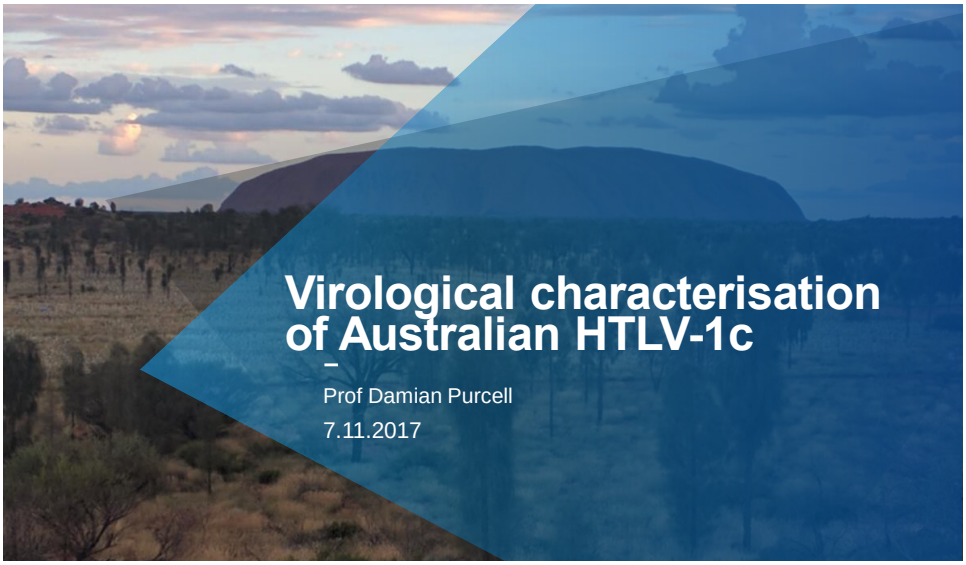

Virological characterisation of Australian HTLV-1c

Prof Damian Purcell

7.11.2017

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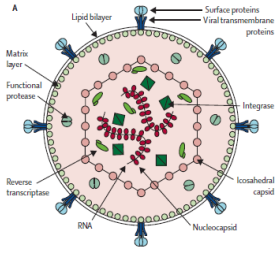
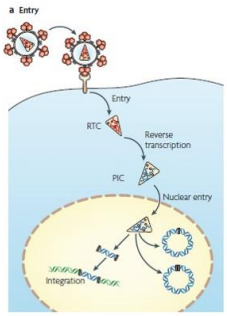
Baker IDI Heart & Diabetes Institute

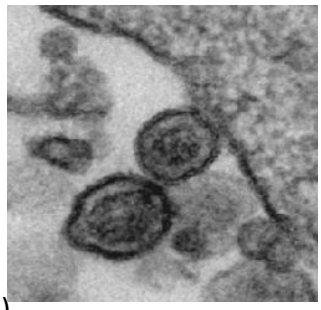
Human T-cell lymphotropic virus (HTLV-1)

- First described Retrovirus infecting humans (1980)
- A cousin of the second described Retrovirus (1983/4)
  - HIV-1 → some similarities but different.
- It's a virus – an evolutionary machine
- Obeys only the laws of evolution and fitness
- Two phases:
  - Virion
    - RNA genome
  - The infected cell
    - Proviral DNA genome





Human T-cell lymphotropic virus (HTLV-1)

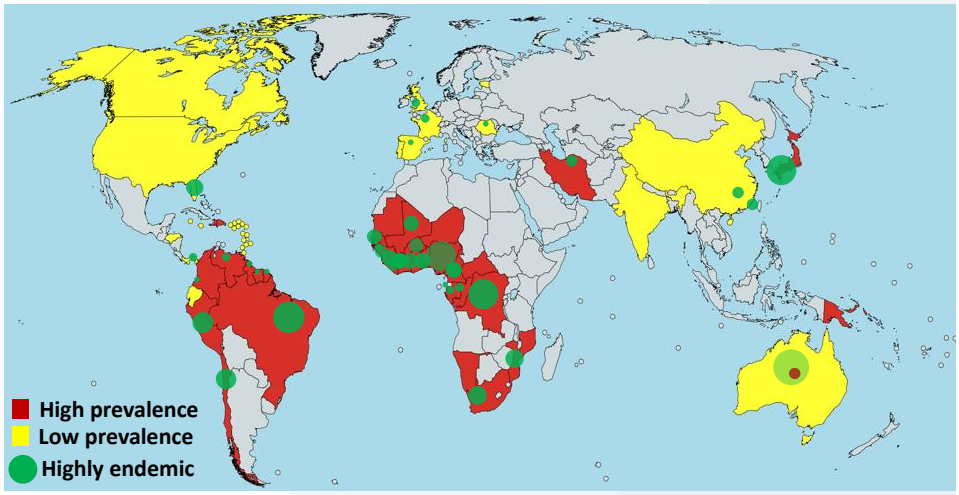


- Lifelong infection upon transmission
- Mutations when viral RNA → cDNA
- Infects primarily by cell-cell transfer
  - Receptor is glucose transporter 1 (Glut-1)
  - Assisted by neuropilin-1 (NRP-1), and
  - heparan sulfate proteoglycans (HSPG).
- Primarily targets T-cells ($CD4^+$ and CD8+)
  - Can infect B-cells, monocytes, DCs, myeloid cells, endothelial cells
- No detectable cell-free virus in plasma
- Proviral loads range 50 – 2,000,000 : million
- Diagnosis by serology, Western blot, proviral PCR (ddPCR)
- No specific drugs, no vaccines

Human T-cell lymphotropic virus (HTLV-1) - similar to HIV-1, but also subtly different

- HIV-1 virions are found in high levels in blood
  - HTLV-1 mostly exists within infected cells
- HIV-1 eliminates targeted T-cells
  - HTLV-1 does not eliminate infected cells
- HIV-1 damages immunity by removing T-cells
  - HTLV-1 damages immunity by altering T-cell function
- HIV-1 disease monitored by levels of virus in blood
  - HTLV-1 disease monitored by % T-cells infected

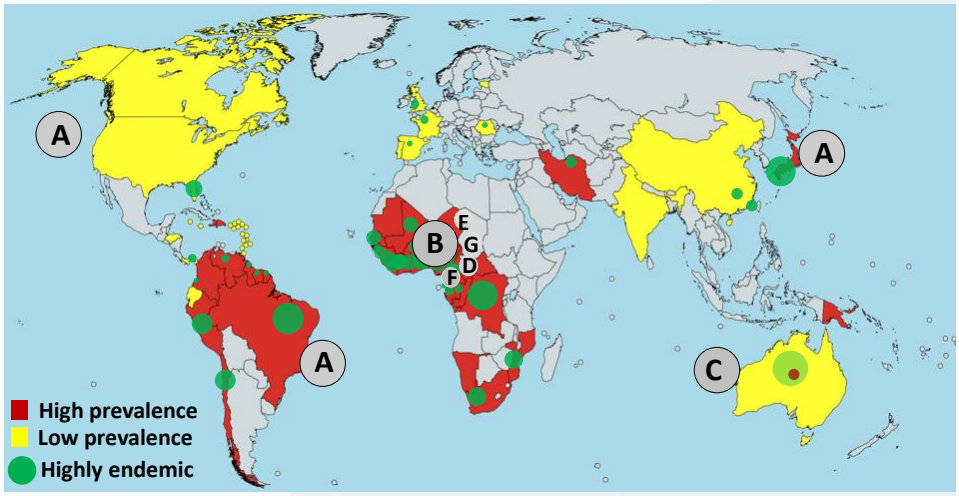
Human T-cell lymphotropic virus type-1 (HTLV-1)



- Infects 10 – 15 million; transmitted by breastfeeding, sexual contact, and blood transfer
- >90% remain asymptomatic
  - 5% develop ATL
  - 1-4% develop HAM/TSP

Adapted from Gessain and Cassar, 2012 and Watanabe, 2011

Human T-cell lymphotropic virus type-1 (HTLV-1)



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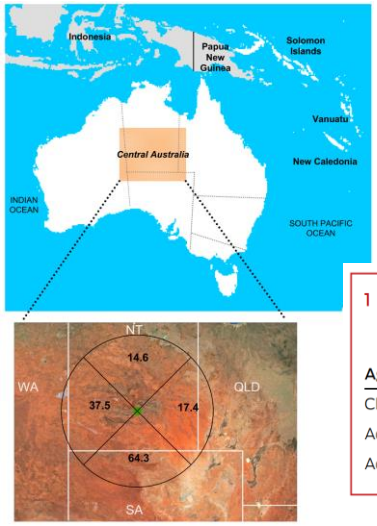
HTLV-1c: High prevalence in remote central Australia



Einsiedel et al
PLoS NTD 2014

- High prevalence in hospital based surveys ~ 43%
  Einsiedel and Woodman, MJA 2010
  Einsiedel et al., PLoS NTD 2014
- 40% prevalence in a community based survey *(Einsiedel et al. MJA 2016)*
  > 60% in others *(Einsiedel et al., Intl. HTLV 2017)*

1 Prevalence of HTLV-1 infection among 97 Indigenous Australian residents of a remote Northern Territory community, according to age group

Age category	Male	Female
Children (1–14 years)	1 of 13 (7%)	0 of 10
Adults (15–34 years)	7 of 24 (29%)	6 of 19 (32%)
Adults (≥ 35 years)	10 of 15 (67%)	7 of 16 (44%)

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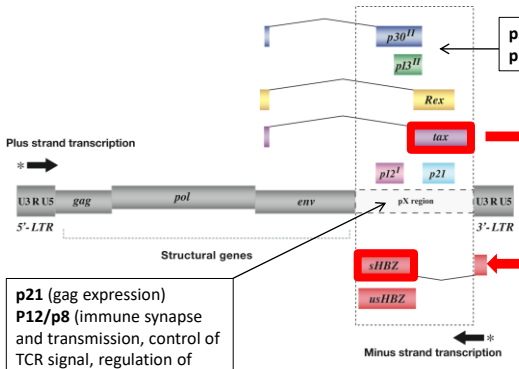
HTLV-1: genetic structure

- complex retrovirus with many regulatory and accessory genes



p30 (viral infectivity)
p13 (viral infectivity)

Tax protein and HBZ mRNA drive proliferation of the infected cell

p21 (gag expression)
P12/p8 (immune synapse and transmission, control of TCR signal, regulation of MHC-1 (immune evasion))

Adapted from Satou and Matsuoka, 2013

Proviral genome sequence divergence of HTLV-1c

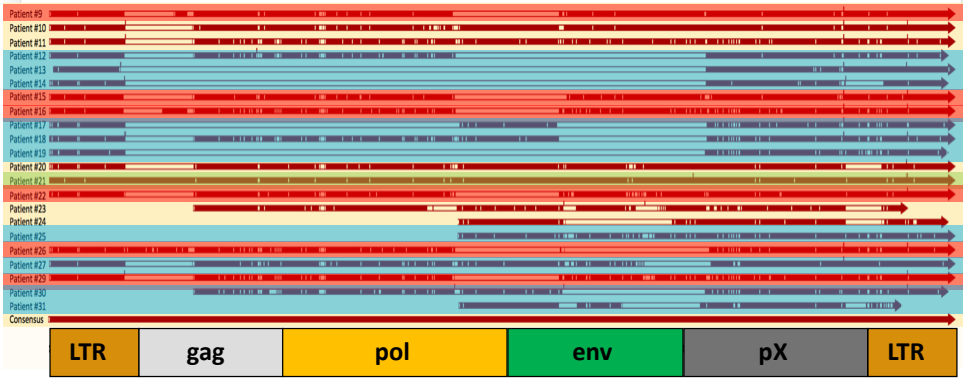
- Papua New Guinea strains, (Yanagihara et al., PNAS 1991)
- Solomon Island strains, HTLV-1 MEL 1 / MEL 5 (Gessain, et al., J. Virol. 1993)
- Australian Indigenous strain, HTLV-1 MSHR-1 (Bastian et al., J. Virol. 1993)
- Four Australian proviruses (Cassar et al., PLoS Negl Trop Dis. 2013)
  - 2 distinct provirus clades
  - LTR, Gag, Tax sequence on 19 others

Several studies identified sequence divergence of p12/X-region

Frozen PBMC provided by Dr. Lloyd Einsiedel (Alice Springs Hospital)

- 22 full proviruses from consenting patients in the ASH HTLV-1c cohort

HTLV-1c sequence alignment of remote Indigenous Australians

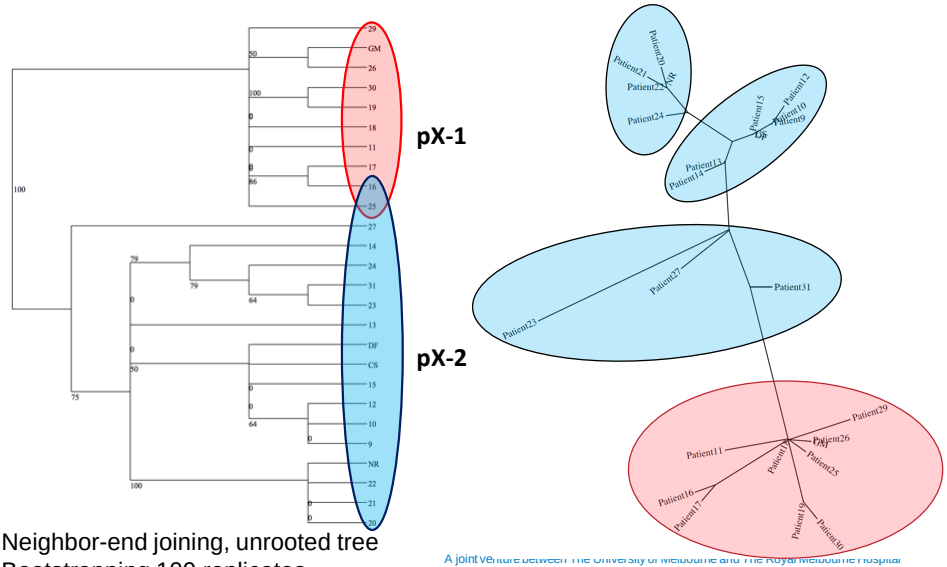


Snapgene software V.4.04

Differences in HTLV-1a vs HTLV-1c?

Genomic Region	Nucleotide Divergence %	Amino Acid Divergence %
Rex	5.26	13.23
Env	6.27	3.07
Pol	6.54	3.91
Tax	6.69	7.65
Pro	6.95	8.97
Gag	7.60	3.96
5'LTR	9.14	n/a
3'LTR	9.40	n/a
pX region	9.50	21.95
p30	10.41	15.68
HBZ	12.36	19.12
p27	12.96	22.35
p8	13.33	18.84
p12	19.39	26.80

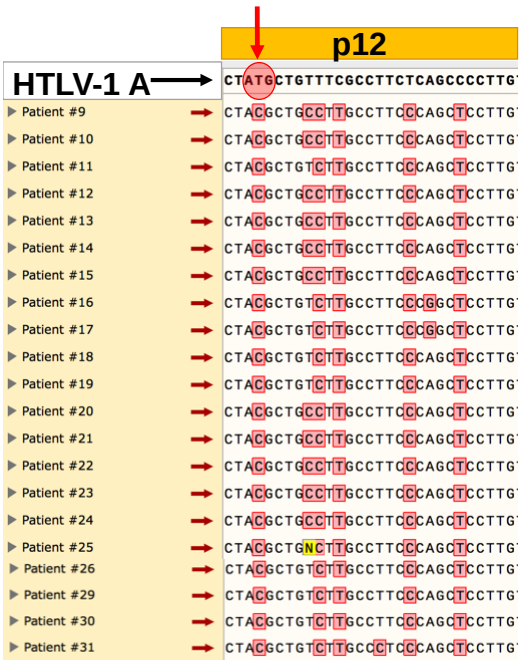
Phylogenetic comparison of HTLV-1c in remote Indigenous Australians



Neighbor-end joining, unrooted tree
Bootstrapping 100 replicates
Pairwise distance used K2P method

Seaview software V.4.5.4

p12 start codon mutation



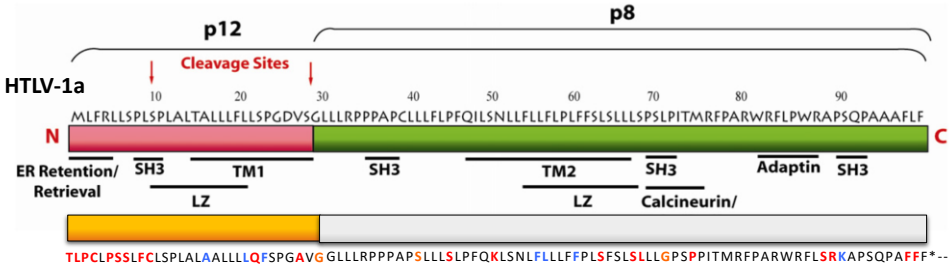
ATG = start codon

A~~T~~G → ACG

methionine → threonine

Snapgene software V.4.04

p12 variation between HTLV-1a and -1c



Red – significant substitution, Orange – similar property, Blue – very similar properties

Adapted from Van Prooyen et al., 2010

- Lack of p12 initiating Met in ALL subtype C proviruses
- Similar mRNA splice acceptor sites compared to HTLV-1a
  - Highly inefficient cryptic splice donor downstream from Tax/Rex SD2 ?
- P12 / p8 ER-processing signals retained
- mRNA or protein for p12/p8 not yet elucidated

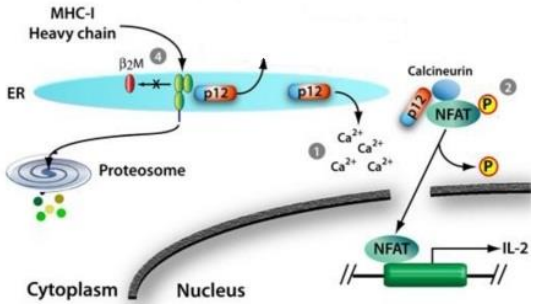
p12 protein functions

Promotes viral replication

Modulates Ca^{2+} release → upregulates TCR signalling

Binds IL-2 receptors → upregulates T-cell proliferation

Re-routes MHC-1 to proteosome for degradation → immune evasion



Adapted from Edwards, 2011. Viruses

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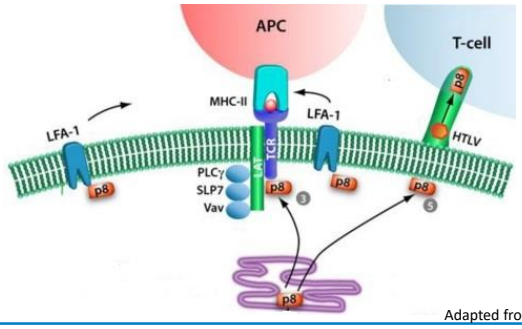
p8 protein functions

Cleaved product of p12, inhibits viral replication

Downregulates TCR signalling →leads to anergy of T-cells

Clusters LFA-1 and ICAM-1 → increases T-cell contact



Adapted from Edwards, 2011. Viruses

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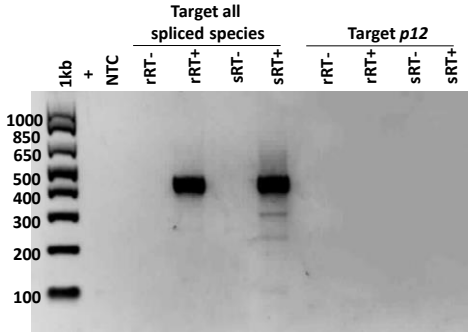
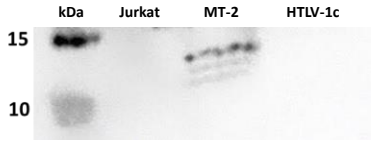
No p12 mRNA or protein detected

- Primers designed to target *p12* mRNA sequence for RT-PCR
- Cloned and sequenced all amplicons
- No amplification of *p12* mRNA



- Probed HTLV-1c proteins with anti-HTLV-1a
- No detection of HTLV-1c p12 protein



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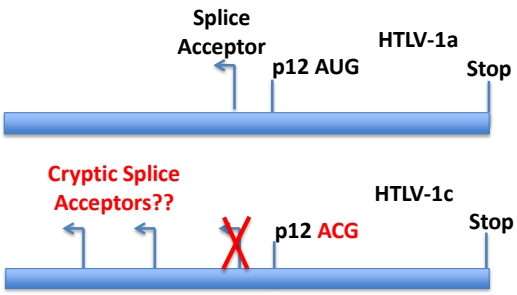
Significant genomic differences of HTLV-1c

Percentage Divergence of HTLV-1c from HTLV-1a

	Nucleotide level	Amino acid level
9kB Genome	8.5%	n/a
LTR	8.2%	n/a
Gag	7.4%	4%
Tax	7%	3%
HBZ	14%	19%
p12	20%	29%



Adapted from Yurick, D., 2016

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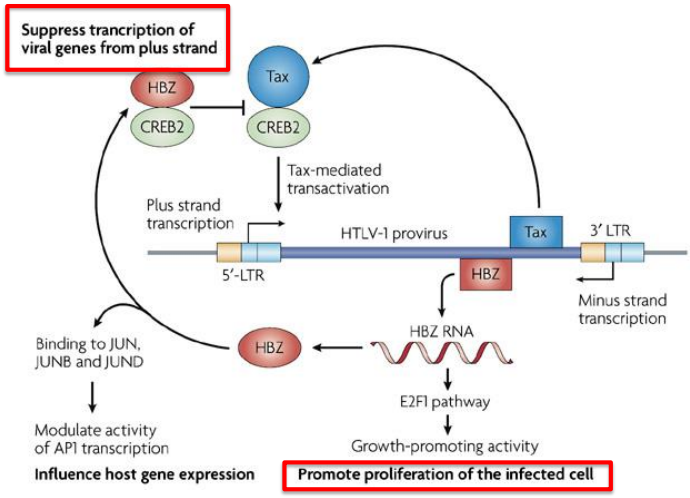
HBZ protein functions



Matsuoka et al. 2007

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Novel HBZ mRNA species detected

- Primers designed to target HBZ mRNA on the antisense strand
- Novel splice donor and acceptor sites detected for HBZ minor
- Deduced protein sequence of HBZ minor is 38 amino acids shorter than HBZ major, all in the activation domain

```
HTLV-1a Major HBZ | MAASGLFRCLPVSCPEDLLVEELVDGLLSLEEELKDK-EEEEAVLDGL
HTLV-1c Major HBZ | MAASGPFRCLPVPRPEDLLVEDLVDGLLSLEDDLKDQREEEESVLDGV
HTLV-1c Minor HBZ | MAASGRA-----------------------------------DGV

HTLV-1a Major HBZ | LSLEEESRGRLRRGPPGEKAPPRGETHRDRQRRAEEKRKRKKEREKE
HTLV-1c Major HBZ | LSLEEESR--LRWGLPGEEAPPRGETHRDRRRAEEKRKRKREREKE
HTLV-1c Minor HBZ | LSLEEESR--LSWGLPGEEAPPRGETHRDRRRAEEKRKRKREREKE
```

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Function of anti-sense viral product HBZ

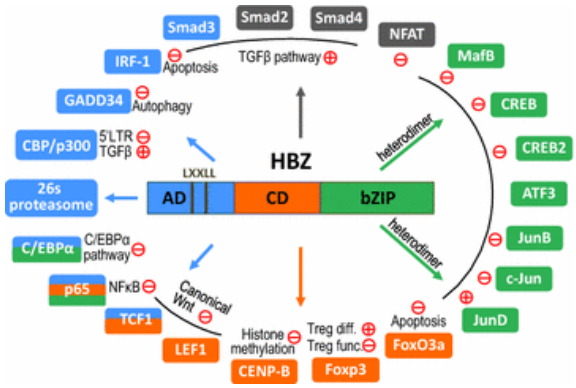
Ma et al. Retrovirology (2016) 13:16
DOI 10.1186/s12977-016-0249-x

Retrovirology

REVIEW Open Access

Multifaceted functions and roles of HBZ in HTLV-1 pathogenesis

Guangyong Ma, Jun-ichirou Yasunaga and Masao Matsuoka*



Conclusions

Relatively higher divergence for genes associated with ATL and HAM-TSP

- p12 / p8 and HBZ

p12 start codon mutation → may be expressed through a novel mechanism

- Chimeric host/virus mRNA, internal initiation?

HTLV-1c may have adapted to not require p12

Novel HBZ mRNA may affect viral pathogenesis

Amino acid deletions in the activation domain of HBZ

- Decreased HBZ-inhibition of Tax expression → immune exhaustion?
- Promote cell survival?

These differences may alter transmission and pathogenicity of HTLV-1c, and contribute to the unique disease outcomes seen in remote Indigenous communities in Central Australia

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Acknowledgements

Alice Springs Hospital/Baker IDI:
- Lloyd Einsiedel
- Hai Pham

South Australian Health & Medical Research Institute:
- James Ward

Doherty Institute:
Damian Purcell Lab –
David Yurick
Georges Khoury
Charlene MacKenzie
Chris Gonelli
Hannah King
Michelle Lee
Behnaz Heydarchi
Natalia Salazar
Leigh Harty
Nicole Tay
Shuo Li
Ashley Hirons

Doherty Institute:
Sharon Lewin and her Lab –
Megan Crane, Jenny Audsley

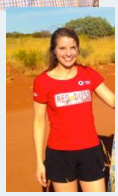
Doherty Institute:
Kedzierska Lab –
Katherine Kedzierska
Bridie Clemens
Sneha Sant
Liyen Loh

National Reference Lab
Kim Wilson
Stirling Dick

VIDRL:
Peter Revill

Burnet Institute:
Gilda Tachedjian

Indigenous Australian volunteers!



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With thanks
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What is the HTLV-1c PVL doing over time?

- Blind study of 83 HTLV-1 patients with > 2 or more time points (gDNA)
- Collection dates from: 09 Mar 2008 – 10 Nov 2015
- Study spans: 7 years, 8 months, 1 day

Clinical profiles of HTLV-1c participants in PVL Longitudinal Study		
	Female n = 98	Male n = 126
Age range (years)	25 - 72	23 - 85
Age, years (mean±SD)	51 ± 10.8	56 ± 12.5
Sex (n%)	33/83 (39.8.0)	50/83 (60.2)
Median HTLV-1 PVL per genome[1]	4.38 x 10^3 (IQR, 3600 - 5.3 x 10^3)	4.5 x 10^3 (IQR, 3610 - 5.48 x 10^3)
Median HTLV-1 PVL per T-cell[1]	2.5 x 10^4 (IQR, 18800 - 3.23 x 10^4)	3.3 x 10^4 (IQR, 29100 - 3.62 x 10^4)
[1]HTLV-1c Tax copy number and interquartile range (IQR) per 10^6 PBMCs		

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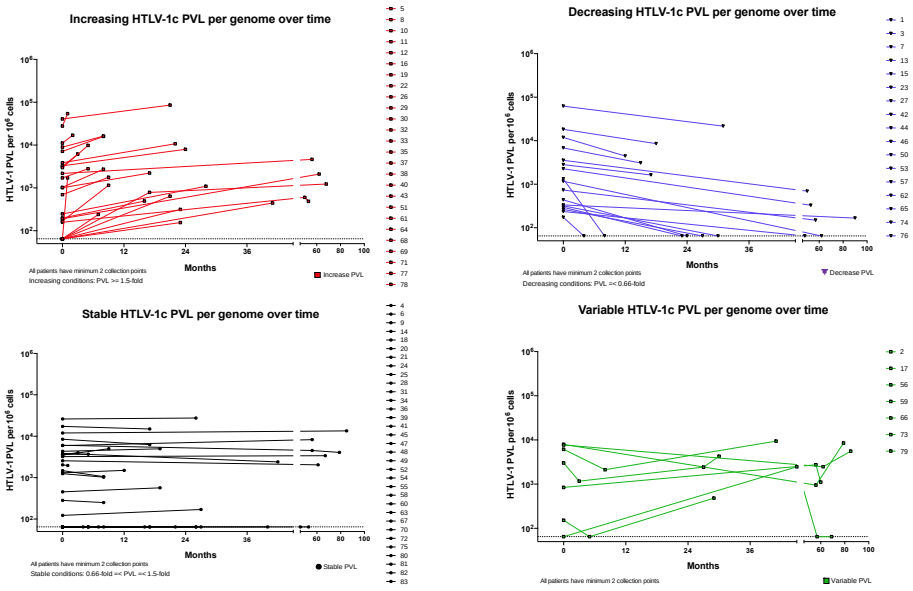
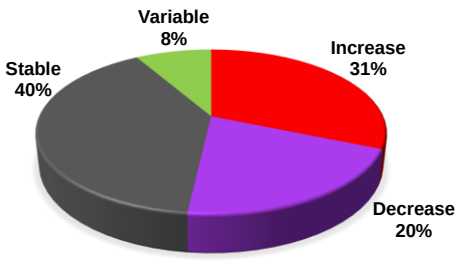
HTLV-1 PVL Over Time

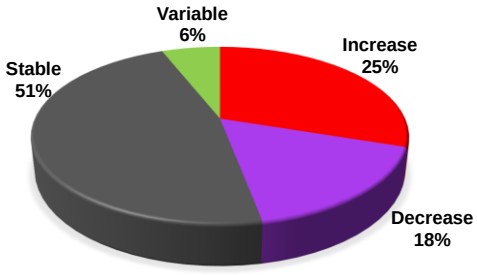


Examination of HTLV-1c PVL changes over time

HTLV-1c PVL per genome



HTLV-1c PVL per T-cell



- HTLV-1c PVL longitudinal study in 83 remote Indigenous central Australians
- Post-hoc analysis generated hypothesis: high HTLV-1c PVL in remote Indigenous Australians increase risk of certain clinical inflammatory diseases

Conclusions: Australian HTLV-1c infections

- HTLV-1c from remote central Australian Aboriginal communities
  - Highly prevalent
  - Efficient community transmission
- Relatively higher divergence for genes associated with ATL and HAM-TSP
  - p12 / p8 and HBZ
- High rates of inflammatory disease (bronchEx) and blood stream infections
  - Lower rates of ATL and HAM-TSP
- Open questions and areas of research:
  - Viral mRNA structure and expression?
  - Disease associations in the longitudinal study
  - Immune mechanisms of “controllers” in high prevalence communities.
  - No current community PREVENTION STRATEGIES, specific drugs or vaccines.