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Background

- HIV notifications are on the rise
  - Against global trends
- Changing HIV subtype diversity patterns
  - Historical associations between geographical location
  - Clinical implications (disease progression, susceptibility to ART).

Aims

- Perform HIV phylogenetic analysis
  - Statistical approach to monitor sequence similarity
- Determine characteristics of sequences similarities between HIV subtypes
- Investigate a link between clinical parameters, HIV viral subtypes and sequence relatedness
  - CD4 T cell counts and HIV RNA

Methods

- De-identified data (Not contact tracing)
  - Gender, age, Notification year
- Clinical data (2000 – 2014)
  - Baseline HIV-1 sequences (RT + PR)
  - HIV subtype
  - CD4 T cell Counts, CD4:8 ratios
  - HIV RNA
- Data analysis
  - Sequence alignments checked
  - Duplicate sequences checked
  - Phylogenetic analysis
  - Network identification


Background: mobility patterns

- ~6.5 million short term arrivals
- 19% from countries where HIV is increasing
- From countries where HIV diversity is increasing

- ~9 million short term resident departures
- 22% to countries where HIV is increasing
- To countries where HIV diversity is increasing

Statistics compiled from the Australian Bureau of Statistics
Methods: Phylogenetic analysis

- MEGA provides the Bootstrap test – reliability of Phylogenetic tree
- Nucleotide sequences are re-sampled 100 times.
- Maximum-Likelihood branch lengths are computed.

Results: Gender, age and clinical parameters at first assessment

<table>
<thead>
<tr>
<th>Subtype</th>
<th>B</th>
<th>Non B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequences #</td>
<td>619</td>
<td>364</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>557</td>
<td>232</td>
</tr>
<tr>
<td>- Female</td>
<td>62</td>
<td>132</td>
</tr>
<tr>
<td>Age (yrs)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>38.8 (12-76)</td>
<td>39.6 (1-74)</td>
</tr>
<tr>
<td>- Female</td>
<td>33.1 (1-74)</td>
<td>32.7 (1-68)</td>
</tr>
<tr>
<td>Viral load (lcpm)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>4.57 (1.6-7)</td>
<td>4.76 (1.6-7)</td>
</tr>
<tr>
<td>- Female</td>
<td>4.33 (1.6-6.23)</td>
<td>4.40 (1.6-7)</td>
</tr>
<tr>
<td>CD4 T cell count (cell/μl)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>440 (3-1792)</td>
<td>362 (2-1710)</td>
</tr>
<tr>
<td>- Female</td>
<td>436 (6-1020)</td>
<td>357 (6-2024)</td>
</tr>
<tr>
<td>CD4/CD8 ratio**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>0.46 (0.01-2.57)</td>
<td>0.41 (0.01-1.92)</td>
</tr>
<tr>
<td>- Female</td>
<td>0.5 (0.04-2.6)</td>
<td>0.39 (0.02-1.21)</td>
</tr>
</tbody>
</table>

Results: Age and gender according to HIV-1 subtype

<table>
<thead>
<tr>
<th>Non B subtype</th>
<th>B subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Std</td>
</tr>
<tr>
<td>Female</td>
<td>33.1</td>
</tr>
<tr>
<td>Male</td>
<td>39.2</td>
</tr>
</tbody>
</table>

Results: HIV subtype diversity over time

Results: Gender and HIV-1 subtypes over time
**Results: Phylogenetic analysis**

A: Radial B subtype tree  
B: Radial non B subtype tree

**Sequences in phylogenetic clusters**

- B-subtype: 195 / 619 (31.5%)  
- Non-B-subtype: 62 / 402 (15.4%)  
- Overall: 257 / 1021 (25.2%)  

**Results: Sequences within clusters**

A: Radial B subtype tree  
B: Radial non B subtype tree

**Sequences in large clusters (>2 in size)**

- B-subtype: 133 / 145 (91.7%)  
- Non-B-subtype: 13 / 145 (8.3%)  
- Overall: 145 / 257 (56.4%)  

**Sequences in pairs (2 in size)**

- B-subtype: 62 / 112 (55.3%)  
- Non-B-subtype: 49 / 112 (44.7%)  
- Overall: 112 / 257 (43.6%)  

**Results: Earliest viral load assessment**

- Strong influence of notification era (p<0.001).
- Higher viral load associated with HIV-1 sequences in large cluster (n=53, p=0.01).
- No association between HIV-1 subtype and viral load (p=0.31).

**Results: Earliest CD4 T cell count**

- No significant association between notification era and CD4 T cell count (p=0.1) or CD4:CD8 ratio (p=0.2).
- Higher CD4 count associated with HIV-1 sequences in large cluster (n=53, p=0.001).
- HIV-1 subtypes C and AE associated with lower CD4 count (p<0.01).

**Results: Earliest Abs CD4 T cell count**

Regression analysis

**Results: Lower CD4 T cell count**

Regression analysis
Results: Large network (n=53) dynamics

- Established in 2008
- Median age of 40 years (range=19-61)
- 52M + 1F
- High baseline VL
- High baseline CD4 count
- Expanded by 12 in 2014
- 4 patients had CD4<200 and mature WB

During the period 2008-2014
- 13 patients had IND 4 WB at notification
- 6 Patients had an earliest CD4<200
- 41/53 (76%) patients achieved VL<40 by 2014

Conclusions

- Increasing genetic diversity of HIV-1 in Western Australia over 15 years
  - Highest proportion of non-B-subtype sequences 2008-2011
  - Distinct trends for males versus females over time

- Phylogenetic analysis
  - Overall 25% of sequences in clusters (similar to AMEN analysis)
    - Greater proportion of B-subtype (31%) vs non-B sequences (15%)
    - B-subtype account for >90% of sequences in clusters of size >2
  - Earliest viral load assessment: No influence of subtype
  - Strong influence of calendar time
  - Earliest CD4 count: Significant effect of subtype
    - Suggests later diagnosis for non-B-subtype HIV-1

Emergence of a large B-subtype cluster in Western Australia from 2008-2014 (n=53)

- Ongoing expansion despite:
  - Early diagnosis in 13 pts (indeterminate WB)
    - Note higher CD4 T cell count and viral load at diagnosis associated with this cluster, suggestive of earlier diagnosis
  - High uptake of treatment among diagnosed cases (71% with VL <40)

- Note 4 cases with advanced HIV at diagnosis
  - Single large cluster in keeping with other studies of transmission networks.
    - Indicates risk is not normally distributed
    - How to reach the hard to reach?

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