UC San Diego
UC San Diego Previously Published Works

Title

Mitochondrial DNA Haplogroups and Neurocognitive Impairment During HIV Infection

Permalink

https://escholarship.org/uc/item/8r39h8rp

Journal

Clinical Infectious Diseases, 61(9)

ISSN

1058-4838

Authors

Hulgan, Todd
Samuels, David C
Bush, William
et al.

Publication Date

2015-11-01

DOI

10.1093/cid/civ527

Peer reviewed

Mitochondrial DNA Haplogroups and Neurocognitive Impairment During HIV Infection

Todd Hulgan,[1] David C. Samuels,[1] William Bush,[1,a] Ronald J. Ellis,[2] Scott L. Letendre,[2] Robert K. Heaton,[2] Donald R. Franklin,[2] Peter Straub,[1] Deborah G. Murdock,[1,b] David B. Clifford,[3] Ann C. Collier,[4] Benjamin B. Gelman,[5] Christina M. Marra,[4] Justin C. McArthur,[6] J. Allen McCutchan,[2] Susan Morgello,[7] David M. Simpson,[7] Igor Grant,[2] and Asha R. Kallianpur[8]; for the CHARTER Group[c]

[1]Vanderbilt University, Nashville, Tennessee; [2]University of California-San Diego, California; [3]Washington University, St. Louis, Missouri; [4]University of Washington, Seattle; [5]University of Texas Medical Branch, Galveston; [6]Johns Hopkins University, Baltimore, Maryland; [7]Icahn School of Medicine at Mount Sinai, New York, New York; and [8]Cleveland Clinic Foundation/Lerner Research Institute and Cleveland Clinic Lerner College of Medicine, Ohio

Background. Neurocognitive impairment (NCI) remains an important complication in persons infected with human immunodeficiency virus (HIV). Ancestry-related mitochondrial DNA (mtDNA) haplogroups have been associated with outcomes of HIV infection and combination antiretroviral therapy (CART), and with neuro-degenerative diseases. We hypothesize that mtDNA haplogroups are associated with NCI in HIV-infected adults and performed a genetic association study in the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) cohort.

Methods. CHARTER is an observational study of ambulatory HIV-infected adults. Haplogroups were assigned using mtDNA sequence, and principal components were derived from ancestry-informative nuclear DNA variants. Outcomes were cross-sectional global deficit score (GDS) as a continuous measure, GDS impairment (GDS $\geq$ 0.50), and HIV-associated neurocognitive disorder (HAND) using international criteria. Multivariable models were adjusted for comorbidity status (incidental vs contributing), current CART, plasma HIV RNA, reading ability, and CD4 cell nadir.

Results. Haplogroups were available from 1027 persons; median age 43 years, median CD4 nadir 178 cells/mm^3, 72% on CART, and 46% with HAND. The 102 (9.9%) persons of genetically determined admixed Hispanic ancestry had more impairment by GDS or HAND than persons of European or African ancestry (P < .001 for all). In multivariate models including persons of admixed Hispanic ancestry, those with haplogroup B had lower GDS (β = −0.34; P = .008) and less GDS impairment (odds ratio = 0.16; 95% confidence interval, .04, .63; P = .009) than other haplogroups. There were no significant haplogroup associations among persons of European or African ancestry.

Conclusions. In these mostly CART-treated persons, mtDNA haplogroup B was associated with less NCI among persons of genetically determined Hispanic ancestry. mtDNA variation may represent an ancestry-specific factor influencing NCI in HIV-infected persons.

Keywords. HIV; AIDS; cognitive disorders; DNA, mitochondrial.

Neurocognitive impairment (NCI) remains an important complication of human immunodeficiency virus (HIV) infection in the combination antiretroviral therapy (CART) era [1]. Severe neurologic complications of HIV are less common, but up to one-half of HIV-infected individuals experience milder forms of NCI that are associated with symptomatic decline [2, 3]. In the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) cohort, more than 50% of the >1500 individuals studied had evidence of NCI at enrollment, with increased rates across three levels of increasing

comorbidity [4]. Given the potential impact of these untreatable disorders in aging CART-treated populations, understanding, predicting, and preventing them is important. Although host factors have been associated with HAND [4, 5], genetic predictors of HAND should be more thoroughly characterized.

Early studies of HAND genetics examined HIV-associated dementia (HAD), AIDS dementia complex, or HIV-associated encephalitis, phenotypes that are uncommon in the CART era, and identified associations with chemokine coreceptor 5 (CCR5) [6] and apolipoprotein E (ApoE) [7] gene variants. Recent analyses have included milder NCI phenotypes and have continued to primarily examine the ApoE ε4 allele, with inconsistent results [8, 9]. Reviews on this topic provide additional background and highlight the limitations of relatively small sample sizes and heterogeneous phenotypes [10]. One genome-wide association study (GWAS) of HAND has been reported in a sample of 1287 adults of predominantly European ancestry enrolled in the Multicenter AIDS Cohort Study [11]. This analysis did not find associations meeting GWAS-level significance ($P < 5 \times 10^{-8}$) but did identify associations between processing speed and 2 single-nucleotide polymorphisms (SNPs) in ion channel transporters (SLC8A1 and NALCN) that approached statistical significance.

The critical and complex roles of mitochondria in energy production, reactive oxygen species (ROS) homeostasis, and apoptotic regulation make them vulnerable targets and key mediators of cellular damage in response to environmental stresses. Given high-energy demands of the central nervous system (CNS), mitochondrial function is critically important in neuronal function and neurodegenerative diseases. Mitochondrial DNA (mtDNA) is maternally inherited and encodes 13 electron transport chain polypeptides. Patterns of mtDNA variants— haplogroups—define maternal ancestry, may influence successful aging, and have been associated with human diseases [12]. The potential importance of mtDNA variation in NCI is supported by the prominence of neurologic phenotypes in inherited mtDNA diseases and associations between mtDNA haplogroups and neurodegenerative diseases [13]. Several studies have reported associations between mtDNA haplogroups and HIV- or CART-associated outcomes, including peripheral neuropathy and neuroretinal disease [14]. Furthermore, haplogroup-defining mtDNA variants have recently been linked to altered expression of inflammation, complement, and apoptosis genes, raising the possibility that mitochondrial-nuclear interactions play a role in immune regulation [15].

We hypothesized that genetic variation in mtDNA modulates susceptibility to or severity of NCI in HIV infection, perhaps through neuroinflammation. There are data in HIV-uninfected populations supporting a link between mitochondrial dysfunction and neuroinflammation in neurodegenerative processes [16], and associations with these diseases and mtDNA variation

cited above provide indirect evidence for this hypothesis. This link is yet to be established in HAND. Characterizing associations between mtDNA haplogroups and NCI in this population may facilitate risk stratification of patients and identify targets for prevention or therapy. Because studies of the role of mtDNA haplogroups in HAND have not been published, we used stored DNA and cross-sectional data from the CHARTER study to address this question, and present the first report of a genetic association.

METHODS

Where appropriate, methods followed recommendations from the STrengthening the Reporting of Genetic Association Studies Statement (Supplementary Table 1) [17].

Study Design and Participants

CHARTER is a prospective, observational study conducted at 6 US locations: Baltimore, Maryland; New York, New York; San Diego, California; Galveston, Texas; Seattle, Washington; and St Louis, Missouri. Institutional review boards at each site approved this research, and each participant provided written informed consent. Data were collected between 2003 and 2007 according to a protocol of comprehensive neuromedical, neurobehavioral, and laboratory assessments that were standardized across sites [4]. The data reported herein are a cross-sectional genetic association analysis of a subgroup of CHARTER participants.

Assessments of Neurocognitive Impairment

Participants were English-speaking and underwent a comprehensive test battery that included seven neurocognitive domains affected by HIV-associated CNS dysfunction [4]. Composite global deficit score (GDS) was derived from standard T-scores using best available normative standards to correct for learning, age, education, sex, and ethnicity, as appropriate. For self-reported Hispanics, 3 of 15 measures were corrected for English-speaking Hispanic normative standards; the remainder was adjusted for Caucasian normative standards [18, 19]. The GDS as a continuous variable reflects the number and severity of neurocognitive deficits across the battery; it is the average of the deficit scores on each test, where $T \geq 40 = 0$ (no deficit), 35–39 = 1(mild deficit), 30–34 = 2 (mild to moderate deficit); 25–29 = 3 (moderate deficit), 20–24 = 4 (moderate to severe deficit), and <20 = 5 (severe deficit). An established cutoff of GDS ≥ 0.50 defines NCI [20]. To further classify presence and severity of HAND, we applied a published objective algorithm that has been shown to yield excellent inter-rater reliability in previous multisite studies [21]. This algorithm conforms to the Frascati criteria for diagnosing HAND [22], requiring at least mild impairment in ≥ 2 of seven domains, and includes functional

assessment by self-report or performance-based criteria or both, as well as exclusions based upon comorbidities (non-HIV related risks for NCI). Categories of HAND include asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HAD. Standardized assessments were performed by physicians, nurse practitioners, or trained nurses and research associates certified by the CHARTER coordinating center. As described previously [4], HAND categorization required a determination that NCI and functional impairment were likely due to HIV-related effects on the brain rather than comorbid conditions. Detailed review by 2 senior CHARTER investigators using published guidelines [22] provided categorization of comorbid conditions for all CHARTER participants as incidental, contributing, and confounding. Several conditions (eg, brain trauma, epilepsy or other seizure history, CNS opportunistic diseases) informed this categorization; detailed information on their frequencies are presented elsewhere [4]. Individuals with confounded neurocognitive comorbidities (15% of the total CHARTER cohort) that precluded an assessment of the contribution of HIV to their NCI were not eligible for a diagnosis of HAND according to Frascati criteria [4, 22] and were excluded from genetic analyses.

Mitochondrial DNA Sequencing and Haplogroup Determination

Isolation of DNA from whole blood samples was performed using PUREGENE (Gentra Systems Inc, Minneapolis, Minnesota). Full mtDNA sequencing was performed using the GeneChip Human Mitochondrial Resequencing Array v2.0 (Affymetrix, Inc, Santa Clara, California).

Array intensity data were processed using the MitoChip Filtering Protocol [23], and variants were called relative to the Revised Cambridge Reference Sequence [24], Haplogroups were assigned using HaploGrep (http://haplogrep.uibk.ac.at/) [25].

Most participants also underwent nuclear DNA genotyping using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Inc, Santa Clara, California). Ancestry-informative markers were analyzed using EIGENSTRAT software [26] to generate principal components (PC) (Supplementary Figure 1). Model-based clustering on the top 3 PCs, using the *mclust* R package, was used to assign individuals to genetic ancestry clusters using an ellipsoidal model [27]. Genetic ancestry clusters showed 97.4% agreement with self-reported race and ethnicity (Supplementary Table 2). All analyses used PC-ancestry based stratifications (European, African, or admixed Hispanic).

Statistical Analyses

Three outcomes were analyzed: (1) Continuous GDS; (2) dichotomous NCI (defined as GDS ≥ 0.50) vs no impairment (GDS < 0.50); and (3) HAND (any HAND category vs normal) [22]. Prior research has shown strong agreement between the latter 2 methods of classifying NCI, such that NCI by GDS criteria virtually guarantees the presence of HAND, but a minority of HAND cases are classified as within normal limits by the GDS [20]. Primary analyses were stratified by PC-derived genetic ancestry. Univariate analyses included nonparametric Wilcoxon tests for continuous variables and Fisher exact tests for dichotomous variables. Multivariable regression of associations between mtDNA haplogroups and GDS and HAND were adjusted for: comorbidity status (incidental vs contributing); current CART use (yes vs no); plasma HIV RNA; reading ability (by Wide-Range Achievement Test-III [WRAT] Reading subtest score); and self-reported nadir $CD4^+$ T-cell count. Adjustments for multiple comparisons were not performed given the exploratory nature of these analyses, the limited number of exposure (genetic) variables within stratified groups, and the relatively limited number of outcomes assessed. Statistical analyses were conducted using R (version 2.15.1) and Stata Statistical Software, Release 13 (College Station, Texas: StataCorp LP).

RESULTS

Participant Characteristics

Haplogroups were available from 1068 participants. Of these, 1027 (96%) had nuclear DNA genotypes that allowed for PC assessment of genetic ancestry (Supplementary Figure 2). The median age for the analysis group (Table 1) was 43 years, median $CD4^+$ T-cell nadir was 178 cells/mm^3, 736 (72%) were on CART at initial assessment, 474 (46%) had HAND, and the median (interquartile range [IQR]) GDS was 0.32 (0.11–0.63). Haplogroup frequencies within each ancestry category were consistent with US population based data [28] (Supplementary Table 3) and did not differ across CHARTER sites (data not shown).

Univariate Analyses of Global Deficit Score and HIV-associated Neurocognitive Disorders

Persons of admixed Hispanic ancestry had a higher median (IQR) GDS (0.53; 0.21–0.94) than persons of African (0.26; 0.11–0.53; P < .0001) or European (0.37; 0.16–0.68; P = .003) ancestry (Supplementary Figure 3A). Persons of admixed Hispanic ancestry also had a greater likelihood of either impaired GDS (52% vs 29% African [P < .0001] and 37% European [P = .009]; Supplementary Figure 3B) or HAND diagnosis (64% vs 40% African [P < .0001] and 48% European [P = .003]; Supplementary Figure 3C and 3D).

Median GDS did not differ significantly by mtDNA haplogroup in participants of African or European ancestry (Figure 1A and 1B). Among the 102 persons of admixed Hispanic ancestry, WRAT-III, GDS, and percentage with GDS impairment or HAND differed significantly across haplogroups in univariate analyses (Table 1). Persons belonging to haplogroup

Table 1. Subject Characteristics at CNS HIV Antiretroviral Therapy Effects Research Entry, Overall, Among Those of European, African, and Admixed Hispanic Ancestry, and by Major Hispanic Mitochondrial DNA Haplogroups

Variable	Overall (N = 1027)	European Ancestry (N = 440)	African Ancestry (N = 485)	Admixed Hispanic Ancestry (N = 102)	Haplogroup A (N = 35)	Haplogroup B (N = 20)	Haplogroup C (N = 14)	Other Hispanic Haplogroups (N = 33)	P Value*
Age, median (range)	43 (18–69)	44 (19–68)	44 (18–69)	40 (21–61)	43 (30–54)	39 (22–61)	42 (32–52)	40 (21–53)	.31
Female sex	235 (23%)	52 (12%)	161 (33%)	22 (22%)	9 (26%)	2 (10%)	4 (29%)	7 (21%)	.50
Genetic Ancestry									
European	440 (43%)	440 (100%)	. . .	. . .	. . .	. . .	. . .	. . .	. . .
African	485 (47%)	. . .	485 (100%)	. . .	. . .	. . .	. . .	. . .	. . .
Admixed Hispanic	102 (10%)	. . .	. . .	102 (100%)	35 (34%)	20 (20%)	14 (14%)	33 (33%)	. . .
CD4 nadir, cells/mm^3	178 (51–307)	193 (78–330)	159 (29–285)	167 (52–279)	110 (52–221)	188 (93–345)	129 (35–187)	205 (60–354)	.23
Plasma HIV RNA, log$_{10}$ copies/mL	2.3 (1.7–4.0)	2.1 (1.7–4.0)	2.5 (1.7–4.0)	2.0 (1.7–3.6)	2.0 (1.7–3.6)	1.7 (1.7–3.5)	1.8 (1.7–2.1)	2.5 (1.7–4.2)	.49
Estimated duration of HIV infection, months	120 (55–184)	120 (47–190)	125 (71–180)	108 (37–164)	124 (58–177)	105 (34–125)	152 (95–178)	49 (17–135)	.10
On CART	736 (72%)	308 (70%)	350 (72%)	78 (76%)	26 (74%)	16 (80%)	12 (86%)	24 (73%)	.83
Estimated time on CART, months	48 (9–88)	53 (8–98)	45 (10–79)	45 (5–95)	65 (32–100)	73 (15–103)	60 (3–138)	17 (1–73)	.05
Comorbidity									.25
Incidental	660 (64%)	304 (69%)	296 (61%)	60 (59%)	18 (51%)	14 (70%)	6 (43%)	22 (67%)	
Contributing	367 (36%)	136 (31%)	189 (39%)	42 (41%)	17 (49%)	6 (30%)	8 (57%)	11 (33%)	
WRAT-III	96 (83–105)	102 (96–109)	87 (76–96)	96 (83–102)	93 (79–105)	100 (96–109)	88 (83–96)	94 (87–100)	.04
HAND									
NCN	553 (54%)	228 (52%)	289 (60%)	36 (35%)	8 (23%)	12 (60%)	2 (14%)	14 (42%)	.01**
Any HAND	474 (46%)	212 (48%)	195 (40%)	66 (65%)	27 (77%)	8 (40%)	12 (86%)	19 (58%)	
ANI	354 (34%)	150 (34%)	158 (33%)	46 (45%)	17 (49%)	6 (30%)	8 (57%)	15 (45%)	.06***
MND	88 (9%)	44 (10%)	30 (6%)	14 (14%)	6 (17%)	2 (10%)	2 (14%)	4 (12%)	
HAD	31 (3%)	18 (4%)	7 (1%)	6 (6%)	4 (11%)	0 (. . .)	2 (14%)	0 (. . .)	
GDS	0.32 (0.11–0.63)	0.37 (0.16–0.68)	0.26 (0.10–0.53)	0.53 (0.21–0.94)	0.68 (0.42–1.0)	0.21 (0.08–0.32)	0.58 (0.43–1.4)	0.53 (0.16–0.89)	.003
GDS impaired	359 (35)	164 (37%)	142 (29%)	53 (52)	24 (69)	4 (20)	8 (57)	17 (52)	.006

Values are N (%) or median (interquartile rages), except where noted.

Abbreviations: ANI, asymptomatic neurocognitive impairment; CART, combination antiretroviral therapy; GDS, global deficit score; HAD, HIV-associated dementia; HAND, HIV-associated neurocognitive disorder; HIV, human immunodeficiency virus; MND, mild neurocognitive disorder; NCN, neurocognitively normal; WRAT, wide-range achievement test.

* P-values by analysis of variance (ANOVA) or exact test comparisons across Admixed Hispanic Ancestry haplogroups except where noted.

** P for comparison of any HAND vs NCN.
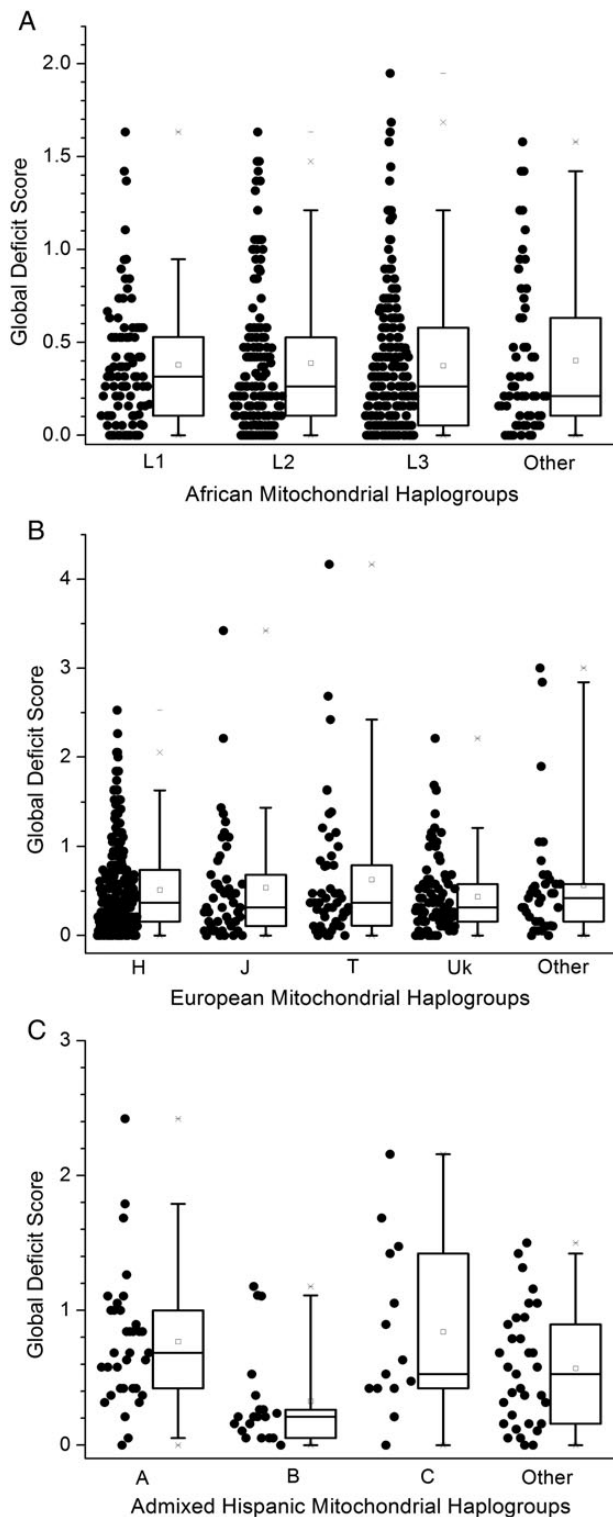
*** P for ANOVA comparison across all HAND categories.

Figure 1. Box and dotplots of global deficit scores among participants of African (panel *A*), European (panel *B*), and Hispanic (panel *C*) ancestry, by major mtDNA haplogroups. Boxes denote median and interquartile range; whiskers denote 95th percentiles. *P*-value = .002 by univariate linear regression for haplogroup B vs other haplogroups among the admixed Hispanic population in Panel *C*. Abbreviation: mtDNA, mitochondrial DNA.
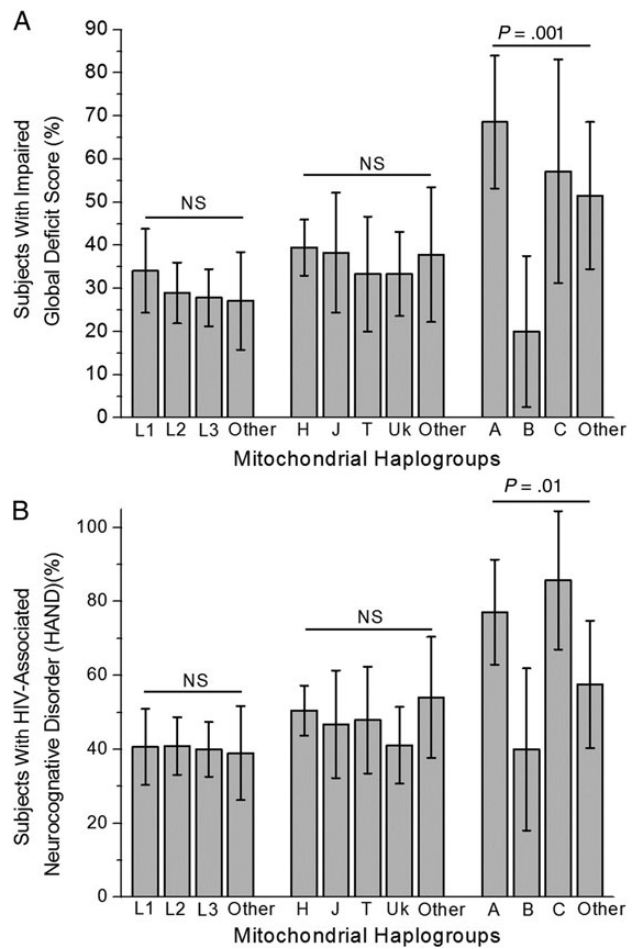

Figure 2. Bar graphs of percentage of participants with global deficit score (GDS) impairment (GDS ≥ 0.50; panel *A*) and HIV-associated neurocognitive disorder (HAND) of any severity (panel *B*), by ancestry and major mtDNA haplogroups. Error bars for frequency estimates were calculated as 2 × Standard Error. *P*-values from univariate logistic regression models are shown. Abbreviations: HIV, human immunodeficiency virus; mtDNA, mitochondrial DNA; NS, not significant.

B (N = 20; 20%) had a lower median GDS (0.21 vs 0.63; *P* = .002; Figure 1*C* and Table 1), and lower likelihood of either GDS impairment (odds ratio [OR] 0.17; 95% confidence interval [CI], .05, .55; *P* = .001; Figure 2*A* and Supplementary Table 4) or HAND (OR 0.27; 95% CI, .10, .75; *P* = .013; Figure 2*B* and Supplementary Table 5) than Hispanic persons with other haplogroups. These relationships persisted in analyses of subgroups on CART, with detectable plasma HIV RNA, and with only incidental neurocognitive comorbidities (data not shown). Among persons of European or African ancestry, there were no statistically significant haplogroup associations with GDS, proportion with GDS impairment, or HAND (data not shown).

Multivariate Analyses of Global Deficit Score and HIV-associated Neurocognitive Disorders

In adjusted models of persons of admixed Hispanic ancestry, haplogroup B was associated with a lower GDS ($\beta = -0.34$; -0.59, -0.09; $P = .008$; Table 2) compared with non-B haplogroups, and a lower likelihood of GDS impairment (adjusted OR 0.16; 0.04, 0.63; $P = .009$; Supplementary Table 4), independent of comorbidity status, WRAT-III score, CART status, nadir CD4$^+$ T-cell count, and plasma HIV RNA level. Although persons with haplogroup B were less likely to have HAND of any severity in univariate analyses, this association was not statistically significant in adjusted analyses (adjusted OR 0.36; 0.11, 1.14; $P = .08$; Supplementary Table 5).

DISCUSSION

In this analysis of chronically HIV-infected, mostly CART-treated CHARTER participants, persons of Hispanic ancestry had more NCI overall, but a common mtDNA haplogroup within the population was associated with significantly lower prevalence of NCI. Specifically, among the almost 20% of persons of admixed Hispanic ancestry having mtDNA haplogroup B, median GDS and the proportion with impaired GDS were significantly lower than in persons with other haplogroups after adjustment for potential confounders. Mitochondrial DNA variants, including haplogroups, have been studied in relation to neurodegenerative diseases in HIV-negative populations. Several studies have explored host genetic risks for HIV-associated NCI [10] and associations between mtDNA haplogroups and non-CNS HIV-related outcomes [14]. This is the first analysis to our knowledge of mtDNA variation and NCI in an HIV-infected population.

Haplogroup B is a major haplogroup seen in persons of East Asian, Native American, and admixed Hispanic ancestry [29, 30], is part of the R superhaplogroup, and includes variations common to the related European haplogroups J and T [31] (Supplementary Figure 4). Haplogroup B has been associated

with nonneurocognitive phenotypes in recent studies [32, 33]. The B haplogroup was also recently associated with changes in epidermal nerve fiber density (ENFD, a measure of small fiber neuropathy) in lower extremity skin biopsies from HIV-infected persons starting CART as part of a clinical trial in Thailand [34]. In that study, persons with the B haplogroup had an increase in ENFD and plasma 8-oxo-deoxyguanosine (a marker of oxidative DNA damage also associated with HAND and mtDNA damage in brain tissue [35]) on CART. Our data cannot address apparently discrepant associations between haplogroup B and what may be an adverse peripheral nerve phenotype in HIV-infected Thais and less NCI in US Hispanics. A recent publication reported an association between subhaplogroup B5a and increased risk for Alzheimer's disease in Han Chinese [36]. Lymphoblastoid and HeLa cells harboring haplogroup B5a and a nonsynonymous SNP (m.8584G > A) found in B5a, respectively, demonstrated adverse mitochondrial phenotypes compared to control cells. Importantly, although B5 was the predominant subhaplogroup in this Han Chinese population and the study of HIV-infected Thais [34], B5 is not represented in our population of admixed Hispanics from the United States. We speculate that this population-specific subhaplogroup could determine whether the B haplogroup is associated with neurodegeneration or neuroprotection.

Potential mechanisms by which mtDNA variation could alter susceptibility to NCI would be similar to those posited for other neurodegenerative processes in aging populations, including differences in ROS production, oxidative stress, inflammation, and apoptotic regulation. In chronic HIV infection, systemic or CNS inflammation or both may contribute to NCI. Given the role of mitochondria in inflammatory processes [37], mtDNA variation could be an additional susceptibility factor. A potential confounder in this population is exposure to older mitochondria-toxic CART drugs, particularly "d-drug" NRTIs zalcitabine, didanosine, or stavudine. Among these CHARTER participants, 13% were taking 1 or more of these at enrollment; 39% had a history of exposure. In secondary

Table 2. Linear Regression of Global Deficit Score in Persons of Admixed Hispanic Ancestry

Variable	Unadjusted Coefficient (95% CI)	P Value	Adjusted Coefficient (95% CI)	P Value
Haplogroup B (vs all others)	-0.37 ($-.61$, $-.13$)	.002	-0.34 ($-.59$, $-.09$)	.008
Comorbidity (incidental vs contributing)	-0.26 ($-.46$, $-.07$)	.008	-0.16 ($-.36$, .04)	.12
WRAT-III score	-0.008 ($-.016$, $-.001$)	.022	-0.003 ($-.01$, .004)	.40
On CART (yes vs no)	0.24 (.009, .464)	.041	-0.02 ($-.36$, .33)	.92
Nadir CD4 (per cell/mm^3)	-0.0006 ($-.0011$, $-.00009$)	.020	-0.0004 ($-.001$, .0002)	.16
Plasma HIV RNA (per log$_{10}$ copies/mL)	-0.09 ($-.17$, $-.01$)	.023	-0.06 ($-.17$, .04)	.23

N = 96, Model P-value = .001, R^2 = 0.16.
Abbreviations: CART, combination antiretroviral therapy; CI, confidence interval; HIV, human immunodeficiency virus; WRAT, wide-range achievement test.

multivariate models, neither d-drug exposure at enrollment nor past exposure were associated with NCI in the admixed Hispanic population, and associations between mtDNA haplogroup B did not change substantially (data not shown). There tended to be variability in the duration of CART exposure by haplogroup among the admixed Hispanic population (Table 1). We therefore also performed a secondary model adjusting for CART duration, and did not observe substantial differences in the haplogroup B results (data not shown).

A strength of our study is the well-characterized and racially and geographically diverse population with NCI and HAND diagnoses and confounders identified in a standardized fashion. Limitations include those inherent to cross-sectional analyses and the lack of an HIV-uninfected control population. Longitudinal approaches to NCI in CHARTER have recently been published [2, 3], and genetic analyses of longitudinal changes in NCI are needed. Although this analysis includes an overall large sample, numbers of persons from minority genetic ancestry groups and within individual mtDNA haplogroups are relatively small. The small sample size of adults of admixed Hispanic ancestry suggests that although the effect sizes identified were large enough to reach statistical significance, the findings may not generalize to other populations of HIV-infected adults of Hispanic ancestry. We did not observe statistically significant associations between mtDNA haplogroup and NCI or HAND in persons of European or African genetic ancestry in CHARTER. Despite relatively large sample sizes, our analyses may be underpowered to detect small but potentially clinically relevant differences, or that subhaplogroups or mtDNA variants not specifically included in our analysis are associated with NCI. The relevance of ANI, the most common HAND category in this population, has been questioned [38]. Recent data, however, have demonstrated that persons with ANI are at risk for symptomatic decline, supporting its clinical and prognostic value [3]. We performed sensitivity analyses of the HAND outcome in admixed Hispanic participants excluding those with ANI (ie, comparing neurocognitively normal vs MND or HAD). With this smaller population (N = 54), the adjusted OR for the B haplogroup was of a similar direction and magnitude (0.22), with an increase of P-value from .08 to .14.

Utility of standardized NCI measures in persons of non-European ancestry is less well established, an important consideration given that we observed both greater prevalence of and haplogroup associations with NCI only in the admixed Hispanic ancestry population. Because specific Hispanic neurocognitive norms exist for only 3 of the tests in the CHARTER battery, use of Caucasian norms for the other tests may have contributed to higher rates of impairment among Hispanics. The choice of norms was based upon evidence that performance of US Hispanics on multiple neurocognitive tests is closer to that of Caucasian than African Americans [39].

In addition, recent longitudinal CHARTER analyses found self-reported Hispanic ethnicity to be a risk factor for decline in neurocognitive performance from baseline over a mean of 36 months of follow-up [2]; those analyses did not use cross-sectional neurocognitive norms and also found Hispanic ethnicity to be associated with NCI. Although self-identified Hispanic participants were drawn from CHARTER sites across the United States, haplogroup frequency did not differ significantly across sites, and the B haplogroup was associated with NCI independent of WRAT-III, a measure of premorbid intelligence and quality of educational background. In addition, birthplace (US or Canada vs other) and primary language (English vs other) did not differ significantly between B and other haplogroups among participants of admixed Hispanic ancestry (data not shown), but there may be other unmeasured or unknown biologic or sociodemographic confounders associated with both mtDNA haplogroup and NCI. Due to genetic diversity and admixture of persons of self-identified Hispanic-American ethnicity, genotype-phenotype associations are complex [40], and definitive conclusions must be drawn cautiously and only after replication.

Future analyses should examine associations between haplogroups and CSF neuroinflammation and non-haplogroup-defining SNPs and NCI, and interactions between mtDNA variants and SNPs in nuclear genes relevant for mitochondrial function and inflammation. Prospective studies may also explore targeted genetic testing for risk-stratification and personalized interventions. Should this association be validated, mtDNA variation may be a novel, ancestry-specific host factor influencing HAND in chronically HIV-infected persons and may provide pathogenic clues that will lead to a better understanding of this condition.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

K. Heaton, PhD (PI), J. Hampton Atkinson, MD, Matthew Dawson; Imaging Component: Christine Fennema-Notestine, PhD (PI), Michael J Taylor, PhD, Rebecca Theilmann, PhD; Data Management Component: Anthony C. Gamst, PhD (PI), Clint Cushman; Statistics Component: Ian Abramson, PhD (PI), Florin Vaida, PhD, Reena Deutsch, PhD; Johns Hopkins University Site: Justin McArthur (PI), Vincent Rogalski; Icahn School of Medicine at Mount Sinai Site: Susan Morgello, MD (Co-PI) and David Simpson, MD (Co-PI), Letty Mintz, NP; University of California, San Diego Site: J. Allen McCutchan, MD (PI), Kaori Phillips, BSN; University of Washington, Seattle Site: Ann Collier, MD (Co-PI) and Christina Marra, MD (Co-PI), Trudy Jones, MN, ARNP; University of Texas, Galveston Site: Benjamin Gelman, MD, PhD (PI), Eleanor Head, RN, BSN; and Washington University, St. Louis Site: David Clifford, MD (PI), Muhammad Al-Lozi, MD, Mengesha Teshome, MD.

References

1. Clifford DB, Ances BM. HIV-associated neurocognitive disorder. Lancet Infect Dis 2013; 13:976–86.

2. Heaton RK, Franklin DR Jr, Deutsch R, et al. Neurocognitive Change in the Era of HIV Combination Antiretroviral Therapy: The Longitudinal CHARTER Study. Clin Infect Dis 2015; 60:473–80.

3. Grant I, Franklin DR Jr, Deutsch R, et al. Asymptomatic HIV-associated neurocognitive impairment increases risk for symptomatic decline. Neurology 2014; 82:2055–62.

4. Heaton RK, Clifford DB, Franklin DR Jr, et al. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. Neurology 2010; 75:2087–96.

5. Heaton RK, Franklin DR, Ellis RJ, et al. HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. J Neurovirol 2011; 17:3–16.

6. van Rij RP, Portegies P, Hallaby T, et al. Reduced prevalence of the CCR5 delta32 heterozygous genotype in human immunodeficiency virus-infected individuals with AIDS dementia complex. J Infect Dis 1999; 180:854–7.

7. Corder EH, Robertson K, Lannfelt L, et al. HIV-infected subjects with the E4 allele for APOE have excess dementia and peripheral neuropathy. Nat Med 1998; 4:1182–4.

8. Morgan EE, Woods SP, Letendre SL, et al. Apolipoprotein E4 genotype does not increase risk of HIV-associated neurocognitive disorders. J Neurovirol 2013; 19:150–6.

9. Panos SE, Hinkin CH, Singer EJ, et al. Apolipoprotein-E genotype and human immunodeficiency virus-associated neurocognitive disorder: the modulating effects of older age and disease severity. Neurobehav HIV Med 2013; 5:11–22.

10. Kallianpur AR, Levine AJ. Host genetic factors predisposing to HIV-associated neurocognitive disorder. Curr HIV/AIDS Rep 2014; 11:336–52.

11. Levine AJ, Service S, Miller EN, et al. Genome-wide association study of neurocognitive impairment and dementia in HIV-infected adults. Am J Med Genet B Neuropsychiatr Genet 2012; 159B:669–83.

12. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet 2005; 39:359–407.

13. Mancuso M, Filosto M, Orsucci D, Siciliano G. Mitochondrial DNA sequence variation and neurodegeneration. Hum Genomics 2008; 3:71–8.

14. Hart AB, Samuels DC, Hulgan T. The other genome: a systematic review of studies of mitochondrial DNA haplogroups and outcomes of HIV infection and antiretroviral therapy. AIDS Rev 2013; 15:213–20.

15. Cristina Kenney M, Chwa M, Atilano SR, et al. Inherited mitochondrial DNA variants can affect complement, inflammation and apoptosis pathways: insights into mitochondrial-nuclear interactions. Hum Mol Genet 2014; 23:3537–51.

16. Trudler D, Nash Y, Frenkel D. New insights on Parkinson's disease genes: the link between mitochondria impairment and neuroinflammation. J Neural Transm 2015; doi:10.1007/s00702-015-1399-z.

17. Little J, Higgins JP, Ioannidis JP, et al. STrengthening the REporting of Genetic Association Studies (STREGA): an extension of the STROBE statement. PLoS Med 2009; 6:e22.

18. Heaton RK, Miller SW, Taylor MJ, Grant I. Revised comprehensive norms for an expanded Halstead-Reitan Battery: demographically adjusted neuropsychological norms for African American and Caucasian adults Scoring Program. Odessa, FL: Psychological Assessment Resources, Inc., 2004.

19. Norman MA, Moore DJ, Taylor M, et al. Demographically corrected norms for African Americans and Caucasians on the Hopkins Verbal Learning Test-Revised, Brief Visuospatial Memory Test-Revised, Stroop Color and Word Test, and Wisconsin Card Sorting Test 64-Card Version. J Clin Exp Neuropsychol 2011; 33:793–804.

20. Blackstone K, Moore DJ, Franklin DR, et al. Defining neurocognitive impairment in HIV: deficit scores versus clinical ratings. Clin Neuropsychol 2012; 26:894–908.

21. Woods SP, Rippeth JD, Frol AB, et al. Interrater reliability of clinical ratings and neurocognitive diagnoses in HIV. J Clin Exp Neuropsychol 2004; 26:759–78.

22. Antinori A, Arendt G, Becker JT, et al. Updated research nosology for HIV-associated neurocognitive disorders. Neurology **2007**; 69: 1789–99.

23. Xie HM, Perin JC, Schurr TG, et al. Mitochondrial genome sequence analysis: a custom bioinformatics pipeline substantially improves Affymetrix MitoChip v2.0 call rate and accuracy. BMC Bioinformatics **2011**; 12:402.

24. Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet **1999**; 23:147.

25. Kloss-Brandstatter A, Pacher D, Schonherr S, et al. HaploGrep: a fast and reliable algorithm for automatic classification of mitochondrial DNA haplogroups. Hum Mutat **2011**; 32:25–32.

26. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet **2006**; 38:904–9.

27. Hall JB, Dumitrescu L, Dilks HH, Crawford DC, Bush WS. Accuracy of administratively-assigned ancestry for diverse populations in an electronic medical record-linked biobank. PloS One **2014**; 9:e99161.

28. Mitchell SL, Goodloe R, Brown-Gentry K, Pendergrass SA, Murdock DG, Crawford DC. Characterization of mitochondrial haplogroups in a large population-based sample from the United States. Hum Genet **2014**; 133:861–8.

29. Li H, Cai X, Winograd-Cort ER, et al. Mitochondrial DNA diversity and population differentiation in southern East Asia. Am J Phys Anthropol **2007**; 134:481–8.

30. Allard MW, Polanskey D, Wilson MR, Monson KL, Budowle B. Evaluation of variation in control region sequences for Hispanic individuals in the SWGDAM mtDNA data set. J Forensic Sci **2006**; 51:566–73.

31. van Oven M, Kayser M. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat **2009**; 30:E386–94.

32. Tiao MM, Liou CW, Huang LT, et al. Associations of mitochondrial haplogroups b4 and e with biliary atresia and differential susceptibility to hydrophobic bile Acid. PLoS Genet **2013**; 9:e1003696.

33. Guardado-Estrada M, Medina-Martinez I, Juarez-Torres E, et al. The Amerindian mtDNA haplogroup B2 enhances the risk of HPV for cervical cancer: de-regulation of mitochondrial genes may be involved. J Hum Genet **2012**; 57:269–76.

34. Hulgan T, Levinson R, Gerschenson M, et al. Epidermal nerve fiber density, oxidative stress, and mitochondrial haplogroups in HIV-infected Thais initiating therapy. AIDS **2014**; 28:1625–33.

35. Zhang Y, Wang M, Li H, et al. Accumulation of nuclear and mitochondrial DNA damage in the frontal cortex cells of patients with HIV-associated neurocognitive disorders. Brain Res **2012**; 1458:1–11.

36. Bi R, Zhang W, Yu D, et al. Mitochondrial DNA haplogroup B5 confers genetic susceptibility to Alzheimer's disease in Han Chinese. Neurobiol Aging **2015**; 36:1604 e7–16.

37. Lopez-Armada MJ, Riveiro-Naveira RR, Vaamonde-Garcia C, Valcarcel-Ares MN. Mitochondrial dysfunction and the inflammatory response. Mitochondrion **2013**; 13:106–18.

38. Nightingale S, Winston A, Letendre S, et al. Controversies in HIV-associated neurocognitive disorders. Lancet Neurol **2014**; 13:1139–51.

39. Casaletto KB, Umlauf A, Beaumont JL, et al. Demographically corrected normative standards for the English version of the NIH Toolbox Cognition Battery. J Int Neuropsychol Soc **2015**; 21:378–91.

40. Levran O, Awolesi O, Shen PH, Adelson M, Kreek MJ. Estimating ancestral proportions in a multi-ethnic US sample: implications for studies of admixed populations. Hum Genomics **2012**; 6:2.