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Accelerated DNA methylation age: Associations with PTSD and neural integrity

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ABSTRACT

Background: Accumulating evidence suggests that posttraumatic stress disorder (PTSD) may accelerate cellular aging and lead to premature morbidity and neurocognitive decline.
Methods: This study evaluated associations between PTSD and DNA methylation (DNAm) age using recently developed algorithms of cellular age by Horvath (2013) and Hannum et al. (2013). These estimates reflect accelerated aging when they exceed chronological age. We also examined if accelerated cellular age manifested in degraded neural integrity, indexed via diffusion tensor imaging.
Results: Among 281 male and female veterans of the conflicts in Iraq and Afghanistan, DNAm age was strongly related to chronological age ($rs \sim .88$). Lifetime PTSD severity was associated with Hannum DNAm age estimates residualized for chronological age ($\beta = .13$, $p = .032$). Advanced DNAm age was associated with reduced integrity in the genu of the corpus callosum ($\beta = -.17$, $p = .009$) and indirectly linked to poorer working memory performance via this region (indirect $\beta = -.05$, $p = .029$). Horvath DNAm age estimates were not associated with PTSD or neural integrity.
Conclusions: Results provide novel support for PTSD-related accelerated aging in DNAm and extend the evidence base of known DNAm age correlates to the domains of neural integrity and cognition.
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1. Introduction

Chronic psychological stress may accelerate cellular aging and lead to early onset of age-related disease, neurodegeneration, and pre-mature mortality (Epel, 2009; Epel et al., 2004; Lindqvist et al., 2015). Posttraumatic stress disorder (PTSD) has been identified as a chronic stress-related condition that may accelerate cellular aging, increasing risk for neuronal cell death via oxidative stress, inflammatory, and other pathophysiological pathways (Lohr et al., 2015; Miller and Sadeh, 2014; Moreno-Villanueva et al., 2013; Williamson et al., 2015). Repeated activation of the hypothalamic–pituitary–adrenal (HPA) axis system, chronic sleep deprivation, and other PTSD-related disturbances are hypothesized to increase reactive oxygen species and decrease the capacity of antioxidants to protect neurons from the toxic effects of oxidative stress (Miller and Sadeh, 2014). Chronic PTSD may also lead to glucocorticoid-mediated increases in peripheral and central nervous system inflammation, and dysregulated autonomic and metabolic processes (Epel, 2009; Lohr et al., 2015; Williamson et al., 2015) thereby degrading cellular integrity and ultimately leading to cell death.

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Within the past two years, important advances have been made in the use of DNA methylation (DNAm) data to index chronological age. Hannum et al. (2013) developed a model of cellular age (DNAm age) based on methylation levels measured in whole blood at 71 DNA loci and found this metric of DNAm age to be highly correlated with chronological age ($r = .96$). The majority of the loci in this algorithm were located in or near genes important for the development of age-related disease, DNA damage and repair, and/or oxidative stress (Hannum et al., 2013). In the same year, Horvath (2013) independently developed a multi-tissue DNAm age algorithm using 353 loci and found this metric to also correlate highly with chronological age at $r = .96$. Despite these impressive associations, the biological mechanism(s) linking epigenetic variation to chronological age remain unclear.

Preliminary cross-sectional evidence suggests that exposure to pathogenic environmental factors may influence DNAm age, yielding higher estimates than would be expected based on chronological age. For example, Horvath et al. (2014) showed that obesity was associated with accelerated DNAm age in human liver tissue. Accelerated DNAm age relative to chronological age has also been linked to indices of disease, including cross-sectional associations with worse performance on measures of fluid intelligence, grip strength, and lung function (Marioni et al., 2015b). Likewise, a meta-analysis of over 4600 older-aged adults found that for every five year-increase in DNAm age relative to chronological age using the Hannum et al. (2013) and Horvath (2013) algorithms, there were 21% and 11% increases, respectively, in all-cause mortality (Marioni et al., 2015a).

To our knowledge, only one published study (Boks et al., 2015) has examined associations between trauma and/or PTSD and DNAm age. In that study of 96 male soldiers, trauma exposure was positively related to DNAm age per the Horvath (2013) algorithm, while self-reported PTSD symptoms unexpectedly predicted decreased DNAm age estimates over the course of approximately one year (Boks et al., 2015). However, chronological age was not included in this analysis so it remains unclear how these findings relate to discrepancies between DNAm age and chronological age. In this study, we aimed to address this limitation by examining the association between the cumulative lifetime burden of PTSD and DNAm age, controlling for chronological age.

We also tested the hypothesis that accelerated DNAm age is correlated with indices of neural integrity in regions known to degrade with normal aging. Gray matter volume and cortical thickness decrease globally with advancing age (Good et al., 2001; Resnick et al., 2003) with these effects most evident in prefrontal regions (Resnick et al., 2003; Salat et al., 2004, 2005b). Studies of white matter microstructural integrity (i.e., diffusion tensor imaging; DTI) have found the most consistent effects using measures of fractional anisotropy (FA) in the prefrontal cortex (Bennett et al., 2010; Burgmans et al., 2010; Pfefferbaum et al., 2000; Salat et al., 2005a) and the genu of the corpus callosum (Bennett et al., 2010; Burgmans et al., 2010; Kochunov et al., 2012; Pfefferbaum et al., 2000; Salat et al., 2005a; Voineskos et al., 2012; Zahr et al., 2009), which connects the right and left prefrontal cortices (Hofer and Frahm, 2006). These regions are involved in higher-order executive functions, such as working memory and response inhibition (Zahr et al., 2009), which also show age-related declines (Park et al., 2002). Based on this, a final aim of this study was to examine possible links between accelerated DNAm age and performance on executive function tasks that depend on these regions of interest (ROIs).

We hypothesized that lifetime PTSD severity (as indexed by a latent variable capturing PTSD severity across three time intervals), would be associated with accelerated DNAm age estimates relative to chronological age. We also expected that advanced DNAm age would be negatively related to microstructural integrity (FA values) in areas of the brain previously linked to age-related degeneration (the frontal cortex and genu) and to performance on executive function tasks mediated by our ROIs.

2. Materials and methods

2.1. Participants

Horvath (2013) and Hannum et al. (2013) DNAm age estimates were available for 289 veterans of the conflicts in Iraq and Afghanistan assessed at the Translational Research Center for TBI and Stress Disorders, a VA RR&D Traumatic Brain Injury Center of Excellence at VA Boston Healthcare System. Exclusion criteria included history of seizures (unrelated to head injury), neurological illness, current bipolar or psychotic disorder, severe depression or anxiety, active homicidal and/or suicidal ideation with intent, cognitive disorder due to general medical condition other than traumatic brain injury (TBI), and unstable psychological diagnosis that interfered with accurate data collection. For the MRI portion of the assessment, additional exclusionary criteria included pregnancy and having a metal implant, shrapnel, aneurysm clip, or pacemaker. Eight participants were excluded from these analyses due to history of moderate or severe traumatic brain injury, yielding a total sample size of 281 for analyses focused on PTSD and DNAm age. Of these, DTI data were available for 241 participants.

Of the 281 veterans with DNAm age data, the mean age was 31.93 years (SD: 8.40, range: 19–58) and 87.9% were male ($n = 247$). Self-reported race and ethnicity was as follows: 70.5% white, 15.3% Hispanic or Latino/a, 8.5% black or African American, 2.1% Asian, 1.1% American Indian (2.5% did not self-report). 55.9% and 74.0% of the sample met DSM-IV-TR diagnostic criteria for current and lifetime PTSD, respectively, per the Clinician Administered PTSD Scale (CAPS; Blake et al., 1995), as defined by the DSM-IV-TR algorithm with clinician frequency ratings ≥ 1 and severity ratings ≥ 2 required for endorsement of the criterion (along with the distress/impairment criteria; Weathers et al., 1999). The mean post-deployment CAPS score was 63.08 (SD = 34.63).

2.2. Procedures

Veterans gave informed consent and then provided fasting blood samples, underwent diagnostic interviewing by a PhD-level clinician, completed a neuropsychological battery, and underwent MRI acquisition. Psychiatric diagnoses were confirmed by an expert consensus team. The protocol was reviewed by the appropriate institutional review boards.

2.2.1. DNA genotyping and methylation

Peripheral whole blood samples were obtained and DNA extracted from buffy coat. Genotyping, relevant here for capturing DNA ancestral variation via principal components (PC) analysis (see Supplementary Methods), was conducted on the hybridized DNA samples using the Illumina Human Omni 2.5–8 microarray and scanned with an Illumina iScan System (Illumina, San Diego, CA). For DNAm quantification, hybridized bisulfite-modified DNA (achieved via Zymo EZ-96 DNA Methylation Kits D5004) were whole-genome amplified, fragmented, precipitated, resuspended, and hybridized to Illumina HumanMethylation 450k beadchips and then scanned with an Illumina iScan System (Illumina, San Diego, CA). The Horvath age estimate was computed using an R script supplied by Dr. Horvath (http://labs.genetics.ucla.edu/horvath/dnamage/). As this script includes a normalization step, non-normalized average beta values as exported from Genomestudio were used as input after removing two subjects with low intensity scores. The Hannum et al. DNAm age estimates were computed in *R* directly using a linear function of the 89