Differential Expression Analysis Reveals Pervasive Transcriptional Changes in the Brains of PWH

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BACKGROUND

Central nervous system (CNS) dysfunction is a common consequence in people with HIV (PWH). However, the ramifications of HIV infection on the human brain remain limited to select cell types and regions. Here, we describe our preliminary efforts to establish cell type specific gene expression signatures across multiple brain regions in PWH using single nuclei transcriptomics.

METHODS

Postmortem samples from 4 groups:

People w/o HIV/OUD (CTR)	People w/ HIV (PWH)	People w/ OUD (OUD)	People w/ HIV & OUD (HIVOUD)

3 regions per individual:

Prefrontal Cortex (PFC)	Ventral Striatum (VST)	Insular Cortex (INS)

136 samples in this poster:

	PFC	VST	INS
CTR	14	6	6
HIV	14	6	6
OUD	14	14	14
HIVOUD	14	14	14

(20 x group x region planned)

10x Multiome



~1M nuclei analyzed (post QC):

- Embedding
- Clustering (Leiden)
- Marker-based annotation

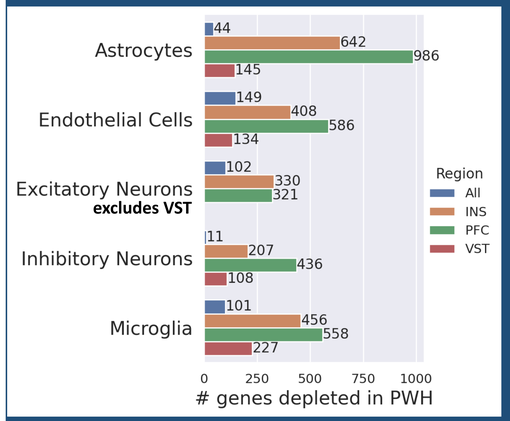
CTR	PWH	OUD	HIVOUD
183,685	157,593	333,656	296,019

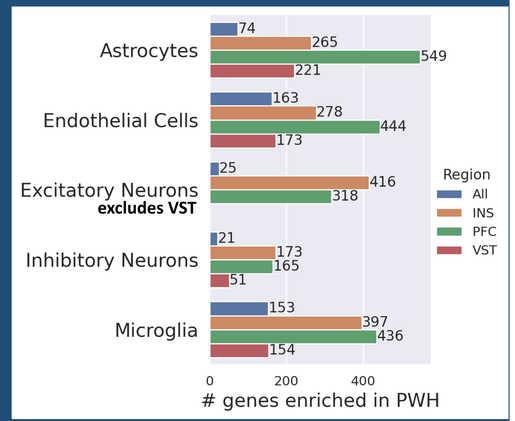
PFC	VST	INS
401,732	274,126	295,095

Differentially expressed gene (DEG) analysis

- EdgeR – GLM pseudobulk • Conditional, regional
- Main effects of HIV:
  - DEG in average of (PWH vs. CTR), (HIVOUD vs. OUD)
  - DEG within a single region & DEG average across regions
- Covariates:
  - PMI • # of nuclei • age • sex • race
- Criteria
  - Absolute Log Fold Change [LFC] >= 1 • False Discovery Rate [FDR] < .01 (Benjamini-Hochberg)

Many depleted and enriched DEGs in PWH *across regions* and *cell types*





- Most cell types exhibited numerous DEGs in all regions
- Prefrontal cortex contained the most significant DEGs

RESULTS

- **Many DEGs span multiple cell types:**

4 DEGs in *VST region in 3 cell types:*

- CERCAM • FOSB • MT.CO1 • SEC14L5

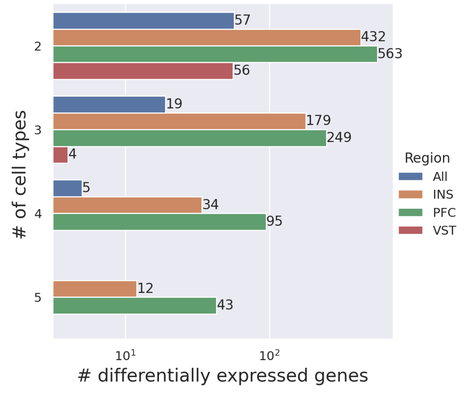
5 DEGs in *all regions in 4 cell types:*

- IL1RL1 • SERPINA3
- SLC17A7 • SST • VGF

12 DEGs in *INS in all 5 cell types*:

* indicates also ***DEG in PFC***

- AC068989.1 • BEX1* • BEX2* • BEX3*
- GNG3* • MZT2B • PLSCR2 • SST*
- CCK* • DDX60L* • TTC9B* • TUBB2A



Top 5 DEG per cell type, sorted by abs(LFC)

astrocytes			endothelial			excitatory neur.			inhibitory neur.			microglia		
Gene	LFC	FDR	Gene	LFC	FDR	Gene	LFC	FDR	Gene	LFC	FDR	Gene	LFC	FDR
SST	-1.9	3.4e-16	GRP	-3.3	2.3e-28	SERPINA3	1.9	3.7e-04	IGHG3	1.6	2.3e-06	ADAMDEC1	2.6	5.5e-07
CXCL10	1.9	3.9e-07	AKR1C8P	2.9	6.3e-08	NPC1L1	1.8	1.7e-03	IGHG1	1.5	4.9e-05	IGLC2	2.4	3.2e-03
IGLC2	1.9	6.6e-04	IGLC3	2.6	7.2e-04	IGF1	-1.8	9.8e-04	IGKC	1.5	6.1e-05	IL1R2	2.4	9.2e-12
IGKC	1.9	5.5e-04	CRH	-2.4	1.0e-05	ST8SIA2	-1.8	7.7e-09	IGLC1	1.4	4.3e-04	TNFRSF11B	2.2	1.3e-17
IGLC1	1.8	1.8e-03	DNAH5	2.3	3.0e-37	GRP	-1.6	3.5e-04	IL1RL1	1.4	7.4e-08	CLEC12A	2.1	2.9e-14

CONCLUSIONS

Here we have shown preliminary efforts to characterize transcriptomic signatures of HIV in the human brain. By analyzing a large, diverse cohort with multiple disease backgrounds, we were able to isolate reproducible and substantial HIV main effects across multiple regions and cell types. Though microglia have been the primary target of many studies of HIV in the brain, our results suggest that disease effects are pronounced across many other cell types that warrant further exploration.

FUTURE DIRECTIONS

- Annotation & analysis of subtypes & states
  - Neuronal subtypes, e.g. VST medium spiny neurons
  - Glial states, e.g. microglial activation, astrocyte role
- Identification of HIV-associated states
- Gene regulatory network analysis
- Alternative DEG methodologies
  - Cluster-free methods such as LEMUR, miloDE
- ATAC-seq analysis
- Dissemination of gene signature resources

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Single Cell Opioid Responses in the Context of HIV
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