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Higher buccal mitochondrial DNA and mitochondrial common deletion number are associated with markers of neurodegeneration and inflammation in cerebrospinal fluid

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Abstract

Human immunodeficiency virus (HIV) infection is potentially associated with premature aging, but demonstrating this is difficult due to a lack of reliable biomarkers. The mitochondrial (mt) DNA “common deletion” mutation (mtCDM) is a 4977-bp deletion associated with aging and neurodegenerative diseases. We examined how mtDNA and mtCDM correlate with markers of neurodegeneration and inflammation in people with and without HIV (PWH and PWOH). Data from 149 adults were combined from two projects involving PWH (n = 124) and PWOH (n = 25). We measured buccal mtDNA and mtCDM by digital droplet PCR and compared them to disease and demographic characteristics and soluble biomarkers in cerebrospinal fluid (CSF) and blood measured by immunoassay. Participants had a median age of 52 years, with 53%

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Conflict of interest The authors Dipesh Solanky, Jerel A. Fields, Jennifer E. Iudicello, Ronald J. Ellis, Donald Franklin, David B. Clifford, Benjamin B. Gelman, Christina M. Marra, Susan Morgello, Leah H. Rubin, Igor Grant, Robert K. Heaton, Scott L. Letendre, and Sanjay R. Mehta declare that they have no conflicts of interest.

white and 81% men. Median mtDNA level was 1,332 copies/cell (IQR 1,201–1,493) and median mtCDM level was 0.36 copies × 10^2/cell (IQR 0.31–0.42); both were higher in PWH. In the best model adjusting for HIV status and demographics, higher mtDNA levels were associated with higher CSF amyloid-β 1–42 and 8-hydroxy-2'-deoxyguanosine and higher mtCDM levels were associated with higher plasma soluble tumor necrosis factor receptor II. The differences in mtDNA markers between PWH and PWOH support potential premature aging in PWH. Our findings suggest mtDNA changes in oral tissues may reflect CNS processes, allowing the use of inexpensive and easily accessible buccal biospecimens as a screening tool for CSF inflammation and neurodegeneration. Confirmatory and mechanistic studies on mt genome alterations by HIV and ART may identify interventions to prevent or treat neurodegenerative complications.

Keywords

Mitochondrial DNA; Amyloid; Neuroinflammation; Neurodegeneration; HIV

Introduction

One of the most widely accepted theories for how organisms age is progressive and cumulative damage to the mitochondrial (mt) genomes in cells (Harman 1956). Damage to mtDNA is at least partially mediated by reactive oxygen species (ROS) generated within the mitochondria themselves as a byproduct of cellular respiration (Harman 1956, 1972). While these molecules can damage all components of the cell, including lipids, proteins and nucleic acids, this study addressed damage to the mitochondrial genomes. Damage to mitochondrial DNA manifests as mutations and deletions which accumulate within the mitochondrial network, and then begin to adversely affect the function of the cell. Some mutations can be passed on to daughter cells upon cellular division, resulting in their propagation and accumulation throughout an organism's lifetime (Fleming et al. 1982; Harman 1972; Miquel et al. 1980; Ludwig et al. 2019). ROS also induce the senescence-associated secretory phenotype in cells, triggering the expression of inflammatory mediators that activate the innate immune system, which, over time, leads to additional tissue damage (Blaser et al. 2016; Martinon 2010).

Mounting evidence supports the notion that people with human immunodeficiency virus (HIV)-1 (PWH) exhibit evidence of premature aging, including those on suppressive antiretroviral therapy (ART)(Horvath and Levine 2015; Mackiewicz et al. 2019; Sundermann et al. 2019; Guarda et al. 1984; Levine et al. 2016; Scott et al. 2011). Depending on the age at which ART is initiated, life expectancy is 10–30 years less in PWH than persons without HIV (PWOH) (Lohse et al. 2007; Antiretroviral-Therapy-Cohort-Collaboration 2008). Aging-related conditions including cardiovascular disease, diabetes mellitus, and osteoporosis are also more common and occur at younger ages in PWH than in the general population(Guaraldi et al. 2011; Deeks and Phillips 2009).

One important aging-related condition is dementia, which affects an estimated 44 million people worldwide (Lane et al. 2018). Alzheimer's disease (AD) is the commonest form, accounting for 50–75% of all acquired dementias (Lane et al. 2018). Neurocognitive

impairment (NCI) in PWH is also well-described, ranging from mild to severe, and can be associated with limitations in everyday functioning and earlier mortality (Clifford and Ances 2013; Eggers et al. 2017). Over 40% of PWH in the United States (U.S.) are over the age of 55 (Smith 2005). In addition to risk of NCI, older PWH are also at risk for AD and its precursor, amnestic mild cognitive impairment (aMCI) (Tan et al. 2013). Evidence of AD-like pathology has been observed in some older PWH (Achim et al. 2009; Alisky 2007; Clifford et al. 2009; Esiri et al. 1998), and one study found that aMCI was more common among PWH than in the general population (Sheppard et al. 2017; Bhatia et al. 2012; Levine et al. 2016). Viral infection has long been suspected as a possible etiology of some cases of sporadic AD (Fulop et al. 2018; Gosztyla et al. 2018). Amyloid-β is a potent antimicrobial agent, spurring the hypothesis that amyloid-β may result from neurological infection rather than be the cause of AD (Gosztyla et al. 2018). While a single pathogen is unlikely to be responsible for AD, the combination of genetic predisposition, environmental factors, and repeated viral exposures could initiate inflammatory cascades upstream of AD pathogenesis, at least in some cases. The evidence for possible premature aging in PWH highlights a need to identify biomarkers that may indicate progression to aging-related diseases such as AD.

The mitochondrial DNA “common deletion” (mtCDM) is a 4977-base pair deletion that has been found at increasing frequency with older age in human tissue extracted from multiple sites, including the brain, heart, liver, kidney and skeletal muscle (Cortopassi and Arnheim 1990; Linnane et al. 1990; Yen et al. 1991; Torii et al. 1992; Zhang et al. 1992), and likely affects mitochondrial function. Similarly, reduction in the number of mtDNA copies per cell is associated with impaired cellular and mitochondrial function (Jeng et al. 2008) as well as frailty(Ashar et al. 2015), cardiovascular disease (Fazzini et al. 2019; Koller et al. 2020), susceptibility to infection (Fazzini et al. 2019), and mortality (Ashar et al. 2015; Mengel-From et al. 2014; Koller et al. 2020). The relationship between neurodegenerative disease (including AD and Parkinson disease) and mitochondrial dysfunction is also well-described (Hou et al. 2019), with evidence of deviations in mtDNA copy number in peripheral blood correlating with neurocognitive impairment in PWH (Hulgan et al. 2019). Given the association of AD, mitochondrial DNA damage, and potentially also HIV with aging, we investigated how mtDNA and mtCDM correlated with biomarkers of inflammation and degeneration in cerebrospinal fluid (CSF) from both PWH and PWOH.

Methods

Participants

Data from 149 adults were combined from two cross-sectional research projects. Data from 78 PWH participants were from the CNS HIV Antiretroviral Effects Research (CHARTER) project, which examined PWH between May 2016 and April 2018 at university-based centers in six US cities (Baltimore, Galveston, New York, St. Louis, San Diego, and Seattle). Eligibility criteria for CHARTER were purposely limited and included only HIV infection and willingness to undergo the study assessments. For the present analysis, participants were excluded if they had a current substance use disorder, untreated hepatitis C infection, or a major neurologic diagnosis unrelated to HIV such as Parkinson disease. Additional details regarding the project have been previously published (Ellis et al. 2020). Data from another

71 participants (46 PWH, 25 PWOH) were from the Translational Methamphetamine AIDS Research Center (TMARC) project, which was a single-center cohort focused on understanding the combined effects of HIV and methamphetamine dependence on brain structure and function. Participants in TMARC included those with or without HIV infection, as well as those with or without methamphetamine use disorder as assessed by the Composite International Diagnostic Interview Version 2.1 (CIDI) (WHO 1997) and based on Diagnostic and Statistical Manual (DSM)-IV criteria. Exclusion criteria for TMARC included current intoxication or withdrawal from any addictive drug besides cannabis, and neurologic or psychiatric conditions known to affect cognitive function other than dementia (e.g., head injury with prolonged loss of consciousness, seizure, stroke, schizophrenia). Participants with current substance use disorders, including methamphetamine use, were also excluded from the present analysis.

Standard protocol approvals, registrations and patient consents

Both projects were approved by local institutional review boards and all participants provided informed consent for the study procedures.

Mitochondrial DNA variables and biomarkers

mtDNA and mtCDM were quantified in copies per cell by DNA extraction from buccal swabs and digital droplet PCR. Specifically, we quantified 1) mtDNA by measuring the copy numbers of the mitochondrial *NADH dehydrogenase 2* (MT-ND2) gene, 2) the cellular control gene *ribonuclease P protein subunit p30* (RPP30) which is present in two copies per cell, and 3) the relative proportion of mtDNA carrying the “common deletion” by designing a primer–probe combination that targets the bridge region on the mitochondrial chromosome before and after the 4,977 bp “common deletion”, as described in our previous work (Var et al. 2016).

Soluble biomarkers in cerebrospinal fluid (CSF) and blood plasma were measured by bead suspension array (Millipore, Billerica, MA) or traditional immunoassay. The biomarkers analyzed were as follows: amyloid β 1–42 (CSF), neurofilament light chain (CSF), 8OH2’-deoxyguanosine [8-OHdG] (CSF and plasma), C-reactive protein [CRP] (plasma), interleukin-6 [IL-6] (CSF and plasma), monocyte chemoattractant protein [MCP] (CSF and plasma), soluble tumor necrosis factor receptor II [sTNFR-II] (CSF and plasma) and soluble cluster of differentiation [sCD-14] (CSF and plasma). This list represents a subset of standard biomarkers of inflammation and neurodegeneration (Simrén et al. 2020; Forloni and Balducci 2018) that were measured in participants across both projects. Biomarkers such as sCD14 and sTNFRII were analyzed for their role in microglial signaling and response to neuronal injury (Probert 2015; Janova et al. 2016). Plasma and CSF 8-OHdG and plasma CRP were analyzed given their associations with dementia, AD and neurocognitive impairment (Beydoun et al. 2018; Ng et al. 2018; Koyama et al. 2013; Engelhart et al. 2004; Mecocci et al. 2002; Gackowski et al. 2008; Kallianpur et al. 2016). Amyloid β 1–42 and neurofilament light chain were analyzed in CSF due to previous findings by our group and others demonstrating association between CSF oxidative damage and these two biomarkers in the CSF, but not in plasma(Ellis et al. 2020; Mehta et al. 2000).

Additional clinical and laboratory assessments

In CHARTER participants, HIV RNA were quantified in CSF and plasma by real-time PCR with a lower limit of 50 copies/mL (Abbott Diagnostics, Des Plaines, IL, USA). HIV serostatus in TMARC participants was confirmed by the MedMira Rapid HIV Antibody Test (MedMira, Halifax, Canada).

Statistics

Demographics, mtDNA, and medical and HIV disease characteristics were summarized using means and standard deviations (SDs), medians and interquartile ranges (IQRs), or counts and percent. Biomarker concentrations were transformed (e.g., base 10 logarithm) to improve distribution symmetry. Correlations among biomarkers were assessed by Pearson *r*. The effect size of the difference between means was quantified using Cohen's d ("d"). A series of multivariable linear regression were conducted to examine associations between mtDNA and mtCDM and soluble plasma and CSF biomarkers after adjusting for the following covariates which were selected using Akaike Information Criterion (AIC): age, sex, ethnicity, education and then, among PWH only, additionally nadir CD4 + T-cell count, estimated duration of HIV infection, and ART exposure, and current CD4 + T-cell count. All associations were corrected using the false discovery rate (FDR) method to account for type 1 error. All statistical analyses were carried out using JMP, Version 14.2.0 (SAS Institute Inc., Cary, NC, USA). Figures were constructed using GraphPad Prism, Version 8.4.3 (La Jolla, CA, USA).

Results

Study participant demographic and HIV characteristics

A total of 149 participants were included (124 PWH, 25 PWOH, Table 1). Among PWH, 96% took antiretroviral therapy (ART, median duration 13.6 years); plasma HIV RNA was ≤ 200 cp/mL in 91%; and median CD4 + T-cell count was 593/μL.

Mitochondrial DNA and "Common Deletion" copies per cell vs. soluble biomarkers

Median mtDNA level was 1,332 copies/cell (IQR 1,201–1,493) and was higher in PWH (d = 1.45, $p < 0.001$) (Fig. 1). In the best model testing of all soluble biomarkers and accounting for demographics (i.e., age, sex, ethnicity, and education) as well as HIV status, higher mtDNA levels were associated with higher CSF Aβ 1–42 levels ($p < 0.001$) as well as HIV infection, fewer years of education, and non-Hispanic ethnicity (model $R^2 = 0.43$, p<0.0001, Table 2). Higher mtDNA levels also trended with higher CSF 8-OHdG (p=0.035, p=0.06 after FDR correction, Table 2). Median mtCDM was 0.36 copies × 10^2/cell (IQR 0.31–0.42) and was also higher in PWH (d=0.75, p<0.001) compared to PWOH. In the best model, higher mtCDM was associated with higher plasma sTNFR-II levels (p=0.036), with HIV infection, older age, and non-Hispanic ethnicity as significant covariates (model R^2=0.28, p<0.001, Table 3). Unless otherwise noted, significant associations held after FDR correction. The AIC selection method retained CSF IL-6 in the best model even though the p value was 0.11.

In the best model analyzing only the PWH subgroup and adjusting for age, absolute and nadir CD4 + T-cell count, duration of HIV infection, duration of ART use, and lifetime dideoxynucleoside analogue exposure, higher mtDNA was again significantly associated with higher CSF Aβ 1–42 ($p = 0.005$) and longer duration of HIV infection, although the latter weakened below statistical significance after FDR correction. Higher mtCDM trended with higher CSF sTNFR-II, but this relationship was also not significant following FDR correction.

Discussion

In this cross-sectional study, we investigated the relationship between mtDNA and mtCDM copy number in buccal swabs with plasma and CSF biomarkers of CNS inflammation and neurodegeneration in PWH and PWOH. We hypothesized that lower levels of mtDNA and higher levels of mtCDM would be associated with higher levels of biomarkers reflective of CNS inflammation and neurodegeneration. Combining data from two well-characterized cohorts, we showed that PWH had higher levels of both mtDNA and mtCDM copies/cell than PWOH and that higher mtDNA were associated with higher Aβ 1–42 levels in the CSF in the total study population. Higher mtCDM levels were also associated with greater levels of plasma sTNFR-II, and trended with other biomarkers of CNS inflammation (CSF IL-6) in the total study population and CSF sTNFR-II among PWH.

One of the pathophysiologic hallmarks of AD is the intracerebral accumulation of amyloid-β plaques which are associated with neuronal and synaptic loss, thereby leading to cerebral atrophy and manifesting as progressive memory impairment (Serrano-Pozo et al. 2011). Accumulation in the brain translates to reduced levels of amyloid-β proteins in CSF—a reflection of decreased clearance from the brain (Tarasoff-Conway et al. 2015). While the exact pathogenesis of AD is unknown, ROS likely play an important role. Oxygen free radicals and peroxides are generated out of proportion to antioxidant defenses in AD, leading to neuronal damage (Harman 1993; Volicer and Crino 1990; Benzi and Moretti 1995). Multiple studies of AD brains have demonstrated preferential accumulation of radical adduct 8-hydroxy-2′-deoxyguanosine (8-OHdG) and oxidized bases in mtDNA over nuclear DNA (Mecocci et al. 1994; Wang et al. 2005; Cheignon et al. 2018), and significantly higher levels overall in AD brains over control brains. The presence of intracellular amyloid-β and its precursor protein can produce mitochondrial structural abnormalities, impairments in mitochondrial metabolism, and increased ROS production (Askanas et al. 1996; Caspersen et al. 2005; Apelt et al. 2004).

Consistent with our hypothesis, higher mtDNA copy number significantly correlated with higher amyloid-β in CSF, which has been associated with reduced Aβ accumulation in the brain in several studies (Andreasen et al. 1999a, b; Hulstaert et al. 1999). This finding of an inverse relationship between brain and CSF Aβ levels has led to the theory that increased clearance of Aβ from the brain leads to higher levels of Aβ in the CSF.

Paradoxically, higher—not lower—levels of mtDNA in buccal swabs were associated with HIV infection in our study. This finding is supported by results from previous cross-sectional studies examining 1) mtDNA from brain tissue from PWH and PWOH (Var et al.

2016) and 2) cell-free mtDNA from CSF in PWH with or without AIDS (Pérez-Santiago et al. 2017). In both cases, higher mtDNA trended with HIV, HIV copy number (Var et al. 2016) and AIDS diagnosis(Pérez-Santiago et al. 2017). In contrast, a study by our group found that mtDNA was lower in brain tissue from the frontal cortex in PWH who had neurocognitive deficits as compared with PWH who did not have neurocognitive deficits (Swinton et al. 2019). Overall, this suggests that the progressive burden of HIV infection and its treatment relates to higher levels of mtDNA, which, in our case, was observed in buccal swabs. The mechanism for this relationship is not entirely clear but may reflect a compensatory response to impaired mitochondrial function due to HIV infection, similarly described in individuals with mitochondrial diseases (Chinnery and Samuels 1999; Durham et al. 2007; Sitarz et al. 2012). The relatively younger median age (52 years) of our cohort may have also influenced our findings, as significantly faster declines in mtDNA have been reported in PWH above age 50 (Sun et al. 2019).

Elevated sTNFR-II is associated with HIV infection and is thought to reflect the degree of activation of the TNFα cytokine system in response to the virus (Aukrust et al. 1994), which predicts rapid disease progression and death (Stein et al. 1997; Godfried et al. 1994). Numerous cytokines, including TNFα and IL-6, have been clearly linked to Aβ-associated neurocognitive disorders such as Alzheimer's disease (Rubio-Perez and Morillas-Ruiz 2012). Multiple studies have also associated TNFα with generation of reactive oxygen species and associated mtDNA damage (Suematsu et al. 2003; Nagakawa et al. 2005; Kim et al. 2010). However, the relationship between the TNF pathway and the mitochondrial "common deletion" mutation in the setting of HIV infection is relatively novel. Here, we found that higher mtCDM copies per cell were associated with higher plasma levels of sTNFR-II, which remained significant after adjusting for HIV status. MtCDM also trended with plasma IL-6 levels. Among PWH, sTNFR-II in the CSF trended with higher mtCDM independent of CD4 + T-cell count and other HIV characteristics. Similarly, in previous work we observed a significant, AIDS-independent, positive association between increased CSF mtDNA content and plasma TNFα levels (Pérez-Santiago et al. 2017) in PWH; however, soluble TNF *receptor* levels were not examined in that study. In other work on the white and gray matter from brains from the National NeuroAIDS Tissue Consortium, we found that higher relative proportions of mtCDM and lower mtDNA in brain cells was associated with worse neurocognitive performance (Var et al. 2016) and NCI in PWH (Swinton et al. 2019). Our findings here complement these prior investigations and further characterize the systemic inflammatory profiles associated with mtDNA damage in the setting of HIV.

This study also carries implications for the utility of buccal swabs as a non-invasive sampling method for mtDNA. While mitochondrial DNA in the buccal mucosa tends to exhibit less heteroplasmy than other tissues (e.g., muscle, liver, or brain) due to its greater turnover (Naue et al. 2015), it appears to still reflect other tissues in the body. Evidence already shows that mtDNA derived from buccal swabs can be successfully used in place of peripheral blood to identify mutations associated with hearing loss (Fan et al. 2017; Wang et al. 2018). The relationships observed between soluble biomarkers of CNS inflammation and mtDNA and mtCDM obtained from buccal swabs in this study mirror findings seen in previous studies examining mtDNA in the brain (Volmering et al. 2016) and CSF (Pérez-

Santiago et al. 2017). Further, reductions in mtDNA copy number measured in peripheral blood has been linked to a variety of neuropsychiatric conditions in PWOH, including worse neurocognitive function (Lee et al. 2010), Parkinson disease (Pyle et al. 2016) and depression (Kim et al. 2011). This is consistent with our finding of *higher* mtDNA content associated with a biomarker profile reflective of *decreased* neurodegeneration (i.e., *more* Aβ in the CSF), as well as higher mtCDM content associated with more CNS inflammation (greater plasma sTNFR-II). Overall, the congruence of our findings with previous work on mtDNA sampled from CSF, and brain tissue indicate that buccal swabs can potentially serve as a non-invasive yet effective sampling source for evaluating relationships between mtDNA and CNS inflammation. Future studies directly comparing associations of soluble CNS biomarkers with mtDNA obtained from the brain or CSF, versus those from buccal tissue, will be necessary to validate this finding.

Interestingly, higher—not lower—mtDNA levels were associated with higher CSF 8-OHdG, and there was no significant association between plasma 8-OHdG and mtDNA or mtCDM after controlling for demographic and HIV-specific variables (data not shown). These findings may be due to a compensatory increase in mtDNA genome production to replace damaged mtDNA as reflected by increased mtDNA in PWH in these cohorts. This proposed compensatory mechanism is tangentially supported by our finding that plasma 8-OHdG was inversely associated with CSF Aβ 1–42 ($p = 0.01$), which is consistent with previous analyses from the CHARTER cohort (Ellis et al. 2020). This previous study also noted the association of CSF biomarkers of neurodegeneration with oxidative markers of DNA—but not protein—damage, suggesting mitochondrial dysfunction as a preceding factor (Ellis et al. 2020). Our findings support the possibility that increased mtDNA is a response to neurodegeneration mediated by oxidative stress downstream of mitochondrial dysfunction.

We also found that participants identifying as Hispanic had lower mean mtDNA compared to black and white ethnicities. This was true in individuals both with and without HIV infection on stratified analysis. Mitochondrial DNA haplogroups corresponding to individuals of genetically determined Hispanic ancestry among PWH have been associated with worse neurocognitive outcomes compared to those of European or African ancestry (Hulgan et al. 2015). The results of our study suggest that this association seen in prior work may be in part mediated by mtDNA copy number, but with only 34 individuals in our cohort identifying as Hispanic, our study was not powered to answer this question.

We recognize several limitations to our study. First, our sample size was relatively small, particularly with regard to the number of people without HIV infection. While the effect sizes were large, this still limits our ability to draw robust conclusions regarding the effects of HIV infection on mtDNA and mtCDM. Also, the cross-sectional, observational nature of our study makes it inherently more prone to bias than longitudinal studies. We lacked simultaneous measure of mtDNA and mtCDM in the plasma, brain and CSF of participants to evaluate for congruence with findings seen with mtDNA and mtCDM from buccal swabs. Lastly, the described relationships lack mechanistic data and are unable to support causal inferences. Future longitudinal studies combined with in vitro and animal experiments will better address these limitations.

In conclusion, this is the first investigation in PWH of the relationships between 1) easily accessible buccal mtDNA levels and CSF Aβ 1–42 protein as well as 2) the mtDNA "common deletion" mutation and biomarkers of neuroinflammation and neurodegeneration. Our data support the conclusions that a) HIV is associated with higher buccal mtDNA, which in turn correlates with higher Aβ 1–42 in the CSF and increased clearance from the brain, and b) accumulation of the "common deletion" is associated with modulation of the TNFα cytokine system. Our findings also support the use of buccal swabs as a non-invasive, informative biospecimen for assessing mtDNA. Additional confirmatory and mechanistic studies on the mitochondrial genome alterations by HIV and ART that build on this work may identify interventions to prevent or treat the neurodegenerative complications of HIV infection.

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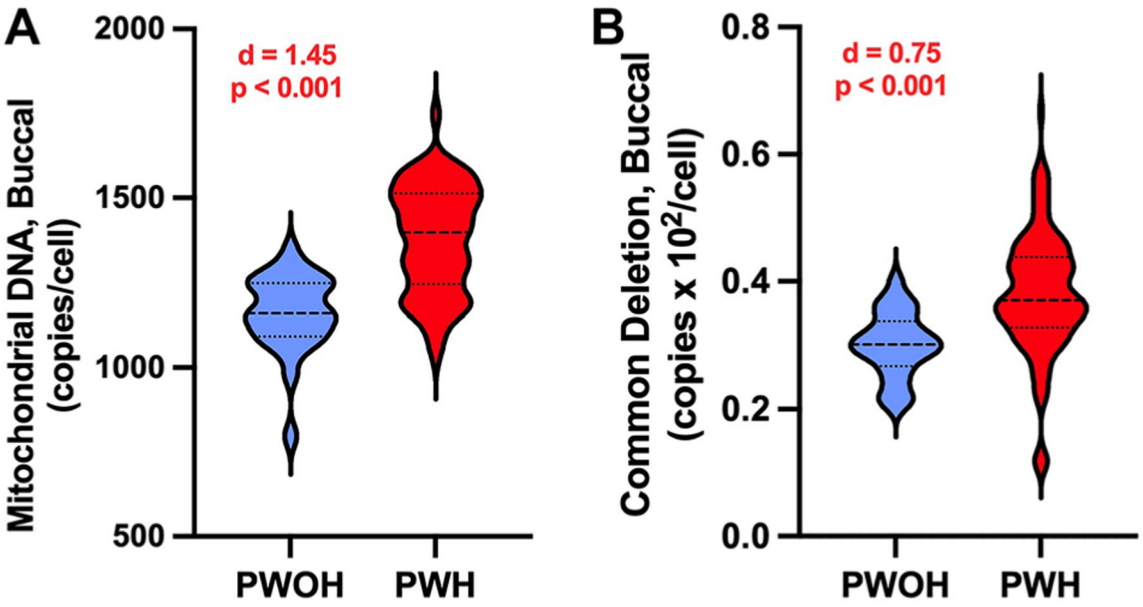

Fig. 1.

PWH had higher buccal mitochondrial DNA copies per cell. Violin plots comparing the distribution of mitochondrial DNA copies per cell (panel A) and common deletion copies per cell (panel B) in participants with and without HIV infection. Data were transformed by Box-Cox transformation and groups were compared using a Student's t-test and the effect size of the difference between means is indicated by Cohen's d ("d"). Abbreviations: HIV = human immunodeficiency virus; DNA = deoxyribonucleic acid; mtDNA = mitochondrial DNA

Table 1

Demographic and HIV disease characteristics of study participants. Distributions between PWOH and PWH were analyzed using a Student's t-test for continuous variables and a chi-square test for categorical variables.

Characteristic	All	PWOH	PWH	*P*-value
Sample Size	149	25	124	-
Age, years[1]	52 (44–60)	53 (35–61)	52 (46–61)	0.066
Female sex[2]	28 (19%)	11 (44%)	17 (14%)	0.001
Non-Hispanic White race/ethnicity[2]	79 (53%)	12 (48%)	67 (54%)	0.786
Antiretroviral Use[2]	-	-	120 (96.8%)	-
HIV RNA, Plasma ≤ 200 copies/mL[2]	-	-	111 (89.5%)	-
Current CD4 + T-cells, /μL[1]	-	-	593 (359–909)	-
CD4 + /CD8 + ratio[1]	-	-	0.8 (0.4–1.2)	-
CD4 + nadir[1]	-	-	181 (36–280)	-
Duration of ART[1]	-	-	13.6 (7.9–18.3)	-
mtDNA, copies/cell[1*]	1,332 (1,201–1,493)	1,160 (1,092–1,248)	1,397 (1,245–1,513)	< 0.001
mtCDM, copies × 10^2/cell[1*]	0.36 (0.31–0.42)	0.30 (0.27–0.34)	0.37 (0.33–0.44)	< 0.001
8-OHdG, CSF, $\log_{10}$ ng/mL[1]	1.07 (0.88–1.23)	0.85 (0.76–0.97)	1.10 (0.94–1.26)	< 0.001
Amyloid β 1–42, CSF, $\log_{10}$ pg/mL[1]	2.86 (2.64–3.05)	2.78 (2.60–2.93)	2.88 (2.66–3.12)	0.013
IL-6, CSF, $\log_{10}$ pg/mL[1]	+ 0.006 (−0.11– + 0.15)	−0.046 (−0.13– + 0.07)	+ 0.016 (−0.10– + 0.18)	0.029
sTNFR-II, CSF, $\log_{10}$ pg/mL[1]	2.60 (2.47–2.71)	2.49 (2.42–2.59)	2.64 (2.48–2.73)	0.0036
sTNFR-II, Plasma, $\log_{10}$ pg/mL[1]	3.69 (3.60–3.86)	3.61 (3.51–3.72)	3.70 (3.61–3.87)	0.018

Abbreviations: PWOH = people without HIV infection; PWH = people with HIV infection; y = years; IQR = interquartile range; N = number; μL = microliter; nadir = lowest historical level of blood CD4 + T cells; ART = anti-retroviral therapy.

Values for either [1]median (IQR) or [2]n (%).

[*]mtDNA and mtCDM values are Box-Cox transformed

Table 2

Regression Table Summarizing Analyses of mtDNA. Multivariable regression adjusted for age, sex, ethnicity, education level, HIV status.

	Univariable		Multivariable			
	β	***P* value**	**β**	***P* value**	**FDR *P* value**	**Risk Direction**
CSF Aβ 1–42	171.38	0.0002	167.03	< 0.0001	0.0003	Higher
CSF 8-OHdG *	164.85	0.0014	98.85	0.035	0.056	Higher
CSF sTNFR-II	159.93	0.029	87.10	0.173	0.230	Higher
HIV Infection	96.42	< 0.0001	73.57	< 0.0001	0.0002	Present
Ethnicity	65.78	0.014	51.08	0.0062	0.016	Non-Hispanic
Education	−16.99	0.0017	−11.95	0.015	0.030	Lower
Age	2.17	0.065	0.177	0.875	0.875	Older
Sex	92.66	0.0089	21.18	0.499	0.571	Male
			Model $R^2 = 0.43$	Model $P < 0.0001$		

P-values adjusted using the false discovery rate (FDR) method.

* Measured in 140 of 149 participants.

Abbreviations: CSF = cerebrospinal fluid, Aβ = amyloid beta, 8-OHdG = 8-hydroxy-2'-deoxyguanosine, sTNFR = soluble tumor necrosis factor receptor. HIV = human immunodeficiency virus

Table 3

Regression Table Summarizing Analyses of mtCDM. Multivariable regression adjusted for age, sex, ethnicity, education level, HIV status.

	Univariable		Multivariable			
	β	***P* value**	**β**	***P* value**	**FDR *P* value**	**Risk Direction**
Plasma sTNFR-II	0.0012	0.0018	0.00097	0.0090	0.036	Higher
CSF IL-6	0.0006	0.036	0.00056	0.069	0.111	Higher
CSF 8-OHdG *	0.0004	0.127	0.000008	0.979	0.978	Higher
HIV Infection	0.0003	0.0023	0.00024	0.019	0.039	Present
Ethnicity	0.0002	0.045	0.0002	0.0071	0.036	Non-Hispanic
Education	0.00005	0.108	−0.00002	0.402	0.460	Lower
Age	0.00002	0.0008	0.00002	0.019	0.039	Older
Sex	0.0004	0.052	0.0002	0.389	0.460	Male
			Model $R^2 = 0.28$	Model $P < 0.0001$		

P-values adjusted using the false discovery rate (FDR) method.

* Measured in 140 of 149 participants.

Abbreviations: CSF = cerebrospinal fluid, sTNFR = soluble tumor necrosis factor receptor, IL = interleukin, 8-OHdG = 8-hydroxy-2’-deoxyguanosine, HIV = human immunodeficiency virus