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# Frailty, sex, and poverty are associated with DNA damage and repair in frail, middle-aged urban adults

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ABSTRACT

Frailty is an age-related syndrome characterized by reduced recovery from stressors and increased risks of morbidity and mortality. Although frailty is usually studied in those over 65 years, our previous work showed that frailty is both present and a risk factor for premature mortality in midlife. We identified altered gene expression patterns and biological pathways associated with inflammation in frailty. Evidence suggests DNA oxidation damage related to inflammation accumulates with age, and that DNA repair capacity (DRC) declines with age and age-related conditions. We hypothesized that inter-individual differences in DNA oxidation damage and DRC are associated with frailty status and poverty level. Using the CometChip assay, we assessed baseline single-strand breaks and hydrogen peroxide ($H_2O_2$)-induced DNA oxidation damage and DRC in non-frail and frail middle-aged African American and White individuals with household incomes above and below poverty. Analysis of baseline single-strand breaks showed no associations with frailty, poverty, race, or sex. However, we identified an interaction between frailty and poverty in $H_2O_2$-induced DNA oxidation damage. We also identified interactions between sex and frailty as well as sex and poverty status with DRC. The social determinant of health, poverty, associates with DRC in men. Baseline DNA damage, $H_2O_2$-induced DNA damage as well as DRC were associated with serum cytokine levels. IL-10 levels were inversely associated with baseline DNA damage as well as $H_2O_2$-induced DNA damage, DRC was altered by IL-4 levels and sex, and by TNF-α levels in the context of sex and poverty status. This is the first evidence that DRC may be influenced by poverty status at midlife. Our data show that social determinants of health should be considered in examining biological pathways through which disparate age-related health outcomes become manifest.

## 1. Introduction

Frailty is a clinical syndrome defined by diminished physiological reserve and reduced ability to recover from stressors [1,2]. Older adults (>65 years old) who are classified as frail are at an increased risk for morbidities and mortality [3]. Estimated prevalence of frailty in older US adults is 14–15 % [4,5]. The prevalence of frailty increases with age and is also influenced by social determinants of health (SDOH) [6]. In the United States, the prevalence of frailty in older adults is significantly higher in African American individuals compared to White individuals [1,5], in women compared to men [1,5,7,8], and in people in lower income groups compared to higher income groups [1,5].

However, frailty is not limited to those over age 65 years; frail individuals younger than 65 years old have been identified in previous

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studies using the FRAIL scale [9,10]. We previously found that middle-aged (45–49 years old) White individuals showed a higher prevalence of frailty than middle-aged African American individuals [9]. The allostatic load, or accumulated weight of persistent stresses and life experiences, results from SDOH and varies over the lifespan [11]. Differences in allostatic load over the lifespan may contribute to differences in frailty prevalence over the lifespan; however, few studies to our knowledge have investigated frailty in individuals younger than 65 years old in cohorts balanced by poverty status, race, and sex [9,12]. Similar to frailty in the elderly, frailty at middle age was associated with higher mortality [9]. Given this association between frailty and mortality at middle age, it is important to determine the underlying mechanisms that drive frailty in middle age and perhaps uncover targets for intervention earlier in the timeline of disability and disablement.

Our previous work identified genome-wide transcriptional changes associated with race and frailty in middle-aged adults [13]. Many of these altered biological pathways and gene expression patterns were related to inflammation [13]. We identified frailty- and race-associated changes in PBMC gene expression of the cytokines interleukin (IL)-6 (*IL6*) and IL-1β (*IL1B*); higher levels of *IL6* were found in White participants compared to African American participants, and an interaction between frailty and race was found for *IL1B* [13]. Specifically, *IL1B* levels were higher in non-frail White individuals compared to frail White individuals, while levels of *IL1B* were higher in frail African American individuals compared to non-frail African American individuals [13]. Inflammation is related to many forms of DNA damage; DNA damage in many different forms also generally accumulates with age [12,14–17]. Frailty in the elderly is associated with oxidative imbalance in the form of higher oxidized glutathione levels, higher protein oxidation levels, higher oxidative damage to lipids, and lower vitamin E levels, among other oxidative stress biomarkers [18–20]. We previously found that circulating levels of the DNA oxidation damage biomarker 8-hydroxy-2'-deoxyguanosine in serum were significantly associated with markers of inflammation, including high sensitivity C-reactive protein in a cohort of middle-aged women [21]. However, to our knowledge, no studies have examined the relationship between DNA oxidation damage, repair, and markers of inflammation in a diverse cohort of frail and non-frail middle-aged individuals.

The traditional comet assay is a well-established technique for examining DNA damage in the form of DNA strand breaks; it has been used in numerous studies to assess DNA damage and repair kinetics in human cells [22–26]. Using the comet assay, we previously found that PBMC γ-radiation-induced DNA damage and repair kinetics differed with age, race, and sex in a cohort of middle-aged individuals from 30 to 64 years old [27,28]. In White women, PBMCs displayed a positive relationship between the rate of the fast component of single-strand break repair and age, while in African American women, the rate displayed an inverse relationship with age; White and African American men did not show any significant relationship between the rate and age [27,28]. However, the comet assay is relatively low throughput, labor intensive, and can be affected by inter-experiment variability [29,30]. Recent technological advancements have improved upon this method with the development of the CometChip Assay, which is a sensitive and high-throughput comet assay with reduced variability as well as simpler visualization and analysis [31,32].

Here, we focused on the associations between cytokines and DNA damage and DNA repair capacity (DRC) in a diverse, middle-aged cohort of frail and non-frail individuals. We evaluated the levels of DNA oxidation damage and repair in our cohort and determined whether there were effects from or interactions among frailty, poverty, race, or sex. We then analyzed serum cytokine levels for our cohort and investigated whether DNA damage or repair associated with specific cytokine levels or cohort demographic factors.

## 2. Materials and methods

The Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study is a prospective, longitudinal study of age-associated health disparities. The HANDLS study consists of 3720 middle-aged (30–64 years old at baseline) African American and White adults from socioeconomically diverse neighborhoods in Baltimore City, Maryland [33]. Approval for HANDLS was obtained from the Institutional Review Board of the National Institutes of Health, and all participants provided written informed consent. Participants in the frailty cohort (N = 52) were balanced across sex, race, and frailty status and were between 45 and 49 years old (Table 1). Participants also varied across socioeconomic level as measured by household poverty status at enrollment (above and below 125 % of the US federal poverty line).

Frailty was assessed using the modified FRAIL scale, which consists of the following domains: fatigue, resistance, ambulation, illnesses, and loss of weight [9,10,13]. The FRAIL scale was modified to include the assessment of weight loss using the responses to item two of the Center for Epidemiologic Studies Depression scale, "Over the past week did you not feel like eating or have a poor appetite?" Loss of weight was considered present when participants responded occasionally (3–4 days a week) or mostly (5–7 days a week). The FRAIL scores range from 0 to 5 positive answers to the five domains; FRAIL scores were dichotomized into "Non-frail" (score of 0–2) and "Frail" (score of 3–5).

Blood samples were obtained from fasting HANDLS participants. Peripheral blood mononuclear cells (PBMCs) were isolated from fasting blood samples within three hours of phlebotomy as previously described [34]. PBMCs were aliquoted and cryopreserved in 40 % RPMI-1640, 50 % FBS, and 10 % DMSO at − 80 °C until use [34]. Fasting blood samples were collected in tubes without anticoagulants, and serum was isolated, aliquoted and stored at − 80 °C until use.

### 2.1. Multiplex cytokine panel

Cohort participant serum samples were thawed and centrifuged at 2000*g* for 3 min. Cytokine serum levels were measured using the V-PLEX Plus Viral Panel 2 Human Kit, which includes IFN-γ, IL-1β, IL-4, IL-6, IL-8, IL-10, and TNF-α, following the manufacturer's guidelines (Meso Scale Discovery). Cytokine levels were log10 transformed before statistical analysis.

### 2.2. CometChip

The alkaline comet assay detects DNA damage in cells embedded in agarose by the increase in DNA migration under electrophoresis [22,23]. The CometChip platform enables increased comet assay throughput through its evenly spaced array of microwells [35,36]. CometChip assays were performed according to detailed protocols in Ge et al. [35]. Briefly, a custom-made polydimethylsiloxane (PDMS) stamp was used to form an array of microwells in 14 mL of molten 1 % normal-melting point agarose in PBS spread on a pre-cut GelBond film (Lonza) [31,35,37]. The PDMS stamp was placed in the molten agarose and left for 3–5

**Table 1**
Demographic information for frailty cohort.

| | White | | African American | |
|---|---|---|---|---|
| | Non-frail, N = 13 | Frail, N = 13 | Non-frail, N = 13 | Frail, N = 13 |
| Age (mean (SD)) | 47.23 (1.26) | 47.58 (1.80) | 47.51 (1.59) | 47.04 (1.62) |
| Sex | | | | |
| Women (%) | 6 (46 %) | 6 (46 %) | 7 (54 %) | 7 (54 %) |
| Men (%) | 7 (54 %) | 7 (54 %) | 6 (46 %) | 6 (46 %) |
| Poverty Status | | | | |
| Above (%) | 10 (77 %) | 8 (62 %) | 7 (54 %) | 5 (38 %) |
| Below (%) | 3 (23 %) | 5 (38 %) | 6 (46 %) | 8 (62 %) |

min at room temperature until the agarose was solidified. 5 mL PBS was pipetted around the agarose to facilitate PDMS stamp removal, and excess agarose was cut away. The CometChip was placed on a glass plate and inspected for microwell quality 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 grown in culture and cryopreserved in one large batch to serve as negative (untreated) controls in each CometChip experiment. TK6 cells were cryopreserved in 90 % FBS and 10 % DMSO in aliquots of 1 mL and concentrations of approximately 100,000 cells/mL. Before each experiment, cryopreserved TK6 cells and cryopreserved PBMCs were briefly thawed in a 37 °C water bath and suspended in 5 mL RPMI culture medium supplemented with 20 % FBS in a 15 mL conical tube. PBMCs were then spun at 1300 rpm for 3 min, the supernatant was removed, and the cells resuspended in 1 mL RPMI

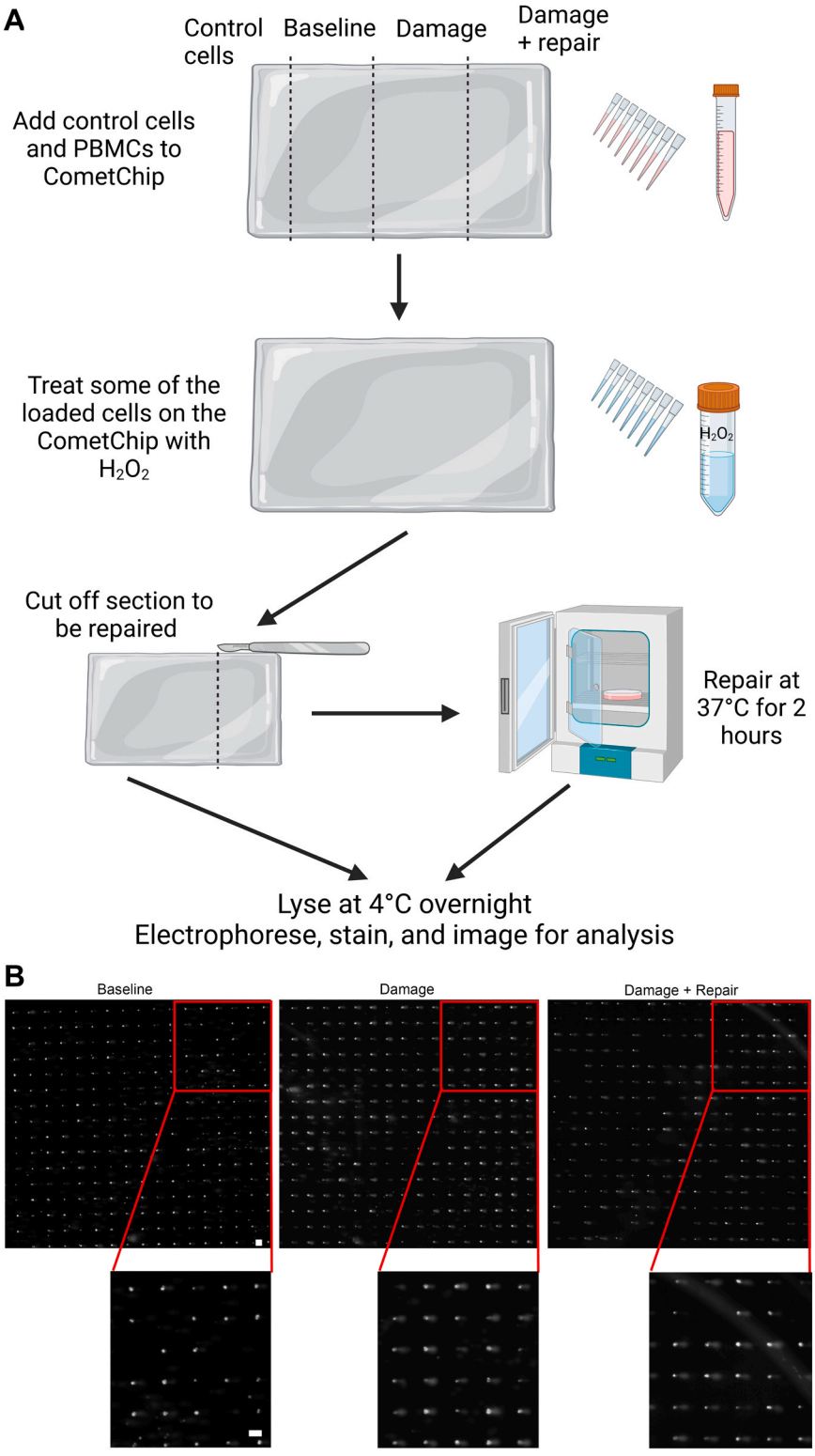


**Fig. 1.** DNA damage assessed by the CometChip. A) Schematic of CometChip experimental workflow. Participant PBMCs and control TK6 cells were added to the CometChip and allowed to settle into the microwells. A section of the CometChip was treated with $H_2O_2$. Part of the CometChip was cut off and placed in a 37 °C incubator for two hours before lysing, while the other section was placed immediately into lysis solution. B) Representative images of individual comets in the CometChip assay of baseline DNA damage, $H_2O_2$-induced damage, and damage remaining after cells were allowed to repair for 2 h. Scale bar = 100 μm.

culture medium supplemented with 20 % FBS.

The overall workflow of the CometChip experiments in this study is shown in Fig. 1A. PBMCs from the cohort of frail and non-frail individuals were thawed and used in the CometChip assay. Potential inter-experiment variability was reduced by normalizing each CometChip experiment with control TK6 cells: each CometChip experiment with varying TK6 cell DNA damage was normalized to the average TK6 DNA damage of all CometChip experiments. 100 μL of cells was loaded into each well of the CometChip-96 well plate. Control TK6 cells were pipetted into one column of the fabricated CometChip-96 well plate, and PBMCs were pipetted in rows into the rest of the plate. Cells were allowed to settle and load into the microwells in the CometChip in a 37 °C and 5 % $CO_2$ tissue culture incubator over at least 30 min, with rocking side-to-side in the middle of the incubation. Microwells were visually inspected for cell loading using a light microscope. Medium was then aspirated from the wells in the 96 well plate and the bottomless 96 well plate was removed. The CometChip was gently washed with 5 mL PBS to remove excess cells 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.

Each condition was run in triplicate. Dosing with $H_2O_2$ was accomplished 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, with PBS serving as a non-treatment control, then placing the CometChip at 4 °C for 20 min. The CometChip was then cut into pieces to allow for multiple repair timepoints. Part of the CometChip (TK6 controls, baseline control, and $H_2O_2$-treated) was placed into lysis buffer (R&D Systems #4250–050–01) and 4 °C immediately, while the other part (Repair) was rinsed with PBS, placed in culture medium supplemented with 20 % FBS, and allowed to repair for two hours at 37 °C before being placed in lysis buffer. Cells were lysed overnight.

The CometChip was allowed to unwind in alkaline electrophoresis solution (NaOH 300 mM, $Na_2EDTA$ 1 mM)[35] for 30 min at 4 °C before running the gel at 22 V and ~300 mA for 30 min in the R&D Comet-Assay Electrophoresis System [37]. The CometChip was then neutralized in neutralization buffer (Tris 400 mM, pH 7.5) for 15 min [35], then neutralization was repeated with fresh buffer for 15 min. The CometChip was stained with 20 mL of 1x SYBR Gold (ThermoFisher) in PBS for one 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.

### 2.3. Statistical analysis

CometChip images were analyzed using the Comet Assay Software from R&D [37]. Data from Comet Assay Software was exported as excel files and imported into and processed in R (Version 4.1.1) using R Studio (Version 1.4.1717) [38,39]. Median % DNA in tail was exported for every well on each CometChip experiment. In each well, an average of approximately 150 comets was analyzed. The mean % DNA in tail was calculated from three technical replicate wells. In order to further reduce inter-experiment variability, we normalized the PBMC DNA damage measurements to control TK6 cells on each CometChip experiment. The data for PBMC % DNA in tail measurements from each CometChip experiment was normalized as follows: an average of % DNA in tail measurements for all untreated TK6 cells in all CometChip experiments was calculated (total average TK6). The average % DNA in tail measurements of TK6 cells was also calculated for each CometChip experiment (CometChip average TK6). Each CometChip average TK6 was compared to the total average TK6, and PBMC % DNA in tail measurements were adjusted by the amount that the CometChip average for that specific experiment differed from the total average TK6.

Measurements of repaired DNA must also take the amount of damage caused in the assay into consideration. A formula previously used for cryopreserved lymphocyte DNA damage was used to calculate the DRC, or percent of DNA damage repaired after two hours for each participant sample [26]:

$$\%\ \text{DNA damage repaired} = 100 \times \frac{(\text{damaged DNA} - \text{DNA damage after 2hr repair})}{\text{damaged DNA}}$$

Multivariable linear regression analyses examined the relationship of % DNA in tail, DRC, and cytokine levels with frailty status, race, sex, and poverty status. Linear regression models were built using backward stepwise selection including all interactions. Models included interactions in order to investigate whether the relationship between the DNA damage or repair outcome and a variable of interest differed depending on the level of another independent variable. A sensitivity analysis was performed by including cryopreserved PBMC viability as a covariate in all linear regression models. We found no differences in model main effects or interactions. A p-value of $< 0.05$ was considered to signify a statistically significant finding.

## 3. Results

To detect whether DNA damage or DRC was associated with frailty in middle-aged adults, we performed high-throughput CometChip experiments using unstimulated, cryopreserved PBMCs from participants balanced across frailty status, race, and sex, and between the ages of 45–49 years old (Table 1). We assessed the baseline DNA damage, damage after $H_2O_2$ treatment and residual damage after PBMCs were allowed to repair the damage for two hours. Representative images of the high-throughput CometChip showing baseline DNA damage, $H_2O_2$-damaged DNA, and residual DNA damage after two hours of repair are shown in Fig. 1B. The % DNA in tail was calculated for each parameter for PBMCs from each individual. The baseline DNA damage for each individual was categorized into low, mid and high tertiles (Fig. 2). Individuals with the lowest tertile of baseline DNA damage also displayed statistically lower amounts of $H_2O_2$-induced DNA damage ($P = 0.021$, Table 2A) and higher DRC compared to individuals in the highest baseline tertile ($P < 0.001$; Table 2B). We performed an additional experiment with repair timepoints at two hours, four hours, and eight hours in order to assess the DRC of cohort cryopreserved PBMCs from longer periods of repair (Fig. S1). All PBMC samples displayed repair of $H_2O_2$-induced DNA damage by the 8-hour timepoint (Fig. S1).

We then investigated the potential associations of frailty, poverty, race, and sex on baseline DNA damage. There were no significant main effects or interactions associated with cohort demographics in baseline DNA damage (Fig. 3, Table 3A). We also tested whether there were any significant differences when baseline DNA damage was categorized into low, medium, and high tertiles. However, there were no significant differences observed (Table 3B).

We analyzed the association of frailty, poverty, race, and sex on DNA damage induced by $H_2O_2$. We identified an interaction between frailty and poverty status for $H_2O_2$-induced DNA damage ($P = 0.023$; Fig. 4A, Table 4A). Frail participants above poverty had lower $H_2O_2$-induced DNA damage compared to non-frail participants who were living above poverty, while frail and non-frail participants living below poverty were not significantly different. In addition, frail participants who were living above poverty also displayed lower $H_2O_2$-induced DNA damage compared to frail participants living below poverty. We observed no significant differences in $H_2O_2$-induced DNA damage with race or sex, however, African American participants had higher amounts of $H_2O_2$-induced DNA damage compared to White participants, but the difference was not significant in the linear regression model ($P = 0.08$; Table 4A).

We then studied the effects of frailty, poverty, race, and sex on the

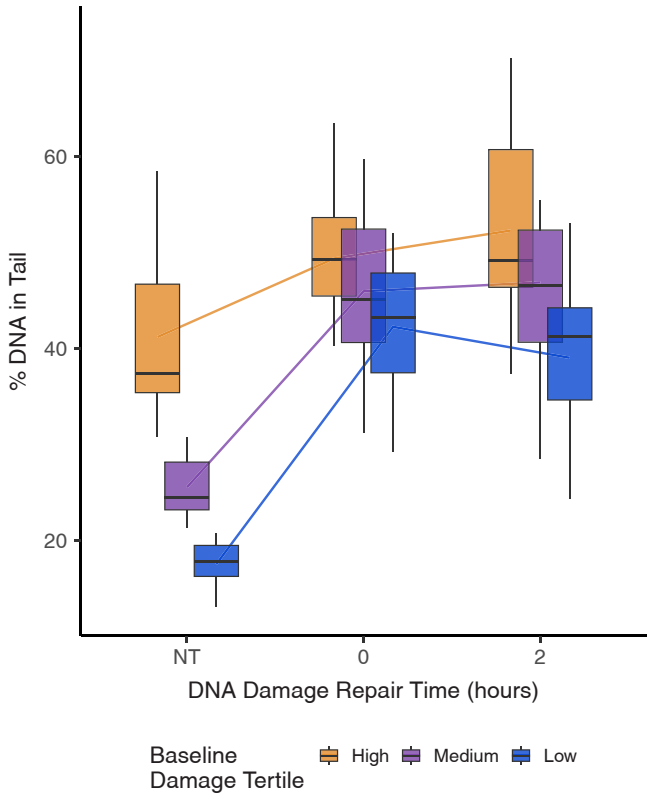


**Fig. 2.** CometChip measurement of DNA damage and repair in cryopreserved human PBMCs. PBMCs were assessed for baseline damage, $H_2O_2$-induced damage, and $H_2O_2$- induced then repaired DNA damage. All participants were sorted into tertiles based on baseline DNA damage. Boxplots represent the summary data for baseline damage, $H_2O_2$-induced damage, and $H_2O_2$- induced then repaired DNA damage for each baseline damage tertile. NT = baseline DNA damage.

**Table 2**
The relationship between tertile levels of baseline DNA damage, $H_2O_2$-induced DNA damage, and residual DNA damage.

| **A) $H_2O_2$-induced DNA Damage (Time 0 hr)** | | |
|---|---|---|
| *Predictors* | *Estimates (CI)* | *P value* |
| Medium Damage Tertile | 3.70 (−2.3 to 9.7) | 0.219 |
| High Damage Tertile | 7.12 (1.1 – 13.1) | **0.021** |
| **B) Residual DNA damage (Time 2 hr)** | | |
| *Predictors* | *Estimates (CI)* | *P value* |
| Medium Damage Tertile | 7.86 (1.6 – 14.1) | **0.015** |
| High Damage Tertile | 13.26 (7.0 – 19.6) | **< 0.001** |

Baseline DNA damage was categorized into tertiles, and the relationships between these tertiles and $H_2O_2$-induced DNA damage (A) as well as residual DNA damage after two hours of repair (B) was analyzed using linear regression. Bold indicates a p value less than 0.05. CI = confidence interval (95 %).

DRC of $H_2O_2$-damaged DNA after two hours. As measurements of repaired DNA damage must also take the amount of damage caused in the assay into consideration, we utilized a previously described formula to calculate the percent of DNA damage repaired after two hours for each participant sample [26]. We observed an interaction between sex and frailty for the percent of DNA damage repaired after two hours. The interaction suggested that frail women had higher DRC compared to non-frail women, while frail and non-frail men were similar. ($P = 0.015$; Fig. 4B, Table 4B). We also identified an interaction between sex and poverty status for percent DNA damage repaired; men living below poverty had higher DRC than men living above poverty. Women who were living above or below poverty displayed similar DRC. Women living above poverty had a higher DRC compared to men living above poverty ($P = 0.019$; Fig. 4C, Table 4B). We observed no significant differences in DRC with race.

Finally, we quantified the serum levels of cytokines IFN-γ, IL-1β, IL-4, IL-6, IL-8, IL-10, and TNF-α in our cohort. We investigated the relationship between DNA damage and serum cytokine levels, frailty status, race, sex, and poverty status; we also investigated potential associations between DRC and serum cytokine levels, frailty status, race, sex, and poverty status. DNA damage and DRC were not associated with any interactions between cytokines IFN-γ, IL-1β, IL-6, IL-8, or cohort demographics. However, levels of IL-10 were inversely associated with baseline DNA damage as well as $H_2O_2$-induced DNA damage ($P = 0.026$ and 0.013, Fig. 5A-B, Table 5A-B). In addition, we observed a significant interaction between IL-4 levels and sex for DRC ($P = 0.022$, Fig. 5C, Table 5C). For men, reduced DRC was associated with higher IL-4 levels, while for women high levels of IL-4 were associated with higher DRC. Finally, DRC also displayed a significant interaction between levels of TNF-α, sex, and poverty status ($P = 0.0480$, Fig. 5D, Table 5D). Women who were living above poverty had a positive relationship between TNF-α and DRC, while men living above poverty did not. However, men and women who were living below poverty displayed similar DRC compared to levels of TNF-α.

## 4. Discussion

In this study, we have examined DNA damage and DRC levels from unstimulated, cryopreserved PBMCs as well as serum cytokine levels from a diverse cohort of frail and non-frail, middle-aged participants. We have shown that baseline DNA damage levels do not differ across frailty status, poverty status, race, or sex in our cohort of middle-aged participants; however, differences in $H_2O_2$-induced DNA damage and DRC are detectable. We found that participants who were living above poverty and non-frail displayed higher amounts of $H_2O_2$-induced DNA damage compared to frail individuals above poverty. In addition, we found two interactions for DRC: for frailty status and sex, women who were non-frail displayed lower DRC than women who were frail; for poverty status and sex, men who were living below poverty displayed higher DRC than men living above poverty. We have found that levels of the anti-inflammatory cytokine IL-10 were inversely related with both baseline DNA damage and $H_2O_2$-induced DNA damage. Finally, we found that men had an inverse relationship with DRC and IL-4 levels while women had a positive relationship. We also observed that men and women above poverty had opposite relationships between DRC and TNF-α levels, while men and women below poverty were similar.

The comet assay is a well-established technique used to investigate many forms of DNA damage and DNA repair kinetics [40]. Higher levels of baseline DNA damage in white blood cells have been found to correlate with occupational exposure to mutagenic compounds, diabetes, renal disease, Alzheimer's Disease, and coronary artery disease in many studies [25,40]. However, there are a limited number of studies utilizing the comet assay to examine the association of frailty status with baseline DNA damage, induced DNA oxidation damage, or DNA repair in PBMCs from human populations [41–43]. DNA damage and DRC was measured via automated fluorimetric alkaline DNA unwinding in the Newcastle 85 + Study, and no association with frailty was found [44]. Teixeira-Gomes et al. found no association between DNA damage in PBMCs via the alkaline and enzyme-modified comet assay and frailty status in older adults in a pilot study [43], as well as a larger study [41]. Another recent study utilizing the enzyme-modified comet assay found no significant relationship between baseline DNA damage levels in PBMCs and frailty status [45]. Our results in midlife are consistent with these previous findings, as we detected no difference in baseline DNA damage between cryopreserved PBMCs from non-frail and frail individuals in our cohort. The difference may appear later in life: Teixeira-Gomes et al. found a significantly higher amount of DNA oxidation damage in PBMCs from pre-frail elderly individuals compared to both the non-frail and frail elderly [41]. DNA repair showed a

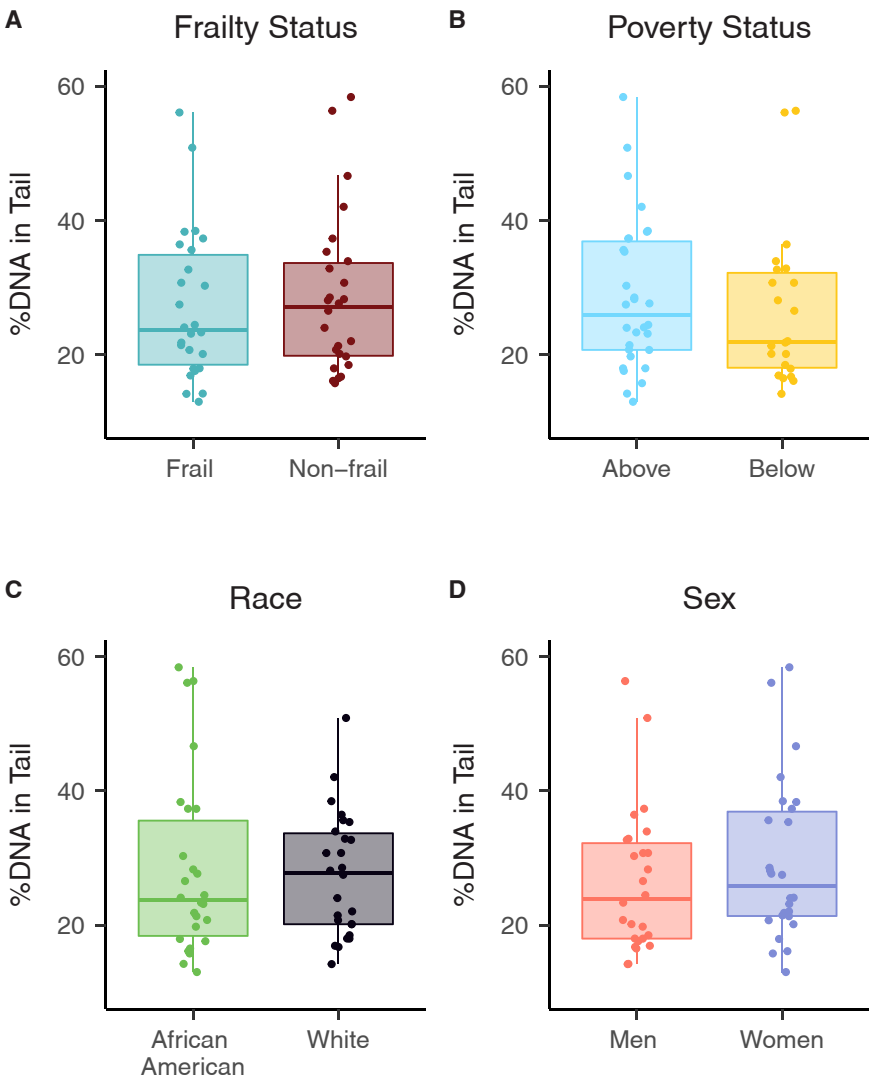


**Fig. 3.** Baseline DNA damage by cohort demographics. For A) frailty status, B) poverty status, C) race, and D) sex, baseline DNA damage datapoints are represented by a scatterplot with a boxplot overlaid; the line in the middle of the box indicates median value, and the box defines values from 25th percentile to 75th percentile. The whiskers extend to the farthest point within 1.5 * the inter-quartile range.

**Table 3**
The relationship between baseline DNA damage or baseline damage tertiles and frailty, race, sex, and poverty.

| **A) Baseline DNA Damage** | | |
|---|---|---|
| *Predictors* | *Estimates (CI)* | *P value* |
| Sex [Men] | 2.5 (−4.2 to 9.2) | 0.456 |
| Race [African American] | -0.1 (−6.8 to 6.6) | 0.968 |
| Frailty Status [Frail] | 1.7 (−4.9 to 8.3) | 0.611 |
| Poverty Status [Below Poverty] | -1.7 (−8.7 to 5.4) | 0.638 |
| **B) Baseline Damage Tertiles 1-Low / 2-Med / 3-High** | | |
| *Predictors* | *Estimates (CI)* | *P value* |
| Sex [Men] | 0.6 (−0.0 to 1.3) | 0.057 |
| Race [African American] | 0 (−0.5 to 0.5) | 0.957 |
| Frailty Status [Frail] | -0.1 (−0.6 to 0.4) | 0.696 |
| Poverty Status [Below Poverty] | 0.4 (−0.4 to 1.1) | 0.334 |
| Sex * Poverty | -0.9 (−1.9 to 0.2) | 0.094 |

The relationships or interactions between baseline DNA damage or baseline damage tertiles and sex, race, frailty, and poverty were analyzed using linear regression. Bold indicates a p value less than 0.05. CI = confidence interval (95 %).

nonsignificant trend to decrease with increasing severity of frailty status in one study looking at bleomycin-induced damage in PBMCs from older adults with the comet assay [46]. Baseline DNA damage results from the same study also showed no association with frailty status [46].

Comet assay measurement of DNA repair in the context of frailty at midlife is limited. Most studies investigating DNA damage via the comet assay have focused on frailty in the elderly, or a comparison of DNA damage between older and younger groups. Several studies, including a meta-analysis by Milić et al., have found that elderly individuals' PBMCs displayed higher baseline DNA damage and/or $H_2O_2$-induced DNA damage when compared to younger individuals [47–50]. In contrast, a study by Hyland et al. found similar levels of DNA damage between nonagenarians and middle-aged control individuals [51]. Humphreys et al. similarly found an increase in DNA damage in older age groups when compared to a younger (20–35 years old) control group; however, the oldest group showed the highest resistance to $H_2O_2$-induced DNA damage [52]. A recent study compared DNA damage and repair via comet assay in younger (<45 years old) adults to older adults and frail older adults [53]. Frail older adults displayed the highest baseline DNA damage and lowest repair capacity; however, frailty at younger ages was not investigated as younger adults served only as healthy controls in the study [53]. Expression of genes encoding DNA repair proteins was found

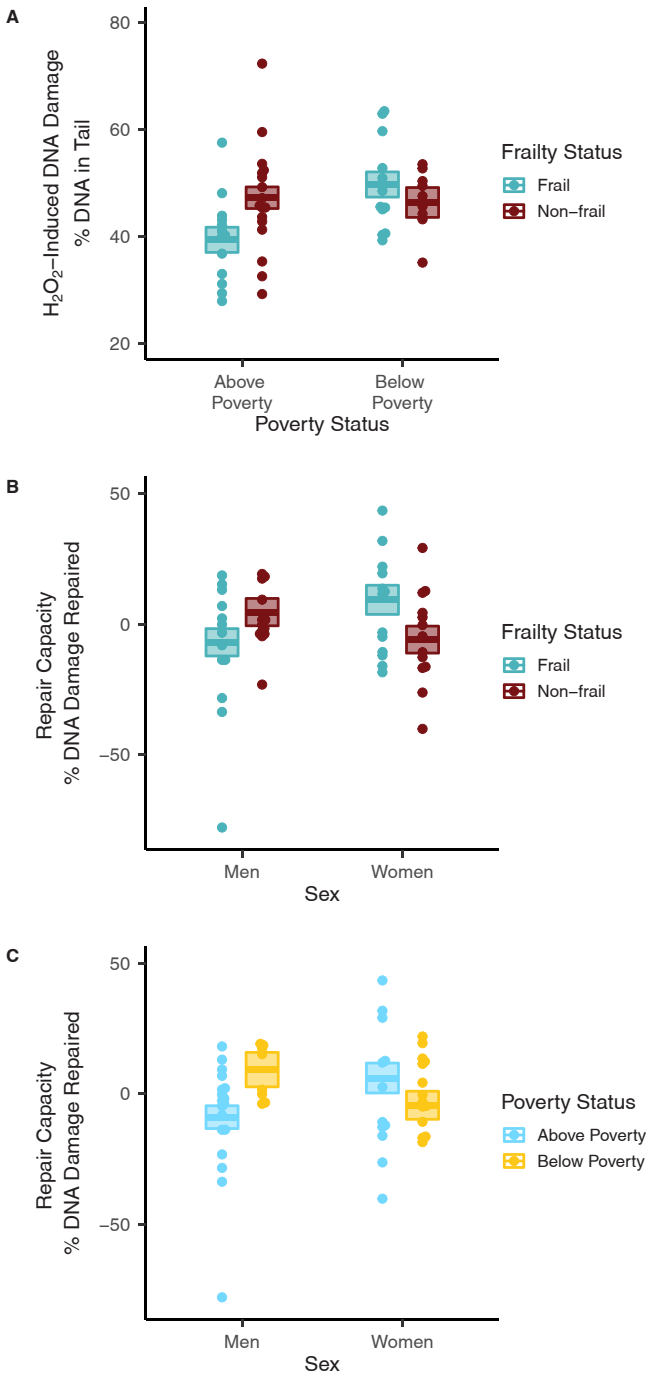


**Fig. 4.** Associations between DNA damage and repair measurements with frailty, sex, and poverty. Linear regression models were built to assess potential relationships between $H_2O_2$-induced DNA damage or DNA repair capacity and cohort demographics. The plots show DNA damage and repair measurements as scatter points with overlaid boxes indicating estimated values from the linear regression model and their standard errors. A) There is a significant interaction between frailty status and poverty status for $H_2O_2$-induced DNA damage ($P = 0.023$). B) There is a significant interaction between frailty status and sex for DNA repair capacity ($P = 0.015$). C) There is a significant interaction between poverty status and sex for DNA repair capacity ($P = 0.019$).

to vary between groups of frail men and frail women in a middle-aged cohort [54]. To the best of our knowledge, there have been no other studies interrogating PBMC DNA damage and repair in the context of frailty in middle-aged individuals. Our results showing significant interactions between $H_2O_2$-induced DNA damage, frailty status, and poverty status; oxidative DRC, frailty status, and sex; as well as oxidative DRC, sex, and poverty in middle-aged adults are thus novel.

**Table 4**
The relationship between $H_2O_2$-induced DNA damage or DRC with frailty, race, sex, and poverty.

| **A) $H_2O_2$-induced DNA Damage** | | |
|---|---|---|
| *Predictors* | *Estimates (CI)* | *P value* |
| Sex [Men] | 3.5 (−1.3 to 8.3) | 0.15 |
| Race [African American] | 4.2 (−0.5 to 9.0) | 0.08 |
| Frailty Status [Frail] | -7.9 (−14.1 to −1.7) | **0.014** |
| Poverty Status [Below] | -0.9 (−7.9 to 6.1) | 0.799 |
| Frailty Status * Poverty Status | 11.2 (1.6 – 20.8) | **0.023** |
| **B) DRC (% DNA Damage Repaired)** | | |
| *Predictors* | *Estimates (CI)* | *P value* |
| Sex [Men] | -1.6 (−18.3 to 15.1) | 0.849 |
| Race [African American] | 9.6 (−1.8 to 21.0) | 0.097 |
| Frailty Status [Frail] | 15.2 (−0.3 to 30.8) | 0.055 |
| Poverty Status [Below] | -10.4 (−27.3 to 6.4) | 0.219 |
| Sex * Frailty Status | -26.8 (−48.2 to −5.4) | **0.015** |
| Sex * Poverty Status | 28.7 (5.0 – 52.3) | **0.019** |

The relationship or interactions between $H_2O_2$-induced DNA damage as well as DRC and sex, race, frailty, and poverty were analyzed using linear regression. Bold indicates a p value less than 0.05. CI = confidence interval (95 %).

As demonstrated by our results, the effect of frailty and poverty on DNA damage and DRC is complex. Our data showing that individuals below poverty displayed smaller differences between frail or non-frail status in $H_2O_2$-induced DNA damage (Fig. 4A) as well as our finding that men below poverty displayed higher DRC than men above poverty (Fig. 4C) could be interpreted as poverty inducing an adaptive response to reduce DNA damage and increase DNA repair mechanisms [55]. The chronic sterile inflammation associated with poverty could lead to oxidative stress and thus an increase in DRC [56–58]. Similarly, our finding that frail women displayed higher DRC than non-frail women may also point towards an adaptive response increasing DRC because of the inflammation associated with frailty [59,60]. Importantly, our study captures a snapshot of disparities in repair and inflammation in at an earlier time than the more commonly studied inflammaging in elderly individuals [60–62]. Our data point toward an interaction between frailty status and SDOH such as sex and poverty status to influence DNA damage and repair at midlife; differences in DNA damage and repair seen at midlife may contribute to health disparities in the elderly.

Elevated serum levels of cytokines such as IL-6 have long been associated with frailty in the elderly [63,64], as well as an overall increased risk of morbidity and mortality later in life [65]. However, Prince et al. observed that PBMC *IL6* gene expression was not associated with frailty at middle age; instead, *IL6* expression was higher in middle-aged White individuals compared to middle-aged African American individuals regardless of frailty status [13]. Of note is the observation that serum cytokine levels were not observed to correlate with gene expression at midlife [13]. A previous study found that serum IL-6 levels at midlife contributed to later development of frailty in the Whitehall Study which was predominantly composed of White participants.[66]. However, in our analysis, serum IL-6 levels were not significantly associated with middle-aged cohort demographics, DNA damage, or DRC.

In this study, there was no detectable main effect of frailty or interaction with frailty and other cohort demographics at midlife. However, we observed two interactions for measurements of DNA damage and the anti-inflammatory cytokine IL-10: in baseline DNA damage and in $H_2O_2$-induced DNA damage (Fig. 5A-B). IL-10 is a central mediator of the anti-inflammation response suppressing cytokine synthesis to offset stressors which threaten homeostasis [67]. Its expression is tightly regulated; however, there is evidence that DNA damage by inducing secretion of TNF-α may lead to the secretion of IL-10 from monocytes [68]. In our overall cohort, higher levels of IL-10 are associated with lower levels of baseline DNA damage as well as lower levels of $H_2O_2$-induced DNA damage, possibly suggesting that IL-10 may be important in preventing

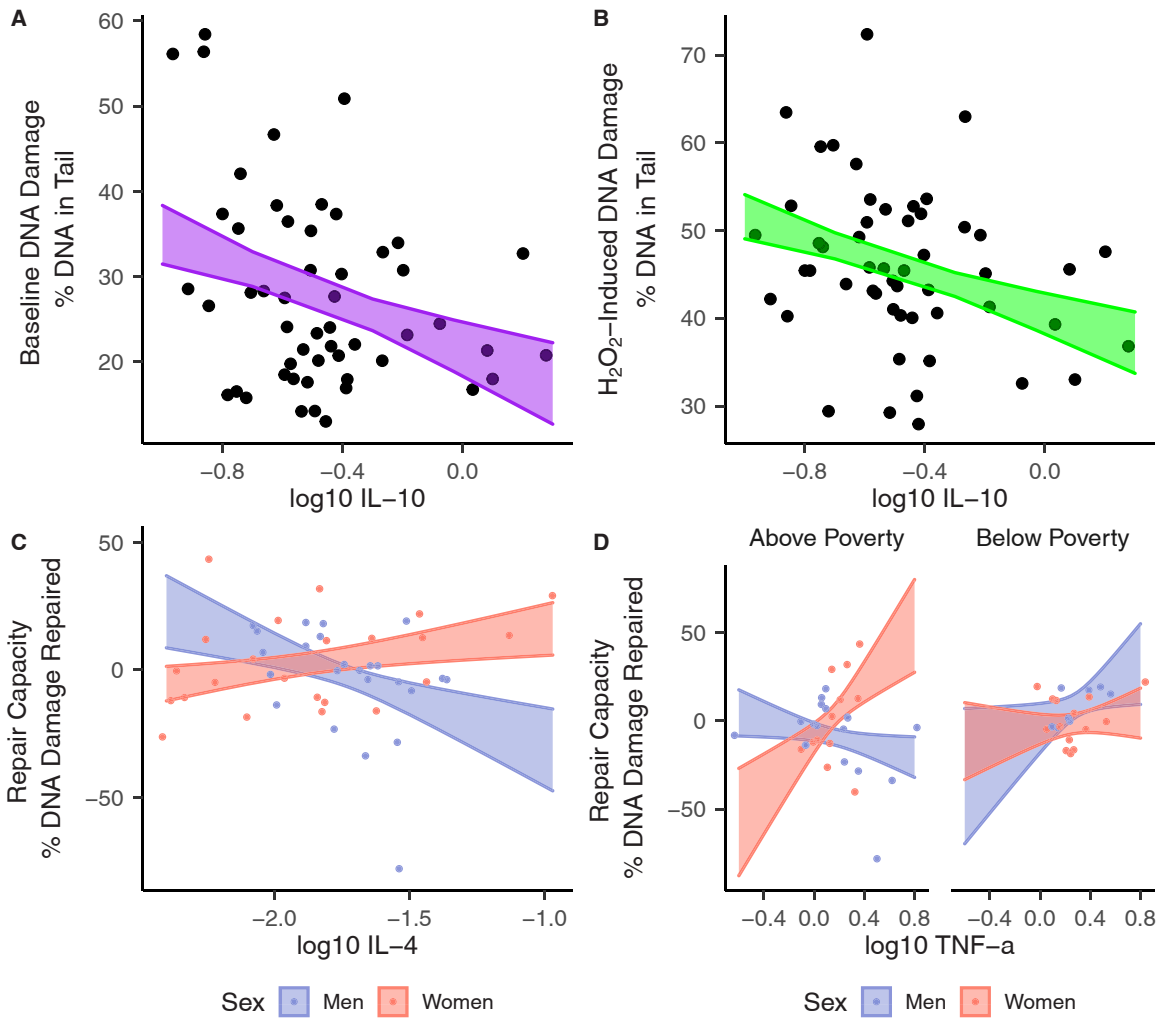


**Fig. 5.** Relationships between serum cytokine levels and DNA damage or repair measurements. Linear regression models were built to assess potential relationships between DNA damage and repair measurements, serum cytokine levels, and cohort demographics. Plots show data scatter points with overlaid ribbons indicating estimated values from the linear regression model and their standard errors. A) There is a significant relationship between IL-10 and baseline DNA damage ($P = 0.026$). B) There is a significant relationship between IL-10 and $H_2O_2$-induced DNA damage ($P = 0.013$). C) There is a significant interaction between sex and IL-4 for DRC ($P = 0.022$). D) There is a significant interaction among sex, poverty status, and TNF-α for DRC ($P = 0.048$).

and responding to genotoxic stressors. Additionally, we detected two interactions between DRC, cytokines, and cohort demographics that may contribute to disparities in frailty. Firstly, DRC displayed an interaction between the cytokine IL-4 and sex. IL-4 is a pleiotropic cytokine associated with both the innate and adaptive immune systems [67]. Exposure to genotoxic agents that damage DNA induces secretion of IL-4 by lymphocytes, basophils and mast cells [69]. In addition, there is evidence that IL-4 not only decreases DNA damage in murine and human cells exposed to genotoxic agents, but also upregulates DNA repair mechanisms in both murine and human cells [70]. IL-4 secretion is partially responsible for modulating the expression of DNA damage response genes in the setting of genotoxic exposures [71]. Sex differences in the production of IL-4 have been reported in human PBMCs and cultured human cell lines [72]. Our finding that higher levels of IL-4 in men are associated with lower levels of DRC are in contrast to women where higher levels of IL-4 are associated with higher DRC, suggesting that IL-4 may modulate DNA repair at the population level and that there may be interindividual and demographic differences. Sex differences in DRC with varying IL-4 levels may point towards a sex-dependent DNA repair response to the cytokine.

We also observed an interaction between levels of TNF-α, sex, and poverty for DRC. TNF-α levels were not associated with DRC among men or women living below poverty. However, women living above poverty displayed higher DRC with higher levels of TNF-α, and the reverse was true for men above poverty. The relationship between TNF-α and DNA damage and repair is complex. Administration of TNF-α in vitro and in vivo is genotoxic [73,74]. DNA damage itself also induces TNF-α which can affect cell cycle kinetics and diminish DNA repair after exposure to certain forms of DNA damage through activation of the PI3K-Akt pathway [75,76]. There are also sex-based differences in TNF-α levels between healthy adult men and women, with men typically displaying higher TNF-α [77]. Among older adults, frailty and pre-frail status are also associated with higher levels of TNF-α [78]. Additionally, studies have shown sex and poverty or socioeconomic level differences in TNF-α levels. Low socioeconomic status has been associated with higher levels of TNF-α in non-diverse European cohorts [79], however, other studies show a non-linear or non-significant association [80,81]. Few studies have investigated potential differences in TNF-α levels and DNA repair in the context of sex, race, and poverty status. Our work supports the idea that poverty as a social stressor may influence the relationship between DRC and TNF-α. Additional studies with larger racially diverse cohorts examining the relationship between DRC, TNF-α, and SDOH are necessary to further clarify the biologic pathways that may be important in the timeline of aging, the development of the frailty phenotype, and the impact of social stressors. Our results just begin to shed light on the social environmental factors and molecular mechanisms that contribute

**Table 5**
The relationship between measures of DNA damage or repair with levels of cytokines and cohort demographics.

| | | |
|---|---|---|
| **A) Baseline Damage** | | |
| *Predictors* | *Estimates (CI)* | *P value* |
| log10 IL-10 | -13.4 (−25.2 to −1.7) | **0.026** |
| Sex [Men] | 0.8 (−5.8 to 7.4) | 0.810 |
| Race [African American] | 1.0 (−5.5 to 7.5) | 0.752 |
| Frailty Status [Frail] | 1.5 (−4.8 to 7.9) | 0.625 |
| Poverty Status [Below Poverty] | -1.7 (−8.4 to 5.1) | 0.622 |
| **B) $H_2O_2$-Induced DNA Damage** | | |
| *Predictors* | *Estimates (CI)* | *P value* |
| log10 IL-10 | -11.0 (−19.6 to −2.4) | **0.013** |
| Sex [Men] | 1.2 (−3.6 to 6.1) | 0.606 |
| Race [African American] | 5.1 (0.3 – 9.9) | **0.037** |
| Frailty Status [Frail] | -3.2 (−7.9 to 1.4) | 0.165 |
| Poverty Status [Below Poverty] | 4.8 (−0.1 to 9.8) | 0.056 |
| **C) DRC (% DNA Damage Repaired)** | | |
| *Predictors* | *Estimates (CI)* | *P value* |
| log10 IL-4 | 15.0 (−5.9 to 35.9) | 0.154 |
| Sex [Men] | -98.8 (−179.2 to −18.4) | **0.017** |
| Race [African American] | 1.1 (−11.7 to 13.9) | 0.866 |
| Frailty Status [Frail] | -3.5 (−15.1 to 8.1) | 0.543 |
| Poverty Status [Below Poverty] | 7.1 (−5.3 to 19.5) | 0.255 |
| log10 IL-4 * Sex | -52.9 (−98.0 to −7.9) | **0.022** |
| **D) DRC (% DNA Damage Repaired)** | | |
| *Predictors* | *Estimates (CI)* | *P value* |
| log10 TNF-α | 79.2 (−0.5 to 158.9) | 0.051 |
| Sex [Men] | 3.5 (−16.6 to 23.5) | 0.730 |
| Race [African American] | 6.7 (−6.4 to 19.8) | 0.307 |
| Frailty Status [Frail] | 1.7 (−9.4 to 12.8) | 0.760 |
| Poverty Status [Below Poverty] | 5.0 (−19.4 to 29.4) | 0.682 |
| log10 TNF-α * Sex | -97.1 (−181.4 to −12.8) | **0.025** |
| log10 TNF-α * Poverty | -67.9 (−161.7 to 26.0) | 0.152 |
| Sex * Poverty | -2.9 (−41.8 to 36.0) | 0.882 |
| log10 TNF-α * Sex* Poverty | 131.0 (1.2 – 260.8) | **0.048** |

The relationship or interactions between serum cytokine levels; Baseline DNA Damage, $H_2O_2$-Induced DNA Damage, or DRC; and sex, race, frailty, and poverty were analyzed using linear regression. Bold indicates a p value less than 0.05. CI = confidence interval (95 %).

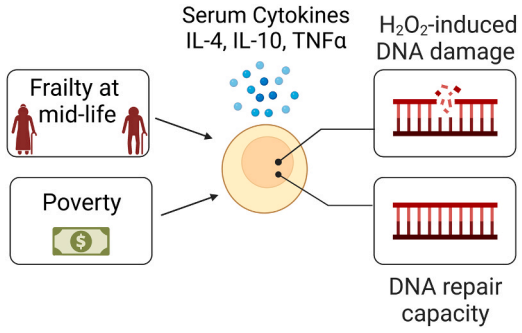


**Fig. 6.** Schematic representation of factors that contribute to interindividual differences in $H_2O_2$-induced DNA damage and DNA repair capacity. Frailty and poverty at mid-life are associated with variation in cytokine levels, $H_2O_2$-induced DNA damage, and DNA repair capacity. This figure was created with BioRender.com.

over time to observed differences between men and women in frailty and old age (Fig. 6).

The high-throughput CometChip assay has been used for genotoxic studies [82–85], however, epidemiological studies utilizing CometChip are still rare [86]. These results show that the CometChip assay is capable of sensing both DNA oxidation damage and repair in unstimulated, cryopreserved human PBMCs. Our findings in baseline DNA damage match previous literature in that we did not find any association with frailty status. However, we have identified significant interactions between $H_2O_2$-induced DNA damage, frailty status, and poverty status; oxidative DRC, frailty status, and sex; and oxidative DRC, poverty status, and sex. Analysis of cytokine levels revealed additional interactions between IL-10 and DNA damage; interactions between DRC, IL-4, and sex; and interactions between DRC, TNF-α, sex, and poverty status. Further validation in combination with other markers of DNA oxidation damage is needed to verify these findings in larger sample sizes and different populations. This study builds upon previous work on frailty at middle age to further clarify the molecular patterns associated with frailty prevalence. This study suggests that differences in DNA damage and repair pathways associated with frailty and SDOH are observable at midlife (Fig. 6). Clarification of the biological pathways that contribute to the development of frailty are important for the mitigation or prevention of frailty.

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## CRediT authorship contribution statement

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

## Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

## Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request through the HANDLS website https://handls.nih.gov/.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dnarep.2023.103530.

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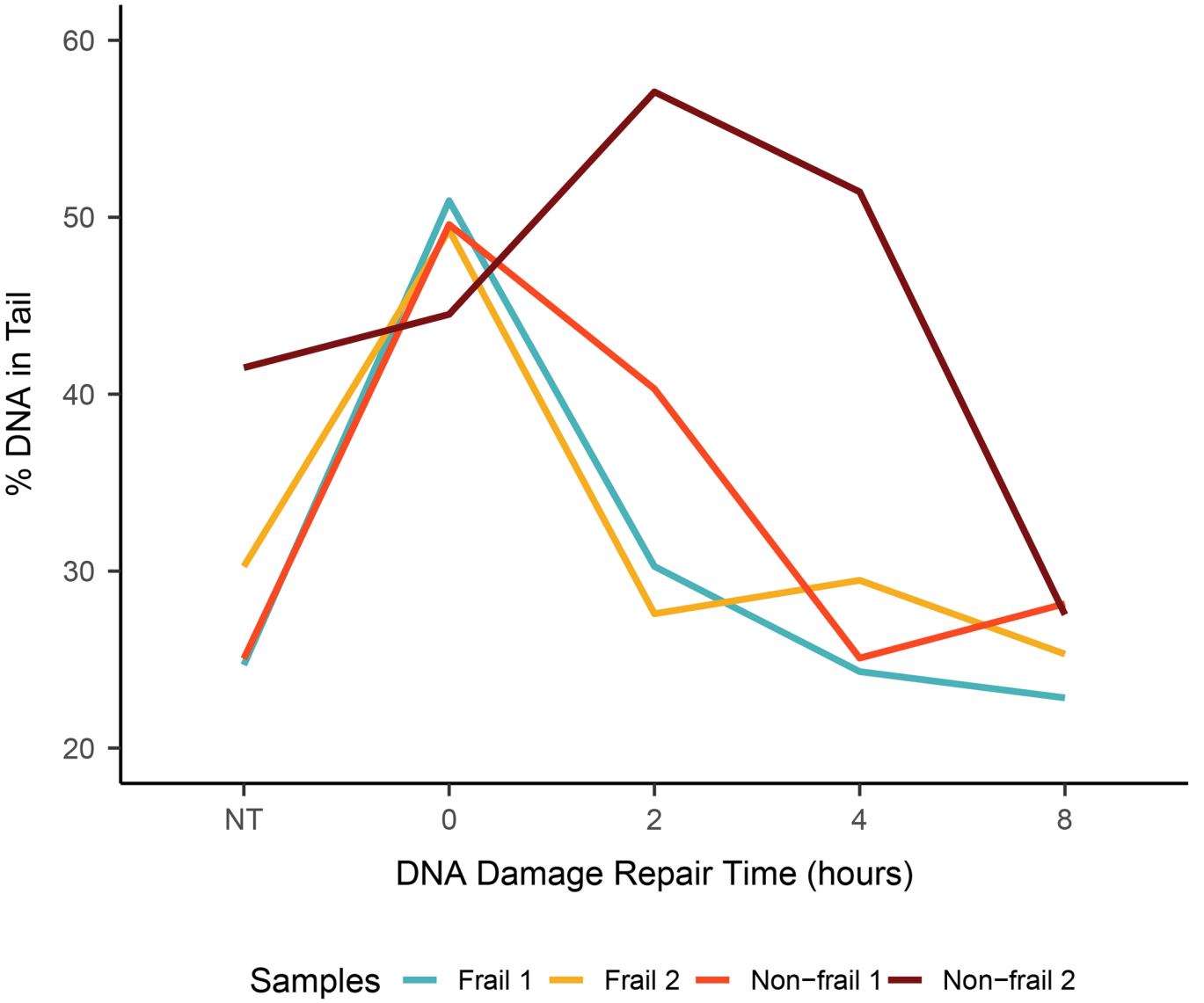


**Supplementary Figure S1: Long-term repair of DNA damage by cryopreserved PBMCs.** PBMCs from non-frail (n=2) and frail (n=4) individuals were prepared and damaged as detailed in Materials and Methods. The baseline and 0 hr repair timepoints were placed into lysis buffer immediately. 2, 4, and 8 hour timepoints were rinsed with PBS, placed in culture medium supplemented with 20% FBS, and allowed to repair for the length of time specified before being placed in lysis buffer. NT = baseline DNA damage