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Mitochondrial Injury and Cognitive Function in HIV Infection and Methamphetamine Use

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Abstract

Objective—In this work we evaluated the association of human immunodeficiency virus (HIV) infection and methamphetamine (METH) use with mitochondrial injury in the brain and its implication on neurocognitive impairment.

Design—Mitochondria carry their genome (mtDNA) and play a critical role in cellular processes in the central nervous system. METH is commonly used in HIV-infected populations. HIV infection and METH use can cause damage to mtDNA and lead to neurocognitive morbidity. We evaluated HIV infection and METH use with mitochondrial injury in the brain.

Methods—We obtained white and gray matter from Brodmann's areas (BA) BA7, BA8, BA9, and BA46 of a) HIV-infected individuals with history of past METH use (HIV+METH+, n=16), b) HIV-infected individuals with no history of past METH use (HIV+METH−, n=11), and c) HIV-negative controls (HIV−METH−, n=30). We used the "common deletion," a 4,977 bp mutation, as a measurement of mitochondrial injury, and quantified levels of mtDNA and "common deletion" by droplet digital PCR, and evaluated in relation to neurocognitive functioning (Global Deficit Score [GDS]).

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Meetings

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Author Contributions

SRV performed DNA extraction, ddPCR quantification experiments, and measurement of soluble and inflammatory markers, performed the data analyses, performed the statistical analyses, and wrote the primary version of the manuscript. TRCD participated in measurement of soluble and inflammatory markers. AV participated in ddPCR quantification experiments, and figure design. DMS participated in data analysis and revision of the manuscript. VS obtained samples, performed brain dissections, and data interpretation. DJM participated in neuropsychological testing, data interpretation, and revision of the manuscript. JPS participated in DNA extraction and statistical analyses. CLA, SRM, and JPS designed the present study, participated in data analysis, and revision of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

SRV, TRCD, AV, VS, DJM, CLA, SRM, and JPS do not have any commercial or other associations that might pose a conflict of interest. DMS has received grant support from ViiV and worked as a consultant for Hologic.

Results—Levels of mtDNA and mitochondrial injury were highest in white matter of BA46. A higher relative proportion of mtDNA carrying the “common deletion” was associated with lower GDS ($p<0.01$) in HIV+METH+ but higher GDS ($p<0.01$) in HIV+METH−.

Conclusions—Increased mitochondrial injury was associated with worse neurocognitive function in HIV+METH− individuals. Among HIV+METH+ individuals, an opposite effect was seen.

Keywords

HIV; mitochondrial DNA; mitochondrial injury; common deletion; neurocognitive impairment; methamphetamine; GDS; droplet digital PCR

Introduction

Mitochondria are the main organelles responsible for synthesizing energy in the form of adenosine triphosphate (ATP) for metabolic and cellular processes throughout the body and in the central nervous system (CNS) [1]. In eukaryotic cellular respiration, post-glycolytic reactions occur in mitochondria, making mitochondria at risk to damage by reactive oxygen species (ROS). Oxidative damage caused by ROS can lead to mitochondrial DNA (mtDNA) mutations and deletions. Previous studies have explored the relationships between mtDNA deletions, neurodegenerative diseases, and mental illnesses [2–4]. However, little is known about mitochondrial and neurocognitive dysfunction in the setting of human immunodeficiency virus (HIV) type 1 infection.

The most frequently identified mtDNA deletion—accounting for about 40% of all mtDNA deletions—is a 4,977 bp deletion that affects RNA transfer and respiratory chain genes known as the “common deletion” [5]. The “common deletion” results in defective mitochondrial function and is often found in higher amounts in the brain tissue of individuals with neurodegenerative diseases, substance abuse, and older age [6–8]. Although each mitochondrion carries multiple copies of its genome in response to the risk incurred by ROS, somatic changes in mtDNA can proliferate within the mtDNA population over time. Accumulation of mtDNA carrying deletions within a cell can lead to reduced energy synthesis and overall mitochondrial network dysfunction.

Neurocognitive dysfunction is a common complication of HIV disease. HIV may enter the CNS and establish a latent infection. In some individuals, productive infection occurs in the CNS leading to inflammation and eventually to HIV-related brain injury, affecting pathways involved in learning, memory, attention/working memory, and executive functioning [9]. Previous studies have also demonstrated that HIV-1-infected macrophages produce factors that suppress axonal growth and induce mitochondrial membrane depolarization. This might lead to reduced ATP production, causing additional damage to neurons [10].

Methamphetamine (METH) use is associated with neurocognitive disorders; the neuropathogenesis of HIV-1 infection may also be exacerbated by METH use [11]. Some of the effects of METH use are apoptosis, oxidative stress, and neuroinflammation [12, 13]. METH is a prominent drug of choice among HIV-infected individuals engaging in high-risk

behaviors [14–17]. METH use during HIV infection is associated with increased viral loads in cerebral spinal fluid (CSF), plasma, or both; enhanced HIV-1 pathogenesis [18]; and greater mtDNA damage [19]. In addition, METH has been shown to increase HIV-1 replication in dendritic cells [20] and monocyte-derived macrophages [21].

In this study, we compared the levels of mtDNA and the relative proportion of the "common deletion" in brain tissue of HIV-infected individuals with or without reported history of past METH use. We also investigated the magnitude of mitochondrial genetic defects in relation to neurocognition. Given our previous work demonstrating the correlation between cell-free CSF mtDNA levels and neurocognitive performance [22], we also investigated cell-free CSF mtDNA in these groups. Elucidation of the relationship between METH use and HIV infection on mtDNA may help improve our understanding of the role of mitochondrial injury in HIV-associated neurocognitive disorders.

Materials and Methods

Study cohorts

Brain tissue samples containing white and gray matter from Brodmann's areas (BA) BA7, BA8, BA9, and BA46 were obtained from the National NeuroAIDS Tissue Consortium (NNTC). The frontal lobe regions (BA8, BA9, BA46) were requested based upon previous work demonstrating the role of oxidative stress in neurodegeneration, methamphetamine effects on the frontal lobe [23–25]. All samples were from unique subjects, except for some paired BA7 and BA8 tissue samples which came from the same participants. Samples were obtained from subjects classified into three groups: a) HIV-infected individuals with reported history of past METH use (HIV+METH+, n=16), b) HIV-infected individuals with no reported history of past METH use (HIV+METH−, n=11), and c) HIV-negative controls (HIV−METH−, n=30). We also obtained CSF samples when available from these subjects. Participants were classified into the same three groups as above: a) HIV+METH+ (n=15), b) HIV+METH− (n=26), and c) HIV−METH− (n=17). Participants with known Alzheimer's disease (HIV+METH− (n=1), HIV+METH+ (n=3), HIV−METH− (n=4)) were excluded from analysis, since Alzheimer's disease is associated with higher levels of mitochondrial DNA deletions [26, 27]. Clinical assessments, socio-demographical variables and neuropsychological performance evaluations were obtained from assessments performed during the pre-mortem period.

Neuropsychological assessments

Participants underwent neuropsychological testing using neuropsychological test battery of seven ability areas summarized by the Global Deficit Score (GDS), a continuous score ranging from 0 (no impairment) to 5 (severest impairment) [28, 29]. An individual with a GDS score of greater than 0.5 was deemed to have neurocognitive impairment [22]. In this study, we analyzed GDS as a continuous variable to measure neuropsychological performance.

Brain removal and storage

All brain specimens were obtained with a postmortem interval of under 24 hours (median = 13.4 hours). The entire brain was removed, weighed, bisected longitudinally, snap frozen, and then sectioned according to NNTC brain processing protocols. The frozen tissue samples were transported on dry ice and stored at −80°C until the samples were processed. Frozen CSF samples were treated in a similar manner.

DNA extraction

Separate DNA extractions were performed from 25 mg of white matter and gray matter from each sample using the QIAamp DNA Mini Kit (Qiagen, Venlo, Holland) from BA7, BA8, BA9, and BA46. The manufacturer's protocol was followed except that the DNA was eluted twice using 200 μl elution buffer each time to maximize DNA recovery. The first elution included a 5-minute incubation before the final spin. Quality of the extraction, both quantification and purity of the nucleic acids, was assessed using the Nanodrop 1000 Spectrophotometer (Thermo Scientific, Waltham, MA) software version 3.7.1 per manufacturer's protocol.

Quantification of mtDNA and mitochondrial injury

DNA quantification was performed using the highly sensitive droplet digital PCR platform [30]. In lieu of a digestion step, we used QIAshredder (Qiagen) cell-lysate homogenizer on 10 ng/μL of extracted DNA in a total volume of 25 μL per manufacturer's protocol. We quantified levels of mtDNA per cell by measuring the copy numbers of the mitochondrial *NADH dehydrogenase 2* (MT-ND2) (Applied Biosystems, Waltham, MA) using a standard Applied Biosystems assay (Cat. # 4331182), and the cellular control gene *ribonuclease P protein subunit p30* (RPP30) (Integrated DNA Technologies, Coralville, IA) which is present in two copies per cell as described in our previous study [22]. The names and sequences of these primer-probe sets are as follows: RPP30-F (5′-GATTTGGACCTGCGAGCG-3′), RPP30-R (5′-GCGGCTGTCTCCACAAGT-3′), and RPP30-P (5′-HEX/CTGACCTGA/ZEN/AGGCTCT/IBFQ-3′). Additionally, we quantified levels of HIV in the brain tissue samples by measuring the copy numbers of conserved regions in HIV-1, such as the long terminal repeat (LTR) and the gag gene (Integrated DNA Technologies, Coralville, IA), to look for any associations between HIV DNA levels in the brain and mitochondrial injury or mtDNA. The names and sequences of these primer-probe sets are as follows: 2LTR-F (5′-TGCCAATCAGGGAAGWAGCCTTG-3′), 2LTR-R (5′-GAACCCACTGCTTAAGCCTCAAT-3′), and 2LTR-P (5′-FAM/CTGACCTGA/ZEN/AGGCTCT/IBFQ-3′) for the LTR gene, and GAG-F (5′-AGTTGGAGGACATCAAGCAGCCATGCAAAT-3′), GAG-R (5′-TGCTATGTCAGTTCCCCTTGGTTCTCT-3′), and GAG-P (5′-HEX/CTGACCTGA/ZEN/AG-GCTCT/IBFQ/-3′) for the gag gene.

In the brain tissue samples, we measured the relative proportion of mtDNA carrying the "common deletion" and used it as our measurement of mitochondrial injury. We designed a primer-probe combination that targets the bridge region on the mitochondrial chromosome before and after the 4,977 bp "common deletion" (CD) (Integrated DNA Technologies, Coralville, IA) (Fig. 1). The names and sequences of these primer-probe sets are as follows:

CD-F (5′-GGCTCAGGCGTTTGTGTATGAT-3′), CD-R (5′-TATTAAACACAAACTACCACCTACC-3′), and CD-P (5′-FAM/ACCATTGGC/ZEN/AGCCTAG/IBFQ-3′). As a result, amplification will only occur in the presence of the deletion. For quantification, we used 50 ng of DNA when measuring the "common deletion," and 50 pg of DNA when measuring MT-ND2. In the extracted DNA from CSF samples, we only measured MT-ND2 and RPP30, given the low levels of DNA present.

Quantification was performed in triplicate using a reaction consisting of 10 μL of 2x Bio-Rad Supermix for probes, either 1 μL of 20x Primer/FAM MT-ND2 mix or 20x Primer/FAM CD mix in combination with 20x Primer/HEX RPP30 mix, 3μL of molecular grade water, and 5 μL of shredded DNA. When we measured levels of HIV, we used a reaction consisting of 10 μL of 2x Bio-Rad Supermix for probes, either 1 μL of 20x Primer/FAM 2LTR mix with 20x Primer/HEX GAG, or 20x Primer/HEX RPP30 mix with 1 μL of molecular grade water, and 8 μL of shredded DNA. The PCR thermal cycling conditions were: (1) initial activation at 95°C for 10 minutes, (2) 55 cycles at 94°C for 30 seconds and 60°C for 1 min (ramp speed 2°C per second), (3) enzyme inactivation at 98°C for 10 minutes, and a 4°C hold. Droplets were read and analyzed using the Bio-Rad QX100 droplet reader and QuantaSoft software version 1.6.6. We calculated the average amount of mtDNA per cell using our cellular control. The amount of cells tested per well were calculated by dividing the amount of RPP30 copies by 2 to adjust for two copies of RPP30 present in one cell. The relative proportion of "common deletion" was calculated by dividing the number of mitochondria carrying the "common deletion" per cell by the total number of mtDNA copies (ND2) per cell. Separate from the brain tissue samples, levels of mtDNA in CSF were expressed in $\log_{10}$ copies/mL of CSF.

Soluble and inflammatory markers in CSF

We measured levels of soluble CD14 (sCD14) and soluble CD163 (sCD163) in our CSF samples using Quantikine ELISA Human sCD14 and sCD163 (R&D Systems, Minneapolis, MN) following manufacturer's protocols. Additionally, we measured neurofilament light (NF-L) and neopterin using the NF-Light (NF-L, Uman Diagnostics, Umea, Sweden) and BRAHMS (GmbH, Hennigsdorf, Germany) kits, respectively. All of these markers have been previously associated with inflammation and neurocognitive impairments in HIV infections [31–33].

Statistical analysis

All statistical analysis was performed using R statistical software Version 3.1.1. Normality of variables was assessed using the Shapiro test. Variables were either log- or square root-transformed to approximate a normal distribution. Differences in the levels of mtDNA levels per cell or relative abundance of common deletion was assessed with either a two-tailed t-test (pairwise comparisons) or by Analysis of Variance (ANOVA) with a Tukey post hoc adjustment for three-class comparisons. Univariate and multivariate association between variables were determined with either fixed-effects or mixed-effects regression analyses adjusting for repeated measures as needed.

Results

Cohort characteristics

The median age at death was 49 years. All participants were male. All HIV-infected patients both in the HIV+METH− group and HIV+METH+ group were on antiretroviral therapy (ART) when clinical and neuropsychological assessments were administered and reported until death. Neuropsychological assessments were performed on all HIV-infected participants prior to the last pre-mortem visit. CSF, blood, lymphocyte profiles, and demographic characteristics were also collected pre-mortem. There were no significant differences between the three study groups in relation to these variables. A summary of cohort characteristics is provided in Table 1. Participants with known Alzheimer's disease were excluded from analysis.

Levels of mtDNA and mitochondrial injury in different brain regions

When comparing the levels of mtDNA in white matter between sampled brain regions BA8, BA9, and BA46 we found that BA46 had significantly higher levels of mtDNA when compared to BA8 ($p<0.01$) and BA9 ($p<0.01$) (Fig. 2A). For a subset of the subjects, we received brain tissues from both BA7 and BA8 from the same participants. As a result of dependent data in the repeated measurements from both BA7 and BA8 for the same participants, we used a mixed model to adjust for variance. We found that there was no statistical difference between the two brain regions. There were no statistical differences between brain regions and mtDNA levels in gray matter (Fig. 2B).

By quantifying the "common deletion," we compared levels of mitochondrial injury in white matter from BA8, BA9, and BA46. We found that BA46 had the highest relative proportion of mitochondrial injury when compared to BA8 ($p<0.01$) and BA9 ($p=0.01$) (Fig. 2C). Again, for a subset of the subjects with brain tissue samples from both BA8 and BA7, we used a mixed model to adjust for variance and found that there were no statistical differences. We did not find any statistical difference between brain regions and mitochondrial injury in gray matter (Fig. 2D), even though higher levels of "common deletion" were present in gray matter rather than white matter. However, we still found that white matter had greater differences between the brain regions and the proportions of mtDNA with the "common deletion" when compared to gray matter in a mixed-model analysis.

Levels of mtDNA and mitochondrial injury in subject groups

Significant differences in mtDNA copy number and mitochondrial injury were found only in white matter rather than gray matter. Additionally, we collected the largest amount of samples from BA8. Because of these reasons, we decided to focus our analysis on white matter from BA8. In this brain region, participants in the HIV+METH+ group had significantly higher levels of mtDNA per cell when compared to HIV+METH− ($p=0.02$) and HIV−METH− ($p<0.01$) (Fig. 3A). HIV+METH+ group showed significantly less mitochondrial injury when compared to the HIV+METH− group ($p=0.02$) and HIV−METH − group ($p=0.08$) (Fig. 3B). When we compared the three study groups by ANOVA followed by the Tukey adjustment, we again found significantly less mitochondrial injury in

the HIV+METH+ group when compared to the HIV+METH− (p=0.01) and HIV−METH− groups (p=0.06). However, we did not find any significant differences in the levels of mtDNA with these additional tests.

Mitochondrial injury is associated with age

Since the relative proportion of mtDNA carrying the "common deletion" is known to be associated with increasing age and mortality [34, 35], we performed fixed-effect regression analysis to determine the association of both measurements of mtDNA copy number and mitochondrial injury with age. After correcting for the subset with measurements from both BA7 and BA8, we found that there was no association between the levels of mtDNA per cell and age (Fig. 3C); however, a higher abundance of the "common deletion" was associated with increasing age (Fig. 3D, p=0.03).

MtDNA and mitochondrial injury is associated with cognitive function

We performed a multivariate analysis to evaluate the association of levels of mtDNA and mitochondrial injury with GDS while adjusting for age and brain region. We used recursive partitioning to determine the effects of age and brain region in the model. If a variable did not contribute significantly to the model, it was removed from the model. In the HIV +METH+ group, we found that a higher abundance of "common deletion" was associated with lower GDS (p<0.01); however, in the HIV+METH− group, higher abundance of "common deletion" was associated with higher GDS (p<0.01) (Table 2).

HIV DNA in brain tissue is associated with mitochondrial injury

We performed a multivariate analysis to evaluate the association between levels of HIV DNA and mitochondrial injury while adjusting for age and brain region. In both HIV+ groups, we found that higher abundance of "common deletion" was associated with higher levels of HIV copies per million cells (p<0.01) found in brain tissue (Table 2). We did not find any associations between levels of HIV DNA and mtDNA.

Levels of cell free mtDNA, soluble and inflammatory markers in CSF

There were no significant associations between cell-free mtDNA in CSF, in relation to subject groups, GDS, or other clinical and immunological variables. There were also no significant associations between soluble and inflammatory markers, such as sCD14, sCD163, NFL, and neopterin, in relation to subject groups and other clinical and immunological variables.

Discussion

This is the first study to measure and compare the levels of mtDNA and the abundance of the "common deletion" in brain tissue in HIV-infected individuals with or without reported history of past METH use. We found significant differences in levels of mtDNA per cell and the abundance of "common deletion" in white matter of brain tissue in HIV+METH+ and HIV+METH− groups. We also found a relationship between the abundance of the "common deletion" with levels of HIV in brain tissue. Previous studies have shown that high levels of mitochondrial injury and cell death are associated with increased environmental stress,

increased production of ROS, and decreased respiration, Ca^{2+} regulation, and electron transport chain activity [36, 37]. A significant increase in ROS is associated with oxidative DNA damage and mtDNA mutations, causing a chain of events that would lead to mitochondrial injury and cell death [38]. In cultured media, toxicity is directly related to Ca^{2+} influx, as Ca^{2+} is a major determinant of glutamate receptor excitotoxicity [39]. A greater abundance of the "common deletion" could indicate a larger amount of mitochondrial injury due to oxidative stress, which may be worsened by HIV infection.

Highest levels of mtDNA and mitochondrial injury were seen in the white matter of BA46 when compared to BA7, BA8, and BA9. We found that only brain tissue from white matter demonstrated statistically significant variation in mtDNA levels and mitochondrial injury. This is supported by previous observations demonstrating that glutamate excitotoxicity and downstream free radical attack by ROS frequently occurs in glial cells like oligodendrocytes and myelinated axons present in white matter [40, 41], while gray matter primarily contains unmyelinated axons [42]. Observable differences in mtDNA levels and injury may vary more between myelinated and unmyelinated axons. In addition, we found that higher abundance of "common deletion" was associated with higher levels of HIV in brain tissue, perhaps indicating a relationship between HIV viral loads in the brain with mitochondrial injury.

We observed that levels of mtDNA and mitochondrial damage in white matter were highest among HIV-infected without METH individuals but lowest in HIV-infected with METH users. In addition, we observed that a higher proportion of mtDNA carrying the common deletion was associated with better neurocognitive function, (i.e., lower GDS), in HIV-infected METH users, but worse neurocognitive function in HIV-infected non-METH users. The reason for the reduced levels of mitochondrial injury in METH users remains unclear, but possible explanations include: 1) A sampling bias from where the brain tissue was sampled; 2) Induction of mitophagy by METH—thus the measureable amount of mitochondrial injury is reduced due to selective degradation of damaged mitochondrial DNA [43, 44]; 3) Neuroprotection by METH—previous work has shown that METH may mediate neuroprotection at low doses through moderate activation of dopamine receptors via the phosphoinositol-3 kinase and protein kinase B (Akt) pathways, both of which are responsible for apoptosis and cell proliferation [45], while dopamine receptor D2 activation plays an important role in protecting the brain against glutamate cytotoxicity through Akt signaling and up-regulation of Bcl-2 expression, an antiapoptotic protein [46, 47]; and 4) Reduced ART exposure—although HIV-infected participants (HIV+METH−, HIV+METH +) in our study were on ART until the last visit prior to death, previous studies have shown that HIV-infected METH users are prone to medication non-adherence [48]. ART has been known to induce oxidative stress [49, 50], and increase mitochondrial toxicity, particularly with the use of nucleoside reverse transcriptase inhibitors [51]. Therefore, reduced ART exposure could lead to lower levels of mitochondrial injury.

As with any evaluation of a clinical cohort, this study has several limitations. Primarily, all participants were enrolled into the NNTC at the time of death. Ante-mortem clinical data was only abstracted from medical records and sometimes acquired through family reports. For example, a urine toxicology test for amphetamine was not administered to everyone in

the HIV+METH+ group. We cannot adequately determine the length of history of the participants' exposure to METH, whether it is past, current, or lifetime, abuse or dependence. We were also limited to only the four BAs that were provided in our study as well as the number of available samples for each group. In addition, in our analysis of HIV viral loads and mitochondrial injury, not all the participants had undetectable HIV viral loads and were in different stages of infection during the time clinical and neuropsychological assessments were administered. The small numbers in our cohort limited our ability to correct for other potential confounders, such as disease stage, ART use, and other demographic factors.

In conclusion, our data indicate that levels of mtDNA per cell and the abundance of "common deletion" are significant in white matter of brain tissue in BA46 when compared to BA7, BA8, and BA9. Participants who were HIV+METH+ had the lowest levels of mitochondrial injury per cell in white matter when compared to the HIV+METH− and HIV −METH− groups, as well as lowest mtDNA copy number. There was also a significant association between greater mitochondrial injury with higher HIV DNA levels in brain tissue. As expected, increased mitochondrial injury was associated with worse neurocognitive function in HIV-infected individuals. However, in those individuals using METH, an opposite effect was seen. Further work is needed to clarify the relationships between the presence of mitochondrial injury and neurocognitive function in HIV-infected individuals and users of METH.

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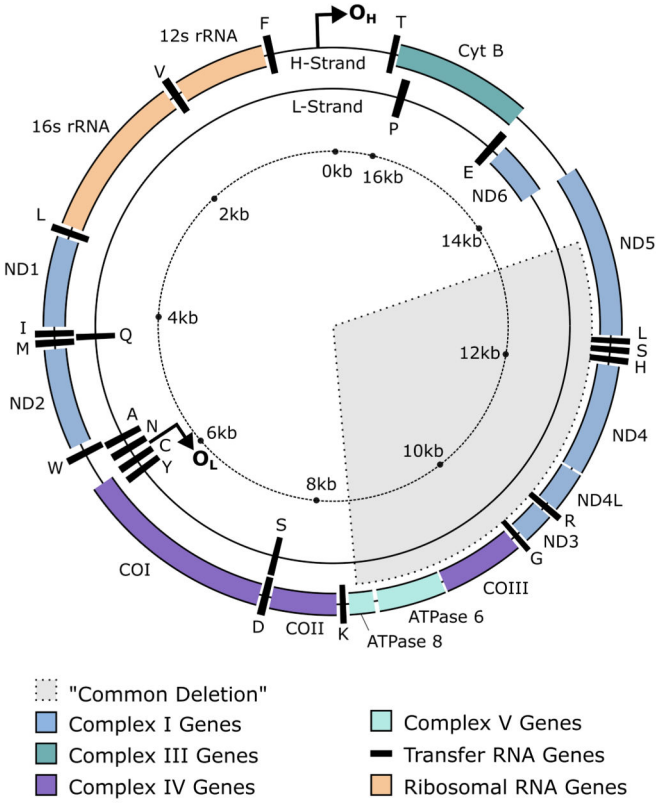

Figure 1. Human mitochondrial DNA (mtDNA)

A diagram of the 4,977 bp “common deletion” and mitochondrial genes in human mtDNA.

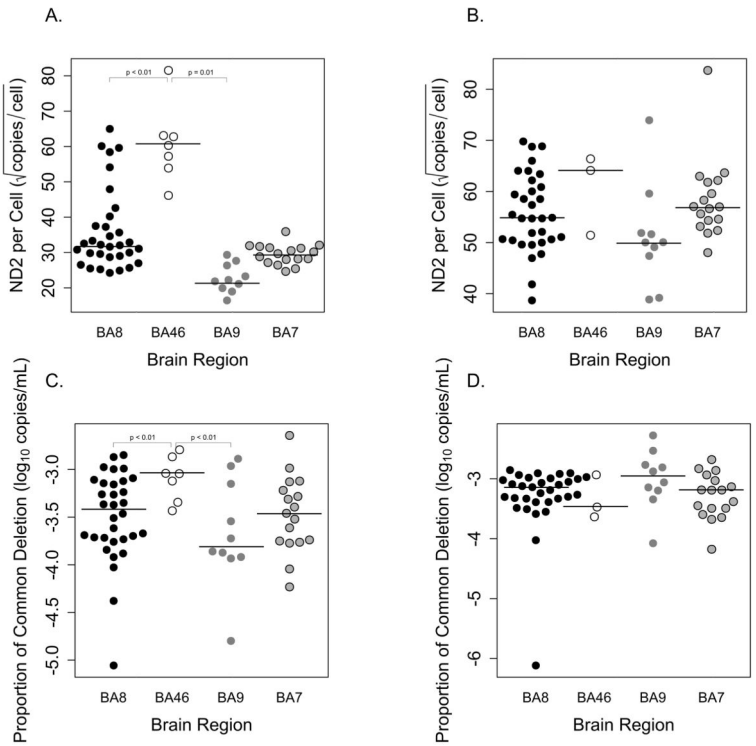

Figure 2. Levels of mitochondrial DNA (mtDNA) and mitochondrial injury in different brain regions

(A, B) Levels of mtDNA per cell and (C, D) the abundance of the "common deletion" in different brain regions in white and gray matter. Significant differences in levels of mtDNA and the abundance of the "common deletion" was seen in white matter. In addition, BA46 showed the highest levels of mtDNA and mitochondrial injury compared to BA8 and BA9. There was no statistical difference in levels of mtDNA and mitochondrial injury in gray matter.

Abbreviations: ND2, NADH dehydrogenase 2; BA, Brodmann Area.

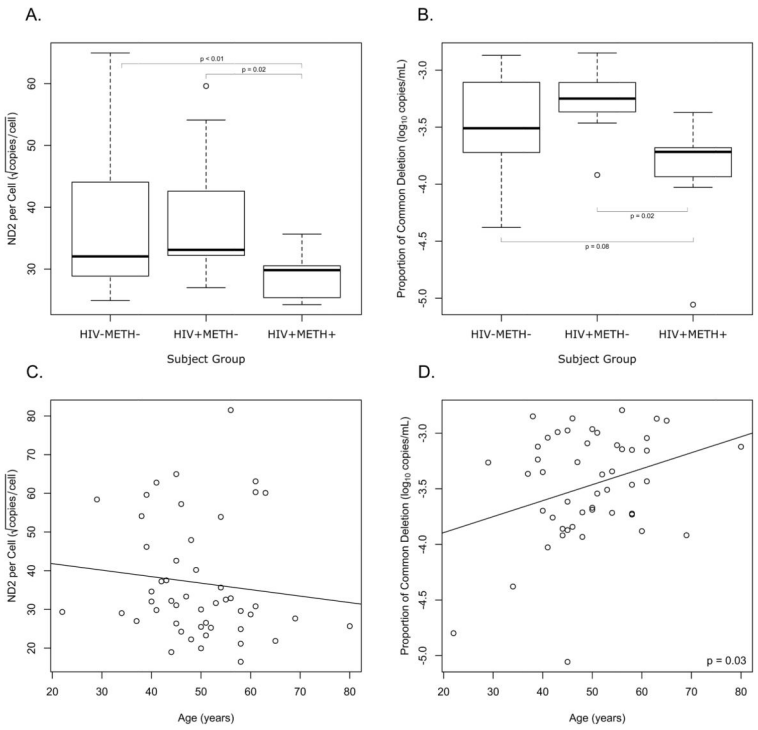

Figure 3. Levels of mitochondrial DNA (mtDNA) and mitochondrial injury in different subject groups and association with age

(A) HIV+METH− participants had significantly higher levels of mtDNA per cell compared to HIV+METH+ and HIV−METH−. (B) However, HIV+METH+ showed significantly lower levels of mitochondrial injury compared to HIV+METH− and HIV−METH−. (C) While there was no association between the levels of mtDNA per cell and age, (D) higher abundance of the "common deletion" was associated with older age.

See "Methods" section for the definitions of HIV+METH+, HIV+METH−, HIV−METH−.

Abbreviations: ND2, NADH dehydrogenase 2.

Table 1

Characteristics of the study participants

	HIV+METH+ (n=16)	HIV+METH− (n=11)	HIV−METH− (n=30)	p-value*
Ethnicity (Hisp:Not Hisp)	2:14	2:9	8:22	0.57
Age	50.2±8.1	46.1±7.5	51.4±12.1	0.32
CSF HIV RNA Levels (log_{10} copies/mL)	1.73±0.86	2.21±1.34	-	0.17
Proportion of undetectability HIV RNA in CSF (<50 copies)	3/13	3/8	-	0.66
Plasma HIV RNA Levels (log_{10} copies/mL)	3.36±1.52	3.27±2.30	-	0.64
Proportion of undetectability HIV RNA in plasma (<50 copies)	2/14	2/9	-	1
CD4 Absolute Counts	117±140	153±231	-	0.40
GDS	1.03±1.07	0.97±0.73	-	0.60

Mean values are shown for each study group.

* Represent the p-value of a double-tailed Mann-Whitney or a Fisher test. See “Methods” section for the definitions of HIV+METH+, HIV+METH−, HIV−METH−.

Abbreviations: Hisp, Hispanic; CSF, cerebral spinal fluid; GDS, global deficit score.

Table 2

P-values of the association of the relative abundance of the “common deletion” with clinical variables

Clinical Variable	HIV+METH+	HIV+METH−
GDS	**0.006**	**0.001**
CSF VL (log_{10} copies/mL)	0.19	0.17
Plasma VL (log_{10} copies/mL)	0.25	0.72
CD4 counts (cells/μL)	0.89	0.86
CD4 percentage (%)	0.59	0.22
CD8 counts (cells/μL)	0.87	0.94
CD8 percentage (%)	0.81	0.55
2-LTR (copy per 1E6 cells)	0.58	0.15
HIV gag (copy per 1E6 cells)	**0.0003**	**0.0001**

See “Methods” section for the definitions of HIV+METH+, HIV+METH−.

Abbreviations: GDS, global deficit score; CSF, cerebral spinal fluid; VL, viral load; LTR, long terminal repeat; 1E6, 1,000,000.