A TRANSCRIPTIONAL SIGNATURE OF INDUCED NEURONS DIFFERENTIATES PWH ON cART FROM PEOPLE WITHOUT HIV

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BACKGROUND

- Neurocognitive impairment (NCI) remains a significant comorbidity in virologically-suppressed people living with HIV (PWH) despite effective antiretroviral therapy (ART)
- Directly induced neurons (iNs) generated by transdifferentiation of skin fibroblasts retain donor-specific disease and age-related features, offering unique opportunities to study neurological disorders
- This study represents the first application of iN technology to investigate HIV effects on neuronal gene expression

METHODS

- Primary dermal fibroblasts were obtained from six PWH on suppressive cART (median age 53 years (range: 27 – 64); 83% Male; 50% White) and seven matched PWoH (median age 55 years (range: 27 – 66); 71% Male; 57% White) (Fig 1).
- Participant fibroblasts were transduced with two lentiviral vectors for the doxycycline-dependent expression of neuronal pioneer transcription factors NGN2, and ASCL1.
- Transdifferentiation into iNs was accomplished by treatment with doxycycline and a cocktail of small molecules.
- At day 21, live iNs were isolated by FACS sorting based on the neuronal marker PSA-NCAM.
- Neuronal identity was validated by scRNA analysis and immunocytochemistry (Fig 2).
- Bulk-RNAseq was performed to compare gene expression between iNs from PWH vs. PWoH (Fig 3).

Figure 1. Study Cohort Characteristics

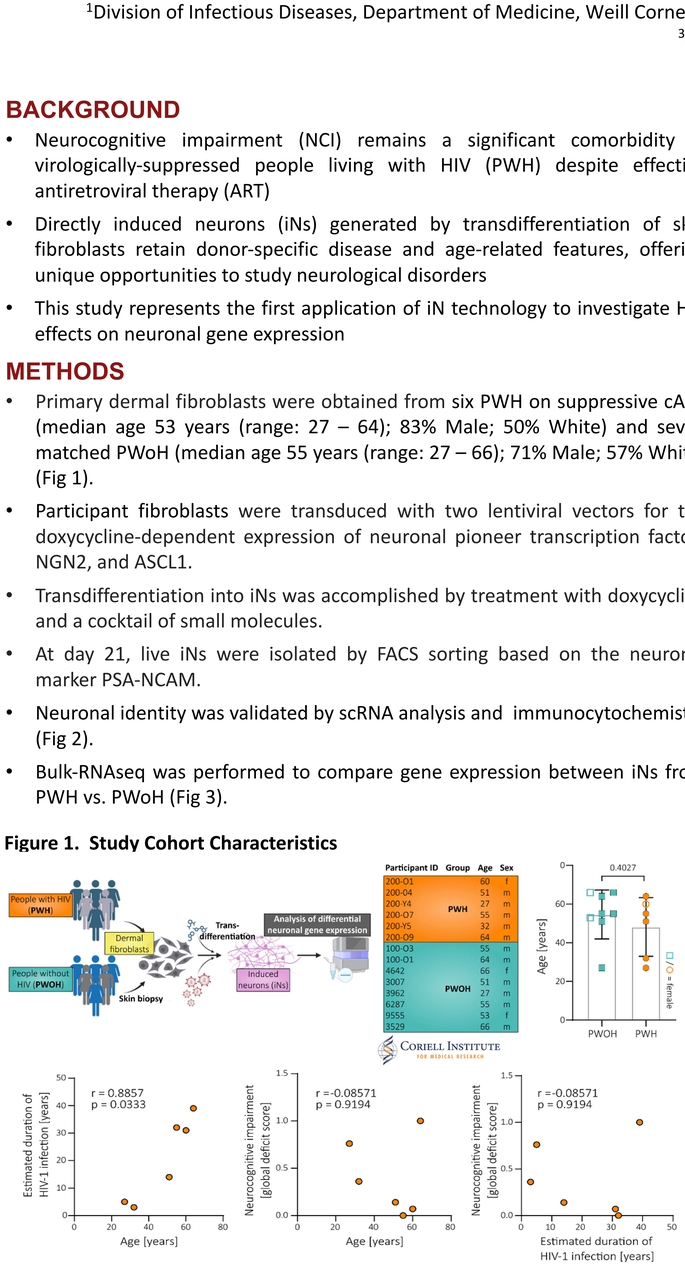


Figure 2. Validation of Neuronal Identity – scRNAseq and IHC

Directly induced neurons (iNs) derived from dermal fibroblasts of virologically-suppressed PWH exhibit a unique gene expression signature different from iNs derived from demographically-matched people without HIV.

These differences support existing concepts of HIV-related neuroinflammation and suggest potential novel mechanisms of NCI.

RESULTS

- Transcriptomic Profiling of PWH vs. PWoH Neurons: 29 genes were significantly differentially expressed (p-adj. < 0.05, log2fc > +/- 0.5) between iNs derived from PWH and PWoH.
  - Of these, 16 genes were downregulated and 13 upregulated in PWH iNs (Fig. 3).
  - 23 DEGs were specific to iNs and not present in matched fibroblasts, suggesting neuron-specific effects of HIV infection.

Figure 3. PWH-derived iNs exhibit statistically significant differentially expressed genes compared to iNs from PWoH

- Pathway Analysis: Protein-protein interaction network mapping revealed that DEGs affect key pathways (Fig. 3).
  - Extracellular matrix organization (COL23A1↓, ENPP2↓)
  - Synaptic transmission (DOC2B↓, RPH3AL↓, OLFM3↑)
  - Alzheimer's-associated pathways (SORCS1↑)
- Validation of Previous Findings: IFI27 was significantly upregulated in PWH-derived iNs, confirming previous observations from three independent post-mortem brain studies (Fig. 4).
- Expression of FOXL2NB-FOXL2-LINC01391 genome locus was significantly reduced in PWH iNs (Fig. 5).
  - Expression levels negatively correlated with the degree of neurocognitive impairment in the PWH cohort

Figure 4. *IFI27* expression levels are increased in PWH-derived iNs and post-mortem brain tissue samples compared to PWoH-derived samples

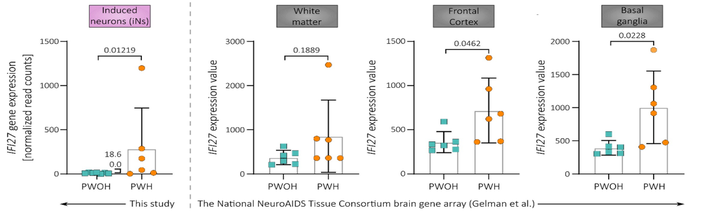
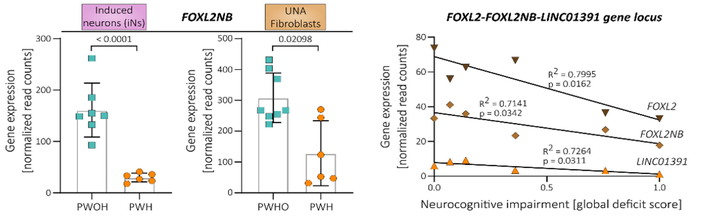


Figure 5. Correlation of gene expression levels of *FOXL2*, *FOXL2NB*, and *LINC01391* with NCI in the PWH study group (n =6)



CONCLUSIONS

- We identified a 29-gene transcriptional signature in iNs that distinguishes PWH from PWoH through direct reprogramming of skin fibroblasts.
- Our findings:
  - Confirm HIV-related neuroinflammation (IFI27 upregulation)
  - Reveal potentially novel mechanisms involving synaptic function and extracellular matrix organization
  - Identify FOXL2NB-FOXL2-LINC01391 locus correlation with neurocognitive impairment severity in PWH
- Participant-derived iNs retain HIV-associated transcriptional changes, providing a valuable model for studying neuronal dysfunction in treated HIV infection.
- These discoveries may guide research into therapeutic targets for HIV-associated neurocognitive disorders in the cART era.

ADDITIONAL KEY INFORMATION

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