APOBEC3-Mediated Editing of HIV Transcripts Protects Brain Injury in ART-Suppressed Individuals (E03) Reservoirs and the Central Nervous System

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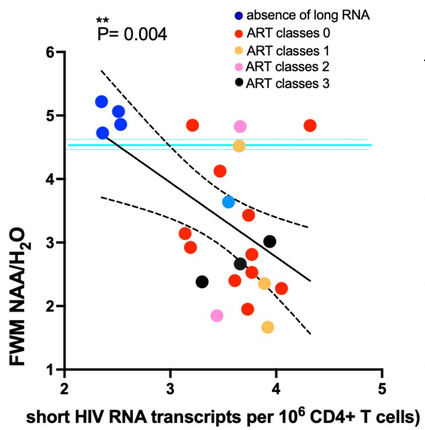
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BACKGROUND

Despite sustained viral suppression with antiretroviral therapy (ART), brain injury remains common in people with HIV. We previously reported that short HIV-1 RNA transcripts in CSF CD4⁺ T cells from virally suppressed individuals were associated with worse brain injury, but the underlying mechanisms remain unclear. To investigate this, we analyzed peripheral viral reservoirs in blood—a more accessible compartment—and examined the roles of APOBEC3-mediated mutations, and drug resistance mutations (DRMs), as markers of low-level productive infection, in brain injury.

METHODS

- Peripheral CD4⁺ T cells from virally suppressed individuals (n=27) were analyzed for HIV-1 gag/pol transcripts (>4.2 kb) and APOBEC3-mediated mutations.
- DRMs were assessed using Oxford Nanopore next-generation sequencing, and translation competence was evaluated through in-vitro activation of CD4+T cells in three participants.
- Neuronal integrity was measured by in vivo proton magnetic resonance spectroscopy (¹H-MRS), quantifying N-acetyl aspartate (NAA) in frontal white matter (FWM).



Note: *Five participants failed to amplify long RNA transcripts (dark blue circles), consistent with full translational inhibition. Solid black line, linear regression; dotted lines, 95% confidence interval. Solid blue line, mean normative FWM NAA at age 56; dotted lines, ages 45 and 67.*

Fig. 1. Inverse correlation between CA short HIV-1 RNA transcript levels and NAA levels in the FWM. Building on prior findings linking short HIV-1 RNA in CSF CD4⁺ T cells to NAA in FWM, we found a similar significant inverse correlation between short RNA transcripts in PBMC-derived CD4⁺ T cells and NAA levels in the FWM,

- Long HIV-1 gag/pol transcripts were detected in CD4⁺ T-cell reservoirs of virally suppressed individuals.
- APOBEC3-mediated premature stop codons (PSCs) commonly appeared in these long transcripts.
- Translation-competent transcripts (without PSCs) were associated with lower frontal white-matter NAA.
- Transcripts containing APOBEC3-mediated PSCs were associated with preserved NAA levels.
- PSC burden in long HIV-1 transcripts may serve as a marker of interferon activity and reservoir impact on the brain.

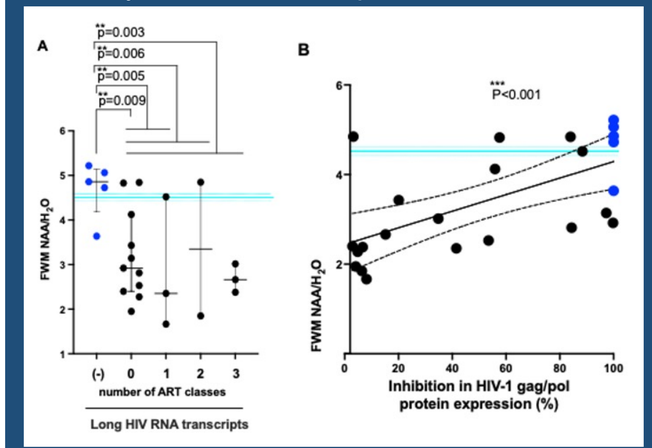


Fig. 2. Dual impact of HIV-1 RNA transcription and translation on brain injury.
(A) FWM NAA levels versus presence/absence of DRMs in the HIV-1 reservoir (number of ART classes indicated).
(B) Effect of APOBEC3-mediated PSCs, which block Gag/Pol translation, on NAA.

Note: *Five individuals failed to amplify long RNA transcripts (dark blue circles), consistent with full translational inhibition. Solid black line, linear regression; dotted lines, 95% confidence interval. Solid blue line, mean normative FWM NAA*

RESULTS

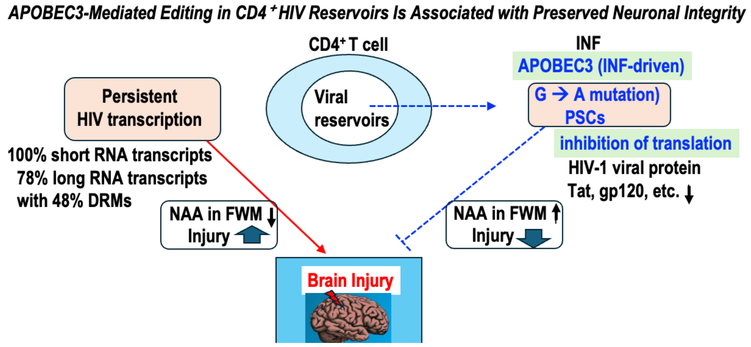
- Persistent in-frame gag/pol transcripts were detected in 78% of participants despite ART suppression.
- Their presence was associated with reduced frontal white matter NAA (p=0.003; Fig.2A), implicating them in axonal injury independent of DRMs.
- In contrast, transcripts with APOBEC3-mediated stop codons may reduce pro-inflammatory Gag/Pol protein expression with increased NAA (p < 0.001; Fig. 2B).
- Among individuals with detectable transcripts, 43% harbored DRMs, including current ART regimen resistance (Fig.3).
- Matching gag/pol sequences from in-vitro reactivated virions confirming their reservoir origin (Fig.3).

CONCLUSIONS

APOBEC3-mediated editing of HIV transcripts appears neuroprotective by blocking viral protein translation and may have a greater influence on brain injury than low-level productive infection in ART-suppressed individuals. Enhancing APOBEC3 activity within reservoir cells could represent a novel strategy to preserve brain health and inform HIV cure interventions.

Note: The graphical abstract illustrates an outline of our findings in this analysis.

Graphical abstract:



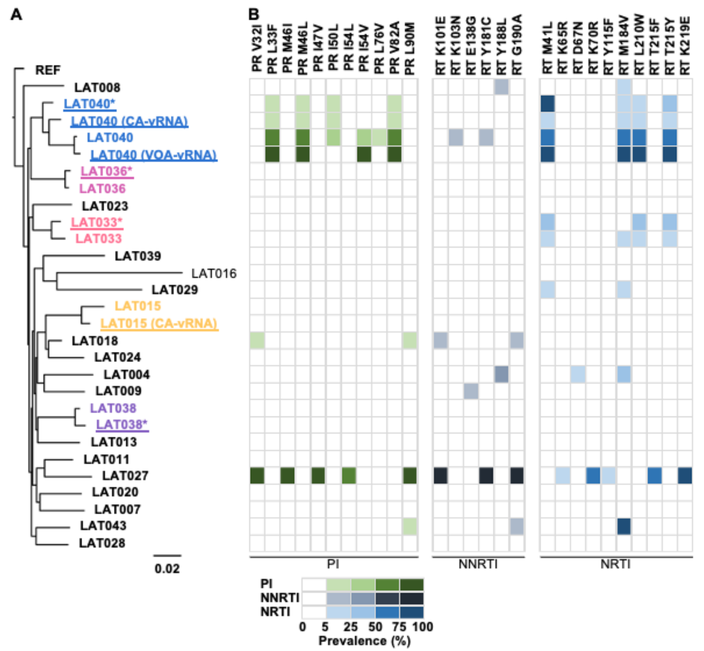


Fig. 3. Phylogenetic relationships and major DRMs.
(A) Approximate maximum-likelihood phylogenetic tree of consensus pol sequences constructed using FastTree, with HXB2 (GenBank accession no. K03455) as the reference.
(B) Prevalence of major DRMs in *pol* sequences lacking PSCs, color-coded by sample. The proportion of sequences containing PSCs in *pol* is also shown.
Long pol transcripts were successfully amplified (n = 21) and analyzed for DRMs. For each sample, the within-sample prevalence of major DRM classes in the pol region is shown, excluding sequences with PSCs. Samples from the same individual, including additional cell-associated vRNA or viral outgrowth assay (VOA)-derived vRNA collected at later time points (18 or 24 months), are color-coded and underlined; asterisks (*) denote additional samples.