The role of mitochondrial DNA variation in age-related decline in gait speed among older men living with HIV

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Running head: mtDNA variation and gait speed in PLWHIV

Summary:

Mitochondrial DNA haplogroup and longitudinal gait speed decline after the age of 50 were studied among white men infected with HIV. Haplogroup J was an independent risk factor for more rapid age-related gait speed decline.

Abstract

Background: Age-related gait speed decline is accelerated in men with HIV. Mitochondrial genetic variation is associated with frailty and mortality in the general population, and may provide insight into mechanisms of functional decline in people aging with HIV.

Material & Methods: Gait speed was assessed semi-annually in the Multicenter AIDS Cohort Study (MACS). Mitochondrial DNA (mtDNA) haplogroups were extracted from genome-wide genotyping data, classifying men ≥ 50 years into 5 groups: mtDNA haplogroup H, J, T, Uk, and other. Differences in gait speed by haplogroups were assessed as: 1) rate of gait speed decline per year; 2) probability of slow gait speed (<1.0 m/s); and 3) hazard of slow gait using multivariable linear mixed-effects models, mixed-effects logistic regression models, and the Andersen-Gill Model, controlling for HCV infection, previous AIDS diagnosis, thymidine analogues exposure, education, body composition, smoking, and peripheral neuropathy. Age was further controlled for in the mixed-effects logistic regression models.

Results: 455 HIV+ white men age ≥ 50 contributed 3,283 person-years of follow-up. Among them, 70% have achieved HIV viral suppression. In fully adjusted models, individuals with haplogroup J had more rapid decline in gait speed (adjusted slopes: 0.018 m/s/year vs. 0.011 m/s/year, $p_{interaction} = 0.012$), and increased risk of developing slow gait (adjusted OR: 2.97, 95% CI: 1.24-7.08) compared to those with other haplogroups.

Conclusions: Among older, HIV-infected men, mtDNA haplogroup J was an independent risk factor for more rapid age-related gait speed decline.

Keywords: mitochondrial genetic; HIV; aging; gait speed.

BACKGROUND

Due to effective antiretroviral therapy (ART), people living with HIV (PLWHIV) have substantially longer life expectancies than in the pre-ART era [1, 2], yet have higher rates of chronic comorbidities and greater risk of premature death than demographically similar adults without HIV [3, 4]. Accelerated functional decline and higher prevalence of frailty among PLWHIV have been observed compared to HIV-uninfected controls [5-8]. Gait speed is an independent measure of physical function and a component of the assessment of frailty, and has been shown to predict functional decline, hospitalization, disability and deaths among older adults [6, 9]. An accelerated rate of decline in gait speed has been noted in older PLWHIV, which could be attributed to multiple factors, including characteristics shared with the general population (reduced energy production[10], compromised energy consumption [10, 11], changes in body composition [12], etc.), as well as unique challenges from HIV chronic infection [13, 14].

Mitochondria—which actively engage in cell energy production [15]—are likely an important contributor to functional aging in PLWHIV. Mitochondrial DNA (mtDNA) is associated with energy production, apoptosis, and inflammation pathways in cells [16], and has been linked to age-related physical function decline [15, 17], frailty and mortality [18]. Conversely, mitochondrial function among PLWHIV could be damaged by HIV chronic infection [14] and long-term usage of ART [13], which trigger proinflammatory responses that could lead to impaired physical function. It is possible

that mtDNA genetic variation interact with multiple mechanisms and confer additional susceptibility to certain individuals.

Mitochondrial DNA haplogroups represent major branch points on the mitochondrial phylogenetic tree, and have been broadly used in studying human evolution and discovering genes involved in complex disease development [19]. Among the HIV-infected (HIV+) population, mtDNA haplogroups have been linked to progression to acquired immunodeficiency syndrome (AIDS) and mortality [20], as well as ART-associated peripheral neuropathy [21], but their association with physical function in PLWHIV has not been investigated. This study aimed to evaluate the association of common European mtDNA haplogroups with gait speed in PLWHIV to better understand the role of mitochondrial genetic background in aging-related declines in physical function.

MATERIALS & METHODS

Study Population

We used data from the Multicenter AIDS Cohort Study (MACS), a long-standing prospective community-based cohort that recruited men with and without HIV who have sex with men at four study sites (Baltimore/Washington DC, Chicago, Pittsburgh, and Los Angeles) starting in 1984. Institutional review boards at each study location approved the study protocol and each participant provided informed written consent. Descriptions of the study design, enrollment, and data collection can be found in previous publications [6, 22]. Briefly, participants were enrolled during three enrollment

periods from 1984-5, 1987-91, and 2001-03, and attend routine semi-annual visits for standardized interviews, laboratory tests, and physical examinations. For purposes of this analysis, subjects were restricted to non-Hispanic white males, because the greatest proportion of MACS participants available for analysis were of non-Hispanic white race/ethnicity. We only included subjects who had two or more visits with physical function measurement and had genetic data available. No mtDNA haplogroup information was available for HIV- men at the time of analysis, thus we only included HIV+ men in the current study. Furthermore, we restricted this analysis to men aged 50 or older, because a previous MACS study demonstrated an accelerated rate of gait speed decline after age 50 among HIV+ participants [6].

Gait Speed

Gait speed was assessed semi-annually in meters per second (m/s) over a 4-meter course, using standard clinical procedures [23]. Participants were asked to walk at their “normal, comfortable pace.” Timing was initiated with a command of “Go” and stopped after the first foot-fall over the finish line. Two measurements were conducted, with the faster measurement [24] used for analysis. A measurement of < 1 m/s was defined as clinically slow gait speed [23, 25]. Gait speeds over 1.7 m/s were recorded as missing (52 visits out of total of 4,031) in the analysis due to potential measurement error, as 1.7 m/s may be considered a transitional speed between walking and running [26, 27].

HIV Serostatus, HIV disease progression, and ART use

All men in this analysis were HIV+, tested by enzyme-linked immunosorbent assay and confirmed by Western blot. HIV disease progression was tracked through history of AIDS and $\log_{10}$ plasma HIV viremia copy-year. HIV viral load was measured using the Roche ultrasensitive assay (limit of detection = 50 copies/mL; Roche Diagnostics, Nutley, NJ). HIV viremia copy-year is a time-varying measurement of cumulative HIV burden since seroconversion [28, 29], and estimates the area under the patient's longitudinal viral load curve from seroconversion to each visit. In the current study, we calculated viremia copy-year for those with and without HIV at baseline using their viral load measured since 2003 or since seroconversion, respectively. Nucleoside reverse transcriptase inhibitors (NRTIs), especially thymidine analogues NRTI has known mitochondrial toxicity [30]. Therefore, we controlled for time-varying exposure to thymidine analogues (stavudine [d4T] and Zidovudine [AZT]) in the final models.

Mitochondrial DNA Haplogroup Determination

Mitochondrial DNA genotyping data were extracted from multiple existing genome-wide genotyping panels (Illumina Human Hap 550, Illumina 1MDuo, Illumina 1M, and TaqMan) in MACS. Comprehensive quality control was completed using PLINK software. All heterozygous calls were set to be missing and SNP strands were flipped to match the mitochondrial reference. mtDNA haplogroups were identified using HaploGrep [19], an application that identifies the most probable mtDNA haplogroups based on the phylogenetic tree of global mtDNA variation (Phylotree: http://www.phylotree.org) [31]. The vast majority of study subjects fit into one of the four

common European haplogroups: H, J, T, and Uk; the remaining were combined into the "other" haplogroup.

Other covariates

Data on sociodemographic status (age, race, and whether or not they have college education) and cigarette smoking (time-varying never vs. ever smoker, time-varying never vs. previous vs. current smoker, or pack-years of smoking) were collected through interviews during enrollment and at each study visit. Hepatitis C infection was identified as anti-HCV seropositive. Peripheral neuropathy was defined as ever reporting pain, burning, numbness, a pins-and-needles sensation in the feet or legs, or inability to detect vibrations in either foot [6].

Statistical Analysis

All statistical analyses were conducted in Stata software version 14 (Statacorp, College Station, TX). Baseline characteristics of participants were stratified by five groups (haplogroup H, J, T, Uk, and other), and differences were evaluated through Fisher's Exact test for categorical variables and Kruskal-Wallis equality-of-populations rank test for continuous variables.

During the exploratory data analysis, scatter plots, linear regression fitted lines (**supplementary Figure 1**), and locally weighted regression smoothers were used to compare gait speed decline for each haplogroup against all other haplogroups. Using longitudinal data, changes in gait speed were evaluated among men from different

haplogroups. Specifically, we conducted the following analyses by haplogroups: (i) the rate of gait speed decline (change in meters per second [m/s] per year) was determined using random effects mixed linear models with random slopes and intercepts after controlling for the following variables: HIV viremia copy-year, ever diagnosed with AIDS, HCV serostatus, weight (kilogram, kg), height (meter, m), ever used any thymidine analogues, peripheral neuropathy (yes/no), college education (yes/no), and time-varying smoking status (never vs. former vs. current). Since we did not observe baseline difference in gait speed by haplogroups, random effects mixed linear models were constructed with the assumption that there was no baseline difference. (ii) The odds of slow gait speed (<1.0 m/s) were identified using mixed effects logistic regression models after controlling for the same covariates. The time unit for longitudinal analysis (i and ii) was one year, starting at age of 50. Because participants could have more than one slow gait speed visit, we estimated (iii) hazard ratios of multiple slow gait visits using the Andersen-Gill Model [32]. Covariates in adjusted models were selected based on their statistical significance ($p<0.05$) and change of magnitude of the association of interest.

RESULTS

Baseline Characteristics

The study sample included 455 HIV+, self-reported non-Hispanic white men aged 50 and older who had $\geq$ 2 study visits between October 1st, 2007 and September 30th, 2016. They contributed a total of 3,283 person-years of follow-up to the analysis (median follow-up: 21 person-years, IQR: 15-23). **Table 1** demonstrates characteristics

of participants at baseline (first visit after October 1st, 2007 when participants were over age 50) by mtDNA haplogroups. Median age was 50 years old, the majority (58.7%) had college education or higher, mean CD4+ cell count was > 500 cells/µL and most (69.5%) had a suppressed HIV-1 RNA.

As presented in **Table 1**, H was the most prevalent haplogroup (41%), followed by Uk, T, and J. Baseline demographic and behavioral characteristics were similar by haplogroup. Mean gait speed at baseline visit was 1.15 m/s overall, and was slightly slower for participants with haplogroup J (not statistically significant, p-value: 0.29).

Gait Speed Decline and mtDNA Haplogroups

In four random effects mixed linear models (**Table 2**), all haplogroups had significant declines in gait speed over time. However, the interaction between haplogroup and time was only significant in the model with haplogroup J, suggesting the rate of gait speed decline over time was only significantly different when comparing haplogroup J vs. non-J (p-value for interaction between time and haplogroup: 0.012). As reflected in the table and **Figure 1**, gait speed declined at a slope of 0.018 m/s/year among haplogroup J vs. 0.011 m/s/year in non-J, after controlling for covariates. Consistent with our previous study performed in MACS [6], HIV viral suppression did not have a significant effect on gait speed. However, having ever been diagnosed with AIDS, higher viremia copy-year, were associated with more rapid decline of gait speed (**Supplemental Table 1**). To prevent bias estimation from dichotomizing exposure (haplogroup) in mixed effect linear regression models, we also constructed a mixed effect linear regression model that fit

all haplogroups as a categorical variable, and assessed the trajectory of gait speed decline in each haplogroup as compared to haplogroup H (**Supplemental Table 2**). All estimations were similar to the results presented in **Table 2**.

Probability of Slow Gait Speed by Haplogroup J

Mixed effects logistic regression models were used to examine differences in the probability of developing clinically slow gait speed between haplogroup J and non-J. Compared to non-J haplogroups, having haplogroup J was associated with 2.97-fold (95% CI: 1.24-7.08) higher risk of developing slow gait speed, after controlling for covariates (**Table 3**). The hazard ratio of slow gait speed among group J vs. non-J from the Andersen-Gill model confirmed these findings (adjusted hazard ratio: 1.60, 95% CI: 1.04-2.47).

DISCUSSION

To our knowledge, this is the first study evaluating the contribution of mitochondrial genetics to physical function in PLWHIV. We found that although there was no difference in gait speed at age 50, gait speed declined significantly faster among participants with haplogroup J than among participants with other haplotypes. In other studies, a minimum clinically meaningful difference in gait speed has generally been taken to be between 0.05 – 0.10 m/s [33]. Studenski, *et. al.* conducted pooled analysis of 9 large cohorts and suggested that the hazard of deaths for older adults decreased by 12% (95% CI: 0.87-0.90) with each increase of 0.1 m/s in gait speed [9]. According to our results, men with haplogroup J, declining 0.006 m/s faster per year than men with

other haplogroups, would reach a difference of 0.05-0.1 m/s after 8 to 16 years (i.e., as early as age 58). Also, participants in haplogroup J had 2.97-fold increased risk of having a study visit with slow gait speed (<1 m/s). Both faster decline in gait speed and higher probabilities of slow gait among haplogroup J were independent from participants' HIV disease state and exposure to thymidine analogues. These findings support the hypothesis that inherited mtDNA variation is associated with physical function decline among PLWHIV.

Previous studies linking mtDNA haplogrous and health outcomes have also noted issues common to haplogroup J. Haplogroup J is known to be associated with increased penetrance of Leber hereditary optic neuropathy (LHON) [34, 35], possibility due to mtDNA defects. Gómez-Durán *et al*. observed that, compared to haplogroup H, cytoplasmic hybrids from haplogroup J contain less mtDNA and RNA, synthesized a smaller amount of mtDNA-encoded polypeptides, and also displayed lower oxygen consumption, lower mitochondrial inner membrane potential, and lower total ATP levels [37], suggesting less efficient mitochondrial function. Lower energy production and reduced energy consumption is associated with faster physical function decline in the general population [10, 11]. In contrast, Suissa, *et al.* observed that individuals with haplogroup J had a 2-fold increase in mtDNA copy number compared to haplogroup H [38]. It is possible that such increase might be due to compensatory response to mitochondrial function damage. Further studies should investigate associations between mtDNA genetics and mitochondrial heteroplasmy to verify these findings.

Within the HIV+ population, haplogroups J and U5 have been observed to be more common among those who display accelerated progression to AIDS and death [20]. The study suggested that both haplogroups (J & U5) contained uncoupling SNPs and were associated with lower ATP production and ROS generation compared to other haplogroups (e.g. H3, H4, H5, and H6) that were more tightly coupled [20], suggesting mtDNA haplogroups could be linked to mitochondrial function variation and play a role in AIDS progression. Our findings suggest that even among men who have achieved HIV viral suppression, such variants as found among haplogroup J could be associated with physical function decline and predict worse functional outcome in PLWHIV.

Studies linking mtDNA variation and physical function decline in the general population have yielded inconsistent findings. One study examined mtDNA variation in frail and non-frail older adults, and suggested that the mt204 C allele, a site that could affect the efficiency of mtDNA replication [39], was associated with a 2-fold increased odds of frailty and decreased grip strength [18]. In contrast, another study found no associations between any tested mtDNA haplogroup and frailty in a very old adult population [40], but did find that haplogroup K (a subgroup of Uk) was associated with a lower hazard of death (HR: 0.52, 95% CI: 0.30-0.91). Although this study was conducted in a relatively large sample with validated measurements, frailty phenotypes were measured at a baseline age of 85+, which may exceed the age at which frailty manifests [41, 42]. None of these studies prospectively evaluated associations between mtDNA genome and the trajectory of physical function decline.

The current study has several limitations. First, no women were studied, and the haplogroups studied were European ancestry-specific. These factors limit the generalizability of our findings. Second, our sample size was relatively small, and the results could be biased by unmeasured confounders leading to false positive findings. We also could not study sub-haplogroups within J, which might have their own specific associations with the outcomes of interest. Further studies are therefore needed to validate our results. Third, very few study visits were captured among individuals with J haplogroup after the age of 67 (total of 17 observations from 3 unique individuals). Thus, our findings may not be representative of those older than 67. Fourth, we were only able to capture viral load since seroconversion among those who were not seroprevalent at study baseline (35.6%) in the viremia copy-year measurement. Thus, we likely underestimated the viremia copy-year for those infected with HIV before they participated in the study. Last, due to the demands of physically attending study visits, men who were lost to follow-up might be sicker, thus we could have understated rate of decline in gait speed with age among all haplogroups.

This study has several strengths. We were able to evaluate the rate and trajectory of gait speed based on validated repeated measurements controlling for HIV disease state and thymidine analogues exposure, and to quantify differential decline of gait speed in PLWHIV with four major European mtDNA haplogroups. Although focused on a HIV+ population, this study may also improve understanding of the impact of inherited mtDNA on functional aging in the general population. Lastly, the MACS cohort recruited

behaviorally and sociodemographically similar individuals, which minimizes confounding and makes it ideal for the study of genetics.

CONCLUSIONS

Individuals with mitochondrial haplogroup J had more rapid gait speed decline compared to persons with other common European mitochondrial haplogroups. These findings suggest a potential pathway through mtDNA to understand the pathophysiology of accelerated function decline among PLWHIV, and may be used to predict risk of function decline in this group if validated prospectively. Investigation into relationships between mitochondrial genetic variation and changes in physical function could bring insights toward development of interventions including personalized long-term disease management strategies that promote healthy aging among PLWHIV.

ACKNOWLEDGEMENTS

We would like to acknowledge support from the multicenter AIDS cohort data coordinating center.

We would also want to acknowledge support for statistical consultant from the National Center for Research Resources and the National Center for Advancing Translational Sciences (NCATS) of the National Institutes of Health through Grant Number 1UL1TR001079.

Funding Sources:

Data in this manuscript were collected by the Multicenter AIDS Cohort Study (MACS) with centers at Baltimore (U01-AI35042): The Johns Hopkins University Bloomberg School of Public Health: Joseph B. Margolick (PI), Jay Bream, Todd Brown, Adrian Dobs, Michelle Estrella, W. David Hardy, Lisette Johnson-Hill, Sean Leng, Anne Monroe, Cynthia Munro, Michael W.

Plankey, Wendy Post, Ned Sacktor, Jennifer Schrack, Chloe Thio; Chicago (U01-AI35039): Feinberg School of Medicine, Northwestern University, and Cook County Bureau of Health Services: Steven M. Wolinsky (PI), Sheila Badri, Dana Gabuzda, Frank J. Palella, Jr., Sudhir Penugonda, John P. Phair, Susheel Reddy, Matthew Stephens, Linda Teplin; Los Angeles (U01-AI35040): University of California, UCLA Schools of Public Health and Medicine: Roger Detels (PI), Otoniel Martínez-Maza (PI), Peter Anton, Robert Bolan, Elizabeth Breen, Anthony Butch, Shehnaz Hussain, Beth Jamieson, John Oishi, Harry Vinters, Dorothy Wiley, Mallory Witt, Otto Yang, Stephen Young, Zuo Feng Zhang; Pittsburgh (U01-AI35041): University of Pittsburgh, Graduate School of Public Health: Charles R. Rinaldo (PI), James T. Becker, Phalguni Gupta, Kenneth Ho, Lawrence A. Kingsley, Susan Koletar, Jeremy J. Martinson, John W. Mellors, Anthony J. Silvestre, Ronald D. Stall; Data Coordinating Center (UM1-AI35043): The Johns Hopkins University Bloomberg School of Public Health: Lisa P. Jacobson (PI), Gypsyamber D'Souza (PI), Alison Abraham, Keri Althoff, Michael Collaco, Priya Duggal, Sabina Haberlen, Eithne Keelaghan, Heather McKay, Alvaro Muñoz , Derek Ng, Anne Rostich, Eric C. Seaberg, Sol Su, Pamela Surkan, Nicholas Wada. Institute of Allergy and Infectious Diseases: Robin E. Huebner; National Cancer Institute: Geraldina Dominguez. The MACS is funded primarily by the National Institute of Allergy and Infectious Diseases (NIAID), with additional co-funding from the National Cancer Institute (NCI), the National Institute on Drug Abuse (NIDA), and the National Institute of Mental Health (NIMH). Targeted supplemental funding for specific projects was also provided by the National Heart, Lung, and Blood Institute (NHLBI), and the National Institute on Deafness and Communication Disorders (NIDCD). MACS data collection is also supported by UL1-TR001079 (JHU ICTR) from the National Center for Advancing Translational Sciences (NCATS) a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical Research. The contents of this publication are solely the responsibility of the authors and do not represent the official views of the National Institutes of Health (NIH), Johns Hopkins ICTR, or NCATS. The MACS website is located at http://aidscohortstudy.org/.JA Schrack was supported by K01AG048765 from NIA. TT Brown was supported in part by R01AI093520 and K24AI120834 from the NIAID. KME was supported in part by K23AG050260 and R01AG054366 from the NIA. This work was also supported by the JHU CFAR (1P30AI094189). Mitochondrial DNA haplogrouping in MACS was supported by R21DK101342 from the NIDDK to Hulgan and Brown.

The contents of this publication are solely the responsibility of the authors and do not represent the official views of the National Institutes of Health (NIH).

Potential Conflict of Interest

T.H. and T.B. report grants from NIH/NIDDK during the conduct of the study. K.E reports grants from NIH, Gilead, Merck, non-financial support from Theratechnologies, and personal fees from EMD Serono, though not directly related to the submitted work. F.P. reports grants and personal fee from Gllead Sciences, Janssen, Merck, and ViiV, though not directly related to the submitted work.

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TABLES

Table 1. Baseline characteristics by mitochondrial genetic haplotype

	Overall	H	J	T	Uk	Others	p-value*
Overall: N (%)	455	188 (41.3)	44 (9.7)	53 (11.7)	108 (23.7)	62 (13.6)	
Age: median (IQR), year	50 (50-54)	50 (50-53)	51 (50-54.5)	50 (50-54)	50.5 (50-54)	52 (50-54)	0.11
Have college degree: N (%)	267 (58.7)	100 (53.2)	26 (59.1)	34 (64.2)	69 (63.9)	38 (61.3)	0.36
Ever Smoking: N (%)	307 (70.7)	119 (68.0)	34 (79.1)	42 (79.3)	72 (68.6)	40 (69.0)	0.38
HCV infection: N (%)	75 (16.5)	29 (15.4)	6 (13.6)	16 (30.2)	16 (14.8)	8 (12.9)	0.11
CD4 cell count at baseline: median (IQR), cells/ µL	523 (354-727)	499 (358-689)	550 (323-795)	597 (371-819)	533 (371-743)	510 (325-689)	0.74
HIV virally suppressed (<200 copies/mL): N (%)	316 (69.5)	129 (68.6)	28 (63.6)	39 (73.6)	72 (66.7)	48 (77.4)	0.49
Peripheral Neuropathy: N (%)	170 (37.4)	70 (37.2)	16 (36.4)	19 (35.8)	44 (40.7)	16 (35.6)	0.96
Weight: median (IQR), kilogram (kg)	78.6 (70.8-86.2)	78.9 (72-85.5)	79.4 (69.1-91)	79.5 (70.8-86.6)	76.3 (69.5-85.3)	79.8 (71.7-85.3)	0.69
Height: median (IQR), meter (m)	1.78 (1.73-1.83)	1.78 (1.73-1.83)	1.80 (1.75-1.80)	1.78 (1.73-1.80)	1.78 (1.73-1.83)	1.80 (1.75-1.85)	0.17
Ever used thymidine analogues: N (%)	51 (11.5)	21 (11.7)	3 (7.0)	7 (13.2)	10 (9.4)	10 (17.0)	0.54
Gait speed: mean (SD), m/s	1.15 (0.19)	1.13 (0.17)	1.07 (0.21)	1.20 (0.21)	1.20 (0.23)	1.19 (0.19)	0.29

*Categorical variables tested by Fisher's Exact test; continuous variables tested by Kruskal-Wallis equality-of-populations rank test
N=number; SD=standard deviation; IQR=Interquartile range

Table 2. Unadjusted and adjusted Longitudinal Association between Age and Gait Speed (modeled as a continuous variable in meters per second), by mitochondrial DNA Haplogroups.

		Crude estimation			Adjusted estimation*		
		Slope (m/s/year)	SE	P	Slope (m/s/year).	SE	P
Halogroup J vs. others	J*age	-0.006	0.003	**0.026**	-0.006	0.003	**0.012**
	gait speed (Non-J)	-0.012	0.001	<0.001	-0.011	0.001	<0.001
	gait speed (group J)	-0.017	0.003	<0.001	-0.018	0.003	<0.001
Haplogroup H vs. others	H*age	0.001	0.002	0.48	0.002	0.002	0.20
	gait speed (Non-H)	-0.013	0.001	<0.001	-0.012	0.001	<0.001
	Gait speed (group H)	-0.011	0.001	<0.001	-0.010	0.001	<0.001
Haplogroup T vs. others	T*age	0.00001	0.002	0.996	-0.00007	0.002	0.98
	Gait speed (Non-T)	-0.012	0.001	<0.001	-0.012	0.001	<0.001
	Gait speed (group T)	-0.012	0.002	<0.001	-0.012	0.002	<0.001
Haplogroup Uk vs. others	Uk*age	-0.0005	0.002	0.78	0.0001	0.002	0.94
	Gait speed (Non-Uk)	-0.012	0.001	<0.001	-0.012	0.001	<0.001
	Gait speed (group Uk)	-0.013	0.001	<0.001	-0.012	0.002	<0.001

*Models adjusted for HCV infection, AIDS diagnosis, Log_{10} viremia copy year, college education, cigarette smoking ł, peripheral neuropathy, weight, height, and time-varying exposure to thymidine analogues (ever vs. never).

ł Cigarette smoking was controlled in models by time-varying smoking status (yes vs. no), time-varying smoking status (never vs. former vs. current), or pack-year smoke. All methods returned similar changes of the magnitude of association between haplogroup and gait speed. Time-varying smoking status (never vs. former vs. current) was selected and used in the final model.

Table 3. Probability of slow gait after age of 50 by independent variables.

	Crude estimation			Adjusted estimation		
	OR	95% CIs	P	OR	95% CIs	P
Haplogroup J	3.35	2.37-8.86	0.015	2.97	1.24-7.08	0.01
age	1.15	1.11-1.21	<0.001	1.14	1.10-1.19	<0.001
HCV infection				1.29	0.56-2.97	0.55
Ever diagnosed with AIDS (yes/no)				1.89	1.04-3.46	0.04
Log_{10} Viremia copy-year, per unit increase				1.25	1.03-1.52	0.02
College education (yes/no)				0.34	0.20-0.60	<0.001
Smoking Never				Ref.	-	-
Former				1.24	0.67-2.28	0.49
Current				1.30	0.64-2.63	0.46
Weight, meter (m)				1.0	0.99-1.01	0.75
Height, kilogram (kg)				0.04	0.0005-3.66	0.17
Exposure to thymidine analogues (ever vs. never)				0.44	0.17-1.15	0.10
Peripheral neuropathy (yes/no)				1.33	1.01-1.76	0.04

FIGURES

Figure 1. Adjusted Predictions of Gait Speed Decline by Haplogroup J with 95% CIs

*Statistics presented in Table 2 and supplemental table 1. Model adjusted for HCV infection, AIDS diagnosis, Log_{10} viremia copy year, college education, cigarette smoking, peripheral neuropathy, weight, height, and time-varying exposure to thymidine analogues (ever vs. never).

Figure 1.