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Hostility and telomere shortening among U.S. military veterans: Results from the National Health and Resilience in Veterans Study

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ABSTRACT

Chronic disorders of aging are critical concerns for the U.S. veteran population, which is, on average, two decades older than the non-veteran population. Characterization of risk factors that may accelerate biological aging is important in identifying targets for prevention and intervention. In the current study, we analyzed data from a contemporary, and nationally representative sample of U.S. veterans to evaluate the relationship between a broad range of sociodemographic, military, and clinical variables, and peripheral telomere length, which is an indicator of biological age and linked to risk for aging-related disorders and mortality. Data from 468 U.S. military veterans who participated in the National Health and Resilience in Veterans Study were analyzed. Telomere length was assessed from cells isolated from saliva using quantitative polymerase chain reaction methods. A multivariable binary logistic regression analysis was conducted to evaluate the relations between hostility and telomere length, while controlling for sociodemographic, military, and clinical variables. Greater scores on a measure of hostility were independently associated with telomere shortening, even after adjustment for a broad range of other variables (odds ratio [OR] = 1.58, 95% confidence interval [CI] = 1.15–2.18). Secondary analyses revealed that this association was driven by difficulties controlling anger (OR = 1.72, 95%CI = 1.14–2.61), which reflect the external manifestation of hostility, rather than aggressive urges or impulses. Hostility, particularly difficulties controlling anger, is associated with peripheral telomere shortening in U.S. military veterans. Prevention and treatment efforts designed to reduce hostility may help mitigate risk for accelerated cellular aging in this growing segment of the U.S. population.

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1. Introduction

Chronic disorders of aging and mortality are critical concerns among the U.S. veteran population, which is on average older than the non-veteran population. In 2014, the median age of male veterans was 64 and the median age of non-veterans was 41 (United States Department of Veterans Affairs: National Center for Veterans Analysis and Statistics, 2016). In addition, more than half (54.6%) of the U.S. veteran population is currently aged 60 or older (United States Department of Veterans Affairs, 2014). During the last decades of life, susceptibility to age-related disorders, such as cardiovascular disease, cancers, and diabetes, dramatically increases. However, there is considerable individual variability in the onset of these disorders. Thus, there is increasing interest in identifying markers of biological aging and factors that influence these markers (Epel et al., 2009), which may help to promote the health of the aging veteran population.

Telomere length is an indicator of biological age that has been repeatedly associated with aging-related medical conditions and mortality (Blackburn et al., 2015). Telomeres are specialized nucleoprotein structures that cap the ends of chromosomes and protect against damage (Sfeir and de Lange, 2012). They are comprised of double-stranded, repetitive TTAGGG deoxyribonucleic acid (DNA) repeats and related proteins that form the shelterin complex (Blackburn, 2001; De Lange, 2005). By protecting against premature replication termination, telomere structure is critical for maintaining chromosomal stability. Telomeres naturally shorten during cell division and as a result of oxidative damage, and when shortened to a critical length, the DNA damage response is initiated,

which in turn leads to apoptosis or genomic instability (Blackburn, 2000). Although average telomere length declines as a function of age, chronological age accounts for less than 10% of variation in human telomere length (Blackburn et al., 2015). Thus, telomere length is a marker of cellular aging and also appears to be an indicator of biological age, which is defined physiologically rather than chronologically (Epel et al., 2004). Further, results of meta-analyses have suggested that short telomere length in humans is associated with medical conditions such as cardiovascular disease, diabetes, and certain types of cancer (D'Mello et al., 2015; Haycock et al., 2014; Weischer et al., 2012; Wentzensen et al., 2011; Willeit et al., 2014; Zhu et al., 2016), and some findings have linked shorter telomere length with premature mortality (Cawthon et al., 2003; Fitzpatrick et al., 2011; Weischer et al., 2012). Although telomere length is partially due to inheritance (estimates range from 30 to 80%), this heritability declines with age, and several clinical and lifestyle factors have been found to impact telomere length in a tissue-specific manner throughout the lifespan (Blackburn et al., 2015; Prowse and Greider, 1995). Sociodemographic factors include advanced age, and ethnic or racial minority status (Blackburn et al., 2015; Diez-Roux et al., 2009); and clinical and lifestyle factors include perceived stress, trauma exposure, psychopathology, cigarette smoking, low physical exercise, poor sleep, and obesity (Cherkas et al., 2006; Epel et al., 2004; Kim et al., 2009; Latifovic et al., 2016; O'Donovan et al., 2011a; Prather et al., 2011; Puterman et al., 2010; Verde et al., 2015; Wolkowitz et al., 2011; Zhang et al., 2014). However, many of these factors have been examined in isolation and results have generally revealed weak associations between them and telomere length, thereby highlighting the need to consider a broad range of possible predictors of telomere shortening in the same sample. Characterization of factors associated with telomere length in aging U.S. military veterans is important, as it can inform prevention and intervention strategies designed to mitigate risk for accelerated cellular aging that are specific to this population.

Hostility, which is a personality trait characterized by aggressive urges or impulses and difficulties controlling anger (Elbogen et al., 2010), is prevalent among veterans (Sippel et al., 2016) and has consistently been found to be associated with aging-related disorders and has also been linked to telomere shortening in a recent study of civilians (Brydon et al., 2012). Meta-analyses have found that greater levels of hostility are associated with age-related disorders such as coronary heart disease, as well as all-cause mortality (Miller et al., 1996; Roberts et al., 2007). Notably, these effects were independent of psychiatric variables such as depression, anxiety, and stress. Researchers have suggested that hostility may promote the activation of biological responses to daily stressors, which then may influence telomere shortening (Brydon et al., 2012; O'Donovan et al., 2012). For example, individuals with greater levels of hostility have elevated circulating levels of inflammatory markers and cortisol (Graham et al., 2006; Ranjit et al., 2007; Smith et al., 2004), which may increase cell turnover and oxidative stress, in turn contributing to shortened telomere length (O'Donovan et al., 2011b; Slavich et al., 2010). However, research examining the influence of hostility on telomere length is limited to a single study. This study found that men who scored higher on a measure of hostility had shorter telomere length than men who were less hostile (Brydon et al., 2012). Although findings from this study provided important initial evidence of the link between hostility and telomere length, it did not control for other psychological factors and stressors that may be associated with age-related disorders and telomere shortening, such as lifetime trauma exposure, posttraumatic stress disorder (PTSD), depression, and current psychological stress (Epel et al., 2004; O'Donovan et al., 2011a; Wolkowitz et al., 2011; Zhang et al., 2014). Further, hostility is a multi-faceted construct characterized by aggressive urges or impulses and difficulties

controlling anger (Elbogen et al., 2010), which may be differentially associated with telomere length. Thus, the purpose of the present study was to evaluate the association between a nuanced model of hostility and telomere length using data from a nationally representative sample of veterans while controlling for relevant covariates that have been previously associated with shorter telomere length. We hypothesized that greater levels of hostility would be related to telomere shortening and that this effect would be independent of sociodemographic, and other clinical and lifestyle variables.

2. Material and methods

2.1. Participants

Participants were a subsample of 468 U.S. military veterans who provided a saliva sample as part of the National Health and Resilience in Veterans Study (NHRVS; Wisco et al., 2014). The NHRVS cohort was recruited from a research panel of more than 50,000 U.S. households developed and maintained by GfK Knowledge Networks, Inc. (Menlo Park, CA, USA). Panel members were recruited using a sampling procedure that includes listed and unlisted phone numbers, telephone, non-telephone, and cell-phone-only households, and households with or without internet access, offering coverage of approximately 98% of U.S. households. Post-stratification weights were applied based on the demographic distribution (age, sex, race/ethnicity, education, Census region, and metropolitan area) of U.S. veterans in the GfK Knowledge Networks survey panel and calibrated against contemporaneous U.S. Census data (2015 Current Population Survey). All participants provided informed consent and the Human Subjects Subcommittee of the Veterans Affairs (VA) Connecticut Healthcare System and VA Office of Research & Development approved the study.

2.2. Assessments

2.2.1. Telomere length assay

Oragene DNA (OG-250) kits were used to collect saliva samples. DNA was extracted and purified from 200 μl of saliva using the Quick-DNA^{TM} Universal 96 Kit. Four volumes of Genomic lysis buffer were added to each sample and column purified followed by elution with 50 uL of elution buffer. DNA concentrations were determined from A_{260} readings using a Nanodrop 2000 spectrophotometer. DNA was brought to a final concentration of 5 ng/uL prior to the telomere real-time PCR assay. The telomere length was measured using a quantitative PCR-based technique (Cawthon, 2002), which expresses the average telomere length as the ratio (T/S) of the telomere repeat copy number (T) to a single copy gene (S). In order to standardize the measurements across PCR plates the T/S ratio is measured relative to a standard DNA used within each assay (Bioline Human DNA). 5, 1.67 fold serial dilutions were made and used as standards in all experiments. The final concentrations of reagents in the PCR were 1 × EvaGreen (Biotium), 1 × Epitaq PCR buffer, 2.5 mM MgCl$_2$, 0.3 mM each dNTP, and 0.4 U EpiTaq HS (Takara/Clonetech) in a 15 uL final volume. The final telomere primer concentrations were: tel 1, 270 nM; tel 2, 900 nM. The final *36B4* (single copy gene) primer concentrations were: 36B4u, 300 nM; 36B4d, 500 nM. The primer sequences (written 5′ → 3′) were: tel 1, GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGT-GAGGGT; tel 2, TCCCGACTATCCCTATCCCTATCCCTATCCCTATCC-CTA; 36B4u, CAGCAAGTGGGAAGGTGTAATCC; 36B4d, CCCATTC-TATCATCAACGGGTACAA.

All PCRs were performed on the Rotor-Gene Q (Qiagen) real-time PCR thermal cycler. The thermal cycling profile for both amplicons began with a 95 °C incubation for 15 min. For telomere PCR, there followed 40 cycles of 95 °C for 15 s, 54 °C for 2 min. For