IMPACT OF RECOMBINATION ON HIV-1 EVOLUTIONARY DYNAMICS IN CSF AND PLASMA

Li Li [1], Leslie Ann St. Bernard [2], Daniel Dunn [2], Douglas F. Nixon [2], Weigang Qiu *[1,2], Teresa H. Evering *[2] (*Equal Contribution)

[1]Graduate Center & Hunter College, City University of New York, New York, NY, USA, [2]Weill Cornel Medicine, New York, NY, USA

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BACKGROUND

- HIV-1 is capable of establishing distinct viral populations within central nervous system, leading to a spectrum of neuronal complications.
- An improved understanding of HIV-1 evolutionary dynamics across the brain and peripheral blood is necessary to inform vaccine and cure efforts and longitudinal assessments are lacking.
- We hypothesized that HIV-1 exhibits distinct evolutionary dynamics in the CSF and plasma due to distinct immune environments in the two compartments.

METHODS

- We used single genome amplification (SGA) to generate full-length HIV-1 *env* (>2.5Kb) clade B variants from the paired CSF and plasma of 13 individuals living with chronic HIV from the CHARTER cohort with no neurocognitive impairment (NCN, N=6) and asymptomatic or mild neurocognitive disease (ANI or MND, N=7).
- Participants were viremic and either treatment naïve (N=6) or experienced (N=7). Each participant contributed viral samples from two time points separated by 6 to 48 months.
- We used phylogenetic analysis to determine viral compartmentalization and genetic divergence. Recombination was detected using both RDP and ClonalFrameML packages. dN/dS analysis was conducted in DnaSP6 software to assess selection force. BEAST software was used to estimate *env* DNA substitution rate.
- Comparisons were made between CSF- and plasma-derived viral populations, and across time points, compartmentalization status, and neurocognitive status. A total of 1300 confirmed single genome sequences were analyzed.

Table 1. Clinical and demographic profiles for study participants

Subj.	NCN1	NCN2	NCN3	NCN4	NCN5	NCN6	ANI1	ANI2	ANI3	ANI4	ANI5	MND1	MND2
Age	52	41	42	41	45	43	61	51	44	34	62	40	32
Sex	M	M	M	M	M	M	M	M	M	M	M	M	F
Race	White	Black	Black	White	Black	White	White	White	White	White	White	Black	Black
Est Dur. Inf (TP1)	59.6	173.1	48.9	214.4	75.3	136.9	154.1	211.7	56.2	59.8	206.6	75	58.5
ARV Status	No ARV	Naïve	No ARV	Naïve	Naïve	No ARV	Naïve	No ARV	Naïve	No ARV	No ARV	Naïve	No ARV
CD4 Nadir	400	350	488	461	297	392	477	220	237	329	215	315	134
CD4 (TP1)	591	802	687	790	420	418	743	220	237	329	466	398	320
Plasma VL (TP1)	65600	749	8620	13900	19700	18900	5320	29800	38600	13000	50100	6250	44900
Plasma VL (TP2)	93200	11238	41846	39500	17900	13700	35100	11700	23500	30400	52300	3530	453943
CSF VL (TP1)	120	120	6600	14700	18300	2910	3210	5930	1320	2430	4330	454	2690
CSF VL (TP2)	3200	5846	47264	13300	11900	2430	7630	9070	489	807	9630	494	6956
Time Between TP1+TP2 (Months)	24	42	48	30	6	12	30	12	6	6	12	24	30

Participant neurocognitive disease classifications are as follows: NCN=Normal neurocognitive performance; ANI=Asymptomatic neurocognitive impairment; MND=Mild neurocognitive disorder. M=male, F=female. Estimated Duration of Infection (Est. Dur. Inf) is shown in months. TP=Time Point, CD4 Nadir and CD4+ T Cell Count (CD4 Count) are in cells/mm^3, plasma and CSF HIV-1 RNA levels (VL) are in copies/mL

Recombination plays a larger role in HIV-1 *env* diversification in the plasma than in the CSF

RESULTS

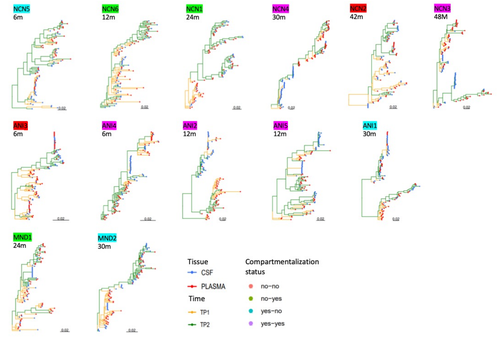
- Clinical and demographic profiles of study participants are shown (Table 1)
- Virologic parameters were well matched across the neurocognitive groups. CD4+ T cell counts were significantly higher in the NCN group compared to the ANI+MND group at time point 1. (Table 2)
- Compartmentalization between CSF and plasma was observed in 11 out of 13 participants in at least one of two time points (Fig 1).
- Compared to their paired CSF-derived counterparts, intra-host plasma-derived HIV-1 *env* showed:
  - Higher rates of sequence diversity in compartmentalized cases and in participants with ANI or MND (Fig 2).
  - Higher nonsynonymous to synonymous substitution rates (dN/dS) (Fig 3).
  - Higher average nucleotide substitution rates (1.16e-02 vs 9.41e-3 sub/site/year, p=0.017)
- Pervasive recombination was observed across HIV-1 *env* in plasma-derived variants (Fig 4).
  - Higher recombination frequency (RF) was noted in plasma- vs. CSF-derived isolates in compartmentalized cases. Greater differences in plasma vs. CSF RF was seen in ANI+MND vs. NCN (Fig 5).
  - The ratio of recombination to point mutation (ρ/θ) was estimated to be 13.86±0.78 in plasma

Table 2. Statistical comparisons of key clinical parameters across study groups

Measure	NCN	TP1 ANI+MND	P-value	NCN	TP2 ANI+MND	P-value
#Males/total	6/6	6/7	N/A	6/6	6/7	N/A
Mean age (years, range)	44 (41-52)	46 (32-62)	1.00	46 (42-54)	47 (35-63)	0.94
Mean Est. Dur. Inf (months, range)	118 (49-214)	117 (56-212)	0.94	140 (74-245)	132 (62-219)	0.62
Mean CD4+ T cell count (cells/mm3, range)	618 (418-802)	388 (220-743)	0.04	562 (305-763)	393 (134-589)	0.19
Mean CD4+ T cell nadir (cells/mm3, range)	432 (297-554)	322 (215-537)	0.10	400 (297-488)	276 (134-477)	0.07
Mean CSF HIV-1 RNA (log copies/mL, range)	3.3 (2.7-4.3)	3.4 (2.6-3.8)	0.72	3.9 (3.4-4.7)	3.4 (2.7-4.0)	1.00
Mean Plasma HIV-1 RNA (log copies/mL, range)	4.0 (3.5-4.8)	4.3 (3.7-4.7)	0.62	4.4 (4.0-5.0)	4.5 (3.5-5.6)	0.22

All p-values were determined by Mann Whitney test. P-values < 0.05 are considered significant

Figure 1. HIV-1 *env* phylogenies display varying degrees of intra-host compartmentalization across time and disease states



Maximum Likelihood trees of SGA sequences

Figure 2. Diversity of HIV-1 *env* in plasma- and CSF-derived isolates by compartmentalization and neurocognitive status

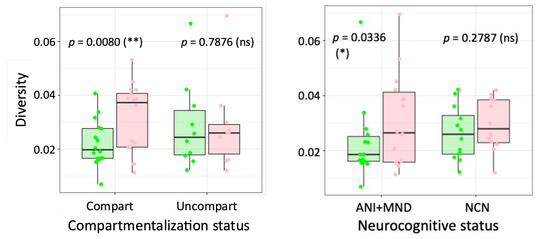


Figure 3. Nonsynonymous and synonymous substitution rates (*dN*, *dS*) of HIV-1 *env* in plasma- and CSF-derived isolates

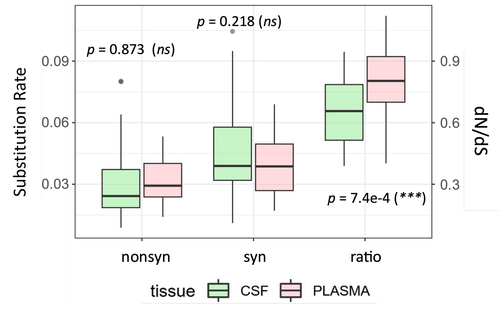
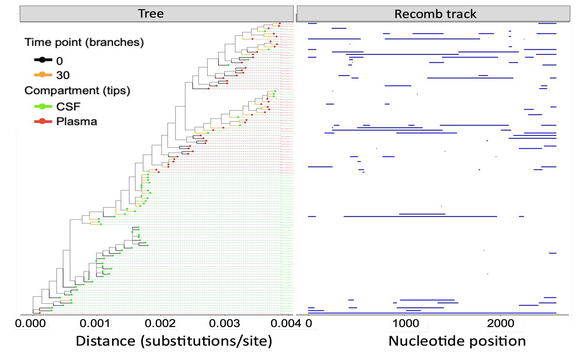
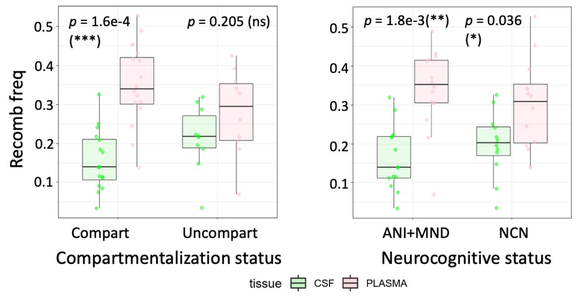


Figure 4. Widespread detection of recombination among intra-host HIV-1 *env* populations



Representative determination of recombination track and lengths in participant NCN4 using ClonalFrameML. The phylogenetic tree was estimated by excluding substitutions introduced by recombination, resulting in much a shorter total tree length (as shown by the tree scale at the bottom)

Figure 5. Recombination Frequency of HIV-1 *env* in plasma and CSF-derived isolates by compartmentalization and neurocognitive status



CONCLUSIONS

- These findings revealed distinct intra-participant HIV-1 evolutionary dynamics between the CNS and plasma and suggest stronger immune selection on HIV-1 *env* in the peripheral blood than in the CNS.
- They also suggest that on average, recombination plays a larger role in HIV-1 *env* diversification in the plasma than in the CSF and is the main driver of viral adaptive evolution in the plasma in this cohort with high rates of compartmentalized virus.
- Findings across neurocognitive disease states warrant additional investigation

ADDITIONAL KEY INFORMATION

Contact Information: Li Li, PhD lli4@gradcenter.cuny.edu,
Teresa H. Evering, MD MS evering@med.cornell.edu

Funding: Robert Wood Johnson Foundation; Harold Amos Medical Faculty Development Program (AMFDP) (THE)

Acknowledgements: We would like to thank the individuals who participated in the research and gratefully acknowledge the CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) study researchers and staff for the provision of de-identified, cryopreserved participant samples for study. CHARTER was supported by award N01MH22005 from the National Institutes of Health.