Analysis of Genetic Linkage of HIV from Couples Enrolled in the HPTN 052 Trial

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Abstract Authors

J Hughes, S Hudelson, A Redd, L Wang, R Debes, Y Chen, S Porcella, E Piwowar-Manning, M McCauley, M Hosseinipour, J Kumwenda, J Hakim, S Chariyalertsak, G de Bruyn, B Grinsztejn, N Kumarasamy, J Makhema, K Mayer, J Pilotto, B Santos, T Quinn, M Cohen, and S Eshleman for the HPTN 052 Study Team
Linkage Analysis

39 transmission events were included in the April 28th DSMB report.

Linkage analysis was performed to identify the subset of transmission events where the index participant was the likely source of the partner’s HIV infection.
The DSMB report included 28 linked transmission events. Those data were used to analyze the impact of immediate ART on HIV prevention.

One additional linked transmission event was identified after the DSMB meeting. This additional event was in the delayed ART arm, further strengthening the association between immediate ART and HIV prevention.
Methods Used for Linkage Analysis

Phylogenetic analysis of *pol* sequences obtained by population sequencing

Statistical (Bayesian) analysis of genetic distances from *pol* sequence pairs

Phylogenetic analysis of *env* sequences obtained by next generation sequencing
Genetic Diversity of HIV

HIV RNA
HIV Diversity in an Infected Person

Genetically related viruses
Establishing Linkage

Index

Partner
Establishing Linkage

Index

Partner

Other individuals in the community
Establishing Linkage

Index
Partner

Other individuals in the community
Establishing Linkage

Index

Partner
Establishing Linkage

Index

Partner

Other individuals in the community
Establishing Linkage

Index Partner

UNLINKED

Other individuals in the community
Establishing Linkage

Index

Partner

Other individuals in the community
Population Sequencing

Consensus sequence
e.g., AACTGATCGGAA...

Consensus sequence
e.g., ATGGGCTACCCGAA...
Methods Used for Linkage Analysis

→ Phylogenetic analysis of \textit{pol} sequences obtained by population sequencing

Statistical (Bayesian) analysis of genetic distances from \textit{pol} sequence pairs

Phylogenetic analysis of \textit{env} sequences obtained by next generation sequencing
Phylogenetic Analysis

Adapted from Margulies et al., Genome Res 2007;17:760-77  ©2007
Phylogenetic Analysis (pol)
Phylogenetic Analysis (pol)
Phylogenetic Analysis ($pol$)
Phylogenetic Analysis (pol)
Phylogenetic Analysis (pol)

Local Controls
Phylogenetic Analysis (pol)
Methods Used for Linkage Analysis

Phylogenetic analysis of *pol* sequences obtained by population sequencing

⇒ Statistical (Bayesian) analysis of genetic distances from *pol* sequence pairs

Phylogenetic analysis of *env* sequences obtained by next generation sequencing
Statistical (Bayesian) Analysis
Comparison of genetic similarity values from paired sequences (*pol*)

Representative data from one transmission event

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<thead>
<tr>
<th>Pair type</th>
<th>Basis</th>
<th>Sample source</th>
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<tbody>
<tr>
<td>“Known”</td>
<td>Two samples from the same person</td>
<td>Index-index</td>
<td>1</td>
<td>99.8</td>
</tr>
<tr>
<td>linked</td>
<td></td>
<td>Partner-partner</td>
<td>1</td>
<td>98.2</td>
</tr>
<tr>
<td>“Known”</td>
<td>Unrelated individuals</td>
<td>10 other index</td>
<td>&gt;50</td>
<td>91.2-95.3</td>
</tr>
<tr>
<td>unlinked</td>
<td></td>
<td>participants / site</td>
<td></td>
<td></td>
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<tr>
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<td>Transmission event</td>
<td>Index-partner</td>
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<td></td>
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## Statistical (Bayesian) Analysis

Comparison of sequence similarity values from paired sequences \((pol)\)

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**Comparison of sequence similarity values from paired sequences (pol)**

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Methods Used for Linkage Analysis

Phylogenetic analysis of *pol* sequences obtained by population sequencing

- Statistical (Bayesian) analysis of genetic distances from *pol* sequence pairs

Phylogenetic analysis of *env* sequences obtained by next generation sequencing
Initial Linkage Assessment

26 events analyzed were clearly linked using both methods – those 26 events were classified as linked.

The linkage status of 12 events could not be definitively determined.
Methods Used for Linkage Analysis

Phylogenetic analysis of *pol* sequences obtained by population sequencing

Statistical (Bayesian) analysis of genetic distances from *pol* sequence pairs

Phylogenetic analysis of *env* sequences obtained by next generation sequencing
Population Sequencing

Consensus sequence
e.g., AACTGATCGGAA...

Consensus sequence
e.g., ATGGGCTACCGGA...
Population Sequencing

Consensus sequence
e.g., AACTGATCGGAA...

Consensus sequence
e.g., ATGGGCTACCGGAA...
Next Generation Sequencing

sequence 1
sequence 2
sequence 3
sequence 4
sequence 5
sequence 6...

sequence 1
sequence 2
sequence 3
sequence 4**
sequence 5
sequence 6...
Example of an Unlinked Event (env)
Example of an Linked Event (env)

Index sample 1
Index sample 2

Partner sample 1
Partner sample 2

0.02
One additional event was confirmed to be linked by next generation sequencing after the April 28th DSMB meeting.

This event was in the delayed study arm, further strengthening the association between immediate ART and HIV prevention.

This partner seroconverted ~1 month after the index started ART. The partner was most likely infected shortly before the index started ART, or shortly after the index started ART before the index was virally suppressed.
Summary of Linkage Analysis

29 linked events
  • In 2 cases, linkage was only apparent using next generation sequencing
  • 1 immediate ART arm; 28 delayed ART arm

7 unlinked events
  • 4 immediate ART arm; 3 delayed ART arm
Summary of Linkage Analysis

29 linked events
- In 2 cases, linkage was only apparent using next generation sequencing
- 1 immediate ART arm; 28 delayed ART arm

7 unlinked events
- 4 immediate ART arm; 3 delayed ART arm

3 additional events
- 2 events were not classified based on available data
- 1 late event is still being analyzed
- All 3 were in the delayed arm, not on ART
Factors Associated with Linkage

Linkage was not associated with:

- Geographic region
- Index gender
- Index CD4 cell count at enrollment
- Years between enrollment and seroconversion
## Factors Associated with Linkage

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<td>1 (3%)</td>
<td>3 (43%)</td>
<td>0.018</td>
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<td>Delayed ART</td>
<td>28 (97%)</td>
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<td><strong>Index on ART at time of SC</strong></td>
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1 linked transmission in the immediate ART arm identified pre-DSMB
1 linked transmission in the delayed ART arm identified post-DSMB
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*Data was missing for one participant
Conclusions

We used a combination of laboratory and statistical methods to determine the linkage status of transmission events in HPTN 052.

These data were used to identify the endpoints used in the analysis of HIV prevention in the HPTN 052 trial.

A manuscript describing the analysis of HIV linkage in HPTN 052 is In Press in the Journal of Infectious Diseases.
Thanks

Craig Martens
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