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

Infectious Pathogen → Host → Disease

Do you get infected?
Do you get disease?
Does it matter? Everyone gets infections.

Heterogeneity

- Tuberculosis: Estimated that 1/3 of the world's 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

Infectious Pathogen

What are you exposed to?

Age, Sex, other covariates

Virulence

Host

Do you get infected?

Age, Sex, other covariates

Do you get disease?

Disease

Genetics

Genetics
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
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

<table>
<thead>
<tr>
<th>Disease</th>
<th>Blood Type</th>
<th>Association</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholera</td>
<td>0</td>
<td>Increased susceptibility</td>
<td>Sircar, 1981</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Clemens, 1989</td>
</tr>
<tr>
<td>Shigella</td>
<td>0</td>
<td>Increased susceptibility</td>
<td>Robinson, 1971</td>
</tr>
<tr>
<td>Norovirus</td>
<td>0</td>
<td>Increased susceptibility</td>
<td>Hutson, 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hennessey, 2003</td>
</tr>
<tr>
<td>P. Falciparum</td>
<td>0</td>
<td>Protected</td>
<td>Rowe, 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cserti, 2007</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>A</td>
<td>Increased Susceptibility</td>
<td>Lima, 1979</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ndamba, 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Camus, 1977</td>
</tr>
</tbody>
</table>
## Twin Studies

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

* 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
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.*

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Parent Dead Before the Age of 50</th>
<th>Parent Dead Before the Age of 70</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO.†</td>
<td>RR</td>
</tr>
<tr>
<td>All causes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biologic</td>
<td>779</td>
<td>1.71$</td>
</tr>
<tr>
<td>Adoptive</td>
<td>913</td>
<td>0.71</td>
</tr>
<tr>
<td>Natural causes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biologic</td>
<td>739</td>
<td>1.98$</td>
</tr>
<tr>
<td>Adoptive</td>
<td>889</td>
<td>0.96</td>
</tr>
<tr>
<td>Infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biologic</td>
<td>641</td>
<td>5.81$</td>
</tr>
<tr>
<td>Adoptive</td>
<td>840</td>
<td>0.73</td>
</tr>
<tr>
<td>Vascular causes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biologic</td>
<td>585</td>
<td>4.52$</td>
</tr>
<tr>
<td>Adoptive</td>
<td>822</td>
<td>3.02</td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biologic</td>
<td>593</td>
<td>1.19</td>
</tr>
<tr>
<td>Adoptive</td>
<td>818</td>
<td>5.16$</td>
</tr>
</tbody>
</table>

*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
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?
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
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

<table>
<thead>
<tr>
<th></th>
<th>0 Week</th>
<th>6 Week</th>
<th>10 Week</th>
<th>14 Week</th>
<th>17 Week</th>
<th>18 Week</th>
<th>39 Week</th>
<th>40 Week</th>
<th>52 Week</th>
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<td>EPI Vaccines</td>
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<td>tOPV</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Anthropometry</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Serum Biomarkers</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteric Co-Pathogens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Study</th>
<th>EH</th>
<th>No EH</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBC</td>
<td>60</td>
<td>252</td>
<td>312</td>
</tr>
<tr>
<td>PROVIDE</td>
<td>110</td>
<td>322</td>
<td>432</td>
</tr>
<tr>
<td>Total</td>
<td>170</td>
<td>574</td>
<td>744</td>
</tr>
</tbody>
</table>
## POTENTIAL COVARIATES

<table>
<thead>
<tr>
<th>Covariate</th>
<th>DBC Mean Controls</th>
<th>DBC Mean cases</th>
<th>DBC P</th>
<th>PROVIDE Mean Controls</th>
<th>PROVIDE Mean cases</th>
<th>PROVIDE P</th>
<th>P het</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAZ_{12} months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of days of exclusive breastfeeding</td>
<td>122.7</td>
<td>130.54</td>
<td>0.42</td>
<td>125.5</td>
<td>123.0</td>
<td>0.69</td>
<td>0.41</td>
</tr>
<tr>
<td>Sex</td>
<td>56.3%</td>
<td>56.7%</td>
<td>0.96</td>
<td>54.7%</td>
<td>52.7%</td>
<td>0.73</td>
<td>0.79</td>
</tr>
</tbody>
</table>
GENETIC ANALYSIS METHODS

Test for association at 6.7 million sites genome-wide.
GENOME-WIDE RESULTS FOR DIARRHEAL EPISODES POSITIVE FOR \textit{E. histolytica}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{genomic_plot.png}
\caption{Genomic plot showing \(-\log P\)-values across chromosomes. A peak at chromosome 9 with a \(-\log P\)-value of \(5 \times 10^{-7}\).}
\end{figure}

Gen Wojcik
**TOP ASSOCIATIONS**

rs58000832
\[ P_{\text{meta}} = 2.4 \times 10^{-9} \]

rs11599920
\[ P_{\text{meta}} = 2.4 \times 10^{-8} \]
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.
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
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
<table>
<thead>
<tr>
<th>SNP</th>
<th>Functional Class</th>
<th>Reference</th>
<th>Disease</th>
<th>Risk Allele (Previous GWAS)</th>
<th>P (Previous GWAS)</th>
<th>OR (Previous GWAS)</th>
<th>Risk Allele (EH)</th>
<th>P (EH)</th>
<th>OR (EH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11010067</td>
<td>Downstream Gene Variant</td>
<td>Liu JZ (2015)</td>
<td>Crohn's Disease</td>
<td>G</td>
<td>1x10^{-26}</td>
<td>1.142</td>
<td>G</td>
<td>4.5x10^{-6}</td>
<td>1.82</td>
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<tr>
<td>rs11010067</td>
<td>Downstream Gene Variant</td>
<td>Jostins L (2012)</td>
<td>Inflammatory Bowel Disease</td>
<td>G</td>
<td>2x10^{-25}</td>
<td>1.115</td>
<td>G</td>
<td>4.5x10^{-6}</td>
<td>1.82</td>
</tr>
<tr>
<td>rs34779708</td>
<td>Intron (CREM)</td>
<td>Liu JZ (2015)</td>
<td>Inflammatory Bowel Disease</td>
<td>2x10^{-25}</td>
<td>-</td>
<td></td>
<td>G</td>
<td>1.2x10^{-6}</td>
<td>1.88</td>
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<tr>
<td>rs12261843</td>
<td>Intron (CCNY)</td>
<td>Anderson CA (2011)</td>
<td>Ulcerative Colitis</td>
<td>G</td>
<td>7x10^{-7}</td>
<td>1.07</td>
<td>G</td>
<td>8.2x10^{-5}</td>
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<td>rs12242110</td>
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<td>Franke A (2010)</td>
<td>Crohn's disease</td>
<td>G</td>
<td>1x10^{-9}</td>
<td>1.15</td>
<td>G</td>
<td>8.4x10^{-5}</td>
<td>1.70</td>
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<tr>
<td>rs17582416</td>
<td>intergenic</td>
<td>Barrett JC (2008)</td>
<td>Crohn's disease</td>
<td>G</td>
<td>2x10^{-9}</td>
<td>1.16</td>
<td>G</td>
<td>4.0x10^{-6}</td>
<td>1.83</td>
</tr>
</tbody>
</table>

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?
Mystery paralysis in children is perplexing parents — and researchers
Paralyzed with Fear: Polio Headlines

Read the stories of Michigan polio survivors
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

1403–1365 BCE
Egyptian Paintings Depict Children with Deformed Limbs

1789
English physician Michael Underwood first describes Polio

1894
First US localized paralytic polio epidemics begin to appear

1908
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
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 departments:
- reviews information from suspect case
- collects medical data
- coordinates specimen transport

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

1894 first US localized paralytic polio epidemics begin to appear

June 1916
NY reports an epidemic of > 27,000 polio cases and > 6,000 fatalities

1940s-1950’s Poliomyelitis cases soar in the US with >15,000 cases per year

2012 first AFM cases reported in California.

2014-2018 AFM occurs across the US
N > 560 cases

2019?
2020?

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)

<table>
<thead>
<tr>
<th>Genus (number of species)</th>
<th>Selected species</th>
<th>Selected serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterovirus (13)</td>
<td>Enterovirus A</td>
<td>Several CV-A types (for example, CV-A6, CV-A10 and CV-A16) and several numbered EVs (for example, EV-A71)</td>
</tr>
<tr>
<td></td>
<td>Enterovirus B</td>
<td>CV-B types, echoviruses and one CV-A (CV-A9)</td>
</tr>
<tr>
<td></td>
<td>Enterovirus C</td>
<td>Polioviruses, several CV-A types (for example, CV-A21 and CV-A24v) and several numbered EVs</td>
</tr>
<tr>
<td></td>
<td>Enterovirus D</td>
<td>Several numbered EVs (for example, EV-D68 and EV-D70)</td>
</tr>
<tr>
<td>Cardiovirus (3)</td>
<td>Cardioivirus A</td>
<td>Encephalomyocarditis virus (2 types)</td>
</tr>
<tr>
<td></td>
<td>Cardioivirus B</td>
<td>Saffold viruses (11 types) and Theiler’s murine encephalomyelitis virus</td>
</tr>
<tr>
<td></td>
<td>Cardioivirus C</td>
<td>Boone cardiovirus</td>
</tr>
<tr>
<td></td>
<td>Foot-and-mouth disease virus</td>
<td>Foot-and-mouth disease virus (7 types)</td>
</tr>
<tr>
<td>Aphthovirus (4)</td>
<td>Bovine rhinitis A virus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bovine rhinitis B virus</td>
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<tr>
<td></td>
<td>Equine rhinitis A virus</td>
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</tr>
<tr>
<td></td>
<td>Parechovirus A</td>
<td>Human parechoviruses (19 types)</td>
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<tr>
<td></td>
<td>Parechovirus B</td>
<td>Ljungan virus</td>
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<tr>
<td></td>
<td>Aichivirus A</td>
<td>Aichi virus 1</td>
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<tr>
<td></td>
<td>Hepatovirus A</td>
<td>Hepatitis A virus</td>
</tr>
</tbody>
</table>

Baggen et al, 2018
What causes AFM? Enterovirus A71 (EV-A71)

First isolated in 1965, causal for aseptic 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

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strength</strong></td>
<td>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.</td>
</tr>
<tr>
<td><strong>Consistency</strong></td>
<td>The credibility of findings increases with repetition of findings, including consistency of study findings across different populations and geographical locations.</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>Causality is more likely if the exposure causes only one specific disease or syndrome, or if a specific location or population are being affected.</td>
</tr>
<tr>
<td><strong>Temporality</strong></td>
<td>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.</td>
</tr>
<tr>
<td><strong>Biological gradient</strong></td>
<td>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.</td>
</tr>
<tr>
<td><strong>Plausibility</strong></td>
<td>A conceivable mechanism for causation between disease and exposure should exist for there to be a causal relationship.</td>
</tr>
<tr>
<td><strong>Coherence</strong></td>
<td>The current association should not contradict any previous knowledge available about the disease and/or exposure.</td>
</tr>
<tr>
<td><strong>Experiment</strong></td>
<td>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.</td>
</tr>
<tr>
<td><strong>Analogy</strong></td>
<td>This criterion uses previous evidence of an association between a similar exposure and disease outcome to strengthen the current argument for causation.</td>
</tr>
</tbody>
</table>
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?

200,000 children injected

- 40,000 with infectious symptoms
- 200 with paralytic polio
- 10 died
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 poliomyelitis 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?

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.
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*

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

*AFM = Acute Flaccid Myelitis*
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 nearly eradicated by vaccines.
  - BUT, we never understood mechanism. If only we did.
### Summary cont.

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