

Stress in relation to biomarkers of Alzheimer's disease

Title: Midlife Stress in Relation to Late-Life Cerebrospinal Fluid Biomarkers of Alzheimer's Disease: A 25-Year Follow-Up Study

Running head: Stress in relation to biomarkers of Alzheimer's disease

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## **Abstract**

**Objective** Psychological stress may increase the risk of dementia. This study aimed to analyze the relationship between midlife stress and late-life cerebrospinal fluid markers of Alzheimer's disease.

**Methods** The study includes 79 non-demented women from the Prospective Population Study of Women in Gothenburg, Sweden. The participants responded to a stress question at the baseline examination in 1968-69 (mean age 49 years), and had a lumbar puncture in 1993-94 (mean age 74 years) where cerebrospinal fluid levels of beta-amyloid ( $A\beta_{1-40}$  and  $A\beta_{1-42}$ ), total tau protein and phosphorylated tau protein were measured.

**Results** Age-adjusted logistic regressions showed that midlife psychological stress was associated with higher cerebrospinal fluid levels of total tau (OR 2.18, 95% CI 1.14 - 4.18,  $p=0.02$ ) and beta amyloid<sub>1-40</sub> (OR 1.80, 95% CI 1.04-3.12,  $p=0.04$ ) in late-life. No associations were found between midlife stress and levels of beta amyloid<sub>1-42</sub> or phosphorylated tau.

**Interpretation** Our findings suggest that midlife stress increases risk of dementia by stimulating unspecific neurodegenerative processes, but not the core processes of Alzheimer's disease, at least not in the early phase of the disease. The higher concentration of total tau protein may reflect a neural disintegration, and higher beta amyloid<sub>1-40</sub> can be an early sign of cerebral amyloid angiopathy or amyloid-beta overproduction.

## Introduction

Longstanding psychological stress may increase the incidence of dementia. We have previously reported associations between midlife longstanding stress and late-life dementia, especially Alzheimer's disease,<sup>1</sup> and late life brain changes such as temporal lobe atrophy,<sup>2</sup> and white matter lesions.<sup>2</sup> We also found that neuroticism<sup>3</sup> and number of midlife life-stressors<sup>4</sup> were related to higher risk of developing late-life Alzheimer's disease. Other longitudinal studies have reported higher risk for dementia and Alzheimer's disease in persons with stress-prone personality (e.g. neuroticism),<sup>5</sup> and in persons with post-traumatic stress disorder<sup>6</sup> and higher number of stressful life-events.<sup>7,8</sup>

There are several hypotheses regarding the mechanisms by which psychological stress increases the risk of brain disease, such as Alzheimer's disease. One is that psychological stress activates the hypothalamic-pituitary-adrenal axis and increases levels of glucocorticoid hormones. Longstanding exposure to stress may lead to atrophy and neurodegeneration in brain areas with large number of glucocorticoid receptors, e.g. in the hippocampus.<sup>9</sup> Hippocampal atrophy can result in impaired memory and spatial functions, and is an early marker for Alzheimer's disease. Animal studies report that high glucocorticoid and/or chronic stress may lead to tau accumulation,<sup>10</sup> trigger tau hyperphosphorylation,<sup>10</sup> and increase the deposition of beta amyloid (A $\beta$ )<sup>11</sup> in the brain. No study has thus far analyzed the effect of psychological stress on levels of tau and A $\beta$  in humans. Two autopsy studies have, however, examined the stress prone personality high neuroticism in relation to tau and A $\beta$  in postmortem brains. One study found that high neuroticism was associated with more advanced stages of neurofibrillary tangle formation,<sup>12</sup> while the other found that neither levels of neurofibrillary tangles nor amyloid plaques were associated with neuroticism.<sup>13</sup>

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Alzheimer's disease is characterized by accumulation of A $\beta$  in brain parenchyma. There are two major variants of A $\beta$ , with either 40 (A $\beta$ <sub>1-40</sub>) or 42 (A $\beta$ <sub>1-42</sub>) amino-acid residues. A $\beta$ <sub>1-42</sub> has the highest tendency for aggregation and plaque formation. Low cerebrospinal fluid A $\beta$ <sub>1-42</sub> levels are related to Alzheimer's disease, and thought to reflect accumulation of senile plaques and A $\beta$  in the brain.<sup>14,15</sup> A $\beta$ <sub>1-40</sub> is the most abundant A $\beta$  peptide in the brain.<sup>16</sup> Its biochemical function is debated, but it has been suggested that high levels of A $\beta$ <sub>1-40</sub> could be a sign of A $\beta$  overproduction and amyloid precursor protein dysfunction.<sup>17</sup> The total concentration of tau (t-tau) reflects intensity of neuronal degeneration. An increase of this marker is seen in the cerebrospinal fluid of Alzheimer's disease patients, but also in e.g. vascular dementia.<sup>18</sup> Increased level of phosphorylated tau (p-tau) is a more specific biomarker for Alzheimer's disease and is thought to reflect accumulation of neurofibrillary tangles.<sup>19</sup> It is not clear whether stress affects these markers of Alzheimer's disease pathology.

Additional understanding is required about risk factors for the pathological processes in dementia disorders. Prospective studies on stress and its associations to the central pathogenic processes of early Alzheimer's disease in the brain are lacking. Therefore, we examined the relationship between midlife psychological stress and late-life cerebrospinal fluid t-tau, p-tau, A $\beta$ <sub>1-40</sub>, and A $\beta$ <sub>1-42</sub> in a sample of women without dementia who were followed over 26 years.

## Methods

### Study population

The sample was drawn from the Prospective Population Study of Women in Gothenburg, Sweden, which was initiated in 1968, and includes several subsequent follow-up examinations over four decades.<sup>1,20</sup> Participants were initially sampled from the Swedish Population Register based on specific birth dates in order to yield a representative sample at the ages

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studied (aged 38 to 60 years). At baseline in 1968-69, a question about psychological stress was included. In the follow-up examination in 1992-94, 590 of the women (born 1908, 1914, 1918 and 1922) participated in an extensive psychiatric examination, and 86 of those consented to a lumbar puncture.<sup>21</sup> Participants and non-participants in the lumbar puncture examination were similar regarding baseline diastolic and systolic blood pressure, body mass index, smoking, and frequency of stroke. The lumbar puncture participants were younger, reported less often distress or depression at baseline, and had less often developed dementia up to 1992, compared to non-participants. According to the Swedish Population Register, lumbar puncture participants had lower 5-year mortality rate compared to non-participants.

The present study includes 79 women without dementia (two women were excluded due to dementia), who responded both to the question about psychological stress at the examination in 1968-69 and took part in the lumbar puncture study in 1993-94. The participants were born in 1908 (n=2), 1914 (n=7), 1918 (n=33), and 1922 (n=37). The Ethics Committee for Medical Research at the University of Gothenburg approved the study and informed consent was obtained from all participants, in accordance with the provisions of the Helsinki Declaration.

### **Assessment of psychological stress**

A question about psychological stress was asked by a physician at the examinations in 1968-69. The question was; "Have you experienced any period of stress (one month or longer) in relation to circumstances in everyday life, such as work, health, or family situation? Stress referred to feelings of irritability, tension, nervousness, fear, anxiety or sleep disturbances." Participants were asked to choose; "0: Have never experienced any period of stress; 1: Have experienced period/s of stress more than five years ago; 2: Have experienced one period of stress during the last five years; 3: Have experienced several periods of stress during the last

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five years; 4: Have experienced constant stress during the last year; or 5: Have experienced constant stress during the last five years". For the purpose of this study, women who acknowledged responses 3, 4 or 5 were considered to have psychological stress.

### **Assessment of cerebrospinal fluid biomarkers**

The lumbar puncture examinations were conducted between January 1993 and March 1994. Cerebrospinal fluid samples (12 ml) were taken through the L3/L4 interspace. To eliminate cells and other insoluble material, the samples were centrifuged at 2000g for 10 minutes. The liquid was then stored at minus 80°C in 1 ml polypropylene vials until analyses were done. Within five years after the lumbar puncture examination, the cerebrospinal fluid was analyzed for biomarkers.<sup>21</sup> Sandwich enzyme-linked immunosorbent assay (ELISA) was used to determine levels of A $\beta$ <sub>1-40</sub>, A $\beta$ <sub>1-42</sub>, t-tau, and p-tau.<sup>21,22</sup> The procedure has been described in details previously.<sup>21</sup>

### **Other measurements**

Information on hypertension, cardiovascular disease, and depressive symptoms were obtained at the baseline examination in 1968-69. Hypertension was defined as systolic blood pressure  $\geq 150$ mmHg and/or diastolic blood pressure  $\geq 90$ mmHg and/or taking antihypertensive medications. Cardiovascular disease was defined as fulfilling one or more of the following criteria: angina pectoris, documented history of myocardial infarction, and/or electrocardiography evidence of ischemia. Major depression was diagnosed according to DSM-5 criteria based on information from the psychiatric interview.<sup>23</sup> In the examination in 1992-94 dementia was diagnosed according to DSM-III-R criteria based on information from psychiatric examinations, and close informant interviews.<sup>24</sup> Apolipoprotein E (*ApoE*) allele

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status was measured in a sub-sample of women (n=51) with genotype data. *ApoE* genotype was dichotomized into presence or absence of the  $\epsilon 4$  allele.

### Statistical analyses

Due to skewed distributions, t-tau and p-tau values were natural log transformed to improve symmetry. Ratios of  $A\beta_{1-42}/A\beta_{1-40}$  were calculated. Means and standard deviations (SD) were determined for all cerebrospinal fluid values. The correlations between age and stress, and age and cerebrospinal fluid markers, were analysed with the Spearman coefficient test. Logistic regression models were used to study the association between psychological stress at baseline 1968-69 and cerebrospinal fluid biomarkers in 1993-94 (t-tau, p-tau,  $A\beta_{1-40}$ ,  $A\beta_{1-42}$ ). The associations are presented as odds ratios (ORs) and 95% confidence intervals (CIs). The models were (i) age adjusted, (ii) age and depression adjusted, and (iii) age and cardiovascular disease/hypertension adjusted. We also examined the ratios of  $A\beta_{1-42}/A\beta_{1-40}$ , which are often used in clinical practice. In persons with DNA information, adjustments for *ApoE*  $\epsilon 4$  genotype was done.

### Results

The 79 participants were between 46 and 60 years at baseline (mean age 49 years; SD 3 years) and between 71 and 85 years at time for the lumbar puncture study (mean age 74 years). At baseline reported 23% of the women frequent psychological stress. Eight women had depression and 13 were diagnosed with cardiovascular disease and/or hypertension. In the subsample of 51 persons with DNA information, 10 were *ApoE*  $\epsilon 4$  carriers. Table 1 present means, minimum/maximum, and median values for t-tau, p-tau,  $A\beta_{1-40}$ ,  $A\beta_{1-42}$ , and  $A\beta_{1-42}/A\beta_{1-40}$  ratio. The data is presented both for both the total sample, and separate for the non-

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stress and stress groups. The participants' age did not correlate with report of stress or levels of cerebrospinal fluid markers.

The relations between psychological stress in 1968-69 and cerebrospinal fluid biomarkers in 1993-94 were analyzed with logistic regressions analyses. In the age adjusted model, stress was associated with higher levels of t-tau (OR 2.18,  $p=0.02$ ) and  $A\beta_{1-40}$  (OR 1.80,  $p=0.04$ ) (Table 2). No associations were found between stress and p-tau,  $A\beta_{1-42}$ , or  $A\beta_{1-42}/A\beta_{1-40}$  ratio. After further adjustment for baseline depression, stress was still associated with higher levels of t-tau (OR 2.36, 95% CI 1.19-4.67) and  $A\beta_{1-40}$  (OR 1.95, 95% CI 1.10-3.46). Stress was associated with higher levels of t-tau (adjusted OR 2.32, 95% CI 1.19-4.52) and  $A\beta_{1-40}$  (OR 1.87, 95% CI 1.07-3.27) also after adjustment for baseline cardiovascular disease/hypertension. In the subsample with DNA information, stress was associated with t-tau (age and *ApoE*  $\epsilon 4$  adjusted OR 2.36, 95% CI 1.05-5.35), but not with  $A\beta_{1-40}$  (OR 1.83, 95% CI 0.89-3.77).

## Discussion

We found that longstanding midlife stress was associated with higher levels of cerebrospinal fluid t-tau and  $A\beta_{1-40}$  in later life, but not with the more specific Alzheimer's disease markers p-tau and  $A\beta_{1-42}$ , in a population-based sample of women without dementia followed over 26 years. The association remained after further adjustments for age, depression, cardiovascular disease and hypertension. To the best of our knowledge, this is the first study to examine midlife psychological stress in relation to late-life levels of tau and  $A\beta$  proteins in cerebrospinal fluid. We have previously reported associations between midlife psychological stress and late-life dementia,<sup>1</sup> Alzheimer's disease,<sup>1</sup> temporal lobe atrophy,<sup>2</sup> and white matter lesions.<sup>2</sup> The new findings shed further light on these findings.



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The finding that midlife stress was related to increased levels of late-life t-tau, suggests that stress may affect the risk of Alzheimer's disease by inducing unspecific neurodegeneration rather than inducing Alzheimer's disease encephalopathy before dementia onset. This is in line with our previous report that midlife stress increases risk of brain atrophy in late-life among women without dementia.<sup>2</sup> It has to be emphasized that our findings occur early in the disease process, before clinical manifestations of dementia. An increase of t-tau in cerebrospinal fluid is found in many studies of dementia disorders.<sup>14</sup> It has been suggested that t-tau increases decades before onset of clinical Alzheimer's disease, but later than the decline in  $A\beta_{1-42}$ .<sup>15</sup> A higher concentration of t-tau can be regarded as a general marker for neural disintegration and degeneration and occurs in brain damage of varied etiology.<sup>8</sup> It may be that stress influences neuronal function, as reflected by diffusion of tau proteins, long before onset of dementia.

In our study, there were no associations between stress and p-tau. Increased concentration of p-tau is a more specific marker for formation of neurofibrillary tangles and is more commonly affected in Alzheimer's disease dementia compared to other dementia disorders, such as vascular dementia.<sup>19</sup> However, high levels of p-tau in cerebrospinal fluid are normally seen in the more developed states of the neurodegenerative processes in Alzheimer's disease, when the disorder is more manifested and after that  $A\beta$  has started to aggregate in plaques.<sup>19</sup> Our findings may thus be due to the cerebrospinal fluid being measured too early in the disease process.

Midlife stress was also associated with higher levels of  $A\beta_{1-40}$  in this study. The function and physiological role of  $A\beta_{1-40}$  is unclear. The less soluble  $A\beta_{1-42}$  is mainly found in the senile plaques, whereas the more soluble  $A\beta_{1-40}$  accumulates as cerebral amyloid angiopathy.<sup>24</sup> A

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higher level of  $A\beta_{1-40}$  may be understood as a failure of perivascular drainage or elimination of  $A\beta$  from the brain<sup>26</sup> or reflecting a general overproduction of  $A\beta$  from cerebral amyloid angiopathy.<sup>18</sup> This indicates that longstanding stress may lead to vascular Alzheimer's disease processes in the brain, which is in line with our previous finding that midlife stress increases risk of late life ischemic white matter lesions.<sup>2</sup> Also,  $A\beta_{1-40}$  is the most abundant form of  $A\beta$  and reflects the total burden of  $A\beta$  in the brain. The accumulation of  $A\beta$  in brain in Alzheimer's disease can theoretically be due to overproduction, as seen in familial Alzheimer's disease,<sup>25</sup> or defective clearance. The available data so far suggest that it is the clearance of  $A\beta$  from the brain that is impaired in symptomatic sporadic Alzheimer's disease, whereas the production seems unaltered.<sup>25</sup> However, no solid data on production and clearance rates in preclinical sporadic Alzheimer's disease exist, and it is possible that also sporadic Alzheimer's disease at an early stage have slightly higher  $A\beta$  production. Increased levels of cerebrospinal fluid  $A\beta_{1-40}$  have been seen in older persons and in Alzheimer's disease patients, in occasional studies.<sup>26</sup>

The amyloid accumulation in senile plaques of Alzheimer's disease brains mainly consists of  $A\beta_{1-42}$ , which has high tendency for aggregation and is the fastest to form plaques. This is reflected by low levels of  $A\beta_{1-42}$  in cerebrospinal fluid.<sup>14</sup> Low concentrations of  $A\beta_{1-42}$  are also found in e.g. vascular dementia and frontotemporal dementia.<sup>14</sup> We found no associations between stress and  $A\beta_{1-42}$ . It has been suggested that levels of  $A\beta_{1-42}$  are higher several decades before dementia onset, and then starts to decline approximately two decades before disease onset and then becomes lower in those afflicted.<sup>15,27</sup> An hypothesis could be that early high levels of cerebrospinal fluid  $A\beta_{1-42}$  mirror a high amyloid production, while the lower levels reflect disturbed metabolism and plaque pathology.<sup>15,28</sup> The levels of  $A\beta$  are thus dependent on 'time to dementia onset'. In our study, all participants were free from dementia, and might thus have variable time until dementia onset.

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The results from this study are difficult to compare to other studies, as few studies have been done on risk factors for cerebrospinal fluid changes in normal individuals. Two autopsy studies examined the effect of neuroticism, a personality trait associated with stress proneness, on Alzheimer's disease encephalopathy. One longitudinal study, with a mean follow-up of 11 years, found that high neuroticism was associated with more advanced stages of neurofibrillary tangles postmortem.<sup>12</sup> In contrast, another study reported that neither levels of neurofibrillary tangles nor amyloid plaques were associated with high neuroticism.<sup>13</sup> Our sample was younger, and all were free from dementia in contrast to these studies.

The strengths of this study include a representative population sample, long follow-up, information already from midlife, and multiple sources of information to exclude dementia. Some methodological issues need to be considered. First, psychological stress was based on a subjective personal response to a single question. Our question on stress has however been used in several previous studies, and are found to be related to hypertension,<sup>27</sup> myocardial infarction,<sup>28</sup> cancer,<sup>29</sup> and Alzheimer's disease.<sup>1</sup> The stress-question has also high correlation to the stress-prone personality trait neuroticism<sup>3</sup> and numbers of life-stressors.<sup>4</sup> Second, all analyses were conducted in relative small samples, resulting in low statistical power. The results may therefore be underpowered to detect small differences between groups. The ability to adjust for possible confounders was therefore also limited. Third, we have only cerebrospinal fluid data from the follow-up examination in 1993-94, and no baseline data on cerebrospinal fluid biomarkers. Fourth, cumulative attrition is a problem in long-term follow-up studies, and the participation in the lumbar puncture study was only 10% of the eligible sample. However, participants and non-participants were similar in a number of variables. Fifth, as cerebrospinal fluid biomarkers were measured in late-life, we cannot exclude the

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possibility of survival effects. Sixth, our study was done in mainly Caucasian women. We can thus not generalize to other groups.

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## **Authors Contributions**

L.J. and I.S. conceived and designed the study. L.J. and I.S. were responsible for data acquisition and analysis of data. All authors drafted the manuscript.

## **Potential Conflicts of Interest**

Nothing to report.

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**Table 1** Biomarker levels in 1993-94, of women with and without midlife psychological stress in 1968-69

	All women		Psychological stress	No psychological stress
	n=79		n=18	n=61
	mean $\pm$ SD	min/max (median)	mean $\pm$ SD	mean $\pm$ SD
t-tau (pg/ml)	332 $\pm$ 194	81/1200 (286)	440 $\pm$ 261	300 $\pm$ 159
p-tau (pg/ml)	22.8 $\pm$ 10.7	10/80 (20)	26.6 $\pm$ 17.7	21.6 $\pm$ 7.4
A $\beta$ <sub>1-40</sub> (pg/ml)	10800 $\pm$ 3299	5417/21331 (9998)	12379 $\pm$ 3570	10334 $\pm$ 3093
A $\beta$ <sub>1-42</sub> (pg/ml)	825 $\pm$ 230	351/1285 (840)	910 $\pm$ 241	800 $\pm$ 223
Ratio A $\beta$ <sub>1-42</sub> /A $\beta$ <sub>1-40</sub>	0.08 $\pm$ 0.03	0.03/0.15 (0.08)	0.08 $\pm$ 0.04	0.08 $\pm$ 0.02

t-tau=total tau, p-tau=phosphorylated tau, A $\beta$ <sub>1-40</sub>=beta amyloid 1-40, and A $\beta$ <sub>1-42</sub>=beta amyloid 1-42

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**Table 2** Midlife psychological stress in relations to late-life cerebrospinal fluid markers, presented as odds ratios (n=79)

	Psychological stress	
	OR (95% CI)*	p-value
t-tau	2.18 (1.14-4.18)	0.02
p-tau	1.23 (0.72-2.11)	0.44
A $\beta$ <sub>1-40</sub>	1.80 (1.04-3.10)	0.04
A $\beta$ <sub>1-42</sub>	1.64 (0.94-2.86)	0.08
Ratio A $\beta$ <sub>1-42</sub> /A $\beta$ <sub>1-40</sub>	1.05 (0.61-1.81)	0.87

t-tau=total tau, p-tau=phosphorylated tau, A $\beta$ <sub>1-40</sub>=beta amyloid 1-40, A $\beta$ <sub>1-42</sub>=beta amyloid 1-42.

Psychological stress measured in 1968-69. Cerebrospinal fluid markers measured in 1993-94

OR=Adjusted for age