Lifetime Stressful Events Associated with Alzheimer's Pathologies, Neuroinflammation and Brain Structure in a Risk Enriched Cohort

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Objective: Along with the known effects of stress on brain structure and inflammatory processes, increasing evidence suggest a role of chronic stress in the pathogenesis of Alzheimer's disease (AD). We investigated the association of accumulated stressful life events (SLEs) with AD pathologies, neuroinflammation, and gray matter (GM) volume among cognitively unimpaired (CU) individuals at heightened risk of AD.
Methods: This cross-sectional cohort study included 1,290 CU participants (aged 48–77) from the ALFA cohort with SLE, lumbar puncture (n = 393), and/or structural magnetic resonance imaging (n = 1,234) assessments. Using multiple regression analyses, we examined the associations of total SLEs with cerebrospinal fluid (1) phosphorylated (p)-tau$_{181}$ and Aβ_{1-42}/$_{1-40}$ ratio, (2) interleukin 6 (IL-6), and (3) GM volumes voxel-wise. Further, we performed stratified and interaction analyses with sex, history of psychiatric disease, and evaluated SLEs during specific life periods.
Results: Within the whole sample, only childhood and midlife SLEs, but not total SLEs, were associated with AD pathophysiology and neuroinflammation. Among those with a history of psychiatric disease SLEs were associated with higher p-tau$_{181}$ and IL-6. Participants with history of psychiatric disease and men, showed lower A$\beta_{1-42/1-40}$ with higher SLEs. Participants with history of psychiatric disease and women showed reduced GM volumes in somatic regions and prefrontal and limbic regions, respectively.

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.26881

Received Aug 9, 2023, and in revised form Jan 22, 2024. Accepted for publication Jan 27, 2024.

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Interpretation: We did not find evidence supporting the association of total SLEs with AD, neuroinflammation, and atrophy pathways. Instead, the associations appear to be contingent on events occurring during early and midlife, sex and history of psychiatric disease.

ANN NEUROL 2024;95:1058–1068

Introduction

Currently, dementia affects around 50 million people worldwide and this number is projected to triple by the year 2050.[1] The most common cause of dementia, Alzheimer's disease (AD), is characterized by the accumulation of β-amyloid (Aβ) and phosphorylated-tau (p-tau) proteins in the brain, neurodegeneration,[2] progressive cognitive decline,[3] and neuroinflammation.[4]

Various psychological risk factors have been identified for AD dementia, including depression,[5] anxiety,[6] and psychological chronic stress.[7] These risk factors may act through shared psychological processes and biological mechanisms[8] that could be the target of early interventions. While studies are starting to link neuropsychiatric symptoms and early AD-related changes,[9,10] less research has placed attention on stressful life events (SLEs) in relation to AD biomarkers.

Definitions of stress vary from objective external threats (eg, SLEs), to individuals' subjective appraisal of the threat, and to the physiological systems that get activated in response to the threats.[11] These definitions have been suggested to represent different stages in a model where SLEs viewed as threatening, activate behavioral and physiological responses that may have possible downstream implications for various diseases.[11] Indeed, the majority of studies, but not all,[12] suggest that experiencing stress/SLEs is associated with an increased risk of dementia[13,14] and cognitive decline, notably among specific populations, such as those with depressive symptoms.[15,16]

Exposure to SLEs could influence AD risk through various mechanisms. First, events that are appraised as threats, will elicit affective responses (eg, anxiety) and alter the functioning of the hypothalamic–pituitary–adrenal (HPA) axis.[11] The immune system[17] gets activated and may promote the synthesis of pro-inflammatory cytokines, such as interleukin 6 (IL-6), which are elevated among patients with AD[18] and have been linked with cognitive decline and AD metabolic alterations.[19] Second, stress may lower resistance to AD pathologies, as higher Aβ burden and p-tau levels have been associated with chronic stress in animal models.[20,21] Finally, as the HPA axis gets activated, it releases glucocorticoids, which may accelerate clinical AD progression[22] and cause structural brain damage.[23,24]

Indeed, there is growing evidence suggesting that exposure to SLEs is cross-sectionally and longitudinally associated with lower volumes in the anterior cingulate, hippocampus, amygdala, and prefrontal regions,[25,26] although larger amygdala volumes have also been reported.[27] However, studies examining SLEs and/or stress in the context of AD pathologies in humans are lacking. The little available evidence shows that midlife stress in women is associated with unspecified neurodegenerative, but not AD processes[28] and that higher perceived stress among African Americans with mild cognitive impairment is associated with higher p-tau.[29]

The general goal of this study was to identify whether SLEs across the life course are associated with AD pathophysiology and brain structure among cognitively unimpaired (CU) individuals at an increased risk of AD dementia. Building upon previous evidence, we examined (1) associations between lifetime SLEs and AD biomarker, neuroinflammation, and brain structure pathways and (2) interaction with and stratification by history of psychiatric disease and sex to test specific vulnerability to SLEs in these groups. As exploratory analyses, we further investigated (3) the associations with SLEs within specific age periods. We hypothesized that lifetime SLEs will be associated with higher Aβ, p-tau, and neuroinflammation and with lower gray matter volume in limbic and frontal areas. We further expected to see stronger effects among participants with a history of psychiatric disease, women and for SLEs experienced in childhood, as early life may be a particularly vulnerable period due to the rapid changes during brain maturation.[23]

Methods

Study Population

The ALFA (for ALzheimer's and FAmilies) is an ongoing longitudinal cohort study. The ALFA parent cohort consists of 2,743 CU participants at an increased risk of AD (86% have at least one parent diagnosed with AD[30]) enrolled between 2013 and 2014. The assessments include clinical interviews and questionnaires related to lifestyle and health, cognitive tests, blood tests for genotyping, lumbar puncture, and magnetic resonance imaging (MRI). Participants were excluded if they reported an active psychiatric disease at baseline, and if an interdisciplinary committee of physicians and a neuropsychologist evaluated the disease likely to interfere with their cognitive abilities (Supplementary Fig S1). Participants with a major psychiatric active disorder (according to DSM-IV-TR), severe auditory and/or visual disorder, neurodevelopmental and/or psychomotor disorder, or a disorder that could

affect cognition at the time of recruitment, were excluded. Further, participants with neurological disorders or brain injuries that could affect cognition, or with a suspected pattern of family history of autosomal dominant Alzheimer's disease, were also excluded. Details of the ALFA project have been previously described in detail elsewhere.[30]

The ALFA study (ALFA-FPM-0311) has been approved by the Independent Ethics Committee "Parc de Salut Mar," Barcelona, and registered at Clinicaltrials.gov (identifier: NCT02485730). Participants provided informed consent at baseline with a form approved by the Independent Ethics Committee "Parc de Salut Mar," Barcelona. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

Cerebrospinal Fluid Measurements

Phosphorylated-tau$_{181}$ (p-tau$_{181}$) and A$\beta_{1–42}$/$_{1–40}$ ratio were chosen to reflect the core AD processes. Based on previous literature,[19] IL-6 was chosen to reflect neuroinflammation.

Lumbar puncture was performed between years 2016 and 2019. Cerebrospinal fluid (CSF) p-tau was measured using the Elecsys® Phospho-tau (181P) CSF electrochemiluminescence immunoassay on a fully automated cobas® e 601 instrument (both Roche Diagnostics International Ltd, Rotkreuz, Switzerland). A$\beta_{1–42}$/$_{1–40}$ ratio and IL-6 were measured with the NeuroToolKit, a panel of robust prototype assays (Roche Diagnostics International Ltd), on a cobas e 411 or e 601 instrument. All CSF measurements were performed at the Clinical Neurochemistry Laboratory in Sahlgrenska University Hospital, Sweden.

Due to non-normality of the residuals in the simple models of A$\beta_{1–42}$/$_{1–40}$ ratio and IL-6 with SLEs, these two variables were log$_{10}$-transformed.

MRI Acquisition and Processing

MRI scanning was performed between 2016 and 2019. The T1-weighted 3D turbo field echo sequence was acquired in a Philips (Best, the Netherlands) 3 T Ingenia CX scanner with a voxel size of 0.75 × 0.75 × 0.75 mm^3, field of view of 240 × 240 × 180 mm^3, sagittal acquisition, flip angle of 8°, repetition time of 9.9 milliseconds, echo time of 4.6 milliseconds, and inversion time of 900 milliseconds. The VBM pre-processing was carried out using Statistical Parametric Mapping (SPM12) software running on MATLAB R2010a. Briefly, after segmentation into gray matter, white matter, and CSF, gray matter maps were normalized using Dartel, then, the images were smoothed by convolving with an isotropic Gaussian kernel of 8 mm full width half maximum. A mean normalized group-level gray matter mask was generated from individual segmented masks and thresholded at gray matter probability >0.3. Cerebellar and vermian areas were removed from the mask.

Assessment of SLEs

Exposure to SLEs was measured through semi-structured interviews containing 18 different pre-defined events adopted from the SNAC-K study,[27] based on Miller and Rahe's list of life change events[31,32] (Supplementary Table S1 and S2). These events have been defined as likely to bring about readjustment-requiring changes in individuals' lives.[32] Some participants went through the SLE interview twice and for these participants their latest assessment was considered. Participants reported whether each event had happened at any point during their lifetime, how many times and the age(s) at which the events occurred. Each event was counted as a single event and events were summed to create a continuous score. For further exploratory analyses, age period SLE variables were calculated by counting the reported ages during which events were reported creating a score per each age period. There was a 6% mismatch between the total SLE scores versus the age period scores, reflecting missing data within one of the two scores.

Semi-structured SLE interviews reduce intra-category variability and have better test–retest reliability than traditional SLE checklists.[33,34] Further, in the current study participants with a current/active psychiatric diagnosis were excluded, which reduces the likelihood of recall bias. We ran test–retest correlations to assess the reliability of our measurement in participants with 2 assessments. The SLE measurements showed a high test–retest correlation within the whole CSF ($r = 0.93$, $p < 0.001$) and brain structure sample ($r = 0.93$, $p = 0.001$; mean time in between assessments = 4.0 and 3.9 years, respectively). The test–retest reliability was similarly high for those with history of psychiatric disease—for whom recall bias could be a concern—in both CSF ($r = 0.94$, $p < 0.001$) and brain structure sample ($r = 0.92$, $p < 0.001$; Supplementary Table S3).

Covariates

All main models/analyses were adjusted for age, sex, years of education in a first step, and additionally for *apolipoprotein E* (*APOE*) ε4 carrier status, categorized as carriers or non-carriers; and self-reported lifetime history of cardiovascular and psychiatric disease (Supplementary Table S4), both categorized as yes or no in a second step. Participants reported their sex/gender using a binary label (woman/man) and therefore we refer to women/men throughout the manuscript.

Statistical Analyses

Characteristics of the sample were summarized as mean and SD for continuous variables and as frequencies and percentages for categorical variables. We first performed

multivariable regression analyses to examine the relationship of the total number of SLEs with Aβ, p-tau, and IL6. Next, in fully adjusted models, we examined the interaction of SLEs with sex and history of psychiatric disease and performed stratified analyses.

In exploratory analyses, to examine the association of SLEs experienced during specific age periods with AD pathophysiology and brain structure, we performed fully adjusted models that were further adjusted for other SLE age periods. For that, we computed the following variables based on previous literature[23,27]: childhood events (at or before the age of 12); adolescence events (ages 13–18); early adulthood events (ages 19–39); middle-adulthood events (ages 40–64); and late-life events (at or after the age of 65; Supplementary Table S1 and S2). Due to skewness, we categorized each period's events into "low level" and "high level of SLEs" based on the median number of events within each period (Supplementary Table S5).

The p-values were 2-sided with statistical significance set at <0.05. All analyses were pre-specified and performed with Stata SE, version 17.0 (StataCorp LLC). Given that all analyses were pre-specified, the hypotheses based on previous literature, and we expected low-to-moderate effects of SLEs in a preclinical sample, we did not use correction for multiple comparison methods as they are too conservative and increase type II error.[35]

Voxel-Wise Statistical Analyses

We used multiple regression in SPM to test for the association of SLEs during the whole life course and during specific age periods with brain structure, including SLEs as a main variable, adjusting for total intracranial volume, age, gender, years of education, *APOE* ε4 status, history of cardiovascular disease, and history of psychiatric disease. We considered significant results surviving an uncorrected p-value of 0.005 at voxel level and a cluster-level $p < 0.05$ family-wise error (FWE) correction. When no significant cluster survived FWE correction, we explored results at an uncorrected voxel-level, p-value of 0.005 and k > 600.

Results

In the current study, brain structure analyses were restricted to participants with data available on SLEs evaluations, brain imaging and across all other variables, resulting in 1234 participants. The AD pathophysiology analyses were restricted to participants with data available on CSF measurements and across all other variables, resulting in 393 participants (overlap of 337 participants). Characteristics of the participants included in the present study are showed in Table 1. The differences in participant characteristics between the ALFA parent cohort (1,743) and current study's participants are shown in Supplementary Table S6. Women and those with a history of psychiatric disease reported more SLEs (Supplementary Tables S7 and S8). A higher number of SLEs was associated with being older at lumbar puncture and MRI (both $p < 0.001$), and with having a history of psychiatric disease in the CSF ($p = 0.003$) and brain structure sample ($p < 0.001$). Both age and history of psychiatric disease were controlled for in the main statistical analyses. Adjusting for all covariates and all SLEs, history of psychiatric disease was not associated with any of the CSF measurements, or with brain structure in voxel-wise analyses at uncorrected 0.005 at voxel level (Supplementary Table S9).

Associations of SLES with AD Pathophysiology

Total SLEs were not associated with $A\beta_{1-42}/_{1-40}$ ratio, p-tau_{181}, or IL-6 (Table 2). Stratified analyses revealed that a higher number of SLEs was associated with lower levels of $A\beta_{1-42}/_{1-40}$ ratio and higher levels of p-tau and IL-6 among those with a history of psychiatric disease (Table 3), and with lower levels of $A\beta_{1-42}/_{1-40}$ ratio among men (Table 4).

There was evidence of an interaction between the total number of events and history of psychiatric disease on $A\beta_{1-42}/_{1-40}$ ratio ($p = 0.027$; Supplementary Table S10 and Supplementary Figure S2) supporting the results of the stratified analyses, while no interaction was observed for sex.

Voxel-Wise Associations of SLEs with Brain Structure

Structural associations with SLEs are shown in Figure. Within the whole sample, there were no clusters surviving FWE correction. However, SLEs were associated with reduced gray matter volume mainly in the left pre and postcentral gyrus; right superior and middle temporal lobe; right amygdala and parahippocampal gyrus; and left amygdala extending to the putamen and the insular cortex at an uncorrected $p = 0.005$ level.

Among women, SLEs were associated with reduced gray matter volume bilaterally in the anterior and posterior cingulate and paracingulate gyri; frontal medial cortices; orbitofrontal cortex; and temporal poles and accumbens; and in the the left hemisphere with reduced amygdala volume extending to the putamen, insular cortex, and hippocampus; at uncorrected 0.005 at voxel level and FWE correction at cluster level. This pattern was similar in interaction analyses, but restricted to medial-frontal regions at uncorrected $p < 0.005$ at voxel level and FWE correction at cluster level (Supplementary Figure S3).

Among those with a history of psychiatric disease, SLEs were associated with reduced gray matter volume in the pre- and postcentral gyrus at uncorrected 0.005 at voxel level and FWE correction at cluster level (Figure). At uncorrected 0.005 at voxel level and FWE correction

Table 1. Characteristics of the Participants in the CSF Sample (n = 393), Brain Structure Sample (n = 1,234), and Full Sample (n = 1,290)

Variable	CSF Sample (n = 393) n (%)	Brain Structure Sample (n = 1,234) n (%)	All Participants (n = 1,290) n (%)
Sex (women)	242 (61.6)	764 (61.9)	792 (61.4)
Ethnicity (White Caucasian)	391 (99.5)	1,225 (99.3)	1,281 (99.3)
History of cardiovascular disease (yes)	160 (40.7)	382 (31.0)	408 (31.6)
History of psychiatric disease (yes)	120 (30.5)	374 (30.3)	390 (30.3)
APOE ε4 carrier (yes)	210 (53.4)	493 (40.0)	525 (40.7)
At least one parent diagnosed with AD Dementia (yes)	348 (88.6)	912 (73.9)	947 (73.4)
	M (SD; range)	**M (SD; range)**	
Age at lumbar puncture (yr)	61.2 (4.7; 49.3–73.6)	–	–
Age at scan (yrs)		59.2 (6.6; 47.6–76.8)	–
Years of education completed	13.5 (3.5; 6–20)	13.6 (3.5; 5–20)	13.6 (3.5; 5–20)
Number of stressors across lifespan	5.2 (2.2; 0–13)	5.4 (2.4; 0–16)	5.4 (2.3; 0–16)
Number of stressors in childhood	0.2 (0.6; 0–4)	0.2 (0.5; 0–4)	0.2 (0.5; 0–4)
Number of stressors in adolescence	0.1 (0.4; 0–3)	0.1 (0.4; 0–3)	0.1 (0.4; 0–3)
Number of stressors in early adulthood	1.3 (1.4; 0–8)	1.5 (1.5; 0–9)	1.5 (1.5; 0–9)
Number of stressors in middle adulthood	3.6 (1.9; 0–9)	3.6 (2.0; 0–14)	3.6 (2.0; 0–14)
Number of stressors in late adulthood	0.5 (0.8; 0–5)	0.5 (0.8; 0–6)	0.5 (0.8; 0–6)
β-amyloid$_{1–42}$/$_{1–40}$ ratio × 100	7.5 (2.0; 2.0–14.2)	–	–
Phosphorylated-tau$_{181}$ (pg/mL)	16.3 (7.6; 7.9–81.1)	–	–
Interleukin 6 (pg/mL)	4.1 (2.0; 1.7–18.4)	–	–

APOE = Apolipoprotein, M = mean, SD = standard deviation.

at cluster level, there was no interaction between psychiatric disease and SLEs on gray matter volume.

Exploratory Analyses: Associations of Life Periods with AD Pathophysiology and Voxel-Wise Associations

Higher SLEs (vs lower) in childhood were associated with higher levels of IL-6 later in life (Supplementary Table S11). Similarly, higher SLEs (vs lower) in middle adulthood were associated with lower levels of Aβ$_{1–42}$/$_{1–40}$ ratio. There were no association of SLEs in specific age periods and brain structure at FWE corrected or uncorrected $p < 0.005$.

Discussion

In cognitively unimpaired older adults at risk for AD, the findings of this study provide moderate support for SLEs to be associated with increased AD pathologies, neuroinflammation, and reduced brain volume depending on history of psychiatric disease, sex, and the timing of exposure to stressors. An association of lifetime SLEs with AD and neuroinflammation pathways was observed only among participants with history of psychiatric disease, while increased Aβ was observed only among men. Childhood and middle adulthood SLEs showed a link with IL6 and Aβ, respectively, within the whole sample. These primary findings, when taken in the context of existing literature linking SLEs with

Table 2. Unstandardized Regression Coefficients (95% Confidence Intervals; *p*-Values) for the Change Per 1-Event Increase in the Total Number of Stressors Across the Life Course in Alzheimer's Disease Pathophysiological Markers (n = 393)

	Model 1 (n = 393)		Model 2 (n = 393)	
	Coeff. (95% CI)	*p*	Coeff. (95% CI)	*p*
AD pathway[a]				
Log_{10} β-amyloid$_{1–42}$/$_{1–40}$ ratio[b]	−0.21 (−0.082 to 0.40)	0.495	−0.55 (−1.13 to 0.04)	0.066
Phosphorylated tau$_{181}$	0.25 (−0.09 to 0.59)	0.145	0.28 (−0.06 to 0.63)	0.109
Neuroinflammation pathway[a]				
Log_{10} interleukin 6[b]	0.71 (−0.06 to 1.48)	0.072	0.71 (−0.08 to 1.51)	0.078

Note: Model 1 adjusted for sex, age, years of education. Model 2 adjusted for Model 1 and *APOE* ε4 carrier status, history of cardiovascular disease and history of psychiatric disease.
AD = Alzheimer's disease.
[a]Adjusted for age at lumbar puncture.
[b]Values of cerebrospinal fluid log_{10} β-amyloid$_{1–42}$/$_{1–40}$ ratio and log_{10} interleukin 6 were multiplied by 100 to facilitate the interpretation of the coefficient.

dementia risk, support further research to evaluate SLEs in relation to AD biomarkers, neuroinflammation and atrophy pathways in cognitively unimpaired older adults at risk for cognitive decline.

Contrary to our hypothesis, SLEs across the life course were not associated with Aβ, p-tau, or IL-6 within the whole sample. A previous study reported an association of midlife stress with unspecific markers of neurodegenerative processes later in life, namely total tau and Aβ$_{1–40}$,[28] but not with Aβ$_{1–42}$ or p-tau. The study suggested that the effects of stress may not be detected in CSF early in the disease progression. The majority of participants of the present study are in the early stages of the AD *continuum* which may have prevented detecting effects on p-tau levels, that occur downstream to Aβ$_{1–42}$/$_{1–40}$ ratio changes.[36]

Although our results showed no overall effect of SLEs (or history of psychiatric disease) on AD pathophysiology within the whole sample, our findings indicate an association of SLEs with AD pathophysiology among those with a history of psychiatric disease, as expected. Further, we showed history of psychiatric disease not to be associated with AD pathophysiology and reported a

Table 3. Unstandardized Regression Coefficients (95% Confidence Intervals; *p*-values) for the Change in Alzheimer's Disease Biomarker and Neuroinflammation Measures per 1-Event Increase in the Total Number of Stressors in the Whole Sample (n = 393) Stratified by History of Psychiatric Disease

	No history of Psychiatric Disease (n = 273)		History of Psychiatric Disease (n = 120)	
	Coeff. (95% CI)	*p*	Coeff. (95% CI)	*p*
AD pathway[a]				
Log_{10} β-amyloid$_{1–42}$/$_{1–40}$ ratio[b]	−0.10 (−0.81 to 0.61)	0.773	−1.54 (−2.57 to −0.51)	0.004
Phosphorylated tau$_{181}$	0.12 (−0.32 to 0.57)	0.585	0.61 (0.04 to 1.18)	0.036
Neuroinflammation pathway[a]				
Log_{10} interleukin 6[b]	0.20 (−0.75 to 0.16)	0.675	1.68 (0.21 to 3.14)	0.025

Note: Adjusted for sex, age, years of education, *APOE* ε4 carrier status, and history of cardiovascular disease.
AD = Alzheimer's disease.
[a]Adjusted for age at lumbar puncture.
[b]Values of cerebrospinal fluid log_{10} β-amyloid$_{1–42}$/$_{1–40}$ ratio and log_{10} interleukin 6 values were multiplied by 100 to facilitate the interpretation of the coefficient.

Table 4. Unstandardized Regression Coefficients (95% Confidence Intervals; *p*-values) for the Change in Alzheimer's Disease Biomarkers, Neuroinflammation, and Brain Integrity Measures per 1-Event Increase in the Total Number of Stressors Stratified by Sex

	Women (n = 242)		Men (n = 151)	
	Coeff. (95% CI)	*p*	Coeff. (95% CI)	*p*
AD pathway				
Log_{10} β-amyloid$_{1–42}$/$_{1–40}$ ratio[a]	−0.42 (−1.12 to 0.28)	0.239	−1.24 (−2.34 to −0.14)	0.028
Phosphorylated-tau$_{181}$	0.45 (0.00 to 0.91)	0.052	0.03 (−0.53 to 0.59)	0.924
Neuroinflammation pathway				
Log_{10} interleukin 6[a]	0.86 (−0.14 to 1.86)	0.093	0.47 (−0.93 to 1.87)	0.508

Note: Adjusted for age at lumbar puncture, years of education, *APOE* ε4-carrier status, history of cardiovascular disease, and history of psychiatric disease.

[a]Values of cerebrospinal fluid β-amyloid$_{1–42}$/$_{1–40}$ ratio and IL-6 were multiplied by 100 to facilitate the interpretation of the coefficient.

cross-over interaction of SLEs and psychiatric disease on Aβ. It is possible that those with a history of psychiatric disease could more susceptible to the effects of SLEs, for example, due to impaired stress-coping abilities[37] thus making them more vulnerable to experiencing psychological stress and tau-burden.[38] Alternatively, experiencing SLEs may contribute to the development of psychiatric diseases[39] which in turn can increase vulnerability to AD pathophysiology. In the present study, participants with a history of psychiatric disease reported more SLEs, supporting this view. As shown by animal studies, stress could exacerbate AD pathogenesis,[20,21] and this could be

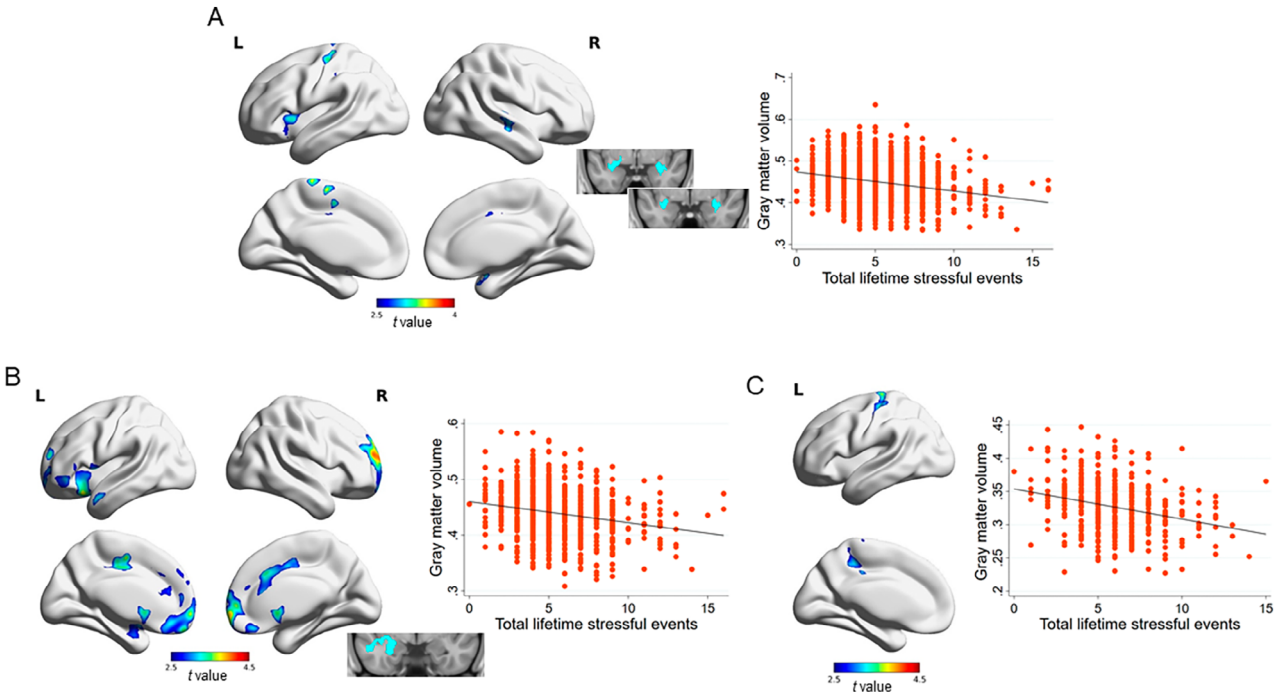

Figure: Results of the voxel-wise analyses showing the association of a higher number of SLEs and reduced regional gray matter volumes. *Y* **axes: average gray matter volume of the significant clusters identified in SPM analyses.** *X* **axes: total accumulated lifetime stressful events. Panel (A) Whole sample: thresholded at $p < 0.005$ at voxel level. Medial temporal results are shown as a binary mask overlaid into a T1 structural image. Panel (B) Women: thresholded at $p < 0.005$ at voxel level and family-wise error $p < 0.05$ at cluster level. Medial temporal results are shown as a binary mask overlaid into a T1 structural image. Panel (C) Participants with a history of psychiatric disease: thresholded at $p < 0.005$ at voxel level and family-wise error $p < 0.05$ at cluster level. All models were adjusted for age, education, total intracraneal volume, *APOE* ε4 carrier status, history of cardiovascular disease, history of psychiatric disease (only Panel A and B) and sex (only Panel A and Panel C). [Color figure can be viewed at www.annalsofneurology.org]**

moderated by psychiatric disease. It is also possible that in the current study those with a history of psychiatric disease were more affected by recall bias. However, we did not observe differences in test–retest scores between those with and without history of psychiatric disease thereby not supporting this hypothesis. Nonetheless, longitudinal studies are needed to disentangle the time relationship between SLEs, onset of psychiatric disease, and AD pathophysiology.

Further, in contrast to our initial hypothesis predicting stronger effects in women, we found an association of SLEs across the life course with Aβ only among men. Of note, the abovementioned study, reporting association of midlife stress with unspecific markers of neurodegenerative processes,[28] was conducted only in women. Since previous studies suggest no sex-differences in Aβ burden in older adults,[40] our results may imply sex-specific effects of stress on Aβ pathology. Also, men might be more inclined to engage in unhealthy coping behaviors compared with women, which may explain the observed sex-differences.[41] Further studies are warranted to assess whether the effects of SLEs on these pathways are seen in people at risk as the disease advances and/or in a sex-specific manner.

Consistent with our hypotheses, we found associations of SLEs with lower gray matter volumes among women and participants with history of psychiatric disease. In the whole sample, these associations were only observed with a more lenient statistical threshold. Thus, within the whole sample, we found some support for our limbic hypothesis, but less clear support for the frontal hypothesis. We showed SLEs to be associated with lower volumes in regions of the limbic system (amygdala, parahippocampal gyrus, and insular cortex) that are known to be involved in stress perception and emotional regulation.[42,43] Previous research in the general population shows SLEs to be associated with lower volumes in the hippocampus,[25] parahippocampal gurys,[25] insular cortex,[26] and anterior cingulate/prefrontal cortex.[25,44] Our results suggesting SLEs to be associated with reduced volume in the anterior cingulate and prefrontal cortex in women, are in line with animal models suggesting that estrogen promotes stress sensitivity in the prefrontal cortex among females.[45] We also found SLEs to be associated with lower volumes of somatic motor areas among those with a history of psychiatric disease, in line with evidence suggesting a disruption of motor cortical brain regions by stress.[46]

In line with our hypothesis, we found that a higher level of childhood SLEs, compared with lower, was associated with higher levels of IL-6. Studies on the general population report an association between chronic stress and systemic inflammation.[47,48] Our results are in line with emerging evidence suggesting childhood trauma to be linked with increased adulthood inflammation[49] and evidence suggesting that the risk of developing chronic inflammation is affected by early development and persists throughout the life course to affect health later in life.[50] Although exploratory, this finding is important, as inflammation has been recognized as a key molecular response in neurodegenerative diseases.[51] Further, we reported a higher level of middle adulthood stressors, compared with lower, to be associated with Aβ. Midlife is known to be a period when AD pathologies start to build up,[52] and studies have established the importance of other risk factors, such as cardiovascular health, in midlife for later-age cognitive functioning.[53] It is therefore possible that midlife could represent a vulnerable period[54] for experiencing psychological stress. It should be noted, however, that more SLEs were reported in midlife and future research should investigate whether specific SLEs or other factors account for the association between midlife stress and amyloid. Taken together, the results involving age periods, should be interpreted with caution due to their exploratory nature, and considered as hypothesis-generating for future studies rather than confirmatory.

This study's strengths include the wide range of biomarkers investigated, the life course approach and the large sample size for stratified analyses. Longitudinal studies investigating SLEs and spanning from decades would be logistically challenging. There are also several limitations. The SLEs checklist might not fully capture life course events and the degree to which the events were subjectively stressful was not considered. The events included in our sum score vary in severity, but were weighted equally in our study. However, using unweighted SLEs increases replicability and comparability across similar studies. Further, recall bias could have affected our results, but the high test–retest score within our sample does not support this. The duration, type, or salience of the events should be considered in future research. Our sample was 99.3% of White Caucasian ethnicity; therefore, the results are not generalizable to other populations at risk. Finally, it is important to highlight that some of the results present a mix of support for the hypotheses. This suggests that future research in the area may benefit from an update in the conceptualization and measurement of SLEs or stress. Also, we did not use correction for multiple comparison methods; therefore, caution should be given about drawing definitive conclusions.

Conclusion

Our findings demonstrate moderate support that in some contexts, SLEs may have a long-lasting impact on brain health through the AD, neuroinflammation, and atrophy pathways decades after exposure to SLEs. However, future research studies are warranted to investigate SLEs in the

context of history of psychiatric disease, sex, and to investigate the timing of events. Psychosocial interventions may represent strategies to improve stress resiliency and stress-coping strategies among those with a high exposure to SLEs who are at risk of cognitive decline. Future research is needed to refine the identification of risk profiles that would most benefit from such type of interventions.

Acknowledgments

This publication is part of the ALFA study (ALzheimer and FAmilies). The authors would like to express their sincerest gratitude to the ALFA project participants, without whom this research would have not been possible. The authors would like to thank the collaborators of the ALFA study:

Cleofé Peña-Gómez, Federica Anastasi, Annabella Beteta, Anna Brugulat-Serrat, Raffaele Cacciaglia, Irene Cumplido-Mayoral, Alba Cañas, Marta del Campo, Carme Deulofeu, Irene Cumplido, Ruth Dominguez, Maria Emilio, Sherezade Fuentes, Patricia Genius, Armand González-Escalante, Laura Hernández, Jordi Huguet, Paula Marne, Tania Menchón, Carolina Minguillon, Paula Ortiz, Wiesje Pelkmans, Albina Polo, Sandra Pradas, Blanca Rodríguez-Ferréndez, Iman Sadeghi, Mahnaz Shekari, Anna Soteras, Laura Stankeviciute, Marc Vilanova and Natalia Vilor-Tejedor.

The research leading to these results has received funding by the Ministry of Science and Innovation (PID2019-111514RA-I00) and the Alzheimer's Association research grants (AARG 2019-AARG-644641, AARG 2019-AARG-644641-RAPID), to EMA-U. EMA-U is supported by the Spanish Ministry of Science and Innovation—State Research Agency (RYC2018-026053-I), co-funded by the European Social Fund (ESF). EP is funded by the Spanish Ministry of Science and Innovation (PRE2020-095827). This work has received funding from "la Caixa" Foundation (ID 100010434), under agreement LCF/PR/GN17/50300004 and the Alzheimer's Association and an international anonymous charity foundation through the TriBEKa Imaging Platform project (TriBEKa-17-519007). Additional support has been received from the Universities and Research Secretariat, Ministry of Business and Knowledge of the Catalan Government under the grant no. 2021 SGR 00913. MS-C is funded by a fellowship from "la Caixa" Foundation (ID 100010434). OG-R is supported by the Spanish Ministry of Science and Innovation—State Research Agency (IJC2020-043417-I/MCIN/AEI/10.13039/501100011033) and the European Union «NextGenerationEU»/PRTR. The Roche NeuroToolKit is a panel of robust prototype assays used for investigational purposes only and not approved for clinical use. COBAS and ELECSYS are trademarks of Roche.

Author Contributions

E.P., M.A., K.F., J.D.G., and E.M.A.-U. contributed to the conception and design of the study; E.P., M.G.-P., K.B., H.Z., M.C., G.K., N.W., C.F., M.S.-C., O.G.-R., G.S.-B., and E.M.A.-U. contributed to the acquisition and analysis of data; E.P., M.A., and E.M.A.-U. contributed to drafting the text or preparing the figures. All authors approved the final version of the manuscript.

Potential Conflicts of Interest

K.B. has served as a consultant and at advisory boards for Acumen, ALZPath, BioArctic, Biogen, Eisai, Julius Clinical, Lilly, Novartis, Ono Pharma, Prothena, Roche Diagnostics, and Siemens Healthineers; has served at data monitoring committees for Julius Clinical and Novartis; has given lectures, produced educational materials and participated in educational programs for Biogen, Eisai, and Roche Diagnostics; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. H.Z. has served on scientific advisory boards for Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies, and CogRx, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS). G.K. and N.W. are full-time employees of Roche Diagnostics GmbH, Penzberg, Germany. M.C. is a full-time employee of Roche Diagnostics International Ltd., Rotkreuz, Switzerland. J.D.G. has received research support from GE Healthcare, Roche Diagnostics, F. Hoffmann—La Roche, speaker fees from Biogen and Philips and consultant fees from Roche Diagnostics. M.S.-C. has served as a consultant and at advisory boards for Roche Diagnostics International Ltd. and has given lectures in symposia sponsored by Roche Diagnostics, S.L.U., Roche Farma, S.A. and Roche Sistemas de Diagnósticos, Sociedade Unipessoal, Lda. O.G.-R. has received research support from F. Hoffmann—La Roche and speaker fees from Roche Diagnostics. E.P., M.A., M.G.-P., C.F., K.F., G.S.-B., and E.M.A.-U. reported no biomedical financial interests or potential conflicts of interest.

Data Availability

Data that support the findings of this study are available on reasonable request from the ALFA Study Investigators.

References

1. Patterson C. The state of the art of dementia research: new frontiers. Alzheimer's Dis Int 2018;1–48.
2. Jack CR, Bennett DA, Blennow K, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement 2018;14:535–562. https://doi.org/10.1016/J.JALZ.2018.02.018.
3. Wilson RS, Leurgans SE, Boyle PA, Bennett DA. Cognitive decline in prodromal Alzheimer disease and mild cognitive impairment. Arch Neurol 2011;68:351–356. https://doi.org/10.1001/ARCHNEUROL.2011.31.
4. Heneka MT, Carson MJ, El KJ, et al. Neuroinflammation in Alzheimer's disease. Lancet Neurol 2015;14:388–405. https://doi.org/10.1016/S1474-4422(15)70016-5.
5. Byers AL, Yaffe K. Depression and risk of developing dementia. Nat Rev Neurol 2011;7:323–331. https://doi.org/10.1038/NRNEUROL.2011.60.
6. Gimson A, Schlosser M, Huntley JD, Marchant NL. Support for midlife anxiety diagnosis as an independent risk factor for dementia: a systematic review. BMJ Open 2018;8:1–9. https://doi.org/10.1136/bmjopen-2017-019399.
7. Bisht K, Sharma K, Tremblay MÈ. Chronic stress as a risk factor for Alzheimer's disease: roles of microglia-mediated synaptic remodeling, inflammation, and oxidative stress. Neurobiol Stress 2018;9:9–21. https://doi.org/10.1016/J.YNSTR.2018.05.003.
8. Marchant NL, Howard RJ. Cognitive debt and Alzheimer's disease. J Alzheimers Dis 2015;44:755–770. https://doi.org/10.3233/JAD-141515.
9. Donovan NJ, Locascio JJ, Marshall GA, et al. Longitudinal association of amyloid beta and anxious-depressive symptoms in cognitively normal older adults. Am J Psychiatry 2018;175:530–537. https://doi.org/10.1176/appi.ajp.2017.17040442.
10. Pichet Binette A, Vachon-Presseau É, Morris JC, et al. Amyloid and tau pathology associations with personality traits, neuropsychiatric symptoms, and cognitive lifestyle in the preclinical phases of sporadic and autosomal dominant Alzheimer's disease. Biol Psychiatry 2021;89:776–785. https://doi.org/10.1016/j.biopsych.2020.01.023.
11. Cohen S, Gianaros PJ, Manuck SB. A stage model of stress and disease. Perspect Psychol Sci 2016;11:456–463. https://doi.org/10.1177/1745691616646305.
12. Sundström A, Rönnlund M, Adolfsson R, Nilsson LG. Stressful life events are not associated with the development of dementia. Int Psychogeriatrics 2014;26:147–154. https://doi.org/10.1017/S1041610213001804.
13. Tani Y, Fujiwara T, Kondo K. Association between adverse childhood experiences and dementia in older Japanese adults. JAMA Netw Open 2020;3:e1920740. https://doi.org/10.1001/JAMANETWORKOPEN.2019.20740.
14. Franks KH, Bransby L, Saling MM, Pase MP. Association of stress with risk of dementia and mild cognitive impairment: a systematic review and meta-analysis. J Alzheimers Dis 2021;82:1573–1590. https://doi.org/10.3233/JAD-210094.
15. Comijs HC, van den Kommer TN, Minnaar RWM, et al. Accumulated and differential effects of life events on cognitive decline in older persons: depending on depression, baseline cognition, or ApoE ε4 status? J Gerontol B 2011;66B:i111–i120.
16. Tschanz JT, Pfister R, Wanzek J, et al. Stressful life events and cognitive decline in late life: moderation by education and age the Cache County study. Int J Geriatr Psychiatry 2013;28:821–830. https://doi.org/10.1002/GPS.3888.
17. McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev 2007;87:873–904. https://doi.org/10.1152/PHYSREV.00041.2006/ASSET/IMAGES/LARGE/Z9J00030724460008.JPEG.
18. Blum-Degena D, Müller T, Kuhn W, et al. Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. Neurosci Lett 1995;202:17–20. https://doi.org/10.1016/0304-3940(95)12192-7.
19. Lyra e Silva NM, Gonçalves RA, Pascoal TA, et al. Pro-inflammatory interleukin-6 signaling links cognitive impairments and peripheral metabolic alterations in Alzheimer's disease. Transl Psychiatry 2021;11:251. https://doi.org/10.1038/s41398-021-01349-z.
20. Sotiropoulos I, Catania C, Pinto LG, et al. Stress acts cumulatively to precipitate Alzheimer's disease-like tau pathology and cognitive deficits. J Neurosci 2011;31:7840–7847. https://doi.org/10.1523/JNEUROSCI.0730-11.2011.
21. Ha Jeong Y, Hyoung Park C, Yoo J, et al. Chronic stress accelerates learning and memory impairments and increases amyloid deposition in APPV717I-CT100 transgenic mice, an Alzheimer's disease model. FASEB J 2006;20:729–731. https://doi.org/10.1096/FJ.05-4265FJE.
22. Csernansky JG, Dong H, Fagan AM, et al. Plasma cortisol and progression of dementia in subjects with Alzheimer-type dementia. Am J Psychiatry 2006;163:2164–2169. https://doi.org/10.1176/AJP.2006.163.12.2164.
23. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci 2009;10:434–445. https://doi.org/10.1038/nrn2639.
24. Arnsten AFT. Stress signalling pathways that impair prefrontal cortex structure and function. Nat Rev Neurosci 2009;10:410–422. https://doi.org/10.1038/NRN2648.
25. Papagni SA, Benetti S, Arulanantham S, et al. Effects of stressful life events on human brain structure: a longitudinal voxel-based morphometry study. 2011;14:227–232. https://doi.org/10.3109/10253890.2010.522279.
26. Ansell EB, Rando K, Tuit K, et al. Cumulative adversity and smaller gray matter volume in medial prefrontal, anterior cingulate, and insula regions. Biol Psychiatry 2012;72:57–64. https://doi.org/10.1016/J.BIOPSYCH.2011.11.022.
27. Gerritsen L, Kalpouzos G, Westman E, et al. The influence of negative life events on hippocampal and amygdala volumes in old age: a life-course perspective. Psychol Med 2015;45:1219–1228. https://doi.org/10.1017/S0033291714002293.
28. Johansson L, Kern S, Zetterberg H, et al. Midlife stress in relation to late-life cerebrospinal fluid biomarkers of Alzheimer's disease: a 25-year follow-up study. Dement Geriatr Cogn Disord 2018;46:90–99. https://doi.org/10.1159/000490885.
29. Trammell AR, McDaniel DJ, Obideen M, et al. Perceived stress is associated with Alzheimer's disease cerebrospinal fluid biomarkers in African Americans with mild cognitive impairment. J Alzheimers Dis 2020;77:843–853. https://doi.org/10.3233/JAD-200089.
30. Molinuevo JL, Gramunt N, Gispert JD, et al. The ALFA project: a research platform to identify early pathophysiological features of Alzheimer's disease. Alzheimer's Dement Transl Res Clin Interv 2016;2:82–92. https://doi.org/10.1016/J.TRCI.2016.02.003.
31. Miller MA, Rahe RH. Life changes scaling for the 1990s. J Psychosom Res 1997;43:279–292. https://doi.org/10.1016/S0022-3999(97)00118-9.
32. Holmes TH, Rahe RH. The social readjustment rating scale. J Psychosom Res 1967;11:213–218. https://doi.org/10.1016/0022-3999(67)90010-4.
33. Gorman DM. A review of studies comparing checklist and interview methods of data collection in life event research. Behav Med 1993;19:66–73. https://doi.org/10.1080/08964289.1993.9937567.
34. Monroe SM. Modern approaches to conceptualizing and measuring human life stress. Annu Rev Clin Psychol 2008;4:33–52. https://doi.org/10.1146/ANNUREV.CLINPSY.4.022007.141207.
35. Streiner DL, Norman GR. Correction for multiple testing: is there a resolution? Chest 2011;140:16–18. https://doi.org/10.1378/CHEST.11-0523.

36. Jack CR, Knopman DS, Jagust WJ, et al. Update on hypothetical model of Alzheimer's disease biomarkers. Lancet Neurol 2013;12: 207–216. https://doi.org/10.1016/S1474-4422(12)70291-0.

37. Orzechowska A, Zajaczkowska M, Talarowska M, Gałecki P. Depression and ways of coping with stress: a preliminary study. Med Sci Monit 2013;19:1050–1056. https://doi.org/10.12659/MSM.889778.

38. Arenaza-Urquijo EM, Przybelski SA, MacHulda MM, et al. Better stress coping associated with lower tau in amyloid-positive cognitively unimpaired older adults. Neurology 2020;94:e1571–e1579. https://doi.org/10.1212/WNL.0000000000008979.

39. Kendler KS, Karkowski LM, Prescott CA. Causal relationship between stressful life events and the onset of major depression. Am J Psychiatry 1999;156:837–841. https://doi.org/10.1176/AJP.156.6.837/ASSET/IMAGES/LARGE/AN5T1.JPEG.

40. Altmann A, Tian L, Henderson VW, Greicius MD. Sex modifies the APOE-related risk of developing Alzheimer disease. Ann Neurol 2014;75:563–573. https://doi.org/10.1002/ANA.24135.

41. Lindquist TL, Beilin LJ, Knuiman MW. Influence of lifestyle, coping, and job stress on blood pressure in men and women. Hypertension 1997;29:1–7. https://doi.org/10.1161/01.HYP.29.1.1.

42. Etkin A, Egner T, Kalisch R. Emotional processing in anterior cingulate and medial prefrontal cortex. Trends Cogn Sci 2011;15:85–93. https://doi.org/10.1016/J.TICS.2010.11.004.

43. Mcewen BS, Gianaros PJ. Central role of the brain in stress and adaptation: links to socioeconomic status, health, and disease. Ann N Y Acad Sci 2010;1186:190–222. https://doi.org/10.1111/J.1749-6632.2009.05331.X.

44. Ringwald KG, Pfarr JK, Stein F, et al. Association between stressful life events and grey matter volume in the medial prefrontal cortex: a 2-year longitudinal study. Hum Brain Mapp 2022;43:3577–3584. https://doi.org/10.1002/HBM.25869.

45. Shansky RM, Glavis-Bloom C, Lerman D, et al. Estrogen mediates sex differences in stress-induced prefrontal cortex dysfunction. Mol Psychiatry 2004;9:531–538. https://doi.org/10.1038/SJ.MP.4001435.

46. Gellner AK, Sitter A, Rackiewicz M, et al. Stress vulnerability shapes disruption of motor cortical neuroplasticity. Transl Psychiatry 2022; 12:91. https://doi.org/10.1038/s41398-022-01855-8.

47. Bourassa KJ, Rasmussen LJH, Danese A, et al. Linking stressful life events and chronic inflammation using suPAR (soluble urokinase plasminogen activator receptor). Brain Behav Immun 2021;97:79–88. https://doi.org/10.1016/J.BBI.2021.06.018.

48. Rohleder N. Stimulation of systemic low-grade inflammation by psychosocial stress. Psychosom Med 2014;76:181–189. https://doi.org/10.1097/PSY.0000000000000049.

49. Baumeister D, Akhtar R, Ciufolini S, et al. Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-α. Mol Psychiatry 2016;21:642–649. https://doi.org/10.1038/mp.2015.67.

50. Miller GE, Chen E, Parker KJ. Psychological stress in childhood and susceptibility to the chronic diseases of aging: moving toward a model of behavioral and biological mechanisms. Psychol Bull 2011; 137:959–997. https://doi.org/10.1037/A0024768.

51. Scrivo R, Vasile M, Bartosiewicz I, Valesini G. Inflammation as "common soil" of the multifactorial diseases. Autoimmun Rev 2011;10: 369–374. https://doi.org/10.1016/J.AUTREV.2010.12.006.

52. Ritchie K, Ritchie CW, Jaffe K, et al. Is late-onset Alzheimer's disease really a disease of midlife? Alzheimer's Dement Transl Res Clin Interv 2015;1:122–130. https://doi.org/10.1016/J.TRCI.2015.06.004.

53. González HM, Tarraf W, Harrison K, et al. Midlife cardiovascular health and 20-year cognitive decline: atherosclerosis risk in communities study results. Alzheimers Dement 2018;14:579–589. https://doi.org/10.1016/J.JALZ.2017.11.002.

54. Vemuri P. "Exceptional brain aging" without Alzheimer's disease: triggers, accelerators, and the net sum game. Alzheimers Res Ther 2018;10:53. https://doi.org/10.1186/S13195-018-0373-Z.