Investigation of Host Genetic Factors in Infectious Disease: Acute Flaccid Myelitis

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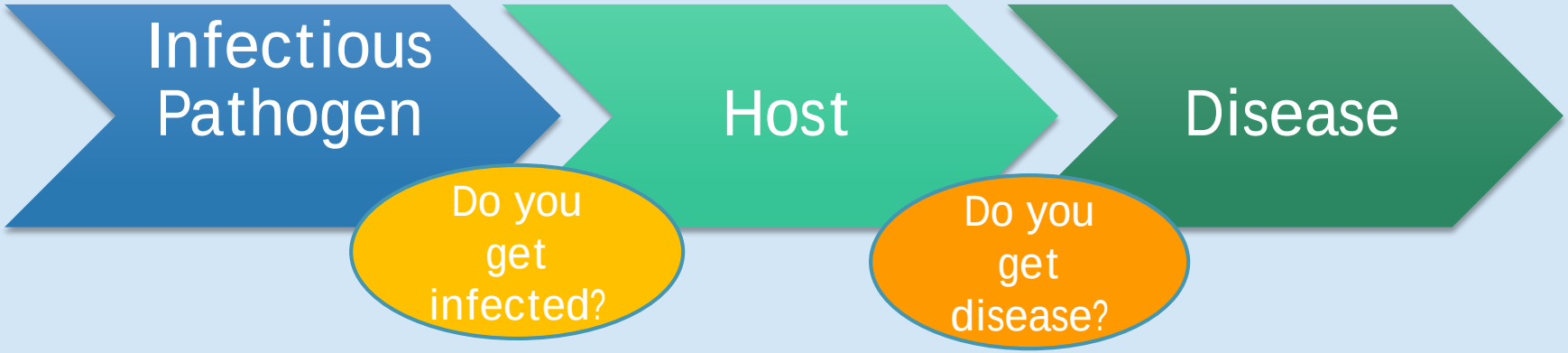
Navigating the Human Genome

- WHY consider genetics in the study of infectious disease?
- HOW do we evaluate host genetic associations?
- WHAT are the implications of host genetic predispositions to infection?
- WHERE are the genes?

Traditional Infectious Path

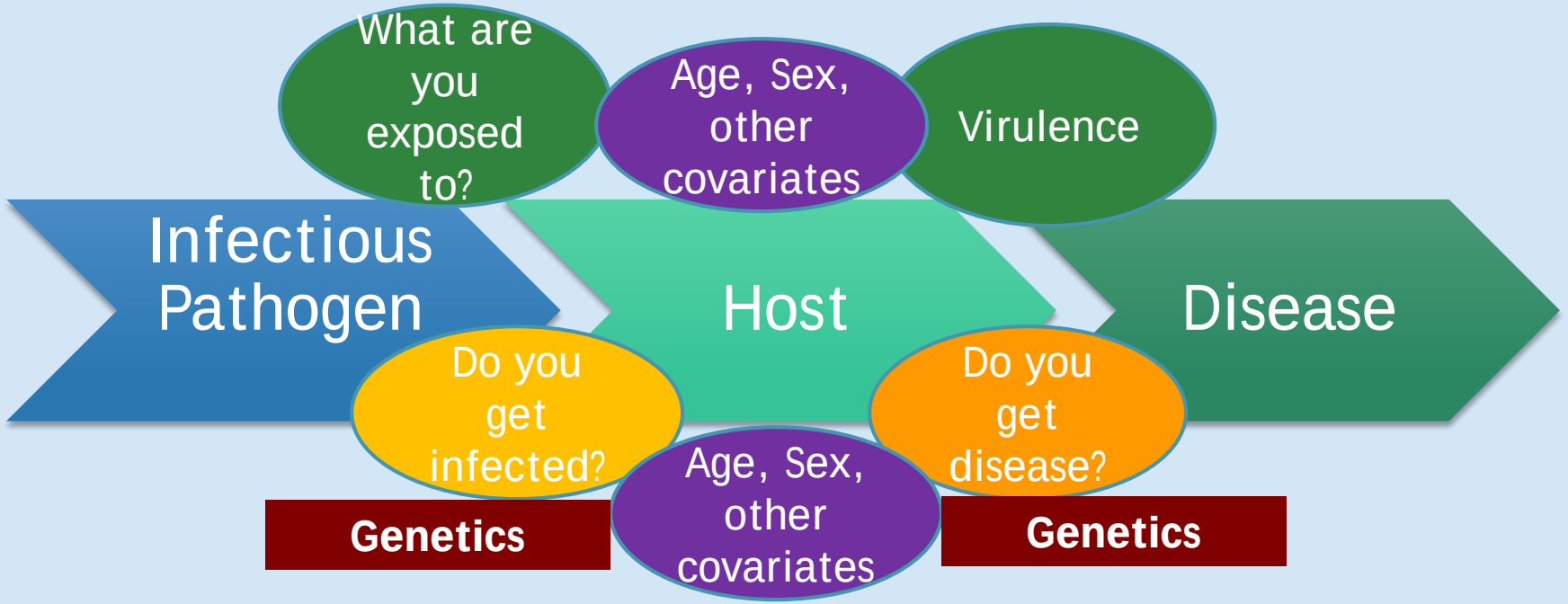


Does it matter? Everyone gets infections.

Heterogeneity

- Tuberculosis: Estimated that 1/3 of the worlds population is infected with mycobacteria, but among those infected only ~10% will develop clinical disease.
- Hepatitis B: 2 billion people (1 out of 3 people) have been infected with hepatitis B in the world, but ~10% will develop chronic disease.
- Lyme Disease: ~10-20% of people have continued symptoms and disease post antibiotic treatment
- HIV/AIDS: variability in time to AIDS (in the pre-HAART and controlled post-HAART era)

Other Factors



Why do we care?

If we can understand why people have different outcomes---we will better understand the immune response and eventually exploit this understanding for therapeutics and vaccines.

Our goals…are still to *better understand disease* so we can prevent and treat.

Infectious Disease: A complex trait?

- Infectious Disease is a perfect example of a complex trait
  - Environmental Exposure: Exposure to a pathogen is critical.
  - Redundancy and Complexity of the immune system suggest multiple genes may be involved in immune response.
  - The pathogen (virus, bacteria, parasite) may also have genetic factors that are important.

Is there evidence of genetics influencing infectious disease?

Examples from the literature

- Rare Mendelian Diseases
- Natural Selection-Population Genetics
- Vaccine controlled examples
- Adoption studies

Rare Disorders of Immunity

Mendelian susceptibility to mycobacterial disease (MSMD)

- Clinically described in 1951
- Highly susceptible to weakly virulent mycobacteria but resistant to most other infectious diseases, except Salmonella.
- Multiple gene mutations in the IL-12/IL-23, IFN-γ mediated immunity pathway.
- This pathway is crucial for host defense of mycobacterium and Salmonella

Early Suggestions with ABO blood groups and infection

Disease	Blood Type	Association	Reference
Cholera	O	Increased susceptibility	Sircar, 1981 Clemens, 1989
Shigella	O	Increased susceptibility	Robinson ,1971
Norovirus	O	Increased susceptibility	Hutson, 2002 Hennessey, 2003
P. Falicparum	O	Protected	Rowe, 2007 Cserti, 2007
Schistosomiasis	A	Increased Susceptibility	Lima, 1979 Ndamba, 1997 Camus, 1977

Twin Studies

Disease	MZ Concordance	DZ Concordance	Country of Study	Reference
Leprosy*	52	22	India	Chakravarti and Vogel 1973
HBV	35	4	Taiwan	Lin, Anticancer Research, 1979
TB	65	25	Germany	Diehl, 1936
	32	14	UK	Comstock, Am, Rev Respir Dis, 1978

* In cases where both twins had leprosy, the type of leprosy—tuberculoid or lepromataus was more likely to be concordant if the pair was monozygotic

** We expect MZ > DZ since they share the same alleles

Genetics or Environment or Pathogen?

However, despite this noticeable heterogeneity in outcome (disease, response, etc) many consider that dose and environment likely explain many of these differences, NOT genetics.

Controlling Dose:
We learn from our mistakes…

- 1926, Lubeck Germany
- 249 babies injected with the same live dose of virulent M. tuberculosis instead of BCG.
- Babies too young to have significant prior exposure to mycobacteria, and not previously vaccinated to BCG.
- All got the same strain, same dose.

76 babies died, 173 babies survived

Dubos, The White Plague 1952

Adoption Studies

GENETIC AND ENVIRONMENTAL INFLUENCES ON PREMATURE DEATH IN ADULT ADOPTEES

Sorensen et al, NEJM 1988

- Followed 960 families that included children born between 1924-1926 who were placed with adoptive parents early in life. The adoptive parents were not related to them.

- Evaluated risk of dying between the ages of 16-58 years for adoptees with a biologic or adoptive parent who died of the same cause before age 50 and before age 70. This was compared to parents who were still alive at the ages of 50 and 70.

Table 4. Effect of the Death of a Biologic or Adoptive Parent on the Rate of Adoptee Mortality from Concordant Causes Assessed by the Proportional-Hazards Regression Model.*

Cause of Death	Parent Dead before the Age of 50			Parent Dead before the Age of 70		
	No.†	RR	95 Percent CL	No.†	RR	95 Percent CL
All causes						
Biologic	779	1.71‡	1.14–2.57	813	1.85‡	1.17–2.92
Adoptive	913	0.71	0.37–1.36	917	0.80	0.55–1.16
Natural causes						
Biologic	739	1.98‡	1.25–3.12	771	1.49	0.92–2.39
Adoptive	889	0.96	0.48–1.90	878	0.96	0.65–1.41
Infection						
Biologic	641	5.81§	2.47–13.7	436	5.00§	1.73–14.4
Adoptive	840	0.73	0.10–5.36	537	1.00	0.34–2.97
Vascular causes						
Biologic	585	4.52§	1.32–15.4	464	1.92	0.78–4.73
Adoptive	822	3.02	0.72–12.8	627	1.50	0.65–3.46
Cancer						
Biologic	593	1.19	0.16–8.99	463	0.87	0.26–2.88
Adoptive	818	5.16¶	1.20–22.2	578	1.49	0.56–3.97

*RR denotes relative risk (i.e., the ratio of the cause-specific mortality rate among adoptees with at least one parent who died of the same cause before the indicated age to that among adoptees whose parents were both alive at that age), and CL denotes confidence limits.

†Number of parent–adoptee pairs in the analysis. A pair is included in the analysis only if a parent died of causes of interest or if both parents were alive at the set age. The numbers of pairs differ mainly because of censoring of parents who died of causes not of interest.

‡P<0.01. §P<0.001. ¶P<0.05.

Is there a place for genetics?

- ✓ Rare Mendelian Diseases
- ✓ Natural Selection-Population Genetics
- ✓ Vaccine controlled examples
- ✓ Adoption studies

Navigating the Human Genome cont.

- WHY consider genetics in the study of infectious disease?
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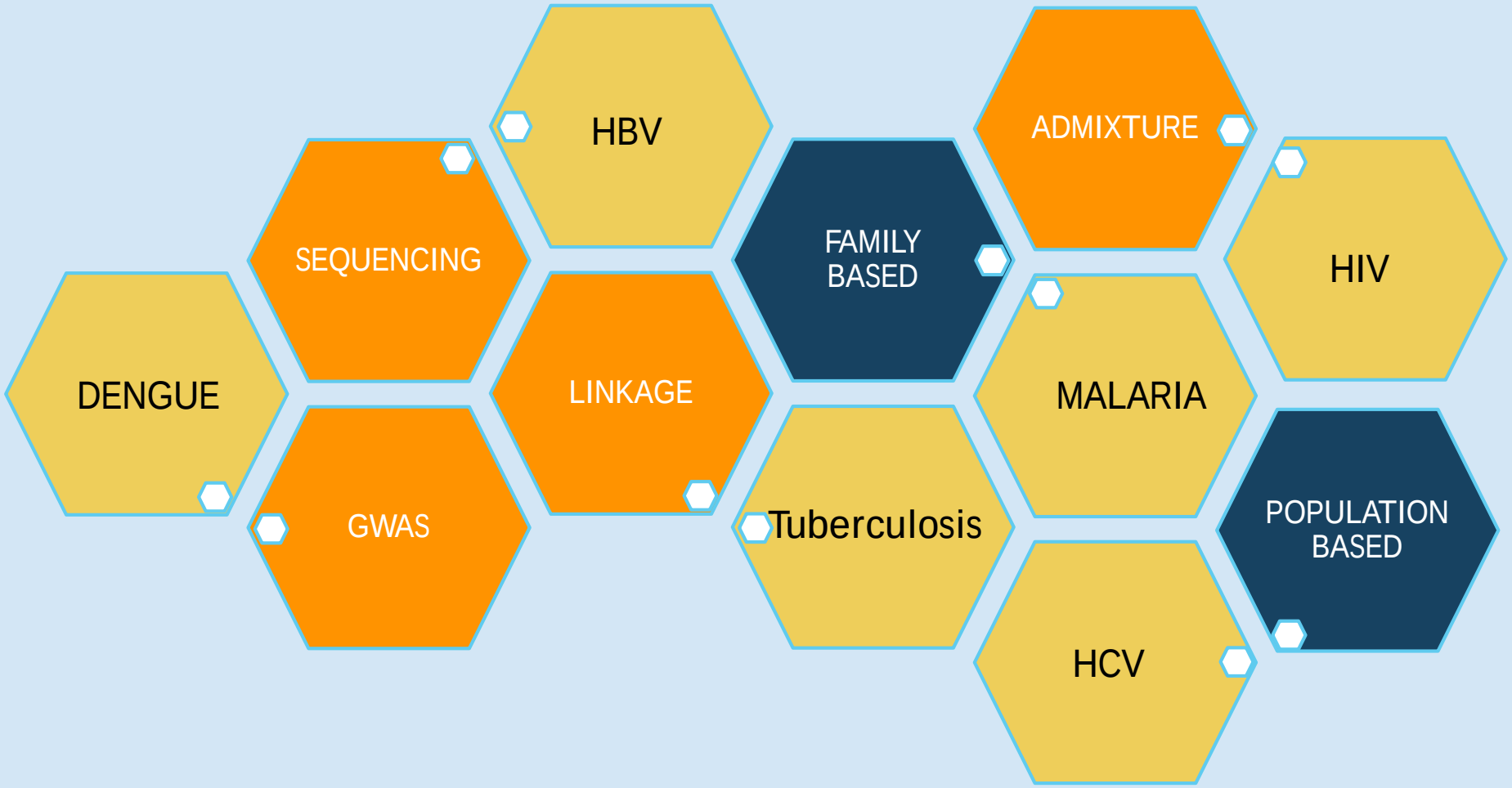
- WHAT are the implications of host genetic predispositions to infection?

How do we evaluate genetic associations?



Genetics and Infectious Disease: What do we already know?

- HIV: *HLA, CCR5, Cullin 5*
- Pulmonary TB: *MFN2, HLA, RPS4XP18*
- Malaria: *HBB, ABO, ATP2B4*
- Dengue: *MICB, PLCE1*
- HCV: *Interferon Lambda, HLA, GPR158*

University of Virginia School of Medicine

icddr,b

Bill & Melinda Gates foundation

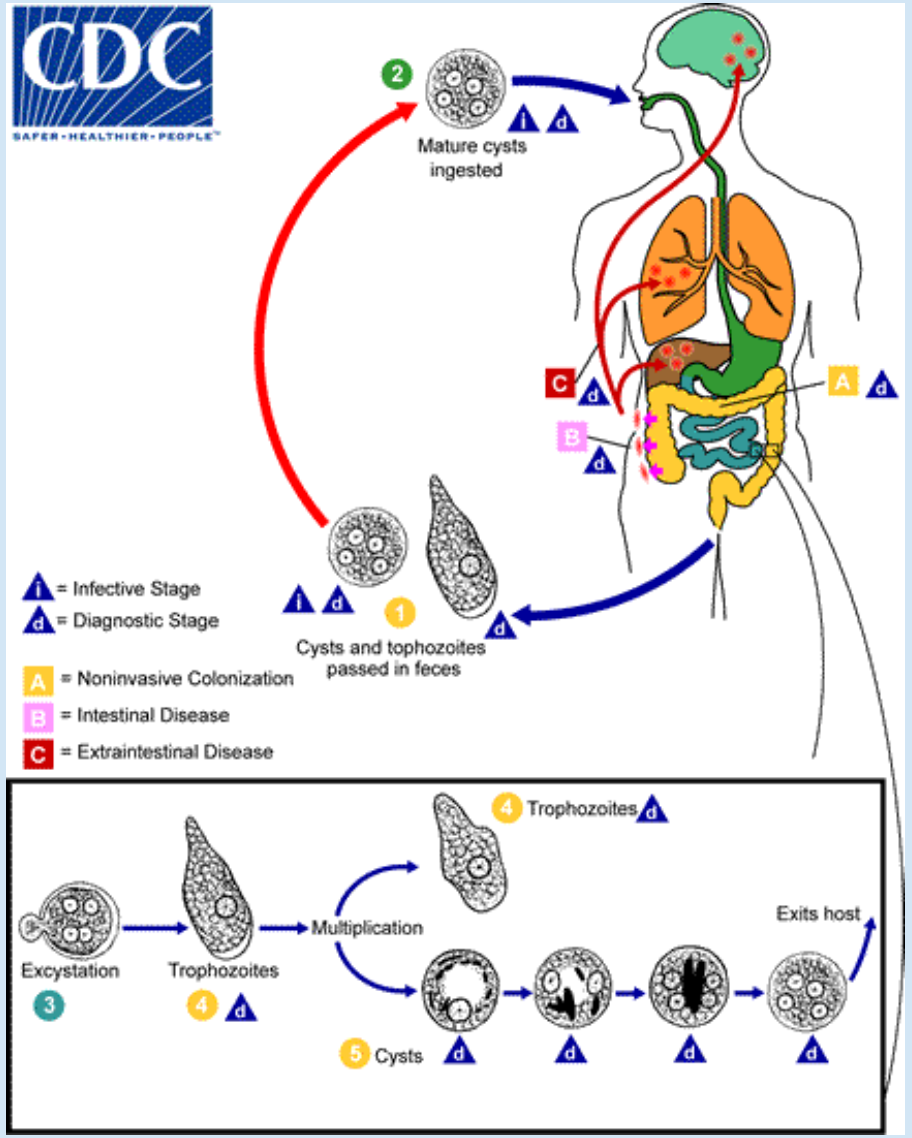
Johns Hopkins Bloomberg School of Public Health

Stanford Medicine

Genetic links between symptomatic *Entamoeba histolytica* infection and inflammatory bowel disease

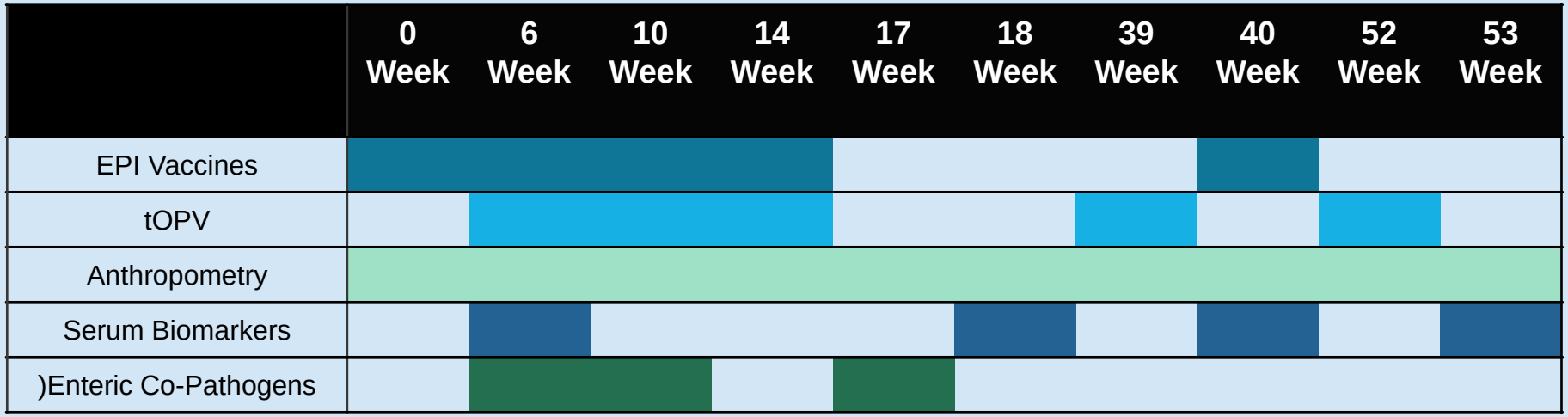
Entamoeba Histolytica

- Protozoan parasite
- Fecal-oral transmission route
- Causative agent of amebiasis
  - Colonic mucosal invasion and tissue destruction



STUDY POPULATIONS

- Birth cohort, with all infants recruited from small area of Mirpur in Dhaka, Bangladesh
- All children received all EPI vaccinations and tOPV
- Biweekly home visits with extensive characterization of symptomatic enteric and respiratory infections
- Anthropometric measurements, including stunting and wasting status
- Biomarkers of environmental enteropathy, including intestinal permeability



STUDY POPULATIONS cont.

Both the NIH Birth Cohort (DBC) and PROVIDE are birth cohorts in Mirpur, Dhaka, Bangladesh.

Case definition: A symptomatic infection (diarrheal episode) within the first year of life, as detected by RT-PCR or ELISA

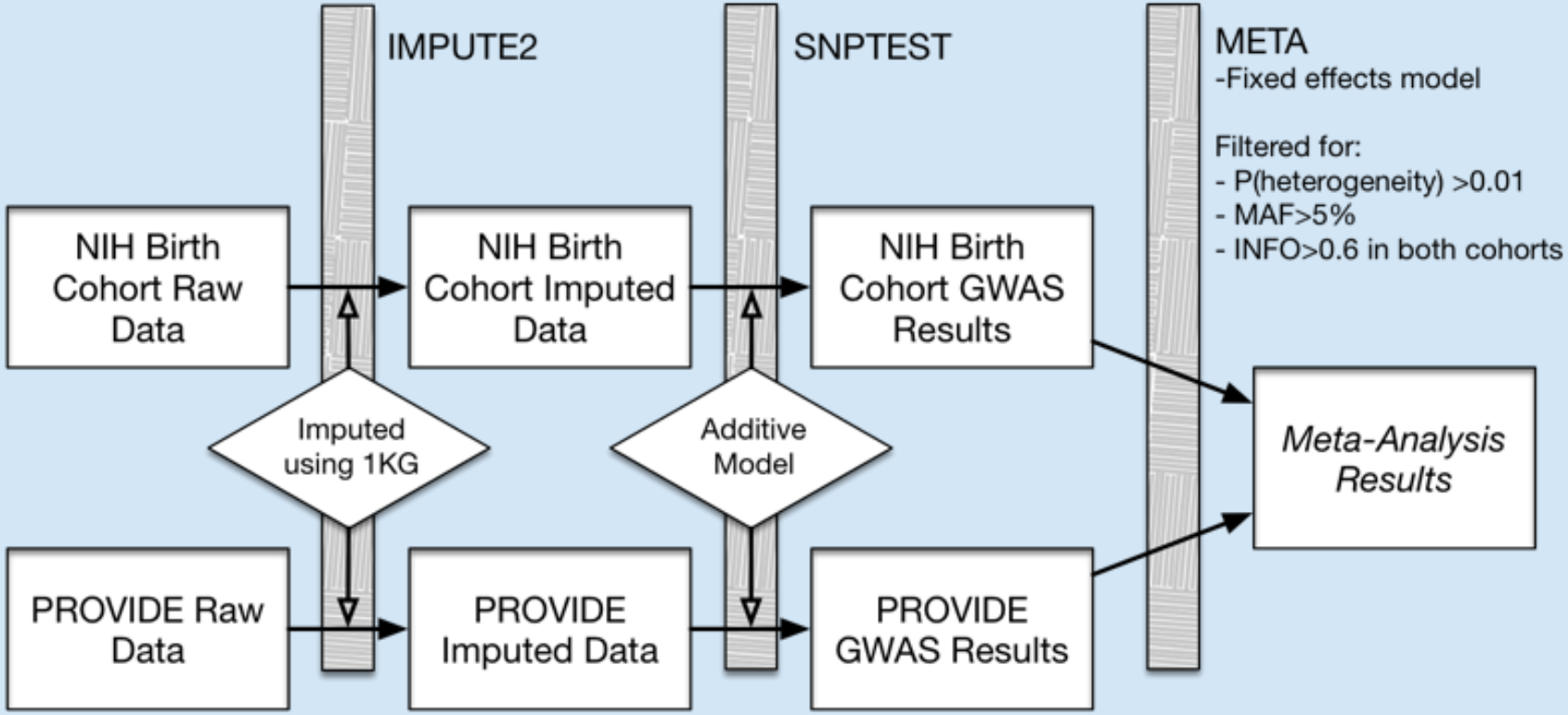
Control definition: No diarrheal episodes or monthly visits positive for E. histolytica within the first year of life

Study	EH	No EH	Total
DBC	60	252	312
PROVIDE	110	322	432
Total	170	574	744

POTENTIAL COVARIATES

Covariate	**DBC** Mean Controls	**DBC** Mean cases	**DBC** P	**PROVIDE** Mean Controls	**PROVIDE** Mean cases	**PROVIDE** P	P_{het}
$HAZ_{12\ months}$	-2.02	-2.02	0.99	-1.45	-1.47	0.83	0.85
Number of days of exclusive breastfeeding	122.7	130.54	0.42	125.5	123.0	0.69	0.41
Sex	56.3%	56.7%	0.96	54.7%	52.7%	0.73	0.79

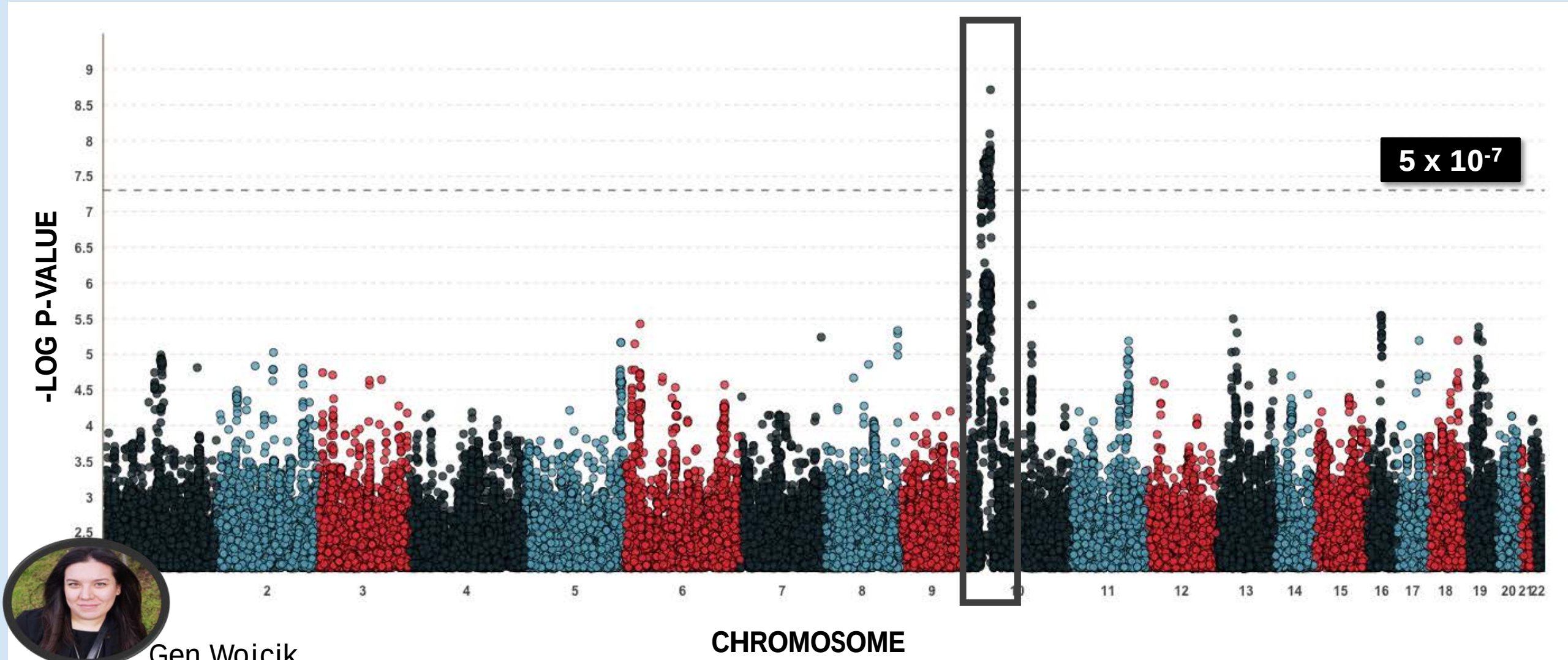
GENETIC ANALYSIS METHODS



Gen Wojcik

Test for association at 6.7 million sites genome-wide.

GENOME-WIDE RESULTS FOR DIARRHEAL EPISODES POSITIVE FOR *E. HISTOLYTICA*

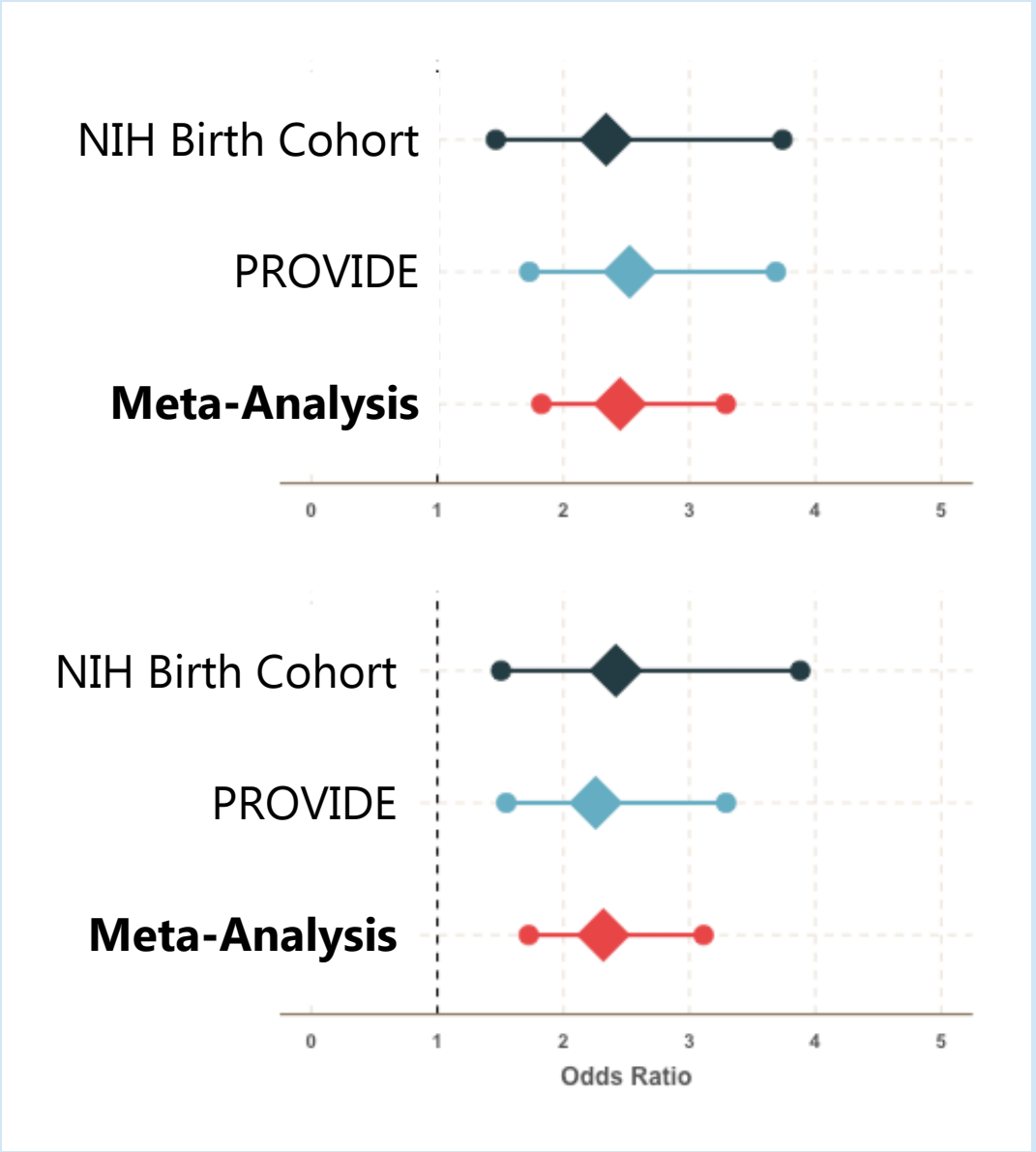


Gen Wojcik

TOP ASSOCIATIONS

rs58000832

$P_{meta}=2.4x10^{-9}$



2.34 [1.46, 3.74]

2.53 [1.73, 3.69]

2.45 [1.83, 3.29]

rs11599920

$P_{meta}=2.4x10^{-8}$

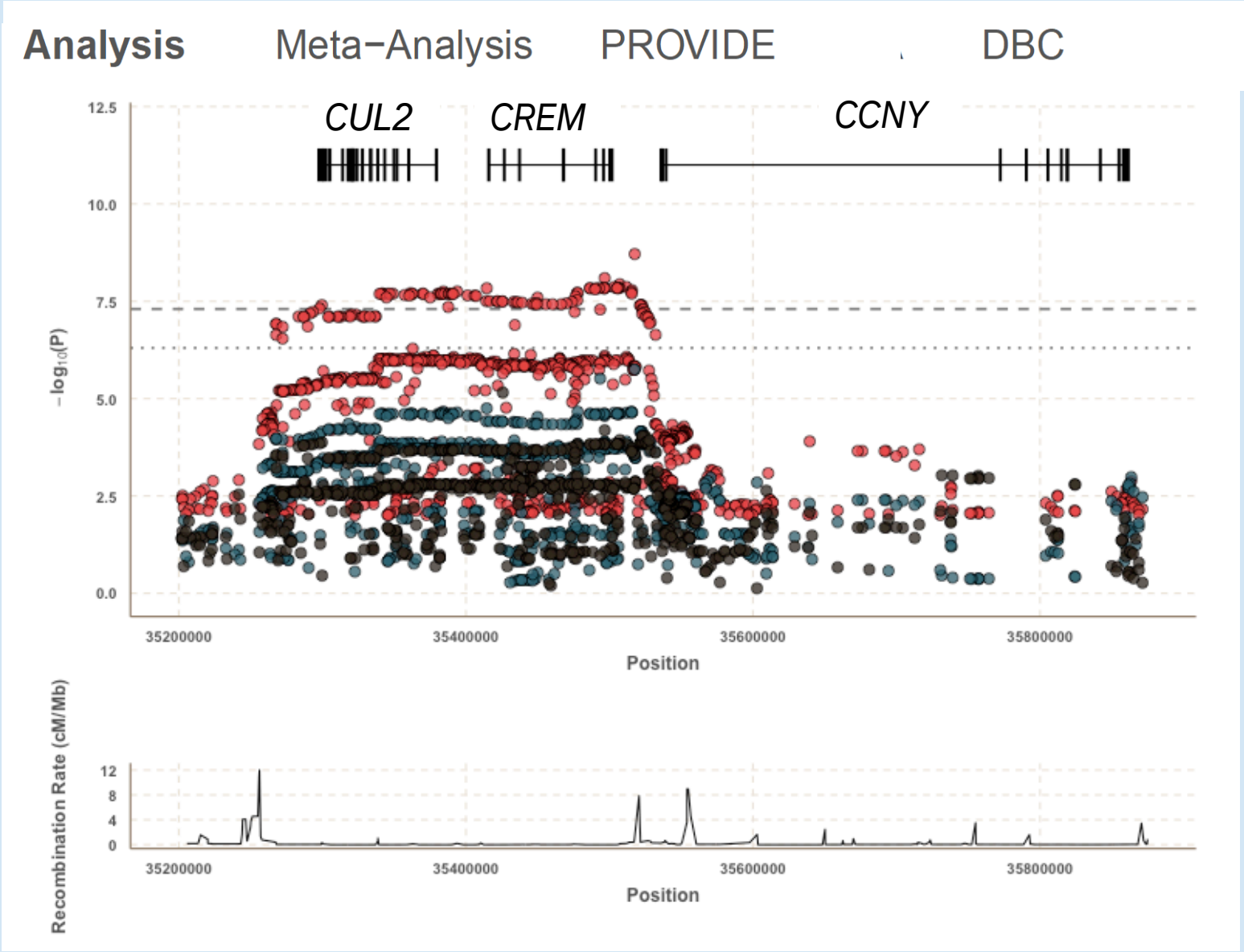
2.42 [1.51, 3.88]

2.26 [1.55, 3.29]

2.32 [1.73, 3.11]

GENETIC RECOMBINATION MAP

ASSOCIATION RESULTS



GENETIC RECOMBINATION MAP

Gen Wojcik

How do we disentangle which gene is responsible for the signal?

We can look to databases of what is known about this genetic region.

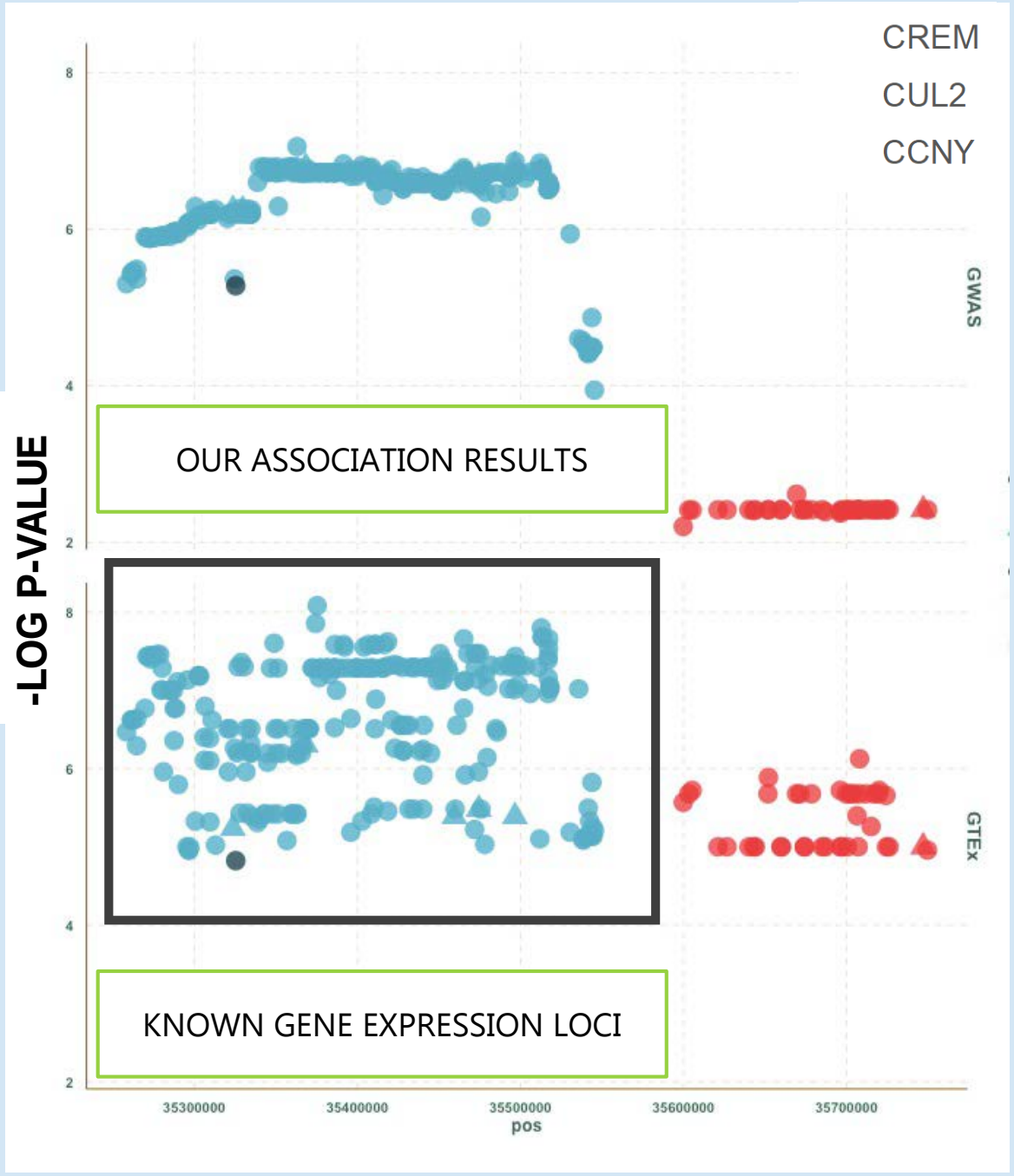
Specifically, are these SNPs known to influence the expression of a specific gene?

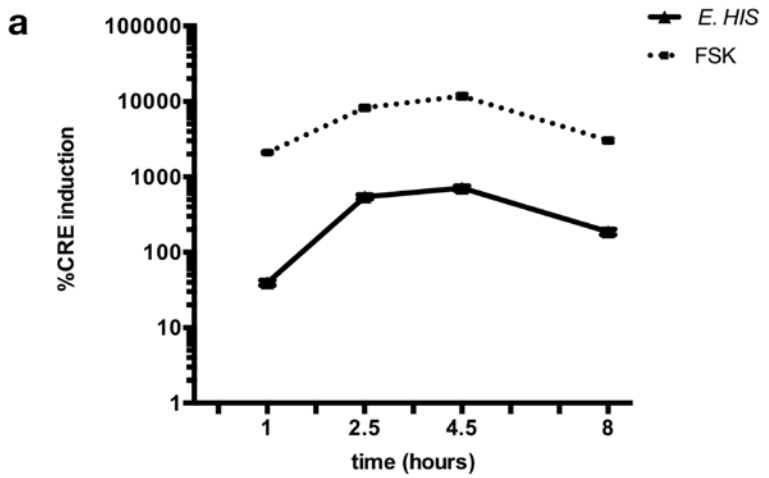
CREM seems more likely.

cAMP responsive element modulator

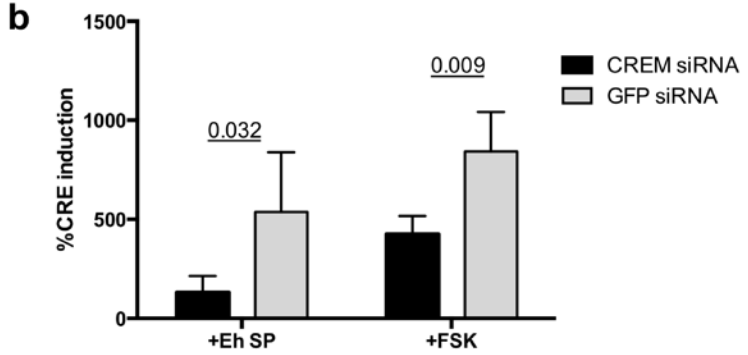
This gene encodes a bZIP transcription factor that binds to the cAMP responsive element found in many viral and cellular promoters.

GTEx Portal

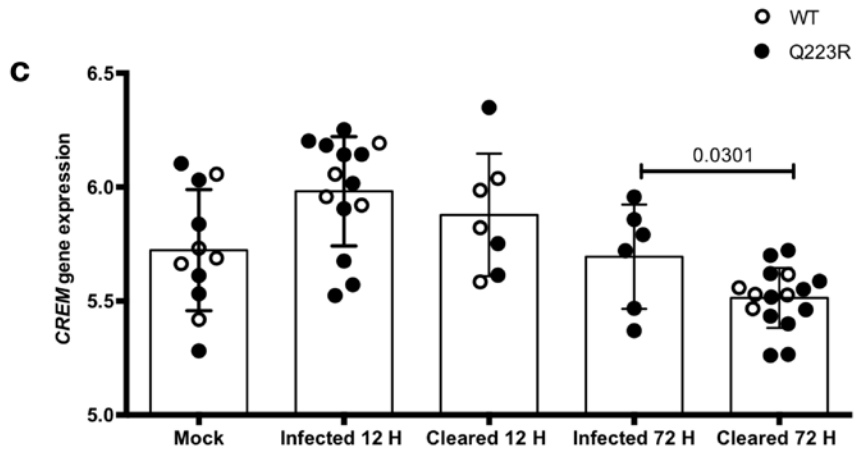




EH activates CRE-regulated gene expression in intestinal epithelial cells

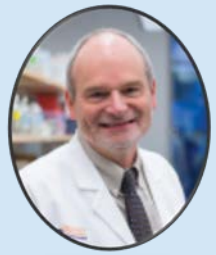


Silencing CREM decreases CRE reporter induction by EH



CREM expression is induced in early infection in mice by EH

Bill Petri and Chelsea Marie

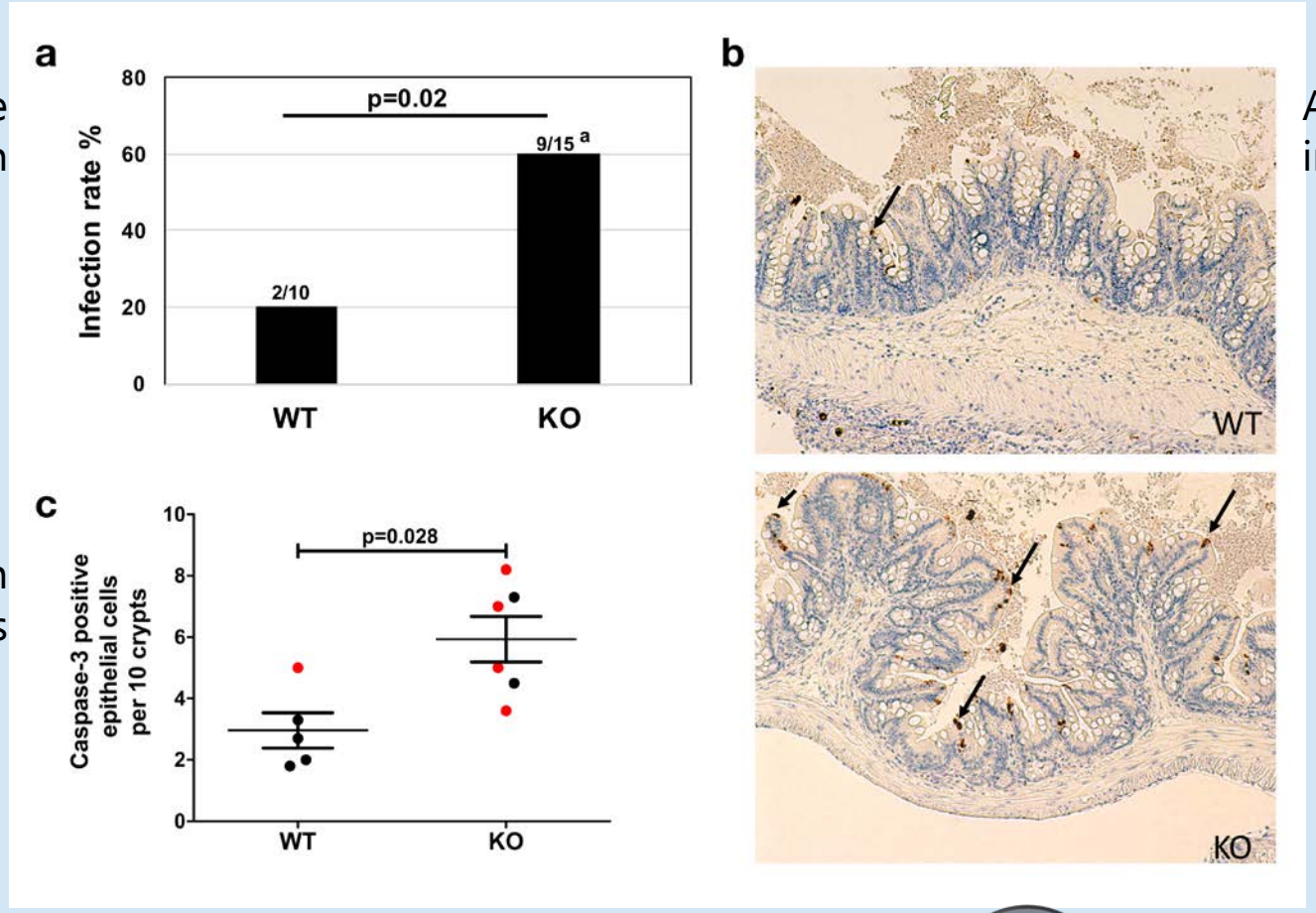


University of Virginia
School of Medicine

CREM$^{-/-}$ MICE ARE MORE SUSCEPTIBLE TO AMEBIC COLITIS

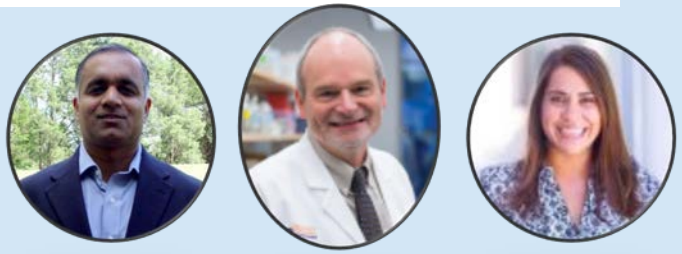
CREM -/- more susceptible to infection

CREM -/- with increased apoptosis



Apoptotic death of cecal intestinal epithelial cells

Mayuresh Abhyankar, Bill Petri, and Chelsea Marie



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KNOWN ASSOCIATIONS OF GENETIC LOCI

SNP	Functional Class	Reference	Disease	Risk Allele (Previous GWAS)	P (Previous GWAS)	OR (Previous GWAS)	Risk Allele (EH)	P (EH)	OR (EH)
rs11010067	Downstream Gene Variant	Liu JZ (2015)	Crohn's Disease	G	$1x10^{-26}$	1.142	G	$4.5x10^{-6}$	1.82
rs11010067	Downstream Gene Variant	Jostins L (2012)	Inflammatory Bowel Disease	G	$2x10^{-25}$	1.115	G	$4.5x10^{-6}$	1.82
rs34779708	Intron (CREM)	Liu JZ (2015)	Inflammatory Bowel Disease		$2x10^{-25}$	-	G	$1.2x10^{-6}$	1.88
rs12261843	Intron (CCNY)	Anderson CA (2011)	Ulcerative Colitis	G	$7x10^{-7}$	1.07	G	$8.2x10^{-5}$	1.69
rs12242110	Upstream (CREM)	Franke A (2010)	Crohn's disease	G	$1x10^{-9}$	1.15	G	$8.4x10^{-5}$	1.70
rs17582416	intergenic	Barrett JC (2008)	Crohn's disease	G	$2x10^{-9}$	1.16	G	$4.0x10^{-6}$	1.83

The same loci predispose both inflammatory bowel disease and amebiasis.

CONCLUSIONS

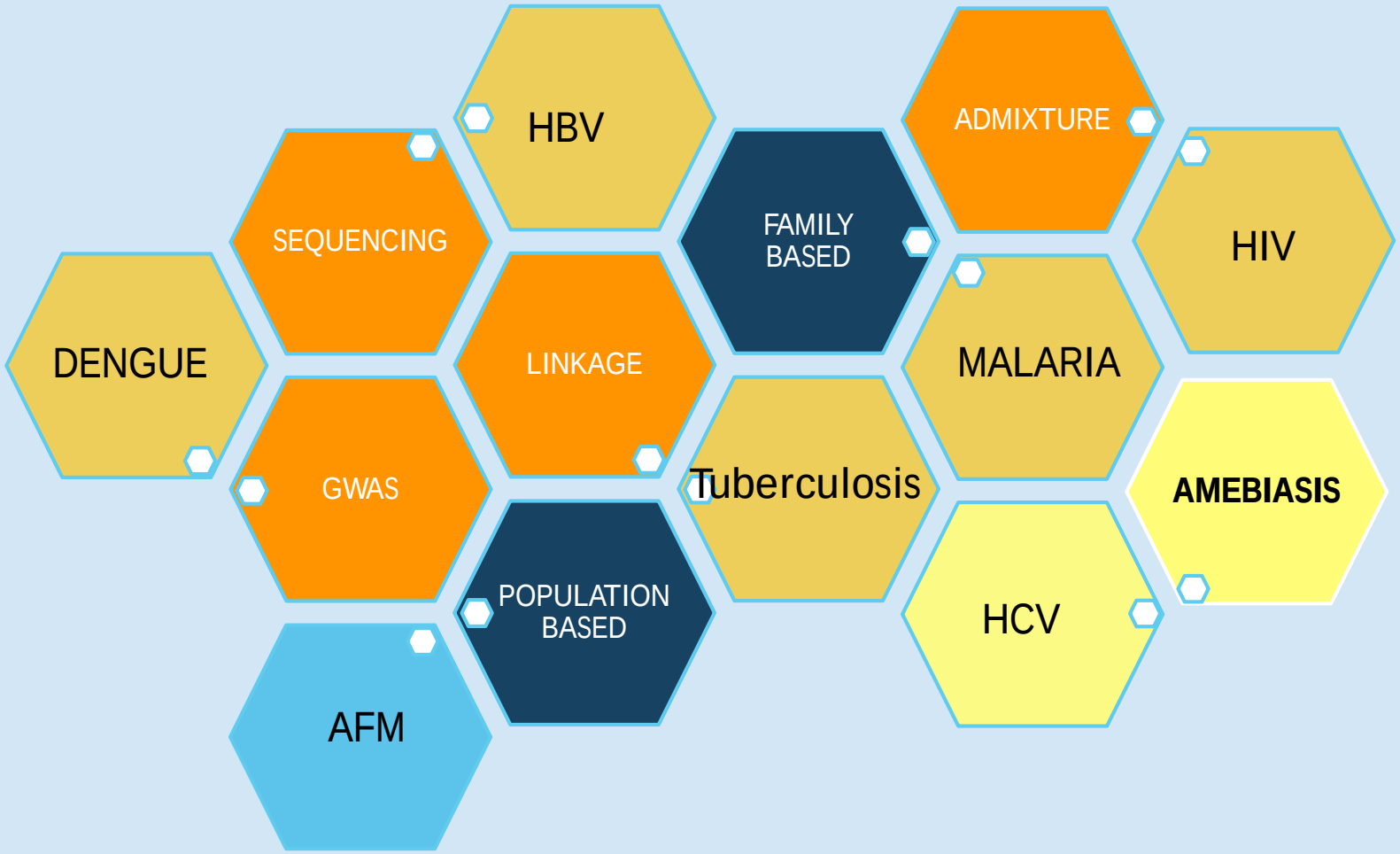
Genome-wide association study (GWAS) shows association of *CREM* with symptomatic *E. histolytica* infection.

Risk alleles also predispose individuals for inflammatory bowel disease (IBD).

Potential role for shared immune response between amebiasis and IBD.

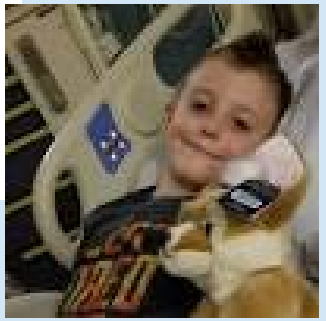
Amebiasis is one infectious disease, many other diarrheal outcomes in this population have resulted in other genome wide associations.

How to we evaluate genetic associations?



Health & Science

Mystery paralysis in children is perplexing parents — and researchers



Paralyzed with Fear: Polio Headlines


Yankees Even World Series By Taking 7-1 Victory

The Daily Tar Heel

POLIO FORCES CANCELATION OF TWO GAMES

Douglas To Be Speaker Here

Eisenhower, McGrath Also May Give Talks

Coeds Note Rush Change

Status Of Cobb Is Left Vague By Legislature

One Grid Player, 3 Others Stricken

Attempts Will Be Made To Re-Schedule Georgia And State Later This Season

Yugoslavian Cosmopolitan Prexy Elect

Dormitory Adviser's Bicycle Goes Astray

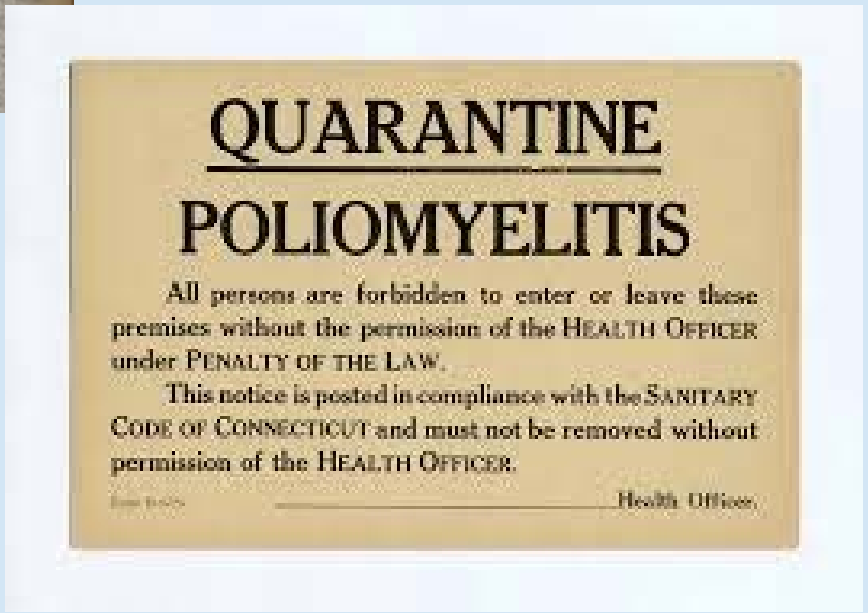

Evening Telegraph

BOY OF 4 MAKES POLIO TOTAL 26

Second Case Outside Schemes

Short Time For NCR


Polio Nursing Institute Set At Hospital


QUARANTINE

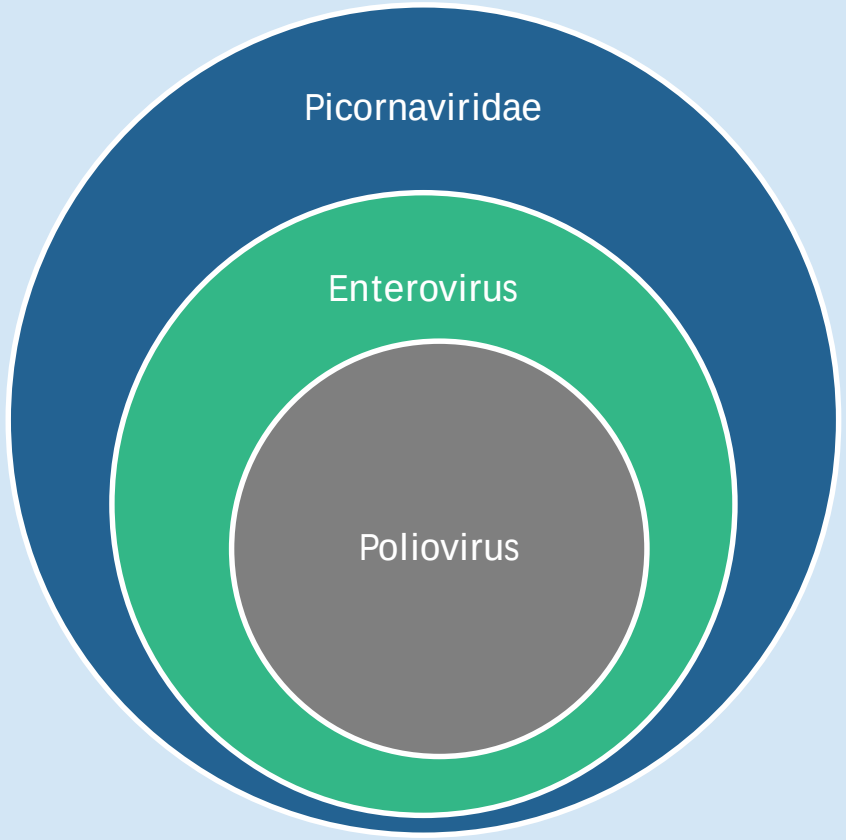
POLIOMYELITIS

All persons are forbidden to enter or leave these premises without the permission of the HEALTH OFFICER under PENALTY OF THE LAW.

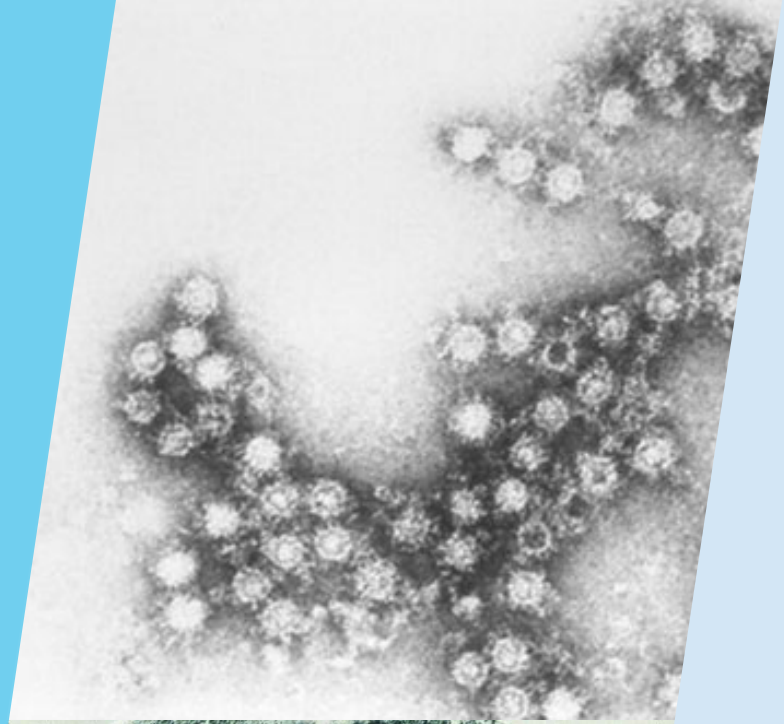
This notice is posted in compliance with the SANITARY CODE OF CONNECTICUT and must not be removed without permission of the HEALTH OFFICER.

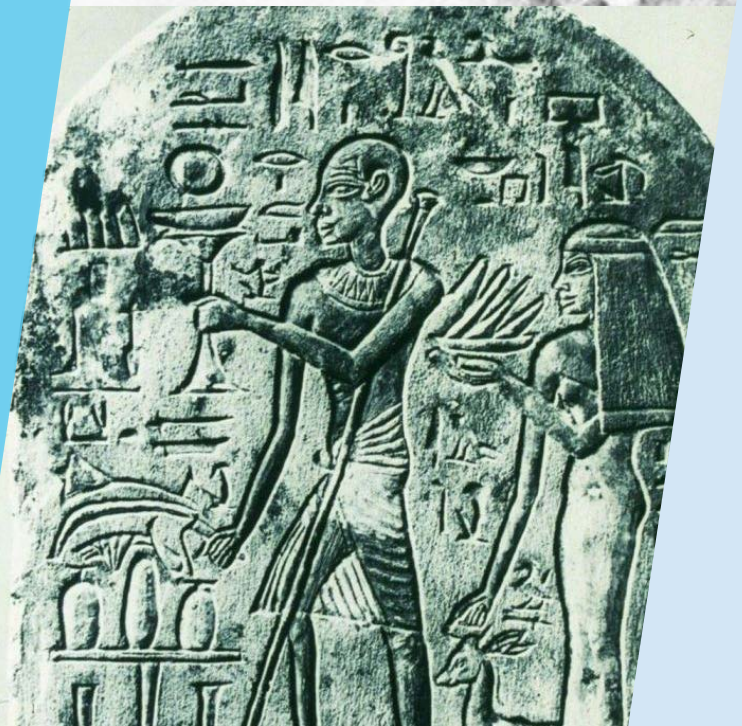
Health Officer.

Poliovirus



- Polio= grey and Myelon= marrow
- Poliomyelitis affects the grey matter, There is extensive damage to the anterior horn cells of the spinal cord. This causes limb paralysis.
- The incubation period ranges from 2 to 35 days.
- Widespread muscular atrophy occurs leading to flaccid paralysis. Death usually occurs due to respiratory paralysis in extreme cases.





Poliomyelitis Epidemic

Egyptian Paintings Depict Children with Deformed Limbs

First US localized paralytic polio epidemics begin to appear

1789

1908

1403--1365 BCE

1894

English physician Michael Underwood first describes Polio

Landsteiner and Popper hypothesize polio may be caused by a virus

Poliomyelitis Epidemic cont.

1916

NY reports an epidemic of > 27,000 polio cases and > 6000 fatalities

1931

Polio viruses 1, 2, 3 identified

1955

Jonas Salk develops inactivated polio vaccine (IPV)

1961

Albert Sabin develops live oral polio vaccine (OPV)

Poliomyelitis cases occurred every summer 1940-1950's

Polio in the world : Eradication and Vaccine

1970’s global surveys identify ”lameness” in developing countries.

Routine immunization is introduced globally

1980’s, polio was paralyzing > 1000 children worldwide every day

Global WHO Eradication efforts began in 1988-→ 3 countries still have wild polio circulating.

As polio eradication is within reach…
…in 2014 a new disease emerged

Racing to understand the polio-like illness paralyzing kids

Mysterious polio-like disease that paralyzes children leaves doctors hunting for answers

What is Acute Flaccid Myelitis?

The term “AFM” was coined in fall 2014 to describe patients ***with sudden onset of limb weakness*** but no known cause

Identical in clinical presentation to poliomyelitis and affects gray matter (neurons) of the spinal cord

AFM may be caused by other viral pathogens:

- non-polio enteroviruses
- flaviviruses (West Nile virus, Japanese encephalitis virus)
- herpesviruses
- adenoviruses

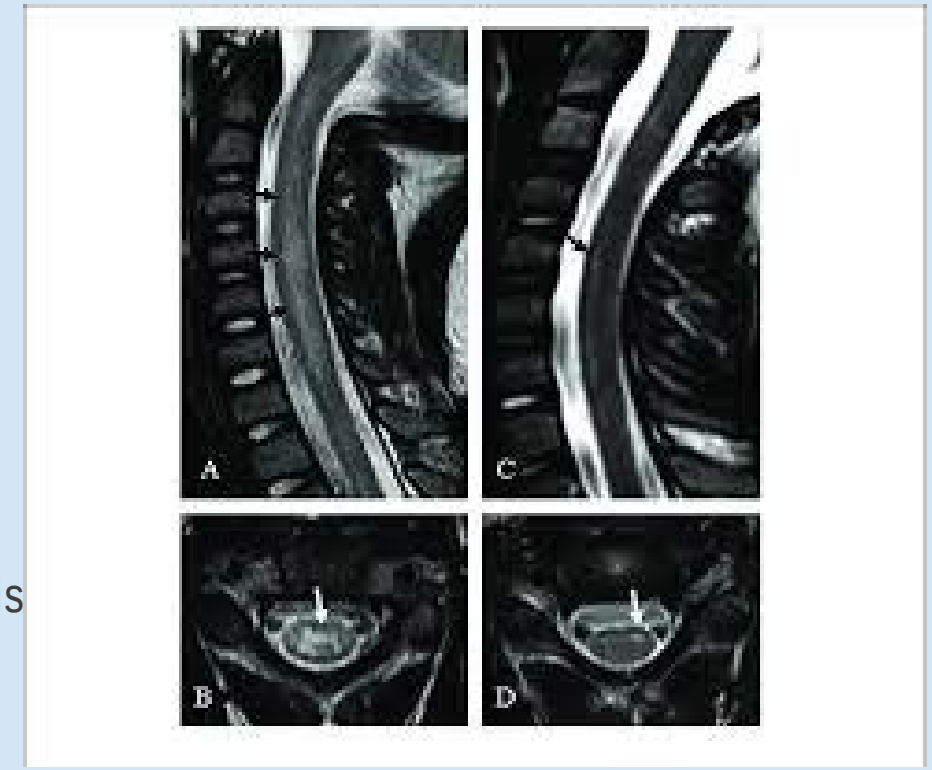


Image Maloney 2015 AJN

AFM Clinical Presentation

- Most patients describe preceding illness 1-2 weeks before weakness onset
- Symptoms include fever, rhinorrhea, cough, vomiting or diarrhea
- Onset of weakness is rapid, within hours to a few days
  - Weakness is in one or more limbs and may be accompanied by stiff neck, headache, or pain in the affected limb(s)
  - Cranial nerve abnormalities may be present
  - Facial or eyelid droop
  - Difficulty swallowing or speaking

Slide courtesy CDC

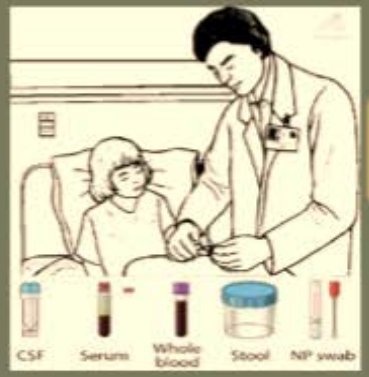
Investigation of AFM in the US, 2014

On September 12, 2014 CDC was notified of 9 children in Colorado with:

- Focal extremity weakness, cranial nerve dysfunction or both
- MRI: multi-level gray matter lesions of the spinal cord, brainstem, or ventral nerve roots
- Temporal Association: A large outbreak of respiratory illness due to enterovirus D-68 (EV-D68) occurred at the same time in Colorado

Nationally > 120 cases reported and confirmed between August-December and 5+ cases in CA, CO, MA, PA and UT

Reporting of cases of suspected AFM



Clinician suspects AFM and:
- performs MRI
- obtains specimens
- completes patient summary form

State and Local Health Department

State and local health departments:
- Reviews information from suspect case
- Collects medical data*
- Coordinates specimen transport

CDC

Confirmed | Probable

Case determination made by expert panel
- Patient form and medical data
- MRI reports and images

Case determination sent to State/ Local Health Department by CDC

Case determination sent to clinician by State/Local Health Department

* Medical data includes: hospital notes, neurology and infectious disease consult notes, MRI reports and images, laboratory test results, vaccination history, and discharge summary

Slide courtesy CDC

What did we learn about AFM cases in 2014?

Who is getting paralyzed?

- Age

Children 3-7 years of age

- Sex

No preference

- Immune status

Healthy kids, no underlying conditions reported

- What characteristics do they share?

Healthy, mild viral infection preceded paralysis

- Is there a common exposure?

?

- Blood samples/CSF samples

Collected and tested for viral pathogens

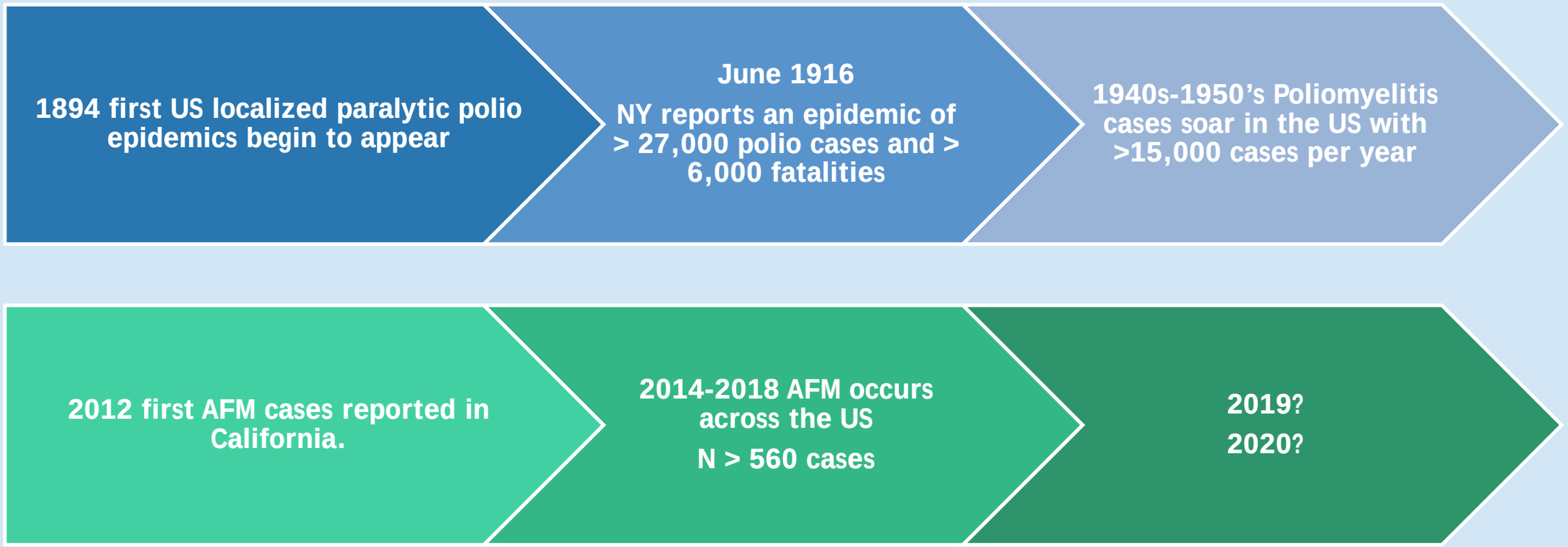
- Clinical information

Heterogeneity in paralysis

- Information from parents about pre-disease exposures, state etc.

Kids were vaccinated for poliovirus; many family members in household had the viral infection, but only 1 paralyzed

Poliomyelitis and AFM in US



AFM cases peak every two years 2012-2018+

Two Main Questions

What causes AFM?

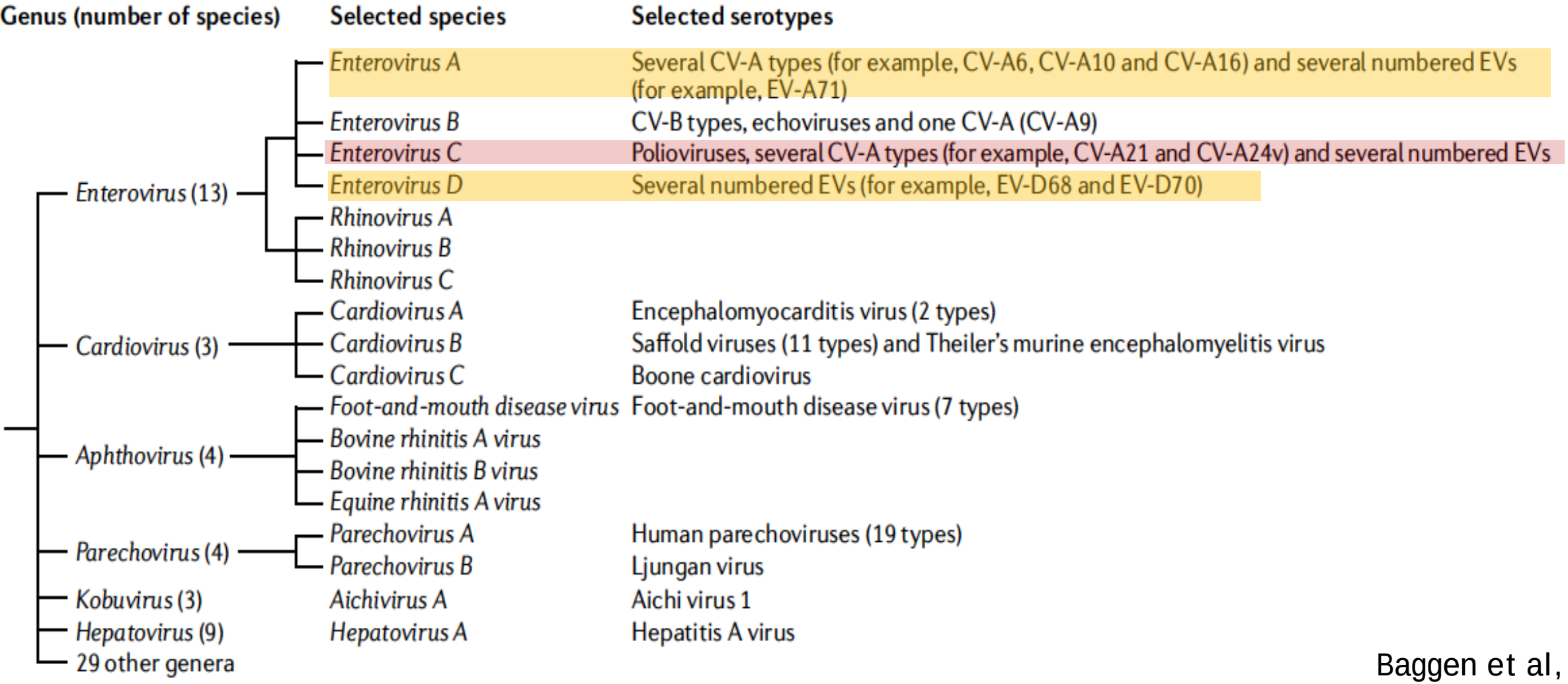
- **If we know the viral cause can we develop a vaccine?**

Why do people get AFM?

Not everyone gets it?
1/100,000- 1-2/million.

But what makes these people different?

Cause: Enterovirus Phylogeny (non enveloped viruses)



Genus (number of species)	Selected species	Selected serotypes
Enterovirus (13)	*Enterovirus A*	Several CV-A types (for example, CV-A6, CV-A10 and CV-A16) and several numbered EVs (for example, EV-A71)
	Enterovirus B	CV-B types, echoviruses and one CV-A (CV-A9)
	Enterovirus C	Polioviruses, several CV-A types (for example, CV-A21 and CV-A24v) and several numbered EVs
	Enterovirus D	Several numbered EVs (for example, EV-D68 and EV-D70)
	Rhinovirus A	
	Rhinovirus B	
	Rhinovirus C	
Cardiovirus (3)	*Cardiovirus A*	Encephalomyocarditis virus (2 types)
	Cardiovirus B	Saffold viruses (11 types) and Theiler's murine encephalomyelitis virus
	Cardiovirus C	Boone cardiovirus
Aphthovirus (4)	*Foot-and-mouth disease virus*	Foot-and-mouth disease virus (7 types)
	Bovine rhinitis A virus	
	Bovine rhinitis B virus	
	Equine rhinitis A virus	
Parechovirus (4)	*Parechovirus A*	Human parechoviruses (19 types)
	Parechovirus B	Ljungan virus
Kobuvirus (3)	*Aichivirus A*	Aichi virus 1
Hepatovirus (9)	*Hepatovirus A*	Hepatitis A virus
29 other genera		

Baggen et al, 2018

What causes AFM? Enterovirus A71 (EV-A71)

First isolated in 1965, causal for asceptic meningitis

Major cause of hand, foot and mouth disease

Usually mild and self-limiting disease, but can cause cardiopulmonary complications

Considered the *most neurotropic non-polio enterovirus*

Major public health threat in 1990's predominantly in Asia

- > 10 million HFMD cases and > 80,000 associated with neuro disease and > 3000 fatalities

Multiple genotypes of EV-A71 have been identified and circulation is regional and temporally restricted

What causes AFM? Enterovirus D-68 (EV-D68)

First isolated from children with respiratory infections in California in 1962 but ***only 26 cases reported before 2006***

Most often associated with mild respiratory infections, but can also result in severe bronchiolitis or pneumonia

In 2014, largest outbreak of EVD68 reported in US with 1,153 confirmed infections and likely millions of untested milder cases.

What causes AFM?
Bradford Hill Criteria

Criterion	Description
Strength	Whether those with the exposure are at a higher risk of developing disease and if so, how much more risk? This criterion suggests that a larger association increases the likelihood of causality.
Consistency	The credibility of findings increases with repetition of findings, including consistency of study findings across different populations and geographical locations.
Specificity	Causality is more likely if the exposure causes only one specific disease or syndrome, or if a specific location or population are being affected.
Temporality	This criterion requires that the exposure must occur before the disease, and not after a latency period that is too long. This criterion must always be fulfilled for causality to be concluded.
Biological gradient	The argument for causality is stronger in the presence of a dose–response relationship, where higher or longer exposure leads to an increased risk of disease.
Plausibility	A conceivable mechanism for causation between disease and exposure should exist for there to be a causal relationship.
Coherence	The current association should not contradict any previous knowledge available about the disease and/or exposure.
Experiment	This criterion can involve scientific experiments and addresses the association of exposure with disease. However, ‘experiment’ relates to the decrease in disease risk when the exposure is removed and often involves animal models.
Analogy	This criterion uses previous evidence of an association between a similar exposure and disease outcome to strengthen the current argument for causation.

Summary

- 7 criteria met for Bradford Hill
- Some overall consensus by scientific researchers that EVD-68 is a causal virus. Although not yet officially stated.
- All acknowledge, that more than 1 virus may be at play
- More work to be done

AFM & Poliomyelitis

Acute Flaccid Myelitis

- Triggered by a viral exposure
- Sporadic cases across the United States
- No familial or neighborhood clustering
- Not everyone exposed has viral symptoms
- Not everyone with viral symptoms develops paralysis
- Paralysis is on a spectrum

Poliomyelitis

- **Triggered by a viral exposure**
- **Initially sporadic cases, then clustered, global**
- **Modest familial clustering**
- **Not everyone exposed had viral symptoms**
- **Not everyone with viral symptoms developed paralysis**
- **Paralysis is on a spectrum**

What can tragedy teach us?

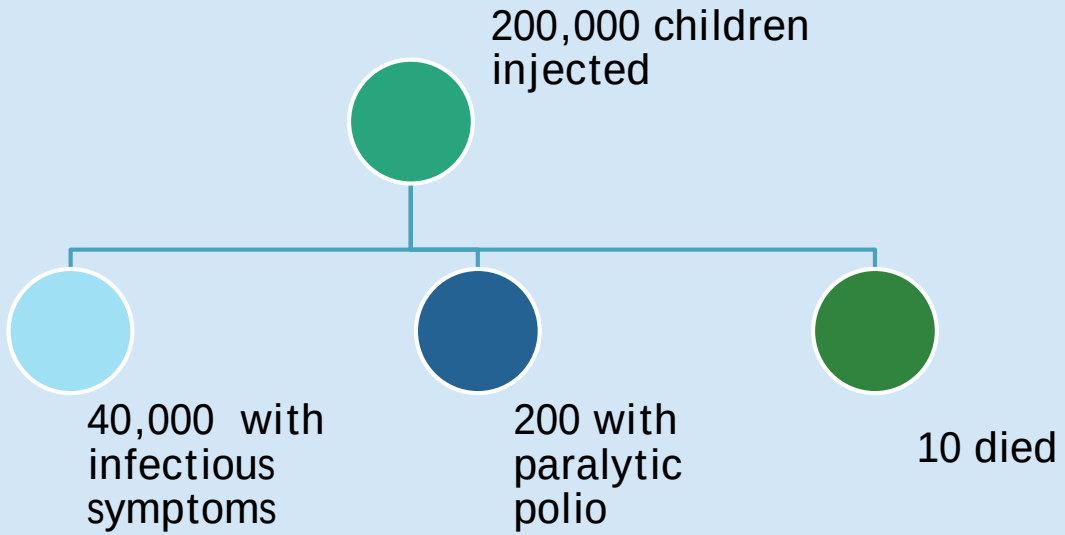
The Cutter Incident

Polio vaccine administered that was not inactivated to school age children

Same active virus administered but with different outcomes

Why, if everyone got a similar infectious dose of live virus did not everyone get infected?

Why did not everyone get paralysis?



Factors affecting clinical heterogeneity of AFM?

Age

Theory: Older individuals have waning immune systems

More children affected. Age may play a role, but not clear what role it is playing.

Sex

Theory: Biologic differences in immune responses due to sex and hormones

Reports suggest no major sex difference in AFM among the children.

Co-infections/Co-morbidities

Theory: co-I or co-morbidities exacerbate disease and susceptibility

Most children reported to have AFM are healthy and in general do not have underlying immune related or neurologic conditions.

What else may be playing a role in the host?

What can poliomyelitis teach us about host genetics?

1942 Addair and Snyder

- Polio cases in West Virginia. All 29 cases over 50 years occurred in 25 related families

1982-1987 Wyatt

- Evaluated original notes of 1,072 Maltese cases of poliomyelitits from 1909-1964. Traced relatives
- 956 polio cases, 54% were related as sibs, 1st or 2nd cousins.
- 13 pairs of sibs where both had paralytic polio. But the younger siblings was born months or years after first sibling was paralyzed suggesting dosage was not a factor.

Lesson for AFM: Familial aggregation of disease, but specifically in 1st and 2nd degree relatives.

What can poliomyelitis teach us about host genetics? Cont.

Twins and Polio (American Journal of Human Genetics 1951)

- Evaluated presumed monozygotic and dizygotic twins.
- This is an established method in genetic epidemiology to determine the genetic contribution to disease or heritability.
  - 5/14 "monozygotic" twin pairs had paralytic polio (36%)
  - 2/33 dizygotic twin pairs had paralytic polio (6%)
  - No parents had a history of poliomyelitis. No known intermarriage
  - Suggested that this was a recessive gene with relatively high frequency in the population.

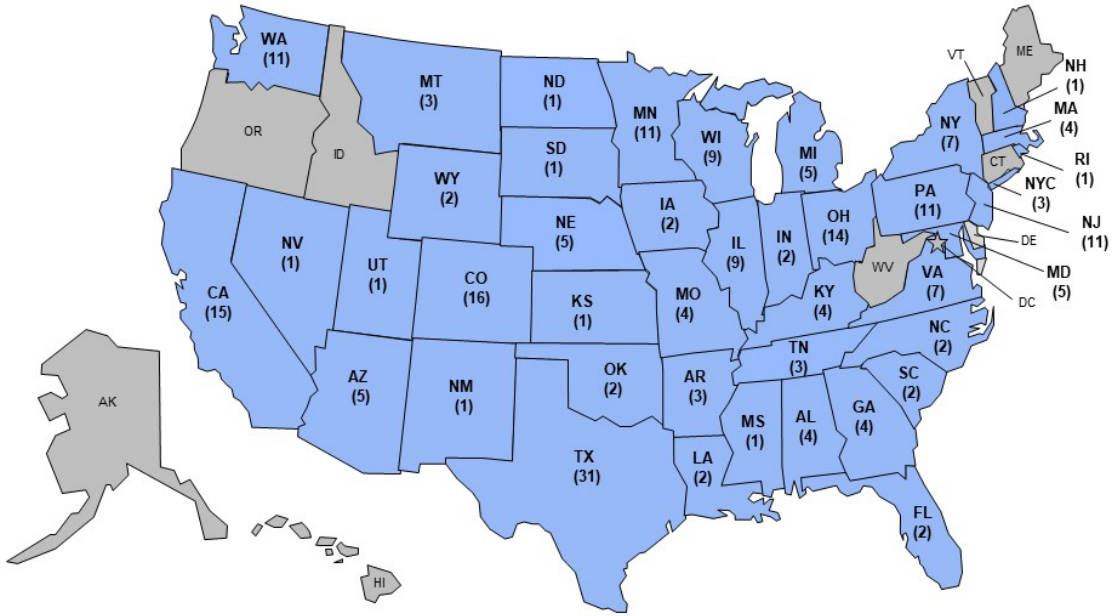
Lesson for AFM: Evidence of genetic heritability for poliomyelitis. We currently have no known monozygotic twins with AFM. Even among those twins, the penetrance of the putative genes is not 100% suggesting it may be modified by other genes, or non-genetic factors. (36%).

What can poliomyelitis teach us?

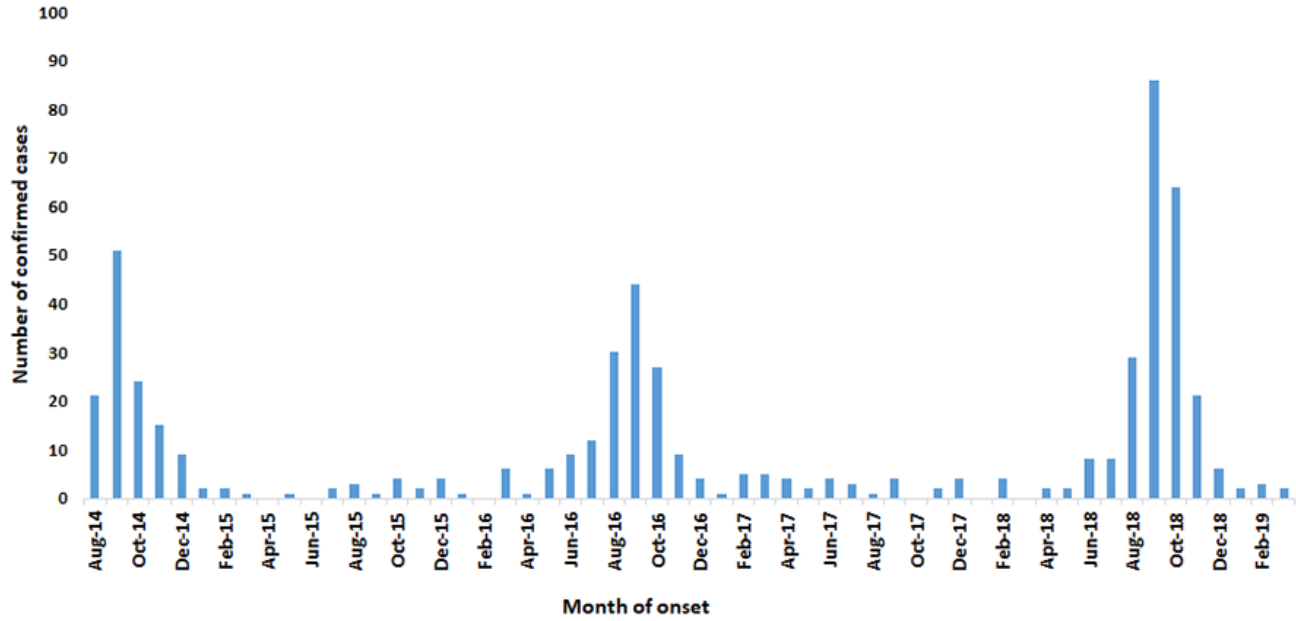
- In a survey of 222 families (Paul, Salinger, Trask 1932) with a child case of poliomyelitis
  - 39% of Siblings 1-4 years of age had mild viral symptoms
  - 32% of Siblings 5-9 years of age had mild viral symptoms
- In 60 control families with no poliomyelitis case
  - 9% of siblings < 10 years of age had mild viral symptoms

Lesson for AFM:. Exposure is important to define a control. Using family based samples will help to insure that a "control" was exposed and still did not develop paralysis.

2018 confirmed cases of acute flaccid myelitis (AFM) by state (N=230)*



Number of confirmed U.S. AFM cases reported to CDC by month of onset, August 2014 - March 2019^*†



Genetics Study Design

- Started in Fall, 2014
- Case-Family design
- Siblings serve as direct controls to the cases
  - Similar in age
  - Parents report similar viral exposure
  - Key that we know the "controls" are also exposed to what the case was exposed to (i.e. enterovirus)
- All first degree relatives are exome sequenced (the coding region of the genome)

AFM Genetics Participant Recruitment

1. **Study Advertisement**

2. **Participants Contact Research Team**

3. **JHU Research Team Consents Family**

4. **JHU Research Team Sends Study Materials**

5. **Family Completes & Returns Study Materials**

6. **Clinical Medical Chart Review**

7. **Extraction of DNA from Saliva Kits**

JHU Genetics Study Design

- Compare the genetic sequence of cases to other cases to identify regions of the genome that are similar among those children with AFM

- Compare the genetic sequence of the cases to their "control" siblings to identify genetic variants that may explain the discordant outcomes.

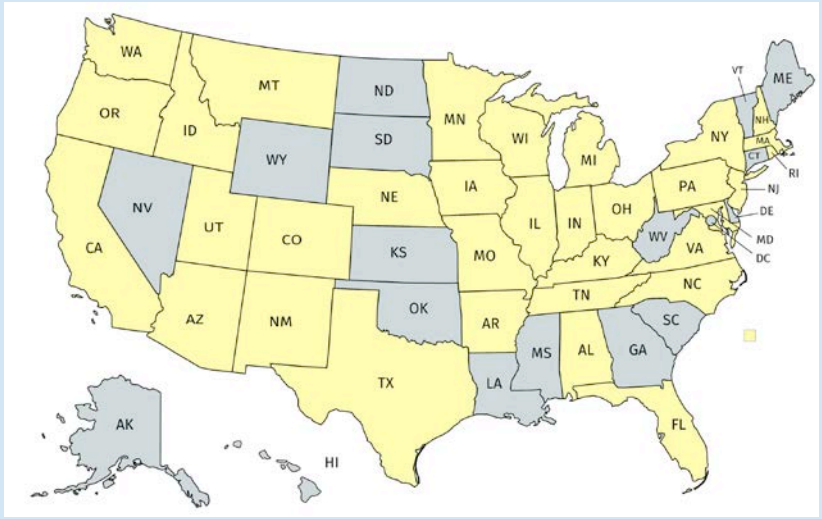
- Use parental genotypes to identify de novo mutations, or those that may be passed on without parental paralysis

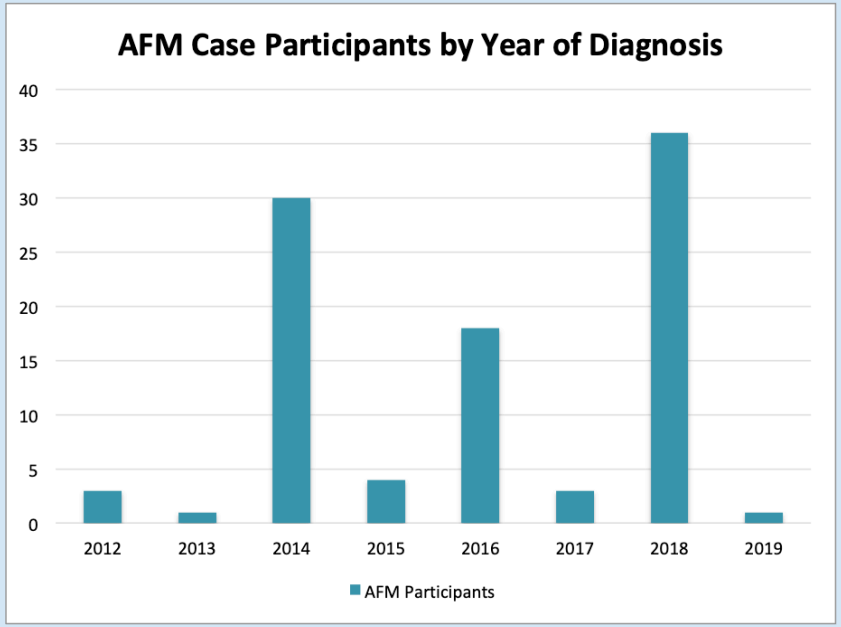
- Compare the genetic sequence of AFM cases with historic Poliomyelitis cases (collected simultaneously)

AFM Genetic Research Study

100 AFM cases 2012-2019*

Mean age of child cases	**5.0 years (SD 4.2)**
Mean age of adult cases (n=4)	30.0 years (SD 1.0)
Sex M:F	61:39
Number with Limb Paralysis	
1-3 limbs	82
All Limbs	18
Self Reported Race/Ethnicity	
White	53%
Black	1%
Asian	2%
Native American	1%
Hispanic	1%
Other/Mixed	42%





Summary of Hopkins Studies

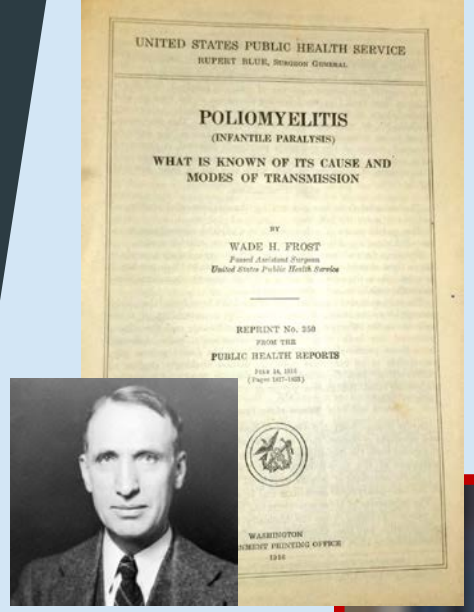
- We established a research definition for AFM using clinical data, and this has been confirmed in 2 separate smaller studies. We are preparing our own validation with enrolled families. (Elrick et al, JAMA Peds 2019)
- We have >100 AFM families enrolled in the genetic study, and we have exome sequenced 45 case/case families
- We have > 80 historic polio cases enrolled in the genetic study
- We are working as a part of the AFM working group to develop protocols and establish clinical and research plans
- We are working to understand viral EVD-68 (Pekosz) and peptide/Immune response for biomarkers (Larman)
- What will we see in 2020 and beyond?
  - Polio was stopped, and now neary eradicated by vaccines.
  - BUT, we never understood mechanism. *If only we did.*

Summary cont.

Not everyone exposed to polio or enteroviruses will be paralyzed.	• Who is at risk? What can we do?
Temporal and Laboratory evidence to suggest that EVD-68 is the causative agent.	• How do we control EVD-68?
Acute Flaccid Myelitis seems to be mirroring what we saw in the early days of poliomyelitis	• Sporadic Global Cases --->will there be a large scale epidemic?
How do we prevent large numbers of AFM cases?	• Poliomyelitis was stopped via vaccine

Acute Flaccid Myelitis Acknowledgements

- Aaron Milstone, David Thomas, Carlos Pardo, Matthew Elrick,Tom Crawford, Elizabeth Dee, Nicole Thornton, Ani Voskertchian, Cristian Valencia, Andy Pekosz, Ben Larman
- Numerous physicians across the US that have been critical to the collection and enrollment of these cases.
- Incredible national AFM working group dedicated to trying to answer these critical questions working with CDC/NIH
- Funding:
  - Johns Hopkins Catalyst Award
  - NIH Sequencing Center


UNITED STATES PUBLIC HEALTH SERVICE
RUPERT BLUE, SURGEON GENERAL

POLIOMYELITIS
(INFANTILE PARALYSIS)
WHAT IS KNOWN OF ITS CAUSE AND MODES OF TRANSMISSION

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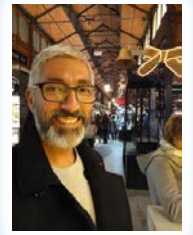

The following papers have been sited:

- Genome-Wide Association Study Reveals Genetic Link between Diarrhea-Associated Entamoeba histolytica Infection and Inflammatory Bowel Disease (on the E. histolytica slides)
- Enterovirus D68 and acute flaccid myelitis-evaluating the evidence for causality (on the bradford-hill slide)