Poster #458

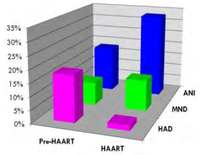
Brain Transferrin Receptor (TFR) RNA Expression is Associated with Neurocognitive Testing Performance in HIV/AIDS

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INTRODUCTION

- The incidence of HIV-associated dementia has declined significantly in the era of combination antiretroviral therapy (CART), but milder forms of HIV-associated neurocognitive disorders (HAND) are increasingly prevalent.[1]
- >50% of all HIV-infected persons on CART have some form of HAND.



ANI, Asymptomatic Neurocognitive Impairment
MND, Mild Neurocognitive Disorder
HAD, HIV-Associated Dementia
Affected domains include: Executive function, speed of information processing, working memory, learning, *etc.*

(*Adapted from*: McArthur *et al*, Ann Neurol 67, 699-714, 2010)

- Brain iron dysregulation occurs in many neurocognitive disorders, and functional neuronal iron deficiency has been implicated in some dementias.[2]
- Chronic inflammation and HIV infection both cause iron sequestration in macrophage-monocyte-lineage cells, likely impeding neuronal iron transport.[3,4]
- Tissue transferrin receptor (TFR) expression is upregulated in iron deficiency and in clinical scenarios of increased or unmet cellular iron demand.

HYPOTHESIS

- **Functional iron deficiency in one of the areas of the brain most affected in HIV-infection (*e.g.*, frontal neocortex) contributes to HAND pathogenesis.**

PATIENTS AND METHODS

Study Population

- National NeuroAIDS Tissue Consortium and Brain Bank
  - U.S.-based resource established in 1998 to bolster research on the effects of HIV infection on the brain by providing high-quality, well-characterized tissues from persons dying of HIV/AIDS.
  - Comprehensive pre-mortem neuromedical and neuropsychiatric/neurocognitive performance data, information on treatment, history of substance abuse, cerebrospinal fluid and plasma viral load, and detailed neuropathological diagnoses.

Diagnosis of HAND and Neurocognitive Testing

- Demographically adjusted T-scores assigned in 7 neurocognitive "domains": abstract executive function, speed of information processing, attention working memory, learning, recall, verbal fluency, & motor skills; converted to standardized deficit scores & averaged as "Global T-Score"
- HAND definition (Frascati criteria[5]): no confounding conditions, impairment in at least 2 domains, presence or absence of functional impairment by self-report and/or performance-based criteria

Laboratory Methods

- Total RNA extracted and purified from frozen brain tissue (frontal neocortex at Brodmann Area 9) in 287 decedents who underwent neurocognitive testing ≤6 months pre-mortem and uniform autopsy and neuropathology protocols at each site; RNA quantified by RT-PCR

Statistical Methods

- Baseline characteristics were compared using the Chi-square test, Student's *t*-test or Wilcoxon rank sum test, as appropriate based on the type and distribution of variables.
- Associations of TFR RNA expression with HAND and individual domain scores assessed by multivariable regression, adjusting for age, race/ethnicity, CD4⁺T-cell nadir, CART, and plasma viral load.

RESULTS

Baseline Characteristics of NNTC Decedent Population

Variable	HAND n=243	No HAND n=44	*p*- value
Age, years (median, IQR)	43 (38, 50)	47 (42, 53)	0.01
Sex (n, % women)	45 (19)	5 (11)	0.29
Race/ethnicity (n, %) Non-Whites Whites	 69 (29) 156 (64)	 8 (18) 35 (80)	 0.11 0.05
CD4⁺ T-cell count, cell/mm³ median (IQR)	31 (8, 115)	88 (17, 273)	0.02
Plasma viral load (median #copies/mL, IQR)	4.6 (3.2, 5.4)	4.2 (2.4, 5.2)	0.15
CSF viral load (#copies/mL, IQR)	2.6 (2.1, 3.6)	2.6 (1.8, 3.7)	0.52
CART era (n, %)	111 (31)	44 (100)	<0.05
Hepatitis C virus+, (n, %)	65 (39)	8 (30)	0.40
Alcohol abuse (n, %)	39 (24)	14 (41)	<0.05
Frontal cortex RNA, median (IQR)	3.9 (3.1, 4.8)	3.1 (2.3, 4.1)	<0.01
Postmortem-freezing interval (PMI), median hours (IQR)	8.5 (5.5, 17)	12 (6, 26)	<0.05
Global T-score, mean ±SD	37 ± 0.65	50 ± 0.82	<0.01
TFR RNA, median (IQR)	0.90 (0.65, 1.2)	0.78 (0.61, 1.0)	0.05

Abbreviations: *TFR*, transferrin receptor; *IQR*, interquartile range; *CART*, combination antiretroviral therapy; *CSF*, cerebrospinal fluid; *HAND*, HIV-associated neurocognitive disorder; *SD*, standard deviation

Neurocognitive Testing Performance across Categories of HAND
Standardized domain T-scores: median (IQR)

Neurocognitive Domains	Normal (n=44)	ANI + MND (n=148)	HAD (n=95)
Abstract Executive Function	53 (50, 61)	41 (34, 48)	35 (27, 45)
Speed of Information Processing	50 (45, 55)	39 (30, 45)	33 (28, 42)
Attention Working Memory	51 (45, 57)	41 (34, 46)	38 (30, 44)
Learning	48 (45, 56)	37 (30, 43)	32 (26, 37)
Memory/Recall	48 (43, 56)	36 (30, 42)	32 (25, 38)
Verbal Fluency	50 (45, 64)	43 (34, 48)	38 (29, 45)
Motor Skills	45 (37, 48)	33 (25, 40)	24 (19, 38)
Global Score	49 (46, 53)	39 (32, 43)	33 (29, 40)

HAND, HIV-Associated Neurocognitive Disorder; *ANI*, Asymptomatic Neurocognitive Disorder; *MND*, Mild Neurocognitive Disorder; *HAD*, HIV-Associated Dementia

Univariable Logistic Regression Analyses:
Associations of TFR RNA Expression with HAND

Variable	Odds Ratios (95% Confidence Interval)		
	ALL HAND (n=243)	ANI + MND (n=143)	HAD (n=95)
TFR RNA level	2.3 (1.2-4.4)*	1.9 (0.97-3.9)¥	2.9 (1.4-6.0)*
Age	0.95 (0.91-0.99)*	0.95 (0.91-0.99)*	0.95 (0.91-1.0)*
White race vs. non-white	0.46 (0.21-1.0))¥	0.46 (0.20-1.0))¥	0.95 (0.90-1.0)*
CD4⁺ T-cell count	0.98 (0.98-0.99)*	0.46 (0.20-1.0))¥	0.46 (0.19-1.1)¥
Total RNA, frontal neocortex	1.4 (1.1-1.9)*	1.4 (1.0-1.8)*	1.5 (1.1-2.0)*
Post-mortem freezing interval	0.98 (0.96-1.0)*	0.98 (0.97-1.01)	0.96 (0.93-0.99)*
Current vs. past alcohol abuse	0.45 (0.21-0.98)*	0.52 (0.23-1.2)	0.31 (0.12-0.85)*

Referent group for all categories: neurocognitively normal subjects

Statistical significance: **p*<0.05; Borderline significance: ¥*p*=0.05-0.06

Abbreviations: HAND, HIV-associated neurocognitive disorder; *MND*, mild neurocognitive disorder; *HAD*, HIV-associated dementia; *CART*, combination antiretroviral therapy

Multivariable Regression Analyses:
TFR RNA Expression is Independently Associated with HAND

Variable	HAND Diagnoses: Odds Ratios (95% CI)¶		
	ALL HAND (n=243)	ANI + MND (n=89)	HAD (n=71)
TFR RNA levels (≥ vs. <median)	2.8 (1.1-7.2)*	2.8 (1.0-7.6)*	3.0 (0.94-9.3)¥
+Hepatitis C virus+	5.2 (1.5-18.1))*	5.9 (1.6-22.3))*	7.7 (1.3-45.6)*
	Neuropsychiatric Domains: β-coefficients (95% CI)§		
	SIP T-Score, n=220	Attention Working Memory T-Score, n=200	Global T-Score n-=215
TFR RNA level	-3.4 (-6.7, -0.06)*	-3.0 (-6.0, -0.02)*	-2.6 (-5.4, 0.26)¥

New Abbreviations: 95% CI, 95% confidence interval; *SIP*, Speed of Information Processing

¶**TOP PANEL:** Logistic regression models are adjusted for age at neuropsychiatric testing, plasma CD4⁺ T-cell count, post-mortem-freezing interval, total frontal neocortex RNA, ±hepatitis C virus (HCV) co-infection status. **Referent=Normal cognition.** No variables in the model other than TFR were statistically significant. Inclusion of self-reported race/ethnicity and alcohol abuse (current vs. past), did not significantly alter results, except that the OR for HAD also became significant (*p*=0.02).

§**BOTTOM PANEL:** Linear regression model is adjusted for post-mortem-freezing interval, current or past alcohol abuse, plasma CD4⁺ T-cell count, and race/ethnicity (based on univariable analyses).

***Statistically significant, *p*<0.05; ¥0.05<*p*≤0.07**

LIMITATIONS

- Brain iron content, TFR protein expression, and brain regions other than frontal neocortex were not assessed in this study.

STRENGTHS

- First study to demonstrate that brain iron demand may be increased in HAND and that the level of iron demand (reflected by TFR RNA expression) may correlate with clinical endophenotypes of HAND (neurocognitive domain scores).

DISCUSSION & CONCLUSIONS

- **Expression of TFR RNA, which is normally tightly regulated in cells according to ambient iron levels and cellular iron demand, is independently associated with milder forms of neurocognitive impairment as well as dementia in HIV-infected persons dying of their disease.**
- Frontal neocortex consists of primarily gray matter (neuronal cell bodies); hence, one may infer that neurons in the this area of brain affected in HAND are functionally iron-deficient. This could be a regional phenomenon.
- The impact of iron transport in the CNS on HAND pathogenesis in subjects with HIV infection and viral suppression on CART warrants comprehensive study.

ONGOING AND PLANNED STUDIES

- Brain iron content, RNA and protein expression studies (including TFR, ferritin and other iron-management gene products) to more completely characterize iron status in the HIV-infected and HAND brain using the NNTC resource
- Evaluation of CSF iron-related biomarkers in HIV-infected subjects with and without HAND and in HIV-uninfected subjects
- Correlation of TFR RNA and CSF biomarker levels and iron-related genomics with quantitative neuroimaging traits (volumetric, metabolic) in HIV-infection.

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ACKNOWLEDGEMENTS & NNTC COLLABORATORS

- **We are grateful to the HIV-infected persons who donated tissues for this research.**
- **NNTC collaborating sites:** University of Texas-Medical Branch, Galveston, TX (Benjamin Gelman); Manhattan HIV Brain Bank, New York, NY (Susan Morgello); California NeuroAIDS Tissue Network, San Diego, CA (Igor Grant); National NeuroAIDS Bank, Los Angeles, CA (Elyse Singer)
- **Funding support:** 1R01 MH095621-03 (to A. Kallianpur and T. Hulgan); Financial support for NNTC provided through the following cooperative agreements with NIH: U01 MH083507, U01 MH083501, U01 MH083500, U01 MH083506, and U01 MH083545

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