Rakai Couples Studies and Molecular Epidemiology Informing HIV Transmission and Pathogenesis

Oliver Laeyendecker MS, MBA, PhD
Epidemiologist, LIR, NIAID, NIH
Assistant Professor of Medicine, SOM, JHU
Assistant Professor of Epidemiology, JHSPH
Objectives

- Viral factors associated with transmission
- Linkage of transmission
- Viral factors associated with pathogenesis
- Population level changes in HIV subtype distribution
- Directionality of transmission and spatial studies

Overall Purpose: To determine viral factors that influence transmission and the health of the host, identify potential targets for interventions - vaccine and therapeutic designs - direct and elucidate the outcome of interventions
Rakai, Uganda

- More than 700 agrarian, fishing and trading communities
- Population ~500,000
- Epicenter of HIV in East Africa
- HIV transmission endemic: prevalence ~15%; incidence ~0.6 per 100 person-years.
Rakai Community Cohort Study (RCCS)

- Population-based HIV incidence cohort in 50 communities in Rakai District
  - Implemented by the Rakai Health Sciences Program
  - ~20,000 study participants in ~8000 households are surveyed at ~18 month intervals

- Ongoing since 1994 (19 survey rounds completed, 20th in progress)
  - ART introduced in 2004
  - Scale-up of male circumcision after 2006

- Study includes census and individual interviews.
  - Household census
  - Sociodemographic & behavioral survey
  - Detailed information on each sexual contact
  - Collection of biological specimens
Impact of Viral Factors on HIV Transmission and How to Determine Linkage of Transmission
Viral Load and Duration of Infection on HIV Transmission

Viral load Set Point on sexual transmission

Transmission per coital act by stage of infection

Quinn et al. NEJM 2000

Wawer... Gray JID 2005
### Viral Linkage and Transmission among Monogamous Couples

#### Factors associated with HIV transmission

<table>
<thead>
<tr>
<th>Observation</th>
<th>RR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VL &gt;75K</td>
<td>13.9 (1.6 to 124.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>VL 75 to 15K</td>
<td>11.2 (1.3 to 95.2)</td>
<td>0.03</td>
</tr>
<tr>
<td>VL 15K to 3.2K</td>
<td>4.40 (0.5 to 40.9)</td>
<td>0.19</td>
</tr>
<tr>
<td>Incident vs. Prevalent</td>
<td>8.08 (2.5 to 25.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AIDS vs. no AIDS</td>
<td>6.18 (2.5 to 15.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>age 15-29 vs. &gt;30</td>
<td>2.78 (1.2 to 6.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>A vs. D</td>
<td>4.43 (1.7 to 11.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>Rec vs. D</td>
<td>1.72 (0.8 to 3.7)</td>
<td>0.17</td>
</tr>
<tr>
<td>GUD vs. no GUD</td>
<td>2.86 (1.4 to 5.8)</td>
<td>0.01</td>
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</table>

#### Monogamous (N = 62)

<table>
<thead>
<tr>
<th>gag</th>
<th>gp41</th>
<th>#</th>
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<tbody>
<tr>
<td>Linked</td>
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<td>36</td>
</tr>
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<td>Linked</td>
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<td>13</td>
</tr>
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<td>No data</td>
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<td>0</td>
</tr>
<tr>
<td>Linked</td>
<td>Not linked</td>
<td>4</td>
</tr>
<tr>
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<td>5</td>
</tr>
<tr>
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<td>No data</td>
<td>2</td>
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<tr>
<td>No data</td>
<td>Not linked</td>
<td>2</td>
</tr>
<tr>
<td>Not Linked</td>
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</table>

Any linkage 93% (58/62)
Any discordance 21% (13/62)

#### Non - Monogamous (N=36)

<table>
<thead>
<tr>
<th>gag</th>
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<tbody>
<tr>
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<td>3</td>
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<tr>
<td>Linked</td>
<td>Not linked</td>
<td>6</td>
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<tr>
<td>Not linked</td>
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<td>2</td>
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<tr>
<td>Not linked</td>
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<td>8</td>
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<tr>
<td>No data</td>
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<td>0</td>
</tr>
<tr>
<td>Not Linked</td>
<td>Not linked</td>
<td>8</td>
</tr>
</tbody>
</table>

Any linkage 56% (20/36)
Any discordance 67% (24/36)

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*Laeyendecker CROI 2004*
Viral Load is a Heritable Trait

Meta-analysis: \( h^2 = 33\% \ (20 - 46\%) \) Fraser et al, Science 2014

Hollingsworth…Fraser PLOS PATH 2010
Fraser et al. Science 2014
Summary – Qualitative Differences of the Virus that impact HIV Transmission and Pathogenesis

• Establishing linkage of transmission can be difficult

• Viral load is the primary predictor of HIV transmission

• Stage of infection is associated with differential risk of transmission
  – Some one who is acutely infected is much more likely to transmit than when they are chronically infected

• Viral load is a heritable trait

• Viral subtype is associated with differential transmission
  – Subtype A is more likely to transmit as subtype D
Natural History of HIV Infection

- **Primary Infection**
  - Wide dissemination of virus
  - Seeding of lymphoid organs

- **Clinical Latency**

- **Opportunistic Diseases**

- **Constitutional Symptoms**

- **Death**

**Graph Details**

- **CD4+ T Lymphocyte Count** (cells/mm³)
  - 0 to 1200 cells/mm³

- **HIV RNA Copies per ml Plasma**
  - 10² to 10⁷ copies/ml

**Timeline**

- **Weeks**
  - 0 to 11

- **Years**
  - 0 to 11

*Source: [HIV Infographic](https://en.wikipedia.org/wiki/HIV#/media/File:Hiv-timecourse_copy.svg)*
Viral Load Distribution by HIV Subtype and Its Impact on Disease Progression

**Box Plots:**
- **Set Point Viral Load (Log10 copies/ml):**
  - **A:**
  - **D:**
  - **M:**
  - **R:**

**Infecting Subtype:**
- **A:**
- **D:**
- **M:**
- **R:**

**KM Survival by Subtype Stratified by Viral Load Setpoint:**
- **Subtype D; VL < 40K**
- **Subtype D; VL > 40K**
- **Subtype A; VL < 40K**
- **Subtype A; VL > 40K**

**References:**
- **Kiwanuka JID 2008**
- **McPhee ARHR 2019**
Viral Tropism Differences between HIV Subtypes

Co-receptor tropism in 1st year post seroconversion

- Subtype A: 37 tested
- Subtype D: 55 tested
- Recombinant: 18 tested

Survival estimates by initial viral tropism

- R5 Only
- X4 or dual tropic
Subtype D and Disease Progression

Log-rank P: 0.065

Cumulative proportion
0 0.2 0.4 0.6 0.8
Years from seroconversion

Subtype A
A: 91, 82, 53, 24, 7
D: 229, 201, 131, 53, 22

Subtype D
A: 54, 49, 34, 18, 7
D: 170, 150, 97, 34, 10

Log-rank P: 0.001

Cumulative proportion
0 0.2 0.4 0.6 0.8
Years from seroconversion

Subtype A
A: 51, 45, 32, 17, 8
D: 177, 158, 113, 52, 22

Subtype D
A: 170, 150, 97, 34, 10
D: 177, 158, 113, 52, 22

Log-rank P: 0.982

Cumulative proportion
0 0.2 0.4 0.6 0.8
Years from seroconversion

Subtype A
A: 177, 158, 113, 52, 22
D: 170, 150, 97, 34, 10

Subtype D
A: 170, 150, 97, 34, 10
D: 177, 158, 113, 52, 22

Log-rank P: 0.936

Cumulative proportion
0 0.2 0.4 0.6 0.8
Years from seroconversion

Subtype A
A: 170, 150, 97, 34, 10
D: 177, 158, 113, 52, 22

Subtype D
A: 170, 150, 97, 34, 10
D: 177, 158, 113, 52, 22

Ng JID 2013

Replication Capacity

Viral Load (Log 10 copies/ml)
Summary – Qualitative Differences that impact HIV Pathogenesis

- Subtype D are more pathogenic than subtypes A
- Subtype D expresses X4 tropic virus more often than Subtype A
- X4 tropic Virus is associated with rapid disease progression
- Higher replication capacity in subtype D than A
- Replication capacity independent of viral load and is predicative of disease progression
Population Level Changes in HIV Subtype Distributions and Viral Linkage
Global Distribution of HIV-1 Group M Subtypes

Hemelaar et al Lancet Inf. Dis 2019
HIV-1 Subtype Distribution: 1994

<table>
<thead>
<tr>
<th>Community cluster</th>
<th>N</th>
<th>A</th>
<th>D</th>
<th>C</th>
<th>R</th>
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</thead>
<tbody>
<tr>
<td><strong>North</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Kalisizo</td>
<td>140</td>
<td>22.1 (31)</td>
<td>66.4 (93)</td>
<td>0.7 (1)</td>
<td>10.7 (15)</td>
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<tr>
<td>Katana</td>
<td>106</td>
<td>23.6 (25)</td>
<td>60.4 (64)</td>
<td>0.9 (1)</td>
<td>15.1 (16)</td>
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<tr>
<td>Buyamba</td>
<td>72</td>
<td>9.7 (7)</td>
<td>77.8 (56)</td>
<td>1.4 (1)</td>
<td>11.1 (8)</td>
</tr>
<tr>
<td>Lwamaggwa</td>
<td>46</td>
<td>30.4 (14)</td>
<td>54.3 (25)</td>
<td>0</td>
<td>15.2 (7)</td>
</tr>
<tr>
<td><strong>All northern region</strong></td>
<td>364</td>
<td>21.2 (77)</td>
<td>65.4 (238)</td>
<td>0.8 (3)</td>
<td>12.6 (46)</td>
</tr>
<tr>
<td><strong>Central</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kyotera</td>
<td>78</td>
<td>15.4 (12)</td>
<td>75.6 (59)</td>
<td>0</td>
<td>9.0 (7)</td>
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<tr>
<td>Kabira</td>
<td>87</td>
<td>13.8 (12)</td>
<td>77.0 (67)</td>
<td>0</td>
<td>9.2 (8)</td>
</tr>
<tr>
<td>Lwanda</td>
<td>87</td>
<td>17.2 (15)</td>
<td>65.5 (57)</td>
<td>1.1 (1)</td>
<td>16.1 (14)</td>
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<tr>
<td><strong>All central region</strong></td>
<td>252</td>
<td>15.5 (39)</td>
<td>72.6 (183)</td>
<td>0.4 (1)</td>
<td>11.5 (29)</td>
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<tr>
<td><strong>South</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kibale-Rakai</td>
<td>42</td>
<td>9.5 (4)</td>
<td>88.1 (37)</td>
<td>2.4 (1)</td>
<td>0</td>
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<tr>
<td>Kakuuto</td>
<td>36</td>
<td>13.9 (5)</td>
<td>72.2 (26)</td>
<td>5.6 (2)</td>
<td>8.3 (3)</td>
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<tr>
<td>Kasasa-Sanjie</td>
<td>79</td>
<td>5.1 (4)</td>
<td>74.7 (59)</td>
<td>0</td>
<td>20.3 (16)</td>
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<tr>
<td><strong>All southern region</strong></td>
<td>157</td>
<td>8.2 (13)</td>
<td>77.7 (122)</td>
<td>1.9 (3)</td>
<td>12.1 (19)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>773</td>
<td>16.7 (129)</td>
<td>70.2 (543)</td>
<td>0.9 (7)</td>
<td>12.2 (94)</td>
</tr>
</tbody>
</table>

Collinson-Streng AIDS Res Hum Retroviruses, 2009
### Table 1. Comparison of Overall Subtype Distribution in 1994 and 2002

<table>
<thead>
<tr>
<th>HIV-1 subtype</th>
<th>1994</th>
<th>2002</th>
<th>Change (%)</th>
<th>Chi-square p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>n = 773, (16.7)</td>
<td>n = 812, (23.3)</td>
<td>6.6</td>
<td>0.005</td>
</tr>
<tr>
<td>C</td>
<td>7 (0.9)</td>
<td>7 (0.9)</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>543 (70.2)</td>
<td>507 (62.4)</td>
<td>-7.8</td>
<td></td>
</tr>
<tr>
<td>Recombinant</td>
<td>94 (12.2)</td>
<td>109 (13.4)</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>
Change in Subtype Proportion by Region Over Time
Geographic Distance and Viral Genetic Distance

Grabowski et al, Plos Med, 2014
Summary – Population Level Changes in HIV Subtype Distributions and Infection Sources

- The frequency of subtype D decreasing, with recombinant and subtypes A increasing
- 39% of transmissions occurred in household sexual partnerships
  - Very high transmission rates (25% among incident cases)
- 40% were from known extra-household sexual partners
  - 62% of these were partners from outside the community.
- 20% unknown sources
Directionality of Transmission
HIV Molecular Clock

Distance in the tree is related to time
HIV exists as a quasispecies, or cloud of related viruses within an individual. The structure of the tree can implicate the direction of the transmission.
HPTN 052: Randomized Clinical Trial of Immediate vs. Delayed ART in Couples

Total HIV-1 Transmission Events: 39

- Linked Transmissions: 28
  - Immediate Arm: 1*
  - Delayed Arm: 27

- Unlinked or TBD Transmissions: 11

96% reduction in HIV transmission (95% CI 73-99%)

*1 transmission in immediate ART arm soon after ART initiation

Cohen 2011 NEJM
52 pairs

Number of index samples

- 30 single infections
- 3 multiple infections
- 9 unlinked infections
- 3 single infections; uncertain linkage**
- 6 had only early index
- 1 had only late index

Linkage

- 36 had evidence of linkage
- 5 unlinked
- 1 linked
- 1 linked

Number of infections

- 3 INC at both time points
- 1 INC at early time point
- 1 INC in late time point
- 1 EQ in both
- 24 Consistent at both time points
- 1 INC in early, unlinked later
- 1 INC at both time points
- 1 EQ in early, consistent in late
- 1 INC at both time points
- 2 Consistent at both time points
- 1 Consistent
- 1 Consistent

Root

- 1 PM/PP
- 2 PP/PP
- 1 PM/PP
- 1 PM
- 8 PM/PM
- 1 PM
- 7 PP/PP
- 1 PP/PP
- 1 PP/PP
- 1 PP/PP
- 1 PP/PP

Topology and root

- 1 MM/MM
- 1 MM/PM (EQ early)
- 1 MM/PM (INC late)
- 1 PP/PP (INC both)
- 1 PM/PP (INC both)
- 1 PM/PP (INC late)
- 1 PM/PP (EQ late)

n=38 linked pairs

Rose JID 2019
Directionality method validated using known transmission pairs from HPTN 052

- 33 pairs where the direction of transmission was known
  - Up to two time points sequenced from the index partner
- 454 pyrosequencing data of gp41
  - 450,336 sequences total
- Predicted direction correctly 67%-74%
**Objective:** Generate 20,000 full-length HIV genomes to improve understanding of HIV transmission dynamics in Sub-Saharan Africa

Pillay et al. Lancet Infec Dis. 2015
Deep sequence viral phylogenetics (phyloscanner)

Wymant et al. Virus Evolution 2018; Wymant et al. MBE 2017; Ratmann, Grabowski et al Nature Com 2019
Are fishing communities a major source of HIV infection among neighboring inland populations in Rakai, Uganda?

Chang, Grabowski et al. Lancet HIV 2016
Reconstructed Transmission Flows

- 293 source-recipient pairs reconstructed
- 232/293 (79%) within inland or within fishing

Ratmann, Grabowski et al. IAS 2018
Reconstructed Transmission Flows

- 7/293 from fishing to inland
- 1/7 involved a source that had moved from fishing to inland
- 21/293 from inland to fishing
- 13/21 involved a source that had moved from inland to fishing

Ratmann, Grabowski et al. IAS 2018
Fishing Sites are Viral Sinks Despite Being Hotspots

A

5.4% [3.3% – 8.4%]

2.8% [1.2% – 5.6%]

7.7% [5% – 11.3%]

41.7% [35.5% – 48%]

2.9% [1.4% – 5.2%]

38.9% [33.3% – 44.8%]

population

- inland communities
- fishing sites
- external

Ratmann, Grabowski et al. IAS 2018
Similar analyses were carried out in Botswana and Zambia

Transmission between Ya Tsie communities in Botswana

Transmission between HPTN 071 communities in Zambia

Magosi et al, Elife 2022

Hall et al, in preparation
Conclusions - Directionality of Transmission and Spatial Studies

• Direction of HIV transmission can be inferred from sequence data most of the time

• High HIV incidence/prevalence (hotspots) are not necessarily the main source of new infections in neighboring low risk areas

• Targeting of hotspots may not benefit to neighboring low risk areas
Conclusions – Overall

• Genetic features of HIV-1 impact its transmission and pathogenies within a human host

• These viral genetic differences can be used to determine how the virus spreads within a population

• Understanding these molecular epidemiologic traits can be used for targeted intervention, both individually and at a population level
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