Behavioral Risk and HIV-1 Molecular Diversity: Making the Connections

Drug Abuse and Risky Behaviors: The Evolving Dynamics of HIV/AIDS
NIDA, NIH, May 8, 2007

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1. Johns Hopkins University; 2. WRAIR; 3. Chiang Mai University; 4. Northern Drug Treatment Center, Chiang Mai, Thailand
Outline

• Introduction
• Molecular epidemiology and segregation by risks
• Risk and complexity
• Associations in the Opiate Users Research Cohort
• Breakpoint analyses and networks
• Conclusions
Introduction: Molecular Epidemiology

From a public health perspective, the advent of molecular epidemiology, which allows tracking of pathogens based on unique genetic sequences or antigenic properties, has revolutionized how epidemiologists investigate and evaluate epidemics and assess endemic diseases.

Introduction: HIV Genetic Diversity

• HIV-1 is a genetically diverse virus with high rates of genetic change: mutation, recombination, dual infection, super-infection

• The genetic diversity of HIV challenges the human immune system, vaccine development, measures of anti-viral drug resistance

• HIV-1 genetic diversity allows for epidemiologic investigations
HIV-1 Molecular Epidemiology: Historical Timeline and Key Milestones

- 1983: First isolation of HIV
- 1988: Variation in HIV-1 populations
- 1992: Subtypes of HIV-1:
- 1995: Recombination in HIV-1 populations
- 1998: Nomenclature for HIV-1 subtypes
- 2001: CRFs (Circulating Recombinant Forms)
- 2002: URF (Unique Recombinant Forms)
- 2003: Co-infection with other viruses

Key Milestones:
- 1988: Nomenclature
- 1995: Recombination
- 2002: URF

Countries and Regions:
- Kenya
- Uganda
- Tanzania

Visual Elements:
- HIV-1 molecular variation
- Subtypic diversity
- Recombination events
- CRF and URF classification
- Co-infection with other pathogens
Global Distribution of Subtypes and Recombinants

Legend:
- A
- B
- C
- D
- CRF01_AE, B
- CRF02_AG, other recombinants
- A, B, AB recombinant
- B, BF recombinant
- B, C, BC recombinant
- F,G,H,J,K,CRF01 other recombinants
- Insufficient data
Uses of Molecular Epidemiology in HIV-1

**Established**
- Powerful tool in understanding epidemic dynamics
- Has regional utility, particularly for **common border epidemics**
- Allows use of the virus to track movements of **people** (truckers, sex workers, migrants, soldiers) and **narcotics**

**Novel**
- Linking diversity to risks could allow for targeting interventions, identifying “hot spots”
- Potential tool for mapping networks
Molecular Epidemiology and segregation by risks
Early Studies of Risks and Subtypes

South Africa 1990s
MSM with B, African Heterosexuals with C

Thailand 1990s
IDU with B, Heterosexuals with E (CRF01-A/E)

Malaysia 1990s
HIV-1 subtypes in Malaysia among different primary risk categories, 1994-1996.

<table>
<thead>
<tr>
<th>Risk</th>
<th>B</th>
<th>E</th>
<th>B/E</th>
<th>B/C</th>
<th>Non-typable</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (N=89)</td>
<td>34 (38%)</td>
<td>48 (54%)</td>
<td>2 (2%)</td>
<td>1 (1%)</td>
<td>4 (4.5%)</td>
</tr>
<tr>
<td>IDU (N=53)</td>
<td>29 (55%)</td>
<td>19 (36%)</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Hetero (N=27)</td>
<td>4 (15%)</td>
<td>23 (85%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SW (N=9)</td>
<td>1 (11%)</td>
<td>6 (67%)</td>
<td>-</td>
<td>-</td>
<td>2 (22%)</td>
</tr>
</tbody>
</table>

* E now called CRF01_A/E

IDU significantly more likely to have HIV-1 subtype B than those with sexual risks (heterosexuals and SW combined) OR 5.9 (95% CI 1.9, 18.5) p < .001.

All of the 3 dually reactive sera were from IDU.

Risk and Complexity

Multi-region Hybridization Assays (MHA)
Dual Infection

Dual infection, more common in high-risk groups, is the engine driving recombination and an important source of HIV diversity.

Many different recombinants can emerge in a dual infected individual, who may transmit them to others; dual infection is an accelerator of HIV diversity in populations.

Many recombinant strains are generated within high risk social networks, which also have high rates of transmission; this coincidence of factors can accelerate the initial spread of new variants.
A clear picture of the evolving HIV-1 epidemics in Asia can only be achieved through the study of large cohorts, using high-throughput and high-resolution subtyping.

Multi-region Hybridization Assay (MHA) to study HIV-1 genetic diversity in Asia

**Principle of MHA**

Real-time PCR with Clade-specific probes

**The MHA family**

Courtesy Dr. F. McCutchan, USMHRP/HJF
Distinguishing HIV-1 molecular forms in Asia
Comparative Epidemiology in Thailand

<table>
<thead>
<tr>
<th>Province</th>
<th>Population</th>
<th>MHA Genotypes (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiang Mai</td>
<td>IDU (OUR)</td>
<td>336</td>
</tr>
<tr>
<td>Lampang</td>
<td>Antenatal Clinic</td>
<td>177</td>
</tr>
<tr>
<td>Rayong-Chon Buri</td>
<td>Vaccine Trial Volunteers</td>
<td>293</td>
</tr>
<tr>
<td></td>
<td></td>
<td>806</td>
</tr>
</tbody>
</table>
## The cohorts

<table>
<thead>
<tr>
<th></th>
<th>RV109</th>
<th>OUR</th>
<th>RV148</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Location</strong></td>
<td>Lampang</td>
<td>Chiang Mai</td>
<td>Rayong Chon Buri</td>
</tr>
<tr>
<td><strong>Participants</strong></td>
<td>180</td>
<td>2,231</td>
<td>26,675</td>
</tr>
<tr>
<td><strong>Cohort</strong></td>
<td>MTCT</td>
<td>Opiate users</td>
<td>Community</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>100% ♂</td>
<td>7% ♂</td>
<td>48% ♂</td>
</tr>
<tr>
<td><strong>Risk factors</strong></td>
<td>heterosexual</td>
<td>IVDU</td>
<td>heterosexual</td>
</tr>
<tr>
<td><strong>HIV sero-prevalence</strong></td>
<td>ca. 3 %</td>
<td>15.6 %</td>
<td>1.6 %</td>
</tr>
<tr>
<td><strong>Genotyped samples</strong></td>
<td>177/180 (98.3%)</td>
<td>336/347 (96.8%)</td>
<td>376/391 (96.2%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>889 / 918 (96.8%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Proportions of Subtypes, Recombinants, Dual Infections

<table>
<thead>
<tr>
<th></th>
<th>Antenatal</th>
<th>Trial Volunteers</th>
<th>IDU</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRF01_AE</td>
<td>94.9%</td>
<td>91.8%</td>
<td>81.8%</td>
</tr>
<tr>
<td>Subtype B</td>
<td>2.3%</td>
<td>2.0%</td>
<td>3.9%</td>
</tr>
<tr>
<td>Recombinant</td>
<td>2.8%</td>
<td>5.5%</td>
<td>9.2%</td>
</tr>
<tr>
<td>Dual</td>
<td>0.0%</td>
<td>0.7%</td>
<td>5.1%</td>
</tr>
</tbody>
</table>
HIV diversity and risks in Thai IDU

336 isolates from Thai IDU in the OUR cohort

- 81.8% CRF01_AE
- 3.9% B
- 9.2% Recombinants: CRF01_AE and B
- 5.1% Dual infections

Subtype B:
- 30 years old or older: OR: 6.9; 95%CI: 1.5-31.7

Dual infection:
- lower education level: AOR=5.0 95%CI: 1.4-17.5
- initiated injecting ≤ 3 years: AOR=3.4 95%CI: 1.2-9.8

Recombinants and duals:
- needle sharing last 3 months: AOR=4.1, 95%CI: 1.41.7
## Comparative Epidemiology in East Africa

<table>
<thead>
<tr>
<th>Country</th>
<th>Cohort</th>
<th>Population</th>
<th>MHA Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanzania</td>
<td>HISIS</td>
<td>High risk ♀ (Sex Workers)</td>
<td>238</td>
</tr>
<tr>
<td>Tanzania</td>
<td>CODE</td>
<td>Urban and rural communities</td>
<td>487</td>
</tr>
<tr>
<td>Uganda</td>
<td>MER</td>
<td>Rural communities</td>
<td>329</td>
</tr>
<tr>
<td>Kenya</td>
<td>Kericho</td>
<td>Agricultural Plantation</td>
<td>366</td>
</tr>
</tbody>
</table>

Total: 1420
Proportions of Recombinant HIV and Dual Infections in A, D, C Subtype Zone

<table>
<thead>
<tr>
<th></th>
<th>Agricultural/Rural</th>
<th>Rural/Urban</th>
<th>Urban High Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>URF</td>
<td>26.4%</td>
<td>29.5%</td>
<td>35.9%</td>
</tr>
<tr>
<td>Dual</td>
<td>7.0%</td>
<td>7.1%</td>
<td>15.0%</td>
</tr>
</tbody>
</table>

**MER**
- URF
- A
- D

**KERICHO**
- URF
- A
- D

**CODE**
- URF
- A
- C

**HISIS**
- URF
- A
- C
Breakpoint Analyses and Networks

Fine mapping of recombinant breakpoints
Describing a Recombinant Strain: Subtypes and Breakpoints

Subtype A

AC Recombinant

Subtype C
Through recombination, parts of the parental strains are lost, and cannot be regained until another dual infection provides opportunity to recombine again.

**Irreversibility lends stability**

Could recombination breakpoints serve as stable markers through many cycles of transmission, permitting mapping of the social networks in which HIV spreads?
Hypotheses

Mapping of shared breakpoints among recombinant strains could provide a new dimension to the molecular epidemiology of HIV-1.

The structure and relationships of recombinant strains may provide information about the social networks in which they spread, providing new focus for interventions.
Recombinant Strains in “Low Risk” Groups

Complete sharing of breakpoints
Recombinant Strains in High Risk Groups

Transmission

- single
- dual
- single
- single
- dual
- single

Sampling

Partial sharing of breakpoints
Recombinant HIV Networks and Risk Groups in Asia

24 CRF01_AE/B recombinants from Thailand and Burma
11 from IDU
13 from heterosexual transmission
Network Visualization Software*

Each strain is a node

Each shared breakpoint is a connection, represented by a line

Highly interconnected strains form dense clusters, with less connected strains at the periphery

*UCiNET and NetDraw by S. Borgatti
Boston College/Analytic Technologies
Networks of Shared Breakpoints Among Recombinant Strains

Asia

East Africa
What can be learned about social networks from the relationships among recombinant strains circulating within them?
Recombinant Networks in Asia

By country:
- China
- Burma
- Thailand

By date:
- 1999 or before
- 2000-2005

By risk:
- IDU
- Het
- n.a.
In Asia

Heterosexual and IDU Networks in Thailand are strongly interconnected and these connections were already established during the first decade of the Thailand epidemic.

Fewer connections across national borders.

Strains from Burma/Myanmar bridge China and Thailand epidemics.
Connections Across National Borders
Contributors

• Participants in cohort development and other studies in Tanzania, Uganda, Kenya, Thailand, China, Myanmar

• Oliver Hoffmann, Steffan Geis, Leonard Maboko, Donan Mmbando, Eluter Samky, Michael Hoelscher, and other members of the Mbeya Medical Research Programme, Tanzania

• David Serwadda, Nelson Sewankambo, Maria Wawer, Ron Gray Makerere University and Uganda Virus Research Institute, Uganda, Columbia University and Johns Hopkins University, USA and other members of the Rakai Project, Uganda

• Carl Mason, USAMRU-K, Monique Wassuna, KEMRI, and other contributors to the Kenya Blood Bank Study

• David Celentano, Chris Beyrer, Vinai Suriyanon, Jaroon Jittiwutikarn, Thira Sirisanthana, Myat Htoo Razak and other contributors to the Opiate Users Research Study, Thailand

• Vilaiwan Gulgolgarn, Manu Wera-arpachai, Chirasak Khamboonrueng, Kenrad Nelson, Nakorn Dabbhasuta and others from the Lampang perinatal transmission cohort study, Thailand

• Supachai Rerks-Ngarm, Sonchai Wattana, Wiwat Wiriyakijja, Sorachai Nitayaphan, Chirapa Eamsila, Jerome Kim, Michael Benenson, Arthur Brown and others for samples from volunteers deferred from enrollment in the Phase III prime-boost vaccine trial in Rayong-Chonburi Provinces, Thailand

• Special thanks to Jocelyn Chiu and her mentors, Sodsai Tovanabutra, and Eric Sanders-Buell, for inspection and analysis of 1,125,000 nucleotides of sequence alignment
Implications for Prevention

Targeting prevention to highest risk groups may be the most important strategy to limit the genetic complexity of the epidemic, both in Africa and in Asia.

Targeting prevention to the most mobile sectors of a given population may also contribute to limiting the overall complexity of strains in an epidemic.

Effective size of the social network in which HIV-1 is spreading in E. Africa may be much larger than in Asia, with implications for dissemination of new strains.

Heroin trafficking routes appear to predict HIV-1 subtype spread and should be priority zones for prevention.
Discussion and Conclusions

Recombinant strains can represent highly informative tools to gain new understanding of the global epidemiology of HIV.

Molecular data is more informative when closely linked to demographic data and becomes more useful when closely and systematically analyzed and when epidemiology and narcotics data are included.

The structure of social networks, particularly the geographic and social mobility of the highest risk groups, can play key roles in the generation and spread of new HIV diversity generated by recombination.