Poster# 446

Abstract# P-G1

21th Conference on Retroviruses and Opportunistic Infections

March 3-6, 2014
Boston, Massachusetts

Mitochondrial DNA in CSF is Associated with Inflammation and Degree of Neurocognitive Deficits in HIV Infection

Josué Pérez-Santiago[1], Rachel D. Schrier[1], Marta Massanella[1], Sara Gianella[1], Mariana Cherner[1], Susanna R. Var[1], Tyler R. C. Day[1], Miguel Ramirez-Gaona[3], Davey M. Smith[1, 2], Ronald J. Ellis[1], Scott Letendre[1], and Sanjay R. Mehta[1]

[1]University of California San Diego, La Jolla, California, USA, [2]Veterans Administration San Diego Healthcare System, San Diego, California, USA and [3]University of Alberta, Edmonton, Alberta, Canada

Josué Pérez Santiago, Ph.D.
Department of Medicine
University of California, San Diego
9500 Gilman Drive
San Diego, CA 92093-0679, USA
E-mail: joperez@ucsd.edu
Phone: (858) 552-8585 x2673
Fax: (858) 642-1620

Background

- HIV invades the central nervous system (CNS) early in infection, often resulting in impairment of neurocognitive function[1].
- Even with antiretroviral therapy (ART), some HIV-infected individuals can experience neurocognitive decline in part due to persistent inflammation[2].
- Persistent inflammation might result in cell death[1, 3, 4]. Cell death would release into the cerebrospinal fluid (CSF) mitochondrial DNA (mtDNA), which itself can cause a potent inflammatory response[5].

Objective

To characterize the relationship between mtDNA in the CSF, inflammation, and neurocognitive function.

Methods

- We quantified mtDNA levels in CSF of 28 HIV-infected patients by droplet digital PCR (ddPCR).
  - ✓ A Global Deficit Score (GDS) reflecting demographically adjusted neuropsychological performance across 7 ability domains was used as a measure of neurocognitive impairment (NCI), with GDS < 0.5 considered neurocognitively normal (NCN)[6].
  - ✓ We measured soluble markers (sCD14, sCD163) in CSF by ELISA and cytokines (TNF-α, IL-6) and chemokines (MCP-1, IP-10) in CSF and blood plasma with a liquid bead suspension array system.
  - ✓ Intracellular cytokine production of TNF-α, IL-2, IFN-γ, and CD107a in $CD4^+$ and $CD8^+$ T cells was quantified by flow cytometry.
- To investigate the relationship between pleocytosis and mtDNA levels, we also included 5 HIV-infected individuals, who had undergone a structured ART interruption.
  - ✓ In these patients, we selected 6 samples for each subject: 2 prior to interruption of ART, 2 within 8 weeks of ART interruption and 2 after re-initiation of ART and quantified levels of mtDNA by ddPCR.
- All statistical analyses were performed in R statistical software.

References

1. Gonzalez-Scarano et al. *Nat Rev Immunol.* 2005.
2. Zhou et al. *Infect Dis Rep*. 2013.
3. Capeau et al. *Clin Infect Dis* 2011.
4. Guaraldi et al. *Clin Infect Dis* 2011.
5. Zhang et al. *Nature*. 2010.
6. Carey et al. J Clin Exp Neuropsychol. 2004

Table 1. Virologic, Immunologic and Neurocognitive Characteristics

	All samples (N=28)	NCI (N=14)	NCN (N=14)	p-value*
CSF VL (log_{10} copies/mL)	2.58	2.25	2.58	0.94
CSF VL >50 copies/mL (%)	62%	57%	67%	0.70
CD4 counts (cells/μL)	386	391	369	0.77
CD4 percentage (%)	22	20	23.1	0.30
CD8 counts (cells/μL)	1006	1112	945	0.08
CD8 percentage (%)	54.6	54.05	54.6	0.65
CD4/CD8	0.41	0.4	0.42	0.34
CD4 Nadir (cells/μL)	244	240	252	0.67
Global Deficit Score	0.49	0.72	0.17	<0.001

Table 1: Characteristics of our study participants. Median values are shown for the entire cohort and by groups defined by neurocognitive performance (NCI vs. NCN). *Represent the p-value of a double-tailed Mann-Whitney or a Fisher test.

Results

Figure 1.Higher mtDNA in CSF is positively associated with worse neurocognitive performance among individuals with NCI

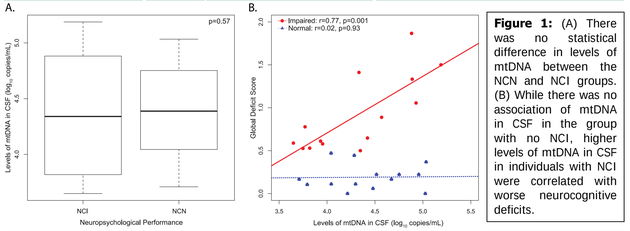

Figure 1: (A) There was no statistical difference in levels of mtDNA between the NCN and NCI groups. (B) While there was no association of mtDNA in CSF in the group with no NCI, higher levels of mtDNA in CSF in individuals with NCI were correlated with worse neurocognitive deficits.

Figure 2. Higher mtDNA in CSF is associated with inflammation in individuals with NCI

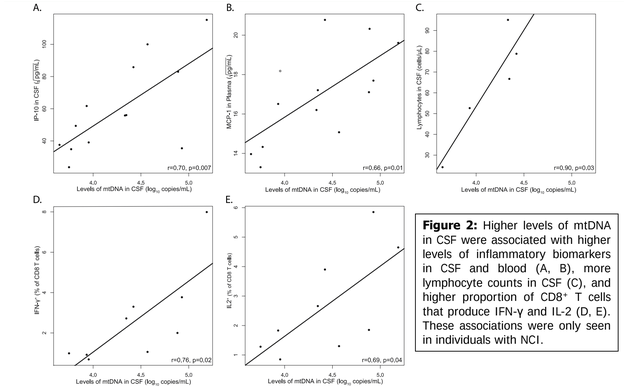

Figure 2: Higher levels of mtDNA in CSF were associated with higher levels of inflammatory biomarkers in CSF and blood (A, B), more lymphocyte counts in CSF (C), and higher proportion of $CD8^+$ T cells that produce IFN-γ and IL-2 (D, E). These associations were only seen in individuals with NCI.

Figure 3. Higher mtDNA in CSF is associated with inflammation if CSF VL is detectable in both NCI and NCN groups

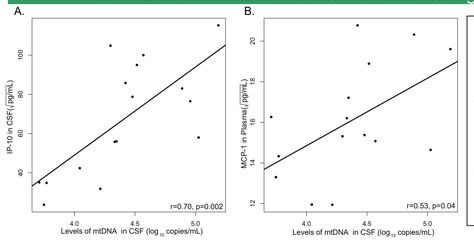

Figure 3: Higher levels of mtDNA in CSF were associated with higher levels of inflammatory markers in CSF (A, B) when participants had detectable CSF VL. Half of the participants with detectable VL in CSF did not have NCI. There were no associations of mtDNA with any variable in individuals with undetectable VL in CSF.

Figure 4. mtDNA in CSF precedes pleocytosis

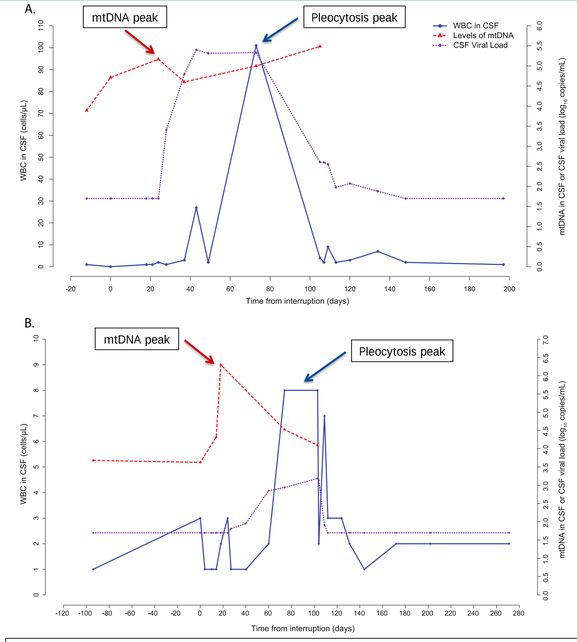

Figure 4: In all cases when ART is interrupted, mtDNA levels and CSF VL rise before the CSF white blood cell count, therefore levels of mtDNA precede pleocytosis. Only 2 of the 5 participants are shown but all showed the same pattern.

Table 2. mtDNA in CSF is strongest correlate of NCI severity

	All participants		NCI		NCN	
	Correlation	p-value	Correlation	p-value	Correlation	p-value
mtDNA	0.29	0.14	**0.77**	**0.001**	0.02	0.94
CSF VL	0.09	0.65	0.2	0.476	-0.55	0.06
CD4 Nadir	-0.06	0.77	-0.19	0.54	0.4	0.19
IP-10 in CSF	0.21	0.3	0.53	0.06	-0.33	0.25
MCP-1 in CSF	-0.07	0.75	-0.01	0.96	-0.38	0.18
CD4 counts	0.08	0.67	-0.07	0.79	0.34	0.25
CD8 counts	0.17	0.39	0.001	0.99	-0.44	0.13
CD4/CD8	-0.1	0.59	-0.07	0.8	0.57	0.04

Table 2: Association of clinical and soluble markers with GDS. Associations were tested with all participants and also by groups defined by neuropsychological performance (NCI vs. NCN). Levels of IP-10 in CSF showed a positive trend with GDS in individuals with NCI but there were no association between GDS and CSF VL, inflammatory markers or lymphocyte profiles.

Conclusions

- ❖ While mtDNA was not associated with the presence of NCI, higher levels of mtDNA were strongly associated with greater severity of impairment among those with NCI (**Figure 1**).
- ❖ Higher levels of mtDNA were associated with more inflammation in the CNS and blood (**Figure 2, 3**), and was the strongest predictor of NCI severity (**Table 2**).
- ❖ Levels of mtDNA in CSF were not a result of pleocytosis as mtDNA peak levels preceded pleocytosis (**Figure 4**).
- ❖ Altogether these data support that mtDNA is a biomarker for severity of NCI. Given that mtDNA is pro-inflammatory and is strongly associated with other markers of inflammation in the CNS (IP-10, $CD8^+$ T cells in CSF producing IFN-γ and IL-2), we hypothesize that either the source of mtDNA in individuals with NCI is from neurons or individuals with NCI have inflammatory mechanisms that are different than individuals who are NCN or both.

Acknowledgments

This work was supported by the Department of Veterans Affairs and grants from the National Institutes of Health: MH85610 (Letendre R21), AI090970 (IAVI), AI100665 (Smith-Training), MH097520 (Smith-Viral Aging), DA034978 (Smith-Avant Garde), MH083552 (Smith-Multisite), AI036214 (Richman-CFAR), AI087164 (Richman-biomarkers), AI007384 (Richman Training Grant), MH062512 (Heaton-HNRC), AI096113 (Margolis-Delaney), AI047745 (Kosakovsky Pond-Dynamics), MH081482 (Gianella-IFRN fellowship), AI027763 (Gianella-CMV), AI106039 (Little-PIRC), AI104283 (Strain R21), MH101012 (Ellis R01), MH94159 (Achim), DA026306 (TMARC Grant PI), MH58076 (Ellis R01), PST-HN39 (Gianella-HNRP developmental grant), PST5TP2 (Pérez-Santiago-TMARC developmental grant), MH097673 (Letendre K24) ; the International AIDS Vaccine Initiative (IAVI); the National Science Foundation DMS0714991; the James B. Pendleton Charitable Trust.