Poster # 461 Session: P-G4

Asha Kallianpur | 9500 Euclid Avenue/NE50, Cleveland Clinic | Cleveland, OH 44195 | (216) 445-2337 | (216) 636-0009 | kalliaa@ccf.org

Iron-Regulatory Genes are Associated with Neuroimaging Traits in HIV Infection

Tricia A. Thornton-Wells[1], Christine Fennema-Notestine[2], Todd Hulgan[1], Scott L. Letendre[2], Ronald J. Ellis[2], Donald R. Franklin[2], Susan Morgello[3], Justin C. McArthur[4], Ann C. Collier[5], Benjamin B. Gelman[6], J. Allen McCutchan[2], and Asha R. Kallianpur[7] for the CHARTER Group.

[1]Vanderbilt University, Nashville, TN; [2]University of California-San Diego, San Diego, CA; [3]Icahn School of Medicine of Mount Sinai, New York, NY; [4]Johns Hopkins University, Baltimore, MD; [5]University of Washington, Seattle, WA; [6]University of Texas Medical Branch, Galveston, TX; [7]Cleveland Clinic/Lerner Research Institute, Cleveland, OH.

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

Objective

To test the hypothesis that genetic variation in iron metabolism is associated with aging-related neuroimaging traits in HIV-infected persons with or without neurocognitive impairment.

Background & Significance

- HIV-Associated Neurocognitive Disorder (HAND) remains a highly prevalent complication of HIV infection, despite viral suppression with combination antiretroviral therapy (CART).
- Persistent neuro-inflammation/immune activation and global mitochondrial dysfunction are implicated in HAND.[1]
- Brain iron dysregulation occurs in aging-related neurocognitive disorders that share neuroimaging traits with HAND, such as white matter damage.[2]
- Immune activation, macrophage-monocyte inflammation and iron metabolism are linked via the hepcidin pathway (**Figure 1**).

Methods I

Study Subjects

- **CHARTER** is a U.S.-based observational study of neuro-HIV outcomes in which ambulatory, HIV-infected adults underwent standardized neuromedical assessments from 2003-2007.
- Comprehensive **neurocognitive testing** (CHARTER battery) in all patients: HAND defined by Global Deficit Score (GDS) impairment [*normal* (GDS<0.5) or *impaired* (GDS≥0.5)]
- High-resolution magnetic resonance (MR) neuroimaging at entry in a subset (N=288)
- This genetic study included subjects with previously acquired **genome-wide genotyping** data from CHARTER who lacked confounding neuropsychiatric comorbidities.
- Evaluated 243 subjects with images of adequate quality, comparable technique

Image Acquisition and Quality Control

- Standardized protocols for image acquisition across all 5 sites
- 21 brain regions of interest (ROI) evaluated
- **Structural MR** imaging (sMRI) at 1.5 Tesla to measure gray matter (GM) and white matter (WM) volumes
- Single-voxel **MR spectroscopy** was sampled in frontal GM, frontal WM, & basal ganglia to measure brain metabolites: *N*-acetyl-aspartate (NAA), choline, creatine, and myoinositol

Gene selection & genotyping (12 candidate genes)

- **Gene selection**: nuclear iron storage and transport; hepcidin pathway (**Figure 1**); mitochondrial iron transport or binding; linked to CNS phenotypes; polymorphic in diverse populations (MAF≥5%)
- Requested nucleotide SNP calls at 250 iron-regulatory gene loci of interest from existing CHARTER GWAS (Affymetrix™ Human SNP Array 6.0)

Macrophage-Monocyte Iron Recycling

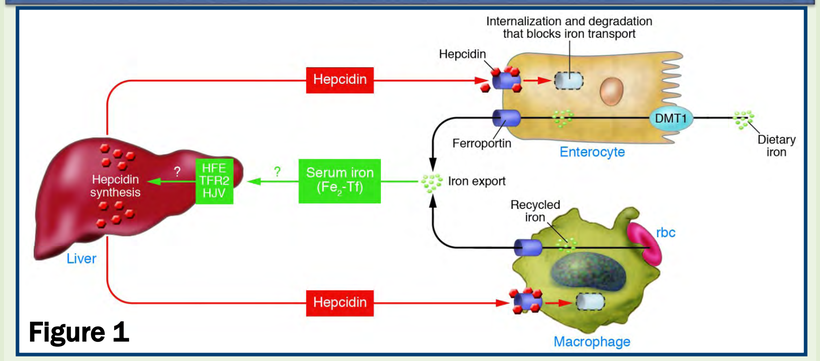


Figure 1

Methods II - Analyses

- Structural volumes were measured using a multi-channel segmentation approach[3,4], and spectroscopy data were analyzed using **LCModel.**[5]
- Imaging measures were normalized and log-transformed
- Additive genetic model; differences between genotype groups determined using Generalized Linear Model Analysis of Covariance (**MANCOVA**) using PLINK
- Adjustment for fixed *plus* trait-specific covariates
- Analyses stratified by RNA detectability and by incidental *vs.* contributing comorbidity
- Haploview™ to assess genomic regions, haplotype blocks

Correction of p-values for multiple-testing at 2 levels:
Level 1 - 37 haplotype blocks (p<0.00135)
Level 2 - 37 haplotype blocks + 21 ROI (p<6.43 e^{-5})

Results

Summary of Significant Models

(after multiple testing correction for 37 haplotype blocks)

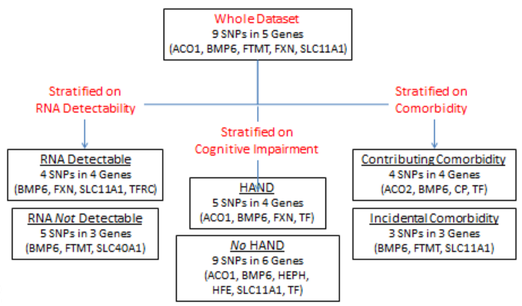


Figure 2

Results

Gene/SNP (Phenotype; subgroup)	Adjusted β-coeffic.	Locus (Variant Type)	Protein Function	*p*-value
TFRC rs17091382 ***(A)*** Subcortical GM volume; detectable viremia	-0.098	3q29 intronic	Iron transport, all cell types	3.2 e^{-5}
SLC11A1 rs7576974 ***(T)*** Frontal GM NAA:		2q35 intronic	Intracellular iron transport	
1) No HAND	0.040			3.3 e^{-5}
2) All subjects	0.040			4.3 e^{-5}
3) Incidental comorbidities	0.053			5.2 e^{-5}
SLC40A1 rs13404407 ***(C)*** Abnormal WM volume; undetectable viremia	0.902	2q32.2 intronic	Macrophage iron export	2.2 e^{-6}
CP rs4974389 ***(A)*** Basal ganglia choline; contributing comorbidities	0.048	3q24-q25 intronic	Copper ferroxidase; inflammation	3.5 e^{-5}
ACO2 rs9611598 ***(A)*** Subcortical GM; contributing comorbidities	-0.316	22q13.2 intronic	Mitochondrial TCA cycle enzyme	5.9 e^{-5}

Table. Genes and SNPs significant at *Level 2* of multiple-testing correction in separate multivariable models of neuroimaging traits

Conclusions

- In HIV-infected individuals, iron-regulatory gene variants were associated with:
  - Metabolic MR-spectroscopy measures, including markers of neuroinflammation (choline) and neuronal integrity (NAA) in basal ganglia & frontal GM
  - Lower subcortical GM volume & greater abnormal WM volume

  (neuroimaging traits that have been linked to neurodegeneration or neuro-inflammation and are potentially HAND endophenotypes).
- Associations involving SNPs in ***TFRC***, ***CP***, and ***SLC11A1*** were statistically robust, but due to the small number of variant alleles, SNPs in ***SLC40A1*** and ***ACO2*** require further study.
- 29 additional interesting associations (p<0.00135) were identified for patient subgroups with/without HAND, un/detectable viremia, and incidental/contributing comorbidity (multiple SNPs in each of ***BMP6***, ***ACO1***, ***TF***, ***FXN***, ***FTMT*** genes).
- *CAVEAT:* Neuroimaging methods sensitive to iron were not performed in this study. Studies incorporating T2*, susceptibility-weighted imaging, and other methods of iron-sensitive neuroimaging are underway.
- These findings require in-depth exploration and replication in other HIV$^+$ populations.

References

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CHARTER NEUROAIDS DATA RESOURCE

Supported by NIH grant MH095621 (PIs: T. Hulgan & A. Kallianpur), contracts N01 MH22005 & HHSN271201000036C (CHARTER; PI: I. Grant). Participating sites: Johns Hopkins University (J. McArthur); Icahn School of Mt. Sinai (S. Morgello & D. Simpson); University of California, San Diego (J. A. McCutchan); University of Texas Medical Branch, Galveston (B. Gelman); University of Washington, Seattle (A. Collier & C. Marra); Washington University, St. Louis (D. Clifford)

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