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Abstract # 100

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HIV Migrates Bi-directionally between Blood and Cerebrospinal Fluid and between Blood and Semen

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Background

- Once HIV infection is established, the virus persists in anatomic compartments, including the blood, central nervous system and genital tract.
- Understanding the dynamics of viral migration between compartments will be important for vaccine design and efforts towards a sterilizing cure.

Hypothesis

Anatomic compartments must be considered not only as a 'sink' for HIV but also as a reservoir that actively contributes to viral replenishment and diversification during the course of HIV infection.

Methods

- Partial HIV *env* sequences were sampled longitudinally from 15 ART- naïve, HIV-infected subjects, including:
  - 12 paired cerebrospinal fluid (CSF) and blood samples (from 4 subjects) [1].
  - 46 paired semen and blood samples (from 11 subjects) [2-5].
- Sequences were filtered and aligned as described in [6], and analyzed by:
  - phylogenetic reconstruction (Bayesian Markov-chain Monte Carlo approach).
  - viral compartmentalization (tree--based and distance--based methods).
  - migration events (discrete Bayesian asymmetric phylogeographic approach of diffusion with Markov jump counts estimation [7]).
- Markov chain describes how a genetically inherited trait changes state over the tree. We estimated the rate of gene flow among compartments using 'Markov jump' counts of location state transitions [7] **Figures 1-3**.
- Potential correlates of viral gene flow across compartments were evaluated using viral gene flow per unit of time and available clinical and virological data.

Results

- Subjects were sampled longitudinally (range: 3-11 timepoints/subject) over a mean duration of 433 days (range: 9-2071 days).
- HIV-1 env sequences were generated by clonal sequencing from:
  - blood plasma (range: 27-152 sequences/subject)
  - seminal plasma (21-85 sequences/subject)
  - CSF supernatant (11-59 sequences/subject)
  - Mean *env* sequence length was 484 nucleotides (range: 333-648)
- Mean CD4+ T--cell count was 541 cells/µl (range: 200-1123).
- Mean HIV RNA $\log_{10}$ copies/ml was 4.6 for blood plasma (range: 2.5- 5.4), 3.4 for seminal plasma (2.5-4.0), and 3.8 for CSF (2.5-5.4).

Acknowledgments

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Bayesian approach of inferred migration events and Slatkin-Maddison test for compartmentalization

Markov jump counts correlated with inferred migration events according to Slatkin-Maddison tree based test of compartmentalization, **Figure 1.**

- Compartmentalization was maintained throughout all sampled timepoints for:
  - 3 of the 4 individuals with paired blood and CSF samples.
  - none of the subjects with paired blood and semen samples.

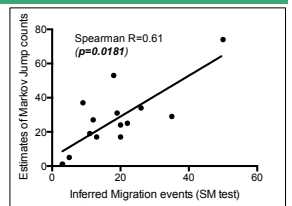

Figure 1. Correlation between estimates of Markov Jump counts and inferred migration events using a Slatkin-Maddison test.

Discrete Bayesian diffusion approach to reconstruct the gene flow between compartments

- By estimating the ancestral spatial locations of HIV-1 variants, we inferred the direction of viral gene flow from blood to anatomic compartment fluid (CSF or seminal plasma) or from anatomic compartment fluid to blood (**Figure 2**).
- In most cases, there was a reciprocal replenishment between blood and anatomical compartments:
  - mean of 15 migration events from blood to semen (range: 0--34) and 8 migration events from blood to CSF (range: 0--16).
  - mean of 14 migration events from semen to blood (range: 1--43) and 11 migration events from CSF to blood (range: 1--15).

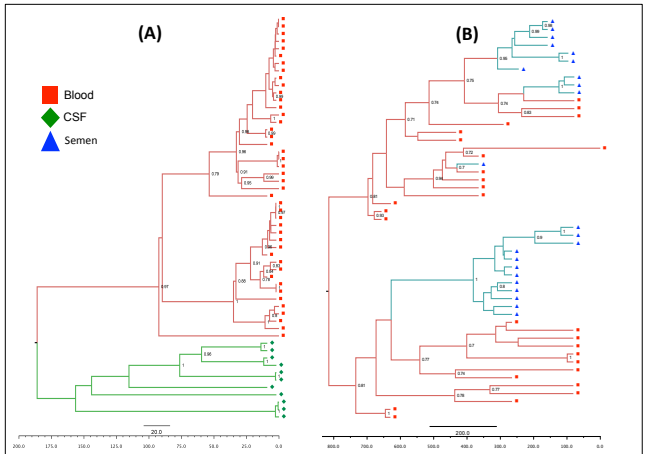

Figure 2. Time scaled Bayesian phylogenetic trees
Branches are colored according to the most probable location state of their descendant nodes respectively in red (blood plasma), blue (seminal plasma) and green (CSF). Squares, diamonds and triangles represent blood, semen and CSF derived variants. Posterior probability >0.70 are indicated at the root of each branch. Time scale (in days before last timepoints) are indicated above each tree.

Patterns of HIV migration and compartment disruption during the course of infection

- We identified different peaks of Markov jump density, reflecting distinct waves of viral migration between compartments during infection.
- These results demonstrate temporality of the bi-directional viral migration events between blood and other anatomical compartments.

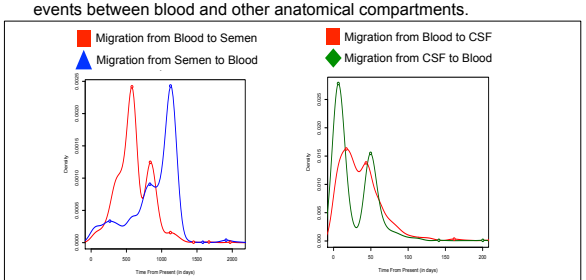

Figure 3. Markov jump count density plot of viral migration between compartments. (**A**) Subject with paired blood and semen samples. (**B**) Subject with paired blood and CSF samples. Migration from BP, CSF and SP are colored in red, green and blue.

Markov jump density plots over time represent distinct waves of viral migrations between compartments. **Figure 3.**

Potential correlates of viral gene flow across anatomical compartments

- Mean viral gene flow was 0.34 migration events/day
- We found trends between:
  - Genital CMV replication and increased compartmentalization in the seminal compartment (p=0.08).
  - CSF pleocytosis and disruption of compartmentalization in CSF (*p*=0.08).

Tree topology for subject **A** argues for well-segregated and independently evolving variants in the different compartments. For subject **B** we identified multiple location state transitions suggesting that viral populations often mix between compartments, **Figure 2.**

Conclusions

- We present a novel Bayesian phylogeographic methods to evaluate the dynamics and determinants of HIV-1 compartmentalization.
- Our results suggest the need to consider anatomical sites not only as restricting viral gene flow but that viral subpopulations migrate back and forth between anatomic compartments.
- Bi-directional flow has profound implications in eradication studies, which should consider anatomic compartments in their design.

Selected References

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