



- Establishment of:
 - Quality metrics
 - Threshold values
- Limits of detection (0.16 to 313 genomic copies or colony forming units per milliliter for each representative organism type)

• Miller S, et al. Genome Research 2019

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Precision Diagnosis of Acute Infectious Diseases (PDAID) Study Design

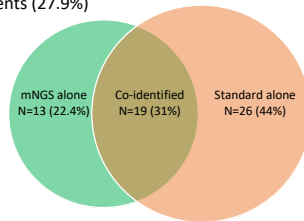
- 8 academic medical centers around the U.S.
- Prospective enrollment of patients with acute meningitis +/- encephalitis or exacerbation of chronic meningitis
- No diagnosis at the time of enrollment
- Adults and children

• Wilson MR, Sample HA, et al. NEJM 2019
 • ClinicalTrials.gov number, NCT02910037

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Results

- 204 patients enrolled
- 58 infections diagnosed in 57 patients (27.9%)



• Wilson MR, Sample HA, et al. NEJM 2019
 • Chiu CY, et al. Emerg Inf Dis 2017
 • Murkey JA, et al. Open Forum Inf Dis 2017

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Results

- 10/13 mNGS-only infections not entertained by treating physicians (St. Louis encephalitis virus, hepatitis E virus, etc) or had been missed

• Wilson MR, Sample HA, et al. NEJM 2019
 • Chiu CY, et al. Emerg Inf Dis 2017
 • Murkey JA, et al. Open Forum Inf Dis 2017

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Results

- 10/13 mNGS-only infections not entertained by treating physicians (St. Louis encephalitis virus, hepatitis E virus, etc) or had been missed
- CSF mNGS 80% sensitive and 98.2% specific for infections identified by direct detection methods in CSF
- Traditional direct detection CSF assays were 67.5% sensitive and 99.4% specific

• Wilson MR, Sample HA, et al. NEJM 2019
 • Chiu CY, et al. Emerg Inf Dis 2017
 • Murkey JA, et al. Open Forum Inf Dis 2017

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Results

- Clinical false negatives (n=26)
 - Diagnosed by serology (n=11)
 - Diagnosed in samples other than CSF (n=7)
 - Low pathogen titer in CSF (n=8)

• Wilson MR, Sample HA, et al. NEJM 2019
 • Chiu CY, et al. Emerg Inf Dis 2017
 • Murkey JA, et al. Open Forum Inf Dis 2017

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Disadvantages of Direct Detection Assays

- Short time windows for detection for some pathogens
- Compartmentalized / focal infections may shed little to no genetic material into the cerebrospinal fluid

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Neuroinvasive viruses diagnosed by CSF serology

- West Nile virus
- Powassan virus
- Japanese encephalitis virus
- Eastern equine encephalitis virus
- Measles virus
- Poliovirus
- Varicella zoster virus
- Rabies virus

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Approaches to Antibody Discovery

What we would like:

- Next-generation sequencing-compatible
- Super cheap
- Plate-based
- Robot friendly
- High signal to noise ratio
- Scalable

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VirScan Design

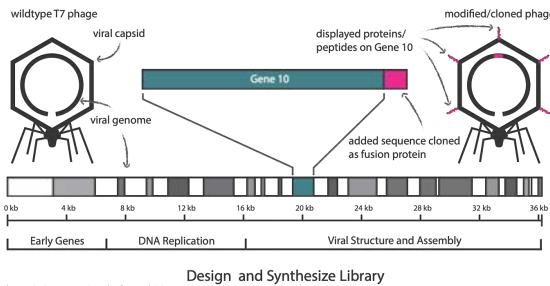


- Final number of representative virus genomes: 76,700
 - All vertebrate, tick and mosquito viruses
 - Collapsed on 98% amino acid identity
- Final number of 62 amino acid peptides overlapping by 14 amino acids: **481,966**

• Xu GJ, Kula T, Xu Q, Li MZ, Vernon SD, Ndung'u T, et al. Science. 2015
 • Johnson TP, et al. Ann Neurol 2019
 • Schubert RO, et al. Nat Med 2019

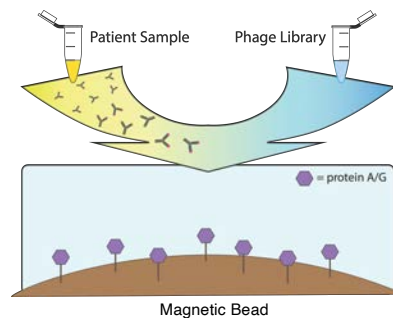
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Utilize Phage to Display Fragments of All Vertebrate and Arboviruses

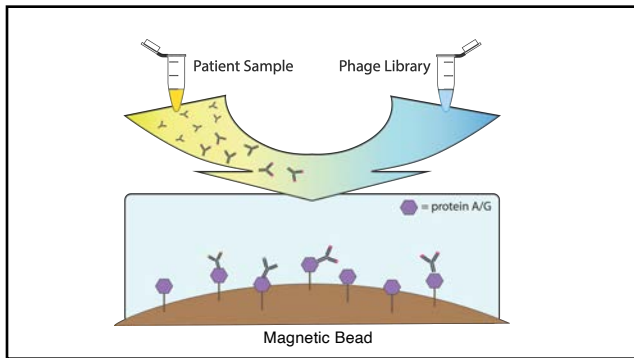


• Xu GJ, Kula T, Xu Q, Li MZ, Vernon SD, Ndung'u T, et al. Science. 2015
 • Schubert RO, et al. Nat Med 2019

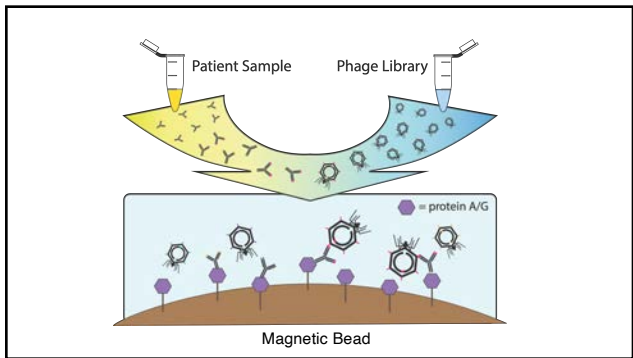
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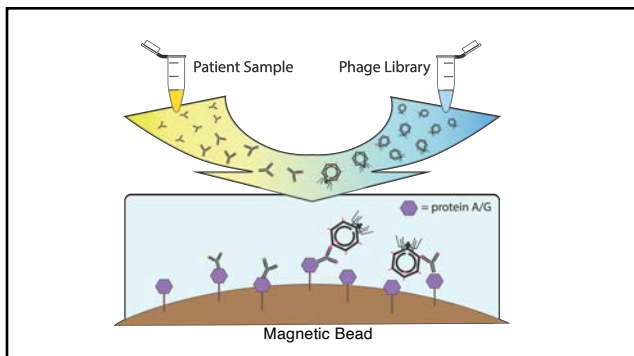
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Acute Flaccid Myelitis

- Upsurge in AFM cases in 2014 (n=120), 2016 (n=149) and 2018 (n=210)
- Baseline incidence of 22-35 cases/year
- Median age of 6 years old
- Slightly more boys than girls
- 80% of cases preceded by a prodromal illness
- Asymmetrical paralysis over hours to days
- Cases now reported in 14 countries on 6 continents

Messacar K, Tyler KL, JAMA 2019


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AFM Controversies


- ~40% of AFM children have EVD68 in body compartments outside the CNS
- Almost none of these children have EV detected in the CSF
- No alternate agent has been identified in 60% of patients

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AFM Etiology Study



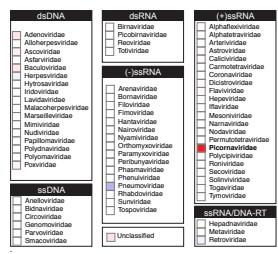
- CSF samples from children with AFM (n=42)
 - Median age: 37.8 months, IQR 11 to 64 months
 - 2018 (n=36), 2014 (n=4), 2016 (n=2)
- CSF samples from children with other neurologic diseases (n=58)
 - Median age: 120 months, IQR, 66 to 174 months
 - Infectious (40%), Autoimmune (38%), Non-inflammatory (10%), Malignancy (5%), Unknown (7%)



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CSF VirScan Detects Enterovirus Ab in AFM

- AFM: mean *Picornaviridae* reads 11,082 rpK, IQR 16,850
- OND: mean *Picornaviridae* reads rpK 1121 IQR 974
- p -adjusted = 6.3×10^{-4} Wilcoxon signed-rank test with Bonferroni adjustment

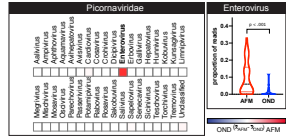


• Schubert RD, et al. Nat Med 2019

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CSF VirScan Detects Enterovirus Ab in AFM

- Enriched *Picornaviridae* peptides belonged almost entirely to the genus *Enterovirus*
- 69% (29/42) of AFM cases versus 7% (4/58) of OND controls considered positive for EV antibodies by VirScan



• Schubert RD, et al. Nat Med 2019

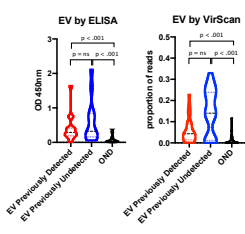
63

AFM CSF antibodies target multiple EV antigens

• Schubert RD, et al. Nat Med 2019
• Mikova N, et al. mSystems 2019

64

No Difference in EV Antibody Detection Based on Presence of Viral Nucleic Acid

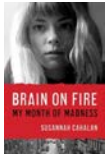


• Schubert RD, et al. Nat Med 2019

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Autoimmune Encephalitis

- Natural experiments with autoantibodies targeting individual proteins important for CNS function
- Can result in dramatic clinical phenotypes that can mimic other diseases



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Encephalitis in the 21st Century: Rise of Autoantibodies

- Incidence in Olmsted County, MN 1995-2015:
 - Infectious Encephalitis **0.8/100,000**
 - Autoimmune **1.0/100,000**
- Autoimmune 1995-2005 **0.4/100,000**
- Autoimmune 2006-2015 **1.2/100,000**

Dubey, et al. Annals of Neurology, 2018

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Neuromyelitis optica

NMDA R Ab encephalitis

LG1 Ab encephalitis

Marignier R, et al. Brain 2010
Zemke J, et al. Ann Neurol 2007
Kochanski A, et al. Neurosci Res 2017

<https://www.youtube.com/watch?v=OyPg6V08ok>

<https://www.youtube.com/watch?v=QIEHp8oYhsQ>

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Antibody Discovery Pipeline

- Rodent brain slice staining
- Immunoprecipitation → Mass spectrometry

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PhIP-Seq: A Rapid Way to Discover Linear Autoimmune Targets

Human Protein X

NCBI RefSeq

O'Donovan BD, Mandel-Brehm C, et al. <https://www.biorxiv.org/content/10.1101/021871> Under review
Larman HB, et al. Nat Biotechnol 2011

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Case 2

- 37 y.o. right-handed man with a history of testicular seminoma status post left orchiectomy in 2012
- Presented with 9 months of vertigo and that acutely worsened with constant double vision for five months
- Patient reports it is like constantly 'walking in a canoe'
- Right hand tremor
- Stopped driving and working because of vision and balance problems
- Not able to pick up his small children

Mandel-Brehm C, Dubey D, et al. NEJM 2019

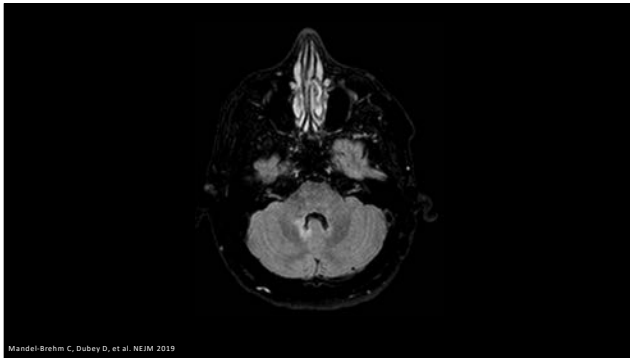
71

Case 2

- CSF
 - WBC 13 (0-5)
 - RBC 11
 - Protein 51 (15-45)
 - Glucose 56
- 14 unique oligoclonal bands
- IgG index 2.1 (<0.6)

Mandel-Brehm C, Dubey D, et al. NEJM 2019

72



73

Case 2

- Dramatic improvement with pulse steroids
- Tumor screen with whole body PET/CT negative
- Started monthly maintenance IVig...BUT

Mandel-Brehm C, Dubey D, et al. NEJM 2019

74



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Autoantibody Discovery

- Phage display identified a candidate autoantigen: Kelch-like protein 11
- Component of a cullin-RING-based BCR (BTB-CUL3-RBX1) E3 ubiquitin-protein ligase complex that mediates the ubiquitination of target proteins

Mandel-Brehm C, Dubey D, et al. NEJM 2019

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Mayo Collaboration

- Additional 12 patients identified
 - All men with a history of testicular cancer
 - 8 men developed their neurologic syndrome before the diagnosis of testicular cancer

Mandel-Brehm C, Dubey D, et al. NEJM 2019

77

Mayo Collaboration

- Additional 12 patients identified
 - All men with a history of testicular cancer
 - 8 men developed their neurologic syndrome before the diagnosis of testicular cancer

Mandel-Brehm C, Dubey D, et al. NEJM 2019

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KLH11 Encephalitis



- Second known autoantigen associated with testicular cancer
- Age-adjusted male-specific prevalence of autoimmune KLHL11 encephalitis in Olmsted County, MN:
 - 2.79/100,000 person-yrs (95% CI 0, 6.65/100,000 person-years)

Mandel-Brehm C, Dubey D, et al. NEJM 2019

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Conclusions

- CSF mNGS is now clinically available with a ~7 day turnaround time
- CSF mNGS increases diagnostic yield in patients with meningitis and encephalitis
- Additional results conveyed in the clinical microbial sequencing board
 - Viral genotyping
 - Accurate species identification
 - Resistance mutations
 - Pathogen reads below the reporting threshold

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Conclusions

- mNGS can be insensitive for detecting infections that are
 - Compartmentalized
 - Serologically diagnosed
 - Very low abundance in the CSF
 - CSF with very high pleocytosis (e.g., >1000 WBC)

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Conclusions

- Comprehensive viral antibody profiling can shed additional light on neuroviral infections, especially when direct detection methods are insensitive
- Autoantibody profiling with phage display can identify paraneoplastic syndromes that defy detection by traditional methods
- Limitations of phage display
 - Conformational epitopes not well represented
 - Lack of post-translational modifications

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