Comprehensive Clinical Biofluid Investigation and Correlation of HIV Associated Subjects

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Introduction

- Systemic HIV infection profoundly affects the central nervous system (CNS) with direct consequences on protein abundance in biofluids such as cerebrospinal fluid (CSF) and saliva.
- HIV associated variables such as antiretroviral therapy, co-infection, illicit drug use, and treatment also directly impact the CNS, however our understanding of their effect upon global protein abundances in the clinical biofluid space is still limited.
- Understanding how the CSF and saliva proteome are influenced by these perturbations enables understanding of pathobiology mechanisms and discovery of relevant protein signatures.
- Presented is a summary of multiple label-free, high mass accuracy LC-MS/MS based proteome studies within well defined clinical cohorts linking neurocognitive, inflammatory, and neuronal injury metrics to protein abundance alterations.

Results

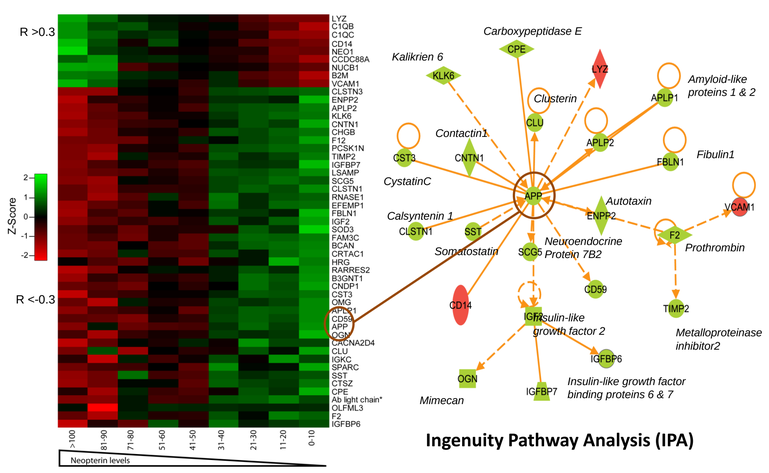


Figure 1. Protein correlations with CSF neopterin and APP centric pathway. Previous studies have identified amyloid precursor protein (APP) as a highly connected network node inversely correlated with neopterin, a general CSF immune activation marker. The heatmap includes significant protein correlations (proteins with R values either >0.3 or <-0.3 by Spearman analysis) with concentrations of CSF neopterin. The network diagram shows results of Ingenuity Pathway Analysis that included the highest number of previously defined relationships of these proteins.[1,2]

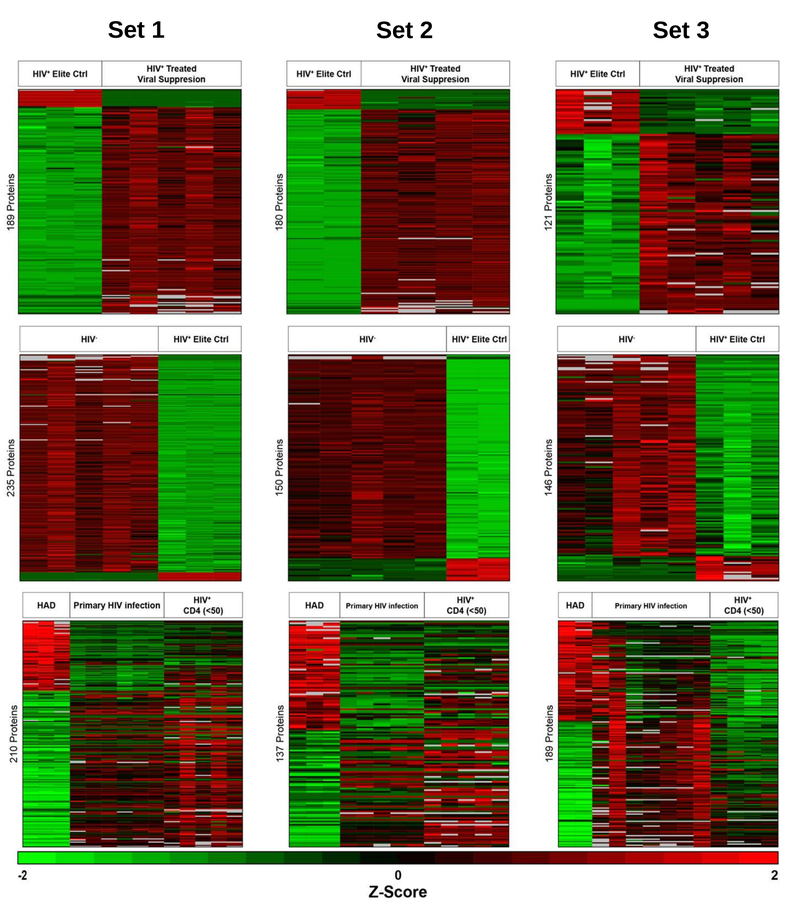
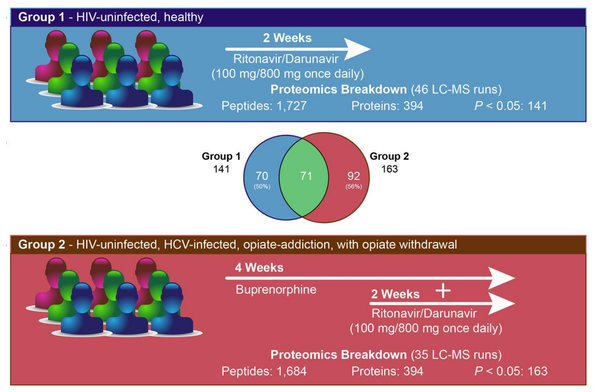


Figure 2. Heat maps of alternations in the CSF proteome across various HIV status cohorts. CSF was collected from a study group of 122 individuals spanning several clinical HIV conditions. Cohorts were evenly distributed and analyzed across three sets for comparison and validation. Shown are proteins at adjusted pvalue <0.01 with fold change >2.0. Observed is the dramatic shift in the CSF proteome comparing HIV+ elite controllers with both HIV+ viral suppressed and HIV-, in addition to differentiation of CSF protein signatures across HAD, primary HIV infection, and HIV+ CD4<50.



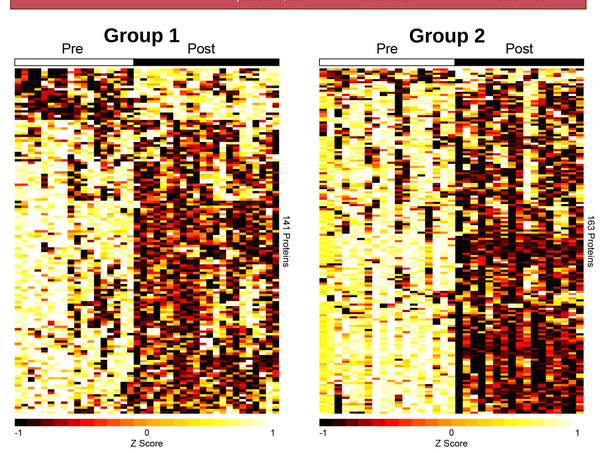
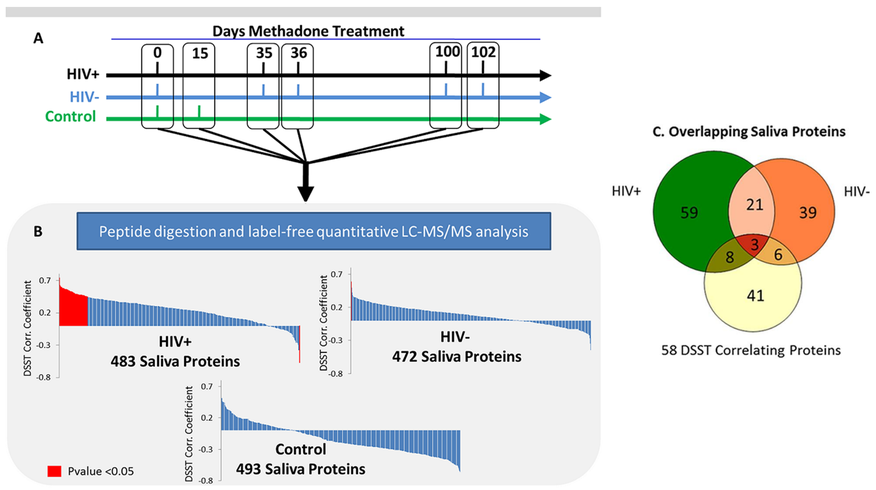


Figure 3. Saliva proteome analysis of HIV- cohorts. Proteome analysis of saliva collected from both a healthy control and opiate addicted, buprenorphine treated cohorts. Both groups include a pre and post ritonavir/darunavir (R/D) treatment time point. At a <0.05 adjusted pvalue, a dramatic down regulation of the saliva proteome is observed between pre and post R/D administration, more pronounced in group 2, and independent of saliva protein concentration

Orthogonal measure	Positive Corr.	Negative Corr.	Total
CSF sAPPB	44	10	54
CSF NFL	5	44	49
CSF neopt	17	27	44
sVCAM	15	29	44
Log_{10}csf_vl	40	3	43
sCD163	22	17	39
sCD14	15	20	35
IL-6	13	19	32
CSF t-tau	4	24	28
TNF-a	12	8	20
IP-10	14	4	18
MCP-1	9	9	18
MMP-9	8	2	10
CSF WBC	8	0	8
CSF sAPPa	3	0	3
TIMP-1	1	0	1
CSF AB1-42	0	0	0
CSF p-tau	0	0	0

Table 1. CSF proteome correlation with additional 18 markers. Listed are the number of proteins which correlate with orthogonal ELISA measurements across all clinical cohorts from Fig. 2. CSF sAPPβ remains a top metric of global proteome shifts in CSF.



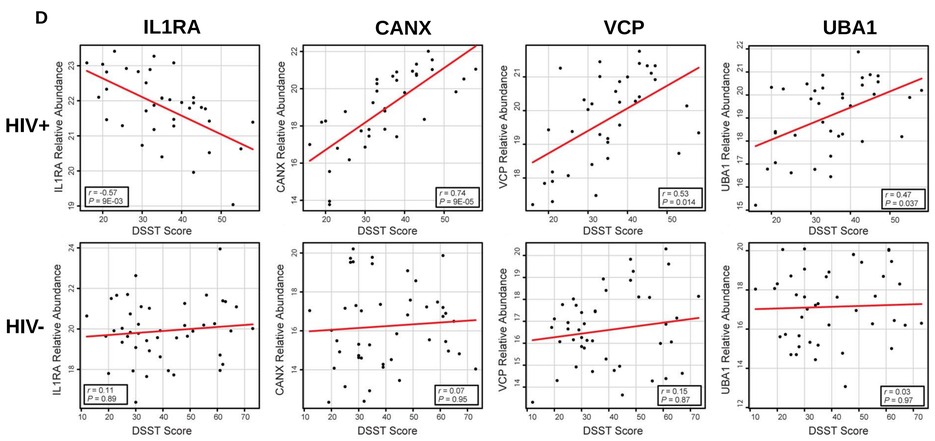


Figure 4. Saliva proteome analyses linking cognition to saliva markers in HIV+ cohort. A) and B) Recent studies utilized HIV-/+ heroin addicted methadone treated cohorts (with non-treated controls) to identify correlations between saliva proteins and cognitive Digit Symbol Substitution Test (DSST) scores. Only saliva proteins from HIV+ individuals demonstrated multiple correlations with cognition. C) Only a limited number of DSST correlating proteins overlap with saliva proteins significantly altered due to methadone treatment, helping to discriminate cognition changes independent of HIV and/or methadone treatment. D) Key proteins showing correlation with HIV+ but not HIV- cohort DSST scores.[3]

Conclusions

- Specific CSF proteins such as APP have been identified which can inform upon CNS pathophysiology of HIV infection and treatment.
- Extensive alterations in the CSF protein composition, even across elite controller, HIV+ suppressed, and HIV- cohorts implies more diverse and dynamic interactions in the CNS space than previously understood.
- Broad saliva proteome alterations were observed as a direct result of R/D treatment (independent of HIV infection). Follow-up studies have implicated ritonavir with inhibition of cell growth and the proteasome complex.
- Saliva protein abundances were shown to correlate with cognition, but only within a HIV+ cohort. The majority of proteins appear decoupled from methadone treatment effects but linked to the disruption of protein quality control pathways.

Methods

All studies utilized a high mass accuracy (LC-MS/MS, VelosLTQ-Orbitrap) approach for label-free, AMT tag based identification and quantification of peptides followed by quantitative protein based statistical analysis with the DanteR package.[1,3]

References

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