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PTSD and the klotho longevity gene: Evaluation of longitudinal effects on inflammation *via* DNA methylation

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ABSTRACT

Background: Longevity gene klotho (*KL*) is associated with age-related phenotypes including lifespan, cardiometabolic disorders, cognition, and brain morphology, in part, by conferring protection against inflammation. We hypothesized that the *KL*/inflammation association might be altered in the presence of psychiatric stress and operate *via* epigenetic pathways. We examined *KL* polymorphisms, and their interaction with posttraumatic stress disorder (PTSD) symptoms, in association with *KL* DNA methylation in blood. We further examined *KL* DNA methylation as a predictor of longitudinal changes in a peripheral biomarker of inflammation (C-reactive protein; CRP).

Methods: The sample comprised 309 white non-Hispanic military veterans (93.5 % male; mean age: 32 years, range: 19–65; 30 % PTSD per structured diagnostic interview); 111 were reassessed approximately two years later.

Results: Analyses revealed a methylation quantitative trait locus at rs9527025 (C370S, previously implicated in numerous studies of aging) in association with a Cytosine-phosphate-Guanine site (cg00129557; B = −.65, p = 1.29 X 10^{-20}), located within a DNase hypersensitivity site in the body of *KL*. There was also a rs9527025 x PTSD severity interaction (B = .004, p = .035) on methylation at this locus such that the minor allele was associated with reduced cg00129557 methylation in individuals with few or no PTSD symptoms while this effect was attenuated in those with elevated levels of PTSD. Path models revealed that methylation at cg00129557 was inversely associated with CRP over time (B = −.14, p = .005), controlling for baseline CRP. There was also an indirect effect of rs9527025 X PTSD on subsequent CRP *via* cg00129557 methylation (indirect B = −.002, p = .033).

Conclusions: Results contribute to our understanding of the epigenetic correlates of inflammation in PTSD and suggest that *KL* methylation may be a mechanism by which *KL* genotype confers risk vs. resilience to accelerated aging in those experiencing traumatic stress.

1. Introduction

Polymorphisms in the gene klotho (*KL*) have been associated with lifespan and age-related phenotypes in numerous preclinical and clinical studies (Kuro-o et al., 1997; Kurosu et al., 2005; Dubal et al., 2014; Abraham et al., 2016). The gene and the protein it encodes were named

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for the mythological Greek goddess, Clotho, who was said to have spun the thread of human life (Hamilton, 2011). The gene and its protein have been linked to a wide variety of age-related processes including cardiometabolic function (Arking et al., 2002), recovery following experimentally-induced cardiac damage (Ramez et al., 2019), kidney function (Drew et al., 2017), late-life cognition (Dubal et al., 2014; Yokoyama et al., 2015), white matter integrity (Chen et al., 2013b; King et al., 2012), motor function (Kuro-o et al., 1997), and lifespan (Kuro-o et al., 1997; Kurosu et al., 2005; Dubal et al., 2014). Greater *KL* expression in old age is thought to confer protection against age-related declines in neural integrity and cognition (Dubal et al., 2014).

KL exerts its neuro- and cardio-protective effects by reducing inflammation (Hui et al., 2017; Maekawa et al., 2009; Xia et al., 2016; Zhu et al., 2018; Zeldich et al., 2019) and oxidative stress (Yamamoto et al., 2005; Zeldich et al., 2014). Inflammation and oxidative stress are well known biomarkers of cellular aging, with increasing levels of each known to play a mechanistic role in age-related adverse outcomes and cellular senescence (Ridker et al., 2002; Frasca and Blomberg, 2016; Fülöp et al., 2016). KL is known to suppress nuclear factor κB (NF-κB) signaling *via* inhibition of IκB phosphorylation and expression of tumor necrosis factor alpha (TNFα; Guo et al., 2018; Maekawa et al., 2009), which in turn reduces oxidative stress and endothelial cell inflammation (Maekawa et al., 2009). Research suggests that, *in vivo*, *KL* expression (*i.e.*, mRNA; Martín-Núñez et al., 2017) and circulating KL proteins (Prystupa et al., 2016) tend to be inversely correlated with pro-inflammatory biomarkers, including C-reactive protein (CRP), while *in vitro* studies have shown KL to reduce inflammatory cytokines (Sedighi et al., 2019). In the choroid plexus, the anatomical barrier between the periphery and brain ventricles, KL has been found to play a pivotal role in preventing peripheral inflammatory molecules from reaching the central nervous system (Zhu et al., 2018). The KL protein also influences endocrine fibroblast growth factor pathways that impact phosphate and calcium metabolism (Kuro-o, 2019) and insulin regulation (Donate-Correa et al., 2016), and KL impacts Wnt signaling, which is critical for determination of cellular fate and progenitor cell senescence (Liu et al., 2007).

Environmental factors are also known to contribute to cellular aging. Traumatic stress and other forms of psychological stress are positively associated with age-related conditions, including inflammation and cardiometabolic pathology (for reviews, see Miller et al., 2018a; Wolf and Morrison, 2017). Posttraumatic stress disorder (PTSD) has been associated with elevated CRP, interleukin (IL)-6, IL1β, and TNFα (Miller et al., 2018a, 2018b; Passos et al., 2015; Michopoulos et al., 2015; Rosen et al., 2017). Its association with CRP, an acute-phase protein that responds quickly to inflammation and helps to activate innate immunity to bacterial infection (Black et al., 2004; Sproston and Ashworth, 2018), is not consistent across all studies (Miller et al., 2018a). However, this association is of particular interest because chronically elevated CRP is a marker for cardiovascular disease risk (Ridker et al., 2002) and a signature of inflammation-related biological aging (Frasca and Blomberg, 2016). A better understanding of the mechanisms by which PTSD relates to CRP could shed light on novel treatment approaches to reduce inflammation and associated age-related comorbidities.

PTSD is also positively associated with epigenome-wide DNA methylation [DNAm] markers of advanced cellular age (*e.g.*, advanced "epigenetic age" also known as "advanced DNAm age," Wolf et al., 2016, 2018a, 2018b; Wolf et al., 2019a). In a recent study that forms part of the basis for this one, we found that the strength of the association between traumatic stress and both advanced epigenetic age and CRP was impacted by *KL* genotype (Wolf et al., 2019b) such that the association was accentuated among those with the minor frequency allele (A) of the *KL* SNP rs9315202. This SNP was also found to interact with PTSD to predict reduced integrity of two white matter tracts that connect prefrontal and limbic regions (Wolf et al., 2019b). The SNP is located just downstream of the gene. The minor allele (C) of a second *KL* polymorphism, rs398655, was associated with reduced epigenetic age among those with chronic pain. That SNP is upstream of the gene and was previously associated with better cognitive function (Mengel-From et al., 2016). The minor allele (C) of a third *KL* SNP, rs9527025 (also known as C370S, located in an exonic coding region), was directly associated with slowed epigenetic age. This latter finding was consistent with many prior studies suggesting that this SNP, which is part of a haplotype comprised of SNPs in 100% linkage disequilibrium (LD) referred to as KL-VS, is associated with improved health in humans across various markers of biological aging, including cognition (Dubal et al., 2014; Yokoyama et al., 2015), prefrontal cortex morphology, and longevity (Arking et al., 2002, 2005; Revelas et al., 2018). One study suggested that the minor allele at rs9527025 altered the protein product of the gene in such a way as to enhance its co-receptor functioning for fibroblast growth factor signaling (Zhou et al., 2013). The direct molecular consequences of rs9315202 and rs398655 are unknown.

To our knowledge, no studies have yet examined the possible role of *KL methylation* in mediating the relationship between *KL* genotype and inflammatory processes associated with aging. Research on *KL* methylation published to date has been limited in scope and focused primarily on cancer, kidney-related, and brain aging phenotypes. Those studies suggest that hypermethylation of the *KL* promotor region is associated with reduced *KL* expression (*e.g.*, Lee et al., 2010; Rubinek et al., 2012; King et al., 2012) and is a signature of cancerous tumors (Lee et al., 2010; Rubinek et al., 2012), cancer-related death (Zhu et al., 2019), and kidney fibrosis (Azuma et al., 2012). One study expanded this work to cognitive aging and found that *KL* promoter hypermethylation was positively associated with mild cognitive impairment in a Chinese sample (Luo et al., 2015). However, this effect was not replicated in a second Chinese sample in the same study. Finally, a study in rhesus monkeys found increased *kl* methylation in the promoter region of prefrontal white matter in older monkeys and also found that this alteration was associated with decreased *kl* transcription, possibly contributing to brain aging phenotypes (*e.g.*, impaired cognition, lack of myelin integrity; King et al., 2012). However, the role of *KL* methylation in human biological aging remains an open question.

1.1. Study aims

The primary aim of this study was to address this question by examining the effects of the three previously implicated *KL* variants (rs9315202, rs398655, and rs9527025) and PTSD on *KL* methylation levels. The second aim was to examine if *KL* methylation, in turn, contributed to changes in a peripheral marker of inflammation (CRP) over time, such that the association between PTSD and CRP was indirect, *via KL* methylation. This study was based on the same sample of white non-Hispanic military veterans with a high prevalence of PTSD in which we previously reported *KL* genotype X PTSD effects on measures of biological aging (Wolf et al., 2019b). This study expanded on that prior cross-sectional investigation in that we examined potential molecular mechanisms (*KL* methylation) that may link *KL* genotype and PTSD to changes in a biomarker of aging (inflammation) over time.

2. Methods

2.1. Participants and procedure

Participants in cross-sectional analyses in this study were 309 white non-Hispanic military veterans (93.5 % male, mean age = 32 years, Table 1) who deployed as part of the Global War on Terror; 111 of these veterans were included in the longitudinal analyses based on a follow-up assessment that was completed on average approximately two years later (M = 1.96 years, SD = .65 years, range = 0.98–4.78 years).[1]

[1] Data collection for this multi-wave protocol is on-going and is released for

Table 1
Participant Characteristics.

	Baseline (n = 309)	Follow-up (n = 111)
Variable	*M* (*SD*) or *n* (%)	*M* (*SD*) or *n* (%)
Male	289 (93.5)	101 (91.0)
Age (years)	32.04 (8.40)	33.97 (8.86)
Current PTSD dx	198 (64.1)	56 (50.5)
Current PTSD sev	52.45 (29.62)	43.51 (29.93)
CRP (mg/dL)	.23 (.24)	.18 (.12)

Note. Raw CRP values are reported here but log-transformed CRP values were analyzed. Dx = diagnosis; sev = severity; CRP = C-reactive protein.

Analyses were restricted to this ancestral group (from a larger baseline sample of n = 458 veterans of mixed ancestry) due to genetic population stratification and insufficient sample size to proceed with these analyses in other ancestral groups. Veterans participated in The Translational Research Center for TBI and Stress Disorders (TRACTS) research protocol (McGlinchey et al., 2017), an on-going longitudinal study of deployment stress and psychological, cognitive, metabolic, and neurological health among veterans aged 18–65 years. Recruitment efforts and exclusion criteria were described in detail previously (McGlinchey et al., 2017). In short, veterans were excluded primarily due to serious active psychopathological processes that posed an immediate risk (*e.g.*, suicidality) or prevented data collection, presence of neurological condition other than that related to mild traumatic brain injury, and due to standard neuroimaging exclusionary criteria. Participants completed psychological interviews and cognitive testing administered by doctoral-level staff; they also had peripheral blood drawn by a certified phlebotomist into 2 X 10 ml EDTA tubes for DNA extraction and metabolic and inflammation panels and underwent magnetic resonance imaging of the brain. The most common trauma exposure type was combat (64.08 %), with the sudden and unexpected loss of a friend or loved one next most common (41.42 %). On average, participants had experienced 1.84 different types of traumatic experiences (*SD*: 1.73). The study was reviewed and approved annually by the hospital institutional review board.

2.2. Measures

2.2.1. PTSD

PTSD was assessed with the Clinician Administered PTSD Scale (CAPS) for *DSM-IV* (Blake et al., 1995). The CAPS was administered by doctoral-level psychologists, and both symptom severity ratings and PTSD diagnosis were tabulated according to standard scoring rules (Weathers et al., 1999). Diagnoses were based on expert consensus team review of the CAPS. Descriptive statistics pertaining to PTSD are provided in Table 1. Analyses focused on PTSD symptom severity ratings in the past 30 days (current PTSD severity).

2.2.2. CRP

As described elsewhere (Miller et al., 2018b), serum CRP levels were obtained from a commercial laboratory (Quest Diagnostics; Cambridge, MA) with a nephelometric assay using CRP monoclonal antibodies. Samples were shipped to the lab the same day they were collected and processed within 24 h. Lab procedures were standardized by the International Federation of Clinical Chemistry and Laboratory Medicine, the Bureau Communautaire de Référence, and the College of American Pathologists. The analytic sensitivity of the test was 0.10 mg/dL. Regrettably, the clinical lab did not provide information regarding inter/intra-assay variation. Descriptive statistics for CRP are listed in Table 1. The reference range for normal values is <.80 mg/dL. The CRP values for one participant at Time 1 (T1) and for a different participant at Time 2 (T2) were set to missing as they were extreme outliers (12 *SD*s beyond the mean).

2.3. DNA-related methods and statistical processing

DNA was extracted from whole blood and genotyped on the Illumina HumanOmni2.5–8 array as described in Logue et al. (2013) and in the Supplementary Materials. We restricted our analysis to genetically confirmed white non-Hispanic subjects identified using SNP weights (Chen et al., 2013a) according to the PTSD-GWAS consortium criteria (Logue et al., 2015). Principal components (PCs) reflecting ancestry were computed within the white non-Hispanic subsample for inclusion as covariates in analyses (see Supplementary Materials). These PCs were included to prevent false positives due to unmeasured population substructure within the white non-Hispanic subjects. The three SNPs that were evaluated in this study were in Hardy-Weinberg equilibrium (all p > .35).

DNAm was interrogated using the Illumina Infinium MethylationEPIC array (approximately 850 K sites) per manufacturer's protocol (Supplementary Materials). For subjects assessed at a single time point (cross-sectional cohort), sample positions on the methylation arrays were balanced for PTSD diagnostic status and sex. For longitudinal DNAm data, sample position on the arrays was balanced for PTSD diagnostic status, sex, and time (*i.e.*, the T1 and T2 DNA samples from the same subject were included on the same array). We applied the Psychiatric Genomics Consortium PTSD Epigenetics workgroup pipeline for methylation data screening, processing, cleaning, and quality control procedures (Ratanatharathorn et al., 2017), which we have used in the past (see *e.g.* Sadeh et al., 2016), and which we will only briefly describe here. Missingness filters were used on CpG sites (>10 %) and subjects (>5 %). Sites cross hybridizing to non-autosomal chromosomes were excluded. DNAm data were normalized with the beta mixture quantile dilation (BMIQ) method (Pidsley et al., 2013), and batch correction was performed using the ComBat method (Leek et al., 2017). The identity of the samples was confirmed by examining genomic variants assessed on the EPIC array and matching genotypes to the corresponding SNP array. The proportions of different white blood cell (WBC) types in each blood sample were estimated from the methylation data, inclusive of CD8-T, CD4-T, natural killer, B-cells, and monocytes (Aryee et al., 2014; Fortin et al., 2017). Additional details pertaining to DNA extraction for the cross-sectional and longitudinal cohorts and the statistical pipelines are provided in Wolf et al. (2016), 2019a, and in the Supplementary Materials.

2.4. Data analyses

We first evaluated the three SNPs as candidate methylation quantitative trait loci (meQTLs; *i.e.* methylation associated SNPs), by testing their association (using hierarchical regression) with 10 *KL* DNAm loci which passed cleaning and variability filters in the cross-sectional dataset of n = 309 white non-Hispanic military veterans. Linear regression was used given the dimensional nature of DNAm data as the response variable; we focused on identification of meQTLs so as to identify SNPs that were regulatory with respect to *KL* DNAm. DNAm probes were selected for analysis if they met all quality control criteria (Wolf et al., 2019a; Ratanatharathorn et al., 2017) and had sufficient variability (range >.10, per Logue et al., 2017). This model included the covariates age, sex, WBCs estimated from the DNAm data, the first three ancestry substructure PCs, as well as the main effect of current PTSD symptom severity in a first step of the model. The SNP was entered in a second step of the model to determine the unique contribution of each SNP. As we found significant correlations across the 10 *KL* DNAm loci, a Bonferroni correction for the evaluation of SNP to DNAm

(*footnote continued*)
data analyses at various points in time. There were no statistical differences in PTSD severity or age among those who did versus did not yet return for follow-up, however, a greater percentage of women (65% vs. 40% of the men) returned for the follow-up by the time of the data release that was used in this study (p = .028). Sex was included as a covariate in all analyses.

associations would be overly conservative. Therefore, we used a permutation procedure to derive multiple-testing corrected *p*-values that accounted for the three SNPs and 10 DNAm loci in the model. This procedure permuted the genotypes randomly between subjects (with 10,000 replicates) while preserving the correlational structure within the SNPs and within the DNAm loci. Each replicate was analyzed, and the minimum *p*-value across SNPs and CpGs was noted. The percentile of the uncorrected *p*-value in this minimum-*p* distribution is the corrected *p*-value. These analyses were conducted in RStudio v.1.0.153 running R version 3.6.1. After determining which SNPs were associated with *KL* DNAm probes in step 2 of the regression, we built upon the model described above by adding a third step to the cross-sectional model that included the interaction between PTSD severity and SNPs identified as meQTLs in the first set of analyses to determine if the association between PTSD symptom severity and DNAm differed as a function of genotype.

A longitudinal path (*i.e.*, regression) analysis was performed next to examine SNP and PTSD severity main effects and SNP X PTSD interactions on T1 DNAm, and to simultaneously examine the intermediate effect of T1 DNAm on T2 CRP, controlling for T1 levels of CRP. This mediation model examined the indirect effects of the SNP, PTSD, and their interactions on T2 inflammation *via* T1 DNAm (*i.e.*, a direct test of our hypothesis that *KL* DNAm was a mechanism linking *KL* genotype X PTSD to later CRP). Because we controlled for baseline levels of CRP, the model predicted *residualized change* in CRP (*e.g.*, levels of the T2 biomarker that were not simply due to the T1 level of the same biomarker, Allison, 1990; Shutz, 1989). The direct effects of the SNP, PTSD, and their interaction on T2 inflammation were also included in path models to account for potential effects that did not operate *via KL* DNAm, as were the direct effects of several covariates (age, sex, top three PCs). Although our primary mediation models focused on residualized change in CRP (as the only T2 variable included in the model), we also conducted additional analyses in which we included T2 PTSD and T2 methylation and allowed for the direct effects of the SNP, T1 CRP, T1 PTSD and T1 methylation on each of these variables in order to more comprehensively assess autoregressive and cross-variable longitudinal associations in the data. Path models were examined in Mplus 8.2 (Muthén and Muthén, 1998-2018) using an estimator that is robust to non-normality (MLR). Model fit was evaluated using established guidelines (Hu and Bentler, 1999). Unstandardized regression coefficients are presented in the text unless otherwise noted. The path models were conducted in the subset of 111 participants with follow-up data.

3. Results

3.1. SNP, PTSD, and SNP X PTSD Effects on KL DNAm: cross-sectional sample

Table 2 shows the descriptive statistics pertaining to *KL* variants and DNAm loci that were evaluated. Table S1 lists the full results for each model predicting *KL* DNAm. Results revealed two meQTLs: The KL-VS SNP (rs9527025) evidenced corrected significant main effect associations with methylation levels at cg02706658[2] (B = −.55, $p = 1.3 \times 10^{-19}$, corrected $p < .0001$) and cg00129557 (B = −.65, $p = 1.29 \times 10^{-20}$, corrected $p < .0001$; Table S1). These loci are 215 bp apart within a DNase hypersensitivity site in the body of *KL* (GRCh37 chr13:33607361-33607915 as identified in the ENCODE-project database wgEncodeRegDnaseClusteredV3 as accessed through UCSC Genome Browser). They are highly correlated ($r = .69$ in these data), hence they likely represent association between rs9527025 and methylation across a broad genomic region, and are not independent

Table 2
Characteristics of Examined KL SNPs and Methylation Loci.

	SNPs			Methylation Loci		
Variable	MAF	Minor/ Major Allele	Location (bp)	Mean (M values)	*SD* (M values)	Range (raw beta values)
SNPs						
rs398655	40.71	C/A	33587652			
rs9527025	17.15	C/G	33628193			
rs9315202	25.64	A/G	33642016			
Methylation						
cg00129557				−3.70	.77	.22
cg02706658				−3.33	.63	.23
cg09886946				−3.03	.38	.13
cg17806623				−2.64	.27	.12
cg18056695				−3.61	.46	.14
cg19154940				3.53	.49	.14
cg20672059				2.48	.23	.11
cg23584087				3.91	.64	.15
cg25223823				2.56	.40	.22
cg26325430				4.06	.58	.12

Note. M values (log 2 logit transformed beta-values) were analyzed in this study. The raw beta range (proportion of methylated DNA) is presented in this table as this was used to exclude loci with insufficient variability. All SNPs examined were located on chromosome 13 and the base pair location is referenced to the GRCH37.p13 build. The MAF of each SNP was consistent with that reported for the CEU reference sample in 1000 Genomes. SNPs = single nucleotide polymorphism; bp = base pair; MAF = minor allele frequency; SD = standard deviation.

effects. Given the high correlation between the two associated *KL* methylation loci, we proceeded to conduct our primary analyses with just cg00129557, which showed the strongest association with rs9527025 (but replicated results with cg02706658 and also examined effects predicting mean methylation values across the two loci as a potential indicator of methylation in this region, see Supplementary Materials for details). Fig. 1 shows the location of the three SNPs, cg00129557, and the DNase hypersensitivity site. Table 3 shows the zero-order correlations between the primary variables in the path model.[3]

The same SNP (rs9527025) also evidenced a nominally significant main effect on cg19154940 (B = .12, $p = .03$, corrected $p = .54$; Table S1). We were not able to detect meQTL effects for the other two SNPs examined, although rs398655 was nominally associated with methylation values at cg00129557, cg02706658, cg17806623, and cg09886946, but not at a level that survived multiple testing correction (Table S1).

We next expanded the model examining rs9527025 in association with cg00129557 by adding the PTSD severity X SNP term in a third step. This analysis revealed a significant interaction between PTSD severity and rs9527025 in association with DNAm at cg00129557 (B = .004, $p = .035$; Table 4; Fig. 2). The minor allele of rs9527025 (C) was associated with less methylation at cg00129557 when PTSD severity was low, but this effect was attenuated at higher levels of PTSD severity. The residuals from the model were examined and found to be normally distributed. Results held when this model was rerun using dominant coding for the SNP, which compared those with 0 copies vs. 1 or 2 copies of the minor allele at this locus (B for SNP = −.69, $p < .001$; B for SNP X PTSD = .006, $p = .015$). In analyses described in the Supplementary Materials, we added potential confounds to our

[2] cg refers to cytosine guanine and is the standard nomenclature for identifying DNAm loci.

[3] We did not have access to a suitable complete dataset to test for replication of the meQTL involving rs9527025 and cg00129557. This locus was also not included in publicly available datasets that we identified. However, we had a subset of genotype and DNAm data available from an on-going study of veterans who screened positive for PTSD ($n = 133$; see Supplementary Materials) and we used this genetic data to examine replication of the meQTL. We found that this SNP and DNAm locus correlated with each other at $r = -.46$, $p = 1.91 \times 10^{-8}$, which is within .01 of the coefficient reported in Table 3.

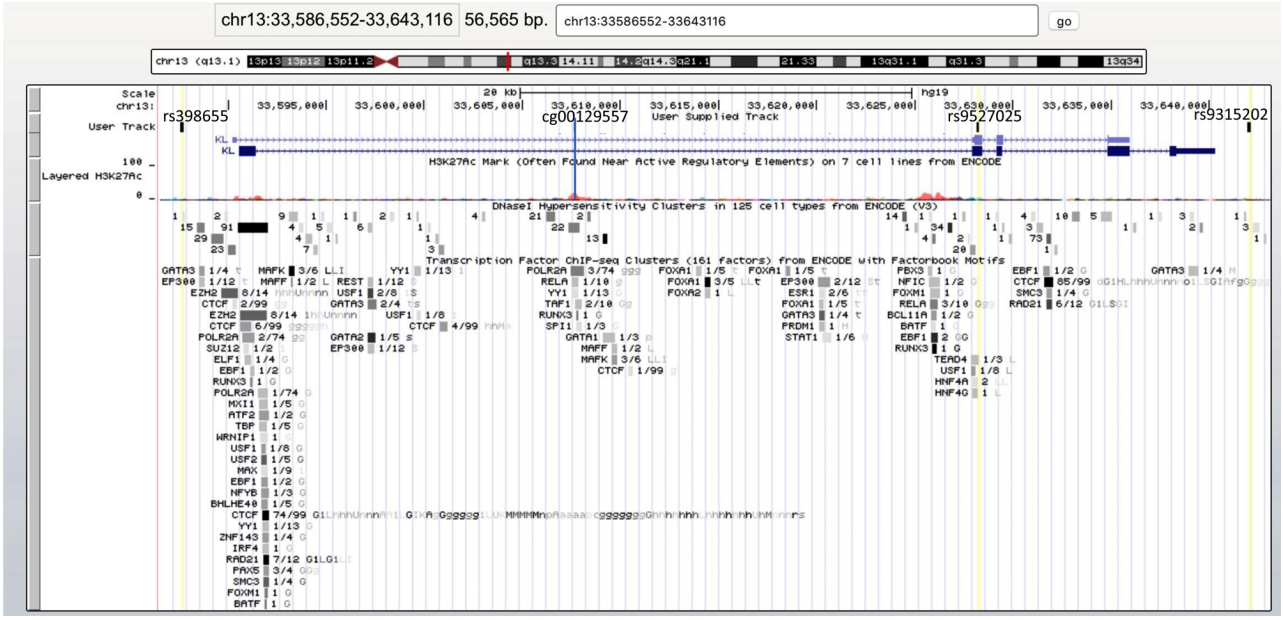

Fig. 1. The figure shows the region of the *KL* gene, including the three SNPs that were evaluated (yellow lines labeled by rs #), cg00129557, and the DNase hypersensitivity site just below this DNAm locus (as indicated by the vertical blue line). The figure was generated using the University of California, Santa Cruz (UCSC) Genome Browser (Kent et al., 2002) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 3
Bivariate Associations Among Key Study Variables.

Variable	1	2	3	4
1. rs9527025				
2. cg00129557 at T1	−.45***			
3. PTSD severity at T1	.08	.06		
4. CRP at T1	−.01	−.09	.17**	
5. CRP at T2	−.05	−.22*	.13	.43***

Note. $N = 309$ for cross-sectional associations and $n = 111$ for longitudinal associations. PTSD = posttraumatic stress disorder; CRP = C-reactive protein, T1 = time 1, T2 = time 2.
*$p < .05$. **$p < .01$. ***$p < .001$.

cross-sectional model (the main and interactive effects of current cigarette use, major depressive disorder, alcohol-use disorder, and body mass index) and found that none accounted for the significant main effect of rs9527025 or the interaction between this SNP and PTSD severity.

3.2. Path models predicting residualized change in peripheral inflammation: longitudinal subsample

The first path analysis evaluated the main and interactive effects of the KL-VS SNP (rs9527025) and PTSD severity on residualized change in CRP values at T2 *via* T1 methylation levels at cg00129557 (*i.e.*, indirect effects), controlling for age, sex, and the first three ancestry substructure PCs. Because the number of participants with two copies of the minor allele at this locus was small in the longitudinal subsample ($n = 2$), these analyses proceeded with dominant allele coding ($n = 78$ with 0 copies of minor allele vs. $n = 33$ with 1–2 copies). The direct effects of the SNP and PTSD (and their interaction) on residualized change in CRP values was also included in the model. Results revealed that T1 methylation at this locus was associated with residualized change in CRP values over time (B = −.14, $p = .005$). Methylation at this locus was predicted by the SNP (B = −1.20, $p < .001$) and by the interaction between the SNP and PTSD severity in this subset of the data (B = .01, $p = .004$; Fig. 3 for standardized results). The indirect effect

Table 4
Effects of rs9527025 and PTSD on KL DNAm at cg00129557.

	cg00129557		
Variable	B	SE	*p*
Step 1: Covariates			
Age	−.01	.01	.054
Sex	−.21	.16	.198
CD8-T	3.24	1.20	.007
CD4-T	4.47	.91	1.63×10^{-06}
NK	6.28	1.40	1.00×10^{-05}
B cells	3.44	1.91	.072
Mono	−1.44	1.72	.404
PC1	−.90	.79	.257
PC2	.69	.79	.382
PC3	−.94	.77	.221
PTSD sev	−.001	.001	.641
Step 2: SNP			
rs9527025	−.65	.07	1.29×10^{-20}
Step 3: IX w/PTSD sev			
rs9527025	.004	.002	.035

Note. Beta values (*i.e.*, regression coefficients) are unstandardized. Main effect results for other SNPs and CPGs that were either not associated with each other or were only nominally significant are shown in Table S1. NK = natural killer; mono = monocytes; PC = principal component; PTSD sev = posttraumatic stress disorder severity; SNP = single nucleotide polymorphism, w = with; IX = interaction.

of the interaction term on residualized change in CRP values *via* methylation at this locus was also significant (B = −.002, standardized β = −.13, $p = .033$). In total, the model explained 30 % of the variance in CRP values at T2 and 24 % of the variance in methylation at cg00129557. We added to this model by including the estimated WBCs as covariates of methylation at this locus. Again, the pattern of results was unchanged with a significant indirect effect of the interaction term on residualized change in CRP values over time (B = −.002, standardized β = −.11, $p = .04$). The fit of the path models is shown in Table S2. Fit statistics suggested that all models were consistent with good model fit.

We also reran this model using additive genotype coding at this

Fig. 2. The figure shows the nature of the interaction between *KL* SNP rs9527025 and PTSD symptom severity on raw (beta) methylation levels at the *KL* locus cg00129557 for $n = 308$ participants in this cross-sectional analyses. The protective effects of the minor allele (C) were attenuated at high levels of PTSD symptom severity. This SNP is located on chromosome 13 at 33,628,193 bp; cg00129557 is located on chromosome 13 at 33,607,618 bp. There were 9 participants homozygous for the minor allele (CC), 88 heterozygous (CG), and 211 (GG) homozygous for the major allele.

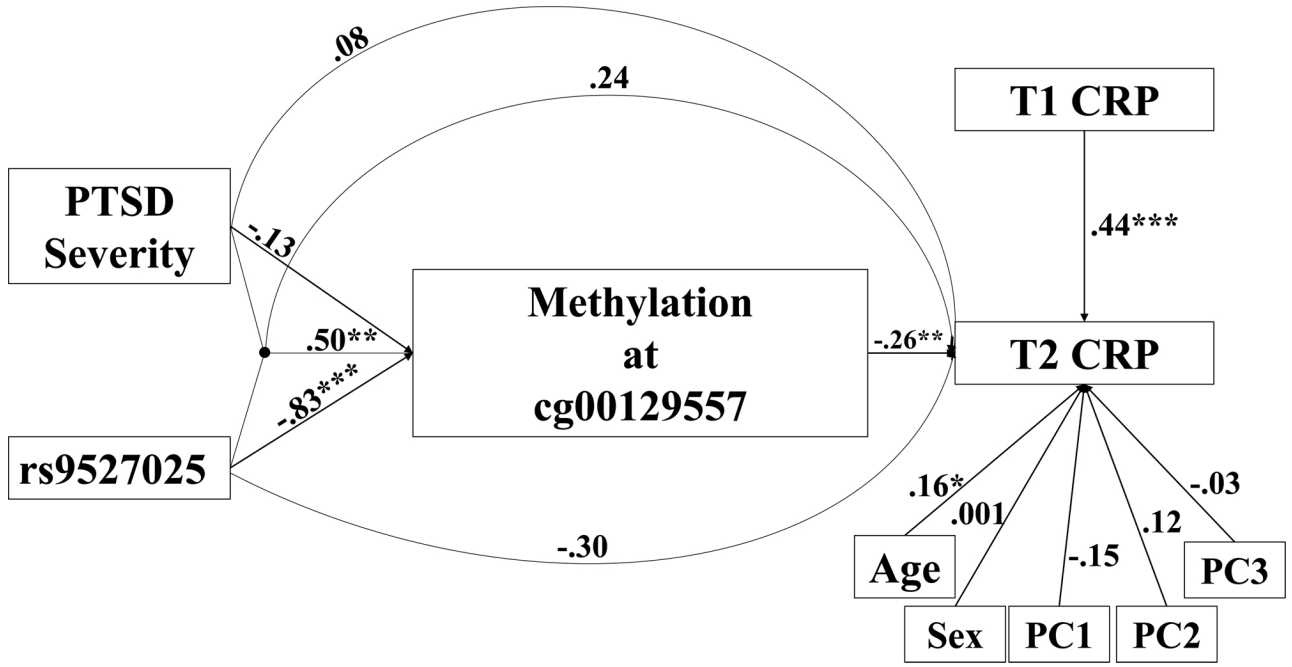

Fig. 3. The figure shows the results of the path model examining the direct and indirect effects of the KL-VS SNP (rs9527025), PTSD severity, and their interaction on residualized changes in CRP levels over the course of approximately two years *via* methylation at the *KL* locus cg00129557 ($n = 111$). Standardized coefficients are presented.
*$p < .05$. **$p < .01$. ***$p < .001$.

locus and found that the results were nearly identical, with the exception of an additional direct effect of rs9527025 on residualized changes in CRP (B = −.26, p = .049). The indirect effect of the SNP X PTSD on residualized changes in CRP *via* methylation at cg00129557 remained unchanged (B = −.002, p = .044; standardized β = −.13, p = .037).

Next, we added T2 methylation and T2 PTSD severity to the model and examined the direct effects of rs9527025, T1 PTSD severity, T1 CRP and T1 cg00129557 levels on these T2 variables. We found that T2 PTSD severity was predicted by T1 PTSD severity (B = .80, p < .001), but not by T1 CRP, methylation, or the SNP (Fig. S1 for standardized path results). T2 methylation at cg00129557 was associated with T1 methylation at this same locus (B = .30, p = .002) and evidenced a residual association with rs9527025 (B = −.48, p = .002) that was not accounted for by the effect of this SNP on T1 methylation at this locus. There were no effects of T1 PTSD or CRP on methylation at this locus at T2. Other than these autoregressive effects and the additional effect of the SNP on T2 methylation, the results of the analysis were otherwise unchanged from that without these T2 variables included in the model. Specifically, the indirect effect of PTSD severity X rs9527025 on residualized change in CRP at T2 *via* T1 methylation at cg00129557 remained significant (B = −.002, p = .042, standardized β = −.13, p = .035). Results for the correlated DNAm locus (cg02706658) are shown in Fig. S2.

4. Discussion

The primary finding of this study was that effects of the *KL* genetic variant rs9527025 and PTSD on residualized changes in inflammation over a two-year interval are mediated, at least in part, by *KL* methylation. Many of the age-related effects of *KL* are thought to operate through inflammatory pathways (Hui et al., 2017; Maekawa et al., 2009), and the results of this study implicate methylation at the cg00129557 locus as a causal factor. Though the minor allele of rs9527025 is known to confer protection against a host of age-related phenotypes (Arking et al., 2002) and was similarly associated with decreased CRP levels over time (and with reduced *KL* methylation at cg00129557) in this study, we found that this SNP also interacted with PTSD severity such that its protective effect was diminished in those with greater PTSD symptom severity. Specifically, those without substantial PTSD symptoms exhibited reduced methylation at this locus, consistent with the main effect of the genotype. In contrast, those with greater PTSD severity evidenced higher levels of methylation at this locus, despite having the protective variant, and this was associated with increased inflammation. These changes likely operate *via* reduced *KL* expression, as supported by data from the Genotype-Tissue Expression (GTEx) Project's online database (version 8 release).[4] Specifically, although gene expression was not examined in this study, the GTEx reference data suggested that rs9527025 is an expression quantitative trait loci (eQTL), with the minor allele predictive of lower *KL* expression in whole blood (normalized effect size [NES] = −.13, p = 8.1 X 10^{-5}) in 670 subjects. Per GTEx, the SNP also showed significant associations with *KL* expression in at least two brain regions, including nucleus accumbens (NES = .18, p = .01, n = 202) and hippocampus (NES = −.11, p = .03, n = 165), further supporting the relevance of this SNP for alterations in *KL* expression in the periphery and brain.

Epigenetic modifications are thought to reflect both genetic and environmental influences and here we found that PTSD interacted with genotype to predict *KL* methylation (and indirectly, residualized CRP values over time). One way to conceptualize this association is to consider symptoms of PTSD as part of the environment that interacts with at risk genotypes (*i.e.*, G X E) to promote cellular aging. Evaluation of genotype in an at-risk PTSD population could provide useful clinical information as to who is at greatest risk for increasing inflammation. This could encourage targeting of lifestyle and pharmacological interventions to those with the greatest need. As well, the role of cg00129557 in linking PTSD and genotype to inflammation may prove useful in developing new pharmacological therapies to reduce inflammation in those at risk (*e.g.*, personalized medicine).

To our knowledge, this is the first study to link DNAm at cg00129557 to inflammation. This locus is located mid-way through the gene in a region identified as a "DNase hypersensitive site" per the Encode project (GRCh37 chr13:33607361-33607915). Hypersensitive sites are regions that are easily cleaved by the nuclease DNAse I. They occur at open ends of chromatin allowing for binding of transcriptional factors and as such, they are thought to be regulatory (Thurman et al., 2012). Increased methylation at these sites is associated with reduced chromatin binding availability, and therefore, reduced expression (Thurman et al., 2012). Thus, methylation at cg00129557 may be a mechanism linking the KL-VS SNPs to various age-related phenotypes identified in the literature previously, but replication in other samples and cell types is clearly needed.

The identification of a *KL* DNase hypersensitivity site that links genotype to inflammation further highlights the importance of pursuing KL-related interventions. Previous studies suggest that circulating KL is increased as a function of exercise (Ramez et al., 2019; Amaro-Gahete et al., 2019), and that *KL* expression is increased by cholesterol-lowering medications (Janić et al., 2019). Targeting *KL* promoter region using CRISPR technology has also been associated with alterations in *KL* expression in neural and kidney cell lines (Chen et al., 2018), suggesting a possible therapeutic approach for increasing KL levels and protecting against neurobiological health decline. Thus, there may be multiple avenues to pursue in the development of interventions to alter the modifiable aspects of *KL* expression and potentially protect against a range of age-related adverse cardiac, renal, and cognitive health outcomes.

The association between *KL* and CRP is important as CRP has been shown to interact with the NF-κB signaling pathway to promote greater inflammation and reactive oxygen species (Zhong et al., 2015). CRP can be used as a marker for "inflammaging" (Frasca and Blomberg, 2016), in which low, but chronic, levels of inflammation increase risk for age-related disease, health decline, and mortality (Franceschi et al., 2000). For example, in a large study of adults aged 65 or older, CRP was found to be negatively associated with functional ability (e.g., activities of daily living), and cognition, and it predicted mortality in survival analyses (Puzianowska-Kuźnicka et al., 2016). Similarly, CRP was one of the strongest correlates of having multiple physical health diagnoses in an epidemiological sample of over 50,000 adults (Stepanova et al., 2015). CRP has also been shown to predict worse psychiatric outcomes in depression (Eswarappa et al., 2019), although this finding is not consistent (Chu et al., 2019; Lamers et al., 2019), suggesting that its predictive value may be more meaningful for physical rather than psychological health outcomes. The association between PTSD and CRP has also been inconsistent in the literature, with some studies finding evidence of this and others reporting null effects (see Miller et al., 2018a for a review). The results of this study raise the possibility that one reason for variability in these findings is that the association between PTSD and CRP is dependent on genotype (see also, Miller et al., 2018b). Collectively, results contribute to our understanding of the importance of CRP as a risk-factor for age-related morbidity and mortality, particularly in populations at risk by virtue of their psychiatric status and genetic makeup.

4.1. Study limitations

There are several limitations to this study that should be considered in evaluating these results. First, although the EPIC array is currently the most comprehensive commercially available BeadChip, assaying more than 850 K sites, its coverage of the genome is not complete, and it may well be that there are other sites near cg00129557 (and

[4] Accessible at: https://www.gtexportal.org/home/.

cg02706658) in the hypersensitivity site that alone, or in aggregate, are causally linked to CRP. Similarly, we could not determine whether the causal SNP in this case was actually rs9527025 or some other genetic variant in LD with this SNP. This may be resolved in the future by examining PTSD cohorts with other ancestries where patterns of LD may differ or *via* sequencing of the entire *KL* gene. DNAm data were obtained from whole blood, thus we could not examine cell type specific methylation patterns and instead (and as is standard) covaried for estimated cell type. It is also unknown if results would generalize to other tissue (*e.g.*, neural tissue). We did not obtain other inflammatory markers, which would allow for a more comprehensive examination of the effects of PTSD and *KL* on inflammation and inflammaging. Results from our cohort of white non-Hispanic mostly male veterans may not generalize to other demographic groups or other PTSD samples. The longitudinal analyses were conducted over a relatively short period of time (approximately two years), which may be too short an interval to observe substantial changes in biomarkers and the follow-up sample was small, limiting our statistical power. As well, there are numerous unmeasured potential confounds (such as new disease onset, unmeasured daily life stressors occurring between T1 and T2, and participation in mental health treatment) that could affect our longitudinal results. We also did not obtain mRNA or KL protein levels on these participants, so could not directly test the impact of *KL* methylation on expression. We did not have access to a suitable replication dataset and it is critical to test if these effects replicate in independent cohorts. Given these concerns, additional research is necessary to examine the replicability of these results and to test their robustness in the broader PTSD population.

4.2. Conclusions

These limitations notwithstanding, our results provide initial evidence of longitudinal effects of *KL* genotype and PTSD symptom severity on residualized changes in CRP over time *via KL* methylation. Results help to address questions regarding individual differences in inflammatory outcomes in PTSD samples by suggesting that associations between PTSD and CRP levels, which are well documented in the literature (Eswarappa et al., 2019; Rosen et al., 2017; Michopoulos et al., 2015), are at least partially dependent on *KL* genotype and methylation status. This highlights the importance of identifying the subpopulations of individuals who are at greatest risk for adverse PTSD-related health outcomes, including premature aging. Efforts to alter the transcriptional effects of circulating levels of KL (that has been shed from transmembrane *KL*; Chen et al., 2018) may ultimately prove beneficial in mitigating the health burden associated with psychiatric stress, and in promoting health and wellbeing into old age.

Disclosures

All authors report no financial or other conflicts of interest in relationship to the contents of this article. Filomene G. Morrison's contribution to this work was completed as a post-doctoral fellow at Boston University. Dr. Morrison is currently an employee of BlackThorn Therapeutics.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2020.104656.

References

Abraham, C.R., Mullen, P.C., Tucker-Zhou, T., Chen, C.D., Zeldich, E., 2016. Klotho is a neuroprotective and cognition-enhancing protein. Vitam. Horm., vol. 101. Academic Press, UK, pp. 215–238. https://doi.org/10.1016/bs.vh.2016.02.004.

Allison, P.D., 1990. Change scores as dependent variables in regression analysis. Sociol. Methodol. 20, 93–114. https://doi.org/10.2307/271083.

Amaro-Gahete, F.J., De-la-O, A., Jurado-Fasoli, L., Espuch-Oliver, A., de Haro, T., Gutiérrez, A., Castillo, M.J., 2019. Exercise training increases the S-Klotho plasma levels in sedentary middle-aged adults: A randomised controlled trial. The FIT-AGEING study. J. Sports Sci. 37, 2175–2183. https://doi.org/10.1080/02640414.2019.1626048.

Arking, D.E., Atzmon, G., Arking, A., Barzilai, N., Dietz, H.C., 2005. Association between a functional variant of the Klotho gene and high-density lipoprotein cholesterol, blood pressure, stroke, and longevity. Circulation 96, 412–418 https://doi.org/10.1161/01.RES.0000157171.04054.30.

Arking, D.E., Krebsova, A., Macek, M., Arking, A., Mian, I.S., Fried, L., Dietz, H.C., 2002. Association of human aging with a functional variant of Klotho. Proc. Natl. Acad. Sci. U. S. A. 99, 856–861. https://doi.org/10.1073/pnas.022484299.

Aryee, M.J., Jaffe, A.E., Corrada-Bravo, H., Ladd-Acosta, C., Feinberg, A.P., Hansen, K.D., Irizarry, R.A., 2014. Minfi: a flexible and comprehensive bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics 30, 1363–1369. https://doi.org/10.1093/bioinformatics/btu049.

Azuma, M., Koyama, D., Kikuchi, J., Yoshizawa, H., Thasinas, D., Shiizaki, K., Kusano, E., 2012. Promoter methylation confers kidney-specific expression of the Klotho gene. FASEB J. 26, 4264–4274. https://doi.org/10.1096/fj.12-211631.

Black, S., Kushner, I., Samols, D., 2004. C-reactive protein. J. Biol. Chem. 279, 48487–48490. https://doi.org/10.1074/jbc.R400025200.

Blake, D.D., Weathers, F.W., Nagy, L.M., Kaloupek, D.G., Gusman, F.D., Charney, D.S., Keane, T.M., 1995. The development of a clinician-administered PTSD scale. J. Trauma. Stress 8, 75–90. https://doi.org/10.1002/jts.2490080106.

Chen, C.-Y., Pollack, S., Hunter, D.J., Hirschhorn, J.N., Kraft, P., Price, A.L., 2013a. Improved ancestry inference using weights from external reference panels. Bioinformatics 29, 1399–1406. https://doi.org/10.1093/bioinformatics/btt144.

Chen, C.D., Sloane, J.A., Li, H., Aytan, N., Giannaris, E.L., Zeldich, E., Luebke, J.I., 2013b. The antiaging protein Klotho enhances oligodendrocyte maturation and myelination of the CNS. J. Neurosci. 33, 1927–1939. https://doi.org/10.1523/JNEUROSCI.2080-12.2013.

Chen, C.D., Zeldich, E., Li, Y., Yuste, A., Abraham, C.R., 2018. Activation of the anti-aging and cognition-enhancing gene Klotho by CRISPR-dCas9 transcriptional effector complex. J. Mol. Neurosci. 64, 175–184. https://doi.org/10.1007/s12031-017-1011-0.

Chu, A.L., Stochl, J., Lewis, G., Zammit, S., Jones, P.B., Khandaker, G.M., 2019. Longitudinal association between inflammatory markers and specific symptoms of depression in a prospective birth cohort. Brain Behav. Immun. 76, 74–81. https://doi.org/10.1016/j.bbi.2018.11.007.

Donate-Correa, J., Martín-Núñez, E., Delgado, N.P., de Fuentes, M.M., Arduan, A.O., Mora-Fernández, C., González, J.F.N., 2016. Implications of Fibroblast growth factor/Klotho system in glucose metabolism and diabetes. Cytokine Growth Factor Rev. 28, 71–77. https://doi.org/10.1016/j.cytogfr.2015.12.003.

Drew, D.A., Katz, R., Kritchevsky, S., Ix, J., Shlipak, M., Gutiérrez, O.M., Sarnak, M., 2017. Association between soluble Klotho and change in kidney function: the health aging and body composition study. J. Am. Soc. Nephrol. 28, 1859–1866. https://doi.org/10.1681/ASN.2016080828.

Dubal, D.B., Yokoyama, J.S., Zhu, L., Broestl, L., Worden, K., Wang, D., Ho, K., 2014. Life extension factor Klotho enhances cognition. Cell Rep. 7, 1065–1076. https://doi.org/

10.1016/j.celrep.2014.03.076.
Eswarappa, M., Neylan, T.C., Whooley, M.A., Metzler, T.J., Cohen, B.E., 2019. Inflammation as a predictor of disease course in posttraumatic stress disorder and depression: a prospective analysis from the mind your heart study. Brain Behav. Immun. 75, 220–227. https://doi.org/10.1016/j.bbi.2018.10.012.
Fortin, J.P., Triche Jr., T.J., Hansen, K.D., 2017. Preprocessing, normalization and integration of the Illumina HumanMethylationEpic array with Minfi. Bioinformatics 33, 558–560. https://doi.org/10.1093/bioinformatics/btw691.
Franceschi, C., Valensin, S., Bonafe, M., Paolisso, G., Yashin, A.I., Monti, D., De Benedictis, G., 2000. The network and the remodeling theories of aging: Historical background and new perspectives. Exp. Gerontol. 35, 879–896. https://doi.org/10.1016/S0531-55650000172-8.
Frasca, D., Blomberg, B.B., 2016. Inflammaging decreases adaptive and innate immune responses in mice and humans. Biogerontology 17, 7–19. https://doi.org/10.1007/s10522-015-9578-8.
Fülöp, T., Dupuis, G., Witkowski, J.M., Larbi, A., 2016. The role of immunosenescence in the development of age-related diseases. Rev. Invest. Clin. 68, 84–91.
Guo, Y., Zhuang, X., Huang, Z., Zou, J., Yang, D., Hu, X., Liao, X., 2018. Klotho protects the heart from hyperglycemia-induced injury by inactivating ROS and NF-κB-mediated inflammation both in vitro and in vivo. Biochim. Biophys. Acta. Mol. Basis. Dis. 1864, 238–251. https://doi.org/10.1016/j.bbadis.2017.09.029.
Hamilton, E., 2011. Mythology: Timeless Tales of Gods and Heroes. Hachette UK, London, UK.
Hu, L.T., Bentler, P.M., 1999. Cutoff criteria for fit indexes in covariance structure analysis: conventional criteria versus new alternatives. Struct. Equ. Model. A Multidiscip. J. 6, 1–55. https://doi.org/10.1080/10705519909540118.
Hui, H., Zhai, Y., Ao, L., Cleveland Jr., J.C., Liu, H., Fullerton, D.A., Meng, X., 2017. Klotho suppresses the inflammatory responses and ameliorates cardiac dysfunction in aging endotoxemic mice. Oncotarget 8, 15663–15676. https://doi.org/10.18632/oncotarget.14933.
Janić, M., Lunder, M., Novaković, S., Škerl, P., Šabovič, M., 2019. Expression of longevity genes induced by a low-dose Fluvastatin and Valsartan combination with the potential to prevent/treat "aging-related disorders" Int. J. Mol. Sci. 20, 1844. https://doi.org/10.3390/ijms20081844.
Kent, W.J., Sugnet, C.W., Furey, T.S., Roskin, K.M., Pringle, T.H., Zahler, A.M., Haussler, D., 2002. The human genome browser at UCSC. Genome Res. 12, 996–1006. https://doi.org/10.1101/gr.229102.
King, G.D., Rosene, D.L., Abraham, C.R., 2012. Promoter methylation and age-related downregulation of Klotho in rhesus monkey. Age(Dordr) 34, 1405–1419. https://doi.org/10.1007/s11357-011-9315-4.
Kuro-o, M., 2019. The Klotho proteins in health and disease. Nat. Rev. Nephrol. 15, 27–44. https://doi.org/10.1038/s41581-018-0078-3.
Kuro-o, M., Matsumura, Y., Aizawa, H., Kawaguchi, H., Suga, T., Utsugi, T., Iwasaki, H., 1997. Mutation of the mouse Klotho gene leads to a syndrome resembling ageing. Nature 390, 45–51. https://doi.org/10.1038/36285.
Kurosu, H., Yamamoto, M., Clark, J.D., Pastor, J.V., Nandi, A., Gurnani, P., Shimomura, I., 2005. Suppression of aging in mice by the hormone Klotho. Science 309, 1829–1833. https://doi.org/10.1126/science.1112766.
Lamers, F., Milaneschi, Y., Smit, J.H., Schoevers, R.A., Wittenberg, G., Penninx, B.W., 2019. Longitudinal association between depression and inflammatory markers: results from the Netherlands study of depression and anxiety. Biol. Psychiatry 85, 829–837. https://doi.org/10.1016/j.biopsych.2018.12.020.
Lee, J., Jeong, D.J., Kim, J., Lee, S., Park, J.H., Chang, B., Kim, K.I., 2010. The anti-aging gene KLOTHO is a novel target for epigenetic silencing in human cervical carcinoma. Mol. Cancer 9, 109. https://doi.org/10.1186/1476-4598-9-109.
Leek, J.T., Johnson, W.E., Parker, H.S., Fertig, E.J., Jaffe, A.E., Storey, J.D., et al., 2017. SVA: surrogate variable analysis r package version. Bioinformatics 3 (10), 10–18129.
Liu, H., Fergusson, M.M., Castilho, R.M., Liu, J., Cao, L., Chen, J., et al., 2007. Augmented Wnt signaling in a mammalian model of accelerated aging. Science 317, 803–806. https://doi.org/10.1126/science.1143578.
Logue, M.W., Amstadter, A.B., Baker, D.G., Duncan, L., Koenen, K.C., Liberzon, I., Ressler, K.J., 2015. The psychiatric genomics consortium posttraumatic stress disorder workgroup: posttraumatic stress disorder enters the age of large-scale genomic collaboration. Neuropsychopharmacology 40, 2287–2297. https://doi.org/10.1038/npp.2015.118.
Logue, M.W., Baldwin, C., Guffanti, G., Melista, E., Wolf, E.J., Reardon, A.F., et al., 2013. A genome-wide association study of post-traumatic stress disorder identifies the retinoid-related orphan receptor alpha RORA gene as a significant risk locus. Mol. Psychiatry 18, 937–942. https://doi.org/10.1038/mp.2012.113.
Logue, M.W., Smith, A.K., Wolf, E.J., Maniates, H., Stone, A., Schichman, S.A., Miller, M.W., 2017. The correlation of methylation levels measured using Illumina 450k and EPIC BeadChips in blood samples. Epigenomics 9, 1363–1371. https://doi.org/10.2217/epi-2017-0078.
Luo, M., Zhou, X., Ji, H., Ma, W., Liu, G., Dai, D., Duan, S., 2015. Population difference in the associations of KLOTH promoter methylation with mild cognitive impairment in Xinjiang Uygur and Han populations. PLoS One 10, e0132156 https:doi.org/10.1371/journal.pone.0132156.
Maekawa, Y., Ishikawa, K., Yasuda, O., Oguro, R., Hanasaki, H., Kida, I., Rakugi, H., 2009. Klotho suppresses TNF-α-induced expression of adhesion molecules in the endothelium and attenuates NF-κB activation. Endocrine 35, 341–346. https://doi.org/10.1007/s12020-009-9181-3.
Martín-Núñez, E., Donate-Correa, J., López-Castillo, Á., Delgado-Molinos, A., Ferri, C., Rodríguez-Ramos, S., Mora-Fernández, C., 2017. Soluble levels and endogenous vascular gene expression of KLOTHO are related to inflammation in human atherosclerotic disease. Clin Sci(Lond). 131, 2601–2609. https://doi.org/10.1042/CS20171242.
McGlinchey, R.E., Milberg, W.P., Fonda, J.R., Fortier, C.B., 2017. A methodology for assessing deployment trauma and its consequences in OEF/OIF/OND veterans: the TRACTS longitudinal prospective cohort study. Int. J. Methods Psychiatr. Res. 26. https://doi.org/10.1002/mpr.1556.
Mengel-From, J., Soerensen, M., Nygaard, M., McGue, M., Christensen, K., Christiansen, L., 2016. Genetic variants in KLOTHO associate with cognitive function in the oldest old group. J. Gerontol. A Biol. Sci. Med. Sci. 71, 1151–1159. https://doi.org/10.1093/gerona/glv163.
Michopoulos, V., Rothbaum, A.O., Jovanovic, T., Almli, L.M., Bradley, B., Rothbaum, B.O., Ressler, K.J., 2015. Association of CRP genetic variation and CRP level with elevated PTSD symptoms and physiological responses in a civilian population with high levels of trauma. Am. J. Psychiatry 172, 353–362. https://doi.org/10.1176/appi.ajp.2014.14020263.
Miller, M.W., Lin, A.P., Wolf, E.J., Miller, D.R., 2018a. Oxidative stress, inflammation, and neuroprogression in chronic PTSD. Harv. Rev. Psychiatry 26, 57–69 https://dx.doi.org/10.1097%2FHRP.0000000000000167.
Miller, M.W., Maniates, H., Wolf, E.J., Logue, M.W., Schichman, S.A., Stone, A., McGlinchey, R., 2018b. CRP polymorphisms and DNA methylation of the AIM2 gene influence associations between trauma exposure, PTSD, and C-reactive protein. Brain Behav. Immun. 67, 194–202. https://doi.org/10.1016/j.bbi.2017.08.022.
Muthén, L.K., Muthén, B.O., 2018. Mplus User's Guide, 8th ed. Muthén & Muthén, Los Angeles, CA.
Passos, I.C., Vasconcelos-Moreno, M.P., Costa, L.G., Kunz, M., Brietzke, E., Quevedo, J., Kauer-Sant'Anna, M., 2015. Inflammatory markers in post-traumatic stress disorder: a systematic review, meta-analysis, and meta-regression. Lancet Psychiatry 2, 1002–1012. https://doi.org/10.1016/S2215-03661500309-0.
Pidsley, R., Wong, C.C., Volta, M., Lunnon, K., Mill, J., Schalkwyk, L.C., 2013. A data-driven approach to preprocessing Illumina 450k methylation array data. BMC Genomics 14, 293. https://doi.org/10.1186/1471-2164-14-293.
Prystupa, A., Dąbrowska, A., Sak, J.J., Tarach, J., Toruń-Jurkowska, A., Lachowska-Kotowska, P., Dzida, G., 2016. Concentrations of fetuin-A, osteoprotegerin and α-Klotho in patients with alcoholic liver cirrhosis. Exp. Ther. Med. 12, 3464–3470. https://doi.org/10.3892/etm.2016.3754.
Puzianowska-Kuźnicka, M., Owczarz, M., Wieczorowska-Tobis, K., Nadrowski, P., Chudek, J., Slusarczyk, P., Mossakowska, M., 2016. Interleukin-6 and C-reactive protein, successful aging, and mortality: the PolSenior study. Immun. Ageing 13, 21. https://doi.org/10.1186/s12979-016-0076-x.
Ramez, M., Rajabi, H., Ramezani, F., Naderi, N., Darbandi-Azar, A., Nasirinezhad, F., 2019. The greater effect of high-intensity interval training versus moderate-intensity continuous training on cardioprotection against ischemia-reperfusion injury through Klotho levels and attenuate of myocardial TRPC6 expression. BMC Cardiovasc. Disord. 19, 118. https://doi.org/10.1186/s12872-019-1090-7.
Ratanatharathorn, A., Boks, M.P., Maihofer, A.X., Aiello, A.E., Amstadter, A.B., Ashley-Koch, A.E., Garrett, M.E., 2017. Epigenome-wide association of PTSD from heterogeneous cohorts with a common multi-site analysis pipeline. Am. J. Med. Genet. B Neuropsychiatr. Genet. 174, 619–630. https://doi.org/10.1002/ajmg.b.32568.
Revelas, M., Thalamuthu, A., Oldmeadow, C., Evans, T.J., Armstrong, N.J., Kwok, J.B., Attia, J.R., 2018. Review and meta-analysis of genetic polymorphisms associated with exceptional human longevity. Mech. Ageing Dev. 175, 24–34. https://doi.org/10.1016/j.mad.2018.06.002.
Ridker, P.M., Rifai, N., Rose, L., Buring, J.E., Cook, N.R., 2002. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N. Engl. J. Med. 347, 1557–1565 https:doi.org/10.1056/NEJMoa021993.
Rosen, R.L., Levy-Carrick, N., Reibman, J., Xu, N., Shao, Y., Liu, M., Marmor, M., 2017. Elevated C-reactive protein and posttraumatic stress pathology among survivors of the 9/11 World Trade Center attacks. J. Psychiatr. Res. 89, 14–21. https://doi.org/10.1016/j.jpsychires.2017.01.007.
Rubinek, T., Shulman, M., Israeli, S., Bose, S., Avraham, A., Zundelevich, A., Wolf, I., 2012. Epigenetic silencing of the tumor suppressor Klotho in human breast cancer. Breast Cancer Res. Treat. 133, 649–657. https://doi.org/10.1007/s10549-011-1824-4.
Sadeh, N., Spielberg, J.M., Logue, M.W., Wolf, E.J., Smith, A.K., Lusk, J., et al., 2016. SKA2 methylation is associated with decreased prefrontal cortical thickness and greater PTSD severity among trauma-exposed veterans. Mol. Psychiatry 21, 357–363.
Sedighi, M., Baluchnejadmojarad, T., Fallah, S., Moradi, N., Afshin-Majdd, S., Roghani, M., 2019. Klotho ameliorates cellular inflammation via suppression of cytokine release and upregulation of miR-29a in the PBMCs of diagnosed Alzheimer's disease patients. J. Mol. Neurosci. 69, 157–165. https://doi.org/10.1007/s12031-019-01345-5.
Shutz, R.W., 1989. Analyzing change. In: Safrit, M.K., Wood, T.M. (Eds.), Measurement Concepts in Physical Education and Exercise Science. Human Kinetics Books, Champaign, IL, pp. 207–228.
Sproston, N.R., Ashworth, J.J., 2018. Role of C-reactive protein at sites of inflammation and infection. Front. Immunol. 9, 754. https://doi.org/10.3389/fimmu.2018.00754.
Stepanova, M., Rodriguez, E., Birerdinc, A., Baranova, A., 2015. Age-independent rise of inflammatory scores may contribute to accelerated aging in multi-morbidity. Oncotarget 6, 1414–1421. https://doi.org/10.18632/oncotarget.2725.
Thurman, R.E., Rynes, E., Humbert, R., Vierstra, J., Maurano, M.T., Haugen, E., Garg, K., 2012. The accessible chromatin landscape of the human genome. Nature 489, 75–82. https://doi.org/10.1038/nature11232.
Weathers, F.W., Ruscio, A.M., Keane, T.M., 1999. Psychometric properties of nine scoring rules for the clinician-administered posttraumatic stress disorder scale. Psychol. Assess. 11, 124. https://doi.org/10.1037/1040-3590.11.2.124.
Wolf, E.J., Logue, M.W., Hayes, J.P., Sadeh, N., Schichman, S.A., Stone, A., et al., 2016.

Accelerated DNA methylation age: associations with PTSD and neural integrity. Psychoneuroendocrinology 63, 155–162. https://doi.org/10.1016/j.psyneuen.2015.09.020.

Wolf, E.J., Logue, M.W., Morrison, F.G., Wilcox, E.S., Stone, A., Schichman, S.A., Miller, M.W., 2019a. Posttraumatic psychopathology and the pace of the epigenetic clock: a longitudinal investigation. Psychol. Med. 49, 791–800. https://doi.org/10.1017/S0033291718001411.

Wolf, E.J., Logue, M.W., Stoop, T.B., Schichman, S.A., Stone, A., Sadeh, N., et al., 2018a. Accelerated DNA methylation age. Psychosom. Med. 80, 42–48. https://doi.org/10.1097/psy.0000000000000506.

Wolf, E.J., Maniates, H., Nugent, N., Maihofer, A., Armstrong, D., Ratanatharathorn, A., et al., 2018b. Traumatic stress and accelerated DNA methylation age: a meta-analysis. Psychoneuroendocrinology 92, 123–134. https://doi.org/10.1016/j.psyneuen.2017.12.007.

Wolf, E.J., Morrison, F.G., 2017. Traumatic stress and accelerated cellular aging: from epigenetics to cardiometabolic disease. Curr. Psychiatry Rep. 19, 75. https://doi.org/10.1007/s11920-017-0823-5.

Wolf, E.J., Morrison, F.G., Sullivan, D.R., Logue, M.W., Guetta, R.E., Stone, A., Miller, M.W., 2019b. The goddess who spins the thread of life: klotho, psychiatric stress, and accelerated aging. Brain Behav. Immun. 80, 193–203. https://doi.org/10.1016/j.bbi.2019.03.007.

Xia, W., Zhang, A., Jia, Z., Gu, J., Chen, H., 2016. Klotho contributes to pravastatin effect on suppressing IL-6 production in endothelial cells. Mediators Inflamm. 2016. https://doi.org/10.1155/2016/2193210.

Yamamoto, M., Clark, J.D., Pastor, J.V., Gurnani, P., Nandi, A., Kurosu, H., Kuro-o, M., 2005. Regulation of oxidative stress by the anti-aging hormone Klotho. J. Biol. Chem. 280, 38029–38034. https://doi.org/10.1074/jbc.M509039200.

Yokoyama, J.S., Sturm, V.E., Bonham, L.W., Klein, E., Arfanakis, K., Yu, L., Dubal, D.B., 2015. Variation in longevity gene KLOTHO is associated with greater cortical volumes. Ann. Clin. Transl. Neurol. 2, 215–230. https://doi.org/10.1002/acn3.161.

Zeldich, E., Chen, C.D., Boden, E., Howat, B., Nasse, J.S., Zeldich, D., et al., 2019. Klotho is neuroprotective in the Superoxide Dismutase (SOD1 G93A) mouse model of ALS. J. Mol. Neurosci. 69, 1–22. https://doi.org/10.1007/s12031-019-01356-2.

Zeldich, E., Chen, C.D., Colvin, T.A., Bove-Fenderson, E.A., Liang, J., Zhou, T.B.T., et al., 2014. The neuroprotective effect of Klotho is mediated via regulation of members of the redox system. J. Biol. Chem. 289, 24700–24715. https://doi.org/10.1074/jbc.M114.567321.

Zhong, Y., Cheng, C.F., Luo, Y.Z., Tian, C.W., Yang, H., Liu, B.R., Liu, S.M., 2015. C-reactive protein stimulates RAGE expression in human coronary artery endothelial cells in vitro via ROS generation and ERK/NF-κB activation. Acta Pharmacol. Sin. 36, 440–447. https://doi.org/10.1038/aps.2014.163.

Zhou, T.B.T., King, G.D., Chen, C., Abraham, C.R., 2013. Biochemical and functional characterization of the klotho-VS polymorphism implicated in aging and disease risk. J. Biol. Chem. 288, 36302–36311. https://doi.org/10.1074/jbc.M113.490052.

Zhu, Y., Cao, X., Zhang, X., Chen, Q., Wen, L., Wang, P., 2019. DNA methylation-mediated Klotho silencing is an independent prognostic biomarker of head and neck squamous carcinoma. Cancer Manag. Res. 11, 1383–1390. https://doi.org/10.2147/cmar.s188415.

Zhu, L., Stein, L.R., Kim, D., Ho, K., Yu, G.Q., Zhan, L., Mucke, L., 2018. Klotho controls the brain-immune system interface in the choroid plexus. Proc. Natl. Acad. Sci. U. S. A. 115, E11388–E11396. https://doi.org/10.1073/pnas.1808609115.