Neuropsychopharmacology www.nature.com/npp

ARTICLE

Klotho, PTSD, and advanced epigenetic age in cortical tissue

Erika J. Wolf[1,2], Ci-Di Chen[3], Xiang Zhao[1,2], Zhenwei Zhou[1,4], Filomene G. Morrison[1,2], Nikolaos P. Daskalakis[5], Annjanette Stone[6], Steven Schichman[6], Jaclyn Garza Grenier[7], Dana Fein-Schaffer[1], Bertrand R. Huber[8,9], Traumatic Stress Brain Research Group, Carmela R. Abraham[3,10], Mark W. Miller[1,2] and Mark W. Logue[1,2,4,11]

This study examined the klotho (*KL*) longevity gene polymorphism rs9315202 and psychopathology, including posttraumatic stress disorder (PTSD), depression, and alcohol-use disorders, in association with advanced epigenetic age in three postmortem cortical tissue regions: dorsolateral and ventromedial prefrontal cortices and motor cortex. Using data from the VA National PTSD Brain Bank ($n = 117$), we found that rs9315202 interacted with PTSD to predict advanced epigenetic age in motor cortex among the subset of relatively older (>=45 years), white non-Hispanic decedents (corrected $p = 0.014$, $n = 42$). An evaluation of 211 additional common *KL* variants revealed that only variants in linkage disequilibrium with rs9315202 showed similarly high levels of significance. Alcohol abuse was nominally associated with advanced epigenetic age in motor cortex ($p = 0.039$, $n = 114$). The rs9315202 SNP interacted with PTSD to predict decreased *KL* expression via DNAm age residuals in motor cortex among older white non-Hispanics decedents (indirect $\beta = -0.198$, $p = 0.027$). Finally, in dual-luciferase enhancer reporter system experiments, we found that inserting the minor allele of rs9315202 in a human kidney cell line HK-2 genomic DNA resulted in a change in *KL* transcriptional activities, likely operating via long noncoding RNA in this region. This was the first study to examine multiple forms of psychopathology in association with advanced DNA methylation age across several brain regions, to extend work concerning the association between rs9315202 and advanced epigenetic to brain tissue, and to identify the effects of rs9315202 on *KL* gene expression. *KL* augmentation holds promise as a therapeutic intervention to slow the pace of cellular aging, disease onset, and neuropathology, particularly in older, stressed populations.

Neuropsychopharmacology (2021) 46:721–730; https://doi.org/10.1038/s41386-020-00884-5

INTRODUCTION

Longevity gene klotho (*KL*) has been linked to multiple biomarkers of cellular aging and adverse health outcomes in both preclinical and clinical studies [1–3]. Of particular interest are studies associating *KL* genotype, expression, and protein levels to phenotypes such as Alzheimer's disease [4], Alzheimer's related biomarkers (e.g., amyloid-beta; [5–7]), memory and cognitive performance [8–12], prefrontal cortical brain volume and neural connectivity [13, 14], hippocampal synaptic plasticity and neurogenesis [8, 9, 15], and axonal myelination [16, 17]. Evidence suggests that *KL* expression serves a protective function against age-related cognitive decline, with effects most evident among older individuals [11, 15, 18], though null effects have also been reported (e.g., [19]).

A separate line of research suggests that traumatic stress is associated with various biomarkers of cellular aging, including advanced epigenetic age, also known as DNA methylation (DNAm) age, relative to chronological age [20–24]. That said, these findings also have not been uniform across studies [25, 26]. Recently, in a sample of veterans with a high prevalence of posttraumatic stress disorder (PTSD), we found that the association between PTSD and several biomarkers of accelerated cellular aging was dependent on the presence of the minor allele at a single nucleotide polymorphism (SNP) in the *KL* gene [27]. Specifically, we examined 43 common SNPs in the *KL* gene and found that carriers of the rs9315202 minor allele (A) evidenced a strong association between PTSD symptom severity and advanced epigenetic age in blood (per the Horvath index), increased peripheral inflammation (C-reactive protein), and decreased white matter integrity in right-lateralized tracts connecting prefrontal to limbic regions. These effects were accentuated in subjects who were aged 30 years or greater (the median age in that sample of veterans returning from the Global War on Terror), consistent with other research suggesting that the effects of *KL* are more evident with increasing age [11, 15, 18].

Though numerous studies have found evidence of associations between traumatic stress and advanced DNAm age measured in blood (e.g., [22]), no study to date has examined associations between traumatic stress and epigenetic aging in brain tissue. Studies of advanced epigenetic age in postmortem brain tissue in association with other psychiatric conditions have yielded mixed results. Specifically, significant associations were reported between a novel epigenetic age acceleration measure and major depression in prefrontal and subgenual regions ($n = 141$; [28]), but

[1]National Center for PTSD at VA Boston Healthcare System, Boston, MA, USA; [2]Department of Psychiatry, Boston University School of Medicine, Boston, MA, USA; [3]Department of Biochemistry, Boston University School of Medicine, Boston, MA, USA; [4]Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA; [5]Department of Psychiatry, Harvard Medical School, McLean Hospital, Belmont, MA, USA; [6]Pharmacogenomics Analysis Laboratory, Research Service, Central Arkansas Veterans Healthcare System, Little Rock, AR, USA; [7]Brigham and Women's Hospital, Channing Division of Network Medicine, Boston, MA, USA; [8]Pathology and Laboratory Medicine, VA Boston Healthcare System, Boston, MA, USA; [9]Department of Neurology, Boston University School of Medicine, Boston, MA, USA; [10]Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, MA, USA and [11]Biomedical Genetics, Boston University School of Medicine, Boston, MA, USA
Correspondence: Erika J. Wolf (erika.wolf@va.gov)

Received: 21 May 2020 Revised: 12 September 2020 Accepted: 29 September 2020
Published online: 23 October 2020

not between the Horvath index and depression in prefrontal cortex (two samples of $n = 40$ and 58; [29]). Null effects were reported for bipolar disorder with Horvath-derived estimates ($n =$ 32 cases and 32 controls; [30]), though there was evidence of bipolar-related advanced DNAm age in hippocampal tissue among those >45 years of age). Studies of schizophrenia [31, 32] and alcohol-use disorders [33] reported no significant associations in frontal and/or temporal regions. Finally, heroin-use was associated with slowed epigenetic age in orbitofrontal cortex relative to controls and non-heroin-related suicide cases [34]. The equivocal pattern of findings across studies may be related to sample size, variability in brain regions evaluated, lack of consideration of genetic (such as *KL*) and demographic (such as age) modifiers of these associations, and/or differences arising from the DNAm age algorithm or psychiatric diagnosis under evaluation. The varied results of these studies highlight the need for further evaluation of stress-related epigenetic age acceleration in brain tissue.

AIMS AND HYPOTHESES

Based on the foregoing, we advanced the following aims and hypotheses.

Aim 1

The first aim was to test the main and interactive effects of PTSD and the *KL* SNP rs9315202 on advanced epigenetic age in postmortem brain tissue obtained from the VA National PTSD Brain Bank. Our primary hypothesis was that rs9315202 would moderate the association between PTSD and epigenetic age (relative to chronological age) in older decedents in the brain bank, consistent with the pattern of results we previously observed in blood and neuroimaging data [24]. To evaluate this, we conducted regression analyses focused on this candidate SNP X PTSD as a predictor of DNAm age residuals, evaluating effects in a subset of the cohort who were age 45 years or greater (the median). Models were also examined in those below the median age and in the sample as a whole. Three brain regions were evaluated: dorsolateral prefrontal cortex (dlPFC), ventromedial prefrontal cortex (vmPFC), and motor cortex. Alterations in dlPFC and vmPFC have commonly been associated with PTSD [35, 36] and aging [37, 38]. Moreover, *KL* has shown particular associations with right dlPFC [13, 14], suggesting this may be a primary locus for *KL*-related neuropathology risk versus resilience. Motor cortex was included given evidence of notable age-related cortical thinning in this area [37] and the role of *KL* in motor disturbances. Specifically, animal studies suggest that genetic manipulation of *KL* leads to early onset of motor pathology, such as gait disturbance [2], and that alterations in motor-related cells (e.g., anterior horn and Purkinje cells) in the central nervous system are key features of *KL* knockout mice [39, 40].

Aim 2

The second aim was to examine the main effects of diagnoses commonly comorbid with PTSD (major depression and alcohol abuse/dependence, representing internalizing and externalizing pathology, respectively; [41]) on epigenetic age in brain tissue relative to chronological age. Based on prior research in blood [24] and brain [28], we hypothesized that PTSD and both depression and alcohol-use disorders would be associated with advanced epigenetic age.

Aim 3

The third aim was to test the hypothesis that advanced epigenetic age in brain tissue is associated with altered *KL* mRNA expression and that the effects of rs9315202 and PTSD on altered *KL* expression are indirect, via advanced epigenetic age. We used mediation analysis to evaluate if rs9315202 X PTSD was associated with altered *KL* expression via advanced epigenetic age in the older cohort.

Aim 4

The final aim was to examine the molecular effect of the candidate SNP, rs9315202, on *KL* regulation, which is thus far unknown. To do so, we cloned genomic DNA containing this SNP into a reporter system, and evaluated the effects of rs9315202 on *KL* gene expression. We hypothesized that the SNP would act as an enhancer that regulates *KL* gene expression.

METHODS

PTSD brain bank

Participants and procedure. Postmortem tissue from $N = 117$ brains was obtained from the VA National PTSD Brain Bank housed at the VA Boston Healthcare System [42], which acquired the tissue and clinical information from the Lieber Institute for Brain Development at Johns Hopkins University [43]. Of this group, 2 were missing genotypes due to quality control concerns and were removed from analyses. A total of $n = 85$ were determined to be of white, non-Hispanic ancestry (per genotype in concert with medical record information) and were the primary sample evaluated in analyses involving *KL* genotype (due to concerns of population stratification). Analyses that did not include genotypes (i.e., evaluation of main effects of PTSD comorbidity and main effects of DNAm age residuals on *KL* expression) were based on the full cohort with complete data regardless of ancestry ($n = 114$ for methylation-only analyses and $n = 92$ for analyses including RNA expression data) to maximize statistical power and generalizability. Left dlPFC (Brodmann Area [BA] 9/46) was taken at the level of the genu of the corpus callosum, left vmPFC (BA 12/32) was taken at the level of the genu of the corpus callosum, and left motor cortex (BA 4) was taken at the superior central sulcus. Neuropathological exams and toxicology evaluations were performed by board-certified neuropathologists. Cause and manner of death were determined by Maryland state medical examiners.

The neuropathology examination and diagnostic assessment included medical record review and next-of-kin interviews to determine medical and psychiatric diagnoses, including administration of MINI International Neuropsychiatric Interview 6.0, the PTSD checklist for DSM-5 (adapted for postmortem studies), and the Lieber Psychological Autopsy Interview, which included interviews with mental health clinicians where possible [43]. Psychiatric diagnoses were further reviewed by at least two independent board-certified psychiatrists, with confidence in PTSD determinations rated on a 1−5 scale [44]. Diagnoses rated 3 or higher were classified as PTSD cases. From these sources, we obtained diagnoses of PTSD, major depression, and alcohol abuse or dependence. Exclusion criteria were neurodegenerative disease, history of severe traumatic brain injury (or neuropathological evidence of such), and neuritic pathology [44].

The full sample was 61.2% male with a mean age at death of 44.79 years (*SD*: 13.93, range: 17.35–92.25; Table 1). The median age at death was 45 years. This median point was used to a *priori* divide the sample into older vs. younger cohorts for age-group specific analyses.

DNA, DNAm, and related statistical procedures. Procedures for DNA extraction are described in Supplementary Materials. Genotypes were determined from DNA extracted from motor cortex samples and were interrogated on the Illumina HumanOmni2.5−8 array. Genotype imputation procedures (for secondary analyses) are described in the Supplementary Materials. DNAm was assessed in the dlPFC, vmPFC, and motor cortex with the Illumina Infinium MethylationEPIC array (Supplementary Materials). The Horvath [45] DNAm age calculation was based on the 335 probes on the EPIC array that overlap those on the 450k array

Table 1. Demographic characteristics of the sample.

	All ($n = 114$) % or *M* (*SD*)	White non-Hispanic ($n = 85$) % or *M* (*SD*)
Sex (male)	61.2	60.0
Ancestry		
White	75.0	100
Black	25.0	
Age at death	44.79 (13.93)	44.72 (13.57)
PTSD	40.5	41.2
Major depression	69.0	75.3
Alcohol Ab/dep	31.0	34.1
Control	24.1	21.2
BMI	30.75 (8.97)	31.00 (9.39)
Smoker	61.72	65.9
Suicide death	16.4	17.6
Substance use death	46.6	49.4
PMI (hours)	28.73 (8.23)	29.05 (8.81)
RIN	7.95 (1.32)	8.00 (1.21)

Self-reported ancestry is included in the table.
PTSD posttraumatic stress disorder, *ab/dep* abuse/dependence, *BMI* body mass index, *PMI* postmortem interval, *RIN* RNA integrity.

used to develop the algorithm [46]. Estimated percentage of neurons from each region was computed using the CETS method [47] and included as covariates to control for cellular heterogeneity (Supplementary Materials). Two sets of ancestry principal components (PCs) were calculated, each from 100,000 randomly chosen common SNPs (at least 5% minor allele frequency; see Supplementary Materials for details). The first set was developed to capture ancestral variation across all ancestries in the sample (global ancestry) and the second set was developed within the white non-Hispanic subsample (the largest in the cohort) to represent any cryptic population substructure within that cohort. The top three PCs were included in each set of analyses as covariates, as appropriate to the composition of the cohort in a given analysis.

RNA sequence data and related statistical procedures. RNA was extracted from each frozen tissue section, and library preparation conducted using the Illumina TruSeq Stranded total RNA kit with globin depletion. Libraries were then sequenced using HIseq 2500 (Supplementary Materials). The pipeline for determining gene expression included adapter removal and quality filter of reads with trimmomatic [48], alignment to the hg38 genome with STAR [49], transcript quantification with Kallisto [50, 51], and quantification of *KL* gene expression with the tximport Bioconductor package and log transformed using the regularized log transformation (rLog) method implemented in DEseq2 [52]. Cell types (astrocytes, endothelial cells, microglia, mural cells, neurons, oligodendrocytes, and red blood cells) were estimated using BrainInABlender and controlled for in all RNA analyses.

Data analyses. We first calculated the Horvath DNAm age estimates and compared them with age at death (i.e., chronological age) using correlation. We then regressed age at death out from the DNAm age estimates to generate "DNAm age residuals" to index a dimension of residuals reflecting advanced to slowed cellular aging for each brain region (i.e., the primary dependent variable in subsequent analyses) and compared these values across brain regions via correlation. Next, to test Aim 1, we conducted multiple linear regression analyses in the white non-Hispanic decedents (separately for each brain region). These analyses were conducted in the older cohort (where we hypothesized an effect) with multiple testing correction across the three regions (see below). We also tested for association in the younger cohort and in the full cohort for completeness, using the same multiple testing correction. In each of these models, we included demographic covariates (the first three ancestry substructure PCs, sex), methodological covariates (postmortem interval, RNA integrity [RIN] value, percentage of neurons the DNA was extracted from as estimated from the DNAm data), and PTSD diagnosis in the first step of the model. The second step added the SNP, and the third step added the interaction term for PTSD X rs9315202. The multiple testing correction used a procedure that permutes PTSD case status and adjusts the *p* value based on the correlational structure among the DNAm age residuals across the three brain regions (e.g., [24]). Significant results were further evaluated in separate models by including additional covariates in the model related to manner of death (death by suicide, death by substance use). Although we were focused on rs9315202 in interaction with PTSD, we wondered if other *KL* variants might also relate to DNAm age residuals or account for effects attributable to the candidate SNP. Therefore, we conducted follow-up regressions (parallel to the models described above) examining common variants in the gene ($n = 211$ variants beyond rs9315202) to determine if they showed significant interactions with PTSD on DNAm age residuals (Supplementary Materials).

To test Aim 2, we entered the main effects of several clinical variables including PTSD comorbidity (major depression and alcohol abuse/dependence diagnoses) and other lifestyle/behavioral factors (body mass index [BMI], cigarette use) that have previously been associated with epigenetic aging in blood [53, 54] in a regression in the full sample, as we did not have a *priori* age-related hypotheses regarding the main effects of psychopathology and lifestyle factors on DNAm age (age-specific hypotheses were only relevant to *KL* analyses and *KL* was not included in this model). We followed the same permutation procedure for multiple testing correction across the three regions as described above.

To test Aim 3, we obtained *KL* expression levels and examined the association between DNAm age residuals and *KL* expression, controlling for 7 cell types the RNA was extracted from, age at death, sex, RIN, PMI, and 3 ancestry PCs in the all-ancestry older (hypothesized) cohort. To be thorough, we also conducted the analysis in the full cohort and the younger cohort. We then conducted a follow-up mediation model (path analysis) in which we examined the indirect effects of rs9315202, PTSD, and their interaction on *KL* expression via DNAm age residuals in the older white non-Hispanic cohort.

Effects of rs9315202 on *KL* regulation

To test Aim 4 and determine the effects of rs9315202 on *KL* regulation, we cloned the rs9315202 containing region into the enhancer region of *KL* 4 kb promoter in firefly luciferase (FLuc) and NanoLuc luciferase (NLuc) coincidence reporter [55]. To do so, the 3.5 kb genomic DNA region flanking the rs9315202 variant was PCR amplified from the human kidney cell line HK-2 genomic DNA and cloned to replace the SV40 enhancer region of pNLCoI1-SV40-KL4000 using Clontech HiFi and In Fusion cloning kit according to the manufacturer's protocol using the following primers:

5′-AAATCGATAAGGATCCATGAGTGGTTGCTAGCTAAT-3′ (Enhancer forward)

5′-ATACGCAAACGGATCCTCTCTATCTGACCCATCCATCTTCC-3′ (Enhancer reverse)

5′-GGATCCGTTTGCGTATTGGGCGCTC-3′ (Vector forward)

5′-GGATCCTTATCGATTTTACCAC-3′ (Vector reverse).

The mutagenesis to produce rs9315202 was performed using Clontech HiFi and In Fusion cloning kit using the following primers:

5′-ATGATAATCTGTCTTACTAGGTAGAGAGGCAGTAAAAC-3′ (T variant forward)

5′-AAGACAGATTATCATTCCTTCCTCTGAATATATAGGATGGAGC-3′ (T variant reverse).

Transfection was accomplished via HEK and HK-2 cells grown on poly-D-lysine-coated plates in 96, 12, or 6-well formats. Twenty-four hours after plating, cells reached 70−80% confluency and were transfected with reporter plasmid or control plasmid. Transfections were carried out using Mirus TransIT-X2 with 100 ng, 1 µg, or 2 µg of total plasmid DNA per well in 96, 12, or 6-well plates, respectively. Transfection medium was removed and replaced with fresh medium after 5 h.

For measurement of FLuc and NLuc expression, the coincidence reporter vector under the KL promoter, Nano-Glo® Dual-Luciferase® Reporter Assay System (cat. N1620, Promega) was used according to manufacturer's instructions. The luminescence was measured using a plate reader (GloMax® Discover System, Promega).

RESULTS

PTSD brain bank: DNAm age estimates

The correlation between Horvath DNAm age estimates and age at death ranged from $r = 0.92$ to $r = 0.93$ ($ps < 0.001$) across the three brain regions. The estimates of DNAm age correlated with each other across brain regions also at $r = 0.92$ to 0.93 ($ps < 0.001$). The DNAm age residuals correlated with each other at $r = 0.50$ to 0.51 ($ps < 0.001$) across the three regions.

Aim 1: rs9315202 X PTSD as a predictor of DNAm age residuals

To test our hypothesis for Aim 1, we entered PTSD, rs9315202, and their interaction as terms in a regression predicting age residuals in each region in the older white non-Hispanic cohort. After controlling for covariates (Table 2), we found a corrected significant positive interaction between PTSD and rs9315202 on DNAm age residuals in motor cortex in the older cohort ($p = 0.002$, corrected $p = 0.014$; Table 2, Fig. 1). The positive association between PTSD status and DNAm age residuals was evident among those with the minor allele (A) but not those with the major allele (G). In follow-up analyses, we additionally controlled for manner of death covariates (death by suicide, death by substance use), and found that neither was associated with DNAm age residuals in motor cortex ($p = 0.15$ and 0.17, respectively), while the interaction term remained significant ($p = 0.005$). As expected, the effect of rs9315202 X PTSD on DNAm age residuals in the motor cortex was specific to the older subset: This effect was not observed in the younger cohort ($p_{motor} = 0.78$), nor was it evident in the combined all-ages sample ($p_{motor} = 0.38$, $p_{dlPFC} = 0.57$, $p_{vmPFC} = 0.56$; Table 2). We then examined additional common variants in *KL* ($n = 211$), and found that while several yielded similar or identical significance values, these were all in high or complete linkage disequilibrium (LD) with rs9315202 and none yielded meaningfully more significant interactions with PTSD (or main effects) in any region or cohort than the rs9315202 X PTSD interaction effect in the older cohort in the motor cortex (Fig. S1 and Supplementary Materials).

Aim 2: PTSD comorbidity as a predictor of DNAm age residuals

We next examined the main effects of PTSD comorbidity and behavioral factors in association with DNAm age residuals in the full sample in each region. Results revealed a nominally significant main effect for alcohol abuse/dependence diagnoses in association with DNAm age residuals in motor cortex ($p = 0.039$, corrected $p = 0.100$; Table S1; Fig. S2). Based on these results, we ran additional analyses in which we included the aforementioned potential time-of-death confounds in the model and found that none of the covariates were significantly associated with DNAm age residuals in this region (smallest $p = 0.102$) while alcohol abuse/dependence remained (nominally) significantly associated ($p = 0.029$). We also examined the same model separately in men and women in motor cortex because our main effects for psychopathology on DNAm age residuals in our previous studies were conducted in veteran samples that were nearly entirely male. Results suggested that the nominally significant effect for alcohol abuse/dependence in the full sample was likely driven by the men as alcohol abuse/dependence was nominally associated with DNAm age residuals among the men ($p = 0.021$; Table S2) but not the women ($p = 0.53$, Table S2). The effect was still significant ($p = 0.012$) when the aforementioned covariates were added to the male-only model.

Aim 3: advanced epigenetic age as a predictor of *KL* expression

We then examined the association between DNAm age residuals in motor cortex and *KL* expression in the same region (i.e., the region where we found an effect for PTSD X rs9315202 on DNAm age residuals), controlling for 7 cell types the RNA was extracted from, age, sex, RIN, PMI, and 3 ancestry PCs in the older cohort (including all subjects, regardless of race as genotype was not included in the model). We found that DNAm age residuals were associated with decreased *KL* expression in motor cortex in the older cohort ($\beta = -0.022$, std $\beta = -0.434$, $p = 0.032$, Table 3, Fig. S3). We next proceeded to test our planned mediation model to examine the indirect effect of PTSD, rs9315202, and their interaction on *KL* expression in motor cortex via DNAm age residuals in motor cortex in the subset of older, white non-Hispanic decedents. We found significant indirect effects of the interaction term on *KL* expression through DNAm age residuals (indirect $B = -0.116$, std indirect $\beta = -0.198$, $p = 0.027$; Fig. S4), controlling for 3 ancestry PCs, age, sex, RIN, and PMI.[1,2] In total, the model explained 37% of the variance in DNAm age residuals in motor cortex and 43% of the variance in *KL* expression in motor cortex.

The effect that we observed for DNAm age residuals as a predictor of *KL* expression in motor cortex in the older cohort was not evident when we ran the same regression model in the younger cohort ($\beta = -0.008$, std $\beta = -0.178$, $p = 0.265$, Table 3). The effect was significant in the combined-age sample ($\beta = -0.010$, std $\beta = -0.216$, $p = 0.042$, Table 3), but this was clearly driven by the older members of the cohort given that the effect was not significant in those below the median age.

Aim 4: molecular effects of rs9315202 on regulation of *KL* expression

The *KL* variant (rs9315202) is located 1734 bps downstream of the *KL* coding region (Fig. S5A). The region that contains the SNP of either wild type (WT, i.e. common allele) or rs9315202 mutant (i.e. minor allele) was cloned into a dual-luciferase enhancer reporter system to evaluate the effects of allelic variation at rs9315202 on *KL* gene regulation. In this system, Fluc and PEST-destabilized NlucP are expressed off the same *KL* 4 kb promoter using ribosome skipping mediated by the P2A peptide (Fig. S5B). We amplified 3.5 kb of genomic DNA sequence from HK-2 cells and cloned into the enhancer region of the FLuc and NLuc luciferase coincidence reporter containing the 4 kb or the 1.8 kb *KL* promoter

[1]Because this was a smaller subgroup analysis of older, white non-Hispanic decedents, we dropped the seven cell type variables from the path model so as not to overfit the model. None of the cell type variables were significantly associated with *KL* expression in motor cortex in the multiple regression models (Table 3).

[2]The path model fit the data well per standard fit indices: X^2 (1, $n = 43$) = 0.17, $p = 0.68$, root mean square error of approximation < 0.001, standardized root mean square residual = 0.012, confirmatory fit index = 1.00, Tucker-Lewis index = 2.057. The model was estimated with maximum likelihood estimation.

Table 2. Interactions between rs9315202 and PTSD on DNAm age residuals across three brain regions.

	Age ≥ 45 years ($n = 42$)						Age < 45 years ($n = 43$)						Combined Sample ($n = 85$)					
Variable	B	SE	Std β	*t*	*p*	*p*-corr	B	SE	Std β	*t*	*p*	*p*-corr	B	SE	Std β	*t*	*p*	*p*-corr
dlPFC																		
Step 1																		
PC1	9.741	4.641	0.343	2.099	0.044	NA	10.686	15.473	0.136	0.691	0.495	NA	9.727	3.877	0.273	2.509	0.014	NA
PC2	3.367	4.991	0.106	−0.675	0.505	NA	4.279	9.206	0.096	0.465	0.645	NA	3.527	3.860	0.099	0.914	0.364	NA
PC3	2.324	6.758	0.058	0.344	0.733	NA	4.332	6.712	0.135	0.645	0.523	NA	1.523	3.935	0.042	0.387	0.700	NA
Sex	1.363	1.444	0.162	0.944	0.352	NA	0.347	1.306	0.047	0.266	0.792	NA	0.999	0.914	0.127	1.093	0.278	NA
% Neurons	−1.180	4.696	−0.040	−0.251	0.803	NA	2.271	6.367	0.069	0.357	0.724	NA	0.746	3.338	0.025	0.223	0.824	NA
RIN	0.337	0.545	0.103	0.618	0.541	NA	0.270	0.545	0.087	0.495	0.624	NA	0.374	0.369	0.117	1.014	0.314	NA
PMI	−0.141	0.075	−0.315	−1.897	0.067	NA	0.019	0.076	0.045	0.252	0.802	NA	−0.060	0.048	−0.136	−1.241	0.218	NA
PTSD	1.600	1.450	0.185	1.104	0.278	NA	0.421	1.380	0.059	0.305	0.762	NA	0.848	0.892	0.108	0.951	0.345	NA
Step 2																		
rs9315202	−0.214	1.001	−0.036	−0.214	0.832	NA	−1.069	0.889	−0.209	−1.203	0.238	NA	−0.526	0.624	−0.094	−0.842	0.402	NA
Step 3																		
SNP × PTSD	1.695	2.017	0.233	0.841	0.407	.822	1.534	2.131	0.276	0.720	0.477	.804	0.722	1.251	0.113	0.577	0.565	.611
Motor																		
Step 1																		
PC1	3.684	4.105	0.147	0.941	0.353	NA	−17.727	17.792	−0.183	−0.996	0.326	NA	1.889	4.236	0.049	0.446	0.657	NA
PC2	−1.302	4.874	−0.044	−0.267	0.791	NA	22.915	10.366	0.418	2.211	0.034	NA	3.436	4.301	0.090	0.799	0.427	NA
PC3	5.916	6.228	0.160	0.950	0.349	NA	17.271	7.759	0.437	2.226	0.033	NA	4.516	4.321	0.117	1.045	0.299	NA
Sex	3.056	1.284	0.394	2.380	0.023	NA	−0.373	1.500	−0.041	−0.249	0.805	NA	1.318	0.996	0.156	1.324	0.190	NA
% Neurons	7.877	5.461	0.234	1.442	0.159	NA	8.402	7.537	0.186	1.115	0.273	NA	6.793	4.375	0.174	1.553	0.125	NA
RIN	0.434	0.500	0.143	0.869	0.391	NA	0.574	0.623	0.149	0.921	0.364	NA	0.481	0.402	0.140	1.197	0.235	NA
PMI	−0.119	0.070	−0.288	−1.706	0.097	NA	0.048	0.084	0.091	0.577	0.567	NA	−0.028	0.053	−0.059	−0.525	0.601	NA
PTSD	0.385	1.343	0.048	0.287	0.776	NA	−1.531	1.571	−0.175	−0.974	0.337	NA	−0.140	0.978	−0.017	−0.143	0.887	NA
Step 2																		
rs9315202	−1.081	0.912	−0.195	−1.186	0.245	NA	−1.041	1.021	−0.165	−1.020	0.315	NA	−0.880	0.683	−0.147	−1.289	0.201	NA
Step 3																		
SNP × PTSD	5.530	1.598	0.820	3.461	0.002	.014	0.676	2.420	0.098	0.279	0.782	.984	1.250	1.413	0.183	0.884	0.379	.426
vmPFC																		
Step 1																		
PC1	−0.885	4.219	−0.036	−0.210	0.835	NA	−3.106	17.687	−0.036	−0.176	0.862	NA	−1.761	3.871	−0.052	−0.455	0.650	NA
PC2	1.332	4.900	0.048	0.272	0.787	NA	−0.846	9.682	−0.018	−0.087	0.931	NA	0.431	3.853	0.013	0.112	0.911	NA
PC3	7.580	6.373	0.222	1.189	0.243	NA	5.993	7.401	0.171	0.810	0.424	NA	6.334	4.005	0.183	1.582	0.118	NA
Sex	0.031	1.384	0.004	0.023	0.982	NA	−0.723	1.425	−0.092	−0.507	0.615	NA	−0.221	0.927	−0.030	−0.239	0.812	NA
% Neurons	−4.841	4.040	−0.218	−1.198	0.239	NA	0.321	5.218	0.012	0.062	0.951	NA	−2.657	2.883	−0.109	−0.922	0.360	NA
RIN	0.113	0.516	0.040	0.220	0.827	NA	0.074	0.590	0.023	0.125	0.901	NA	0.101	0.365	0.033	0.278	0.782	NA
PMI	−0.019	0.071	−0.049	−0.268	0.790	NA	0.040	0.082	0.084	0.489	0.628	NA	0.014	0.050	0.033	0.282	0.779	NA
PTSD	0.563	1.380	0.075	0.408	0.686	NA	−0.381	1.449	−0.051	−0.263	0.794	NA	0.037	0.903	0.005	0.041	0.968	NA

Table 2. continued

	Age ≥ 45 years ($n = 42$)					Age < 45 years ($n = 43$)					Combined Sample ($n = 85$)				
Variable	B	SE	Std β	*t*	*p*	*p*-corr	B	SE	Std β	*t*	*p*	*p*-corr	B	SE	Std β	*t*	*p*	*p*-corr
Step 2																		
rs9315202	−1.787	0.898	−0.345	−1.990	0.055	NA	−0.206	0.976	−0.038	−0.211	0.834	NA	−0.812	0.623	−0.153	−1.303	0.196	NA
Step 3																		
SNP × PTSD	−1.262	1.757	−0.199	−0.718	0.478	.880	−0.229	2.124	−0.039	−0.108	0.915	.999	−0.727	1.237	−0.120	−0.588	0.559	.609

Sample was limited to the white non-Hispanic subjects due to concerns of population stratification and the PCs that were employed for these analyses were ancestry substructure PCs.
SE standard error, *std* standardized, *corr* corrected, *dlPFC* dorsolateral prefrontal cortex, *PC* principal component, *RIN* RNA integrity number, *PMI* postmortem interval, *PTSD* posttraumatic stress disorder, *SNP* single nucleotide polymorphism, *vmPFC* ventromedial prefrontal cortex, *NA* not applicable.

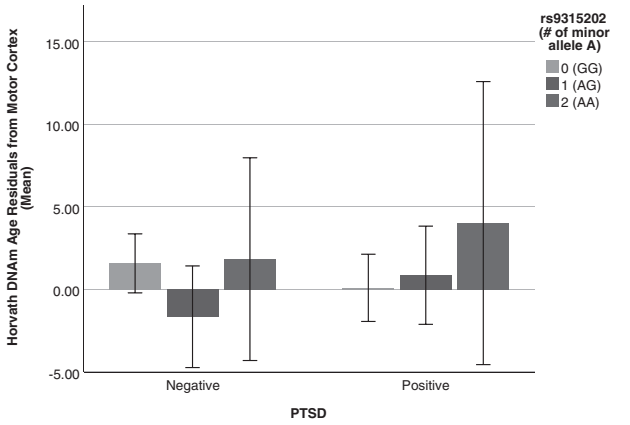

Fig. 1 PTSD is associated with advanced epigenetic age in motor cortex as a function of rs9315202. The figure shows mean (error bars represent +/−1.96 SE) DNAm age residuals in the motor cortex in the older cohort as a function of PTSD diagnosis and genotype at rs9315202.

(Fig. S5B). We examined the enhancer activities of rs9315202 minor allele and WT constructs by transfecting the dual-luciferase reporter into HK-2 cells that express *KL* endogenously. The results showed that single point C > T mutation (reverse strand as that reported for brain bank cohort) at the SNP position resulted in significant reduction in dual-luciferase reporter activities for both *KL* 4 and 1.8 K reporter systems (Fig. 2). These results demonstrate that the rs9315202 variant drives a change in *KL* transcriptional activities.

The 3.5 kb genomic DNA from HK-2 cells was then sequenced (Fig. S6). By searching ncRNA database in RNAcentral.org using the sequence flanking SNP rs9315202, we found an ncRNA with ID HSALNG0096254 that is 1729 bases in length (Fig. S6). We refer to this ncRNA as ncRNA1729 for its length.[3] These data indicate that the ncRNA1729 within the enhancer region alters *KL* gene expression, suggesting that variation at rs9315202 may affect the ncRNA function in *KL* gene expression.

DISCUSSION

The *KL* gene was named for the Greek goddess, Clotho, who was said to spin the web of life and thereby determine human lifespan. Our results underscore the apt naming of the gene, as Clotho transcends mythology to promote accelerated epigenetic aging in brain tissue. Specifically, we showed that the *KL* SNP rs9315202 moderated the association between PTSD and advanced epigenetic age in motor cortex, such that PTSD was associated with advanced DNAm age among those with the minor frequency allele (A) at this locus. The effect was evident only in relatively older decedents in the sample, consistent with our prior results in living subjects which found that the impact of rs9315202 on the association between PTSD symptom severity and advanced epigenetic age in blood was accentuated in older adults [27]. No other *KL* variant (out of an additional 211 imputed genotypes) evidenced a more significant association with DNAm age residuals (alone or in interaction with PTSD) in any brain region or cohort. Rather, variants in high LD with the candidate SNP showed equivalent patterns of interaction with PTSD. These follow-up analyses bolster our decision to focus on our candidate SNP in the older cohort.

[3]The brain bank RNA sequence data did not have coverage of this transcript thus we were unable to examine this ncRNA in the brain bank data directly.

Table 3. Effects of DNAm age residuals on KL expression in motor cortex.

	Age ≥ 45 years ($n = 39$)					Age < 45 years ($n = 53$)					Combined Sample ($n = 92$)				
Variable	B	SE	Std β	*t*	*p*	B	SE	Std β	*t*	*p*	B	SE	Std β	*t*	*p*
Astrocyte	0.031	0.118	0.067	0.264	0.794	−0.021	0.105	−0.046	−0.200	0.843	−0.029	0.070	−0.064	−0.416	0.678
Endothelial	0.179	0.263	0.266	0.679	0.504	0.141	0.143	0.242	0.983	0.332	0.144	0.106	0.234	1.362	0.177
Microglia	−0.009	0.123	−0.018	−0.074	0.942	−0.004	0.069	−0.010	−0.053	0.958	−0.020	0.050	−0.048	−0.403	0.688
Mural	−0.526	0.329	−0.641	−1.596	0.124	−0.299	0.154	−0.443	−1.947	0.059	−0.369	0.125	−0.505	−2.962	0.004
Neuron	−0.016	0.134	−0.038	−0.121	0.905	0.033	0.146	0.056	0.223	0.825	0.008	0.089	0.016	0.091	0.928
Oligodendrocyte	0.088	0.089	0.219	0.984	0.335	0.114	0.072	0.343	1.600	0.118	0.097	0.049	0.273	2.007	0.048
Red blood cell	−0.042	0.136	−0.064	−0.309	0.760	0.045	0.099	0.076	0.451	0.655	0.023	0.070	0.037	0.322	0.748
Age @ death	−0.004	0.008	−0.103	−0.532	0.600	−0.002	0.005	−0.075	−0.429	0.670	−0.001	0.002	−0.070	−0.618	0.539
Sex	0.132	0.079	0.308	1.660	0.110	0.010	0.070	0.025	0.143	0.887	0.055	0.044	0.133	1.257	0.213
RIN	−0.041	0.059	−0.168	−0.700	0.491	0.022	0.036	0.097	0.613	0.543	−0.003	0.026	−0.013	−0.121	0.904
PMI	−0.001	0.005	−0.045	−0.227	0.822	−0.002	0.005	−0.076	−0.431	0.669	−0.003	0.003	−0.125	−1.145	0.256
PC1	0.262	0.520	0.102	0.504	0.619	−0.342	0.334	−0.166	−1.023	0.313	−0.178	0.230	−0.079	−0.775	0.441
PC2	0.599	0.475	0.243	1.262	0.220	−0.169	0.366	−0.069	−0.460	0.648	0.105	0.249	0.043	0.423	0.673
PC3	−0.068	0.716	−0.017	−0.095	0.925	−0.070	0.379	−0.029	−0.186	0.854	−0.227	0.282	−0.081	−0.806	0.423
DNAm Age Res	−0.022	0.010	−0.434	−2.278	0.032	−0.008	0.007	−0.178	−1.132	0.265	−0.010	0.005	−0.216	−2.069	0.042

SE standard error, *std* standardized, *RIN* RNA integrity number, *PMI* postmortem interval, *PC* principal component, *DNAm* DNA methylation, *Res* residuals.

The interaction between rs9315202 and PTSD in these data was indirectly linked to reduced *KL* mRNA expression in motor cortex via advanced epigenetic age in this same region. This effect, too, was driven by older decedents in the sample. Reduced *KL* expression is thought to be a risk factor for weakened synaptic activity and cognitive decline (e.g., [9]), particularly at older ages where *KL* enhancement is needed to protect against age-related neurocognitive loss. Examination of the molecular effects of allelic variation at rs9315202 further supported our hypothesis that this SNP exerts regulatory effects on *KL* expression as the polymorphism was found to be associated with alterations in *KL* transcription via ncRNA. Specifically, the 3.5 kb downstream region of *KL* may act as an enhancer to regulate *KL* gene expression.

Long ncRNAs are significant in that they regulate gene expression without directly transcribing the gene. These transcripts are common throughout the genome and one of their major roles is to alter chromatin structure, which has downstream effects on gene expression [56]. This commonly occurs at DNAasel hypersensitivity sites, which are located at open (and therefore modifiable) ends of chromatin, known for their role in transcriptional binding and gene regulation [57]. Thus, rs9315202 may exert effects on *KL* expression in older, stressed populations through intermediate effects on chromatin structure modifications that promote accelerated aging. This is likely to be tissue-specific given that the effect of this SNP on *KL* expression in the brain bank data was indirect and moderated by PTSD. Additional molecular work, such as identifying the expression patterns and cell types of ncRNA1729 in brain tissue and using CRISPR gene editing technology to study the effect of rs9315202 on *KL* gene regulation via ncRNA1729 in cell lines, is needed to further characterize the effects of rs9315202 on ncRNA1729 function and on *KL* gene regulation.

In addition to replicating the results from our prior work in blood demonstrating a PTSD X rs9315202 interaction on advanced epigenetic age, we also replicated results from a distinct longitudinal study [24] in which we found effects for alcohol abuse/dependence on an accelerated pace of epigenetic aging over time [24]. In this study, there was a nominally significant association between alcohol abuse/dependence diagnoses and advanced epigenetic age in motor cortex. Other labs have also found evidence (albeit not consistently so) of associations between alcohol misuse and advanced epigenetic age in blood [33, 58, 59] and these results suggest that this effect may be parallel across blood and brain tissue.

All the effects we observed were only in motor cortex, which was not expected given prior evidence of associations between both *KL* [13, 14] and PTSD [60] with dlPFC. However, motor cortex shows substantial age-related changes, such as decreased cortical thickness [37, 61]. As well, *KL* has known effects on age-related motor phenotypes [2], including in a mouse study of Parkinson's disease [62] which found that KL augmentation ameliorated Parkinson's disease pathophysiology and improved performance on motor tasks in rodents. Similarly, enhancing KL expression in a mouse model of amyotrophic lateral sclerosis, a fatal disease defined by progressive and rapid skeletal muscle wasting and paralysis, resulted in delayed disease onset, slowed disease progression, longer lifespan, and reduced expression of a number of inflammatory genes in motor cortex via reduced microglial activation, both of which are thought to be major contributors to progression in the disease [63]. The combined results highlight the importance of examining multiple brain regions in postmortem evaluation of accelerated biological aging, as differences in the pace of epigenetic aging across regions may help to explain the inconsistent pattern of results in prior studies of psychiatric disorders and epigenetic age in postmortem brain tissue.

Study limitations and strengths

These results should be interpreted in light of several study limitations. First, though our sample size in the PTSD brain bank was among the largest to date to examine associations between psychiatric conditions and epigenetic age in postmortem tissue, the sample size was, overall, small and most of our effects were in yet smaller subsets of the sample (e.g., the older cohort), which can increase the risk of false-positive (and negative) results. We attempted to mitigate this concern by testing specific hypotheses in our analyses that were based on the results of prior studies. Similarly, given the difficulty in obtaining PTSD-associated postmortem brain tissue, we did not have access to a second cohort to test for replication. We were also limited in the brain regions available for analysis and, thus, we could not examine other regions that might be of particular interest to aging, PTSD, and *KL*, such as the hippocampus. Only left hemisphere tissue was

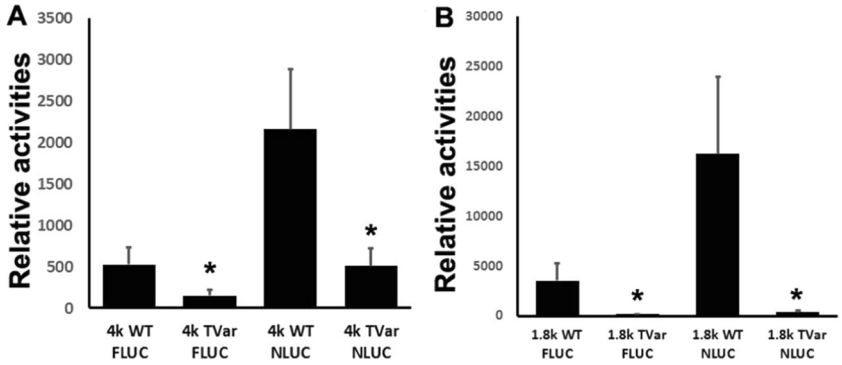

Fig. 2 Analysis of the effects of mutation on rs9315202 by a dual-luciferase reporter system in HK-2 cells. Firefly (FLUC), NLUC coincidence reporter system under control of either KL 4Kb (**a**) or 1.8 kb (**b**) promoter. The enhancer activities of WT and rs9315202 T variant (Tovar) constructs were analyzed by dual-luciferase assay.

obtained in the brain bank, thus we could not examine potential right-lateralized effects. We also did not conduct cloning experiments outside the rs9315202 region, thus it is possible that other genotypes are also critical for *KL* expression in the brain. These limitations are arguably off-set by the strengths of the study, including analysis of genotype, DNAm, and mRNA expression in a single study, evaluation of multiple psychiatric and behavioral factors thought to relate to cellular aging, consideration of potential confounds and covariates that might account for our primary associations of interest, and the examination of the molecular effects of rs9315202 on *KL* regulation.

CONCLUSIONS

Results further highlight the importance of *KL* and rs9315202 for the study of accelerated cellular aging in stressed populations. The SNP moderated the association between PTSD and advanced epigenetic age in those 45 years of age and older and indirectly predicted reduced *KL* mRNA expression in motor cortex via advanced epigenetic age, with experimental evidence suggesting that the SNP influenced *KL* regulation. KL augmentation holds promise as a therapeutic intervention to slow the pace of cellular aging and stem the tide of premature disease onset and neuropathology [55, 64, 65], especially in the populations at greatest risk by virtue of their advanced age and psychiatric history. The evidence that psychiatric stress accelerates aging and is associated with early onset of age-related disease is substantial. The time has come to extend beyond these observations to determine the pathophysiology of advanced cellular aging and to develop personalized therapeutics that will effectively lengthen Clotho's thread and enhance health and wellness.

FUNDING AND DISCLOSURES

This work was supported by the National Institute on Aging grant number 1R21AG061367 to EJW, and VA BLR&D Merit Award grant number 1I01BX003477 to MWL. This work was also supported by a Presidential Early Career Award for Scientists and Engineers (PECASE 2013A) to EJW, as administered by U.S. Department of Veterans Affairs Office of Research and Development and by the National Center for PTSD. FGM's contribution to this work was supported by National Institute of Mental Health award number 5T32MH019836-16. Genotype and methylation data were generated with the support of resources at the Pharmacogenomics Analysis Laboratory (Research Service, Central Arkansas Veterans Healthcare System, Little Rock, Arkansas), a core research laboratory funded by the Cooperative Studies Program, Research and Development, Department of Veterans Affairs. The contents of this manuscript do not represent the views of the U.S. Department of Veterans Affairs, the National Institutes of Health, or the United States Government.

All named authors report no financial or other conflicts of interest in relationship to the contents of this article. FGM contribution to this work was completed as a post-doctoral fellow at Boston University School of Medicine and the National Center for PTSD. Dr. Morrison is currently an employee of BlackThorn Therapeutics.

Traumatic Stress Brain Research Group member Ronald Duman has received consulting, speaking fees and/or grant support from Naurex, Taisho, Johnson & Johnson, Lilly, Lundbeck, Sunovion, Navitor, and Forest. Traumatic Stress Brain Research Group member John Crystal was a consultant for AstraZeneca Pharmaceuticals, Biogen, Idec, MA, Biomedisyn Corporation, Bionomics, Limited (Australia), Boehringer Ingelheim International, Concert Pharmaceuticals, Inc. Epiodyne, Inc., Heptares Therapeutics, Limited (UK), Janssen Research & Development, L.E.K. Consulting, Otsuka America Pharmaceutical, Inc., Perception Neuroscience Holdings, Inc., Spring Care, Inc., Sunovion Pharmaceuticals, Inc., Takeda Industries, and Taisho Pharmaceutical Co., Ltd. He is on the scientific advisory board of Bioasis Technologies, Inc., Biohaven Pharmaceuticals, BioXcel Therapeutics, Inc. (Clinical Advisory Board), Cadent Therapeutics (Clinical Advisory Board), Psycho-Genics, Inc., Stanley Center for Psychiatric research at the Broad Institute of MIT and Harvard, and Lohocla Research Corporation. He owns stock in ArRETT Neuroscience, Inc, Biohaven Pharmaceuticals, Sage Pharmaceuticals, and Spring Care, Inc. He has stock options in Biohaven Pharmaceuticals Medical Sciences, BlackThorn Therapeutics, Inc., Storm Biosciences, Inc. He is an editor for Biological Psychiatry, and has received research support from AstraZeneca Pharmaceuticals provides the drug, Saracatinib, for research related to NIAAA grant "Center for Translational Neuroscience of Alcoholism [CTNA-4]. He has the following patents: (1) Seibyl JP, Krystal JH, Charney DS. Dopamine and noradrenergic reuptake inhibitors in treatment of schizophrenia. US Patent #:5,447,948.September 5, 1995. (2) Vladimir, Coric, Krystal, John H, Sanacora, Gerard—Glutamate Modulating Agents in the Treatment of Mental Disorders US Patent No. 8,778,979 B2 Patent Issue Date: July 15, 2014. US Patent Application No. 15/695,164: Filing Date: 09/05/2017. (3) Charney D, Krystal JH, Manji H, Matthew S, Zarate C., - Intranasal Administration of Ketamine to Treat Depression United States Application No. 14/197,767 filed on March 5, 2014; United States application or Patent Cooperation Treaty (PCT) International application No. 14/306,382 filed on June 17, 2014. (4): Zarate, C, Charney, DS, Manji, HK, Mathew, Sanjay J, Krystal, JH, Department of Veterans Affairs "Methods for Treating Suicidal Ideation", Patent Application No. 14/197.767 filed on March 5, 2014 by Yale University Office of Cooperative Research. (5) Arias A, Petrakis I, Krystal JH.—Composition and methods to treat addiction. Provisional Use Patent Application no.61/973/961.

April 2, 2014. Filed by Yale University Office of Cooperative Research. (6): Chekroud, A., Gueorguieva, R., & Krystal, JH. "Treatment Selection for Major Depressive Disorder" [filing date 3rd June 2016, USPTO docket number Y0087.70116US00]. Provisional patent submission by Yale University. (7) Gihyun, Yoon, Petrakis I, Krystal JH—Compounds, Compositions and Methods for Treating or Preventing Depression and Other Diseases. U. S. Provisional Patent Application No. 62/444,552, filed on January 10, 2017 by Yale University Office of Cooperative Research OCR 7088 US01. (8) Abdallah, C, Krystal, JH, Duman, R, Sanacora, G. Combination Therapy for Treating or Preventing Depression or Other Mood Diseases. U.S. Provisional Patent Application No. 047162-7177P1 (00754) filed on August 20, 2018 by Yale University Office of Cooperative Research OCR 7451 US01. None of the authors or remaining members of the Traumatic Stress Brain Research Group have any conflicts to disclose. The authors have no conflict of interest.

ACKNOWLEDGEMENTS

The Traumatic Stress Brain Research Group are Matthew Friedman, M.D., Ph.D.—PTSD BB, Director, Neil Kowall, M.D., PI, VABBB Director, Christopher Brady, Ph.D., Co-I, VABBB Director of Scientific Operations, Ann McKee, M.D., Chief Neuropathologist, Thor Stein, M.D., Ph.D., Neuropathologist, BRH, M.D., Ph.D., Neuropathologist, Victor Alvarez, M.D., Neuropathologist, David Benedek, M.D., Director VA PTSD BB Assessment Core, Robert J. Ursano, MD, Director Center for the Study of Traumatic Stress, Department of Psychiatry, Uniformed Services University, Douglas Williamson, PhD, Site Director, Dianne Cruz, M.S., Co-investigator, Keith Young, PhD, Senior Advisor, Ronald Duman, PhD, Director VA PTSD BB Intramural Research Program, John Krystal, MD, Chair Department of Psychiatry, Deborah Mash, MD, Director, Brain Bank, Melanie Hardegree, RN, Co-Investigator, William Scott, Ph.D. Executive Director of the UM Brain Endowment Bank, David Davis Ph.D UM Brain Endowment Bank, Matthew Girgenti Ph.D., Co-Investigator., Brian Marx, PhD, Deputy Director, National Center for PTSD, Behavioral Science Division and Professor of Psychiatry, Boston University School of Medicine and Paul Holtzheimer, MD, Deputy Director for Research, National Center for PTSD and Associate Professor of Psychiatry and Surgery, Geisel School of Medicine at Dartmouth.

AUTHOR CONTRIBUTIONS

EJF, CDC, MWL, FGM, CRA, and MWM provided substantial contributions to the conception and design of the work; and the acquisition, analysis, and interpretation of data for the work. XZ, ZZ, NPD, AS, SS, JGG, DFS provided substantial contributions to the analysis and interpretation of data for the work. BRH and the Traumatic Stress Brain Research Group provided substantial contributions to the conception of the work and the acquisition and interpretation of data for the work. EJF, CDC, MWL, CRA, ZZ, and MWM drafted the work and revised it critically for important intellectual content. FGM, XZ, NPD, AS, SS, JGG, DFS, BRH, and the Traumatic Stress Brain Research group critically revised the work for important intellectual content. All authors provided final approval of the version submitted for publication and all authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ADDITIONAL INFORMATION

Supplementary Information accompanies this paper at (https://doi.org/10.1038/s41386-020-00884-5).

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Arking DE, Krebsova A, Macek M, Macek M, Arking A, Mian IS, et al. Association of human aging with a functional variant of klotho. Proc Natl Acad Sci. 2002;99:856–61.
2. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature. 1997;390:45–51.
3. Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, et al. Suppression of aging in mice by the hormone klotho. Science. 2005;309:1829–33.
4. Semba RD, Moghekar AR, Hu J, Sun K, Turner R, Ferrucci L, et al. Klotho in the cerebrospinal fluid of adults with and without Alzheimer's disease. Neurosci Lett. 2014;558:37–40.
5. Erickson CM, Schultz SA, Oh JM, Darst BF, Ma Y, Norton D, et al. KLOTHO heterozygosity attenuates APOE4-related amyloid burden in preclinical AD. Neurology. 2019;92:e1878.
6. Kuang X, Chen Y-S, Wang L-F, Li Y-J, Liu K, Zhang M-X, et al. Klotho upregulation contributes to the neuroprotection of ligustilide in an Alzheimer's disease mouse model. Neurobiol Aging. 2014;35:169–78.
7. Zeng C-Y, Yang T-T, Zhou H-J, Zhao Y, Kuang X, Duan W, et al. Lentiviral vector–mediated overexpression of Klotho in the brain improves Alzheimer's disease–like pathology and cognitive deficits in mice. Neurobiol Aging. 2019;78:18–28.
8. Dubal DB, Yokoyama JS, Zhu L, Broestl L, Worden K, Wang D, et al. Life extension factor klotho enhances cognition. Cell Rep. 2014;7:1065–76.
9. Dubal DB, Zhu L, Sanchez PE, Worden K, Broestl L, Johnson E, et al. Life extension factor klotho prevents mortality and enhances cognition in hAPP transgenic mice. J Neurosci. 2015;35:2358–71.
10. Massó A, Sánchez A, Bosch A, Giménez-Llort L, Chillón M. Secreted αKlotho isoform protects against age-dependent memory deficits. Mol Psychiatry. 2018;23:1937–47.
11. Leon J, Moreno AJ, Garay BI, Chalkley RJ, Burlingame AL, Wang D, et al. Peripheral elevation of a klotho fragment enhances brain function and resilience in young, aging, and α-synuclein transgenic mice. Cell Rep. 2017;20:1360–71.
12. Shardell M, Semba RD, Rosano C, Kalyani RR, Bandinelli S, Chia CW, et al. Plasma klotho and cognitive decline in older adults: findings from the InCHIANTI study. J Gerontol Ser A. 2016;71:677–82.
13. Yokoyama JS, Sturm VE, Bonham LW, Klein E, Arfanakis K, Yu L, et al. Variation in longevity gene KLOTHO is associated with greater cortical volumes. Ann Clin Transl Neurol. 2015;2:215–30.
14. Yokoyama JS, Marx G, Brown JA, Bonham LW, Wang D, Coppola G, et al. Systemic klotho is associated with KLOTHO variation and predicts intrinsic cortical connectivity in healthy human aging. Brain Imaging Behav. 2017;11:391–400.
15. Laszczyk AM, Fox-Quick S, Vo HT, Nettles D, Pugh PC, Overstreet-Wadiche L, et al. Klotho regulates postnatal neurogenesis and protects against age-related spatial memory loss. Neurobiol Aging. 2017;59:41–54.
16. Chen C-D, Sloane JA, Li H, Aytan N, Giannaris EL, Zeldich E, et al. The antiaging protein klotho enhances oligodendrocyte maturation and myelination of the CNS. J Neurosci. 2013;33:1927–39.
17. Zeldich E, Chen C-D, Avila R, Medicetty S, Abraham CR. The anti-aging protein klotho enhances remyelination following cuprizone-induced demyelination. J Mol Neurosci. 2015;57:185–96.
18. Mengel-From J, Soerensen M, Nygaard M, McGue M, Christensen K, Christiansen L. Genetic variants in KLOTHO associate with cognitive function in the oldest old group. J Gerontol Ser A. 2016;71:1151–9.
19. Porter T, Burnham SC, Milicic L, Savage G, Maruff P, Lim YY, et al. Klotho allele status is not associated with Aβ and APOE ε4–related cognitive decline in preclinical Alzheimer's disease. Neurobiol Aging. 2019;76:162–5.
20. Jovanovic T, Vance LA, Cross D, Knight AK, Kilaru V, Michopoulos V, et al. Exposure to violence accelerates epigenetic aging in children. Sci Rep. 2017;7:1–7.
21. Wolf EJ, Logue MW, Hayes JP, Sadeh N, Schichman SA, Stone A, et al. Accelerated DNA methylation age: associations with PTSD and neural integrity. Psychoneuroendocrinology. 2016;63:155–62.
22. Wolf EJ, Maniates H, Nugent N, Maihofer AX, Armstrong D, Ratanatharathorn A, et al. Traumatic Stress and Accelerated DNA Methylation Age: A Meta-Analysis. Psychoneuroendocrinology. 2018;92:123–34.
23. Wolf EJ, Logue MW, Stoop TB, Schichman SA, Stone A, Sadeh N, et al. Accelerated DNA methylation age: associations with posttraumatic stress disorder and mortality. Psychosom Med. 2018;80:42–48.
24. Wolf EJ, Logue MW, Morrison FG, Wilcox ES, Stone A, Schichman SA, et al. Posttraumatic psychopathology and the pace of the epigenetic clock: a longitudinal investigation. Psychol Med. 2019;49:791–800.
25. Verhoeven JE, Yang R, Wolkowitz OM, Bersani FS, Lindqvist D, Mellon SH, et al. Epigenetic age in male combat-exposed war veterans: associations with posttraumatic stress disorder status. Mol Neuropsychiatry. 2018;4:90–9.
26. Zannas AS, Arloth J, Carrillo-Roa T, Iurato S, Röh S, Ressler KJ, et al. Lifetime stress accelerates epigenetic aging in an urban, African American cohort: relevance of glucocorticoid signaling. Genome Biol. 2015;16:266.
27. Wolf EJ, Morrison FG, Sullivan DR, Logue MW, Guetta RE, Stone A, et al. The goddess who spins the thread of life: Klotho, psychiatric stress, and accelerated aging. Brain Behav Immun. 2019;80:193–203.
28. Han LKM, Aghajani M, Clark SL, Chan RF, Hattab MW, Shabalin AA, et al. Epigenetic aging in major depressive disorder. Am J Psychiatry. 2018;175:774–82.
29. Li Z, He Y, Ma X, Chen X. Epigenetic age analysis of brain in major depressive disorder. Psychiatry Res. 2018. 2018;269:621–4.

30. Fries GR, Bauer IE, Scaini G, Valvassori SS, Walss-Bass C, Soares JC, et al. Accelerated hippocampal biological aging in bipolar disorder. Bipolar Disord. 2019;00:1–10.
31. Voisey J, Lawford BR, Morris CP, Wockner LF, Noble EP, Young RM, et al. Epigenetic analysis confirms no accelerated brain aging in schizophrenia. Npj Schizophr. 2017;3:1–3.
32. McKinney BC, Lin H, Ding Y, Lewis DA, Sweet RA. DNA methylation evidence against the accelerated aging hypothesis of schizophrenia. Npj Schizophr. 2017;3:1–3.
33. Rosen AD, Robertson KD, Hlady RA, Muench C, Lee J, Philibert R, et al. DNA methylation age is accelerated in alcohol dependence. Transl Psychiatry. 2018;8:1–8.
34. Kozlenkov A, Jaffe AE, Timashpolsky A, Apontes P, Rudchenko S, Barbu M, et al. DNA methylation profiling of human prefrontal cortex neurons in heroin users shows significant difference between genomic contexts of hyper- and hypomethylation and a younger epigenetic age. Genes. 2017;8:152.
35. Fonzo GA, Goodkind MS, Oathes DJ, Zaiko YV, Harvey M, Peng KK, et al. PTSD psychotherapy outcome predicted by brain activation during emotional reactivity and regulation. Am J Psychiatry. 2017;174:1163–74.
36. Hayes JP, Hayes SM, Mikedis AM. Quantitative meta-analysis of neural activity in posttraumatic stress disorder. Biol Mood Anxiety Disord. 2012;2:9.
37. Salat DH, Buckner RL, Snyder AZ, Greve DN, Desikan RSR, Busa E, et al. Thinning of the cerebral cortex in aging. Cereb Cortex. 2004;14:721–30.
38. Salat DH, Tuch DS, Greve DN, van der Kouwe AJW, Hevelone ND, Zaleta AK, et al. Age-related alterations in white matter microstructure measured by diffusion tensor imaging. Neurobiol Aging. 2005;26:1215–27.
39. Anamizu Y, Kawaguchi H, Seichi A, Yamaguchi S, Kawakami E, Kanda N, et al. Klotho insufficiency causes decrease of ribosomal RNA gene transcription activity, cytoplasmic RNA and rough ER in the spinal anterior horn cells. Acta Neuropathol (Berl). 2005;109:457–66.
40. Shiozaki M, Yoshimura K, Shibata M, Koike M, Matsuura N, Uchiyama Y, et al. Morphological and biochemical signs of age-related neurodegenerative changes in klotho mutant mice. Neuroscience. 2008;152:924–41.
41. Miller MW, Greif JL, Smith AA. Multidimensional personality questionnaire profiles of veterans with traumatic combat exposure: Externalizing and internalizing subtypes. Psychol Assess. 2003;15:205–15.
42. Friedman MJ, Huber BR, Brady CB, Ursano RJ, Benedek DM, Kowall NW, et al. VA's national PTSD brain bank: a national resource for research. Curr Psychiatry Rep. 2017;19:73.
43. Mighdoll MI, Deep-Soboslay A, Bharadwaj RA, Cotoia JA, Benedek DM, Hyde TM, et al. Implementation and clinical characteristics of a posttraumatic stress disorder brain collection. J Neurosci Res. 2018;96:16–20.
44. Morrison FG, Miller MW, Wolf EJ, Logue MW, Maniates H, Kwasnik D, et al. Reduced interleukin 1A gene expression in the dorsolateral prefrontal cortex of individuals with PTSD and depression. Neurosci Lett. 2019; 692:204–9.
45. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14:3156.
46. Logue MW, Smith AK, Wolf EJ, Maniates H, Stone A, Schichman SA, et al. The correlation of methylation levels measured using Illumina 450K and EPIC BeadChips in blood samples. Epigenomics. 2017;9:1363–71.
47. Guintivano J, Aryee MJ, Kaminsky ZA. A cell epigenotype specific model for the correction of brain cellular heterogeneity bias and its application to age, brain region and major depression. Epigenetics. 2013;8:290–302.
48. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20.
49. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2013;29:15–21.
50. Bray NL, Pimentel H, Melsted P, Pachter L. Near-optimal probabilistic RNA-seq quantification. Nat Biotechnol. 2016;34:525–7.
51. Bray NL, Pimentel H, Melsted P, Pachter L. Erratum: near-optimal probabilistic RNA-seq quantification. Nat Biotechnol. 2016;34:888.
52. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550.
53. Quach A, Levine ME, Tanaka T, Lu AT, Chen BH, Ferrucci L, et al. Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. Aging. 2017;9:419–37.
54. Gao X, Zhang Y, Breitling LP, Brenner H. Relationship of tobacco smoking and smoking-related DNA methylation with epigenetic age acceleration. Oncotarget. 2016;7:46878–89.
55. Chen C-D, Zeldich E, Li Y, Yuste A, Abraham CR. Activation of the anti-aging and cognition-enhancing gene klotho by CRISPR-dCas9 transcriptional effector complex. J Mol Neurosci. 2018;64:175–84.
56. Mercer TR, Mattick JS. Structure and function of long noncoding RNAs in epigenetic regulation. Nat Struct Mol Biol. 2013;20:300–7.
57. Thurman RE, Rynes E, Humbert R, Vierstra J, Maurano MT, Haugen E, et al. The accessible chromatin landscape of the human genome. Nature. 2012;489:75–82.
58. Fiorito G, McCrory C, Robinson O, Carmeli C, Rosales CO, Zhang Y, et al. Socioeconomic position, lifestyle habits and biomarkers of epigenetic aging: a multicohort analysis. Aging. 2019;11:2045–70.
59. Luo A, Jung J, Longley M, Rosoff DB, Charlet K, Muench C, et al. Epigenetic aging is accelerated in alcohol use disorder and regulated by genetic variation in APOL2. Neuropsychopharmacology. 2020;45:327–36.
60. Miller MW, Wolf EJ, Sadeh N, Logue M, Spielberg JM, Hayes JP, et al. A novel locus in the oxidative stress-related gene ALOX12 moderates the association between PTSD and thickness of the prefrontal cortex. Psychoneuroendocrinology. 2015;62:359–65.
61. Ziegler DA, Piguet O, Salat DH, Prince K, Connally E, Corkin S. Cognition in healthy aging is related to regional white matter integrity, but not cortical thickness. Neurobiol Aging. 2010;31:1912–26.
62. Baluchnejadmojarad T, Eftekhari S-M, Jamali-Raeufy N, Haghani S, Zeinali H, Roghani M. The anti-aging protein klotho alleviates injury of nigrostriatal dopaminergic pathway in 6-hydroxydopamine rat model of Parkinson's disease: involvement of PKA/CaMKII/CREB signaling. Exp Gerontol. 2017;100:70–6.
63. Zeldich E, Chen C-D, Boden E, Howat B, Nasse JS, Zeldich D, et al. Klotho is neuroprotective in the superoxide dismutase (SOD1G93A) mouse model of ALS. J Mol Neurosci. 2019;69:264–85.
64. Abraham CR, Chen C, Cuny GD, Glicksman MA, Zeldich E. Small-molecule Klotho enhancers as novel treatment of neurodegeneration. Future Med Chem. 2012;4:1671–79.
65. Abraham CR, Mullen PC, Tucker-Zhou T, Chen CD, Zeldich E. Chapter Nine—Klotho is a neuroprotective and cognition-enhancing protein. In: Litwack G (ed) Vitamins & Hormones. Academic Press; 2016. pp. 215–238.