Molecular Epidemiology of HCV among PWID: New Insights into HCV Transmission

INHSU Oct 7, 2015

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Disclosures

• Research contracts/grants
  • Roche
  • Siemens
  • Merck
  • Hologic (Gen-Probe)
  • Boehringer Ingelheim

• Honoraria donated to the BCCDC Foundation for Population & Public Health
Many HCV epidemiological tools
  • Social and sexual networks
  • Require lots of data collection

Sintchenko & Holmes (BMJ 2015) – posit that whole genome sequencing (WGS) will transform molecular epidemiology:
  1. High-throughput, rapid, accurate and affordable,
  2. Compare data locally/nationally/internationally,
  3. Link genomics with clinical and epidemiological metadata.

Transform outbreak management!
Sacks-Davis et al. PloS One 2012
- Phylogenetic clustering is associated with social networks and injecting relationships

Jacka et al. Hepatology 2014
- 1/3 of PWID in the VIDUS cohort demonstrated phylogenetic clustering

- Reinfections and spontaneous clearance

Bretana et al. 2015
- Ongoing HCV transmissions amongst high-risk prisoners (HITS-p team)

Lamoury et al. 2015
- Genomic region used is important for transmission cluster discrimination

Jacka et al. submitted 2015
- Young injectors are seeded from many transmission events between HCV-infected older and younger injectors
7 Things Molecular Epidemiology Can do for You!

1. Recreate the transmission dynamics of an outbreak without fully knowing the traditional surveillance epidemiology
2. Determine if infection is incident or prevalent & time the infection
3. Determine transmission directionality: privacy implications
4. Determine if a transmission cluster is expanding or stable
5. Identify: mixed infection; treatment relapse vs reinfection
6. Detect Resistance Associated Variants (RAVs)
7. Study early infection events – vaccine design?
Cloning – precise – labour intensive

PCR-based – average sequence – miss admixtures if <20%

NGS of a single PCR product – detect “all” the variants in the sample + artifacts

WGS (NGS) – sequencing multiple fragments – assembled by computer – “all” variants + artifacts

Long read whole genome – early days - errors
Time

"Parental" viral genome

1<sup>st</sup> replication cycle

Genome variants with point mutations

2<sup>nd</sup> replication cycle

Quasispecies "Cloud"

n replication cycle

Echeverria et al. WJH 2015
Molecular evolution of HCV within a given host

Source “Transmitter”

“Founder” virus

Stage 1: Founder effect 1st bottleneck
Stage 2: Incremental evolution 2nd bottleneck
Stage 3: Emergence of new populations
Stage 4: Settlement

Preciado et al. WJG 2014
- Molecular epidemiology can characterize the transmission history of an epidemic
- Genetic diversity and transmissions unfold at the same time
- People → similar sequences → transmission cluster
Phylogenetics is the study of phylogeny
For viruses – understanding evolutionary history and relationships
Anti-D cohort HCV contaminated Rhogram plasma - Ireland

Time A
(Inoculation)
1977-1978
>500 women received Anti-D immunoglobulin from a single acutely HCV-infected source

Time B
1995-1997
Cohort subject plasma isolated

Time C
1999-2002
Cohort subject plasma isolated

1. Recreate the transmission dynamics of an outbreak without fully knowing the traditional surveillance epidemiology.
Molecular phylodynamics can be integrated with traditional epidemiology to estimate transmission dynamics in a HCV viral epidemic.

NeT using genetic data (Bayesian skyline plot) versus N (estimated from surveillance data using back calculation).

Plots were truncated after 1990 - to characterize HCV transmission prior the virus' discovery in 1989.

As of August 9, 2015 at the BCCDC, Vancouver, Canada:

• 1,472,830 individuals tested for anti-HCV

• 77,010 anti-HCV+
  • Includes 8,736 seroconverters
  • 4,314 within 24 months
2. Determine if Infection is Incident or Prevalent & Time the Infection - Diversity

Low diversity $\rightarrow$ New Infection

High diversity $\rightarrow$ Old infection

![Graph showing Shannon Entropy](image)

- **Acute**: AUROC = 0.86
- **Chronic**: $p < 0.001$

![Graph showing SNV Counts over time](image)

Montoya et al. Hepatology 2015
Determine if the Infection is Incident or Prevalent: Use Temporal Sequence Relatedness to Time the Infection
Greater the Depth of Sequencing = Better Characterization of Sequence Relatedness Over Time

Looks at a distribution of cluster relatedness - cloning

Prosperi et al. Nat Com 2011

Distribution of pairwise patristic distances for PCR product NGS from the NS5b derived from 32,641 reads (n=93)

The intra-individual patristic distances are shown in red and the inter-individual patristic distances in blue

(Montoya – unpublished data)
3. Determine Transmission Directionality: Implications on Privacy?

- Superior Court of the State of Washington ruled transmission direction can be established from blinded case samples.
- The close paraphyly relationship of viral sequences was used to convict an HIV index case for 17 counts of first degree assault:
  - paraphyletic relationships - source viral sequences are more closely related to all recipient sequences than to other source sequences.
- Sentenced to 2,137 months.

Scadutoa et al., PNAS, 2010

- Use a combination of sequence diversity in the sample to determine if the infection is acute or chronic.
- Combine with sequence relationship to demonstrate directionality (Montoya – unpublished data).
4. Determine if a transmission cluster is expanding or stable

Cluster 55: an ‘actionable’ cluster

April 2014 to June 2014

July 2014 to January 2015

HIV drug resistance
no resistance
undetectable viral load

August 7, 2014

Formal outbreak investigation

Poon et al. IAS 2015
Sequence Ebola in real time? There’s an app for that!
http://ebola.nextflu.org

The gap suggests that several undiagnosed people passed on the infection.
5. Identify: Mixed Infection; Treatment Relapse vs Reinfection

- Co-infection
- Superinfection
- Genotype-specific treatment
- Relapse or viral persistence in mixed infection
- Clearance
- Reinfection

• Global phylogeny of hepatitis C virus showing lineages possessing the Q80K polymorphism in nonstructural protein 3 (NS3)
  • The Q80K polymorphism has been associated with reduced susceptibility to the direct acting antiviral inhibitor simeprevir
  • Occurs predominantly in HCV geno 1a, high prevalence in the United States
  • 96% of HCV infections carrying Q80K descend from a single lineage which occurred around the 1940s in the United States, implying that this polymorphism is highly transmissible
### EC50 values of baseline RAVs within NS3, NS5A and NS5B

<table>
<thead>
<tr>
<th>Position</th>
<th>Variant</th>
<th>HCV region</th>
<th>EC50 [fold-change] (subtype)</th>
<th>Resistance Level</th>
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</tr>
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Personalized medicine based on pathogen genome
7) Study Early Infection Events – Vaccine Design?

- Characterize ‘transmitter/founder’ viruses at the earliest infection time points
- Full length molecular clones responsible for initiating hepatocyte infection and eliciting the initial immune responses
- Strategy to identify drivers of the early immune response & potential conserved epitopes for vaccine development

Source
Transmitter

Founder virus

New host

Genetic diversity

Stoddard et al. mBio 2015, Mitchell et al. mBio 2015

Preciado et al. WJG 2014
Molecular Epidemiology of the Future
1st Generation “Dore-Bot”

Not only what virus you have, but:

1) Determines the transmission dynamics with incomplete surveillance data
2) Determines if infection is incident or prevalent and times the infection
3) Determines infection directionality - serious privacy implications?
4) Identifies transmission clusters needing attention
5) Identifies treatment relapses or a reinfection and mixed infections
6) Enables personalized drug selections for patients harbouring RAVs
7) Determines early infection events and informs vaccine design
Acknowledgements

Dr. Andrea Olmstead
Vincent Montoya
Iris Luo
Dr. Art Poon
Dr. Jeffrey Joy
Dr. Jason Grebely
François Lamoury
Brendan Jacka
Dr. Tanya Applegate
Dr. Richard Harrigan