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Epidermal nerve fiber density, oxidative stress, and mitochondrial haplogroups in HIV-infected Thais initiating therapy

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Abstract

Objective—We explored associations between mitochondrial DNA (mtDNA) haplogroups, epidermal nerve fiber density (ENFD), and HIV-associated sensory neuropathy (HIV-SN) in a randomized trial of Thai patients initiating antiretroviral therapy (ART).

Design—The South East Asia Research Collaboration with Hawaii 003 study evaluated toxicity of nucleoside reverse transcriptase inhibitors (stavudine vs. zidovudine vs. tenofovir). We present secondary analyses of mtDNA haplogroups and ENFD changes.

Methods—ENFD, peripheral blood mononuclear cell mitochondrial complex I and IV, and 8-oxo-deoxyguanine (8-oxo-dG) were quantified. Peripheral blood mononuclear cell mtDNA sequences were obtained for haplogroup determination. Multivariate regression of ENFD change was performed.

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Conflicts of interest

N.P. serves as an academic advisory committee member of the Thai Government Pharmaceutical Organization.

J.A. has received honoraria and speaker's fee from Gilead. She serves as an academic advisory committee member of the Thai Government Pharmaceutical Organization.

J.C.M. is an unpaid consultant for Biogen-Idec and Allergan, has received research funding for clinical trials from Pfizer and Biogen-Idec, and holds stock options in Revelare and Gliamed for serving on Scientific Advisory Boards.

C.M.S. received research and training funding support from Gilead Sciences, ViiV and Mitosciences. She is also on the speaker's bureau for ViiV.

T.H. has served as PI of research grants to Vanderbilt University from Merck & Co.

For the remaining authors no conflicts were declared.

Results—Paired ENFD was available from 118 patients. Median age, CD4⁺ cell count, and height at entry were 34 years, 172 cells/μl, and 162 cm, respectively. Major haplogroups included M (42%), F (21%), and B (16%). Baseline ENFD, CD4⁺ cell count, randomized ART, and biomarkers did not differ by haplogroup. Haplogroup B patients were older ($P=0.02$) at baseline, and had an increase in median ENFD (+1.5 vs. -2.9 fibers/mm; $P=0.03$) and 8-oxo-dG break frequency (+0.05 vs. 0.00; $P=0.05$) compared to other haplogroups. In a multivariate model, haplogroup B was associated with increased ENFD ($\beta=3.5$, $P=0.009$) at week 24, whereas older age ($P=0.02$), higher baseline CD4⁺ cell count, ($P=0.03$), higher complex I level ($P=0.03$), and higher ENFD ($P<0.001$) at baseline were all associated with decreased ENFD. Three of the six HIV-SN cases were haplogroup B ($P=0.05$).

Conclusions—Thai persons belonging to mtDNA haplogroup B had increased ENFD and 8-oxo-dG on ART, and were more likely to develop HIV-SN. These results suggest that mtDNA variation influences early oxidative damage and ENFD changes.

Keywords

antiretroviral agents; DNA; genetics; HIV; medical; mitochondrial; peripheral nervous system diseases

Introduction

HIV-associated sensory neuropathy (HIV-SN) is a common complication of HIV infection and its treatment is characterized by symmetric lower-extremity pain and numbness. Both HIV infection and antiretroviral therapy (ART) are associated with development of HIV-SN, and both have been linked to mitochondrial toxicity in peripheral nerves [1]. In particular, treatment including the nucleoside reverse transcriptase inhibitor (NRTI) stavudine (d4T) [2–4], which is still a component of ART in some settings [5,6], is a known risk factor for HIV-SN, probably due to mitochondrial toxicity. Neuropathy risk is also increased in advanced HIV disease as demonstrated by associations with low CD4⁺ cell count and high HIV RNA level [7,8]. Few studies have carefully examined subclinical peripheral nerve damage in asymptomatic ART-naïve patients prior to, and after the initiation of therapy, or the role of host factors associated with such damage. Mitochondrial function, and thus, risk of ART toxicity, may be influenced by host genetic variation in mitochondrial DNA (mtDNA) [9–13]. Previous studies have identified associations with mtDNA variants and HIV-SN in persons of European and African descent [14–18].

Determination of epidermal nerve fiber density (ENFD) involves a skin punch biopsy and quantitation of the number of small nerve fibers in the epidermis after immunohistochemical staining. Reduced distal-leg ENFD has been associated with small unmyelinated nerve fiber damage and neuropathy risk in various diseases including HIV [19–21], but longitudinal ENFD assessments in clinical trials have not been performed in resource-limited settings. In a clinical trial in Thailand – the South East Asia Research Collaboration with Hawaii (SEARCH) 003 study – baseline ENFD prior to ART was higher than that seen in normative US populations, but lower ENFD was associated with previously identified risk factors including lower CD4⁺ cell count, older age, and increased height and BMI [22]. Because ART-associated ENFD changes and HIV-SN may be manifestations of mitochondrial

damage, peripheral blood mononuclear cell (PBMC) oxidative phosphorylation (OXPHOS) was assessed as part of SEARCH 003 by measuring mitochondrial complex I (reduced nicotinamide adenine dinucleotide [NADH] dehydrogenase) and complex IV (cytochrome c oxidase) activities and protein levels [22]. Higher baseline complex IV activity was associated with lower baseline ENFD in prior analyses [22]. Mitochondrial oxidative stress was determined by measuring 8-oxo-deoxyguanine (8-oxo-dG) levels [23].

In the SEARCH 003 study, changes in ENFD 24 weeks after initiation of one of the three NRTI combinations [d4T, zidovudine (ZDV), or tenofovir (TDF), each with lamivudine] were associated with baseline ENFD, monocyte HIV DNA, and PBMC complex I activity/levels, but were not associated with NRTI combination [24], and overt HIV-SN was rare in this population. Our objective was to determine if mtDNA haplogroups were associated with baseline and/or 24-week changes in ENFD in this clinical trial population. To our knowledge, this is the first study of mtDNA haplogroups in Asian HIV-infected individuals initiating ART, and the first assessing genetic predictors of changes in ENFD over time.

Methods

Study participants

The study reports baseline and 24-week findings in ART-naïve patients from SEARCH 003 (NCT00669487) [25]. SEARCH 003 was a 72-week clinical trial conducted at the Thai Red Cross AIDS Research Centre Anonymous Clinic in Bangkok, Thailand, and at the Queen Savang Vadhana Memorial Hospital in Chonburi, Thailand. The study compared a d4T 'switch' regimen [initial d4T-based (30mg twice daily) therapy switching to ZDV-based (250 mg twice daily) therapy at week 24 of the study] to continuous ZDV-based or continuous TDF-based (300 mg daily) therapies; each arm was combined with either lamivudine or emtricitabine and nevirapine at standard doses. The SEARCH 003 study was approved by the Chulalongkorn University Institutional Review Board (IRB) and the Queen Savang Vadhana Memorial Hospital IRB as the primary IRBs of record, and by the University of Hawaii Committee on Human Subjects and by the University of California San Francisco IRB as secondary IRBs. All research was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. Anonymized data were used in these secondary analyses.

Detailed study methods for SEARCH 003 are reported elsewhere [22,25]. Briefly, inclusion criteria were documented HIV infection, age at least 18 years, CD4⁺ cell count below 350 cells/μl and ART-naïve status. Women with prior ART exposure during pregnancy were allowed to enroll provided the exposure was at least 3 months prior to entry. Exclusion criteria included active AIDS-defining or other illness, current use of immunomodulatory or experimental therapy, pregnancy or breast-feeding, and the presence of active malignancies. Patients underwent a neuropathy examination during the screening process utilizing the AIDS Clinical Trials Group (ACTG)/Neurology and Neurologic AIDS Research Consortium (NARC) methodology [20]. One patient with asymptomatic neuropathic signs (diminished sensation by any modality – vibratory, pin, or temperature sensation or contact allodynia – detected bilaterally in the lower extremities, or absent or diminished ankle reflexes relative to knees) at baseline was excluded from the study because of the potential

risk of randomization to the d4T-containing arm. Baseline medical history and general physical examination, and routine safety labs, CD4⁺ cell count, HIV RNA and fasting metabolic assays including glucose levels were performed as described previously [22,25]. Patients were assessed as described above for development of neuropathy during follow-up. HIV-SN was defined as presence of neuropathic signs (defined above) or peripheral neuropathy (reduced vibration sensation in both great toes or diminished ankle reflexes bilaterally relative to knees).

Epidermal nerve fiber density assessment

Skin punch biopsies for ENFD were performed as optional assessments in the SEARCH 003 study prior to initiation of ART using skin punch biopsy technique and processing as recommended by the Cutaneous Nerve Laboratory at Johns Hopkins University [26]. Briefly, following a 1% lidocaine injection subcutaneously and utilizing sterile techniques, a 4-mm skin punch biopsy was performed on the distal leg at the level of the ankle, with an additional skin punch biopsy of the upper lateral thigh as an internal control. Skin specimens were preserved on site and then forwarded, via the University of Hawaii, to the Cutaneous Nerve Laboratory at Johns Hopkins for PGP9.5 immunostaining. The biopsies were sectioned with a sliding microtome into 50- μ m thick frozen vertical free-floating sections for immunostaining. Four sections at fixed intervals from each biopsy were selected, washed in Tris-buffered saline (TBS), and nonspecific binding of secondary antibodies was blocked with 5% normal goat serum, 1.0% Triton X-100, 0.5% nonfat powdered milk in TBS, pH 7.4. The sections were incubated overnight with primary antibody PGP 9.5 (AbD SeroTec, Raleigh, North Carolina, USA; dilution 1 : 5000) followed by biotinylated secondary antibody (goat antirabbit IgG) at room temperature. Endogenous peroxidase was blocked with 1% H₂O₂ in 30% methanol/phosphate-buffered saline and incubated with Avidin-Biotin Complex solution (Vector Laboratories, Burlingame, California, USA) for 1 h, followed by substrate incubation for 8–10 min (blue/gray chromophore; Vector Laboratories).

Stained sections were examined to ensure acceptable specimen quality, and the slides were blinded and clinical information was masked for quantitation. The number of unmyelinated nerve fibers per mm of epidermis (include nerve fragments in epidermis that do not cross the epidermis) was quantified by a single investigator (G.J.E.) using a standardized counting rule that was routinely followed in JHU laboratory [27,28] (see Figure, Supplemental Digital Content 1, <http://links.lww.com/QAD/A521> for an example of ENFD quantitation).

Assessment of peripheral blood mononuclear cell mitochondrial parameters

Viably preserved PBMCs were obtained for analyses as described previously [22]. Briefly, mtDNA copies/cell were assayed by real-time PCR (RT-PCR) and by measuring the 90 base-pair mtDNA-encoded NADH dehydrogenase (subunit 2) and the nuclear DNA-encoded 98 base-pair Fas Ligand gene [29]; PBMC complex I and complex IV protein and activities were measured by thin layer chromatography and immunoassays [30,31] and reported as optical density (OD)/ μ g protein $\times 10^3$; and mitochondrial 8-oxo-dG was assessed using a gene-specific repair assay [23] and reported as break frequency.

Mitochondrial DNA sequencing and haplogrouping

Human DNA was isolated from cryopreserved PBMCs using standard extraction techniques and RT-PCR. mtDNA sequencing was performed using the GeneChip Human Resequencing Array v2.0 (Affymetrix Inc., Santa Clara, California, USA) [32]. Sequence data were used to assign haplogroups and subhaplogroups based on Phylo-Tree classification [33] using HaploGrep software [34]. Analysis groups were determined by major branch points in the human mtDNA phylogenetic tree and to optimize group sample sizes (see Figure, Supplemental Digital Content 2, <http://links.lww.com/QAD/A521>).

Statistical methods

Univariate correlations and associations between ENFD outcome variables and parameters including mtDNA haplogroups were assessed by Spearman correlation, Kruskal–Wallis and Wilcoxon rank-sum tests. Adjusted associations were tested using multiple linear regression. Predictors of decreased ENFD (any negative change from baseline) at week 24 as a dichotomous outcome were assessed by logistic regression. For multivariate analyses of neuropathy outcomes, randomized ART regimen was dichotomized as the d4T-containing arm vs. a combined group of those randomized to ZDV or TDF-containing regimens. Analyses of 24-week outcomes were considered intent-to-treat with respect to NRTI randomization arm. Covariates were selected based on previous report of their association with neuropathy and predictors of baseline and 24-week ENFD changes from primary SEARCH 003 analyses [22,24].

Results

Baseline characteristics and haplogroup frequencies

The SEARCH 003 study included 150 randomized participants, 118 of whom had baseline and 24-week ENFD data (Fig. 1). Baseline demographic and clinical characteristics of these participants are shown in Table 1. All participants were ethnic Thais. The sex distribution (56% women) was consistent with that of the HIV epidemic in Thailand. The percentage of participants with other common causes of peripheral neuropathy (history of isoniazid use, concomitant infection with hepatitis C, and/or presence of diabetes) was small (<5%). Median [interquartile range (IQR)] baseline ENFD was 20.9 (16.5, 25.6) fibers/mm.

Prevalences of the major Asian haplogroups used in analyses are shown in Fig. 1 and Table 1. The M macro-group was most frequent (42%), with haplogroups F and B comprising 21 and 16% of the population, respectively. Other N and R macro-groups made up the remaining 20%. These frequencies are similar to those seen in HIV-negative Thai populations [35–37]. Since DNA was available for all study participants, including those without ENFD, we were able to confirm that distribution of major mtDNA haplogroups did not differ significantly between those with and without ENFD data (data not shown). Detailed subhaplogroup frequencies within each analysis group are shown in the table (Supplemental Digital Content 3, <http://links.lww.com/QAD/A521>).

Baseline characteristics by major mtDNA haplogroup are shown in Table 1. Individuals having mtDNA haplogroup B were older (median 40 years) at baseline compared with other

haplogroups (median 34 years; Kruskal–Wallis $P=0.08$; Wilcoxon $P=0.02$ for haplogroup B vs. all others). No other baseline characteristics differed significantly by mtDNA haplogroup, including baseline distal-leg ENFD. In prior analyses, statistically significant predictors of lower baseline ENFD included older age, higher BMI, greater height, lower CD4⁺ cell count, and higher PBMC complex IV activity [22].

Twenty-four-week changes in epidermal nerve fiber density

In patients who underwent ENFD assessment, 36, 33, and 31% were randomized to d4T, ZDV, and TDF-containing regimens, respectively, and major mtDNA haplogroups did not differ significantly by arm (chi-square $P=0.23$). Ninety (76%) patients had undetectable plasma HIV RNA levels (< 40 copies/ml) at week 24. Median changes in distal-leg ENFD by major haplogroups at week 24 are shown in Fig. 2a. Overall, there was a median decrease of 2.3 (IQR $-6.8, +2.1$) fibers/mm at week 24. In primary analyses of 24-week ENFD changes, higher baseline ENFD, monocyte HIV DNA content, and PBMC complex I protein levels were significant predictors of decreased ENFD [24]. NRTI randomization arm was not significantly associated with ENFD changes in primary analyses or in the subgroup analyzed here ($P=0.47$). However, patients belonging to mtDNA haplogroup B had a median ‘increase’ in ENFD of 1.5 (IQR $-2.3, +5.1$) fibers/mm at week 24 compared with a ‘decrease’ of 2.9 ($-7.2, +1.5$) fibers/mm among those belonging to other haplogroups ($P=0.03$; Fig. 2a). A total of 75 participants (64%) had a decrease in ENFD from baseline. Of the 19 participants belonging to the mtDNA haplogroup B, 6 (32%) had a decrease in ENFD over 24 weeks compared with 69 of 99 (70%) of those belonging to other haplogroups ($P=0.002$).

In multivariate analyses of 24-week change in distal-leg ENFD (Table 2), older age ($P=0.02$), higher CD4⁺ cell count ($P=0.03$), greater PBMC complex I protein level ($P=0.03$) and greater ENFD ($P<0.001$) at baseline were all significantly associated with lower ENFD after 24 weeks of ART. mtDNA haplogroup B was significantly associated with an ‘increase’ in ENFD ($P=0.009$), after adjustment for the factors above, as well as for sex, height, and randomization arm. Similar associations were seen in a multivariate logistic model of any decrease in ENFD from baseline to week 24 (Table 3). Patients who had a decrease in ENFD at week 24 were significantly less likely to belong to the mtDNA haplogroup B [adjusted odds ratio 0.10; 95% confidence interval (CI) 0.02, 0.37; $P<0.001$].

Twenty-four-week changes in peripheral blood mononuclear cell mitochondrial parameters

There were no statistical differences in baseline PBMC mitochondrial parameters by mtDNA haplogroups (data not shown). Overall, 8-oxo-dG did not change significantly after 24 weeks of ART (median break frequency change=0.00), but among individuals belonging to haplogroup B, 8-oxo-dG break frequency increased marginally compared to those from other haplogroups ($+0.05$ vs. 0.00 change; $P=0.05$; Fig. 2b). Although baseline PBMC complex I protein levels were associated with ENFD changes in multivariate analyses (Tables 2 and 3), changes in mitochondrial parameters at 24 weeks did not correlate with ENFD changes.

HIV-associated sensory neuropathy

HIV-associated sensory neuropathy was rare in this population after 24 weeks of ART. A total of six individuals (5%) developed HIV-SN defined as reduced vibration sensation in both great toes or diminished ankle reflexes bilaterally relative to knees. Persons who developed HIV-SN were older at baseline than those without HIV-SN (median age 41 vs. 34 years, respectively), but as in the primary analyses, there was no statistically significant association between HIV-SN and ART arm. Of these six individuals, three belonged to haplogroup B; thus the rate of HIV-SN among haplogroup B individuals was 16% (3/19) compared with 3% (3/99) of persons belonging to other haplogroups ($P=0.05$). Neither ENFD nor PBMC mitochondrial parameters differed significantly between patients with and without HIV-SN, but small sample sizes limited the power of these comparisons.

Discussion

In a clinical trial of HIV-infected Thai patients initiating ART, we found that longitudinal changes in distal-leg ENFD over a 24-week period – a surrogate measure of peripheral nerve damage – differed by mtDNA haplogroup. The median ENFD was higher (>20 fibers/mm) in these ART-naïve patients than that typically seen in the US populations, consistent with differences seen in healthy (HIV-uninfected) US and Thai patients, and highlighting ethnic differences in ENFD [38]. A longitudinal study found that ENFD 10 fibers/mm or less was associated with the greatest risk of progression to symptomatic HIV-SN [39], perhaps explaining the low rates of HIV-SN in this population. Patients belonging to the mtDNA haplogroup B were significantly older (median age >6 years older), and, unexpectedly, actually showed an increase in distal-leg ENFD during the first 24 weeks of ART. Given that the same group demonstrated a significantly greater increase in 8-oxo-dG, which is a marker of mtDNA oxidative damage, and were more likely to manifest HIV-SN, these results suggest that in this population, increased ENFD during the first 24 weeks of ART may represent a compensatory response to nerve damage. Specifically, this increase in ENFD may reflect regenerative regrowth and increased intraepidermal branching of nerve fibers (Figure, Supplemental Digital Content 1, <http://links.lww.com/QAD/A521>). Additional studies will be required to address this hypothesis, as well as possible explanations for why these changes might differ by mtDNA haplogroup.

Reasons for persons from a given haplogroup being older at baseline (without significant differences in CD4⁺ cell count or HIV RNA levels) are unknown. Estimates of length of HIV infection, or time from infection to diagnosis, are not available for these patients, but future studies in this or related populations might explore differences in HIV acquisition or disease progression by haplogroup. Similar studies from HIV-infected persons of European ancestry have reported such differences [40,41]. Although population-based data from Thailand are limited, mtDNA haplogroup frequencies have been similar to those previously reported [35–37], arguing against a significant impact of haplogroup on HIV acquisition risk. Nonetheless, the similar ENFD at baseline and ENFD gain on ART among the B haplogroup despite being older (and independent of other risk factors for ENFD change in this population) are notable and merit further study.

Other notable findings from these data are the unexpected relationship between higher baseline CD4⁺ cell count and greater decrease in ENFD on ART. In baseline analyses of ENFD, lower baseline CD4⁺ cell count was associated with lower baseline (pre-ART) ENFD [22]. The majority of published studies have reported lower baseline CD4⁺ cell count to be a predictor of clinically diagnosed HIV-SN on ART, but few of these included ENFD [19]. These data suggest that there may be differences in the role CD4⁺ cell count plays as a marker and/or mediator of ENFD changes – and subsequent HIV-SN risk – before and during ART. There are longitudinal ENFD data that suggest a progressive loss of epidermal nerve fibers over time in HIV-uninfected diabetic populations (unpublished data), and that women may regenerate epidermal nerve fibers more quickly than men following standardized nerve injury [42]. Few studies to date have carefully examined ENFD changes over time in ART-naïve, HIV-infected persons starting treatment in a randomized fashion; so little is known about the dynamics of ENFD in this setting. Height was included in multivariate analyses based on prior studies (including in Asian populations) demonstrating increased height as a risk factor for HIV-SN [43]. Although shorter stature could be a protective factor in some populations, height was not independently associated with ENFD changes in this analysis, nor did it explain the association between mtDNA haplogroup B and ENFD changes.

The trial included ex-vivo measures of mitochondrial biogenesis (mtDNA copy number), function (complex I and complex IV activity and levels) and mtDNA damage (8-oxo-dG) in PBMC. Prior analyses identified associations between higher baseline complex IV activity and lower baseline ENFD [22], and between baseline complex I levels and 24-week ENFD changes [24]. It is notable both that these measures of mitochondrial function did not appear to differ by haplogroup, and that complex I level at baseline was associated with ENFD change independent of haplogroup. This suggests that mechanistic links between mtDNA haplogroup and peripheral nerve damage may not primarily localize to complex I or complex IV, which were not significantly associated with ENFD changes in these analyses. Of note, mtDNA polymorphisms defining haplogroup B are either noncoding or synonymous (as is true for most haplogroups), and are not located in complex I or complex IV genes [33]. mtDNA haplogroup B is found primarily in East Asia, and is also a major haplogroup seen in Native Americans and persons of self-reported Hispanic ethnicity [44,45]. Haplogroups B and F are part of the R superhaplogroup, with some variations common to the closely related European haplogroups J and T [33]. Haplogroup B has been associated with several phenotypes in recently published studies [46–49], though none of these are obviously related to neuro-degeneration or peripheral nerve damage.

Despite the fact that this is the largest analysis of mtDNA haplogroups in an Asian HIV-infected population to date, these results are limited by relatively small haplogroup sample sizes. Taken together, these data suggest that paradoxically increased ENFD measured early after ART initiation may be an adverse, compensatory response to ART. However, measures of mitochondrial function (complex I and complex IV), biogenesis (mtDNA copy number), or mtDNA damage (8-oxo-dG) in PBMCs did not correlate with ENFD changes, and, with the exception of 8-oxo-dG, were not associated with mtDNA haplogroup; thus we can only speculate about mechanisms of haplogroup-associated risk. It is important to note that the mitochondrial functional measures and mtDNA quantification were from PBMC, which may

correlate only minimally, or not at all, to damage at the tissue (peripheral nerve) level. A lack of association with PBMC mtDNA quantification has also been seen in studies of lipodystrophy [29,50–52]. Similarly, ENFD was not significantly associated with clinically evident HIV-SN in this population, although this outcome was rare at 24 weeks. Reliable assessment of HIV-SN is difficult, thus it is possible that our cases were misclassified, and given the small numbers of cases, these results should be interpreted cautiously. Additionally, the question of relevance of findings from studies using older ART (e.g. d4T, ZDV) must be considered. The SEARCH 003 study was notable for the relative infrequency of HIV-SN during 24 weeks of ART [25], and that HIV-SN and ENFD at week 24 were not associated with initial ART. These findings may have been influenced by the use of lower-dose d4T (30mg twice daily), which has been associated with fewer adverse effects, including peripheral neuropathy [53,54]. The association between ENFD and mtDNA haplogroup B did not appear to be related to ART, thus may be relevant for populations that include this haplogroup irrespective of ART. Finally, given the relatively small number of genetic variants tested ($N=4$ haplogroups), we did not formally correct our results for multiple comparisons; however, the adjusted association between haplogroup B and ENFD decrease would remain significant after multiple testing correction. Analyses using longer-term follow-up data from this population, and replication studies in other cohorts or trial populations, are needed.

In conclusion, these data are unique in that they are from the only study combining mtDNA haplogroups, ex-vivo mitochondrial measures, and ENFD in a non-US HIV-infected population. That NRTIs were given in a randomized fashion is also a strength of these data. Although the results cannot be considered definitive, they suggest that after 24 weeks of ART, increased ENFD and/or PBMC 8-oxo-dG may be indicators of peripheral nerve damage, independent of specific ART, and that a relatively common Asian mtDNA haplogroup was associated with these measures independent of other known risk factors for HIV-SN.

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Fig. 1. Flowchart of patient distribution and mitochondrial DNA haplogroup frequencies
ENFD, epidermal nerve fiber density; mtDNA, mitochondrial DNA; wk, week.

Fig. 2. Plot of 24-week epidermal nerve fiber density (panel a) and 8-oxo-deoxyguanine (panel b) changes, by major mitochondrial DNA haplogroups

Boxes are median, 25th and 75th percentiles; lines are 5th and 95th percentiles. Outliers are shown. P -values are Wilcoxon rank-sum P for haplogroup B vs. all others. 8-oxo-dG, 8-oxo-deoxyguanine; ENFD, epidermal nerve fiber density.

Table 1

Baseline characteristics, overall and by major mitochondrial DNA haplogroup.

	Analysis group (N=118)	Major haplogroups				<i>P</i> ^b
		B (N=19)	F (N=25)	M (N=50)	Other ^a (N=24)	
Age (years)	34 (29–41)	40 (35–42) ^c	34 (29–39)	33 (28–38)	35 (29–46)	0.08
Female sex [N (%)]	66 (56)	10 (53)	12 (48)	31 (62)	13 (54)	0.68
Height (cm)	162 (155–168)	162 (156–171)	163 (160–173)	160 (153–165)	164 (157–167)	0.46
BMI (kg/m ²)	21.6 (19.5–23.8)	20.4 (18.4–22.8)	22.1 (19.2–27.3)	21.5 (19.7–23.6)	21.7 (20.3–23.6)	0.59
CD4 ⁺ cell count (cells/ μ l)	172 (69–232)	134 (81.5–189)	201 (97–250)	138 (66–228)	189 (94–231)	0.38
HIV RNA (log ₁₀ copies/ml)	4.9 (4.5–5.3)	5.2 (4.7–5.8)	4.8 (4.5–5.1)	4.9 (4.5–5.3)	5.0 (4.5–5.5)	0.24
HIV DNA (log ₁₀ copies/10 ⁶ cells)	2.3 (1.8–2.8)	2.2 (1.8–2.4)	2.1 (1.8–3.0)	2.3 (1.9–2.8)	2.4 (1.9–2.7)	0.74
Distal Leg ENFD (fibers/mm)	20.9 (16.5–25.6)	20.1 (17.8–23.2)	23.4 (18.5–29.6)	20.8 (15.9–24.0)	20.7 (15.3–27.7)	0.64
8-oxo-dG (BF)	0.05 (0.00–0.14)	0.01 (0.00–0.12)	0.07 (0.00–0.14)	0.04 (0.00–0.14)	0.06 (0.00–0.17)	0.84
mtDNA quantity (copies/cell)	181 (140–233)	193 (176–237)	175 (127–219)	180 (141–239)	173 (137–215)	0.61
PBMC Complex I activity (OD, $\times 10^3/\mu$ g protein)	93.7 (75.3–114.9)	88.6 (80.3–104.1)	92.6 (69.8–109.8)	94.2 (78.7–117.1)	94.2 (71.3–108.6)	0.84
PBMC complex I protein level	91.6 (76.9–113.1)	94.6 (79.9–119.0)	90.0 (78.1–105.1)	96.9 (73.9–116.1)	90.3 (80.8–113.4)	0.92
PBMC complex IV activity	93.9 (73.6–115.3)	91.2 (78.9–107.9)	95.0 (70.5–109.0)	101.4 (73.4–118.0)	88.3 (72.6–110.0)	0.77
PBMC complex IV protein level	76.7 (54.0–96.9)	87.5 (65.5–105.7)	66.9 (48.5–89.3)	74.6 (52.8–95.8)	81.4 (59.1–89.9)	0.49

Data are median [interquartile range (IQR)] unless otherwise noted. 8-oxo-dG, 8-oxo-deoxyguanine; BF, break frequency; ENFD, epidermal nerve fiber density; mtDNA, mitochondrial DNA; OD, optical density; PBMC, peripheral blood mononuclear cell.

^a Other=R (N=15) and N (N=6) macro-groups, and U haplogroup (N=3).

^b Kruskal–Wallis or chi-square test.

^c Wilcoxon rank-sum *P*=0.02, haplogroup B vs. all others.

Table 2

Multivariable linear model of change in epidermal nerve fiber density from baseline to week 24.

Variable	Parameter estimate (95% CI)	P-value
Age (per year increase)	−0.2 (−0.3, −0.03)	0.015
Height (per cm increase)	0.06 (−0.23, 0.12)	0.52
Baseline CD4 ⁺ cell count (per cell/μl unit)	−0.01 (−0.02, −0.001)	0.03
Sex (women vs. men)	−0.24 (−3.3, 2.8)	0.87
mtDNA haplogroup B (vs. others)	3.5 (0.9, 6.2)	0.009
ART (stavudine vs. zidovudine or tenofovir)	1.2 (−0.8, 3.2)	0.23
PBMC complex I protein level (per log ₁₀ OD×10 ³ /μg)	−3.6 (−6.7, −0.5)	0.03
Baseline ENFD (per fiber/mm)	−0.4 (−0.6, −0.3)	<0.001

ART, antiretroviral therapy; CI, confidence interval; ENFD, epidermal nerve fiber density; mtDNA, mitochondrial DNA; OD, optical density; PBMC, peripheral blood mononuclear cell.

Table 3

Multivariable logistic model of predictors of decreased epidermal nerve fiber density from baseline to week 24.

Variable	Odds ratio (95% CI)	P-value
Age (per year increase)	1.1 (0.99, 1.2)	0.12
Height (per cm increase)	1.0 (0.95, 1.1)	0.39
Baseline CD4 ⁺ cell count (per cell/ μ l unit)	1.0 (1.002, 1.01)	0.01
Sex (women vs. men)	1.8 (0.4, 7.8)	0.43
mtDNA haplogroup B (vs. others)	0.1 (0.02, 0.37)	<0.001
ART (stavudine vs. zidovudine or tenofovir)	1.2 (0.5, 3.4)	0.71
PBMC complex I protein level (per log ₁₀ OD $\times$ 10 ³ / μ g)	3.1 (0.6, 17.6)	0.19
Baseline ENFD (per fiber/mm)	1.2 (1.1, 1.3)	<0.001

ART, antiretroviral therapy; CI, confidence interval; ENFD, epidermal nerve fiber density; mtDNA, mitochondrial DNA; OD, optical density; PBMC, peripheral blood mononuclear cell.