Title

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Peripheral blood mitochondrial DNA copy number obtained from genome-wide genotype data is associated with neurocognitive impairment in persons with chronic HIV infection

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Abstract

Background: Mitochondrial DNA (mtDNA) copy number varies by cell type and energy demands. Blood mtDNA copy number has been associated with neurocognitive function in persons without HIV. Low mtDNA copy number may indicate disordered mtDNA replication; high copy number may reflect a response to mitochondrial dysfunction. We hypothesized that blood mtDNA copy number estimated from genome-wide genotyping data is related to neurocognitive impairment (NCI) in persons with HIV.

Methods: In the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) study, peripheral blood mtDNA copy number was obtained from genome-wide genotyping data as a ratio of mtDNA SNP probe intensities relative to nuclear DNA SNPs. In a multivariable regression model, associations between mtDNA copy number and demographics, blood cell counts, and HIV disease and treatment characteristics were tested. Associations of mtDNA copy number with the global deficit score (GDS), GDS-defined NCI (GDS≥0.5), and HIV-associated neurocognitive disorder (HAND) diagnosis were tested by logistic regression, adjusting for potential confounders.

Results: Among 1,010 CHARTER participants, lower mtDNA copy number was associated with longer antiretroviral therapy duration ($p<0.001$), but not with d-drug exposure ($p=0.85$). mtDNA copy number was also associated with GDS ($p=0.007$), GDS-defined NCI ($p<0.001$), and HAND

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(p=0.002). In all analyses, higher mtDNA copy number was associated with poorer cognitive performance.

Conclusions: Higher mtDNA copy number estimated from peripheral blood genotyping was associated with worse neurocognitive performance in adults with HIV. These results suggest a connection between peripheral blood mtDNA and NCI, and may represent increased mtDNA replication in response to mitochondrial dysfunction.

Keywords

DNA; Mitochondrial; HIV; Neurocognitive Disorders

INTRODUCTION

Effective antiretroviral therapy (ART) in persons with chronic HIV infection has greatly decreased the incidence of the most severe neurocognitive complication, HIV-Associated Dementia (HAD)[1]. Despite this improvement, milder forms of neurocognitive impairment (NCI) remain common in this population. The overall prevalence of HIV-Associated Neurocognitive Disorder (HAND) remains 30–50%[2], and some studies suggest that asymptomatic forms of NCI confer risk of progression to symptomatic HAND[3]. The prevalence of NCI in persons with HIV may increase as the average age of the population continues to increase[4, 5].

A major determinant of neuronal function is energy production from mitochondria[6, 7]. Mitochondria contain their own genome, with many copies of mitochondrial DNA (mtDNA) per cell. In general, the amount of mtDNA per cell closely relates to cellular energy demands, so cells with higher energy requirements (*e.g.* neurons and muscle cells) have more mtDNA copies[8]. Very high or very low mtDNA copy numbers may indicate mitochondrial dysfunction[9–11]. High mtDNA copy numbers are observed in patients with mitochondrial diseases who have impaired oxidative phosphorylation[9, 10]. This phenomenon is generally attributed to a cellular compensatory mechanism, raising mitochondrial biogenesis in response to impaired mitochondrial function[12]. Conversely, impairment of the mitochondrial biogenesis process itself, including mtDNA replication, causes low mtDNA levels[11].

Different methods are used to quantify mtDNA copy number in a blood or tissue sample. Most include quantitative polymerase chain reaction (PCR) and involve a comparison of the amount of mtDNA to the amount of nuclear DNA. In general, these methods do not give absolute counts of mtDNA molecules per cell, but are instead relative measures with higher values indicating higher mtDNA copy number per cell[13]. The mtDNA copy number in peripheral blood, normalized by a nuclear DNA copy number measure, has often been used as a general proxy for the mitochondrial function in an individual. Cross-sectional population studies of blood mtDNA copy number show that the relationship to age is complex, with lower blood mtDNA copy number found in either the young or the old compared to middle-aged persons[14–17]. Peripheral blood mtDNA copy number has been linked to several neurological and neurocognitive phenotypes in adults without HIV[18–25].

Studies of peripheral blood mtDNA copy number in persons with HIV have predominantly focused on the relationship between mtDNA copy number and HIV treatment effects[26–30]. No previous studies have compared peripheral blood mtDNA levels (either directly measured or estimated) and neurocognitive function in a population with HIV. We used a new method to estimate peripheral blood mtDNA copy number using the fluorescence intensity of mtDNA single-nucleotide polymorphism (SNP) probes on a genome-wide genotyping platform[14]. This method could allow for estimation of relative mtDNA copy number in larger populations than traditional quantitation methods. Our objective was to determine factors associated with relative mtDNA copy number, and our hypothesis was that since the central nervous system (CNS) is particularly vulnerable to mitochondrial damage, mtDNA copy number in peripheral blood would be associated with NCI in adults with HIV.

METHODS

Participants

The CNS HIV Antiretroviral Therapy Effects Research (CHARTER) is a prospective cohort study of neurologic complications of HIV infection and treatment conducted at six U.S. locations: San Diego, California; Baltimore, Maryland; Galveston, Texas; New York, New York; Seattle, Washington; and St Louis, Missouri. Institutional review boards at each site approved the study, and each participant provided written informed consent. All data utilized for these analyses were de-identified. Data were collected between 2003 and 2007 according to a protocol of comprehensive neurological, behavioral, and laboratory assessments that were standardized across sites[2].

Assessment of Neurocognitive Impairment

Participants were English-speaking and underwent a comprehensive test battery that included seven neurocognitive domains[2]. Composite Global Deficit Score (GDS) values were derived from standardized *T*-scores using best available normative standards to correct for practice effects[31], as well as age, education, sex, and ethnicity, as appropriate. For persons of self-reported Hispanic ethnicity, three of 15 measures were corrected for English-speaking Hispanic normative standards; the remainder were adjusted for non-Hispanic European normative standards[32]. 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[33].

To further classify presence and severity of HAND, a published algorithm that has excellent inter-rater reliability for the presence of NCI was used[34]. This algorithm conforms to the Frascati criteria for diagnosing HAND[35], requiring at least mild impairment in at least two of seven domains, and includes functional assessment by self-report, performance-based criteria, or both, as well as exclusions based on comorbidities (non-HIV-related risks for NCI). The assessment of HAND status in CHARTER participants required a determination that any NCI was likely due to HIV-related effects on the brain rather than comorbid conditions[2]. Detailed review by two senior CHARTER investigators using published

guidelines[35] provided categorization of neuropsychiatric comorbid conditions for all CHARTER participants as either incidental (minimal), contributing (mild-moderate), or confounding (severe) with respect to neurocognitive performance. Participants with confounding neurocognitive comorbidities, which precluded an assessment of the contribution of HIV infection to their neurocognitive performance (*e.g.*, brain trauma, seizures, or CNS opportunistic infections), were not eligible for a diagnosis of HAND according to Frascati criteria[2, 35] and were excluded from CHARTER genetic studies. Categories of HAND include asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HAD. Standardized assessments were performed by trained study personnel certified by the CHARTER coordinating center. For this analysis, we combined these three groups (ANI, MND and HAD) into a single "HAND" group, and also considered ANI separately from MND and HAD.

Genetics

Whole blood samples were collected in PAXgene tubes, and isolation of genomic nuclear DNA was performed using Puregene (Gentra Systems Inc., Minneapolis, MN, USA). Study participants had genome-wide DNA genotyping available using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Inc., Santa Clara, CA, USA). Ancestry-informative markers were extracted from the autosomal DNA genotypes and were analyzed using EIGENSTRAT software[36] to generate principal components (PC). In addition to genome-wide nuclear genotyping, full mtDNA sequencing was also performed on study participants using the GeneChip Human Mitochondrial Resequencing Array v2.0 (Affymetrix, Inc., Santa Clara, CA, USA). These data were processed using the MitoChip Filtering Protocol (MFP)[37], variants were called relative to the Revised Cambridge Reference Sequence (rCRS)[38], and mtDNA haplogroups were assigned using the HaploGrep program (http://haplogrep.uibk.ac.at/)[39]. Genome-wide genotyping data was used for the mtDNA copy number assessment as described below. Data from the mtDNA-specific genotyping chip was only used for assessing mtDNA haplogroups, not for the mtDNA copy number assessment.

MtDNA copy number measurement

Relative mtDNA copy number can be estimated from the genotyping SNP probe intensity data of genome-wide genotyping arrays[14]. The Affymetrix Genome-Wide Human SNP Array data were analyzed using the Affymetrix Genotyping Console. Only samples with call rates >95% were selected for further processing. For each homozygous SNP determination in an individual, the fluorescence intensity in the SNP was used, minus the fluorescence intensity in the uncalled allele as a measure of the background fluorescence "noise" level. Then, the median of this value for the mtDNA SNP probes and the median for the autosomal SNP probes were calculated. The ratio of these medians was used as an estimate of the relative copy number of the mtDNA compared to the nuclear DNA. A variant of this mtDNA copy number estimate method based on 25 genotyped mtDNA SNPs was tested against the standard quantitative PCR method[40]. That study concluded that the microarray-based method for mtDNA copy number was valid, with a Spearman r of 0.51 compared with PCR.

Since mtDNA copy number per cell varies greatly depending on cell type, the distribution of cell types in the peripheral blood can strongly affect the mtDNA copy number in peripheral blood. Since platelets contain mtDNA but no nuclear DNA, higher platelet count has been shown consistently to increase mtDNA copy number in peripheral blood[16, 41, 42]. Conversely, higher white blood cell (WBC) counts correlate with relatively lower peripheral blood mtDNA copy number per cell[16], because WBCs also contain nuclear DNA. We included the platelet/WBC ratio as a covariate in the statistical models to correct for both, as described previously[43].

Clinical covariates

For the analysis of the relationship between mtDNA copy number in peripheral blood and HAND, several clinical covariates were included. To represent the course of the disease treatment, we included nadir CD4+ T-cell count, estimated duration of HIV infection, duration of ART exposure, and plasma HIV RNA. We also adjusted for duration of exposure to dideoxynucleoside ART drugs (stavudine [d4T], didanosine [ddI], or zalcitabine [ddC]), collectively referred to as d-drugs, since this class has been particularly associated with mitochondrial toxicity, at least in part through disruption of mtDNA replication[44–47]. All covariates were collected at the same CHARTER visit as the DNA collection.

Statistical analyses

Univariate comparisons were performed by t-test for continuous variables and chi-squared test for dichotomous variables. Associations were also tested using multivariable logistic regression for dichotomous outcomes (GDS-defined NCI and HAND) and by linear regression for continuous outcomes (continuous GDS and mtDNA copy number). HAND outcomes were dichotomized as neurocognitively normal, or as impairment of any severity (ANI, MND, or HAD). Considering the importance of comorbidity to NCI, we performed stratified analyses by comorbidity severity (incidental vs. contributing; participants with severe comorbidity were excluded). We also tested for an association of mtDNA copy number with mtDNA haplogroups, with participants stratified into European, African, and Hispanic ancestry based on nuclear DNA-derived PC, as previously described[48]. All analyses were performed using the latest version of R.

RESULTS

Of 1,010 CHARTER participants with genome-wide genotyping and full clinical covariates available, most (78%) were men, with a median age of 44 years (Table 1). Median estimated duration of HIV infection was just over 10 years, and the median nadir and current CD4+ T-cell counts were 173 and 437 cells/mm^3, respectively. Among the 748 (74%) on ART, the median ART duration was 5.6 years and 451 (60%) had plasma HIV RNA <50 copies/mL. Approximately 45% of participants had at least past exposure to d-drugs, with 122 (12% of the total; 16% of those on ART) receiving a d-drug at the study visit. At the time of the mtDNA copy number estimation, 35% of the participants had GDS values in the impaired range, and 45% of the participants were classified as having HAND.

The relative mtDNA copy number estimate from whole blood genotyping in the population was normally distributed (p=0.19 by Shapiro-Wilk test for normality). Consistent with previous reports in populations without HIV[14–16], relative mtDNA copy number values differed significantly by sex (p<0.001), with females having higher values (Supplementary Figure). Multivariable linear regression modeling of the mtDNA copy number was adjusted for platelet/WBC ratio, age, sex, genetic PC 1–3, nadir CD4+ T-cell count, estimated duration of HIV, and duration of ART and d-drug exposure (Table 2). Sex remained highly significantly associated with mtDNA copy number (p=0.001). The most significant association with relative mtDNA copy number was with platelet/WBC ratio; higher copy number was associated with higher platelet/WBC ratio, as expected[16]. Participant age and the first three ancestry PC (representing the racial and ethnic diversity of the cohort) were not significantly associated with mtDNA copy number. Relative mtDNA copy number was also not significantly associated with mtDNA haplogroups (data not shown).

For the HIV-specific clinical covariates, neither estimated duration of HIV infection nor duration of exposure to d-drug ART was significantly associated with mtDNA copy number (Table 2). In contrast, longer total estimated duration of ART was significantly associated with lower mtDNA copy number (p<0.001).

Higher relative mtDNA copy number was associated with GDS-defined NCI (p<0.001, Figure 1 and Table 3). In a subgroup analysis of participants on ART with plasma HIV RNA ≤ 50 copies/mL, the association between relative mtDNA copy number and impaired GDS persisted (p=0.006; data not shown). As expected, nadir CD4+ T-cell count (p=0.02) was associated with higher GDS (poorer performance), as was the Hispanic ancestry PC (p<0.0001), a finding previously observed in CHARTER and other populations with HIV[48–51]. The association of NCI with Hispanic ancestry was independent of relative mtDNA copy number and was consistent across all adjusted models of the NCI outcomes. We also carried out a secondary analysis using the continuous GDS value instead of the dichotomized GDS impairment measure (Supplementary Table 1). In this analysis, whole blood mtDNA copy number was also significantly associated with continuous GDS values (p=0.007), and higher mtDNA copy number was associated with higher GDS scores (poorer neurocognitive performance).

Higher whole blood mtDNA copy number was also significantly associated with a greater likelihood of HAND (p=0.002; Table 4). Median whole blood mtDNA copy number was significantly greater in persons with ANI (N=283) vs. those without HAND (N=553; Wilcoxon p=0.0002), but not in those with MND or HAD (N=174 [141 with MND; 33 with HAD]; p=0.35). In fully-adjusted logistic regression models, higher mtDNA copy number was associated with greater likelihood of ANI (p<0.001) but not MND/HAD (p=0.68).

Considering the importance of comorbidities in assessing HIV-associated NCI, we performed a stratified analysis by comorbidity status. In adjusted analysis of participants with incidental comorbidities (N=651), those with GDS-defined NCI (N=191) had higher median relative mtDNA copy number than those with an unimpaired GDS (p<0.001; Figure 1 and Supplementary Table 2). In participants with comorbidities that were considered potentially contributing to NCI (N=359), the difference in mtDNA copy number by GDS

impairment was of a lower magnitude and not statistically significant (p=0.08; Figure 1 and Supplementary Table 3).

The analyses reported above tested for linear associations of mtDNA copy number with NCI measures. To test for a possible non-linear relationship, we carried out a quintile analysis, separating participants into five equally sized groups based on their mtDNA copy number. The proportion of participants with GDS impairment across mtDNA copy number quintiles was significantly non-uniform (p=0.001 by chi-square test; Figure 2), with a greater proportion of participants with GDS impairment seen in the higher quintiles of relative mtDNA copy number. Participants with the lowest mtDNA copy numbers (quintile 1) also showed an increased proportion with GDS impairment, consistent with the hypothesis that both extremes of relative mtDNA copy number may be associated with NCI.

To further investigate the effect of ART on mtDNA copy number, we tested for an association between mtDNA copy number and the ART regimen at the time of sample collection in the subgroup of study participants on ART with controlled HIV infection, defined by plasma HIV RNA ≤50 copies/mL (N = 449). Drugs for which >5% of the participants were treated were analyzed, for a total of 10 different medications. Each drug was tested in a multivariable linear regression model, using 1 or 0 for current exposure to the drug or not, adjustting for the platelet/WBC ratio, age, and sex (Figure 3). The only significant association with mtDNA copy number was nevirapine (NVP) exposure, which was associated with lower mtDNA copy number (p=0.003). This association was not changed when all of the ART drugs were included simultaneously in a single model (NVP coefficient ± 2x standard error = −0.46 ± 0.32, p=0.004).

DISCUSSION

In this large analysis of a well-characterized cohort, higher relative mtDNA copy number in blood was associated with a greater probability of NCI in CHARTER participants. The association was not explained by potentially relevant covariates such as age, prior use of d-drug ART, or comorbidities that might have contributed to NCI, and was robust in analyses of multiple measures of NCI. Interestingly, in analyses of secondary HAND outcomes, the association between higher blood mtDNA copy number and HAND was predominantly seen in persons with ANI, not in those with more functionally significant forms of NCI, MND or HAD. These findings may suggest a biologic correlate of asymptomatic NCI that is either lost with more severe forms of NCI, or that we were underpowered to detect in these subgroups. While these cross-sectional data cannot assess temporal relationships, future studies will need to address the relationship between mtDNA copy number and transition from ANI to MND or HAD over time.

Significant associations between peripheral blood mtDNA copy number and neurocognitive phenotypes have been reported in several studies. Lower mtDNA copy number in blood compared to controls has been reported for Huntington's disease[25] and Parkinson's disease[18]. While not identical to neurocognitive impairment or neurodegenerative diseases, data in neuropsychological phenotypes are mixed: significantly lower blood mtDNA levels have been reported in elderly women with depression[19], but not in young adults[20]. A study

on bipolar disorders in young adults found only a borderline significant association of lower blood mtDNA levels with more severe forms of bipolar disorder, and no association with milder bipolar disorder[21]. Related studies in healthy, elderly Korean women reported that lower blood mtDNA levels were significantly associated with poorer performance on the Mini-Mental State Examination[22] and the Geriatric Depression Scale[23]. In contrast to all of these studies, which showed worse phenotypes with lower blood mtDNA level, higher blood mtDNA copy number has been significantly associated with an increased likelihood of childhood autism[24].

In this analysis, we used a novel method to estimate relative peripheral blood mtDNA copy number from available genome-wide genotype data. This method has recently been used to demonstrate associations between lower mtDNA copy number and increased risk of sudden cardiac death[52], incident cardiovascular disease and stroke[53], and chronic kidney disease[40] in populations without HIV. While studies of direct peripheral blood mtDNA quantitation have been performed in persons with HIV for many years, this is the first study to assess this particular measure in a population with HIV, and the first to assess relationships of any mtDNA copy number estimate with carefully characterized neurocognitive performance. The presence of expected relationships between this measure and sex, platelet count, and WBC provided a degree of validation, although the expected association with age was absent. While multiple studies have shown decreasing blood mtDNA copy number with age in the elderly[14, 15], studies over a broader range of ages show a more complex relationship, with blood mtDNA either stable or rising with age in the young and middle-aged, then dropping in the elderly[16, 17]. The age distribution of our study population is in the range that has little age-related variation in peripheral mtDNA copy number, consistent with our results[16].

We found an unexpected association between NVP and lower relative mtDNA copy number. ART has generally been associated with lower mtDNA copy number in peripheral blood when measured by PCR[29, 54]. A small study of 32 patients showed that treatments containing either NVP or 3TC were associated with significantly higher peripheral blood mononuclear cell (PBMC) mtDNA copy number compared to regimens containing ddI[28]. Another study of 47 participants examined changes in PBMC mtDNA copy number after switching to a nucleoside reverse transcriptase inhibitor (NRTI)-sparing ART regimen, and found that the group switched from NRTIs (d-drug and/or zidovudine) to NVP plus a protease inhibitor (lopinavir/ritonavir) had an increase in PBMC mtDNA over 48 weeks[55]. A recent analysis of pregnant women/infant pairs with or without HIV found lower blood mtDNA copy number in HIV-exposed infants than unexposed[56]. While unadjusted mtDNA copy number was lower in NVP-exposed than unexposed infants, after multivariable adjustment the difference was statistically significant only in infants exposed to the thymidine analog NRTI zidovudine, but not those exposed to NVP. NVP has also been implicated in increasing mitochondrial depolarization to levels sufficient to induce apoptosis[57]. We found no statistically significant associations between mtDNA copy number and NRTIs zidovudine or d4T, each of which are associated with mitochondrial toxicity in patients[58–61], although our probe-intensity method of estimating relative mtDNA copy number could partially explain differences across studies. However, recent data have found

good correlation between probe-intensity measurements from exome sequencing data and direct RT-PCR quantitation of mtDNA copy number in the same cell lines[62].

Limitations of our study include those inherent to cross-sectional, observational analyses, including that they are more prone to bias than longitudinal studies. We did not have simultaneous measures of relative or directly measured mtDNA copy number from CSF, or CSF biomarker data from a large enough sample to include in these analyses. Thus, mechanistic insights are necessarily limited and will need to be addressed in future studies. The CHARTER study population includes a large proportion of individuals treated with earlier-generation ART drugs and so our findings may not generalize well to younger populations who have only been exposed to newer drugs. We included data from 94 persons (9% of the total population) of self-reported Hispanic ethnicity. At the time of data collection, normative population standards for correction were limited, thus NCI could be overestimated in this population. There was no control population without HIV for comparison, so we cannot draw conclusions regarding the contribution of HIV infection itself to mtDNA copy number. We did not adjust for all factors potentially related to mtDNA biogenesis and NCI, including substance use or concomitant non-ART medications. Finally, due to the exploratory nature of analyses with this novel measure, we did not adjust for multiple comparisons, but many p-values for adjusted NCI associations were robust to levels well below the p=0.05 threshold.

In summary, we present the first analysis of relative mtDNA copy number derived from genome-wide genotyping data in adults living with HIV. This is also the first analysis linking peripheral blood mtDNA copy number to HIV-associated NCI. We found a consistent association between higher relative mtDNA copy number in blood and poorer neurocognitive performance, defined by either the GDS or Frascati criteria. These results can inform longitudinal studies of NCI risk and progression in HIV by providing a novel biomarker that may already be available in population-level genotyping data. They also provide mechanistic insights, affirming a possible role for mitochondria in the pathogenesis of HIV-associated NCI. More studies are needed to assess longitudinal changes in mtDNA copy number, correlate peripheral blood and CSF or tissue-level measures of mtDNA, and explore mechanisms underlying associations between mtDNA and HIV-associated NCI. Studies like these could inform future clinical studies of NCI prediction or prognosis and mitochondria-targeted interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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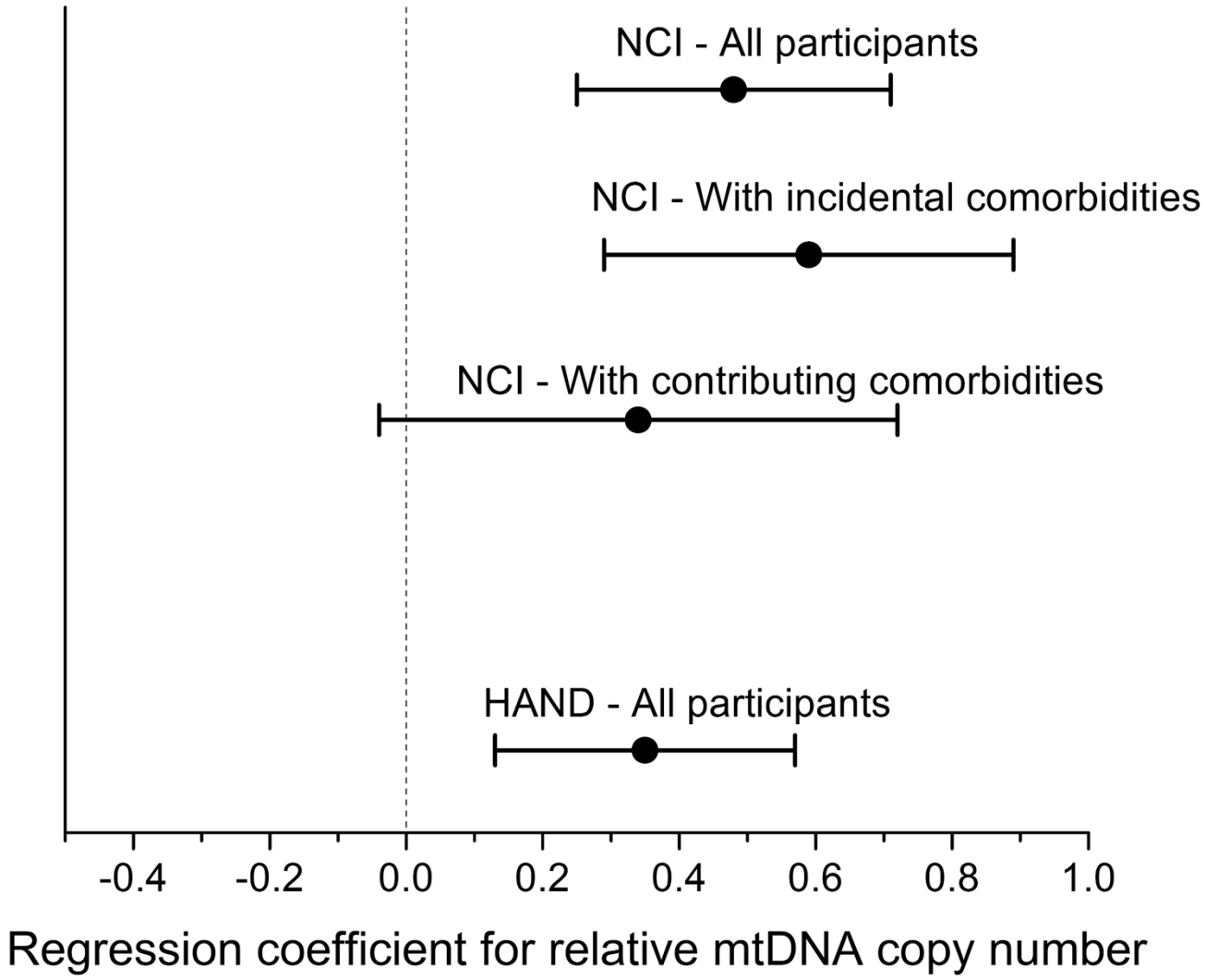

Figure 1.

Forest plot of regression coefficients (± 2x standard error) for associations between relative mtDNA copy number and either neurocognitive impairment (NCI; GDS≥0.5) or HAND in models adjusting for nadir CD4+ T-cell count, estimated duration of HIV infection, duration of ART and d-drug exposure, and plasma HIV RNA level. Plots for associations between mtDNA copy number and NCI for subgroups stratified by baseline comorbidity status are also shown.

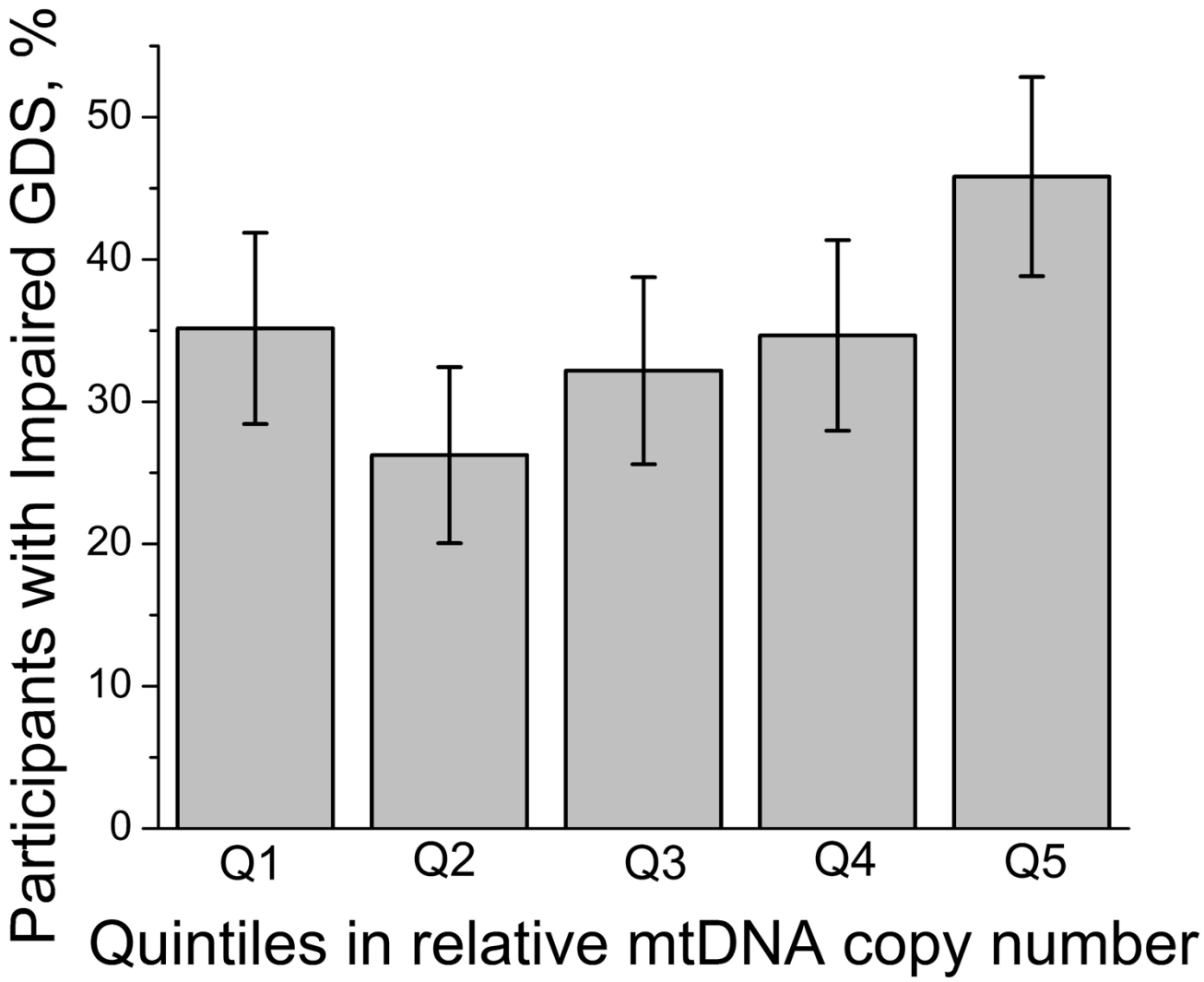

Figure 2.

Percentage of CHARTER participants with impaired GDS within each quintile of the blood mtDNA copy number. Q1 is the lowest mtDNA copy number group and Q5 is the highest. Error bars for proportion p were calculated as 2 x SQRT(p(1-p)/N), where N is the sample size. P value=0.001 by chi-squared test.

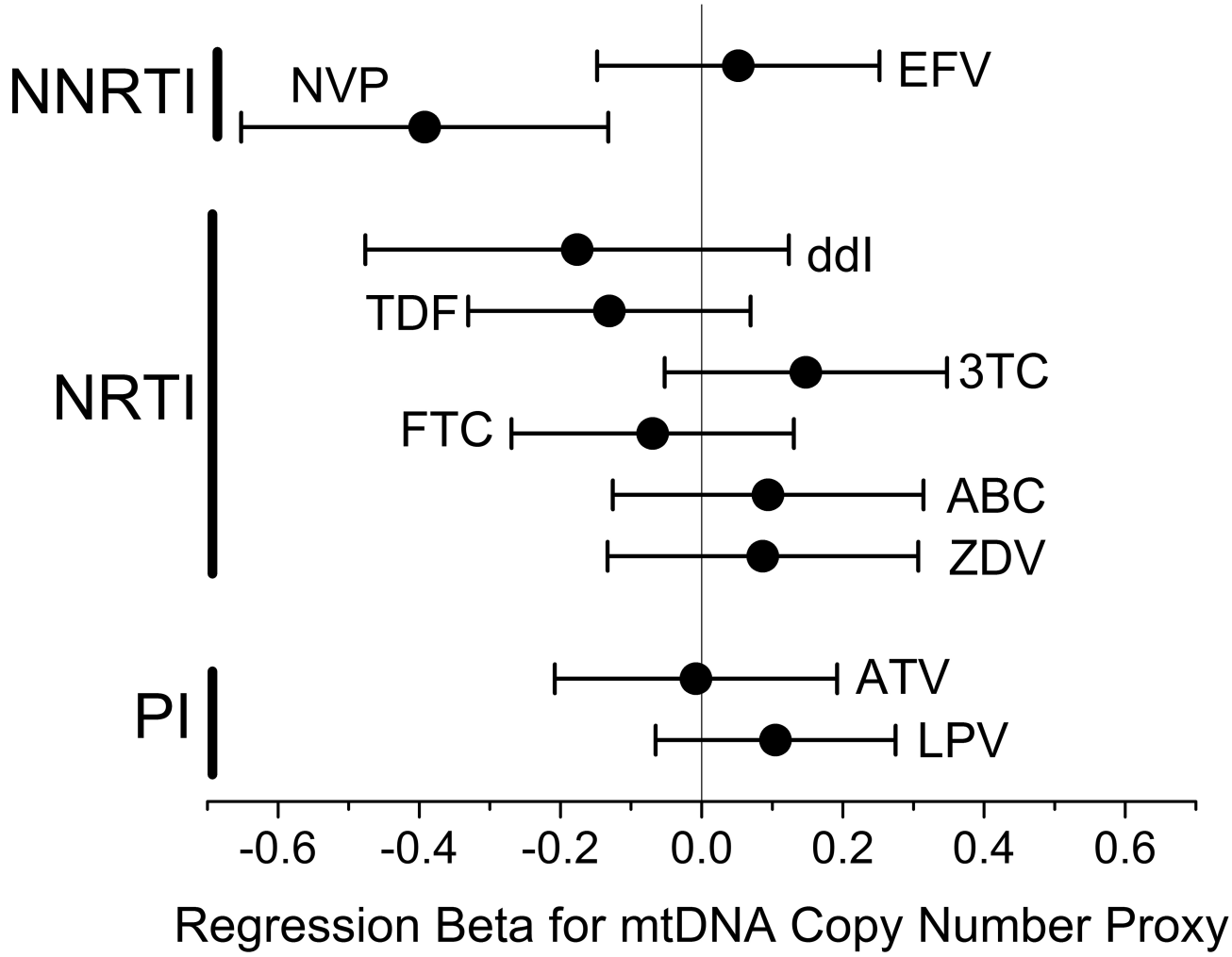

Figure 3.

Regression coefficients (± 2x standard error) for associations between duration of exposure to specific ART on the relative mtDNA copy number in peripheral blood. All ART drugs were tested in a single multivariable linear regression model, adjusting for the platelet to WBC ratio, age at measurement, and sex. (Abbreviations: NNRTI=Non-Nucleoside Reverse Transcriptase Inhibitors; NRTI=Nucleoside Reverse Transcriptase Inhibitors; PI=Protease Inhibitors; EFV=efavirenz; NVP=nevirapine; ddI=didanosine; TDF=tenofovir disoproxil fumarate; 3TC=lamivudine; FTC=emtricitabine; ABC=abacavir; ZDV=zidovudine; ATV=atazanavir; LPV=lopinavir/ritonavir.)

Table 1.

Study demographics, clinical variables, and neurocognitive outcomes.

Variable	
Total N	1,010
Female, N (%)	227 (23)
Age in years, median (IQR)	44 (39–49)
Non-Hispanic Black, N (%)	469 (46)
Non-Hispanic White, N (%)	429 (43)
Hispanic, N (%)	94 (9)
Other race/ethnicity, N (%)	18 (2)
Estimated duration of HIV in months, median (IQR)	127 (60–191)
Currently on ART, N (%)	748 (74)
Estimated total duration of ART in months, median (IQR) *	68 (28–103)
Ever d-drug exposure, N (%)	458 (45)
Duration d-drug exposure in those exposed in months, median (IQR)	36 (17–66)
Current d-drug use, N (%) *	122 (16)
Current CD4+ T-cell count, median (IQR) cells/mm^3	437 (283–610)
Nadir CD4+ T-cell count, median (IQR) cells/mm^3	173 (50–297)
Plasma HIV RNA <50 copies/mL, N (%) *	451 (60)
Contributing comorbidities, N (%)	359 (36)
GDS, median (IQR)	0.33 [0.11–0.67]
GDS impairment (GDS ≥ 0.5), N (%)	352 (35)
HAND, N (%)	457 (45)

*Among those participants on ART at the study visit. IQR=interquartile range; GDS=global deficit score; HAND=HIV-associated neurocognitive disorder

Table 2.

Adjusted associations between demographic and clinical variables and relative mtDNA copy number.

Variable	Coefficient (±2 SE)	P value
Platelet / WBC ratio	0.01 ± 0.001	<0.001
Age (per 10 years)	−0.03 ± 0.05	0.18
Female sex	0.16 ± 0.09	0.001
Genetic PC1	1.40 ± 1.55	0.07
Genetic PC2	−0.96 ± 1.51	0.20
Genetic PC3	−1.01 ± 1.47	0.17
Nadir CD4+ T-cell count (per 100 cells)	0.008 ± 0.02	0.44
Estimated duration of HIV (per year)	0.0005 ± 0.01	0.89
Estimated duration of ART (per year)	−0.02 ± 0.01	0.001
Estimated d-drug exposure (per year)	−0.001 ± 0.01	0.85

Table 3.

Adjusted associations between demographic and clinical variables, relative mtDNA copy number, and neurocognitive impairment by global deficit score (GDS ≥0.5).

Variable	Coefficient (±2x SE)	P value
Peripheral blood mtDNA copy number	0.48 ± 0.23	<0.0001
Platelet / WBC ratio	−0.01 ± 0.01	0.02
Age (per 10 years)	0.04 ± .09	0.68
Female sex	0.23 ± 0.34	0.18
Genetic PC1	−1.23 ± 5.46	0.65
Genetic PC2	10.63 ± 5.40	<0.0001
Genetic PC3	1.39 ± 5.25	0.60
Estimated duration of HIV (per year)	0.01 ± 0.02	0.56
Contributing comorbidity	0.67 ± 0.28	<0.0001
Nadir CD4+ T-cell count (per 100 cells)	0.10 ± 0.08	0.02
Plasma HIV RNA ≤50 copies/mL	0.09 ± 0.28	0.52

Table 4.

Adjusted associations between demographic and clinical variables, relative mtDNA copy number, and any HAND.

Variable	Coefficient (±2x SE)	P value
Peripheral blood mtDNA copy number	0.35 ± 0.22	0.002
Platelet / WBC ratio	−0.01 ± 0.01	0.001
Age (per 10 years)	0.01 ± 0.16	0.86
Female sex	0.11 ± 0.32	0.50
Genetic PC1	3.04 ± 5.51	0.27
Genetic PC2	9.71 ± 5.34	0.0003
Genetic PC3	0.25 ± 5.22	0.92
Estimated duration of HIV (per year)	0.01 ± 0.02	0.28
Contributing comorbidity	0.77 ± 0.27	<0.0001
Nadir CD4+ T-cell count (per 100 cells)	0.06 ± 0.04	0.10
Plasma HIV RNA ≤50 copies/mL	0.03 ± 0.14	0.83