THE JOURNAL OF
CLINICAL PSYCHIATRY
THE OFFICIAL JOURNAL OF THE AMERICAN SOCIETY OF CLINICAL PSYCHOPHARMACOLOGY

Supplementary Material

Brief Report Title: Association of Symptoms of Posttraumatic Stress Disorder and GrimAge, an Epigenetic Marker of Mortality Risk, in US Military Veterans

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DOI Number: 10.4088/JCP.21br14309

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Supplementary Methods

Participants

The National Health and Resilience in Veterans Study (NHRVS) is a nationally representative survey of U.S. military veterans. The NHRVS sample was drawn from KnowledgePanel, a research panel of more than 50,000 households that is maintained by Ipsos, a survey research firm. KnowledgePanel® is a probability-based, online non-volunteer access survey panel of a nationally representative sample of U.S. adults that covers approximately 98% of U.S. households. Panel members are recruited through national random samples, originally by telephone and now almost entirely by postal mail. Households are provided with access to the Internet and computer hardware if needed. KnowledgePanel® recruitment uses dual sampling frames that include both listed and unlisted telephone numbers, telephone and non-telephone households, and cell-phone-only households, as well as households with and without Internet access.

Of the 4,750 veterans who were in KnowledgePanel® when the NHRVS was fielded (veteran status was assessed using a general demographic questionnaire), 3,408 (71.7%) responded to an invitation to participate and completed a screening question to confirm their study eligibility (current or past active military service). Of these respondents, 3,199 (93.5%) confirmed their current or past active military service, 3,157 (92.6%) complete a confidential, 60-minute online survey, and 2,397 (70.3%) consented to and provided a saliva sample for genotyping. Given that the vast majority of U.S. veterans are male and European-American and to increase statistical power and avoid confounding analyses with low numbers of female and ethnically diverse veterans, epigenetic analyses were limited to male European-American veterans in the genotyped cohort (n=1,132).

Demographic data of survey panel members are assessed regularly by Ipsos using the same set of questions used by the U.S. Census Bureau. To permit generalizability of study results to the entire population of U.S. veterans, the Ipsos statistical team computed post-stratification weights using the following benchmark distributions of U.S. military veterans from the 2011 Current Veteran Population Supplemental Survey of the U.S. Census Bureau's American Community Survey: age, gender, race/ethnicity, Census Region, metropolitan status, education, household income, branch of service, and years in service. An iterative proportional fitting (raking) procedure was used to produce the final post-stratification weights. All participants provided informed consent and the study was approved by the Human Subjects Committee of the VA Connecticut Healthcare System.

Assessments

Cumulative trauma burden. The Trauma History Screen (THS)[1] was used to assess exposure to the lifetime occurrence of 14 potentially traumatic events; the NHRVS additionally assessed exposure to life-threatening illness or injury. The sum of potentially traumatic events endorsed, ranging from 0–15, was used as an index of lifetime trauma burden.

PTSD symptoms. PTSD symptoms were assessed using the PTSD Checklist-Specific Stressor Version (PCL-S); score ≥ 35, which have been recommended for general population samples,[2] were indicative of a positive screen (Cronbach's α in the current sample=0.94). Participants were asked to respond to PCL-S in relation to their 'worst' trauma endorsed on the THS.

Combat veteran. Combat veteran status was assessed with the following question: "Did you ever serve in a combat or war zone?" and the Combat Exposure Scale,[3] a 7-item self-report measure that assesses wartime stressors experienced by combatants.

Childhood physical or sexual abuse. Childhood physical or sexual abuse was assessed using two items from the THS: "Hit or kicked hard enough to injure – as a child" and/or "Forced or made to have sexual contact – as a child." A three-level variable was created based on responses to these items: No physical or sexual childhood abuse; physical abuse; and physical and sexual abuse.

Years since index trauma. Years since index trauma was assessed by subtracting current age from age of index trauma.

Number of medical conditions. Sum of number of medical conditions endorsed in response to question: "Has a doctor or healthcare professional ever told you that you have any of the following medical conditions?" (e.g., arthritis, cancer, diabetes, heart disease, asthma, kidney disease). Range: 0-24 conditions.

Body mass index (BMI). BMI was calculated based on self-reported height and weight using the standard formula weight (kg)/height (m^2). Obesity was defined as BMI ≥30.00, consistent with CDC guidelines.[4]

Current major depressive disorder. Major depressive disorder symptoms were assessed using the two depressive symptoms items of the PHQ-4,[5] which assessed symptoms occurring in the past two weeks; score ≥ 3 was indicative of a positive screen for major depressive disorder (Cronbach's α in the current sample=0.90).[5]

Current alcohol use disorder. Alcohol use disorder was assessed using the Alcohol Use Disorders Identification Test-Consumption (AUDIT-C), a validated measure used to screen for alcohol use disorder.[6] The AUDIT-C consists of 3 questions that assess severity of alcohol consumption and yield a total score ranging from 0 to 12. A score of 5 or higher was considered as indicative of probable alcohol use disorder.[7,8]

Current smoker. Current smoking status was assessed using a question that asked whether veterans had ever smoked cigarettes; response options were "Yes, in the past;" "Yes, currently;" and "Never."

GrimAge calculation

GrimAge is a composite epigenetic biomarker based on the DNAm surrogates of plasma proteins that are known to be associated with mortality or morbidity, and a DNAm-based estimator of

smoking pack-years. A two-stage procedure was performed to develop GrimAge. First, DNAm-based surrogate biomarkers of smoking pack-years and 88 plasma proteins previously identified to be linked to mortality were identified. Second, time-to-death was regressed on chronological age, sex, and DNAm-based biomarkers of smoking pack-years and the 12 plasma proteins that exhibited a correlation r>0.35 with their respective DNAm-based surrogate marker in step 1. The plasma protein surrogates that were selected by an elastic net regression model were leptin, cystatin C, tissue inhibitor metalloproteinases 1 (TIMP1), adrenomedullin (ADM), beta-2-microglobulin (B2M), growth differentiation factor-15 (GDF-15), and plasminogen activation inhibitor 1 (PAI-1). The resultant mortality risk estimate of the regression model was then transformed linearly into units of years. The rationale for selecting these proteins and details of analytical procedures that generate GrimAge have been described in detail previously.[9]

In the current study, we operationalized accelerated GrimAge as a residual GrimAge acceleration of 5 or more years relative to chronologic age (mean in full sample=8.3 years, SD=2.2, range=5-16). This magnitude difference is clinically meaningful (i.e., approximates an average 5-10 year greater acceleration of epigenetic relative to chronological aging) and permits comparability to prior studies.[10-12] We calculated DNAm age for each individual based on salivary DNA samples profiled with the Illumina Infinium EPIC array and Horvath age estimation algorithm.[13] In the present sample, chronological age correlated strongly with GrimAge (r=0.91; p<0.001).

Cell proportion estimation analysis was conducted using a modified version of the Houseman method,[14] which yielded estimates of each cell type proportion (e.g., CD14, CD34, and buccal cells) in the peripheral saliva samples. Principal component analysis was conducted to adjust for population stratification using the Barfield method;[15] the first 10 principal components were included in analyses.

Data Analysis

First, we compared baseline characteristics of veterans with or without PTSD using chi-square and independent-samples t-tests. Second, we conducted a multivariable relative risk regression analysis to identify variables associated with accelerated GrimAge variables associated with accelerated GrimAge at the p<0.05 level in bivariate analyses were entered into this analysis; PTSD screening status and variables shown in Table 1, as well as cell type proportions (CD34, CD14, and buccal) and 10 ancestry principal components, were entered into this analysis. Given that GrimAge includes a measure of smoking pack-years, analyses were first conducted without adjusting for smoking status; we then conducted a sensitivity analysis to determine whether any significant associations were robust to smoking status. Third, we conducted a second regression analysis with individual PTSD symptoms entered as independent variables to identify symptom(s) that were independently associated with accelerated GrimAge; alpha for this analysis was Bonferroni-corrected to 0.0029 (0.05/17 symptoms). Fourth, we conducted a multivariate analysis of covariance to evaluate how PTSD related to component aspects of GrimAge; this analysis adjusted for variables identified as significant correlates of accelerated GrimAge in the regression model.

Supplement References

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Supplementary Table 1. DNAm surrogate markers by PTSD screening status

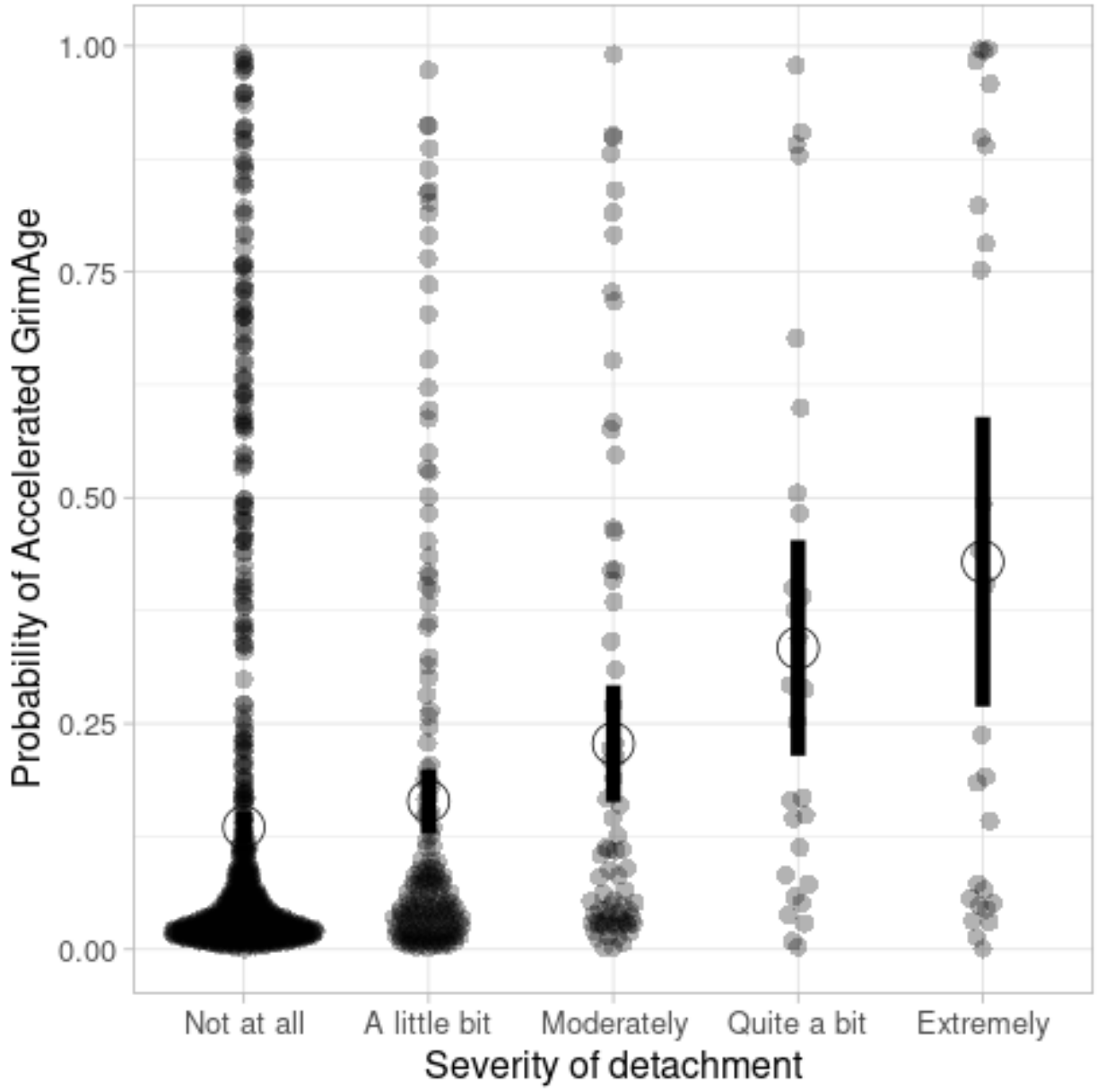
	No Positive Screen for PTSD N=1,019 (weighted 88.5%)	Positive Screen for PTSD N=113 (weighted 11.5%)	Bivariate test of difference
	Weighted mean (SE)	Weighted mean (SE)	F
DNAm PACKYRS	6.45 (0.46)	7.20 (0.93)	0.61
DNAm Cystatin C	2718.12 (1036.58)	2698.06 (2100.63)	0.00
DNAm Leptin	597.09 (251.26)	114.67 (509.18)	0.85
DNAm TIMP-1	101.37 (26.10)	219.29 (52.89)	4.71*
DNAm ADM	2.19 (0.70)	3.31 (1.42)	0.59
DNAm B2M	1886.92 (3892.29)	21033.80 (7887.71)	8.01**
DNAm GDF-15	17.71 (6.77)	53.80 (13.72)	6.56*
DNAm PAI-1	284.29 (131.89)	160.71 (267.28)	0.20

Note. SE=standard error of the mean; PTSD=posttraumatic stress disorder; DNAm=DNA methylation; PACKYRS= smoking pack-years; TIMP-1=tissue inhibitor metalloproteinase 1; ADM=adrenomedullin; B2M=beta-2 microglobulin; GDF-15=growth differentiation factor-15; PAI-1=plasminogen activation inhibitor 1.

Analysis is adjusted for education, current smoking status, cell type proportions (CD34, CD14, and buccal), and top 10 ancestry principal components.

Significant difference: *=$p<0.05$; **$p<0.01$.

Supplementary Figure 1. Probability of accelerated GrimAge as a function of severity of detachment symptoms of PTSD



Note. Circles represent mean probabilities; error bars represent 95% confidence intervals. Mean probability of accelerated GrimAge in the sample was 0.18.