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Epigenetic Age in Male Combat-Exposed War Veterans: Associations with Posttraumatic Stress Disorder Status

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Abstract

DNA methylation patterns change with age and can be used to derive an estimate of "epigenetic age," an indicator of biological age. Several studies have shown associations of posttraumatic stress disorder (PTSD) with worse somatic health and early mortality, raising the possibility of accelerated biological aging. This study examined associations between estimated epigenetic age and various variables in 160 male combat-exposed war veterans with (n = 79) and without PTSD (n = 81). DNA methylation was assessed in leukocyte genomic DNA using the Illumina 450K DNA methylation arrays. Epigenetic age was estimated using Horvath's epigenetic clock algorithm and Δage (epigenetic age-chronological age) was calculated. In veterans with PTSD (Δage = 3.2), Δage was on average lower compared to those without PTSD (Δage = 5.0; p = 0.02; Cohen's d = 0.42). This between-group difference was not explained by race/ethnicity, lifestyle factors or childhood trauma. Antidepressant use, however, explained part of the association. In the PTSD positive group, telomerase activity was negatively related to Δage

J.E.V. and R.Y. share first authorship.

($\beta = -0.35$; $p = 0.007$). In conclusion, veterans with PTSD had significantly lower epigenetic age profiles than those without PTSD. Further, current antidepressant use and higher telomerase activity were related to relatively less epigenetic aging in veterans with PTSD, speculative of a mechanistic pathway that might attenuate biological aging-related processes in the context of PTSD.

Introduction

Aging can be seen as a time-dependent functional decline that affects living organisms [1]. Whereas chronological aging is indexed by calendar time, biological aging is indexed by multiple cellular and molecular processes such as genomic instability, telomere attrition and mitochondrial dysfunction [1]. A recent indicator of biological aging is based on DNA methylation patterns [2]. Essentially, DNA methylation is characterized by the addition of a methyl group to cytosine at cytosine-phosphate-guanine (CpG) sites, thereby regulating gene expression. Recent studies identified several genes and genomic regions that become either hypomethylated or hypermethylated with age [2, 3], which, together, can be used to develop a so-called epigenetic clock. In 2013, Horvath [3] developed such an epigenetic age predictor using 8,000 samples from multiple tissues, identifying 353 CpG sites that predict chronological age with high accuracy [3]. Since then, several studies have applied this epigenetic clock to a variety of conditions, showing associations between accelerated epigenetic aging and insomnia [4], Down syndrome [5], obesity [6], Parkinson's disease [7], and all-cause mortality [8, 9].

Indicators of biological age, rather than chronological age, are of increased interest in the study of stress-related psychiatric disorders, because of their associations with increased risks for somatic illnesses commonly seen with advanced age [10, 11] and an almost twofold higher risk of mortality [12]. In recent years, telomere length, one of the most robust indicators of cellular aging, has been extensively studied and was found to be shorter across a variety of psychiatric disorders [13, 14], including posttraumatic stress disorder (PTSD) [15]. Associations with the epigenetic clock, which may also be linked to the telomere/telomerase system [16], remain relatively unexplored in psychiatric settings. So far, few studies have examined the epigenetic age in PTSD. Boks et al. [17] found that in 96 male veterans, combat trauma exposure was associated with accelerated epigenetic aging based on

Horvath's algorithm, but in contrast, the development of PTSD symptoms during follow-up was associated with *less* epigenetic aging. In a study comprising 281 male and female veterans, Wolf et al. [18] found no associations between Horvath's epigenetic age estimate and lifetime PTSD, but alternatively they found that accelerated epigenetic age was associated with lifetime PTSD based on an another epigenetic estimate using 71 CpG sites by Hannum et al. [19]. In a follow-up study, Wolf et al. [20] found an association between accelerated epigenetic age (as estimated by Hannum et al. [19]) and PTSD hyperarousal symptoms in 339 trauma-exposed veterans but not with total PTSD severity. Last of all, Zannas et al. [21] found that lifetime stressors, but not current PTSD symptomatology, were associated with accelerated epigenetic aging in 392 highly traumatized African American individuals.

Overall, the literature examining associations of epigenetic age with PTSD symptomatology or diagnosis is scarce and mixed [17, 18, 20, 21]. The current study examined epigenetic age in white blood cells based on Horvath's algorithm in 79 male combat-exposed war veterans with PTSD and 81 male combat-exposed war veterans without PTSD. To our knowledge, this is the first study to compare trauma-exposed veterans with a clinically verified current PTSD diagnosis with a control sample of veterans matched for age and sex without PTSD. Furthermore, data of a subsample ($n = 44$) were available for follow-up longitudinal testing, which allowed us to examine the stability of epigenetic age estimates over time.

Patients and Methods

Ethical Statement
The Institutional Review Boards of Icahn School of Medicine at Mount Sinai (ISMMS; New York, NY, USA), the James J. Peters Veterans Administration Medical Center (JJPVAMC; Bronx, New York, NY, USA), New York University Medical Center (NYU; New York, NY, USA), the US Army Medical Research and Materiel Command, and the University of California, San Francisco, Medical Center (UCSF; San Francisco, CA, USA) approved this study. Study participants gave their written informed consent to participate. Participants were compensated for their participation. The study was conducted in accordance with the provisions of the Helsinki Declaration.

Recruitment Procedures and Study Sample
One hundred sixty-six veterans from Operation Iraqi Freedom and Operation Enduring Freedom were recruited by NYU and ISMMS/JJPVAMC. Participants were recruited from the Mental Health Services of the Manhattan, Bronx and Brooklyn Veterans

Affairs Medical Centers, other regional VA medical centers, Veterans Service Organizations, National Guard, reservist agencies and organizations and from the general community. Recruitment methods included flyers, in-person presentations, media advertisements, Internet postings (e.g., Craigslist), and referral from clinicians. Criteria for inclusion were (a) having served in war zones; (b) current age between 20 and 60; (c) males; and (d) proficient in the English language. Exclusion criteria included: (a) history of alcohol dependence within the past 8 months; (b) history of drug abuse or dependence (except nicotine dependence) within the past year; (c) lifetime history of any psychiatric disorder with psychotic features, bipolar disorder, or obsessive-compulsive disorder; (d) those who were currently being exposed to recurrent trauma or had been exposed to a traumatic event within the past 3 months; (e) prominent suicidal or homicidal ideation; (f) neurologic disorder or systemic illness affecting central nervous system function; (g) history of anemia or recent blood donation in the past 2 months; (i) veterans who were not stable for at least 2 months on psychiatric medication, anticonvulsants, antihypertensive medication, or sympathomimetic medication; (j) veterans who were classified with a moderate or severe traumatic brain injury (TBI) on the Ohio State University TBI Identification Method-Short Form [22].

All study participants had exposure to a combat-related PTSD Criterion A trauma and participants in the PTSD+ group were also required to have a current (past month) Clinician Administered PTSD Scale for DSM-IV (CAPS) score [23] ≥40 and to meet full DSM-IV criteria for PTSD; in the non-PTSD group, all veterans were required to have a current CAPS score ≤20 and they could never have met criteria for PTSD in the past. The CAPS cut-off scores of ≤20 and ≥40 were selected to create stringent and distinct groups of PTSD negative and positives participants. The average duration from any self-reported PTSD symptoms was 72 months for PTSD positive and 29 months for PTSD negative veterans ($p < 0.001$). The 166 combat-exposed male veterans were recruited for a larger overall study examining biomarkers of PTSD. Of these, 6 were removed because there were no DNA methylation data available ($n = 4$), hepatitis was diagnosed ($n = 1$) and a psychiatric diagnosis in the control group was made ($n = 1$), respectively, leaving 79 participants with PTSD and 81 without PTSD. Forty-four of the veterans with PTSD and none of the combat-exposed controls fulfilled the criteria for major depressive disorder (MDD). Additionally, longitudinal follow-up data were available for a subsample of 44 participants (28 PTSD+ veterans and 16 controls). The average follow-up duration was 3.3 years (SD = 1.1; range 1.1–5.1).

Clinical Assessments

To establish psychiatric diagnoses, a Structured Clinical Interview for DSM-IV disorders (SCID) was conducted by doctoral-level psychologists, was audio recorded and calibrated weekly with a senior clinician [24]. Current (past month) and lifetime PTSD symptom severity was assessed with the CAPS. Depression symptom severity was assessed with the self-rated Beck Depression Inventory-II (BDI-II) [25]. The Perceived Stress Scale was used to measure the perception of psychological stress in the past month [26]. Exposure to early life trauma was evaluated using the Early Trauma Inventory-Self Report Short Form [27]. Antidepressant use was assessed by self-report.

Data from the Deployment Risk and Resilience Inventory-2 (DRRI-2) were available for 90 participants (35 with and 55 without PTSD) [28]. The DRRI-2 is a suite of 17 individual scales that assesses key deployment-related risk and resilience factors. For the purpose of assessing the degree of combat exposure in this sample, we used the Combat Experiences subscale (section D). Further, the number of tours of deployment was assessed, including deployment to regions other than Iraq and Afghanistan.

Blood Draw and DNA Methylation Assessment

Blood was drawn in the morning after a night of fasting. Genomic DNA was isolated from blood collected in PAXgene Blood DNA Tubes using the PAXgene Blood DNA Kit according to the manufacturer's protocol (Qiagen, Germantown, MD, USA). The DNA yield was assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher, Wilmington, DE, USA). The A260/280 and A260/230 ratios were measured by spectrophotometric assay, and quality controlled samples were selected if both ratios were larger than 1.75. The methylated DNA fraction was selected from the whole genome via immunocapture performed on a 200 µL fragmented pooled DNA sample.

The whole blood genomic DNA (500ng) from each sample was bisulfite-converted using the EZ96 DNA methylation kit (Zymo Research, Orange, CA, USA) following the manufacturer's standard protocol with instructions specific for Illumina arrays. The bisulfite-treated DNA was then analyzed using HumanMethylation450 BeadChip (Illumina Inc., USA) according to the manufacturer's protocol. To minimize the potential batch effects, the samples were fully randomized in each experimental step, and all samples were processed with the same technician and machines. The samples were de-identified, the labs that performed the assays were blind to all other measurements, and case and control samples were randomly distributed over 4 plates.

Epigenetic Age

Epigenetic age was calculated from DNA methylation data using the online calculator by Horvath [3]. In short, normalization of the relevant CpG methylations levels was conducted using a mixed effect model and based on the DNA methylation levels. We used a customized R code, according to the calculator's pipeline and used the "BMIQ method using a Gold standard" [3] method for normalization. Horvath predicted ages of tissue based on regression coefficients obtained from training sets encompassing over 13,000 subjects, described in the original article [3]. This epigenetic profile correlated strongly with chronological age assessed across tissues and cell types, and across multiple studies and populations.

Cell Counts

Cell counts were estimated by Horvath's epigenetic clock software [29]. We also used complete blood count to confirm the estimations. The correlations between cell type percentages using Complete Blood Count and the estimations are presented in online Supplementary Table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000491431). Leukocyte cell type counts had no significant associations with any of the CAPS scores, PTSD status or combat exposure. To adjust for cell count in analyses with epigenetic age, Horvath's estimates were used (see Statistical Analyses).

Telomere Length and Telomerase Activity

The assay of telomere length from leukocytes was adapted from the published original method [30] as reported elsewhere [31]. The same reference DNA was used for all PCR runs. Telomerase activ-

ity in leukocytes was assessed with an optimized assay on the basis of the commercially available kit TRAPeze (Chemicon, Temecula, CA, USA) reported in more detail elsewhere [32]. In short, 2 concentrations (5,000 and 10,000 cells) were used for Telomeric Repeat Amplification Protocol (TRAP) reactions to ensure that the assay for each sample is in the linear range. In all, 293T cells are used as positive controls. In addition to the 293T cancer cell extract as the standard for telomerase activity quantification, we also include 2 positive control extracts from the human cancer cell line Hela. The average intra assay variability of PBMC samples ($n = 6$, assayed in triplicates) is 8% and the inter-assay variability of PBMC samples ($n = 24$, assayed on 2 different days) is 6.7%. More details of both methods can be found in the study by Lin et al. [33].

Statistical Analyses

Baseline characteristics were compared between PTSD+ and PTSD– veterans using chi-square statistics and analyses of variance. ΔAge was calculated for all participants by subtracting actual chronological age from estimated epigenetic age (epigenetic age minus chronological age) to reflect accelerated (positive Δage values) or decelerated (negative Δage values) epigenetic aging. PTSD+ and PTSD– groups were matched for chronological age on a group level and therefore age was not added as a covariate in subsequent analyses. Analyses of covariance were used to determine differences in Δage between veterans with and without PTSD. All analyses with Δage were controlled for abundance measures of cell types estimated by Horvath's epigenetic clock software: naïve CD8 and CD4 T-cells, CD8+CD28-CD45RA T-cells, plasma blast cells, natural killer cells, monocytes, and granulocytes (model 1); additionally, analyses were controlled for race/ethnicity, alcohol use, smoking status, and BMI (model 2). In secondary analyses, we tested the extent to which between-group differences were explained by antidepressant use, childhood trauma, comorbid MDD and number of deployment tours. Regression analyses further tested associations between Δage and symptom severity scales, antidepressant use, childhood trauma and number of deployment tours in the total sample. To gain insight into relationships of epigenetic age with other measures of biological aging, we tested associations between Δage and telomere length and telomerase activity.

As a validation check for using Δage as our main outcome variable, we performed additional analyses in which we regressed DNA methylation age on chronological age and saved the unstandardized residuals. Positive DNA methylation age residuals reflect accelerated epigenetic age, while negative residuals reflect decelerated epigenetic age. All analyses were performed with both Δage and unstandardized residuals as outcome variables, both analyses yielded highly similar results (results not shown).

Results

Sample Characteristics

The mean chronological age of the total study sample was 32.8 years (SD = 7.9, range 20–59). Characteristics for veterans with and without PTSD are presented in Table 1. The groups did not differ significantly in age, education, race/ethnicity, and alcohol use. Veterans with PTSD had a higher average BMI, reported more childhood trauma and were more often current smokers. Further, the groups differed significantly on all clinical variables, with the PTSD positive group having more severe symptoms, comorbid MDD diagnoses and antidepressant users, as expected. The groups did not differ in the number of deployment tours (range 1–4).

Epigenetic age was strongly correlated with chronological age across the total sample ($r = 0.812$; $p < 0.001$); see online Supplementary Figure 1. ΔAge ranged from –10.8 to 19.5, and the average Δage across the entire sample was 4.1 (SD = 4.9). ΔAge was negatively related to chronological age ($r = -0.261$; $p = 0.001$), but was not associated with race/ethnicity ($p = 0.192$), alcohol use ($p = 0.564$), smoking status ($p = 0.846$) or BMI ($p = 0.453$). Those participants who currently used antidepressant medication ($n = 26$) had lower Δage than those without antidepressants ($p = 0.017$) across the total sample.

Between-Group Comparisons of ΔAge

The PTSD positive group had an average Δage of 3.2 (SD = 4.5), while the PTSD negative group had a Δage of 5.0 (SD = 4.6). This showed that those with a PTSD diagnosis had an average lower Δage than that of the group without PTSD (model 1: $F[1,150] = 6.85$; $p = 0.022$; Cohen's d = 0.42, Fig. 1a), indicating significantly less acceleration of epigenetic age in those with PTSD. Adjusting for race/ethnicity (including Hispanic status), smoking, alcohol, and BMI did not impact the group difference (model 2: $p = 0.019$).

In sensitivity analyses, we tested whether clinical and deployment characteristics were explanatory factors in the between-group comparison. The between-group difference remained significant when adjusting for childhood trauma ($p = 0.032$) or number of tours ($p = 0.023$). When adjusting for current antidepressant use, the between-group association between PTSD and Δage became non-significant ($p = 0.105$). Interestingly, dividing the PTSD positive group into 2 groups based on antidepressant medication use showed that compared to the PTSD negative controls (Δage = 5.0), those with PTSD that used antidepressant medication use (Δage = 2.1; $p = 0.012$), but not those without medication use (Δage = 3.6; $p = 0.120$) had significantly lower Δage (model 1: overall effect: $F[2,150] = 3.59$; $p = 0.030$, Fig. 1b; model 2: $p = 0.015$). Within the PTSD group, there was no significant difference in Δage between those with and without antidepressant medication ($p = 0.187$).

As a post hoc analysis, we tested whether the association between PTSD status and Δage was mediated by an-

Table 1. Sample characteristics (mean ± SD or % [n]) by PTSD status ($n = 160$)

	PTSD negative ($n = 81$)	PTSD positive ($n = 79$)	p value*
Sociodemographic characteristics			
Age	32.6 (8.0)	33.0 (7.8)	0.790
Years of education	13.4 (5.1)	13.0 (3.9)	0.649
Race/ethnicity, % (n)			0.170
Hispanic	32.1 (26)	45.6 (36)	
Non-hispanic black	23.5 (19)	26.6 (21)	
Non-hispanic white	33.3 (27)	24.1 (19)	
Asian	6.2 (5)	1.3 (1)	
Other	4.9 (4)	2.5 (2)	
Lifestyle characteristics			
Body mass index	28.5 (4.8)	30.1 (5.1)	0.042
Hazardous alcohol use (AUDIT)	2.9 (2.4)	3.2 (3.8)	0.612
Smoking (% yes, n)	11.1 (9)	34.2 (27)	<0.001
Clinical characteristics			
Clinician administered PTSD scale (CAPS), current	3.7 (5.2)	68.8 (16.9)	<0.001
Clinician administered PTSD scale (CAPS), lifetime	9.3 (8.5)	91.1 (15.7)	<0.001
Beck depression inventory (BDI-II)	5.6 (6.2)	25.1 (10.9)	<0.001
Perceived stress scale	1.8 (0.6)	3.1 (0.7)	<0.001
Comorbid major depressive disorder (% yes, n)	0 (0)	55.7 (44)	<0.001
Current antidepressant use (% yes, n)	4.9 (4)	27.8 (22)	<0.001
Childhood trauma inventory score	2.2 (1.9)	2.8 (2.2)	0.079
Deployment characteristics			
Combat experiences (DRRI-D score; $n = 90$)	31.5 (12.4)	53.2 (18.4)	<0.001
Number of military tours (range 1–4)	1.8 (0.9)	1.8 (0.8)	0.882

* p values are based in one-way ANOVA for continuous variables and chi's square test for categorical variables.

tidepressant medication, using the Sobel method [34], which estimates the direct and indirect effects of the independent variable on the dependent variable through the mediator variable. Results showed that current antidepressant use was – albeit reducing the direct effect of PTSD on Δage from $c = -1.8$ (se = 0.8) to $c' = -1.3$ (se = 0.8) – not a significant mediator ($p = 0.11$).

Associations of ΔAge and Clinical Characteristics

Current and lifetime severity of PTSD symptoms, measured with the CAPS, was negatively associated with Δage across all participants (Table 2). Similar negative associations were found across all participants for the BDI-II and the Perceived Stress Scale. Associations were, however, not significant within the 2 groups separately, indicating that severity measures did not further differentiate within the PTSD group and results might thus reflect the group difference. Those with a MDD diagnosis, further, had significantly lower Δage than those without MDD ($p = 0.013$). ΔAge was not associated with childhood trauma (Early Trauma Inventory; $β = -0.05$; $p = 0.512$) across all participants and within the 2 groups separately. We found no associations between Δage and number of deployment tours ($β = -0.05$; $p = 0.517$) or severity of combat experiences ($β = -0.08$; $p = 0.448$). Adjusting for chronological age did not change any of the results.

Associations of ΔAge and Telomere Length and Telomerase Activity

ΔAge was not related to telomere length in the whole sample (model 1: $p = 0.377$, Table 2) or in the PTSD positive ($p = 0.592$) or negative ($p = 0.925$) samples separately. However, we did find a significant negative association between Δage and telomerase activity in in the full sample (model 1: $β = -0.238$; $p = 0.012$, Table 2). This association was driven by the group with PTSD ($β = -0.352$; $p = 0.007$), while no association was found in the controls ($β = -0.147$; $p = 0.371$), showing that in the PTSD group, higher telomerase activity was associated with relatively less epigenetic

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Table 2. Univariate linear regression analyses of Δage with various characteristics in the total sample ($n = 160$)

	B	SE	β	p value
Clinical characteristics				
Clinician administered PTSD scale (CAPS), current	−0.03	0.01	−0.21	0.007
Clinician administered PTSD scale (CAPS), lifetime	−0.02	0.01	−0.19	0.014
Beck's depression inventory (BDI-II)	−0.08	0.03	−0.21	0.008
Perceived stress scale	−1.34	0.43	−0.24	0.002
Antidepressant use (no = reference)	−2.48	1.03	−0.19	0.017
Early Trauma Inventory score	−0.05	0.08	−0.05	0.512
Deployment characteristics				
Number of military tours	−0.30	0.46	−0.05	0.517
Combat experiences (DRRI-D; $n = 90$)	−0.02	0.02	−0.08	0.448
Other cellular aging characteristics				
Telomere length[1]	0.35	0.40	0.07	0.377
Telomerase activity[1]	−1.01	0.42	−0.24	0.012

Univariate regression analyses adjusted abundance measures of cell types (model 1). [1] Additionally adjusted for chronological age.
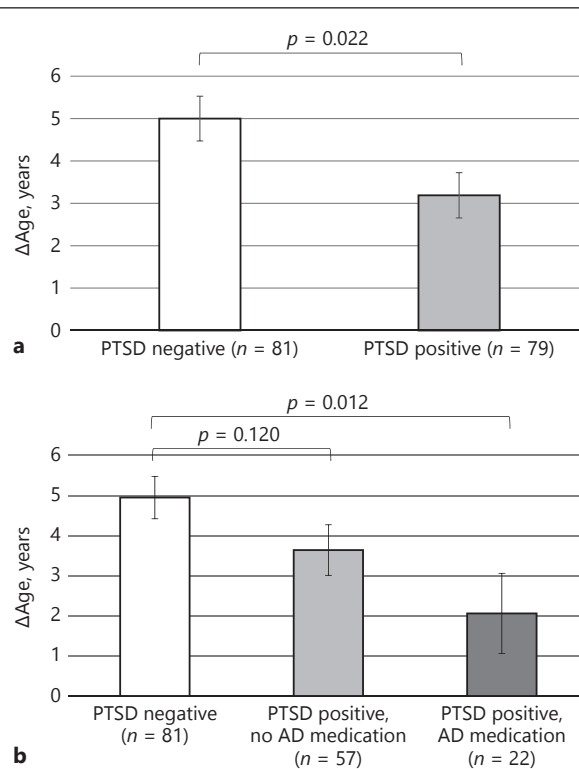

Fig. 1. Mean Δage (epigenetic age – chronological age) by (**a**) PTSD status and (**b**) PTSD and current antidepressant medication use. Note. Estimated means (and standard error) of Δage from analysis of covariance, adjusted for abundance measures of cell types (model 1); AD medication = antidepressant medication.

age acceleration (Fig. 2). The interaction effect of PTSD-by-telomerase activity was trending to be significant ($p = 0.83$; $n = 107$). In the current study sample, neither telomere length ($p = 0.263$) nor telomerase activity ($p = 0.761$) was significantly associated with PTSD status, in analyses corrected for chronological age. More detailed associations with PTSD status and telomere length or telomerase activity in a larger study sample are being explored in a more comprehensive analysis and are beyond the scope of the current paper (Kang et al., in preparation).
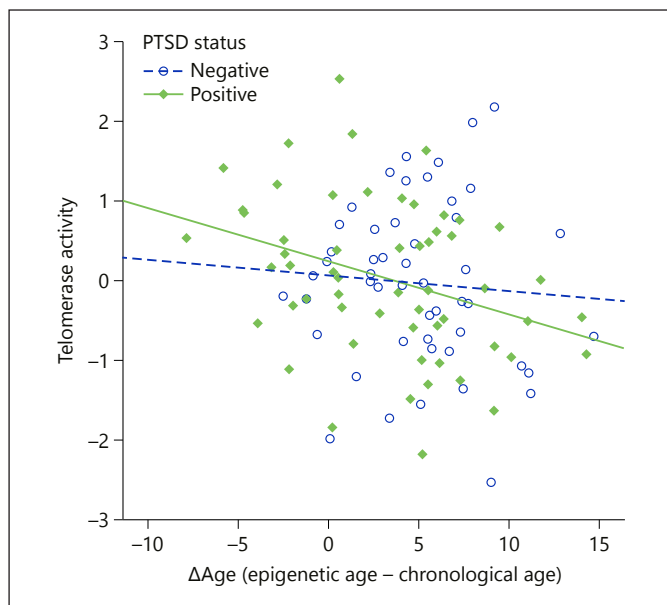
Fig. 2. Scatterplot of telomerase activity by Δage for PTSD groups separately. Telomerase activity is only significantly associated with Δage in the PTSD positive group (β = –0.35; p = 0.007) and not in the PTSD negative group (β = –0.15; p = 0.371).

Stability of ΔAge Over Time

Follow-up data were available for a subsample of 44 individuals, including 28 veterans with PTSD and 16 without PTSD. Epigenetic age at baseline correlated highly with epigenetic age at an average of 3-year follow-up in this subsample (r = 0.91; p < 0.001), demonstrating a high within-person stability. Correlations between Δage at baseline and follow-up were similarly high (r = 0.68; p < 0.001). Results of a between-group comparison of Δage at follow-up were in the same direction as at baseline, albeit non-significant (PTSD positive Δage = 3.3, PTSD negative Δage = 4.0).

Discussion

This study examined epigenetic age acceleration based on leukocyte DNA methylation levels in male combat-exposed war veterans. Contradicting our expectations, those with PTSD showed less accelerated epigenetic aging than those without PTSD. In other words, those with PTSD showed a relatively "younger" epigenetic profile than PTSD negative controls, according to Horvath's epigenetic age estimator [3]. The significant between-group difference in epigenetic aging was not explained by race/ethnicity, BMI, smoking or alcohol use, childhood trau-

ma, or number of tours of duty. Differences were also not accounted for by proportional differences in leukocyte subtype composition. Interestingly, the between-group difference became non-significant when adjusting for current antidepressant use, suggesting that part of the association might be explained by mechanisms secondary to antidepressant use. Further, analyses suggested that veterans with the most severe psychiatric profiles, such as those with high PTSD symptom severity and MDD comorbidity, had the lowest Δage. Lastly, in those with PTSD, but not in those without PTSD, telomerase activity was negatively related to Δage, indicating that the higher the telomerase activity, the younger the epigenetic age.

The finding of relatively less accelerated DNA methylation profiles in combat-exposed veterans with PTSD compared to veterans without PTSD was unexpected. Meta-analyses show that PTSD is associated with a higher risk of all-cause mortality [12, 35] as well as several aging-related diseases [36, 37]. Further, recent work showed that epigenetic age is independently associated with mortality [9], which would logically lead to the hypothesis of relatively more accelerated epigenetic aging in those with PTSD compared to those without PTSD. However, most previous studies on PTSD and somatic health or mortality compared PTSD individuals to non-traumatized healthy controls [12, 36, 37], making it difficult to separate the effects of trauma exposure from those of PTSD status per se. Our data suggest that individuals with combat trauma-exposed PTSD showed less epigenetic acceleration than those without PTSD. While this finding was unexpected, another study also found indications of decelerated epigenetic aging in PTSD: Boks et al. [17] found that the development of more PTSD symptoms over 13 months was associated with *decelerated* DNA methylation aging, as estimated with Horvath's metric. In line with our results, they found that trauma exposure per se (i.e., combat-exposure) was associated with increased epigenetic aging, but the reverse was true for PTSD development in 96 war veterans who were evaluated pre-deployment and again 6 months later. On the other hand, Wolf et al. [20] found no associations in 281 male and female veterans between epigenetic age according to Horvath's algorithm and "latent lifetime PTSD severity," a construct they developed with factor analysis from pre-military, post-military, and current PTSD symptoms. However, they did not report whether associations were present in the current PTSD group compared to those without PTSD, which makes it difficult to compare with the current study. It should be noted that Wolf et al. [20]

did find an association between PTSD and another estimate of epigenetic age by Hannum et al. [19] In a follow-up study, Wolf et al. [20] found an association between accelerated epigenetic age according to Hannum's estimation and PTSD hyperarousal symptoms in 339 trauma-exposed veterans, but not with PTSD severity overall. In that study, Horvath's estimation was not examined. It is interesting to note that analyses with Horvath's and Hannum's estimates have shown mixed results in the literature up until now, indicating that both clocks might capture different (epigenetic) traits. Both clocks have only 6 CpG sites in common and are associated with different genetic loci affecting age acceleration [38]. Although beyond the scope of the present study, future studies should estimate whether the clocks reflect meaningful differences in the context of PTSD (see e.g., [39, 40]).

Currently, we can only speculate on the reasons why we find relatively less accelerated epigenetic age in PTSD compared to the non-PTSD group. One possible explanation is the generation of a compensatory mechanism in response to stress and subsequent PTSD development, an explanation also proposed by Boks et al. [17] Interestingly, we showed that high telomerase activity was associated with lower Δage only in the PTSD group, which is in line with earlier studies that found elevated telomerase in response to acute psychological stress in human caregivers [41, 42], in unmedicated individuals with MDD [32], and chronically stressed rats [43]. Telomerase has several important functions in addition to its role in telomere maintenance, including promotion of cell growth and cell longevity [44, 45], and might thus induce mechanisms that reverse or decelerate cellular aging. For example, elevated telomerase has been found to prevent age-related decrease in DNA 5-methylcytosine transferase I (DNMT1) expression that could contribute in the maintenance of a relatively "younger" epigenetic profile [45, 46]. Further, a recent GWAS revealed a critical role for the human TERT gene in epigenetic aging rates and showed TERT-specific and independent effects on both longer telomere length and epigenetic age acceleration [16]. Like other studies, we found no associations between epigenetic aging and telomere length [47]. A recent study, however, did find an inverse association between telomere length and advanced epigenetic aging, possibly as a result a high memory CD8+ T cells/naive CD8+ T cells ratio [48].

Another possible pathway is that epigenetic changes may interact with altered glucocorticoid responses in PTSD [49], as a recent study found that 85 of the 353 epigenetic clock CpG sites from Horvath's clock were located within glucocorticoid response genes [21]. Activation of the glucocorticoid receptor as a consequence of cortisol secretion might drive changes in DNA methylation and other epigenetic modifications [50]. This may be especially relevant here, since combat-veterans with PTSD showed lower methylation of the promoter of the glucocorticoid receptor gene [51]. Further, we found a suggestion that our results were partly explained by antidepressant medication use. Currently used antidepressants drugs may exert direct epigenetic effects which might impact epigenetic aging [52, 53]. It should be noted, however, that it remains to be explored if our finding regarding antidepressant medication is an effect of antidepressants per se, or rather of severity, since those who used antidepressants also had higher MDD comorbidity levels and CAPS and BDI scores (p values <0.001).

The current study found no evidence of an association between epigenetic age and early life adversities. This is in line with 2 recent studies that found no associations between epigenetic age and childhood maltreatment in 96 male veterans [17] and 392 individuals from an African American population cohort [21]. Interestingly, the latter study found that in those with high childhood abuse, the associations between personal life stressors and accelerated epigenetic age were attenuated, which the authors proposed to be the consequence of some "resilience mechanisms" triggered by childhood trauma [21]. Kananen et al. [54] found that epigenetic age showed great stability over 4-year and 25-year follow-ups, with hardly any changes in individuals' rank orders. This is consistent with the stability in epigenetic age that we found in a subset of participants tested at 2 time-points. It remains to be explored whether epigenetic age can be used as a pre-deployment vulnerability marker by future longitudinal studies, including the time before the occurrence of traumas and the onset of PTSD.

Study strengths are the relatively large sample size with extensive clinical phenotyping, including information on clinical severity, combat trauma exposure, childhood trauma, MDD, antidepressant use and a wide variety of lifestyle factors. Further, methylation patterns were all assayed simultaneously in the same lab and randomized over batches, thus minimizing potential batch-related noise. Some limitations of this study should, however, also be noted. First, both groups in this study consisted of male war veterans, selected as relatively young and healthy individuals without traumatic brain injury, thus the generalizability of our findings in samples with and without PTSD in both men and women remains to be explored. It should thus be noted that results might be different for

older groups with more comorbidity. Also, those without PTSD in our sample had also experienced combat traumas but did not develop PTSD, which may indicate that they are highly resilient group, as opposed to a traditional healthy control group. Second, our suggestion of overall accelerated epigenetic aging in the current sample should be considered within the parameters of this specific population. We found a suggestion that both veteran groups on average showed older DNA methylation age compared to their chronological age. However, we withhold from interpreting this finding, since we cannot rule out other explanations such as a mean error in the estimation of epigenetic age causing the mean Δage to be off by a certain constant. Third, the estimate of cellular aging used in this study is designed to provide an accurate predictor of chronological age, rather than to explore mechanistic pathways that lead to alterations in cellular aging. DNA methylation age predicts all-cause mortality in later life [9], suggesting an association with a process related to aging, but it is unknown if the methylation changes at the 353 CpGs used in the clock model play a direct causal role in the aging process. Fourth, we are, to our knowledge, the first to report a negative association between telomerase activity and Δage in the PTSD, this finding should therefore be considered preliminary, and future research in independent samples should find if this association can be replicated. Fifth, DNA methylation data were collected from mixed blood cell types, while DNA methylation patterns are known to differ between cell types [3]. However, we controlled for abundance measures of cell types as provided by Horvath's online calculator. Lastly, we cannot rule out pre-deployment differences. Future studies could add substantial knowledge by exploring these associations longitudinally and, in the context of veterans, including pre-deployment data.

This study found that combat trauma-exposed veterans with PTSD, showed lower Δage values, indicating relatively less accelerated epigenetic aging, based on Horvath's "epigenetic clock," compared to a well-matched control group of combat trauma-exposed veterans without PTSD. This association was partly explained by antidepressant use. Higher telomerase activity was related to relatively lower Δage only in PTSD patients, suggesting a mechanistic pathway that might attenuate cellular aging-related processes in response to stress. Future research remains to explore the long-term health consequences of these changes in epigenetic age.

Disclosure Statement

All authors declare that there are no conflicts of interest to disclose.

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Disclaimer

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