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Poster # 460

Mitochondrial DNA Haplogroups and CSF Neuroinflammation in the CHARTER Cohort

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CHARTER
CNS HIV Anti-Retroviral Therapy Effects Research
CNS HIV ANTI-RETROVIRAL THERAPY EFFECTS RESEARCH

Objective

To determine associations between mitochondrial DNA haplogroups and cerebrospinal fluid (CSF) biomarkers of neuroinflammation.

Background & Significance

- Neurocognitive impairment (NCI) remains an important complication in the combination antiretroviral therapy (CART) era, and may reflect effects of chronic neuroinflammation.
- Host factors – including genetic variants – that influence neuroinflammation are not completely known.
- Mitochondrial DNA (mtDNA) haplogroups are ancestry-specific patterns of single nucleotide polymorphisms in mtDNA that have been associated with differential mitochondrial function and human disease risk.
- Haplogroups have been associated with HIV- and ART-associated outcomes (including peripheral neuropathy and NCI), and with neurodegenerative diseases in HIV-uninfected populations.
- To our knowledge, mtDNA haplogroups and neuroinflammation or iron-related CSF biomarkers in HIV have not previously been studied.
- Understanding genetic factors associated with NCI can lead to better diagnostic, prevention, and therapeutic strategies.

Methods

- CHARTER is a U.S.-based observational study of ambulatory HIV-infected adults who underwent standardized neuromedical assessments from 2003 to 2007.
- A subset of unselected CHARTER subjects underwent lumbar puncture with CSF collection and cryopreservation.
- CHARTER genetic studies excluded persons with confounding neurologic cormorbidities.
- mtDNA genotyping was obtained from whole blood using the GeneChip Human Mitochondrial Resequencing Array v2.0 (Affymetrix, Inc., Santa Clara, CA, USA). Array intensity data were processed using the MitoChip Filtering Protocol (MFP) under a haploid model, and variants called relative to the Revised Cambridge Reference Sequence (rCRS).
- Haplogroups were assigned using the HaploGrep program (http://haplogrep.uibk.ac.at/) based on full mtDNA sequences.
- Outcomes were $\log_{10}$-transformed CSF neuroinflammation biomarkers **IL-6**, **IL-8**, **TNF-alpha** (by high-sensitivity immunoassay), **VEGF**, **IP-10**, and soluble markers of iron homeostasis **ceruloplasmin** and **haploglobin** (by standard immunoassay).
- Ancestry was determined using principal component (PC) analysis of genome-wide nuclear genotyping (Affymetrix SNP6.0), and primary analyses were stratified by – and limited to – participants of European, African, and admixed Hispanic ancestry.
- Multivariable regression of associations between mtDNA haplogroups and CSF biomarkers were performed adjusting for: age, sex, current CART status (yes vs. no), CSF HIV RNA detectability (yes v. no), and nadir CD4 T cell count.

Results

- Haplogroups were assigned for 385 CHARTER participants with CSF (**Table 1**).
- Most were male (80%), on CART (74%), and had undetectable CSF HIV RNA (69%). 11% were of admixed Hispanic ancestry and were younger than those of European or African ancestry (p=0.007). Other statistical differences between ancestry groups are noted in **Table 1**.
- CSF biomarker distribution by ancestry and haplogroups are shown in the **Figure**. There were no significant associations with IL-8, VEGF, or haptoglobin (not shown).
- In adjusted models, IP-10 was higher among persons of admixed Hispanic ancestry with mtDNA haplogroup B than other haplogroups (β=0.2; p=0.03), independent of other factors including CSF HIV RNA detectability (**Table 2**).
- CSF ceruloplasmin was higher in persons of African ancestry with mtDNA haplogroups L1 or L2 than L3 or other African haplogroups (**Table 2**).
- CSF biomarkers did not differ by haplogroup in persons of **European ancestry**.

Table 1 . Clinical and demographic characteristics.

	ALL N=385	European Ancestry N=174	African Ancestry N=168	Admixed Hispanic N=43
Age	43 (39-49)	44 (39-50)*	43 (39-48)	40 (34-46)*
Male	309 (80)	153 (88)*	120 (71)*	36 (90)
On CART	283 (74)	132 (76)	118 (70)	33 (77)
Nadir CD4	175 (54-308)	181 (74-330)	175 (35-283)	107 (52-240)
CSF HIV RNA <50 cps/mL	266 (69)	131 (75)*	107 (64)	28 (65)

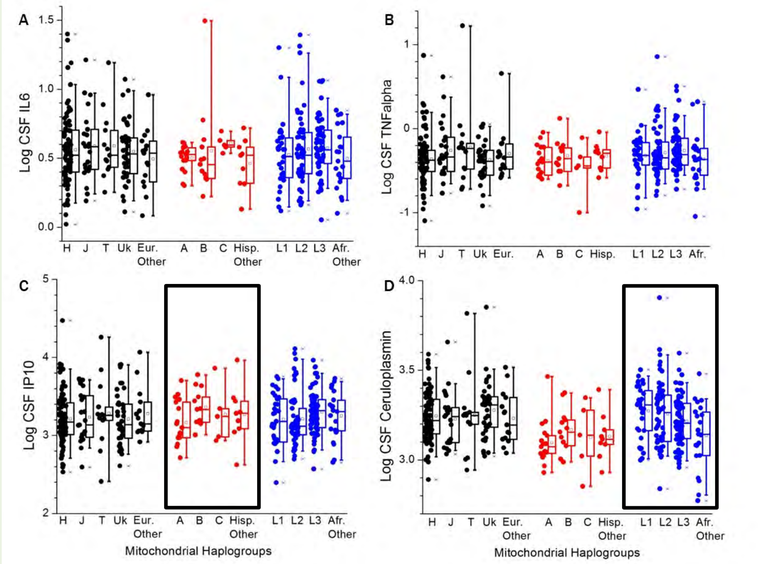
Data are median (IQR) or N (%); *Wilcoxon p<0.05 for comparison with other ancestry groups combined.

Table 2. Multivariate models of CSF biomarkers ceruloplasmin and IP-10 in African and admixed Hispanic ancestry subgroups, respectively.

	African ancestry Ceruloplasmin		Admixed Hispanic ancestry IP-10	
	β (95% CI)	P-value	β (95% CI)	P-value
Age	0.006 (0.002-0.009)	0.002	-0.01 (-0.02- -0.002)	0.02
Sex (F vs. M)	-0.025 (-0.08-0.03)	0.38	-0.08 (-0.27-0.12)	0.45
On CART	0.05 (-0.03-0.12)	0.22	0.08 (-0.15-0.31)	0.49
Nadir CD4*	-0.005 (-0.02-0.01)	0.56	0.01 (-0.03-0.06)	0.53
CSF HIV RNA <50 cps/mL	-0.05 (-0.11-0.02)	0.14	-0.42 (-0.61- -0.24)	<0.001
Haplogroup**	0.11 (0.02-0.2)	0.02	0.2 (0.02-0.37)	0.03

*per 100 cells/mm3; **haplogroup L1 for African; haplogroup B for admixed Hispanic

Figure. Selected CSF biomarker plots, by genetic ancestry and mitochondrial DNA haplogroups. Boxes denote medians, 25th and 75th %tiles; whiskers represent 5th and 95th %tiles.



Conclusions

- Ancestry-specific associations between mtDNA haplogroups and CSF biomarkers **IP-10** (interferon gamma-induced protein-10, or CXCL10) and **ceruloplasmin** (a hepatic protein involved in iron metabolism) were observed in persons of admixed Hispanic and African ancestry, respectively.
- Haplogroup B was statistically associated with IP-10 only after adjustment for age and CSF HIV RNA detectability.
- African haplogroups L1 and L2 were associated with higher CSF ceruloplasmin.
- IP-10 has been studied previously in CSF from HIV-infected persons. Ceruloplasmin has not, but was elevated in serum of HIV-infected subjects with HAD compared to those without dementia (Rozek, et al. 2008; PMCID: PMC3406605).
- Prior analyses in CHARTER reported less NCI in admixed Hispanic persons with mtDNA haplogroup B.
- Analyses to assess relationships between mtDNA haplogroups, CSF biomarkers, other comorbidities, and NCI are ongoing.

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