The challenges of diversity: HIV-1 subtype distribution and transmission networks within the Australian Molecular Epidemiology Network-HIV (AMEN-HIV).

**Australian Molecular Epidemiology Network (AMEN-HIV)**

**Background**

- HIV-1 is a genetically variable and rapidly mutating virus
  - HIV-1 sequencing has been performed in routine HIV laboratories throughout Australia for >10 years
  - Drug resistance testing
  - HIV-1 subtype
    - B subtype
    - C subtype

**Methods**

- Australian Molecular Epidemiology Network (AMEN) formed December 2013
  - All states and territories represented (NT + Tas → Vic)
- Ethics and governance framework established
  - De-identified HIV-1 sequence results
    - Gender, age, state
    - Year of sequencing
    - HIV-1 sequences (RT + PR)
      - 2005 – 2012
    - Data analysis at one site
      - Sequence alignments checked
      - Duplicate sequences checked
    - Complete dataset other than NSW

**Overall:** 4873 sequences
Evidence of transmission by association:

**CLUSTERING CRITERIA**

- Bootstrap ≥98%
- Genetic distance ≤1.5%

How likely is it that sequences will cluster together statistically in repeated analyses? (≥ 98 out of 100 times)

How genetically similar are the sequences? (≥ 98.5% identical)

### Results: Proportion of HIV-1 sequences committed per State to the AMEN study

<table>
<thead>
<tr>
<th>State</th>
<th>Non B (%)</th>
<th>B (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>QLD</td>
<td>283 (17.9%)</td>
<td>1296 (82.1%)</td>
<td>1579</td>
</tr>
<tr>
<td>SA</td>
<td>111 (31.6%)</td>
<td>240 (68.4%)</td>
<td>351</td>
</tr>
<tr>
<td>VIC</td>
<td>407 (24.4%)</td>
<td>1261 (75.6%)</td>
<td>1668</td>
</tr>
<tr>
<td>WA</td>
<td>297 (41.0%)</td>
<td>427 (59.0%)</td>
<td>724</td>
</tr>
<tr>
<td>W NSW</td>
<td>144 (26.1%)</td>
<td>407 (73.9%)</td>
<td>551</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1242 (25.5%)</td>
<td>3631 (74.5%)</td>
<td>4873</td>
</tr>
</tbody>
</table>

### Results: HIV-1 subtype distribution over time

- B subtype: 829 / 3631 (22.8%)
- Non B subtype: 306 / 1242 (24.6%)
- Overall: 1135 / 4873 (23.3%)

### Results: HIV-1 subtype distribution per state in more detail

<table>
<thead>
<tr>
<th>Subtype</th>
<th>WA</th>
<th>SA</th>
<th>Vic</th>
<th>W NSW</th>
<th>Qld</th>
</tr>
</thead>
<tbody>
<tr>
<td>B Subtype (%)</td>
<td>59.0</td>
<td>68.4</td>
<td>75.6</td>
<td>73.9</td>
<td>82.1</td>
</tr>
<tr>
<td>CRF01_AE (%)</td>
<td>19.2</td>
<td>10.8</td>
<td>10.4</td>
<td>7.8</td>
<td>5.7</td>
</tr>
<tr>
<td>C Subtype (%)</td>
<td>15.1</td>
<td>10.0</td>
<td>9.2</td>
<td>9.8</td>
<td>7.8</td>
</tr>
<tr>
<td>CRF02_AG (%)</td>
<td>2.4</td>
<td>5.7</td>
<td>0.6</td>
<td>3.8</td>
<td>1.3</td>
</tr>
<tr>
<td>D Subtype (%)</td>
<td>0.7</td>
<td>0.3</td>
<td>0.2</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Other Subtypes (%)</td>
<td>3.2</td>
<td>2.6</td>
<td>2.9</td>
<td>2.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Total number of sequences n=4873</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Results: Age and gender according to HIV-1 subtype

- Non B subtype
- B subtype

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Std</td>
<td>N</td>
</tr>
<tr>
<td>Non B</td>
<td>32.5</td>
<td>11.9</td>
</tr>
<tr>
<td>B</td>
<td>38.5</td>
<td>13.2</td>
</tr>
</tbody>
</table>

**p<0.001**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>p&lt;0.012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non B</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
</tr>
</tbody>
</table>

**Results: Phylogenetic analysis distribution**

- B-subtype: 629 / 3631 (22.8%)
- Non-B-subtype: 306 / 1242 (24.6%)
- Overall: 1135 / 4873 (23.3%)
Results: Detection of HIV subtype pairs and networks over time

- **Detection of 2° sequence in a pair**
  - National results - All sequences
  - B subtype
  - Non B

- **Detection of 3° sequence in a network size >2**
  - National results - All sequences
  - B subtype
  - Non B

**Results: Gender distribution in HIV networks for a given subtype**

- 76% of male only groups
- 88% B subtype groups are only males
- 53% AE subtype groups are only males
- 17% C subtype groups are only males

**Results: Distribution of network size for a given HIV subtype**

- **C Subtype**
  - Sequences: CRF01_AE, B Subtype
  - Percentages: Pairs, Network of 3, Network of 4, Network of ≥5

Significantly more non B pairs than non B larger networks (p=0.003)
Larger network size (>2) with B subtypes than the non B subtypes (p=0.021).

**Results: Comparison of interstate vs intra-state clusters**

Sequences in interstate versus ‘intra-state’ clusters (>2 in size)
B-subtype: 190 / 438 (43.4%)
Non-B-subtype: 52 / 115 (45.2%)
Overall: 242 / 553 (43.8%)

Sequences in interstate versus ‘intra-state’ clusters (pairs)
B-subtype: 78 / 384 (20.3%)
Non-B-subtype: 34 / 186 (18.3%)
Overall: 112 / 570 (19.6%)

**Results: Proportion of B and Non-B sub-type networks according to gender**

- **B-network sequences**
- **Non-B-network sequences**

**Results: Age and gender according to HIV-1 subtype and network size**

- **Non-B - singleton**
- **Non-B - pairs**
- **Non-B-network (>2 in size)**

- **B - singleton**
- **B - pairs**
- **B-network (>2 in size)**

**Results: Comparison of interstate vs intra-state clusters**

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Conclusions

- AMEN collaboration has established a national approach to HIV-1 molecular epidemiology studies
  - Ethics and Governance frameworks are in place
  - National database: prospective as well as retrospective dataset
    - Inclusion of NSW data underway
  - Platform for research collaboration: non-affiliated.
- Not a contact tracing analysis
  - De-identified baseline viral sequencing data
- HIV-1 subtypes in Australia
  - Overall ~25% of sequences are non-B-subtype
    - Increasing proportions across all states, with a broad range of subtypes reflecting geographic origins.
    - Reflects a growing influence of migration and travel
  - Wide age distribution for both B- and non-B-subtype virus
    - More pediatric cases with non-B subtypes
    - More females with non-B subtypes (esp. C-subtype)
    - B-subtype sequences remain predominantly associated with males

Conclusions

- HIV-1 phylogenetic clusters in Australia
  - Overall ~23% sequences classified within clusters
    - Similar proportions of B-subtype (23%) and non-B-subtype (25%) sequences within clusters
    - Higher proportion of male/female clusters in non-B
      - C-subtype (S Africa, India): 90%
      - AE-subtype (SE Asia): 50%
      - B-subtype: 12%
  - No obvious clustering according to state.
  - Significant proportion of clusters involve sequences from more than 1 state (20% of pairs, 44% of larger clusters)
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  - Overall ~23% sequences classified within clusters
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Conclusions

- Highlights the national structure of HIV-1 in Australia
  - Common challenges to all states
  - Growing impact of travel and migration
  - Benefits of engagement with international partners
  - Provides a basis for creating and monitoring prevention strategies
- Valuable data: now and for future
  - Collaborative network: facilitated engagement with clinical, scientific and community organizations

Acknowledgements

- Western Australia
  - George Guelfi
  - Mina John
- South Australia
  - VIDRL
    - Megan Gooey
    - Mike Catton
- NSW
  - Tony Kelleher
  - Angie Pinto
  - Roger Garsia
- Queensland
  - Helene Johanson
- STI/HIV conference (ISSTDR scholarship)

Australian Molecular Epidemiology Network

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Results: Proportion of sequences from interstate or intra-state networks according to size

- Networks with a B-subtype
- Networks with a Non-B-subtype

Results: Factors associated with a sequence being classified into a cluster (y,n:>18yo)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall</th>
<th>WA</th>
<th>SA</th>
<th>VIC</th>
<th>NSW</th>
<th>QLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td>0.003</td>
<td>-0.005</td>
<td>-0.002</td>
<td>-0.001</td>
<td>-0.008</td>
<td>-0.003</td>
</tr>
<tr>
<td></td>
<td>p=0.001</td>
<td>p=0.001</td>
<td>p=0.3</td>
<td>p=0.34</td>
<td>p=0.001</td>
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<tr>
<td>SEQ ERA</td>
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<td>0.05</td>
<td>0.07</td>
<td>0.03</td>
<td>0.02</td>
<td>0.04</td>
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<tr>
<td></td>
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<td>p=0.001</td>
<td>p=0.002</td>
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</tr>
<tr>
<td>Gender</td>
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<td>-0.02</td>
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<tr>
<td></td>
<td>p=0.023</td>
<td>p=0.17</td>
<td>p=0.03</td>
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<td>Non-B vs B</td>
<td>0.05</td>
<td>-0.07</td>
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<td>0.05</td>
<td>-0.02</td>
<td>0.07</td>
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<tr>
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</table>

Variables associated with the classification of sequences into larger network size 2-3 or >3. (>18yo, not pairs)

<table>
<thead>
<tr>
<th>Variable</th>
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<th>NSW</th>
<th>QLD</th>
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<tbody>
<tr>
<td>AGE</td>
<td>0.002</td>
<td>-0.001</td>
<td>-0.001</td>
<td>-0.001</td>
<td>-0.003</td>
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<td>p=0.77</td>
<td>p=0.04</td>
<td>p=0.01</td>
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<tr>
<td>SEQ ERA</td>
<td>0.03</td>
<td>0.09</td>
<td>-0.003</td>
<td>-0.003</td>
<td>0.003</td>
<td>0.03</td>
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<tr>
<td></td>
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<td>p=0.004</td>
<td>p=0.96</td>
<td>p=0.88</td>
<td>p=0.02</td>
<td>p=0.13</td>
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<tr>
<td>Gender</td>
<td>-0.12</td>
<td>-0.02</td>
<td>0.03</td>
<td>-0.4</td>
<td>0.13</td>
<td>-0.04</td>
</tr>
<tr>
<td></td>
<td>p=0.008</td>
<td>p=0.86</td>
<td>p=0.001</td>
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<td>p=0.17</td>
<td>p=0.6</td>
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<tr>
<td>Non-B vs B</td>
<td>-0.03</td>
<td>-0.34</td>
<td>0.07</td>
<td>-0.07</td>
<td>0.09</td>
<td>0.03</td>
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<tr>
<td></td>
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<td>p=0.001</td>
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<td>p=0.021</td>
<td>p=0.12</td>
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</tr>
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<td>p=0.7</td>
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