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Original Article

Geriatric Frailty Is Associated With Oxidative Stress, Accumulation, and Defective Repair of DNA Double-Strand Breaks Independently of Age and Comorbidities

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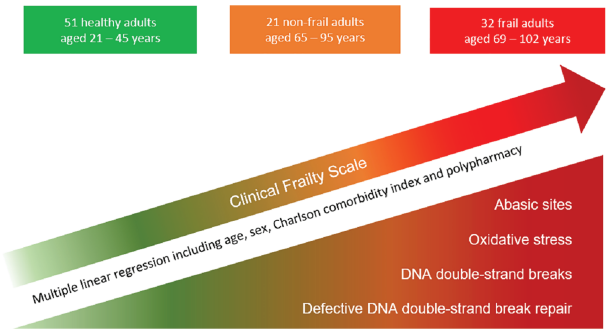
Abstract

Defects in the DNA damage response and repair (DDR/R) network accumulate during the aging process. Physical frailty, a state of reduced physiological function and decreased resilience to biological stressors, is also exacerbated by aging, but its link with DDR/R aberrations beyond the effect of age and comorbidities is unclear. Fifty-three community-dwelling older adults, aged 65–102 years, who underwent frailty classification according to the Rockwood Clinical Frailty Scale (CFS), and 51 healthy adults younger than 45 years were examined in parallel. The following DDR/R parameters were determined in their peripheral blood mononuclear cells (PBMCs): (a) oxidative stress and abasic (apurinic/apyrimidinic; AP) sites, (b) endogenous DNA damage (alkaline comet assay olive tail moment [OTM] indicative of DNA single-strand breaks [SSBs] and double-strand breaks [DSBs] and γH2AX levels by immunofluorescence [DSBs only]), (c) capacity of the 2 main DNA repair mechanisms (DSB repair and nucleotide excision repair). Older individual-derived PBMCs displayed reduced-to-oxidized glutathione ratios indicative of increased levels of oxidative stress and increased AP sites, as well as increased accumulation of endogenous DNA damage (OTM and γH2AX) and defective DSB-repair capacity, compared with younger controls. These DDR/R aberrations were more pronounced in frail versus nonfrail older adults. Notably, oxidative stress, AP sites, DSBs, and DSB-repair capacity were associated with individual CFS levels after adjusting for chronological age, sex, Charlson Comorbidity Index, and polypharmacy. Geriatric frailty is independently associated with increased DNA damage formation and reduced DSB-R capacity, supporting further research into these measures as potential frailty biomarkers.

Graphical Abstract



Keywords: DNA damage response and repair network, DNA double-strand break repair, Frailty, Oxidative stress

Geriatric physical frailty is a relatively novel concept established in routine medical practice over the recent years, constructed to entify reduced physiological function and decreased resilience to biological stressors, which afflicts a subset of older adults, beyond the effects of chronic diseases (1). The prevalence of geriatric frailty in the community ranges between 4% and 17% with female predominance, and increases with advancing age. Frail individuals are at higher risk of functional dependence and increased mortality when exposed to acute illness (2) or even minor ailments, hence, the early identification, prevention, and management of frailty have become a pressing need for health care systems worldwide (3). Although frailty often accompanies multimorbidity and polypharmacy (4), and is more prevalent in patients with certain chronic diseases, such as congestive heart failure or chronic obstructive pulmonary disease, it presents with distinct clinical features affecting individuals beyond the impact of organ-specific comorbidities.

Phenotypically, the physical frailty syndrome presents as a constellation of exhaustion, decreased physical strength, weight loss, slow gait, and reduced activity, which advances along a continuum of severity (5). Several validated instruments exist for its diagnosis and classification both in the primary care and inpatient settings; among them, the Rockwood Clinical Frailty Scale (CFS) is a simple, intuitive tool applied to grade individuals on a 9-level scale according to their levels of physical fitness and ability to function independently (6). CFS scores of 1–3 denote normal individuals ranging from very athletic to unrestricted ordinary daily functioning despite potential coexisting chronic diseases, a score of 4 is assigned to people managing to live independently notwithstanding mild symptoms of frailty in their usual activities, and scores 5–7 indicate advancing severity of frailty and dependence on others for basic care. The highest scores are reserved for people considered to be near the end of life either due to extreme debility or because they have been diagnosed with a specific disease state limiting their life expectancy to <6 months (6).

Although frailty was traditionally viewed as a degenerative process resulting from the accumulation of comorbidities, recent evidence suggests that it comprises a distinct biological entity. At the cellular level, oxidative stress and/or a defective DNA damage response/repair (DDR/R) system (7) have attracted significant attention as contributing factors to the frailty phenotype. Frailty status has been associated with increased oxidative stress levels and defective repair of DNA double-strand breaks (DSBs) (8), whereas other traditional measures of DNA damage, such as the alkaline comet assay, which measures overall DNA damage (single-strand breaks [SSBs] and DSBs), failed to show an association with frailty (8). Nevertheless, previous studies utilized only univariate and/or age-adjusted analyses and did not control for the confounding effect of multiple comorbidities (8). In the present study, we aimed to investigate whether frailty may be associated with measures of DNA damage formation and/or DNA repair capacity in older individuals beyond the effect of age, multimorbidity, and polypharmacy.

Method

Patient Population

All consenting consecutive adults >65 years old requesting a geriatric consultation with the research group's geriatrician (E.K.) were evaluated for inclusion in the study; after excluding patients with active malignancy, history of autoimmune disease, active infection, or other acute illness, participants underwent a geriatric assessment for recording comorbidities and chronic prescription drugs, calculation of the Charlson Comorbidity Index (CCI) (9), determination of their CFS score (6), and venipuncture for isolation of peripheral blood mononuclear cells (PBMCs). PBMCs were also extracted from 51 healthy adults younger than 45 years, who visited our department's primary care clinic for health maintenance and consented to serve as controls in this study.

Peripheral Blood Mononuclear Cell Isolation

PBMCs were isolated and purified using the Ficoll gradient centrifugation, as previously described (10). Cells were resuspended in a freezing medium (90% fetal bovine serum [FBS], 10% dimethyl sulfoxide [DMSO]; AppliChem, Germany) and stored at −80°C until further processing.

Oxidative Stress Measurements and Detection of Abasic Sites

Oxidative stress was quantified using a luminescence-based system that measures the ratio of reduced glutathione (GSH) over oxidized glutathione (GSSG), according to manufacturer's experimental protocol (GSH/GSSG-Glo Assay, Promega, UK) (10). Abasic sites were evaluated using the OxiSelect Oxidative DNA Damage Quantitation Kit (AP sites) according to manufacturer's experimental protocol.

Endogenous DNA Damage Accumulation

Endogenous DNA damage accumulation in PBMCs was quantified using 2 methods: (a) single-cell gel electrophoresis (comet assay) under alkaline conditions, which measures both SSBs and/or DSBs in DNA (11,12) and (b) immunofluorescence quantification of γH2AX (H2AX phosphorylated at Ser139; #9718T, Cell Signaling Technology), which can be used as a surrogate for monitoring DSBs only (13,14). Detailed methodology for both techniques has been previously described (15). Briefly, for the comet assay, PBMCs were resuspended in low-melting agarose and spread onto slides precoated with 1% standard agarose. Afterwards, cellular membranes were lysed using a lysis solution, and cells were incubated in prechilled electrophoresis buffer. After electrophoresis was performed, slides were placed in neutralization buffer and distilled water and left to dry overnight. Gels were stained with SYBR Gold Nucleic Acid Gel Stain (Thermo Fisher Scientific, Waltham, MA) and studied with a fluorescence microscope (Zeiss Axiophot, Oberkochen, Germany). olive tail moments (OTMs) of at least 200 cells/experiment were evaluated.

DSB-Repair Capacity

To measure DSB-repair (DSB-R) capacity, freshly isolated PBMCs were treated with 100 μg/mL melphalan for 5 min at 37°C in RPMI medium containing 10% FBS, 100 units/mL penicillin, 100 mg/mL streptomycin, and subsequently incubated in drug-free medium for 0, 8, and 24 h, adhered to coverslip, fixed, and analyzed. Following subtraction of the baseline γH2AX levels, the DSB-R capacity was measured by calculating the area under the curve (AUC) of melphalan-induced γH2AX during the whole experiment (0–24 h) (10,15).

Nucleotide Excision Repair Capacity

Nucleotide excision repair (NER) capacity was measured at cellular level after UVC irradiation, as previously described (10,16).

Freshly isolated PBMCs were directly resuspended in PBS and UVC-irradiated with a total dose of 5 J/m^2. PBMCs were centrifuged after UVC irradiation, passed in complete RPMI medium, and incubated in a humidified CO_2 incubator (37°C, 5% CO_2) for 1, 2, and 6 h. At each time point, cells were stored in freezing medium (90% FBS, 10% DMSO) at −80°C until further processing. Following subtraction of the baseline DNA damage levels, the NER capacity was measured by calculating the AUC of UVC-induced DNA damage during the whole experiment (0–6 h).

Statistical Analysis

Normality of variable distribution was assessed graphically and by the Shapiro–Wilk test. Student's *t*-test or Mann–Whitney *U* test was applied to examine pairwise differences between older adults and younger healthy controls depending on variable distribution. Comparison among 3 groups (young healthy adults, nonfrail older adults, and frail older adults) was performed by ANOVA with Bonferroni correction or Kruskal–Wallis with Dunn's multiple comparisons test depending on variable distribution. Univariate associations between the CFS, chronological age, the CCI, and polypharmacy, were explored using linear regression analysis.

Among older participants, 6 separate multiple linear regression models were applied for the association of the clinical frailty scale to GSH/GSSG, AP sites, log-transformed OTM, γH2AX, DSB-AUC (DSB-R capacity), and UVC-induced DNA damage AUC (NER capacity). Each linear regression model consisted of the clinical frailty scale as the dependent variable, only one of the above cellular biomarkers under investigation as the independent variable, and potential confounders associated with frailty in the relevant literature (8,17), including age, sex, polypharmacy (defined as ≥4 chronic prescription drugs) and the CCI score, which were all forced into the model as cofactors. All variables were continuous except for sex and polypharmacy, which were binary.

All tests were 2 sided with α set at .05. STATA v.14 (StataCorp LLC, College Station, TX) was used for all analyses, and GraphPad Prism v. 7.05 was used to create all graphs.

Results

Among the 53 older adults spanning ages 65–102 years, 34 (64%) were female, and 32 (60%) were frail (CFS ≥ 4), with a higher prevalence of age-related comorbidities in frail persons, as expected (Table 1). Among the older adults, CFS was univariably associated with increasing age using linear regression (β-coefficient [95% CI]: 0.11 [0.07–0.15] per 1-year increase, $p < .001$), with polypharmacy (β-coefficient [95% CI]: 2.17 [1.35–2.98], and with higher CCI (β-coefficient [95% CI]: 0.412 [0.25–0.57] per 1-unit increase, $p < .001$).

Oxidative Stress Is Increased in Frail Older Adults, Beyond the Effects of Chronological Age and Comorbidities

Analysis of GSH/GSSG ratio revealed increased oxidative stress in the PBMCs of folder adults compared with the younger healthy controls ($p < .001$; Figure 1A). Further analysis by frailty status revealed that nonfrail older adults had higher levels of oxidative stress compared with young healthy controls, and frail older adults had even higher levels compared with nonfrail older adults (both $p < .001$; Figure 1B). Similar results were obtained when we examined the presence of abasic sites (Figure 1C and D), the common DNA

Table 1. Univariate Associations of Clinical Parameters and DNA Damage Response and Repair Measurements With the Older (>65 y) Participants' Frailty Status According to the Rockwood Clinical Frailty Scale

Parameter	Young Healthy Controls[◦] n=51	Nonfrail Older Adults (CFS < 4) n=21	Frail Older Adults (CFS ≥ 4) n=32	*p*-Value (Nonfrail Older Adults vs Controls/Frail Older Adults vs Controls)	*p*-Value (Frail Older Adults vs Nonfrail Older Adults)
Age (y, mean ± *SD*)	31 ± 7	76 ± 9	86 ± 8	<.001/<.001[#]	<.001*
Female sex (%)	47	76	56	.024/.415[§]	.943[§]
CFS	N/A	2.5 ± 0.5	5.4 ± 1.2	N/A	<.001*
CCI (mean ± *SD*)	N/A	4 ± 2	6 ± 2	N/A	<.001*
Dementia (%)	N/A	10	47	N/A	.004[§]
CHF	N/A	10	44	N/A	.008[§]
Polypharmacy (≥4 prescription drugs)	N/A	88.9	96.4	NA	.384[§]
Active smoking (%)	20	19	12.5	.957/.400[§]	.515[§]
Alcohol consumption (1 portion daily vs 0)	N/A	24	22	N/A	.869[§]
CKD stage 3 or higher	N/A	5	39	N/A	.008[※]
GHS/GSSG ratio	76 ± 5.8	56 ± 2	44 ± 2	<.001/<.001*	<.001
Baseline OTM	4.6 ± 1.6	7.7 ± 3.0	10.3 ± 4.4	<.001/<.001[#]	.037[¶]
OTM AUC (NER)	51 ± 18	39 ± 23	28 ± 19	.017[#]/.002[#]	.098[¶]
DSB	11 ± 2	17 ± 4	20 ± 3	<.001*	.019*
DSB-AUC	122 ± 63	348 ± 13	389 ± 10	<.001/<.001[#]	.017*

Notes: AUC = area under the curve; CCI = Charlson Comorbidity Index; CFS = Clinical Frailty Scale; CHF = congestive heart failure; CKD = chronic kidney disease; DSB = double-strand breaks; N/A = not applicable; OTM = olive tail moment.

[◦]Adults <45 y without acute or chronic health problems.

*Student's test.

[§]χ^2 test.

[#]Kruskal–Wallis test.

[※]Fisher's exact test.

[¶]Wilcoxon test.

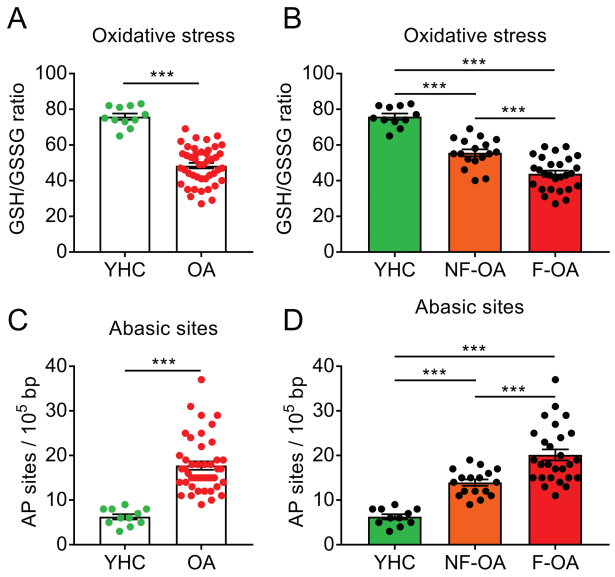


Figure 1. DNA damage formation in PBMCs of frail and nonfrail older adults relative to young healthy controls. (**A**) GSH/GSSG ratio in PBMCs of older adults (OA; aged >65 years) versus young healthy controls (YHC; adults <45 years without acute or chronic health problems). (**B**) GSH/GSSG ratio in PBMCs of nonfrail older adults (NF-OA), frail older adults (F-OA), and young healthy controls. A lower ratio indicates higher oxidative stress levels. (**C**) Abasic sites in PBMCs of older adults (aged >65 years) and YHC. (**D**) Abasic sites in PBMCs of nonfrail older adults, frail older adults, and YHC. *p*-Values are derived from Student's *t*-test/Mann–Whitney *U* test for pairwise comparisons (**A, C**) and from ANOVA with Bonferroni correction or Kruskal–Wallis with Dunn's multiple comparisons test for comparison of 3 groups (**B, D**).

lesions, which can be produced either spontaneously or as a result of genotoxic insults, such as oxidative stress (18).

Of interest, linear regression analysis revealed an independent association between oxidative stress levels/AP sites and the older adults' clinical frailty scale score, beyond the effect of other clinical parameters with significant associations to geriatric frailty, such as age, sex, the CCI, and polypharmacy (Table 2, Models A and B).

Increased DNA Damage Accumulation in Older Adults Is Associated With Geriatric Frailty

Next, we examined whether endogenous DNA damage accumulation was increased in older individuals and especially in frail older adults. Indeed, both OTM, indicative of the presence of DNA SSBs and DSBs (Figure 2A and B), and γH2AX levels, indicative of DSBs (Figure 2D and E), were increased in the PBMCs of older adults. Moreover, both frail and nonfrail older adults had higher levels of OTM and γH2AX compared with healthy young individuals (Figure 2C and F), while frail older adults had higher levels of DSBs compared with nonfrail older adults (Figure 2F).

However, when we controlled for the effect of additional relevant biological parameters on geriatric frailty such as chronological age, sex, comorbidities, and polypharmacy, only DSBs as surrogated by elevated γH2AX levels retained their association with geriatric frailty (Table 2, Models C and D).

Geriatric Frailty Is Associated With Defective DSB Repair

We then examined whether the 2 main DNA repair mechanisms, namely DSB-R and NER, were affected in older adults, focusing especially on those with frailty. Indeed, PBMCs of older adults showed defective DSB-R capacity, which was more pronounced in frail individuals (Figure 3A–C). On the other hand, NER capacity was found deregulated in older adults, showing increased NER rates compared with young healthy controls (p = .008; Figure 3D and E) due to the significant elevation of the NER capacity of frail individuals (p = .002 vs young healthy controls; Figure 3F). After adjusting for age, sex, the presence of comorbidities, and polypharmacy, DSB-R capacity was independently associated with the patients' frailty status (Table 2, Model E), whereas NER capacity was not (Table 2, Model F).

Discussion

In the present study, we show that frailty is associated with increased DNA damage formation (oxidative stress and abasic sites), DNA damage accumulation in the form of DSBs, and defective repair of DSBs independent of age, sex, and the presence of comorbidities or polypharmacy. These results confirm previous reports linking clinically measured frailty with oxidative stress in humans (17,19–21). Additionally, we suggest that frailty is associated with impaired DSB repair rather than UVC-induced damage repair, when the confounding effect of chronological age, sex, comorbidity burden, and polypharmacy is taken into consideration.

Previous studies have shown that oxidative stress is associated with both advanced age and degree of injury or illness (22,23). According to the "free radical theory of ageing," oxidative stress has a central role in the aging process, through free radical-mediated damage accumulation overtime; however, this theory has failed to fully explain the clinically observed decline of physical function that sometimes accompanies aging. Newer gero-science evidence connect abundance of free radicals and oxidative stress with frailty, rather than mere chronological age and the aging process per se (7,21,24–28). Suggested underlying biological mechanisms for this association are related to modulation of multiple intracellular signaling cascades by ROS (29), immune system dysregulation (30–33), and musculoskeletal damage, such as increased proteasomal activity leading to elevated intracellular calcium, muscle degradation, and declining populations of myoblasts and muscle cells, all leading to impaired muscle function (34,35). However, the few large clinical studies conducted to date have yielded conflicting results: In line with our data, demonstrating that frailty (assessed by Rockwood CFS) is associated with increased oxidative stress (as expressed by the GSH/GSSG ratio) independent of confounders, the Framingham Offspring Study (19) had also found a cross-sectional association of frailty (Fried criteria) and slower gait speed with isoprostanes, critical markers, and mediators of oxidative stress (36), independent of age, sex, body mass index, smoking, and major comorbidities. Similar, albeit unadjusted for common confounders, associations between frailty (Fried criteria) and oxidative stress levels were demonstrated in older (20,21), and more recent reports (17) using either oxidized glutathione, serum 8-hydroxy-2′-deoxyguanosine, or auto-oxidation of oxysterols, respectively. Of note, these cohorts all consist of participants ≥60–65 years old. By contrast, in the Newcastle 85+ cohort, no correlation was found between frailty (by the Rockwood frailty index) and oxidative stress using isoprostanes as markers of lipid peroxidation (37). This contradiction implies that different mechanisms may be operative across the wide age range of older adults, underlining the need for further studies in the ever expanding aging population.

Furthermore, the association of physical frailty with markers of endogenous DNA damage accumulation (eg, comet assay, γH2AX

Table 2. Multivariate Linear Regression-Derived Estimates of the Association Between Components of the DNA Damage Response and the Clinical Frailty Scale of 53 Older Adults (>65 y), After Adjusting for Chronological Age, Sex, and Comorbidity Index

Outcome: Clinical Frailty Scale*	Coefficient	95% CI	*p*-Value
Model A—Independent association between oxidative stress levels and geriatric frailty (adj. R^2 = .52)			
Age (per year)	0.04	(−0.02) to 0.11	.145
Female sex	0.38	(−0.52) to 1.28	.399
Charlson Comorbidity Index (per score unit)	0.17	−0.05 to 1.28	.119
Polypharmacy	0.81	(−0.21) to (1.83)	.117
GHS/GSSG	−0.05	(−0.10) to (−0.01)	.031
Model B—Independent association between AP sites and geriatric frailty (adj. R^2 = .54)			
Age (per year)	0.07	0.01 to 0.15	.17
Female sex	0.32	(−0.58) to 1.23	.472
Charlson Comorbidity Index (per score unit)	0.16	(−0.06) to 1.23	.150
Polypharmacy	0.68	(−0.33) to 1.69	.178
AP sites	0.08	0.01 to 0.15	.024
Model C—Lack of association between baseline OTM and geriatric frailty (adj. R^2 = .52)			
Age (per year)	0.06	0.01 to 0.10	.011
Female sex	0.43	(−0.38) to 1.24	.289
Charlson Comorbidity Index (per score unit)	0.20	0.01 to 0.40	.054
Polypharmacy	0.96	0.04 to 1.89	.041
OTM at baseline	0.08	(−0.01) to 0.16	.083
Model D—Independent association between baseline DSBs and geriatric frailty (adj. R^2 = .53)			
Age (per year)	0.06	0.01 to 0.12	.027
Female sex	0.66	(−0.25) to 1.57	.150
Charlson Comorbidity Index	0.22	0.01 to 0.44	.047
Polypharmacy	−0.93	(−0.12) to 1.98	.082
DSBs at baseline	0.11	0.01 to 0.22	.049
Model E—Independent association between DNA double-strand break repair capacity and geriatric frailty (adj. R^2 = .57)			
Age (per year)	0.06	(0.01) to 0.11	.021
Female sex	0.34	(−0.56) to 1.24	.445
Charlson Comorbidity Index	0.24	(0.03 to 0.46)	.030
Polypharmacy	0.83	(−0.17) to 1.83	.102
DSB AUC	0.01	0.01 to 0.02	.027
Model F—Lack of association between NER and geriatric frailty (adj. R^2 = .52)			
Age (per year)	0.07	0.01 to 0.12	.028
Female sex	0.50	(−0.50) to 1.50	.318
Charlson Comorbidity Index	0.27	0.01 to 0.53	.041
Polypharmacy	0.67	(−0.46) to 1.79	.237
NER AUC	−0.01	(−0.03) to 0.01	.340

Notes: AUC = area under the curve; DSB = double-strand breaks; GSH/GSSG, ratio of reduced glutathione over oxidized glutathione; NER = nucleotide excision repair; OTM = olive tail moment.

*Scored by a geriatrician after performing a comprehensive geriatric assessment.

phosphorylation, micronucleus accumulation) is unclear (7). Basic science studies have supported increased DSBs, as detected by accumulated γH2AX foci, in senescing human cell cultures (38,39) and in aging mice (40,41). Translational human studies attempting to link DNA damage and repair with older adult frailty have been undertaken previously using DSB accumulation by γH2AX (8,17), alkaline comet assay (8), ionizing radiation-induced DNA damage and repair (37), and cytotoxic damage-related DNA repair (8). As in our data, irradiation-induced SSBs and their repair (37) as well as comet assay results (8) were not associated with frailty in multivariable analysis. A declining repair capacity of cytotoxic DNA damage was also reported by Valdiglesias and colleagues, which did not reach statistical significance (8); this was measured by comparing comet assay results in bleomycin-treated cell lines before and after incubation to allow repair, rather than γH2AX kinetics, as in our study.

In previous research investigating associations of geriatric frailty with DSB accumulation, elevated γH2AX levels were observed in participants with frailty relative to participants with prefrailty and young individuals, to a statistically significant degree in univariate analysis, congruent with our findings (8,17). Only one of the 2 studies also undertook a multivariable analysis (8), where again in agreement to our data the association between DSBs accumulation and frailty persisted while adjusting for chronological age, sex, and smoking; in our multivariable analysis, we confirm these observations while also controlling for the effect of multimorbidity and polypharmacy.

But although the CFS was associated with defective DSB-R in our results, UV-related (NER) damage repair capacity was preserved in older adults and even increased in frail individuals, in accordance with findings from a large aging cohort in the United Kingdom using low-dose (5 Gy) gamma-irradiation, which is estimated to lead to DNA SSBs (37). This distinction between the DSB-R and repair of smaller DNA lesions underlines the need to examine the effect of frailty on the diverse DNA repair mechanisms separately. Moreover, while defective DNA repair can lead to DNA damage accumulation and apoptosis, aberrant/excessive DNA repair activity may also

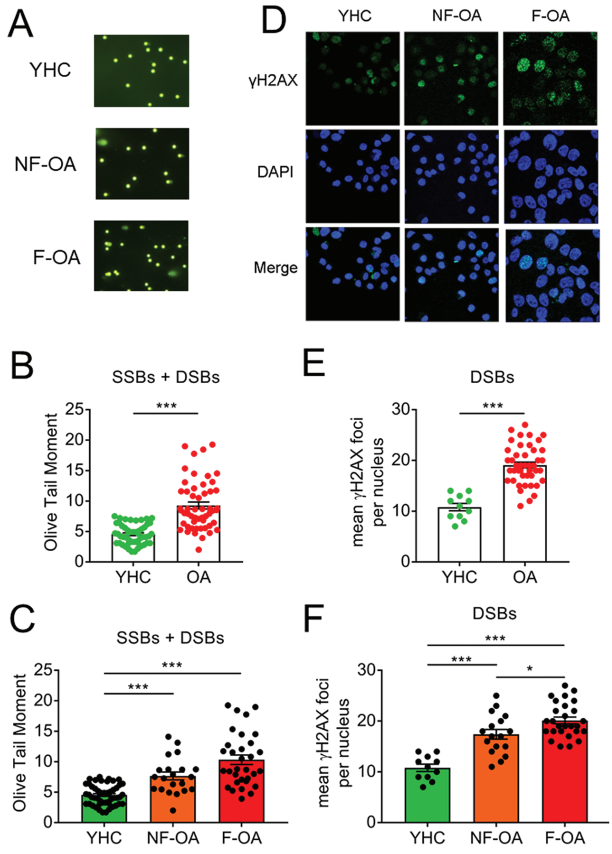


Figure 2. Endogenous DNA damage accumulation in PBMCs of frail and nonfrail older adults relative to young healthy controls. (**A**) Representative comet assay images of untreated PBMCs from a young healthy control (YHC; adults <45 years without acute or chronic health problems), a nonfrail older adult (NF-OA), and a frail older adult (F-OA). Scale bar: 20 μm. (**B**) Olive tail moment (OTM) measurements, representative of SSBs and/or DSBs quantified by comet assay, in PBMCs of young healthy controls *vs* older adults. Scale bar: 25 μm. (**C**) Olive tail moment measurements, representative of SSBs and/or DSBs quantified by comet assay, in PBMCs of NF-OA and YHC. (**D**) Confocal microscopy images showing γH2AX staining from a representative aYHC, a NF-OA, and a F-OA. Upper, immunofluorescence γH2Ax staining; middle, cell nuclei labeled with DAPI; bottom, merged. (**E**) Average number of γH2Ax foci per cell nucleus, representing DSBs, in PBMCs of YHC *vs* older adults. (**F**) Average number of γH2Ax foci per cell nucleus, representing DSBs, in PBMCs of NF-OA, F-OA, and YCH. *p*-Values are derived from Student's *t*-test/Mann–Whitney *U* test for pairwise comparisons (**B, E**) and from ANOVA with Bonferroni correction or Kruskal–Wallis with Dunn's multiple comparisons test for comparison of 3 groups (**C, F**).

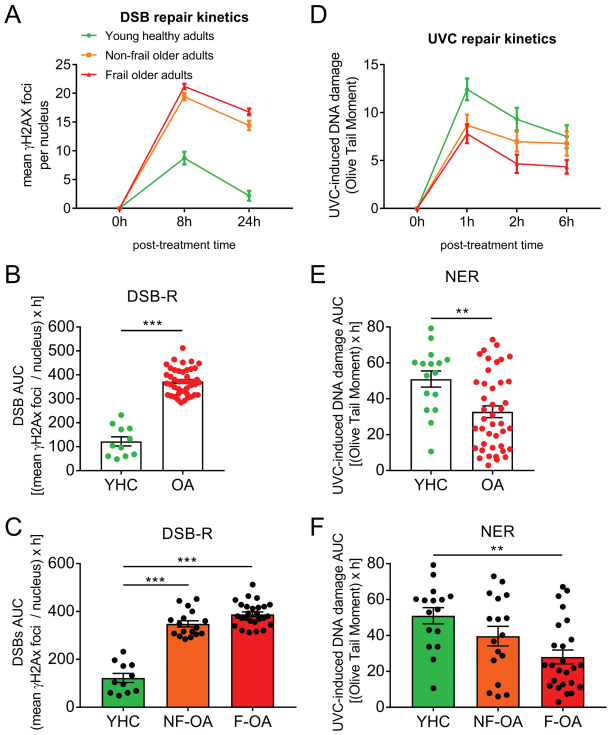


Figure 3. Evaluation of DNA repair capacity in PBMCs of frail and nonfrail older adults relative to young healthy controls. (**A**) Kinetics of γH2AX formation and removal 0–24 h after melphalan treatment of freshly isolated PBMCs derived from young healthy controls, nonfrail older adults, and frail older adults. (**B, C**) DSB accumulation (AUC; γH2Ax immunofluorescence) 24 h after treatment of PBMCs with melphalan, after subtracting initial DSB levels, in young healthy controls (YHC; adults <45 y without acute or chronic health problems) versus older adults (OA) (**B**), and in nonfrail older adults (NF-OA) versus frail older adults (F-OA) versus young healthy controls (**C**). Higher DSB accumulation is indicative of worse DSB-R capacity. (**D**) Kinetics of OTM (representing both DSBs and SSBs) at baseline, 1 h, 2 h, and 6 h after ex vivo irradiation of freshly isolated PBMCs with 5 J/m² UVC. (**E, F**) SSBs/DSBs accumulation (AUC; comet assay-OTM) 6 h after UVC irradiation of PBMCs, after subtracting initial OTM levels, in YHC versus older adults (**E**), and in NF-OA versus F-OA versus YHC (**F**). Higher SSB and/or DSB accumulation is indicative of worse NER capacity. *p*-Values are derived from Student's *t*-test/Mann–Whitney *U* test for pairwise comparisons (**B, E**) and from ANOVA with Bonferroni correction or Kruskal–Wallis with Dunn's multiple comparisons test for comparison of 3 groups (**C, F**).

contribute to genomic instability (42), resulting in accumulation of senescent cells, organ-system dysfunction, and frailty.

Our study has certain limitations. First, the number of participants is relatively low and therefore does not allow firm interpretation of negative results or adjustments for additional potential confounders in extensive multivariate models, such as cigarette smoking and alcohol consumption, despite previous reports linking them to oxidative stress (43,44) and DNA damage accumulation (45,46). However, they were not expected to have a significant impact to our results as they did not manifest univariate associations with frailty in our cohort. In addition, since this is a cross-sectional experiment, the direction of associations is not clear: Geriatric frailty may either be a cause, or a result of deregulated DNA repair mechanisms. At the same time, our study's strengths include a well-characterized geriatric population that is representative of community-dwelling older adults' physical frailty levels, age range, and prevalence of comorbidities. Our data support that, in addition to the Fried frailty phenotype, an intuitive (47), accurate (48), and widely adopted (49) frailty classification tool such as the Rockwood CFS is also correlated with molecular biomarkers of aging. In our view, this finding corroborates the concept of the CFS as a surrogate for biological age (37) and substantiates it as an additional measure at the disposal of researchers interested in the cellular mechanisms underlying pathological aging in humans.

To conclude, our results show that clinical frailty is associated with measures of increased DNA damage formation, including oxidative stress levels and AP sites, as well as accumulation and defective repair of the toxic DNA DSBs, beyond the effect of age, sex, comorbidities, and polypharmacy. Future studies are warranted to examine the potential role of deregulated DDR/R factors

as biomarkers for diagnosis and/or prognosis of frailty, as well as their potential as therapeutic targets in frailty and aging-related pathologies.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None declared.

Author Contributions

Study conception: P.P.S., E.K., P.A.N., V.L.S. Patient recruitment: E.K., P.A.N. Experiments: P.A.N., V.L.S., M.P. Drafting of manuscript: E.K., P.A.N., N.I.V., P.P.S. Critical review of manuscript: all authors. All authors contributed to the article and approved the submitted version.

Ethics Approval

The study has been approved by the Laikon General Hospital Scientific Council (approval no. 1110).

Patient Consent

All participants have signed an informed consent document prior to participation in the study.

Data Availability

The data set used for the analyses presented in this manuscript will be made available upon reasonable request, following publication of the submitted work.

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