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ORIGINAL ARTICLE

Association of mitochondrial DNA haplogroup, oxidative DNA damage and inflammatory markers in middle-aged adults

Jessica T. Smith, Nicole Noren Hooten, Nicolle A. Mode, Alan B. Zonderman, Ngozi Ezike and Michele K. Evans

Laboratory of Epidemiology and Population Sciences, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA

ABSTRACT

Purpose: Previous literature suggests that African American adults have higher levels of systemic oxidative stress than White adults. Mitochondrial DNA (mtDNA) variants can be traced to geographic origins of populations and classified into haplogroups. Emerging evidence indicates that mtDNA haplogroup may affect cellular stress responses such as the response to irradiation or oxidative stress. However, little is known about whether mtDNA haplogroup influences systemic baseline or induced levels of oxidative DNA damage.

Materials and Methods: We determined mtDNA haplogroup (African American or European) in 104 middle-aged adults living above and below poverty to investigate the association of mtDNA haplogroup and inflammatory markers with baseline and H_2O_2-induced DNA damage in peripheral blood mononuclear cells. DNA damage was measured using a high-throughput CometChip assay. Several inflammatory markers were measured in serum. Linear regression examined if variables were related to DNA damage in interactions or independently.

Results: mtDNA haplogroup alone did not influence levels of baseline or H_2O_2-induced DNA damage. However, baseline and H_2O_2-induced DNA damage were associated with poverty, sex, age, and markers of inflammation. Baseline DNA damage was related to IL-6 levels differentially based on poverty status. Baseline DNA damage was inversely associated with levels of IL-8. Men showed overall higher levels of H_2O_2-induced DNA damage. H_2O_2-induced DNA damage was also related to IL-6 levels but differentially based on age. mtDNA haplogroup did affect both baseline and H_2O_2-induced DNA damage through differential effects on two markers of inflammation.

Conclusion: These data suggest that mtDNA haplogroup plays an indirect role in the cellular response to endogenous and exogenous oxidative stress. Sex and age may also contribute to levels of inflammation in the DNA damage response. Finally, the social determinant of health poverty status, appears to play a role in interactions with inflammatory markers for baseline DNA damage at mid-life.

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Introduction

African American adults have been observed to have increased levels of systemic oxidative stress biomarkers compared to other racial or ethnic groups (Watters et al. 2009; Feairheller et al. 2011; Morris et al. 2012; Szanton et al. 2012; Deo et al. 2015; Strath and Sorge 2022). Oxidative stress arises from increased production of reactive oxygen species (ROS) or from reduced ROS intrinsic protection mechanisms. Oxidative stress leads to oxidative damage to DNA and other cellular components, which can in turn lead to inflammation and further increased levels of oxidative stress (Wautier et al. 2001; Pálmai-Pallag and Bachrati 2014). However, it is unclear whether the observed increase in oxidative stress in African Americans is associated with an increase in nuclear DNA damage as measured by the comet assay; previous studies have instead found lower oxidative DNA damage in African Americans (Watters et al. 2007; Watters et al. 2009; Divekar et al. 2024). Differences in levels of oxidative stress across varying populations may contribute to the development of health disparities in minority populations (Noren Hooten et al. 2022). The multi-level interacting pathways influencing these observed differences in oxidative stress across populations, as well as other factors that may be associated with oxidative damage, are incompletely understood.

Inflammation and oxidative stress can result in oxidatively damaged nuclear DNA and mitochondrial DNA (mtDNA) (Richter et al. 1988; Yakes and Van Houten 1997; Meira et al. 2008; Lonkar and Dedon 2011; Herst et al. 2017; Kay et al. 2019; Shimura et al. 2025). The 16 kb mitochondrial genome is susceptible to damage and mutations, given its lack of either introns or histones as well as its proximity to ROS (Soares et al. 2009; Roy et al. 2022). Ionizing

CONTACT Michele K. Evans me42v@nih.gov National Institute on Aging National Institutes of Health NIH Biomedical Research Center, 251 Bayview Boulevard, Suite 100 Room 4C-222 Baltimore, MD 21224, USA

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radiation can both directly and indirectly damage the mitochondrial genome, as 'bystander' mtDNA damage effects in nearby, non-irradiated cells have been documented after irradiation (Murphy et al. 2005; Averbeck and Rodriguez-Lafrasse 2021). Both inherited and novel mutations in the mitochondrial genome can affect mitochondrial function (Cann et al. 1987; Hagström et al. 2014). Cellular damage, including nuclear DNA damage, can also lead to mitochondrial stress and dysfunction (Hiebert et al. 2015; Fang et al. 2016; Wang et al. 2023; Shimura et al. 2025). Mitochondrial dysfunction can lead to impaired oxidative phosphorylation system activity, increased mtDNA oxidative damage, a reduction in mitochondrial quality, changes in mitochondrial dynamics, and increased oxidative stress (Hällberg and Larsson 2014; Herst et al. 2017; Xu et al. 2025). Oxidative stress can lead to increased inflammation; thus, mitochondrial dysfunction can result in a feedback loop of oxidative stress and chronic inflammation (Mills et al. 2017; Ju et al. 2025). Mitochondrial dysfunction is further linked to aging and to many age-related diseases such as cardiovascular disorders, diabetes mellitus, and cancer (Herst et al. 2017; Kraja et al. 2019; Li et al. 2019).

Mitochondrial function, which is influenced by mitochondrial genetic ancestry, may lead to observed variation in oxidative stress, as mitochondria are a major source of ROS in the cell (Balaban et al. 2005; Hällberg and Larsson 2014; Kenney et al. 2014b; Herst et al. 2017). Race is a social construct; self-identified race is not sufficient to determine mitochondrial genetic ancestry, especially in recently admixed populations such those identifying as African American (Torres-Gonzalez and Makova 2022). Consideration of ancestry as well as race is thus necessary to identify the possible molecular origins of disparities in systemic oxidative stress. As mtDNA is maternally inherited with little to no recombination, the presence or absence of certain polymorphisms indicate branch points in the human phylogenetic tree (Chen et al. 1995; van Oven and Kayser 2009). An individual's set of inherited mtDNA polymorphisms is their specific mitochondrial haplotype, and a set of similar haplotypes in a population which all descend from a common ancestor is termed an mtDNA haplogroup (van Oven and Kayser 2009). Specific haplogroup-defining mtDNA variants have been linked with an increased risk of age-related diseases such as cardiovascular disease (Bray and Ballinger 2017). Increased levels of systemic oxidative stress in African American adults may result from the influence of African L mtDNA haplogroups, which have been found to be present in the majority of the African American population, or perhaps through the presence of circulating cell-free mtDNA (ccf-mtDNA) in the circulation (Feairheller et al. 2011; Johnson et al. 2015; Dela Cruz and Kang 2018; Nakayama and Otsu 2018). Krzywanski et al. found that cell lines with the African L mtDNA haplogroup were prone to more oxidative stress than cell lines with the Eurasian H mtDNA haplogroup (Krzywanski et al. 2016). In addition, the molecular effects from discordance between mtDNA ancestry and self-identified race or nuclear DNA ancestry (mitonuclear discordance) are still unclear. Mitonuclear discordance in populations that were recently admixed, such as African American individuals, has been linked with lower mtDNA gene expression (Torres-Gonzalez and Makova 2022). Studies in cytoplasmic hybrid (cybrid) cell lines, which compared two or more cell lines that share nuclear DNA sequence but differ by mtDNA sequence, show that mtDNA also affects the silencing and expression of nuclear DNA (Fetterman and Ballinger 2019; Kopinski et al. 2019; Atilano et al. 2022). Studies in cybrid cells have shown that cybrids with African mtDNA haplogroups had differentially expressed genes related to inflammation when compared with European cybrids (Kenney et al. 2014b). African and European cybrids differ when exposed to exogenous stressors as well; mitochondrial function declined in only African cybrids in response to the cellular stressor amyloid-β (Atilano et al. 2022). Damaged or dysfunctional mitochondria can release mtDNA into the cytosol and circulation (Giordano et al. 2025; Shimura et al. 2025). Ccf-mtDNA, which resembles bacterial DNA, is recognized by DNA-sensing receptors that trigger inflammatory responses (Giordano et al. 2025). The release of ccf-mtDNA, and its detection in the cytoplasm or extracellular space, is associated with aging, cancer, stroke, and many other diseases and conditions (Nakahira et al. 2015). Based on data suggesting that mtDNA haplogroups are associated with inflammation and oxidative stress, we previously investigated mtDNA haplogroups and inflammatory protein levels and found that mtDNA haplogroups were related to levels of ccf-mtDNA carried in extracellular vesicles (EVs). EV mtDNA levels were highest in African American participants with African mtDNA ancestry. Also, EV mtDNA levels were associated with inflammatory markers (tumor necrosis factor alpha trimer, sRAGE) differentially across mtDNA haplogroup (Byappanahalli et al. 2024). However, the relationship between mtDNA haplogroup, inflammation, and oxidative stress remains unclear, and little is known about downstream effects such as nuclear DNA damage.

Although cybrid studies suggest that there may be an association between mtDNA haplogroup and oxidative stress, as well as downstream effects from oxidative stress such as oxidative DNA damage, there is limited experimental evidence from population-based studies connecting them. Thus, we sought to examine how mtDNA haplogroup and inflammatory markers are related to baseline and H_2O_2-induced DNA damage in peripheral blood mononuclear cells (PBMCs). Establishing whether mtDNA haplogroups are associated with DNA damage will contribute to the understanding of mechanisms that may drive aging and age-related diseases in different populations.

Methods

Cohort design

The HANDLS study was initiated in 2004 and is a prospective, longitudinal study of aging among 3,720 middle-aged (30–64 years old at baseline) self-identified African American and White adults from Baltimore City, Maryland (Evans et al. 2010). Participant data were collected through physical examinations and structured medical history interviews during visits to medical research vehicles. Participants in this

study sample (n = 104) had a physical examination, clinical laboratory assays, provided a blood sample, and were at least 40 years old at wave 4 (2013–2017). Poverty status was defined as household income above or below 125% of the 2004 US Federal Poverty Guidelines (US Department of Health and Human Services 2004). Participants with Hepatitis B, Hepatitis C, AIDS, or who were HIV positive were excluded. The sample was chosen randomly from those matching these criteria and balanced across sex at birth, hypertension diagnosis status (yes/no), and mtDNA haplogroup (Table 1). Approval for HANDLS was obtained from the Institutional Review Board of the National Institutes of Health, and all participants provided written informed consent.

Determination of mitochondrial haplogroups

mtDNA haplogroups were ascertained using allelic discrimination PCR assays performed by the Department of Ophthalmology Research, University of California Irvine (Kenney et al. 2014a). ABI Assay-by-Design (Applied Biosystems) was used to synthesize ten allelic discrimination primers that displayed the greatest coverage of European and African mtDNA haplogroups. Samples were run with TaqPath ProAmp Master Mix on a QuantStudio 5 system (Applied Biosystems). Data were analyzed with QuantStudio software, TaqMan Genotyper software, Microsoft Excel, and were compared with a simplified map of mtDNA lineages from www.mitomap.org to assign haplogroups to participants.

In order to match previous studies and identify factors that possibly affect the observed difference in oxidative stress in African Americans compared to other populations, participants were grouped into African, European, or Other mtDNA haplogroup ancestries (Venter et al. 2019; Torres-Gonzalez and Makova 2022; Zaidi et al. 2023; Byappanahalli et al. 2024). L0,1,2,4,5,6 and L3 were grouped into the African mtDNA haplogroup ancestry group; H, HV, J, K, T, U, and UK were grouped into the European mtDNA haplogroup ancestry group; and B-P-F-R, M, and N-A-Y-W-I-X were grouped into the Other mtDNA haplogroup ancestry group. Only participants whose self-identified race matched their mtDNA haplogroup ancestry were included in this study to rule out potential effects from mitonuclear discordance (Torres-Gonzalez and Makova 2022).

Table 1. Mitochondrial DNA haplogroup study sample (N = 104).

	African haplogroup (N = 52)	European haplogroup (N = 52)	Missing N (%)
Men, N (%)	26 (50)	26 (50)	0 (0)
African American, N (%)	52 (100)	0 (0)	0 (0)
Below poverty, N (%)	30 (58)	12 (23)	0 (0)
Age (years), mean (SD)	54.1 (8.0)	53.3 (7.4)	0 (0)
IFN-γ, mean (SD)	16.0 (74.3)	3.8 (5.8)	1 (1)
IL-6, mean (SD)	0.8 (2.2)	1.9 (7.7)	11 (11)
IL-8, mean (SD)	17.2 (14.9)	27.5 (24.3)	1 (1)
IL-10, mean (SD)	0.5 (2.2)	0.4 (0.7)	1 (1)
TNF-α, mean (SD)	0.5 (0.2)	0.7 (0.5)	1 (1)
High-Sensitivity C-Reactive Protein (mg/L), mean (SD)	4.6 (4.5)	3.3 (3.9)	0 (0)
Ferritin (ng/mL), mean (SD)	118.7 (114.3)	138.3 (248.3)	0 (0)
Uric acid (mg/dL), mean (SD)	5.5 (1.2)	5.4 (1.4)	0 (0)
White blood cell (count*10^9/L), mean (SD)	6.6 (1.8)	7.2 (2.0)	4 (4)

N: number; SD: standard deviation.
Demographic characteristics and clinical laboratory assay values for participants in the study sample.

Inflammatory proteins panel

Morning blood samples were collected from participants into BD Vacutainer® serum separator tubes. Samples were centrifuged at room temperature at 1142 *g* for 15 minutes with the brake on. Serum was then aliquoted into cryotubes and stored at − 80 °C until use. Serum samples were thawed, and 70 μl was centrifuged at 2,000 *g* for 3 minutes to remove any particulates. The supernatant was moved to a new tube, and 60 μl was used in the assay. Inflammatory proteins in serum were measured using the V-PLEX Proinflammatory Panel 1 Human Kit (K15049D-1), which includes IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α, following the manufacturer's guidelines (Meso Scale Discovery) on a MESO QuickPlex SQ 120 machine. Inflammatory proteins were skewed and were thus $\log_{10}$ transformed before statistical analysis. Proteins were removed from further analysis if more than 25% of sample values were below the limit of detection. The inflammatory proteins included in statistical analyses were IFN-γ, IL-6, IL-8, IL-10, and TNF-α.

Clinical laboratory assays

High-sensitivity C-reactive protein (hsCRP), ferritin, serum uric acid, and white blood cell (WBC) count were measured by Quest Diagnostics (Nichols Institute, Chantilly, VA). WBC counts were categorized as 'normal' or 'high' WBC levels. In order to take into account the lower neutrophil counts observed in African Americans, we utilized separate reference ranges for WBC counts from participants with African mtDNA haplogroup ancestry and WBC counts from participants with European mtDNA haplogroup ancestry (Coates et al. 2020). Participants with African mtDNA haplogroup ancestry were categorized as 'normal' levels if WBC counts were between 2.7–7.3 × 10^9/L, and 'high' levels if above 7.3 × 10^9/L. Participants with European mtDNA haplogroup ancestry were categorized as 'normal' levels if WBC counts were between 3.0–9.1 × 10^9/L, and 'high' levels if above 9.1 × 10^9/L. No participants had values lower than the given normal ranges.

CometChip assay

CometChip assays were performed as previously described (Ge et al. 2012; Smith et al. 2023). Molten 1% normal-melting point agarose in PBS (14 ml) was spread on a precut GelBond film (Lonza) and a custom-made polydimethylsiloxane

(PDMS) stamp was used to form an array of microwells for 3–5 minutes at room temperature (Wood et al. 2010; Ge et al. 2012). The PDMS stamp was removed with the addition of 5 ml PBS and excess agarose was removed. Microwell quality and integrity was assessed by placing the CometChip on a glass plate and visualizing under a bright field microscope. A bottomless 96-well plate (VWR) was placed over the CometChip and clamped in place with binder clips. Cells were immediately loaded into the CometChip as detailed below.

TK6 lymphoblastoid cells were used as controls in each CometChip experiment. TK6 cells were cultured and cryopreserved in one large batch in 90% FBS and 10% DMSO in aliquots of 90 μl and concentrations of approximately 100,000 cells/ml. Prior to each experiment, cryopreserved TK6 cells and cryopreserved PBMCs were briefly thawed in a 37 °C water bath and suspended in 5 ml RPMI medium supplemented with 20% FBS in a 15 ml conical tube. Cells were then centrifuged at 1,300 rpm for 3 min, the supernatant was removed, and cells were resuspended in 1 ml RPMI with 20% FBS.

CometChip experiments were performed as previously described (Smith et al. 2023). Each CometChip experiment contained both untreated and H_2O_2-treated TK6 cells in order to serve as inter-assay controls and standardize PBMC DNA damage between all CometChip experiments. In brief, PBMCs from the sample cohort as well as control TK6 cells were thawed and used in the CometChip assay. Cells (100 μl) were loaded into each well of the CometChip-96 well plate. Cells in the CometChip were embedded into the microwells by incubating at 37 °C and 5% CO_2 for 30 min. Cell loading in microwells were visually inspected using a light microscope. Medium was then removed, along with the bottomless 96 well plate. Excess cells were removed by gentle washing with 5 ml PBS and then inspected under the bright field microscope to confirm that >70% of the microwells still contained cells. 37 °C molten 1% low-melting point agarose in PBS was pipetted gently over the CometChip in 1 ml increments to overlay the microwells and allowed to gel at room temperature for 3 min, then at 4 °C for 5 min.

Triplicate wells were run for each participant PBMC sample and experiment condition. Cells were treated with H_2O_2 by placing another bottomless 96-well plate over the CometChip and pipetting a H_2O_2 solution (50 μM, diluted in PBS) into treatment wells, then placing the CometChip at 4 °C for 20 min. PBS was used as a non-treatment control. The CometChip was then placed into lysis solution (R&D Systems #4250-050-01) at 4 °C. Cells were lysed overnight.

The CometChip was incubated in alkaline electrophoresis solution (NaOH 300 mM, Na_2EDTA 1 mM) (Ge et al. 2012) for 30 min at 4 °C before running the gel at 22 V and ~300 mA for 30 minutes in the R&D CometAssay Electrophoresis System (Sykora et al. 2018). The CometChip was then neutralized two times by the addition of neutralization buffer (Tris 400 mM, pH 7.5) for 15 min (Ge et al. 2012). DNA was stained with 20 ml of 1x SYBR Gold (ThermoFisher) in PBS for 1 hour at 4 °C, then imaged in a Keyence BZ-X800 microscope at 4x magnification. A standard exposure was used for all CometChip experiments. Individual (>800) images of each CometChip at 4x were stitched together using Keyence software to obtain a full image of each CometChip assay. Full images of each well were then extracted from CometChip assay pictures to be analyzed.

Statistical analysis

CometChip images were analyzed using the Comet Assay Software from R&D (Sykora et al. 2018). Data were exported and processed in R (Version 4.5) (R Core Team 2025). Median % DNA in tail was used as a measure of DNA damage level for every CometChip well. In each well, an average of approximately 150 comets were analyzed. The mean was calculated for median % DNA in tail for each participant from three technical replicate wells. Median % DNA in tail measurements for control TK6 cells had a mean intra-assay coefficient of variation (CV) of 7.7% and inter-assay CV of 16.8%. We reduced inter-experiment variability by standardizing the PBMC DNA damage measurements to control TK6 cells on each CometChip experiment. The data for participant PBMC % DNA in tail measurements from each individual CometChip experiment was standardized across the whole cohort using the following equation:

$$\text{Standardized Participant \% DNA in tail} = \frac{\text{Participant PBMC \% DNA in tail} - \text{Mean Chip PBMC baseline \% DNA in tail}}{\text{Mean Chip TK6 } H_2O_2 \text{ \% DNA in tail} - \text{Mean Chip TK6 baseline \% DNA in tail}}$$

The average cohort PBMC baseline % DNA damage in tail was subtracted from all individual participant PBMC baseline % DNA damage in tail values, and then this value was divided by the difference between the H_2O_2-treated TK6% DNA damage in tail values and untreated TK6% DNA damage in tail values (Trzeciak et al. 2008).

We utilized multivariable linear regression to examine if mtDNA haplogroup and other variables of interest were related to baseline and H_2O_2-induced DNA damage as measured by standardized % DNA in tail. The other variables of interest were inflammatory markers (IFN-γ, IL-6, IL-8, IL-10, TNF-α, hsCRP, uric acid, WBC levels, ferritin), sex, poverty status, and age. Positively skewed variables were $\log_{10}$ transformed for analysis (IFN-γ, IL-6, IL-8, IL-10, TNF-α, hsCRP, ferritin) and age was modeled in decade units from age 50 ((age − 50)/10). For both baseline and H_2O_2-induced DNA damage, two sets of regression models were examined in order to understand potential interactions among the variables. The first set started with the potential three-way interaction of mtDNA haplogroup, sex, and poverty status along with age (haplogroup * sex * poverty status + age), using backwards elimination to remove non-significant interactions. The second set started with all possible two-way interactions between the main covariates and each inflammatory marker (*marker**haplogroup + *marker**sex + *marker**poverty status + *marker**age), using backwards elimination to remove non-significant interactions. A p-value of <0.05 was considered statistically significant.

Results

To detect whether baseline DNA damage or H_2O_2-induced DNA damage were associated with mtDNA haplogroup and inflammatory markers in middle-aged adults, we performed high-throughput alkaline CometChip experiments using unstimulated, cryopreserved PBMCs from 104 participants that were between the ages of 40 and 71 years old and balanced across mtDNA haplogroup and sex (Table 1). The CometChip assay is a high-throughput method that measures DNA damage including DNA breaks, alkaline labile sites, and transient repair sites in cells embedded in agarose (Ostling and Johanson 1984; Singh et al. 1988). When run under alkaline conditions, damaged DNA will migrate during electrophoresis to form a comet 'tail'. CometChip utilizes an evenly spaced array of microwells, enabling a more high-throughput assay compared to the original comet assay (Ge et al. 2012; Ge et al. 2014). Additionally, we measured inflammatory markers; IFN-γ, IL-6, IL-8, IL-10, TNF-α, hsCRP, ferritin, uric acid, and WBC level. We conducted two sets of regression analyses. First, we examined how mtDNA haplogroup, sex, poverty status and age were related to DNA damage. Next, we explored how the inflammatory markers alone or along with mtDNA haplogroup, sex, poverty status, and age were related to DNA damage.

Baseline DNA damage is associated with inflammatory markers differentially by mtDNA haplogroup, sex, poverty status, and age

First, we examined if mtDNA haplogroup, sex, poverty status, and age were related to baseline DNA damage in interactions or independently. We did not find any significant associations for mtDNA haplogroup, sex, poverty status, and age with baseline DNA damage (Table S1A). Next, we examined if inflammatory markers were related to baseline DNA either independently or differentially by mtDNA haplogroup, sex, poverty status, or age (Table S1B). IL-6 was related to baseline DNA damage depending on poverty status (Figure 1A, $P = 0.020$). Participants living below poverty displayed an inverse relationship between baseline DNA damage and IL-6 levels where high IL-6 levels were associated with lower baseline DNA damage. Participants living above poverty has similar levels of baseline DNA damage independent of IL-6 values. WBC level was also related to baseline DNA damage depending on poverty status; among participants with high WBC levels, those living above poverty had higher average baseline DNA damage than those living below poverty (Figure 1B, $P = 0.016$). However, participants with normal WBC levels had similar amounts of baseline DNA damage for those above and below poverty status. IL-8 values were inversely related to baseline DNA damage with high IL-8 values associated with low baseline DNA damage (Figure 1C, $P = 0.016$). Although baseline DNA damage was not associated with mtDNA haplogroup, our inflammatory marker findings indicated that poverty status is associated with differential baseline DNA damage.

Sex ($P = 0.014$) and age ($P = 0.012$) were related to baseline DNA damage depending on WBC level (Figure 2A and 2B). Among participants with high WBC levels, men had higher baseline DNA damage than women, while women and men with normal WBC levels were similar. Participants with high WBC levels had an inverse relationship between baseline DNA damage and age, while participants with normal WBC levels did not (Figure 2B, $P = 0.012$). Finally, mtDNA haplogroup was related to baseline DNA damage only in the context of measured serum uric acid (Figure 2C, $P = 0.033$). Participants with African mtDNA haplogroups displayed a positive relationship between uric acid and baseline DNA damage, while participants with European mtDNA haplogroups did not. Our results are in agreement with previous studies reporting sex differences in baseline DNA damage (Møller 2019; Milić et al. 2021). The findings indicating altered baseline DNA damage with high WBC levels suggest that inflammation affects DNA damage pathways differentially with sex and age. Our finding that baseline DNA damage varies with serum uric acid and mtDNA haplogroup is novel and suggests that mtDNA ancestry plays a role in relationship between DNA damage and uric acid.

H_2O_2-induced DNA damage is associated with inflammatory markers differentially by mtDNA haplogroup, sex, poverty status, and age

First, we examined if mtDNA haplogroup, sex, poverty status, and age were related to H_2O_2-induced DNA damage in interactions or independently (Table S1A). Sex was related to H_2O_2-induced DNA damage with men having higher mean H_2O_2-induced DNA damage than women (Figure 3A, $P = 0.006$). We did not find any significant associations for mtDNA haplogroup, poverty status, or age alone with H_2O_2-induced DNA damage. Next, we examined if inflammatory markers were related to H_2O_2-induced DNA either independently or differentially by mtDNA haplogroup, sex, poverty status, or age (Table S1C). Similar to the pattern observed in baseline DNA damage, WBC level was related to H_2O_2-induced DNA damage depending on poverty status (Figure 3B, $P = 0.005$). Among participants with high WBC levels, those living above poverty had higher average H_2O_2-induced DNA damage than those living below poverty. Among participants with normal WBC levels, the pattern was reversed with those living above poverty having less average H_2O_2-induced DNA damage than those living below poverty. IL-6 was related to H_2O_2-induced DNA damage but differentially by age where younger participants displayed a positive relationship between IL-6 and H_2O_2-induced DNA damage and older participants displayed an inverse relationship (Figure 3C, $P = 0.026$). Finally, mtDNA haplogroup was related to H_2O_2-induced DNA damage only in the context of measured ferritin (Figure 3D, $P = 0.024$). Participants with African mtDNA haplogroups showed a positive relationship between ferritin and H_2O_2-induced DNA damage, while participants with European mtDNA haplogroups did not. Our finding that men displayed higher H_2O_2-induced DNA damage is novel and consistent with reported sex differences in

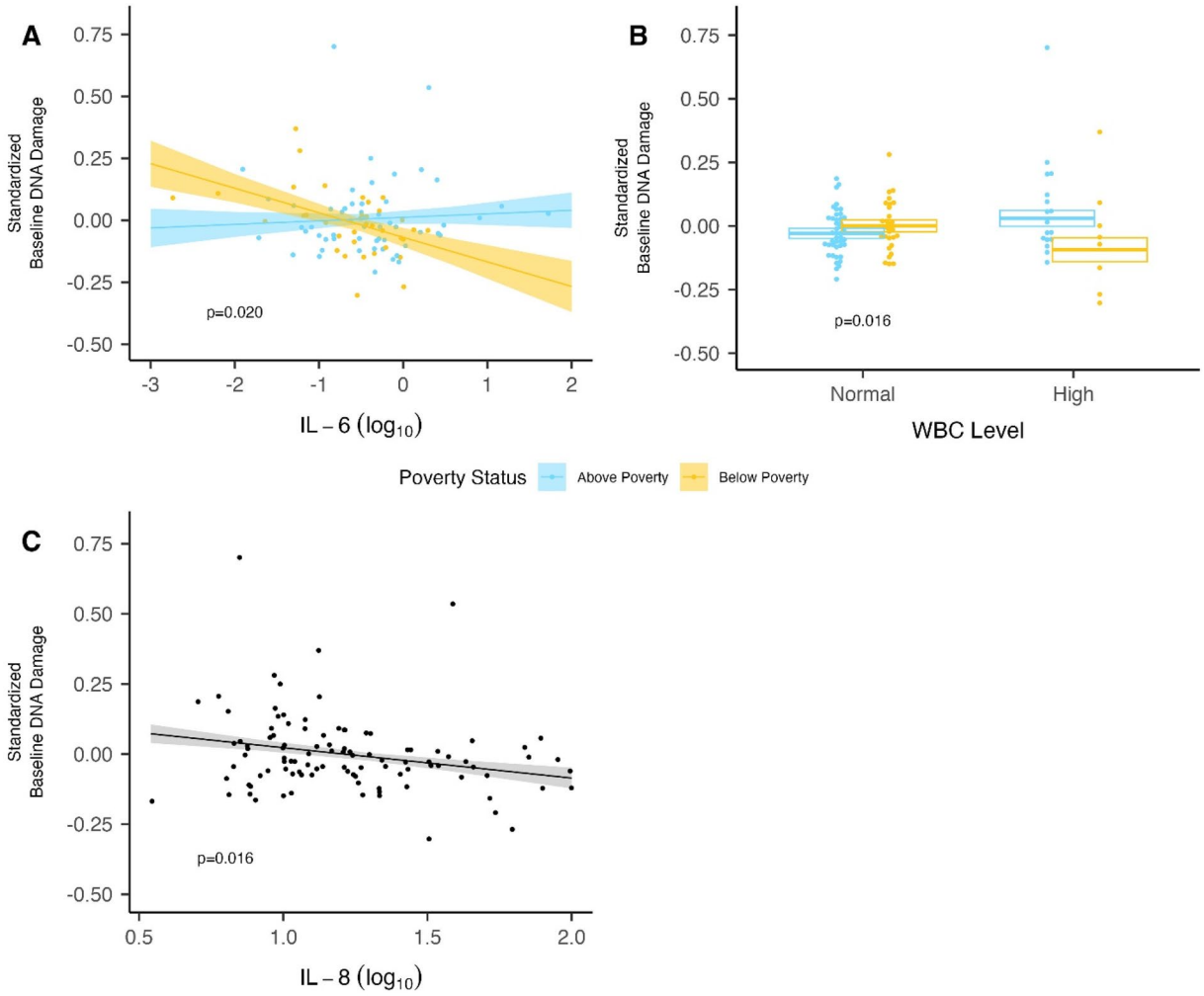

Figure 1. Baseline DNA damage is associated with poverty status, IL-6, IL-8, and WBC level. Linear regression examined if inflammatory markers were related to baseline DNA either independently or differentially by mtDNA haplogroup, sex, poverty status, or age. Plots show measured data values (points) along with estimated means (center line) and standard error of the mean (colored band or edge of boxes) from the regression models. (A) There is a significant interaction between IL-6 and poverty status for baseline DNA damage ($P = 0.020$). (B) There is a significant interaction between WBC level and poverty status for baseline DNA damage ($P = 0.016$). (C) There is a significant relationship between IL-8 and baseline DNA ($P = 0.016$).

baseline DNA damage (Møller 2019; Milić et al. 2021). Results pointing toward lower baseline and H_2O_2-induced DNA damage in participants living below poverty with high WBC levels suggest altered DNA damage pathways in response to poverty. Our results showing differential H_2O_2-induced DNA damage responses with age and IL-6 levels point toward different responses to the multifunctional cytokine over the lifespan. Finally, our findings suggest that African mtDNA ancestry may confer sensitivity to DNA damage with high ferritin levels.

Discussion

In this study, we examined the relationship between inflammatory markers and baseline and H_2O_2-induced DNA damage in unstimulated, cryopreserved PBMCs in a cohort of middle-aged participants balanced across sex and mtDNA haplogroup. In order to limit potentially confounding effects from mitonuclear discordance, we limited our cohort to participants whose self-identified race matched their mtDNA haplogroup ancestry. Although previous reports suggest that higher levels of oxidative stress correlate with self-reported race, we did not find an association between African mtDNA ancestry and oxidative DNA damage as measured by the alkaline comet assay (Feairheller et al. 2011; Morris et al. 2012; Szanton et al. 2012; Strath and Sorge 2022). We found that men displayed overall higher levels of H_2O_2-induced DNA damage than women. We also showed that both baseline DNA damage and H_2O_2-induced DNA damage were affected by interactions between WBC level and poverty status. We found an overall inverse association between levels of IL-8, a pro-inflammatory cytokine, and baseline DNA damage in our cohort. Additionally, we identified that age played a role in interactions with WBC levels and IL-6 for baseline DNA damage and H_2O_2-induced DNA damage, respectively. Finally, we found that baseline and H_2O_2-induced DNA damage were affected by interactions involving mtDNA haplogroup and the inflammatory markers uric acid and ferritin, respectively. Participants with African mtDNA haplogroups displayed positive relationships between DNA damage and uric acid or ferritin levels; participants with European mtDNA haplogroups displayed flat or inverse relationships.

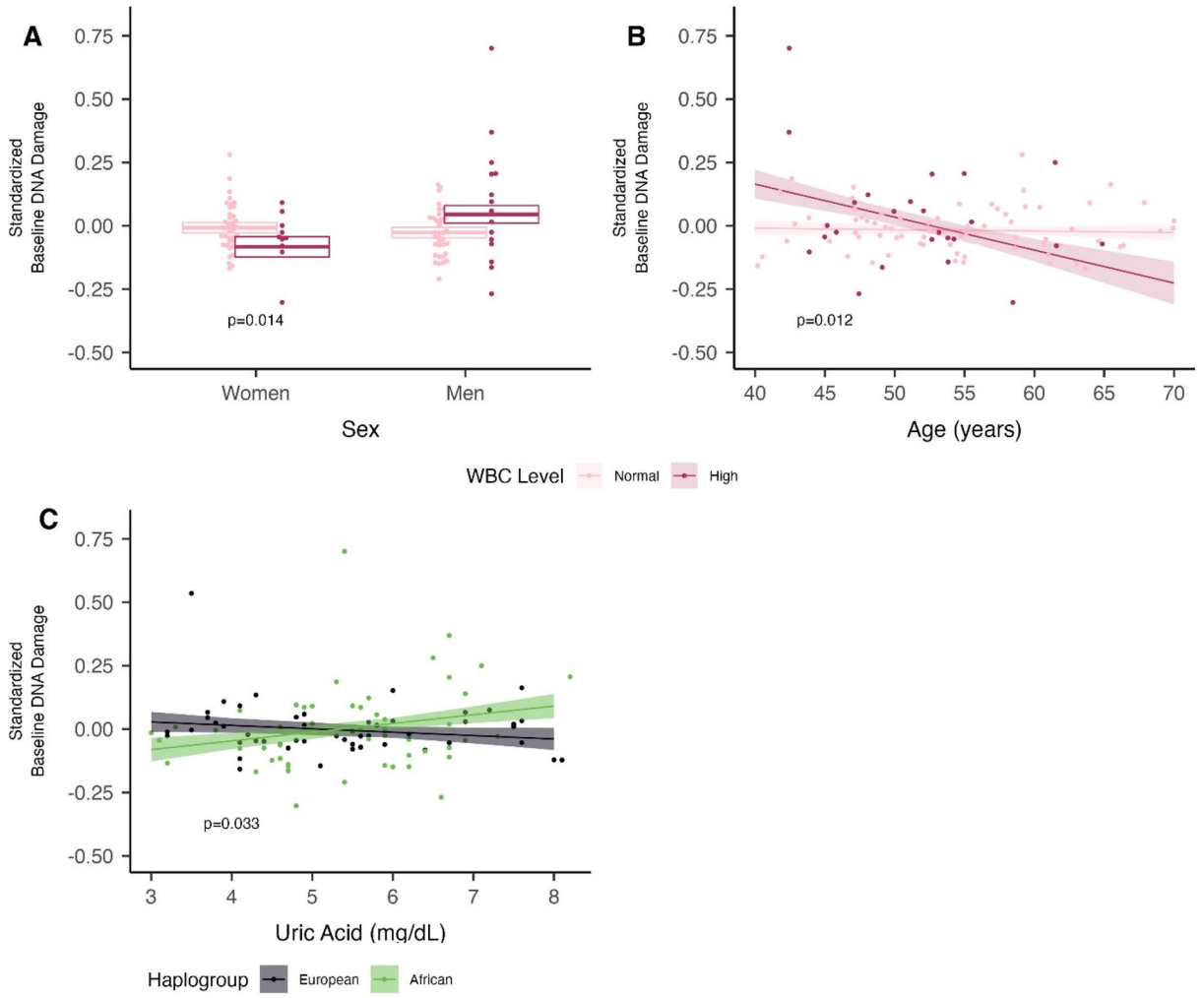

Figure 2. Baseline DNA damage is associated with sex, age, WBC level, uric acid, and mtDNA haplogroup. Linear regression examined if inflammatory markers were related to baseline DNA either independently or differentially by mtDNA haplogroup, sex, poverty status, or age. Plots show measured data values (points) along with estimated means (center line) and standard error of the mean (colored band or edge of boxes) from the regression models. (A) There is a significant interaction between sex and WBC level for baseline DNA damage ($P = 0.014$). (B) There is a significant interaction between age and WBC level for baseline DNA damage ($P = 0.012$). (C) There is a significant interaction between mtDNA haplogroup and uric acid for baseline DNA damage ($P = 0.033$).

In our cohort, we found that men displayed overall higher levels of H_2O_2-induced DNA damage than women. We did not find an association between sex and baseline DNA damage. Studies on DNA damage via the alkaline comet assay generally indicate that men display slightly higher or similar amounts of baseline DNA damage than women (Møller 2019; Milić et al. 2021). However, to the best of our knowledge, our study is the first to examine baseline and H_2O_2-induced DNA damage in men and women in the context of African and European mtDNA haplogroups. The difference in induced damage between men and women may point to varying antioxidant status in our cohort; supplementation with antioxidant- or nutrient-rich foods or food products has been shown to reduce induced DNA damage in several studies, both in vitro and in vivo (Duthie et al. 1996; Duthie and Dobson 1999; Collins et al. 2003; Fenech et al. 2023). A recent review including analysis of 25 studies utilizing the comet assay to measure DNA damage found a slight increase in baseline DNA damage in men compared to women, although the effect was not robust in adjusted analyses (Møller 2019). Sex differences have also been found in DNA repair; however, assessing the true effect of sex on DNA repair is similarly difficult due to conflicting results or cohort differences (Azqueta et al. 2019; Zheng et al. 2025). Sex differences in DNA damage and repair may help explain some of the observed sex differences in cancer incidence (Payne 2007; Scelo et al. 2018; Dong et al. 2020). This data suggests that baseline and induced DNA damage is affected by several interacting factors. To the best of our knowledge, our study is the first to report higher H_2O_2-induced DNA damage in men compared to women.

The impact of poverty status and age on DNA damage assessed by the alkaline comet assay is unclear (Soares et al. 2014; Møller 2019; Milić et al. 2021). Studies have shown that poverty status is associated with increased inflammation and an increase in the rate of aging (Shen et al. 2023; Dalecka et al. 2024; Arnold et al. 2025). In our study, lower baseline and H_2O_2-induced DNA damage in participants with high WBC levels living below poverty compared to above poverty could be interpreted as an adaptive response to stressors that reduces DNA damage and increases DNA repair pathways (Crawford and Davies 1994; Taylor 2010;

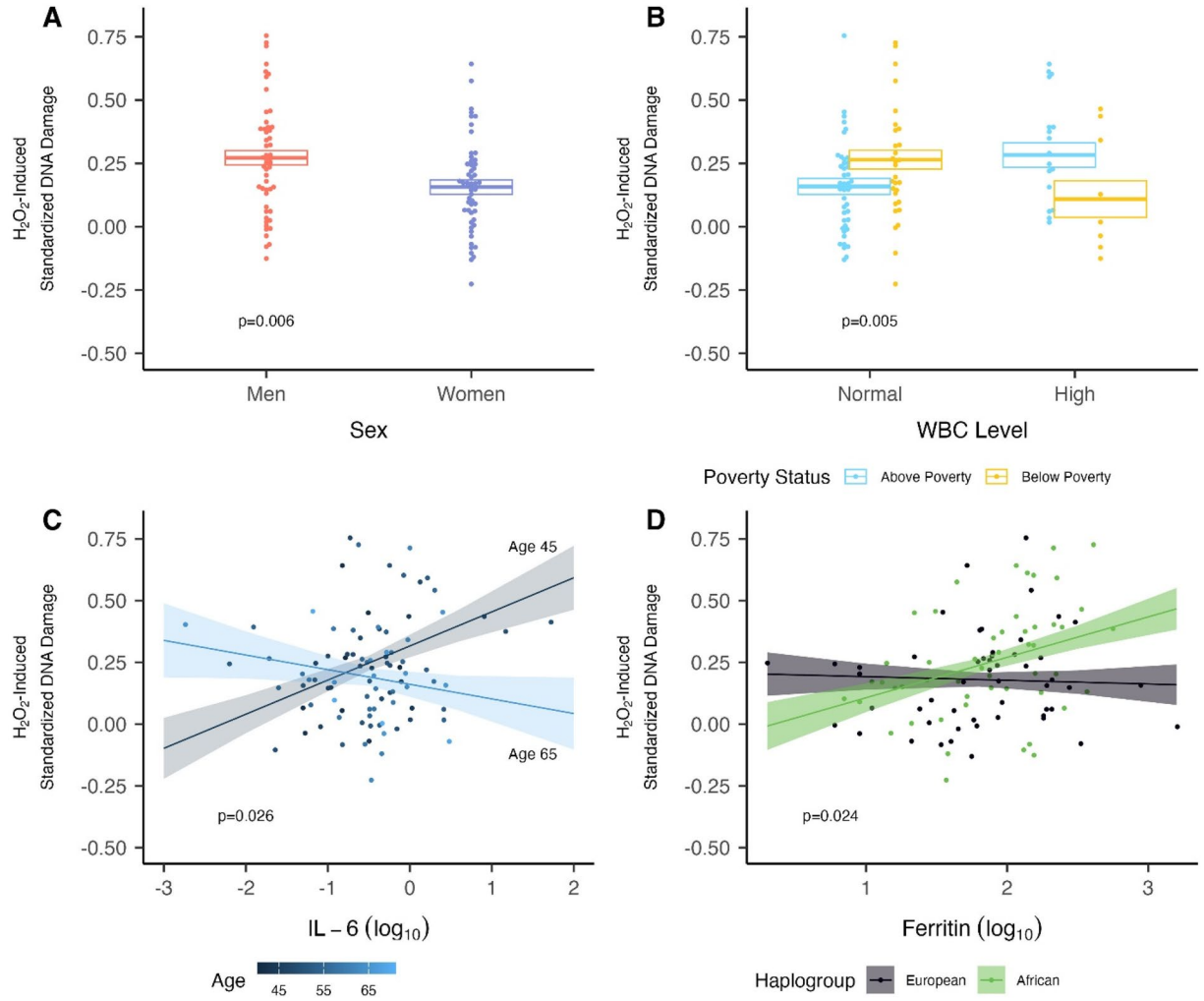

Figure 3. H_2O_2-Induced DNA damage is associated with sex, poverty status, age, WBC level, IL-6, and ferritin. Linear regression examined if inflammatory markers were related to H_2O_2-induced DNA damage either independently or differentially by mtDNA haplogroup, sex, poverty status, or age. Plots show measured data values (points) along with estimated means (center line) and standard error of the mean (colored band or edge of boxes) from the regression models. (A) There is a significant effect of sex for H_2O_2-induced DNA damage ($P = 0.006$). (B) There is a significant interaction between poverty status and WBC level for H_2O_2-induced DNA damage ($P = 0.005$). (C) There is a significant interaction between age and IL-6 levels for H_2O_2-induced DNA damage ($P = 0.026$). Scatter points color indicates participant age, and estimated values from the linear regression are shown for age 45 yrs and 65 yrs. (D). There is a significant interaction between mtDNA haplogroup and ferritin levels for H_2O_2-induced DNA damage ($P = 0.024$).

Miller and Chen 2013; Noren Hooten and Evans 2019; Noren Hooten et al. 2022). Several studies have also shown that DNA damage increases with age (Rübe et al. 2011; Soares et al. 2014; Beerman 2017). However, meta-analyses have suggested that increased DNA damage with age may be due to confounding or moderator variables such as lifestyle factors compounding over time instead of a direct effect of age (Soares et al. 2014; Møller 2019). The inclusion of other cohort variables such as poverty status in the analysis of DNA damage may help explain some of the inconsistencies in the reported effect of sex or age on DNA damage.

Inflammation and DNA damage are closely connected; the DNA damage response leads to inflammation, and persistent oxidative stress caused by chronic inflammation is linked to DNA damage (Pálmai-Pallag and Bachrati 2014; Li and Chen 2018; Kay et al. 2019). WBC level, a biomarker of systemic inflammation, interacted with sex or poverty status for baseline and H_2O_2-induced DNA damage. High WBC level was associated with differing levels of DNA damage across cohort variables such as sex and poverty status, while normal WBC level was similar across cohort variables. Such varying DNA damage levels point toward varying DNA damage responses to high levels of inflammation. Sex, poverty status, and several mtDNA haplogroups have also been associated with inflammation and differential levels of inflammatory markers (Kenney et al. 2014a; Kenney et al. 2014b; Muscatell et al. 2020; Lam et al. 2021). In our cohort, H_2O_2-induced DNA damage was related to the inflammatory marker IL-6 differentially based on the poverty status and age of the participant. The multifunctional cytokine IL-6 increases with age; higher levels of IL-6 are associated with chronic disease and mortality (Ershler and Keller 2000; De Martinis et al. 2005; Tylutka et al. 2024). IL-6 is also associated with activation of the DNA damage response (Rodier et al. 2009; Kumari et al. 2016). Circulating IL-6 levels have been shown to be higher in men than in women, and similarly, cells from men produce higher levels of IL-6 upon stimulation in vitro than women (Ter Horst et al. 2016;

Bernardi et al. 2020). In addition, we found an overall association of the proinflammatory cytokine IL-8 with decreasing levels of baseline DNA damage. Few studies have investigated potential connections between IL-8 and DNA damage levels; however, IL-8 is associated with the DNA damage response (Rodier et al. 2009). Differing levels of baseline DNA damage with IL-6 and IL-8 levels in our cohort suggest that DNA repair is activated heterogeneously in our cohort. The inclusion of poverty status in future studies may help to clarify the relationship between inflammatory markers and DNA damage.

We found that uric acid and ferritin levels played a role in interactions with mtDNA haplogroup affecting both baseline and H_2O_2-induced DNA damage. There was a positive relationship between baseline DNA damage and uric acid levels only in participants with the African mtDNA haplogroup. Elevated serum uric acids levels can cause gout, which is the most common inflammatory arthritis, and is associated with various other conditions including cardiovascular and kidney disease (Kuo et al. 2015; Li et al. 2017; Ndrepepa 2018; Chilunga et al. 2020). Therefore, uric acid can promote oxidative stress and inflammation. Recent data from the MaPLE (Gut and Blood Microbiomics for Studying the Effect of a Polyphenol-Rich Dietary Pattern on Intestinal Permeability in the Elderly) study in Italy showed a positive relationship between uric acid levels and H_2O_2-induced DNA damage in PBMCs (Del Bo et al. 2021). However, little is known about the relationship between uric acid and DNA damage in populations of different ancestries. We also observed a relationship between mtDNA haplogroup and ferritin levels for H_2O_2-induced DNA damage. Both high and low levels of ferritin have been associated with cardiovascular as well as all-cause mortality (Kadoglou et al. 2017; Cornelissen et al. 2019). However, to the best of our knowledge, this is the first study that examines oxidatively-damaged DNA in the context of mtDNA haplogroup and ferritin levels. These results may indicate that African mtDNA haplogroups, but not European mtDNA haplogroups, confer sensitivity to oxidative DNA damage through heterogeneous inflammatory responses. Differences in the amount of oxidative DNA damage visible at midlife may result in varying health outcomes later in life.

Our study has several limitations. Due to the inherent limitations of cross-sectional studies, we are unable to infer DNA damage differences over time or cause-and-effect relationships between variables. However, our data serve as an important first step toward assessing the impact of mtDNA ancestry on inflammatory states and the potential for divergence in DNA damage measurements. While the comet assay is widely used in human biomonitoring and clinical studies as a rapid and relatively simple method to evaluate DNA damage, the use of multiple biomarkers of oxidative DNA damage would present a more complete estimate (Watters et al. 2009; Cadet et al. 2011; Neri et al. 2015; Cordelli et al. 2021). Furthermore, our study focuses on the relationship between mtDNA ancestry, inflammation, and nuclear oxidative DNA damage. Future studies on mtDNA oxidative damage or other mitochondrial endpoints are merited in order to characterize the mechanisms and pathways potentially linking mtDNA ancestry and mitochondrial damage. Future investigations will center on physical biochemical data that links mtDNA haplogroups and mtDNA damage and/or repair. We limited the cohort to participants whose self-identified race matched their mtDNA haplogroup ancestry in order to remove potential confounding effects from mitonuclear discordance. Future studies focused on participants with differing mtDNA ancestry and self-identified race would greatly contribute to the understanding of mitonuclear interactions in diverse populations (Zaidi and Makova 2019). Finally, although we accounted for the race-based differences in total WBC counts, a potential limitation of our analysis is the use of the total WBC population, not specific cell subtypes. WBC cellular heterogeneity may influence comet assay results. To overcome this population heterogeneity, we scored a higher number of cells for each sample. However, future studies examining the specific WBC component populations (lymphocytes, granulocytes, monocytes) may provide unique insights.

Conclusion

mtDNA haplogroup has been linked with disease; however, in this cohort we did not find a direct link between mtDNA haplogroup and baseline and/or H_2O_2-induced DNA damage. We identified a novel effect of sex on H_2O_2-induced DNA damage. The interaction of mtDNA haplogroups and inflammatory pathways as well as their potential effect on DNA damage may be investigated in future studies.

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Authors' contributions

CRediT: **Jessica T. Smith**: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft; **Nicole Noren Hooten**: Conceptualization, Formal analysis, Investigation, Writing – review & editing; **Nicolle A. Mode**: Data curation, Formal analysis, Writing – review & editing; **Alan B. Zonderman**: Data curation, Formal analysis, Project administration, Writing – review & editing; **Ngozi Ezike**: Investigation, Methodology, Writing – review & editing; **Michele K. Evans**: Conceptualization, Formal analysis, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Disclosure statement

The authors report no competing interests.

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Notes on contributors

Jessica T. Smith, Ph.D., did this work as a postdoctoral fellow in the Laboratory of Epidemiology and Population Sciences at the National Institute on Aging Intramural Research Program, Baltimore, Maryland, USA. She is currently a Pharmaceutical Scientist, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, Maryland, USA.

Nicole Noren Hooten, Ph.D., is a Senior Associate Scientist in the Laboratory of Epidemiology and Population Sciences, National Institute on Aging Intramural Research Program, Baltimore, Maryland, USA.

Nicolle A. Mode, M.S., is a Senior Statistician in the Laboratory of Epidemiology and Population Sciences, National Institute on Aging Intramural Research Program, Baltimore, Maryland, USA.

Alan B. Zonderman, Ph.D., is a Senior Scientist in the Laboratory of Epidemiology and Population Sciences, National Institute on Aging Intramural Research Program, Baltimore, Maryland, USA.

Ngozi Ezike, M.D., is a Staff Clinician in the Laboratory of Epidemiology and Population Sciences, National Institute on Aging Intramural Research Program, Baltimore, Maryland, USA.

Michele K. Evans, M.D. is the Deputy Scientific Director of the National Institute on Aging and Acting Chief, Laboratory of Epidemiology and Population Sciences, National Institute on Aging Intramural Research Program, Baltimore, Maryland, USA.

ORCID

Jessica T. Smith http://orcid.org/0000-0001-7884-923X
Nicole Noren Hooten http://orcid.org/0000-0002-1683-3838
Nicolle A. Mode http://orcid.org/0000-0002-8193-0554
Alan B. Zonderman http://orcid.org/0000-0002-6523-4778
Ngozi Ezike http://orcid.org/0009-0000-7382-9352
Michele K. Evans http://orcid.org/0000-0002-8546-2831

Data sharing statement

Data are available to researchers after approval of a research proposal and after they have agreed to confidentiality as required by the National Institutes of Health Institutional Review Board. Policies and forms are available at: https://handls.nih.gov.

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Sup Table 1. Regression result p-values from final models after removal of non-significant interaction terms

A.

Outcome	Haplogroup (African)	Sex (Men)	Poverty Status (Below)	Age††
Baseline DNA damage	0.769	0.364	0.556	0.223
H_2O_2-induced DNA damage	0.124	**0.006**	0.510	0.375

††Age is expressed as decade units from age 50 ((age – 50)/10)

B.

Outcome	Marker Name	Marker	Haplogroup (African)	Sex (Men)	Poverty Status (Below)	Age	Haplogroup* Marker	Sex* Marker	Poverty Status* Marker	Age* Marker
Baseline DNA damage	IFN-γ†	0.342	0.786	0.361	0.635	0.276	-	-	-	-
Baseline DNA damage	IL-6†	0.622	0.655	0.250	0.061	0.233	-	-	**0.020**	-
Baseline DNA damage	IL-8†	**0.016**	0.669	0.293	0.672	0.249	-	-	-	-
Baseline DNA damage	IL-10†	0.591	0.857	0.374	0.634	0.210	-	-	-	-
Baseline DNA damage	TNF-α†	0.063	0.650	0.449	0.521	0.521	-	-	-	-
Baseline DNA damage	hsCRP†	0.416	0.890	0.291	0.559	0.190	-	-	-	-
Baseline DNA damage	Ferritin†	0.837	0.779	0.457	0.564	0.222	-	-	-	-
Baseline DNA damage	Uric Acid	0.361	**0.045**	0.456	0.704	0.165	**0.033**	-	-	-

Baseline DNA damage	WBC level (high)	0.528	0.561	0.532	0.360	0.770	-	**0.014**	**0.016**	**0.012**

C.

Outcome	Marker Name	Marker	Haplogroup (African)	Sex (Men)	Poverty Status (Below)	Age	Haplogroup* Marker	Sex* Marker	Poverty Status* Marker	Age* Marker
H_2O_2-induced DNA damage	IFN-γ†	0.793	0.182	**0.008**	0.404	0.425	-	-	-	-
H_2O_2-induced DNA damage	IL-6†	**0.013**	0.419	**<0.001**	0.146	**0.026**	-	-	-	**0.026**
H_2O_2-induced DNA damage	IL-8†	0.579	0.258	**0.007**	0.396	0.416	-	-	-	-
H_2O_2-induced DNA damage	IL-10†	0.930	0.185	**0.008**	0.419	0.419	-	-	-	-
H_2O_2-induced DNA damage	TNF-α†	0.411	0.342	**0.009**	0.437	0.570	-	-	-	-
H_2O_2-induced DNA damage	hsCRP†	0.148	0.209	**0.003**	0.504	0.285	-	-	-	-
H_2O_2-induced DNA damage	Ferritin†	0.789	0.079	**0.023**	0.573	0.267	**0.024**	-	-	-
H_2O_2-induced DNA damage	Uric Acid	0.360	0.134	**0.015**	0.505	0.353	-	-	-	-
H_2O_2-induced DNA damage	WBC level (high)	**0.036**	0.159	**0.005**	**0.039**	0.296	-	-	**0.005**	-

† Values were log base 10 transformed for analysis due to positively skewed distribution