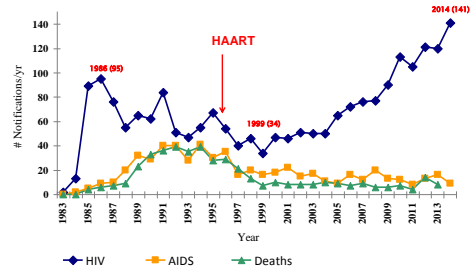## Slide 1

Longitudinal trends in Western Australian HIV-1 sequence diversity and viral transmission networks and their influence on clinical parameters: 2000 – 2014.

Alison Castley, Silvana Gaudieri, Ian James, Laila Gizzarelli, George Guelfi, Mina John, and David Nolan

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Slide 2

Background

- HIV notifications are on the rise
  - □ Against global trends
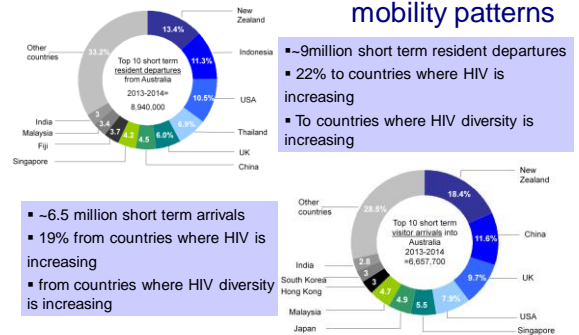

Slide 3

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).

1: Siemeiniuk R, CJIDMM 2013, 2: UK Collaborating Gp, AIDS 2014, 3: Chaix M CID 2013, 4:Castley 2015 submitted, 5 Gaf Vir 2013. 6 Chen Plos One 2010

Slide 4

Background: mobility patterns

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

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

Statistics compiled from the Australian Bureau of Statistics

Slide 5

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

Slide 6

Methods

- Ethics and governance frameworks established
  - ■ 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

Methods: Phylogenetic analysis

Evidence of phylogenetic cluster
(similar HIV-1 sequences)

CLUSTERING CRITERIA
Bootstrap ≥98%
Genetic distance ≤1.5%

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

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)

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## Results: Gender, age and clinical parameters at first assessment

Subtype		B	Non B	N=1021 sequences
Sequences #		619	364	
**Gender	-Male	557	232	
	-Female	62	132	
Age (yrs)	-Male	38.8 (12-76)**	39.6 (1-74)**	
	-Female	33.1 (1-74)	32.7 (1-68)	
Viral load (lcpm)				
	-Male*	**4.57** (1.6-7)	**4.76** (1.6-7)	*p<0.05, **p<0.001
	-Female	4.33 (1.6-6.23)	4.40 (1.6-7)	
CD4 T cell count (cell/uL)	-Male**	**445** (3-1792)	**362** (2-1710)	
	-Female	436 (6-1020)	357 (6-2024)	
CD4:CD8 ratio				
	-Male	0.46 (0.01-2.57)	0.41 (0.01-1.92)	
	-Female*	**0.5** (0.04-2.6)	**0.39** (0.02-1.21)	

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Results: Age and gender according to HIV-1 subtype

Australian Molecular Epidemiology Network
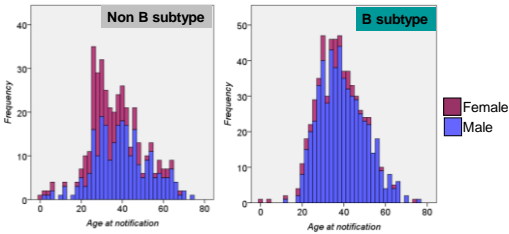
	Non B subtype (p<0.001)			B subtype (p<0.001)			
	Mean	Std	N	Mean	Std	n	
Female	33.1	12.2	145	33.1	12.8	62	p=0.99
Male	39.2	13.4	257	38.9	10.9	557	p=0.7

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Results: HIV subtype diversity over time
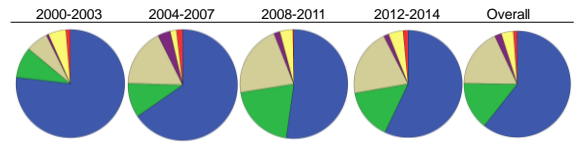
2000-2003 2004-2007 2008-2011 2012-2014 Overall

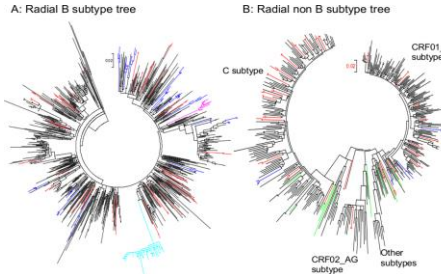B subtype ▪ C subtype ▪ CRF01_AE subtype ▪ CRF02_AG subtype ▪ Inter-subtype recombinant ▪ Other non-B subtypes

10
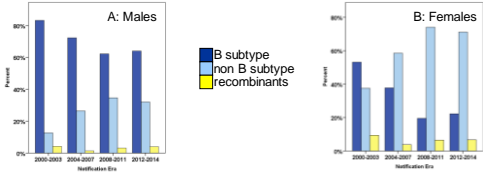
Results: Gender and HIV-1 subtypes over time

Results: Gender and HIV-1 subtypes over time


12
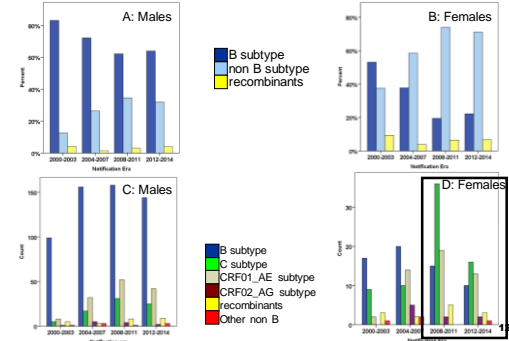
Results: Phylogenetic analysis

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

C subtype

CRF01_AE subtype

CRF02_AG subtype   Other subtypes

Sequences in phylogenetic clusters
B-subtype: 195 / 619 (31.5%)
Non-B-subtype: 62 / 402 (15.4%) *p<0.001*   *Overall:* 257 / 1021 (25.2%)

Results: Sequences within clusters

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

C subtype

CRF01_AE subtype

CRF02_AG subtype   Other subtypes
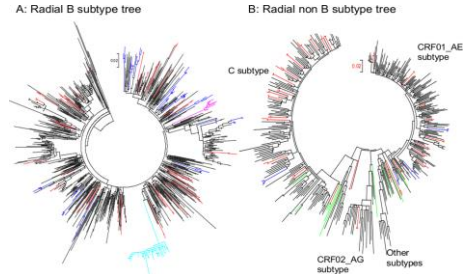
Sequences in large clusters (>2 in size)
B-subtype:133/145 (91.7%)
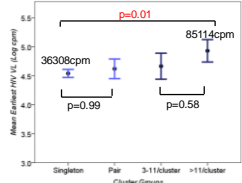Non-B-subtype: 13/145 (8.3%) *p<0.001*
Overall: 145/257 (56.4%)

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
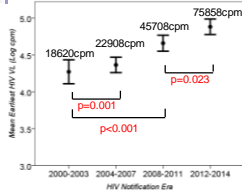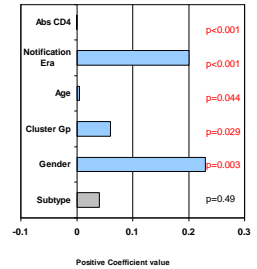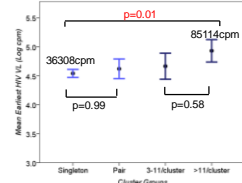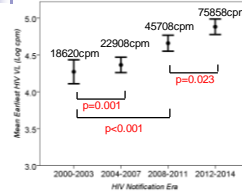

Strong influence of notification era (p<0.001).

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).
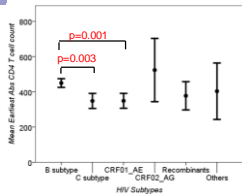
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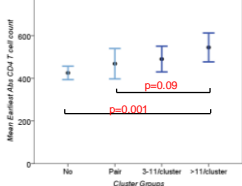## Results: Earliest viral load assessment Regression analysis

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Results: Earliest CD4 T cell count

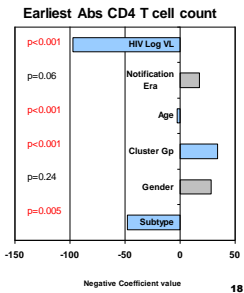No significant association between notification era and CD4 T cell count (p=0.1) or CD4:CD8 ratio (p=0.2)
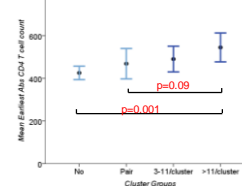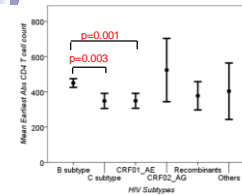
Higher CD4 count associated with HIV-1 sequences in large cluster (n=53, p=0.001)

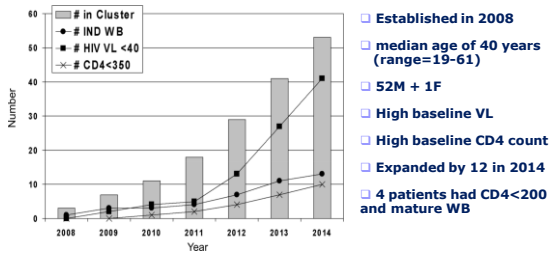HIV-1 subtypes C and AE associated with lower CD4 count (p<0.01).

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Results: Lower CD4 T cell count Regression analysis

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Results: Large network (n=53) dynamics

Legend:
- # in Cluster
- # IND WB
- # HIV VL <40
- # CD4<350

Y-axis: Number
X-axis: Year (2008, 2009, 2010, 2011, 2012, 2013, 2014)

- ❑ **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

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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

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Conclusions

- ■ 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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Acknowledgements

- ■ Staff involved in RPH-DCI PathWest Lab service
  - ❑ Steve Pummer
  - ❑ Linda Smith (dec)

- ■ RPH clinical service
  - ❑ Nurses and staff

- ■ STI/HIV conference (ISSTDR scholarship)

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