Towards precision medicine for dementia: a biopsychosocial approach

Emma Lindsay Twait

when you say the word
'alzheimers'

i know you must be thinking of
the tangles and the plaques that reside in the brain,
the tau and the beta-amyloid that cause such a disease.

but when you say the word
'alzheimers'

i think of my grandmother,
how when her frontal lobe disintegrated,
she'd curse at eight year old me,
and she'd eat goldfish for every meal.

and when you say the word
'alzheimers'

i think of my grandmother,
how she'd move from house to house,
not knowing which one would be home.

and when you say the word
'alzheimers'

i think of my grandmother,
the way she forgot who her husband was.
the way she forgot how to use the restroom.
the way she forgot us.

i think of my grandmother,
all the times she didn't know how to think at all.

i think of my grandmother,
how the last thing she could do before she died,
was sing the same song she learned when she was five.

so when you say the word
'alzheimers'

i hope you think of everyone's
grandmother
mother
wife
daughter
son
husband
father
grandfather
that this disease inflicts.
i hope you think of the smiles that fade
because life doesn't quite seem like anything at all.
i hope you think beyond the plaques and the tangles,
and you think of the people, the lives of the people behind it.

so when you say the word
'alzheimers'
do not shake your head in sympathy.

when you say the word
ALZHEIMERS
i hope you take the anecdotes,
to untangle those tangles
and to clear the plaque
because it's more than just that.

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(met een samenvatting in het Nederlands)

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PART 3: MOVING TOWARDS PRECISION MEDICINE FOR DEMENTIA

Chapter 1

General introduction

Dementia is a neurodegenerative disease, characterized by cognitive decline, currently with no cure. Dementia presently affects 50 million people worldwide, and this number is estimated to triple by 2050 (1). The most common cause of dementia is Alzheimer's disease (AD). There is a prolonged preclinical stage of AD that allows for the possibility to assess which risk factors are present and which biomarkers may be targets for early disease modification and treatment (2). The two defining features of AD include amyloid-beta plaques and neurofibrillary tangles. However, most patients with AD show mixed pathology, multiple types of brain changes contributing to the clinical syndrome of dementia (3). Specifically, the most common form of mixed pathology is with vascular pathology. Vascular pathology can be observed via magnetic resonance imaging (MRI) measures, such as white matter hyperintensities (WMH) and lacunes (4). WMH are hyperintense regions on T2-weighted MRI sequences or as isointense or hypointense regions on T1-weighted sequences (4). Previous studies have suggested a relationship between both vascular and AD pathologies (5-8); however, most studies have been done using expensive and invasive methods such as positron emission topography (PET) or cerebrospinal fluid (CSF) for assessment of AD pathology.

Recent advancements assessing AD pathology in blood plasma have made measuring Alzheimer's pathology much more accessible. Highly-sensitive blood biomarker assays are now available to assess amyloid-beta and phosphorylated tau (p-tau), as well as other AD-related pathology, such as neurodegeneration and astrocyte activation, assessed via neurofilament light (NfL) and glial fibrillary acidic protein (GFAP), respectively (9). The ability to assess AD pathology in blood plasma allows for large-scale studies to assess the relations between AD pathology in vivo with other relevant biological processes, such as vascular pathology. As the relationship between AD pathology and vascular pathology has been hampered by the invasiveness and costs associated with CSF and PET measurements, one of the aims of the current study was to explore the association between these two pathologies within blood plasma.

Dementia and depression: mapping the psychosocial to the biological

Dementia is a multifaceted disease that is influenced by a wide range of factors, not only amyloid-beta and vascular pathology. For example, studies have shown one of the main contributors to dementia is depression (10-12). However, the mechanistic relationship between depression and dementia is still not yet fully understood (13). One explanation for this association could be that depression and dementia share biological mechanisms (14), such as through neurodegeneration (15), AD pathophysiology (16-18), neurotoxicity (19), or vascular pathology (20).

One of the leading hypotheses regarding the relationship between depression and dementia is the neurotoxicity hypothesis (21). This hypothesis stipulates that chronic stress in depression leads to hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and then downstream increases of cortisol. Hypercortisolemia has been related to depression (22), as well as neurodegeneration (23), specifically in the hippocampus (24). Further, the hippocampus is a heterogeneous structure, consisting of multiple subfields that show a differential response to stress. Specifically within the cornu ammonis (CA) 3 and the dentate gyrus, studies have shown neurogenesis inhibition post-stress exposure compared to other subfields within the hippocampus (25).

The second hypothesis regarding the relation between depression and dementia is the vascular hypothesis. The vascular depression hypothesis states that the relation between depression and dementia could be due to shared vascular risk factors. Small vessel changes in the brain due to vascular pathology, specifically in mood-regulating areas, could explain the link with depression (20). In line with the vascular hypothesis, the association of depression with subsequent vascular dementia is larger than that with Alzheimer's disease (26).

Another explanation could be that late-life depression is a reaction to AD-related pathophysiology during the prolonged preclinical stage of dementia, termed the amyloid hypothesis for depression (17). Increased levels of cortical amyloid-beta have been found in those with major depressive disorder (27). Further, a systematic review and meta-analysis found an association between plasma amyloid-beta levels and depression (28).

Alongside depression, other psychosocial factors have also been linked to dementia, such as low social support (29), anxiety (30), and early-life adversity (31), possibly through hippocampal loss as mediator (32-34). However, studies assessing these other psychosocial factors have been more scarce. Therefore, another aim of this thesis was to explore the associations between psychosocial factors, with multiple biological mechanisms that overlap with dementia (i.e., vascular pathology, neurotoxicity, AD pathophysiology, and hippocampal [subfield] atrophy).

Precision medicine and dementia

The emerging field of precision medicine, which aims to take into account individual differences across biological and psychosocial systems, has potential for improving dementia prevention (35). With a better understanding of an individual's risk profile, tailored methods for prevention and treatment could be created and utilized for dementia.

While multiple biological and psychosocial factors have been linked to dementia, these factors are primarily studied in isolation. However, complex relationships exist between these factors (36). With the recent advancements in data-driven statistical methodologies, the potential to create a holistic etiological picture of the biopsychosocial risk factors for dementia becomes increasingly possible. One way to assess biomarker profiles is to use a multisystem approach that allows for the possibility to assess the complex interactions that may exist between biomarkers (37, 38). One framework that encompasses multiple systems of biomarkers (e.g., cardiovascular, metabolic, inflammatory, and stress) is allostatic load (AL). AL refers to the damaging physiological responses to stress that the body performs throughout our lifetime (39). While these responses on the short-term are adaptive (termed 'allostasis'), chronic stress can lead to dysregulation across multiple biomarker systems. Further, by using a multisystem approach, one can assess if one biomarker system (e.g., metabolic) may be more of a driving system for disease risk than another (e.g., cardiovascular) or if some biomarkers systems co-exist more frequently in tandem. Lastly, by understanding possible multisystem risk profiles, clinicians could then further assess a patient's possible risk as well as implement more personalized prevention techniques (35).

Another novel statistical technique that could aid in precision medicine for dementia is machine learning. Thus far, prognostic models for dementia have shown poor calibration and performance when externally validated (40, 41). This could be due to complex interrelationships between predictors or nonlinear relationships and interactions that 'traditional' statistical methods, such as logistic or Cox regression, are unable to take into account. With the rise of more advanced statistical modeling techniques for disease prediction, these complex relationships could then be used instead to increase a model's predictive performance (42).

General objective

The aim of this thesis was to investigate the biological underpinnings of dementia by assessing the relations between amyloid-beta and vascular pathology, as well as with psychosocial factors, such as depression. We further aimed to investigate a biopsychosocial approach to classifying at-risk individuals by incorporating both biological and psychosocial aspects using data-driven profiles and risk prediction using machine learning (see Figure 1).

Outline of this thesis

The **first section** focused on the two main biomarkers for dementia: amyloid-beta deposition and vascular pathology. In **chapter 2,** we explored the cross-sectional association between amyloid burden and white matter hyperintensities in older adults without cognitive impairment using a systematic review and meta-analysis. In **chapter 3,** we explored the association between novel blood plasma markers for AD pathophysiology with MRI markers of vascular pathology and neurodegeneration. The **second section** dove into possible psychosocial factors influencing dementia risk. In **chapter 4,** we assessed psychosocial factors such as depressive symptoms, anxiety symptoms, early-life and late-life adversity, and social support, in relation to hippocampal (subfield) atrophy. In **chapter 5 and chapter 6,** we attempted to elucidate possible mechanisms explaining the relation between depression and dementia. We first performed a systematic review and meta-analysis on amyloid-beta pathology and depression in older adults without dementia in **chapter 5**. In **chapter 6,** we performed an IPD meta-analysis on 8 Dutch cohorts assessing the relation between novel AD plasma biomarkers and depressive symptoms. Finally, **chapter 7** explored if the neurotoxicity hypothesis or the vascular hypothesis explained the relation between depression and dementia. The **final section** of the thesis examined the use of data-driven techniques on moving towards precision medicine of dementia by using this biopsychosocial approach. **Chapter 8** used a data-driven clustering technique to explore if allostatic load risk profiles explained the relationship between depression and dementia. **Chapter 9** implemented machine learning techniques that allow for complex statistical interactions between biological and psychosocial factors to assess the 12-year risk for incident dementia in individuals without any cognitive impairment.

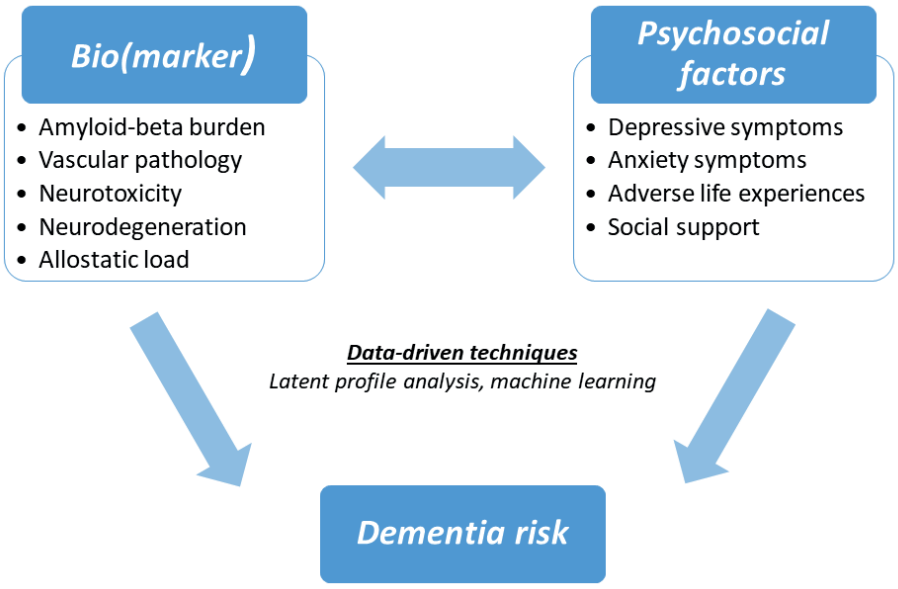

Figure 1. Schematic representation of the topics discussed in this thesis.

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PART 1

BIOMARKERS OF DEMENTIA

Chapter 2

The cross-sectional association between amyloid burden and white matter hyperintensities in older adults without cognitive impairment: a systematic review and meta-analysis

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Abstract

Alzheimer's disease (AD) is the most common cause of dementia, characterized by the aggregation of amyloid-beta (Aβ) proteins into plaques. Individuals with AD frequently show mixed pathologies, often caused by cerebral small vessel disease (CSVD), resulting in lesions such as white matter hyperintensities (WMH). The current systematic review and meta-analysis investigated the cross-sectional relationship between amyloid burden and WMH in older adults without objective cognitive impairment. A systematic search performed in PubMed, Embase, and PsycINFO yielded 13 eligible studies. Aβ was assessed using PET, CSF, or plasma measurements. Two meta-analyses were performed: one on Cohen's d metrics and one on correlation coefficients. The meta-analyses revealed an overall weighted small-to-medium Cohen's d of 0.55 (95% CI: 0.31–0.78) in CSF, an overall correlation of 0.31 (0.09–0.50) in CSF, and a large Cohen's d of 0.96 (95% CI: 0.66–1.27) in PET. Only two studies assessed this relationship in plasma, with an effect size of – 0.20 (95% CI: –0.75 to 0.34). These findings indicate a relationship between both amyloid and vascular pathologies in cognitively normal adults in PET and CSF. Future studies should assess the possible relationship of blood amyloid-beta and WMH for broader identification of at risk individuals showing mixed pathology in preclinical stages.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by declining cognitive functioning and quality of life, accounting for 60-80% of all dementia cases (1). The pathogenesis of AD begins two to three decades before the onset of clinical symptoms, providing opportunity for prevention and intervention. Further, AD pathology rarely occurs in isolation and is typically due to mixed pathology, in which brain changes are associated with multiple causes contributing to dementia (2).

AD pathology includes aggregation of amyloid-beta (Aβ) protein in the brain as well as vascular lesions, where blood vessels in the brain and/or brain tissue are damaged due to not receiving enough oxygen, blood, or nutrients. Cerebral small vessel disease (CSVD) refers to a group of diseases that affect these small cerebral blood vessels (3). One marker of CSVD, visualized on magnetic resonance imaging (MRI), includes white matter hyperintensities (WMH). WMH are common in healthy older adults and are associated with increasing age (4). Additionally, WMH are more frequently present in individuals with AD (5) and are also associated with an increased risk of AD (6). The severity of WMH increases faster over time in individuals with AD compared to healthy older adults (7) and are also associated with worse cognitive functioning (8, 9). In individuals with AD, amyloid burden is associated with WMH (6, 10, 11). Moreover, AD is associated with increased WMH long before the expected onset of symptoms (12), suggesting WMH may play a role in the preclinical stage of AD. However, previous literature assessing the association between amyloid burden and WMH has shown conflicting results in older adults both with and without cognitive impairment. While some studies found that higher amyloid burden was related to more WMH (11, 13-17), other studies did not detect such a relationship (11, 17-25). While AD pathology and vascular pathology could be independent processes, a previous literature review (26) highlighted that a relation may still exist and is blurred by differences in methods between studies. Further, there have been conflicting results when assessing amyloid in different modalities or within different isoforms. For example, van Westen, Lindqvist (11) reported a significant association only for plasma Aβ38 and Aβ40, but not for plasma Aβ42 or when using 18F positron emission topography (PET). Brickman, Guzman (13) found a significant association only when observing amyloid categorically, not continuously. Therefore, a systematic review and meta-analysis that explores these methodological differences and their impact on the association between amyloid-beta and WMH is warranted.

Three previous systematic reviews (6, 26, 27), one including a meta-analysis (6), investigated the cross-sectional association between amyloid and WMH in older adults without cognitive impairment. Two reviews found no association between

amyloid and WMH in cognitively unimpaired older adults (6, 26). However, one review (26) only included studies using PET imaging to assess amyloid burden; while the other review (6) only included two studies in the meta-analysis. The most recent review (27) included 14 studies that assessed amyloid, and all but two found a significant relationship between the two pathologies. Further, only three studies solely focused on cognitively unimpaired individuals. By focusing on cognitively unimpaired individuals in the current review and multiple modalities of amyloid assessment, we can better quantify the relationship between the two leading pathologies of AD during its extended preclinical stage. In this current systematic review and meta-analysis, we investigated the cross-sectional relationship between amyloid burden and WMH in older adults without objective cognitive impairment. Systematic evidence for the presence or absence of an association between amyloid and WMH in cognitively unimpaired older adults could provide more insight into the pathogenesis of AD and its relationship with CSVD in the preclinical stage of AD.

Methods

This systematic review and meta-analysis was performed following the PRISMA guidelines (28) (Supplementary Info 1).

Search and study selection

A search string for studies that investigated the association between amyloid and WMH in older adults without cognitive impairment was developed in consultation with a librarian (P.W., acknowledgments) for PubMed, and it was subsequently translated to Embase and PsycINFO (Supplementary Info 2). On May 7, 2021, the MEDLINE, Embase and PsycINFO databases were searched, after which duplicates were removed with EndNote (v. 20.2) (29) reference management software. Subsequently, two reviewers (E.T. and B.M.) independently screened titles and abstracts using the Rayyan app (30) to assess eligibility. Full texts of the remaining articles were retrieved and screened against eligibility criteria. Any disagreements were resolved by discussion. Snowballing and reverse snowballing were performed by scanning the reference lists of the included articles for any other publications of interest as well as searching Scopus for other works that cited the included articles.

An updated search was performed on February 7, 2022 and the same procedures as listed above were performed independently by two reviewers (E.T. and M.B.) for the additional articles.

Eligibility criteria

Studies eligible for inclusion reported a cross-sectional association between amyloid burden—measured by PET imaging, cerebrospinal fluid (CSF), or blood plasma assays—and WMH, as measured by MRI or CT scan. Studies had an observational cross-sectional or longitudinal design with reported baseline characteristics and associations. Clinical trials were excluded. Only articles reporting associations in older adults without objective cognitive impairment were included. Therefore, studies on individuals who reported subjective cognitive impairment may have been included. No criteria for age of the participants, language, or publication date were set.

Studies reporting only spatial (e.g., only deep or periventricular) measurements of WMH on MRI, as our focus was on total WMH volume, or only longitudinal associations were excluded. Moreover, studies were excluded if they included only participants with the same amyloid status or if there was insufficient information to calculate an effect size. If the same study cohort was used in multiple articles, the study with the largest sample size was included.

Data extraction and risk of bias assessment

Information about the size of the cohort, participant demographics and characteristics, measurements, amyloid method (PET, CSF, or plasma), amyloid isoforms (Aβ40 and Aβ42), metrics (continuous or categorical), WMH assessment, and associations between amyloid and WMH were extracted from the selected articles.

The risk of bias assessment was performed using an adjusted version of the Newcastle-Ottawa Quality Assessment Scale for Cohort Studies (Supplementary Info 3), where the included studies were rated with stars based on nine criteria within the following sections: quality of participant selection, comparability of cohorts based on the design or analysis, and quality of outcome assessment.

Statistical analysis

Statistical analysis was performed using R version 4.0.3 (31). The outcomes of the individual studies were transformed into Cohen's d using means and standard deviations, point-biserial correlations, and Cohen's f using the *esc* package in R (32), if the data was available. If correlation coefficients were reported, they were included in a separate meta-analysis. Effect sizes were reversed if amyloid burden was measured by CSF or blood plasma, as lower amyloid levels in CSF or plasma represent a higher amyloid burden in the brain (33, 34). Therefore, all studies with a positive effect size (i.e., Cohen's d or correlation) represent a relation between more WMH with higher amyloid burden. A random-effects model was used to calculate the

pooled estimates from the Cohen's d and correlation coefficients separately using the *meta* and *metafor* packages (35, 36). We chose a random-effects model over a fixed-effects model because in the presence of heterogeneity, a random-effects meta-analysis weights the studies relatively more equally than a fixed-effect analysis (37).

As some studies had multiple amyloid metrics from the same subjects (i.e., reporting both Aβ40 and Aβ42, reporting continuous and categorical scales, reporting both adjusted and unadjusted results), some analyses were not included in the calculation of the pooled estimate for the overall meta-analysis to avoid those studies getting weighted multiple times in the meta-analysis. Preference was given to continuous data, the isoform Aβ42, and analyses adjusted for covariates.

Heterogeneity was tested using Cochran's Q test and I^2 statistic. Moderate heterogeneity was rated as 30-60%, substantial heterogeneity as 50-90%, and considerable heterogeneity as more than 75% based on the Cochrane Handbook (38). The risk of publication bias was assessed by visual inspection of funnel plots and the Egger's t-test. To explore heterogeneity, subgroup analyses were performed based on amyloid assessment method (PET, CSF, or plasma), covariate adjustment, amyloid classification (continuous or categorical), and WMH assessment (Fazekas score or volumetric). As APOE e4 genotype can influence the relation between AD pathology and WMH (26), we also performed a meta-regression on prevalence of APOE e4 genotype per study. The statistical significance threshold was set at $p < 0.05$.

Results

Search results

A total of 1287 articles were found after duplicate removal, of which 43 full-text articles were assessed for eligibility (Figure 1). After full-text screening, 13 studies were included in the meta-analysis (13-16, 20-25, 39-41) (Figure 1).

The demographics of the subjects of the included studies are shown in Table 1. The included studies consisted of a total of 2649 participants, with a mean age ranging from 59-85 years, the percentage of females ranging from 13-65%, and a mean education ranging from 14-18 years, if reported. Six studies (46.2%) reported APOE ε4 allele positivity, with a range of 21-34% of participants having at least one APOE ε4 allele. Six studies (46.2%) measured amyloid with PET imaging, two studies (15.4%) measured amyloid in plasma, and five studies (38.5%) measured amyloid in CSF. In the studies that used PET imaging, half of the studies used the 11C-PiB PET tracer

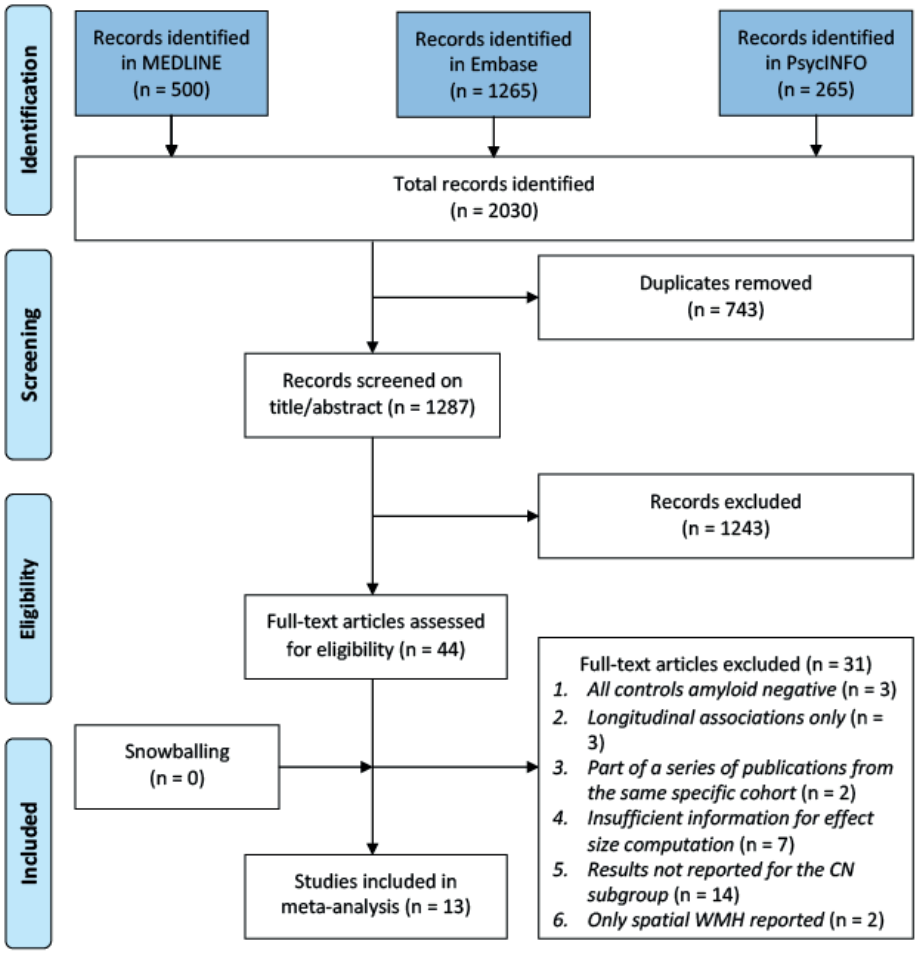

Figure 1. PRISMA flow chart of the original literature search.

and the other half used a 18F PET tracer. Most of the studies looked globally with the cerebellar cortex as reference. All CSF studies used an ELISA assay. For plasma, one study assessed amyloid via endothelial-derived exosomes, and the other used the Luminex xMAP assay. Nine of the 13 studies (69.2%) assessed WMH volumes using automated procedures. Three (42.9%) of the seven studies using CSF or blood plasma measured only Aβ42, while four studies (57.1%) measured both Aβ40 and Aβ42. Eleven studies (84.6%) reported a continuous scale for amyloid burden, two studies (15.4%) reported only a categorical scale, and one study (7.7%) reported both. For the categorical studies, Kaffashian, Tzourio (22) used tertiles to assess amyloid-beta

levels and Kandel, Avants (14) reported that 31% of the sample was amyloid-positive. All studies determined WMH on MRI, except for one study (7.7%) that used a CT scan. Moreover, five studies (38.5%) separated participants into groups based on their WMH burden, while the other studies (61.5%) used WMH burden as a continuous outcome. While three studies (23.1%) used the Fazekas score for WMH assessment, the other 10 studies (76.9%) used volumes. Covariates (e.g., age, sex/gender, education, or other factors) were controlled for in eight studies (61.5%). Five studies (38.5%) reported correlation coefficients, whereas the rest of the studies reported metrics that could be converted into a Cohen's d.

Table 1. Characteristics of the participants of the included studies in the meta-analysis.

Study	Cohort	Cohort origin	Sample size	Age (Mean ± SD in years)
Abner, Elahi (39)	Sander-Browns Center on Aging of University of Kentucky, Memory and Aging Center of University of California	Memory clinic	42	73.5 ± 1.6
Brickman, Guzman (13)	Washington Heights Inwood Columbia Aging Program (WHICAP)	Population	14	82.5 ± 3.3
Gokcal, Horn (40)	Massachusetts General Hospital	Research center	38	70 ± 7.1
Hedden, Mormino (20)	Harvard Aging Brain Study	Population	109	73.5 ± 5.8
Jonsson, Zetterberg (21)	Leukoaraiosis and disability in the elderly (LADIS) project	Hospital	53	74 ± 4.8
Kaffashian, Tzourio (22)	Three-City Dijon Study	Population	1693	72.4 ± 4.1
Kandel, Avants (14)	Alzheimer's Disease Neuroimaging Initiative (ADNI)	Population	158	73.5 ± 6.1
Kester, Goos (41)	Amsterdam Dementia Cohort	Memory clinic	337	59 ± 9
Osborn, Liu (15)	Vanderbilt Memory & Aging Project	Population	77	72 ± 7
Schreiner, Kirchner (23)	Hospital for Psychogeriatric Medicine at University of Zurich	Hospital	27	70.3 ± 5.7
Skoog, Kern (16)	Individuals living in Gothenburg	Population	30	85.4 ± 0.1
van Waalwijk van Doorn, Ghafoorian (24)	Biomarkers for Alzheimer's and Parkinson's Disease (BiomarkAPD) project	Hospital	52	61.1 ± 8.9
Yi, Won (25)	Keimyoung University Dongsan Medical Center	Memory clinic	19	62.5 ± 5.5

Note: CSVD = cerebral small vessel disease; CAA = cerebral amyloid angiopathy.

Risk of bias within and across studies

Studies scored between four and nine stars on the risk of bias assessment (Table 2). Regarding selection criteria, four studies lost stars as their sample was not representative of an older community-dwelling adult without dementia (30.8%). Moreover, five studies (38.5%) did not adjust for any covariates (e.g., age, sex/gender, education, or other). One study (7.7%) did not measure WMH via MRI; therefore, it lost a star regarding ascertainment of the outcome. One study (7.7%) used median cut-offs for amyloid assessment, losing a star regarding the exposure. One study scored all nine stars. The funnel plot to assess publication bias was not fully symmetric

Sex/gender distribution (% women)	Education (Mean ± SD in years)	APOE ε4 positive, %	Vascular burden
52%	18 ± 1	-	52% deemed having CSVD based on Fazekas
43%	-	-	Participants had an average of 1.8 vascular risk factors (i.e., diabetes, hypertension, or heart disease)
13%	-	-	All participants had CAA, 64% having hypertension
-	-	-	-
47%	-	-	-
61%	60% high school or less	21%	77% had hypertension, 8% had diabetes, 6% had prior cardiovascular disease
52%	16 ± 3	30%	-
42%	-	34%	26% had hypertension, 10% had diabetes, 3% had myocardial infarction
29%	17 ± 2	29%	48% on anti-hypertensives, 13% had diabetes
41%	16 ± 2	30%	Vascular risk factors were low (no uncontrolled hypertension/hyperlipidemia, no diabetes, no smoking)
53%	-	-	13% had a stroke
65%	-	-	-
63%	14 ± 3	21%	-

with Cohen's d metrics as four dots lie outside the funnel. However, there was a symmetrical funnel plot for the studies reporting correlation coefficients (Figure 2). The Egger's t statistic did not confirm a publication bias (Cohen's d: bias = 1.95, SE = 1.37, t(6) = 1.42, p = 0.20; correlation coefficient: bias = 1.60, SE = 1.08, t(3) = 1.48, p = 0.24).

Meta-analysis

The study characteristics and effect sizes (Cohen's d and correlation coefficients) of the included studies are shown in Table 3. The meta-analysis of the eight studies resulted in an overall weighted Cohen's d of 0.45 (95% CI: 0.07-0.82, p = 0.02). An overall weighted correlation coefficient on the four other studies was 0.17 (95% CI: 0.03-0.31, p = 0.02) (Figure 3). There was substantial heterogeneity in the pooled estimate for Cohen's d (Q(7) = 50.83, p < 0.001, I^2 = 86.2%) (42). However, there was little to no heterogeneity for the studies reporting correlation coefficients (Q(4) = 3.40, p = 0.49, I^2 = 0.0%).

Table 2. Risk of bias assessment using the adjusted Newcastle-Ottawa Quality Assessment Scale Cohort Studies.

	Selection		
Study	**Representative**	**Selection**	**Exposure**
Abner, Elahi (39)	-	*	*
Brickman, Guzman (13)	*	*	*
Gokcal, Horn (40)	-	*	*
Hedden, Mormino (20)	*	*	*
Jonsson, Zetterberg (21)	*	*	*
Kaffashian, Tzourio (22)	*	*	-
Kandel, Avants (14)	*	*	*
Kester, Goos (41)	-	*	*
Osborn, Liu (15)	*	*	*
Schreiner, Kirchner (23)	*	*	*
Skoog, Kern (16)	*	*	*
van Waalwijk van Doorn, Ghafoorian (24)	*	*	*
Yi, Won (25)	-	*	*

Note: In Gokcal et al. (2022), presence of intracerebral hemorrhage was also included as a confounder. In Kaffashian et al. (2014), adjustments were also done for prior cardiovascular disease, diabetes mellitus, body mass index, hypertension, low-density and high-density lipoprotein cholesterol, triglycerides, uric acid, serum creatinine, and APOE ε2 and ε4 allele presence. In Osborn et al. (2018), models were also adjusted for race/ethnicity, intracranial volume, cognitive diagnosis, a modified Framingham Stroke Risk Profile, and APOE ε4 allele presence. In van Waalwijk van Doorn et al. (2021), models were also corrected for research center.

Six out of the 13 studies reported information regarding the prevalence of APOE e4 genotype. There were too little studies reporting APOE e4 genotype in the correlation coefficient meta-analysis to perform a meta-regression. However, for the meta-analysis on Cohen's d studies, a meta-regression on those studies did not reveal that the prevalence of an APOE e4 allele had an impact on the meta-analysis ($p = 0.05$).

Subgroup analyses

To explore heterogeneity, subgroup analyses were performed. When assessing a difference across methods for amyloid burden, there was a significant subgroup difference in the Cohen's d studies ($Q(2) = 13.97$, $p < 0.001$). The meta-analysis of the three studies in CSF resulted in an overall weighted effect size of 0.55 (95% CI: 0.31-0.78, $p < 0.001$) (Figure 4). For the three PET studies, an overall weighted effect size of 0.96 (95% CI: 0.66-1.27, $p < 0.001$) was found. For the two plasma studies, an effect size of -0.20 (95% CI: -0.75-0.34, $p = 0.47$) was found. There was substantial heterogeneity in the plasma studies ($Q(1) = 3.27$, $p = 0.07$, $I^2 = 69.5\%$) (42). There was no heterogeneity found in the CSF studies ($Q(2) = 0.79$, $p = 0.64$, $I^2 = 0\%$) or in the PET studies ($Q(2) = 0.88$, $p = 0.64$, $I^2 = 0\%$).

	Comparability			Outcome		Overall (max. 9)
Age	Sex/gender	Education	Other factors	Outcome	Same method	
-	-	-	-	*	*	4
-	-	-	-	*	*	5
*	*	-	*	*	*	7
*	-	-	-	*	*	6
-	-	-	-	*	*	5
*	*		*	*	*	7
*	*	*	-	*	*	8
*	*	-	-	*	*	6
*	*	*	*	*	*	9
-	-	-	-	*	*	5
-	-	-	-	-	*	4
*	*	-	*	*	*	8
*	*	-	-	*	*	6

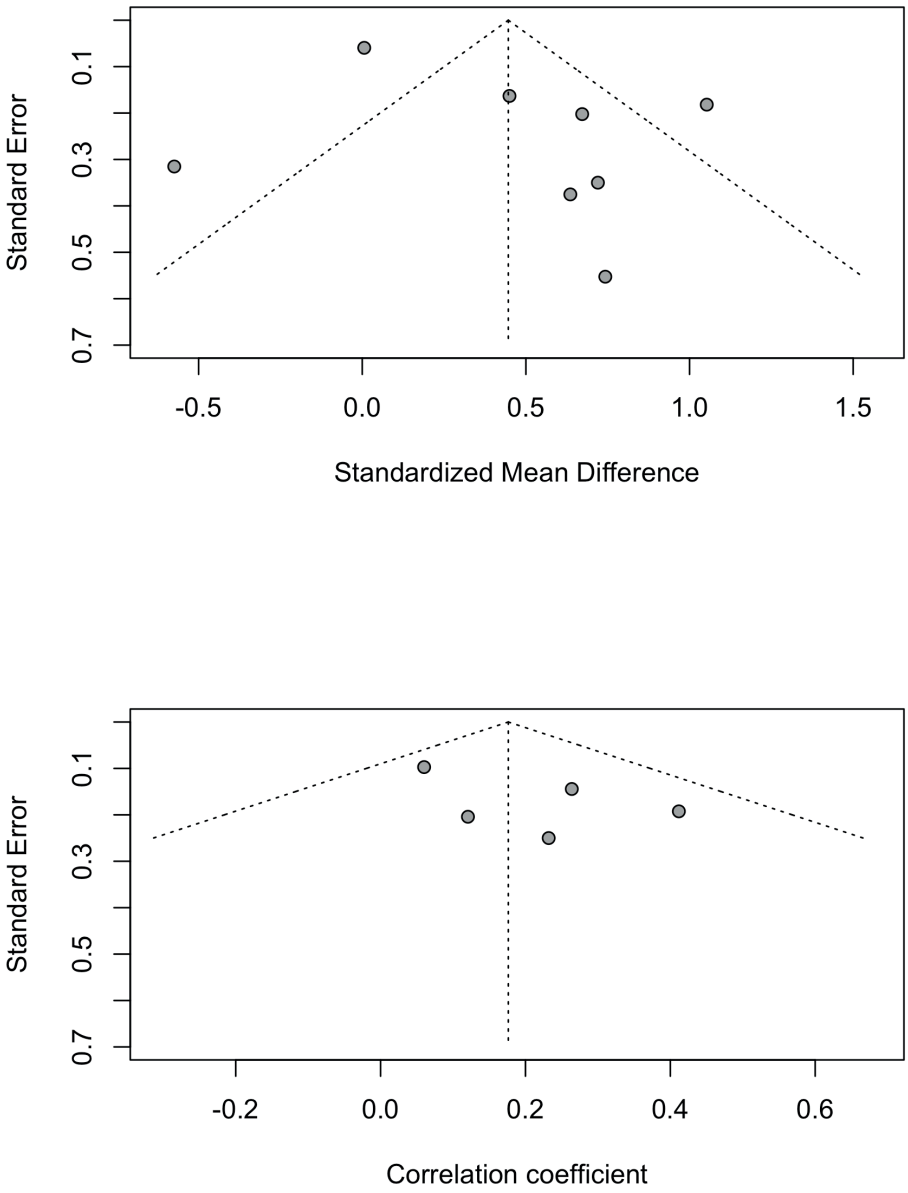

Figure 2. Funnel plot of the 8 studies converted to Cohen's d and the 5 studies using correlation coefficients.

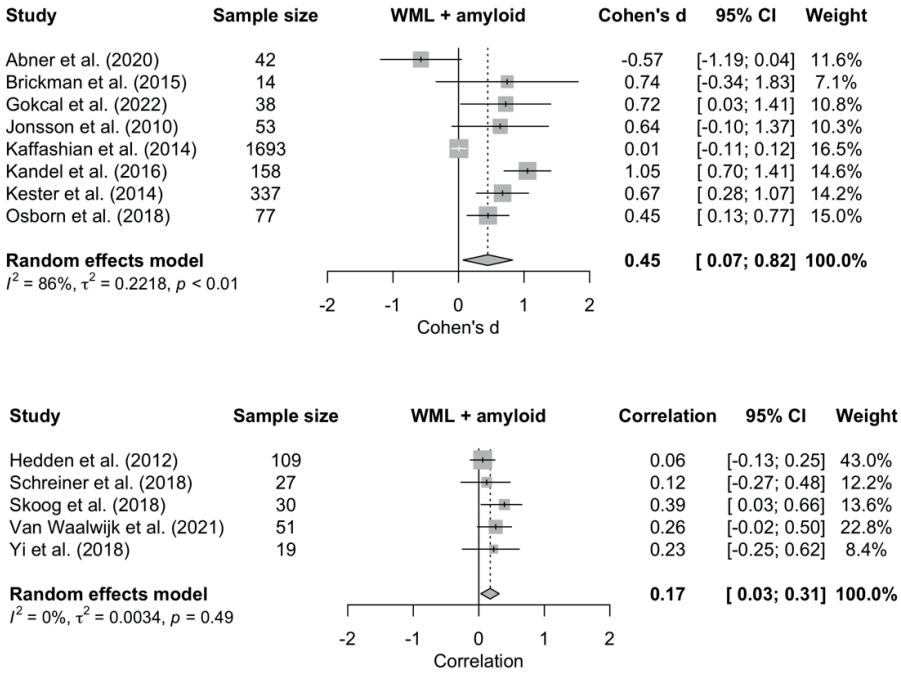

Figure 3. Forest plot of the two meta-analyses for a total of 13 studies of the relationship between amyloid and WMH in cognitively normal older adults. The effect sizes of the individual studies are represented by the squares, of which the size is proportional to the weight of the study. The diamond represents the pooled estimate. The effect sizes of analyses using continuous data, Aβ42, and adjusted for covariates were used for the meta-analysis, when a study reported multiple analyses.

For the studies reporting correlation coefficients, there was not a subgroup difference between studies using PET or CSF (Q(1) = 2.58, p = 0.11). The overall weighted correlation coefficient for CSF studies was 0.31 (95% CI: 0.09; 0.50, p = 0.01). The overall weighted correlation coefficient for PET studies was 0.09 (95% CI: -0.07; 0.25, p = 0.28) (Figure 4). Neither PET nor CSF studies showed heterogeneity.

The studies that adjusted for covariates showed more variance compared to studies that did not adjust for covariates (Supplementary Figure 1). The confidence interval of the pooled effect size as well as the heterogeneity of the studies using a categorical scale were greater than studies using a continuous scale (Supplementary Figure 2); however, this subgroup analysis could only be performed in the Cohen's d meta-analysis. This pattern was also seen for studies using the Fazekas score compared

Table 3. Study characteristics and effect sizes.

Study	N	Amyloid scale	Amyloid method
Abner, Elahi (39)	42	Continuous	Plasma, endothelial-derived exosomes, ELISA assay
Brickman, Guzman (13)	14	Continuous Categorical	^{18}F PET in either frontal, temporal, parietal, posterior cingulate, or occipital cortices
Gokcal, Horn (40)	38	Continuous	^{11}C-PiB PET, globally with cerebellar cortex as reference
Hedden, Mormino (20)	109	Continuous	^{11}C-PiB PET, only in the frontal, lateral parietal and temporal, retrosplenial cortices
Jonsson, Zetterberg (21)	53	Continuous	CSF, MSD Multi-Array ($A\beta_{40}$) & Luminex xMAP ($A\beta_{42}$)
Kaffashian, Tzourio (22)	1693	Categorical	Plasma, Luminex xMap
Kandel, Avants (14)	158	Categorical	^{18}F PET, globally with cerebellar cortex as reference
Kester, Goos (41)	337	Continuous	CSF, ELISA
Osborn, Liu (15)	77	Continuous	CSF, ELISA
Schreiner, Kirchner (23)	27	Continuous	^{11}C-PiB PET, posterior cingulate and precuneus
Skoog, Kern (16)	30	Continuous	CSF, ELISA
van Waalwijk van Doorn, Ghafoorian (24)	52	Continuous	CSF, ELISA
Yi, Won (25)	19	Continuous	^{18}F PET, globally with cerebellar cortex as reference

Note: WMH = white matter hyperintensities. ELISA = enzyme-linked immunosorbent assay. PiB = Pittsburgh compound B. PET = positron emission tomography. SUVR = standardized uptake value ratio. CSF = cerebrospinal fluid. Aβ = amyloid-beta.

to studies assessing WMH volumes (Supplementary Figure 3). This pattern could be explained by less studies in the subgroups with wider confidence intervals and larger heterogeneity.

Nonetheless, no significant subgroup differences were found when the studies were stratified by covariate adjustment (Cohen's d: 3 vs. 5 study groups, Q(1) = 0.44, p = 0.51; correlation coefficients: 2 vs. 3 study groups, Q(1) = 0.65, p = 0.42), amyloid scale (Cohen's d: 2 vs. 6 study groups, Q(1) = 0.03, p = 0.87), or WMH assessment (Cohen's d: 6 vs. 2 study groups, Q(1) = 0.55, p = 0.46).

WMH assessment	Covariate controlled	Cohen's d±SE or correlation coefficient
Fazekas score	No	-0.24 ± 0.31 (Aβ40)
		-0.57 ± 0.31 (Aβ42)
Volumetric, automated	No	0.74 ± 0.55
		2.31 ± 0.69
Volumetric, automated	Age, sex, presence of intracerebral hemorrhage	0.72 ± 0.35
Volumetric, automated	Age	0.06 ± 0.10
Volumetric, automated	No	0.05 ± 0.19 (Aβ40)
		0.64 ± 0.19 (Aβ42)
Volumetric, automated	Age and sex	0.02 ± 0.06 (Aβ40)
		0.01 ± 0.06 (Aβ42)
		-0.08 ± 0.06 (Aβ42/40)
Volumetric, automated	Age, sex/gender, education	1.05 ± 0.18
Fazekas score	Age, sex, medial temporal lobe atrophy	0.67 ± 0.20 (Aβ42)
Volumetric, automated then confirmed manually	Age, sex, race/ethnicity, education, intracranial volume, cognitive diagnosis, APOE4, vascular risk factors	0.45 ± 0.16 (Aβ42)
Volumetric, automated	No	0.12 ± 0.19
Volumetric, via CT scan	No	0.46 ± 0.15 (Aβ40)
		0.39 ± 0.16 (Aβ42)
Volumetric, automated with a ML algorithm then checked manually	Age, sex, and research center	0.26 ± 0.13 (Aβ42)
Fazekas score	Age and sex	0.23 ± 0.22

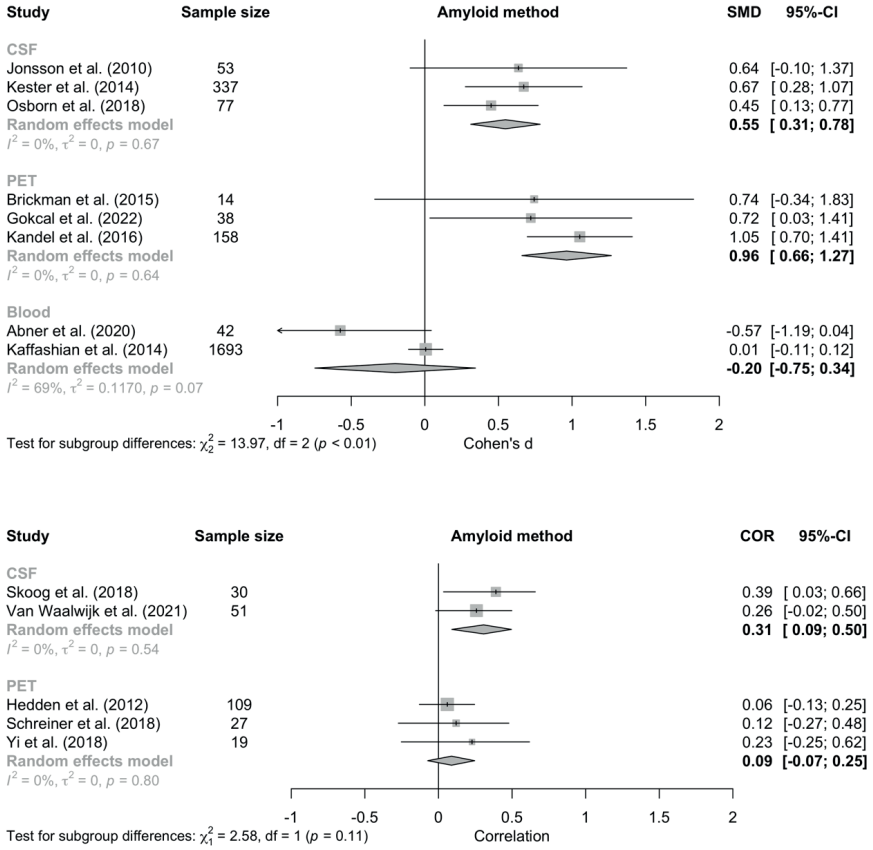

Figure 4. Forest plot of the subgroup meta-analyses based on amyloid assessment method (PET, CSF, or plasma) for a total of 13 studies. The effect sizes of the individual studies are represented by the squares, of which the size is proportional to the weight of the study. The diamond represents the pooled estimate. The horizontal lines represent the 95% confidence intervals of the individual effect sizes. The effect sizes of analyses using continuous data, Aβ42, and adjusted for covariates were used for the meta-analysis, when a study reported multiple analyses.

As WMH are more common in women (43), we also performed a sensitivity analysis removing Hedden, Mormino (20), as they did not report the sex distribution in the analytical sample on WMH. However, our meta-analysis on correlation coefficients remained similar (0.26; 95% CI: 0.08-0.42). Further, we performed a sensitivity analysis removing the studies that did not adjust for age (13, 16, 21, 23, 39). Results remained similar in the overall Cohen's d (Cohen's d: 0.45, 95% CI: 0.07-0.82). However, in the overall correlation meta-analysis, there was only a trend towards significance (r: 0.14, 95% CI: -0.02-0.29).

Discussion

This systematic review and meta-analysis included 13 studies that explored the cross-sectional association between amyloid burden in CSF, PET, and plasma and WMH in older adults without objective cognitive impairment. The meta-analysis using Cohen's d yielded an overall effect size of 0.45, which is considered small- to medium-sized (44). The meta-analysis on correlation coefficients yielded a pooled correlation of 0.17. When stratified by amyloid assessment method, a Cohen's d of 0.55 for the CSF studies and 0.96 was found for the PET studies and a pooled correlation of 0.31 was found for the CSF studies. No association was found for the plasma studies. Almost half of the studies did not adjust for covariates which increased the risk of bias regarding comparability. The funnel plot and Egger's t-test did not reveal evidence for publication bias. Moreover, subgroup analysis revealed that the overall substantial heterogeneity (42) in the Cohen's d meta-analysis was driven by amyloid burden assessment method. However, substantial heterogeneity remained in the plasma studies.

Although many of the included studies did not find evidence for an association between amyloid burden and WMH, this meta-analysis revealed the presence of a small-to-medium sized cross-sectional relationship between the two pathologies which is also in line with the findings of a recent systematic review (27). Among the included studies, one reported a negative association and the remaining 12 reported a positive association. However, 8 of the 13 studies reported non-significant associations. By reducing the variance of the individual studies and increasing power, this meta-analysis was able to reach more precision, whereas most of the individual studies could not. Further, our findings are in line with a recent study of more than 500 individuals that also found an association between WMH and amyloid-beta burden in cognitively unimpaired individuals (45). These findings suggest a possible role in the prevention of CSVD in delaying AD and pathological aging.

In the meta-analysis on correlation coefficients, studies measuring amyloid in CSF showed a significantly higher association with WMH than amyloid measured with PET imaging or in plasma. Amyloid in CSF is a more sensitive marker for early disease stages of AD than amyloid PET imaging (46)—the current meta-analysis only included cognitively unimpaired older adults, which may explain this subgroup difference. Low but present amyloid burden may not have been accurately detected with PET imaging, resulting in only detecting a relationship of WMH with amyloid pathology using CSF in cognitively unimpaired older adults. This hypothesis might also explain why Roseborough, Ramirez (26) did not find a cross-sectional association

between amyloid burden and WMH in cognitively unimpaired older adults, as they only included studies assessing amyloid burden with PET in their systematic review. This could also explain the discrepancy in results between PET and CSF modalities. However, this was only seen in the meta-analysis using correlation coefficients. For the meta-analysis on studies where Cohen's d could be calculated, PET studies showed a slightly higher effect size. This could have been due to the large effect size from Kandel, Avants (14), which may be explained by their categorical classification of amyloid burden. Lastly, while the largest included study that used plasma assessment (22) did not find a cross-sectional association, a longitudinal association was found. With the development of more sensitive assays for plasma Aβ since this study was performed, future studies should assess if plasma amyloid burden may be a more prognostic marker for vascular burden.

Further, substantial heterogeneity was found within the studies using plasma assessment in the Cohen's d meta-analysis. This may be due to differing performance between plasma amyloid assays (47) leading to different results. In addition, while plasma and CSF amyloid have shown positive correlations amongst varying assays (34, 47), some differences have been seen in the relationship between plasma amyloid and AD and plasma amyloid and vascular disease. For example, one study reported higher plasma amyloid in association with vascular risk factors and lower plasma amyloid across the continuum of subjective cognitive decline, mild cognitive impairment, and AD, whereas consistent results were seen with CSF (34). However, as there were only two studies that assessed amyloid in plasma, we could not perform further subgroup analysis. As assessing amyloid burden through plasma is a relatively new modality, further studies need to be done to fully understand the relationship between plasma amyloid burden and WMH.

Some studies could not be included in the meta-analysis due to insufficient information for effect size calculation. Although, these studies also reported higher WMH associated with amyloid burden in PET (19, 48, 49) and CSF (11, 49-51). However, some studies found no association (18, 52, 53). As a previous study found that 18F predominantly labels vascular amyloid (54), we reason that the null finding in Koncz, Thalamuthu (52) could be due to that they combined 11C and 18F PET tracers in their methodology. Further, Dupont, Bocti (18) measured amyloid with 11C PET, possibly explaining the null finding. Methodological discrepancies could also explain the null finding in Marchant, Reed (53), as they characterized vascular burden by not only WMH but also by infarct presence. Studies that only reported spatial WMH also confirmed our findings on PET (55, 56) and CSF (55). However, one study assessing plasma amyloid burden did find an association with periventricular

and subcortical WMH (57); whereas, in our meta-analysis no association was found for amyloid burden in plasma and WMH. Longitudinal studies also reported similar findings (7, 58), where baseline Aβ42 predicted WMH (58) as well as baseline WMH predicting amyloid PET (7).

There are some limitations of the current review and meta-analysis. Only one article (24) included participants with subjective cognitive decline, thus no subgroup analysis could be performed based on cognitive status. Moreover, only a small number of studies could be analyzed in some subgroup analyses (amyloid assessment scale, covariate adjustment, and WMH assessment), giving less precise estimates of the effect sizes and less power for determining significant subgroup differences. There is also a possibility that the association of amyloid burden on WMH is mediated by other vascular factors, such as hypertension. However, as we focused on cross-sectional studies, mediation analyses would have limitations and none of the included studies assessed possible mediation through other factors. Further, as we chose to focus on cross-sectional associations to reduce complexity and heterogeneity, future research should explore the longitudinal relationship between amyloid burden and WMHs to assess their temporal relationship as well as any additive effects. Due to some studies reporting multiple metrics, such as continuous and categorical data, some subjective decisions methodologically were made for the meta-analysis which could have introduced some bias. There was some discrepancy for Aβ40 and Aβ42 in Abner, Elahi (39) and Jonsson, Zetterberg (21), with stronger associations found for Aβ42. This difference is in line with previous studies showing stronger associations with Aβ42 than Aβ40 with cognitive decline and later dementia. However, most studies that reported multiple metrics showed similar directional associations between them. Of note, most of the included studies did not report education level of the participants or included those mostly highly educated. Further, only one study (15) reported the ethnicity of participants, which was 93% White. This is of importance as these findings are not generalizable due to the homogeneity of included individuals regarding education and ethnicity. As most research has traditionally focused on White participants with high education, future studies should include historically marginalized individuals to ensure generalizability. This systematic review and meta-analysis also was not registered on PROSPERO as data extraction had already been performed. However, one of the key reasons for review registration is to prevent duplication, and no current protocols in PROSPERO were on the same topic as the current review. Lastly, the current study did not have an age restriction, and as WMH are common with aging, the age range could have obscured the relation between amyloid burden and WMH.

A suggestion for future studies is to further investigate the nature of the relationship between amyloid burden and WMH and to determine the underlying mechanisms of how vascular damages to small cerebral vessels may affect amyloid burden in early stages of AD, or vice versa. Future research should also examine other pathologies of AD (e.g., tau and neurodegeneration) and their association with CSVD neuroimaging markers (e.g., WMH, lacunes, cerebral microbleeds, enlarged perivascular spaces). Since it is hypothesized that the impairment of the glymphatic system, which includes perivascular spaces, plays a role in amyloid burden (59), a future direction could be to investigate the relationship between enlarged perivascular spaces and amyloid burden. To increase the number of possible included studies, we decided to only include studies that reported total WMH volume. However, we realize that this choice could have introduced bias into the study towards the null. Previous studies have highlighted region-specific associations between parietal WMH and amyloid-beta burden (60-62). We assume if we would have differentiated between spatial regions of WMH that we would have a higher effect estimate. Interestingly, previous studies have found that the spatial topography of WMH matches the deposition of cortical amyloid. Future studies could also assess if parietal WMH hold stronger associations with CSF and plasma amyloid burden. Moreover, examining the longitudinal association between amyloid and WMH could provide more insight in the progression of amyloid burden and WMH over time and their mechanistic pathways in the development of AD (63-65).

In conclusion, this meta-analysis demonstrated a small to medium-sized cross-sectional association between amyloid burden and WMH in CSF and PET in older adults without objective cognitive impairment. As the number of individuals suffering from dementia is expected to increase over the next decades, studying the preclinical stage of AD is of great importance for prevention and potential intervention. The current study highlights the possible use of CSF and PET to assess comorbid amyloid and vascular pathology during the preclinical stage of AD. While no association was found between amyloid burden in blood and WMH, future studies should still consider estimating a possible relation for broader implementation using a cost-effective assessment for amyloid burden. The continued study of the mixed pathologies across the continuum from healthy aging to dementia may provide more insight in the development of the disease and the origins of its heterogeneity.

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Conflict of interest

The authors have no conflict of interest to report.

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SUPPLEMENTARY MATERIAL

Supplemental Info 1: PRISMA Checklist

Section and Topic	Item #	Checklist item	Location where itemis reported
TITLE			
Title	1	Identify the report as a systematic review.	Title page
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	2-4
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	3-4
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	4-5
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	4
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Supplement
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	4
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	5-6
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	5-6
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	5

Supplemental Info 1: Continued

Section and Topic	Item #	Checklist item	Location where itemis reported
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	5
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	5-6
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	4-5
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	5-6
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	5-6
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	6
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	6
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	9-10
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	8
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	8-10

PRISMA 2020 Checklist: Continued

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where itemis reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	7, Fig 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	7, Fig 1
Study characteristics	17	Cite each included study and present its characteristics.	7-8
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	8
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	24, 27
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	22-23
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	8
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	9-10
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	9-10
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	8
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	8
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	10-13
	23b	Discuss any limitations of the evidence included in the review.	13-14
	23c	Discuss any limitations of the review processes used.	13-14
	23d	Discuss implications of the results for practice, policy, and future research.	15

PRISMA 2020 Checklist: Continued

Section and Topic	Item #	Checklist item	Location where itemis reported
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	14
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	14
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NA
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	16
Competing interests	26	Declare any competing interests of review authors.	16
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	24

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. Doi: 10.1136/bmj.n71

For more information, visit: http://www.prisma-statement.org/

Supplementary Info 2: Search strategies for Pubmed, Embase and PsycINFO

Pubmed

((“Amyloid”[Mesh] OR “Plaque, Amyloid”[Mesh] OR amyloid*[Title/Abstract] OR AB[Title/Abstract] OR AB40[Title/Abstract] OR AB42[Title/Abstract] OR AB 40[Title/Abstract] OR AB 42[Title/Abstract] OR BA[Title/Abstract] OR BA40[Title/Abstract] OR BA42[Title/Abstract] OR BA 40[Title/Abstract] OR BA 42[Title/Abstract] OR B amyloid[Title/Abstract] OR Abeta[Title/Abstract] OR Abeta40[Title/Abstract] OR Abeta42[Title/Abstract] OR abeta 40[Title/Abstract] OR abeta 42[Title/Abstract] OR PIB[Title/Abstract] OR Pittsburgh compound B [Title/Abstract] OR flutemetamol[Title/Abstract] OR florbetapir[Title/Abstract] OR florbetaben[Title/Abstract] OR senile plaque*[Title/Abstract])

AND

(“Positron-Emission Tomography”[Mesh] OR PET[Title/Abstract] OR Positron emission tomograph*[Title/Abstract] OR “Cerebrospinal Fluid”[Mesh] OR CSF[Title/Abstract] OR cerebrospinal fluid*[Title/Abstract] OR cerebro spinal fluid*[Title/Abstract] OR plasma[Title/Abstract] OR plasmas[Title/Abstract] OR athologylogy*[Title/Abstract] OR amyloid athology*[Title/Abstract] OR “Neuropathology”[Mesh]))

AND

(“Leukoaraiosis”[Mesh] OR white matter athologylogy*[Title/Abstract] OR white matter hyper athology*[Title/Abstract] OR WMH[Title/Abstract] OR white matter lesion*[Title/Abstract] OR WML[Title/Abstract] OR white matter change*[Title/Abstract] OR white matter athol*[Title/Abstract] OR white matter signal abnormalit*[Title/Abstract])

Embase

((amyloid*:ti,ab,kw OR ab:ti,ab,kw OR ab40:ti,ab,kw OR ab42:ti,ab,kw OR ‘ab 40’:ti,ab,kw OR ‘ab 42’:ti,ab,kw OR ba:ti,ab,kw OR ba40:ti,ab,kw OR ba42:ti,ab,kw OR ‘ba 40’:ti,ab,kw OR ‘ba 42’:ti,ab,kw OR ‘b amyloid’:ti,ab,kw OR abeta:ti,ab,kw OR abeta40:ti,ab,kw OR abeta42:ti,ab,kw OR ‘abeta 40’:ti,ab,kw OR ‘abeta 42’:ti,ab,kw OR pib:ti,ab,kw OR ‘pittsburgh compound b’:ti,ab,kw OR flutemetamol:ti,ab,kw OR florbetapir:ti,ab,kw OR florbetaben:ti,ab,kw OR ‘senile plaque*’:ti,ab,kw OR ‘amyloid’/exp OR ‘amyloid plaque’/exp)

AND

(pet:ti,ab,kw OR 'positron emission tomograph*':ti,ab,kw OR csf:ti,ab,kw OR plasma:ti,ab,kw OR plasmas:ti,ab,kw OR athologylogy*:ti,ab,kw OR 'amyloid athology*':ti,ab,kw OR 'positron emission tomography'/de OR 'cerebrospinal fluid'/exp OR 'neuropathology'/exp))

AND

('white matter athologylogy*':ti,ab,kw OR 'white matter hyper athology*':ti,ab,kw OR wmh:ti,ab,kw OR 'white matter lesion*':ti,ab,kw OR 'white matter change*':ti,ab,kw OR 'white matter athol*':ti,ab,kw OR 'white matter signal abnormalit*':ti,ab,kw OR wml:ti,ab,kw OR 'leukoaraiosis'/exp OR 'white matter lesion'/exp)

PsycINFO

((Amyloid or "Plaque, Amyloid").mh. or amyloid*.ti,ab. Or AB.ti,ab. Or AB40.ti,ab. Or AB42.ti,ab. Or "AB 40".ti,ab. Or "AB 42".ti,ab. Or BA.ti,ab. Or BA40.ti,ab. Or BA42.ti,ab. Or "BA 40".ti,ab. Or "BA 42".ti,ab. Or "B amyloid".ti,ab. Or Abeta.ti,ab. Or Abeta40.ti,ab. Or Abeta42.ti,ab. Or "abeta 40".ti,ab. Or "abeta 42".ti,ab. Or PIB.ti,ab. Or "Pittsburgh compound B".ti,ab. Or flutemetamol.ti,ab. Or florbetapir.ti,ab. Or florbetaben.ti,ab. Or "senile plaque*".ti,ab.)

and

("Positron-Emission Tomography".mp. or PET.ti,ab. Or "Positron emission tomograph*".ti,ab. Or "Cerebrospinal Fluid".mp. or CSF.ti,ab. Or "cerebrospinal fluid*".ti,ab. Or "cerebro spinal fluid*".ti,ab. Or plasma.ti,ab. Or plasmas.ti,ab. Or athologylogy*.ti,ab. Or "amyloid athology*".ti,ab. Or Neuropathology.mp.)

and

(Leukoaraiosis.mp. or "white matter athologylogy*".ti,ab. Or "white matter hyper athology*".ti,ab. Or WMH.ti,ab. Or "white matter lesion*".ti,ab. Or WML.ti,ab. Or "white matter change*".ti,ab. Or "white matter athol*".ti,ab. Or "white matter signal abnormalit*".ti,ab.)

*Supplementary Info 3: Adjusted version of the Newcastle-Ottawa Quality Assessment Scale Cohort Studies*Note: A study can be awarded a maximum of one star for each

numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability. Total maximum number of stars is nine.

Selection

1. Representativeness of the exposed cohort (amyloid positive/WMH presence)

a. truly representative of the average older adult without dementia in the community (i.e., community-based cohort and mix of nondemented individuals with and without subjective complaints) *
b. somewhat representative of the average older adult without dementia in the community (e.g., if a certain selection is made which makes the individuals 'more' cognitively normal, e.g., only nondemented individuals without subjective complaints) *
c. selected group of users, e.g., volunteers, memory clinic visitors, only individuals at higher risk (only subjective complaints, only depressive symptoms, only APOE e4 carriers)
d. no description of the derivation of the cohort

2. Selection of the non-exposed cohort (amyloid negative/no WMH)
a. drawn from the same community as the exposed cohort *
b. drawn from a different source
c. no description of the derivation of the non-exposed cohort

3. Ascertainment of exposure
a. continuous measurement *
b. categorized based on established or published cut-offs *
c. categorized based on non-established cut-offs (e.g., z-score cut-off, mean split, median split)
d. no description

Comparability

1. Comparability of cohorts on the basis of the design or analysis
- study controls for age *
- study controls for sex/gender *
- study controls for education *
- study controls for any additional factor *

Outcome (WMH)

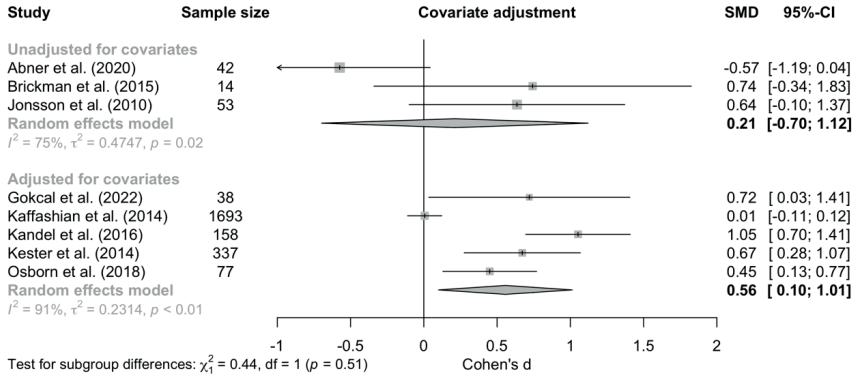
1. Ascertainment of outcome
   (48) Via MRI scan *

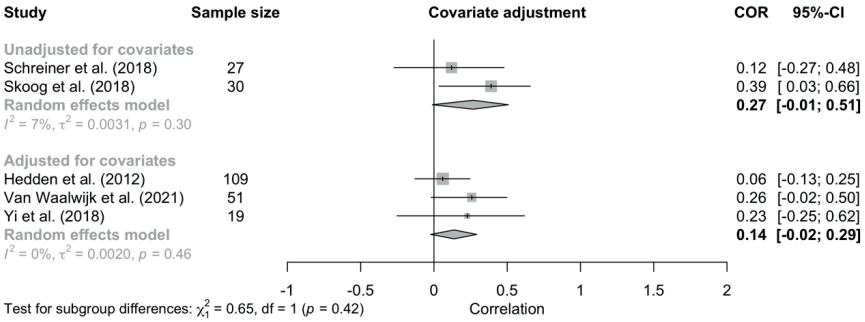
c. no description

2. Same method of assessment for cases (amyloid positive) and controls (amyloid negative)

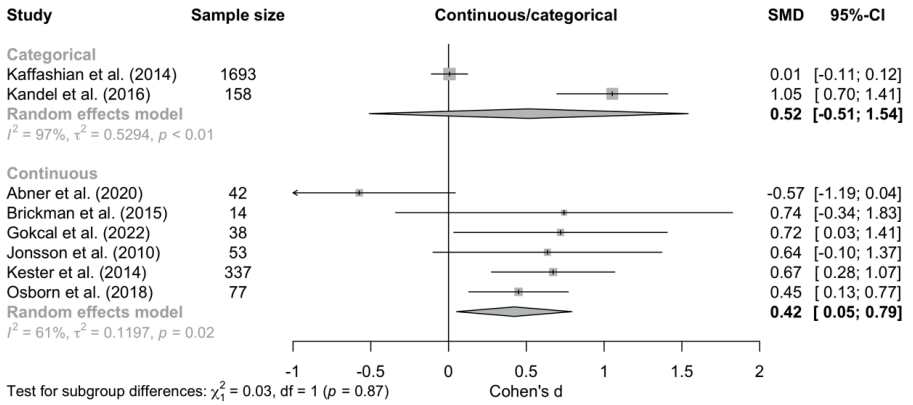
a. yes *

b. no

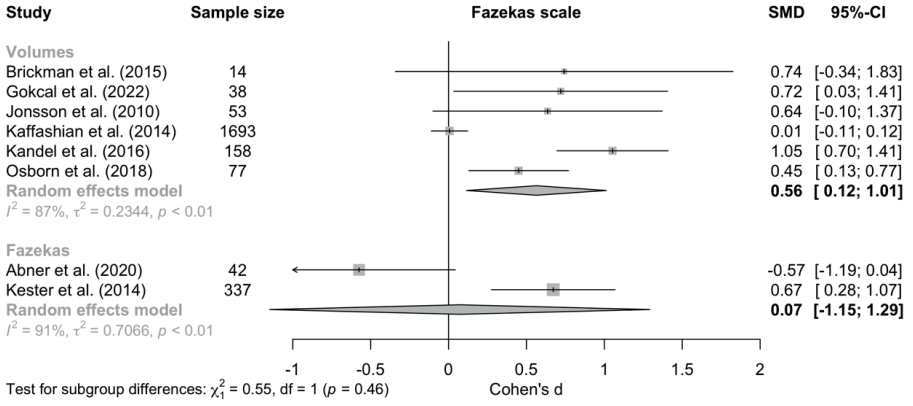




Supplementary Figure 1. Forest plot of the subgroup meta-analysis for a total of 13 studies including analyses unadjusted and adjusted for covariates. The effect sizes of the individual studies are represented by the squares, of which the size is proportional to the weight of the study. The diamond represents the pooled estimate. The horizontal lines represent the 95% confidence intervals of the individual effect sizes.



Supplementary Figure 2. Forest plot of the subgroup meta-analysis based on amyloid assessment scale for a total of 8 studies. The effect sizes of the individual studies are represented by the squares, of which the size is proportional to the weight of the study. The diamond represents the pooled estimate. The horizontal lines represent the 95% confidence intervals of the individual effect sizes.



Supplementary Figure 3. Forest plot of the subgroup meta-analysis based on WMH assessment (volumes or Fazekas score) for a total of 8 study groups. The effect sizes of the individual studies are represented by the squares, of which the size is proportional to the weight of the study. The diamond represents the pooled estimate. The horizontal lines represent the 95% confidence intervals of the individual effect sizes.

Chapter 3

Plasma Alzheimer's disease markers and MRI load of vascular pathology and neurodegeneration: the SMART-MR Study

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Lotte Gerritsen
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Charlotte E. Teunissen
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UCC-SMART Study Group

on behalf of the NCDC Consortium

Submitted, JAHA

Abstract

Background: Two of the main causes for dementia are Alzheimer's disease (AD) and vascular pathology, with most patients showing mixed pathology. Plasma biomarkers for AD-related pathology have recently emerged, including amyloid-beta (Aβ), phosphorylated (p-)tau, neurofilament light (NfL), and glial fibrillary acidic protein (GFAP). There is a current gap in the literature if there is an association between these plasma biomarkers with vascular pathology and neurodegeneration. We investigated this association in individuals with manifest arterial disease and without dementia.

Methods: Data from 594 individuals (mean (SD) age: 64 (8) years; 17% female) were included from the SMART-MR Study, a prospective cohort study of individuals with a history of vascular disease. AD-related plasma markers (Aβ42/40, ptau-181, NfL, and GFAP) were assessed using Single Molecular Array assays (Quanterix). Vascular and neurodegenerative MRI markers included WMH volume, presence of infarcts (yes/no), total brain volume (TBV), and hippocampal volume (HV) assessed on 1.5T MRI. Linear regressions were performed for each standardized plasma marker with WMH volume, TBV, and HV as separate outcomes, correcting for age, sex, education, and intracranial volume. Logistic regressions were performed for the presence of lacunar and cortical infarcts.

Results: Higher ptau-181 was associated with larger WMH volume (b per SD increase=0.16, 95% CI=0.06; 0.26, p=0.015). Higher NfL (b=-5.63, 95% CI=-8.95; -2.31, p=0.015) was associated with lower TBV and the presence of infarcts (OR=1.42, 95% CI=1.13; 1.78, p=0.039). Higher GFAP levels were associated with cortical infarcts (OR=1.45, 95% CI=1.09; 1.92, p=0.010). No other associations were found.

Conclusions: These findings highlight the role of mixed AD and vascular pathology in individuals with manifest vascular disease.

Introduction

Two of the main causes of dementia are Alzheimer's disease (AD) and vascular pathology (1). The presence of AD pathology, i.e. amyloid-beta (Aβ) plaques and neurofibrillary tangles, can be established using cerebrospinal fluid (CSF) or position emission topography (PET). Vascular pathology is typically assessed via MRI measures, such as white matter hyperintensities (WMHs) and lacunes (2). Most patients with cognitive decline and dementia have mixed pathology (3), as well as hippocampal and global brain atrophy (4). The relationship between AD pathology with vascular pathology and neurodegeneration is not yet well known and has been hampered by the invasiveness and costs associated with CSF and PET measurements (5-8).

Recent advancements in the development of high-sensitivity plasma assays have allowed for the assessment of biomarkers for Aβ and phosphorylated tau (p-tau) pathology, as well as neurodegeneration (neurofilament light; NfL) and astrocyte activation (glial fibrillary acidic protein; GFAP) in large-scale studies (9). Previous studies have highlighted a possible relation between these AD-related plasma biomarkers, specifically p-tau181, NfL, and GFAP with vascular pathology on MRI (10-15). However, other studies found no association (16-18). Regarding neurodegeneration on MRI, most studies have found that higher levels of p-tau181 and NfL are associated with greater atrophy, either globally (19, 20) or specifically in the hippocampus (10, 16, 18, 19, 21-23).

However, few studies focused on patients with vascular disease. Neurodegeneration (24) and WMH are common in vascular patients, and many patients show mixed AD and vascular pathology (25, 26). By focusing on a population with vascular disease, it is possible to shed light on the role of AD pathology in the neurodegeneration and WMH seen in vascular patients. We took as a starting point the SMART-MR cohort, a population all with manifest vascular disease. We aimed to examine if blood-based biomarkers (i.e., Aβ42/40, p-tau181, NfL, and GFAP) were associated with MRI measures of cerebrovascular disease and neurodegeneration in individuals with manifest vascular disease and without dementia diagnosis. We hypothesized that NfL, as a general marker of neurodegeneration, would be related to global and hippocampal atrophy, while p-tau181, Aβ42/40, and GFAP, as more specifically related to amyloid pathology, would be related to vascular pathology based on previous studies in non-vascular populations.

Methods

Design of study and sample

Data were obtained from the Second Manifestations of ARTerial disease-Magnetic Resonance (SMART-MR) study. The SMART-MR study is a prospective cohort study which aimed to investigate brain MRI changes in patients independently living with symptomatic atherosclerotic disease (27, 28). All patients that were recently referred to the University Medical Center Utrecht in the Netherlands with manifest cerebrovascular disease, coronary artery disease, peripheral arterial disease, or an abdominal aortic aneurysm and without any MRI contraindications were invited to participate between May 2001 and December 2005. For this study, we used cross-sectional data from the second wave of the SMART-MR study (n=754) (27, 29). A further selection was done for biomarker assessment, particularly being 50 years or older, having a brain MRI scan, and available cognitive measurements (n=594). MRI brain scans, physical examinations, blood sampling, and questionnaires were all performed during a one-day visit at the hospital. Written informed consent was obtained from all participants. A local ethics committee approved the SMART-MR study.

This study was reported in accordance to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist (Supplemental Methods).

Plasma assessment

Briefly, participants underwent venipuncture under overnight-fasting conditions. Plasma was then centrifuged for 10 minutes at 1,800 x g within 2 hours. Then, in polypropylene tubes, plasma was aliquoted in 0.5-mL aliquots and stored at -80^{o}C until use. Aβ40, Aβ42, NfL, and GFAP were all assessed using the Neurology 4-plex E kit (Quanterix) (30). P-tau181 was assessed using the V2 Advantage kit (Quanterix). Measurements were performed according to manufacturer's instructions, using automated sample dilution on board of the Simoa HD-X analyzer. The Neurology 4-plex E kit was ran in singlicates, and the P-tau181 V2 kit was ran in duplicates. We calculated Aβ42/40, to adjust for between-person differences in production rates and to correct for pre-analytical sample handling effects (31). All plasma markers were z-score standardized for analysis.

MRI protocol

Brain MRI was performed using a 1.5 Tesla whole-body system (Gyroscan ACS-NT, Philips Medical System, Best, The Netherlands) (29). A transversal T1-weighted gradient-echo (repetition time (TR)/echo time (TE): 235/2 ms), T2-weighted (TR/TE: 2200/11 ms), fluid-attenuated inversion recovery (FLAIR) (TR/TE/inversion time (TI):

6000/100/2000 ms), and T1-weighted inversion recovery (IR) (TR/TE/TI: 2900/22/410 ms) sequences were acquired with a voxel size of 1.0 x 1.0 x 4.0 mm^3 as part of the protocol (29). For measurement of hippocampal volume, a sagittal T1-weighted 3D fast field-echo sequence was obtained (TR/TE: 7/3.2 ms) (32).

Brain segmentation

WMH and brain volumes were segmented using an automated segmentation program on the T1-weighted gradient-echo, the IR sequence, and the FLAIR sequences. More details regarding the probabilistic segmentation technique can be found here (33, 34). The hippocampus was manually outlined by two trained investigators, blinded to all clinical information (32). The hippocampus proper, subiculum, fimbria, alveus, and dentate gyrus were all included on an average of 40 slices (35). TBV was defined as the sum of both gray and white matter, WMH, and infarct volumes. Total intracranial volume (ICV) was the sum of both TBV as well as the volumes of sulcal and ventricular CSF. Hippocampal volume was the sum of both right and left hippocampi, which were defined by multiplying the total number of voxels by the volume of a voxel (i.e., 1.0 x 0.94 x 0.94 mm).

Infarcts and WMH

Infarcts were rated visually by both an investigator and a neuroradiologist. Both were blinded to any clinical characteristics and re-evaluated in a consensus meeting(36). WMHs were defined as periventricular and deep lesions and were summed to represent the total volume of WMHs using a fully automated technique and visually checked (29, 36).

Infarcts were described as focal hyperintensities on T2-weighted images more than 3mm in diameter and presumed of vascular origin (37). If the T2 hyperintensities were within white matter, they also had to be hypointense on T1-weighted and FLAIR images to be differentiated from WMHs. They were then characterized as lacunar or cortical infarcts. According to the Standards for Reporting Vascular Changes on Neuroimaging criteria (2), lacunar infarcts were defined as focal lesions between 3 and 15mm. Cortical infarcts were defined as being an area of tissue necrosis 4mm or larger in cortical or cortico-subcortical areas(38). Presence of any infarct was categorized as a dichotomous outcome (i.e., yes/no). Lacunar and cortical infarcts were also categorized as any or none.

Covariates

Age, sex, education, smoking status, and alcohol use were given based on self-report. Education was categorized into less than high school education, at least some high

school education, or college/university education based on the Dutch education system. Smoking status was categorized as never, former, or current smoker. Alcohol use was categorized as <1 drinks per week, 1-10 drinks per week, 11-20 drinks per week, and >20 drinks per week. Diabetes mellitus was defined as a (self-)reported history of diabetes, registered use of glucose-lowering medication (i.e., insulin or oral antidiabetic drugs), or a glucose level of seven mmol/L or higher. Hypertension was defined as self-reported use of antihypertensive medication, a mean systolic blood pressure of more than 140 mmHg, or a mean diastolic blood pressure of more than 90 mmHg.

Data analysis

To address missing values (max: 10% missing on hippocampal volume), multiple imputation was performed using the *mice* package in R. Both outcomes and predictors were imputed if needed, using covariate information as predictors in the imputation (39). We chose 10 imputed datasets, as a complete case analysis would be on 90% of the original sample size (40). Pooled results are shown. Kruskal-Wallis tests were done to assess possible differences between sexes in plasma marker levels, and Pearson correlations were performed to assess the relationship between the plasma marker levels, brain volumes, and age. Linear regressions were performed to estimate the association between each standardized plasma marker and log-transformed WMH volume, TBV, and HV, with age, sex, education, and intracranial volume as covariates. Logistic regressions were performed for the presence of infarcts (yes vs no), corrected for age, sex, and education. An additional model was performed correcting for further cardiovascular risk factors (i.e., diabetes mellitus, hypertension, smoking status, and alcohol use). A sensitivity analysis was performed assessing the difference between lacunar and cortical infarcts by performing logistic regressions separately on cortical and lacunar infarcts. Another sensitivity analysis was done on the models of Aβ42/40 by adding 1/Aβ40 and Aβ42 as main effects, as suggested by previous work on the complexities of using a ratio in regression analyses (41). We also report individual associations of Aβ40 and Aβ42 on all outcomes. Assumptions for both linear and logistic regressions were checked and met; therefore, no plasma markers were log-transformed. As there was moderate correlation between our outcomes, we used the Hommel method (42) for multiple comparison adjustment (43).

Results

The characteristics of the study population are shown in Table 1. The mean age of the study population was 64 ± 8 years. Around 17% of the individuals were women and 10% had an education level of a college/university degree. Eight percent met Petersen criteria(44) for mild cognitive impairment. A Pearson correlation matrix on age, the AD plasma markers, and the MRI markers can be found in Supplementary Figure 1. Kruskal-Wallis tests showed no sex differences in Aβ42/40 and p-tau181. Higher levels of NfL and GFAP were seen in women compared to men.

Table 1. Baseline characteristics of the study population.

	Study sample (n=594)
Demographics	**Mean (SD) or *n* (%)**
Age (years)	64 (8)
Sex, women	101 (17%)
Education, college/university	57 (10%)
Current smoker	127 (21%)
Alcoholic drinks per week	1.14 (0.97)
Diabetes mellitus	131 (22%)
Hypertension	401 (68%)
Petersen criteria	48 (8%)
Plasma levels (pg/ml)	
Aβ40	113.19 (30.64)*
Aβ42	6.79 (1.94)*
Aβ42/40	0.06 (0.01)*
P-tau181	1.37 (0.79)*
NfL	13.82 (9.56)*
GFAP	86.33 (53.98)*
MRI markers	
White matter lesion volume, ml	1.24 (2.50)*
Hippocampal volume, ml	5.98 (0.75)
Total brain volume, ml	1137.43 (105.14)
Infarct presence	206 (35%)
Lacunar infarct presence	137 (23%)
Cortical infarct presence	83 (14%)
Model 1	

Note: 1% missing on education, 1% missing on smoking, 1% missing on alcohol use, 1% missing on diabetes, 1% missing on hypertension, 3% missing on Petersen criteria, and 10% missing on hippocampal volume. * shown as median (IQR).

All plasma markers were non-normally distributed. The respective median (10%-90% range) for Aβ42/40, p-tau181, GFAP, and NfL were 0.06 (0.05-0.07), 1.37 (0.85-2.54), 86.33 (47.28-151.97), and 13.82 (7.86-28.59) (shown in pg/mL). For the MRI markers, only WMH volume was non-normally distributed. The mean (SD) for hippocampal volume and TBV were 5.98 mL (0.75) and 1137 mL (105), respectively. The median (10-90% range) for WMH volume was 1.24 (0.29-8.12) mL. Thirty-five percent of our study population had at least one infarct, with 23% having at least one lacunar infarct and 14% having at least one cortical infarct.

Associations with MRI markers of vascular pathology

Linear regression analysis showed that higher plasma p-tau181 was associated with higher WMH volume (b per standard deviation increase: 0.16, 95% CI: 0.06; 0.26, p = 0.015), which remained after further correction (Table 2). Higher NfL showed a trend towards higher WMH volume, but it did not survive correction for multiple comparisons. No other biomarkers were associated with WMH, also when adding 1/Aβ40 and Aβ42 as main effects or assessed individually (Table S1).

Regarding infarcts, higher plasma NfL was associated with higher odds of having an infarct (OR: 1.42, 95% CI: 1.13; 1.78, p = 0.039); however, this did not remain after further covariate adjustment (Table 3). When looking specifically at lacunar infarcts, higher plasma NfL was associated with higher odds of a lacunar infarct (OR: 1.36, 95% CI: 1.06; 1.73, p = 0.014). Regarding cortical infarcts, both higher plasma NfL and GFAP were associated with higher odds of having a cortical infarct (respectively OR: 1.58, 95% CI: 1.20; 2.08, p = 0.001 and OR: 1.45, 95% CI: 1.09; 1.92, p = 0.010) (Table 3). No other plasma markers were associated with the presence of infarcts (Table 3; Table S2).

Associations with MRI markers of neurodegeneration

Higher plasma NfL was associated with lower TBV (b: -5.63, 95% CI: -8.95; -2.31, p = 0.015), which remained after further covariate adjustment (Table 2). There were no associations between plasma markers and hippocampal volume, albeit an association was found for higher Aβ40 and lower hippocampal volume (Table S1).

Table 2. Associations between the plasma AD markers and MRI markers of vascular disease and neurodegeneration.

Plasma levels (per SD increase)	WMH volume, B (95% CI), adjusted p-value	TBV, B (95% CI), adjusted p-value	HV, B (95% CI), adjusted p-value
Model 1			
Aβ42/40	-0.03 (-0.13; 0.06), $p = 0.827$	-1.39 (-4.00; 1.23), $p = 0.827$	0.01 (-0.04; 0.07), $p = 0.827$
P-tau181	**0.16 (0.06; 0.26), $p = 0.015$**	-2.21 (-5.05; 0.63), $p = 0.750$	-0.02 (-0.07; 0.05), $p = 0.827$
NfL	0.17 (0.06; 0.29), $p = 0.052$	**-5.63 (-8.95; -2.31), $p = 0.015$**	-0.05 (-0.12; 0.02), $p = 0.781$
GFAP	0.07 (-0.04; 0.18), $p = 0.827$	-1.14 (-4.31; 2.04), $p = 0.827$	-0.04 (-0.11; 0.03), $p = 0.827$
Model 2			
Aβ42/40	-0.04 (-0.13; 0.05), $p = 0.785$	-1.26 (-3.81; 1.29), $p = 0.785$	0.01 (-0.04; 0.07), $p = 0.785$
P-tau181	**0.15 (0.06; 0.25), $p = 0.028$**	-1.76 (-4.54; 1.01), $p = 0.785$	-0.01 (-0.07; 0.05), $p = 0.785$
NfL	0.17 (0.05; 0.29), $p = 0.052$	**-5.50 (-8.75; -2.24), $p = 0.016$**	-0.05 (-0.12; 0.02), $p = 0.785$
GFAP	0.09 (-0.03; 0.20), $p = 0.738$	-2.07 (-5.23; 1.09), $p = 0.785$	-0.05 (-0.12; 0.02), $p = 0.738$

Note: Model 1 is adjusted for age, sex, education, and intracranial volume. Model 2 adds diabetes mellitus, hypertension, smoking status, and alcohol use. WMH volume is log-transformed. P-values are adjusted using the Hommel method.

Table 3. Associations between the plasma AD markers and infarcts.

Plasma levels (per SD increase)	Infarcts,OR (95% CI), adjusted p-value	Lacunar infarcts, OR (95% CI), p-value	Cortical infarcts, OR (95% CI), p-value
Model 1			
Aβ42/40	1.18 (0.86; 1.63), $p = 0.827$	1.30 (0.84; 2.02), $p = 0.240$	1.04 (0.85; 1.26), $p = 0.723$
P-tau181	1.16 (0.96; 1.40), $p = 0.729$	1.22 (1.00; 1.50), $p = 0.055$	1.17 (0.92; 1.50), $p = 0.202$
NfL	**1.42 (1.13; 1.78), $p = 0.039$**	**1.36 (1.06; 1.73), $p = 0.014$**	**1.58 (1.20; 2.08), $p = 0.001$**
GFAP	0.98 (0.79; 1.21), $p = 0.827$	0.86 (0.68; 1.10), $p = 0.224$	**1.45 (1.09; 1.92), $p = 0.010$**
Model 2			
Aβ42/40	1.17 (0.83; 1.64), $p = 0.785$	1.27 (0.82; 1.95), $p = 0.288$	1.01 (0.84; 1.23), $p = 0.883$
P-tau181	1.13 (0.93; 1.37), $p = 0.785$	1.19 (0.97; 1.47), $p = 0.101$	1.13 (0.88; 1.45), $p = 0.342$
NfL	1.41 (1.12; 1.79), $p = 0.052$	**1.36 (1.05; 1.74), $p = 0.018$**	**1.54 (1.16; 2.03), $p = 0.003$**
GFAP	1.03 (0.82; 1.30), $p = 0.785$	0.87 (0.68; 1.13), $p = 0.297$	**1.56 (1.16; 2.10), $p = 0.003$**

Note: Model 1 is adjusted for age, sex, and education. Model 2 adds diabetes mellitus, hypertension, smoking status, and alcohol use. P-values are adjusted using the Hommel method.

Discussion

In a sample of individuals with manifest vascular disease and WMH, we found that higher NfL was associated with infarcts and global brain atrophy, higher p-tau181 was associated with more WMH volume, and GFAP was related to the presence of one or more cortical infarcts. None of the biomarkers were associated with hippocampal volume.

Higher levels of plasma NfL were associated with infarcts, which is in line with previous literature (12). As NfL has been linked to axonal damage (45), the relationship between NfL and infarcts could be explained by persisting axonal damage due to vascular pathology (46). Additionally, we found an association between higher NfL and lower TBV, which is in line with a previous longitudinal study (20). However, we did not find an association of plasma NfL with hippocampal atrophy, whereas some studies found an association (18, 22, 23, 47). Further, in this cohort, memory has not been associated with hippocampal volume (48). Hippocampal atrophy in vascular patients may have a different pathological mechanism than global atrophy and may not show a clear relation to memory decline. As this is the first study to our knowledge focusing on plasma AD biomarkers and brain volume in individuals with vascular disease, this needs to be validated in other studies of a similar population. We did not find an association after correction for multiple comparisons between NfL and WMH, which is in line with a former study on a vascular population (17). In contrast, studies in MCI and AD populations have reported such an association (13, 14, 49, 50). This apparent contradiction may be explained by being in a later stage of cognitive impairment or may be due to a loss of power in the current study, as our findings were approaching significance. As studies assessing NfL and MRI markers of vascular disease are scarce, further studies need to be performed to replicate this result.

However, higher p-tau181 was associated with more WMH volume, which is in line with previous studies (10, 11). One study found that amyloid PET pathology was associated with WMH in the general population, with cerebral amyloid angiopathy (CAA) possibly explaining this role (51). As p-tau181 in plasma is associated with amyloid PET positivity (52), CAA could explain the relation between AD-specific biomarkers and WMH. Surprisingly, we did not find an association with p-tau181 and hippocampal volume, even though many previous studies have found an association (10, 16, 19, 21, 22). However, two studies also found a null association (53, 54). Hippocampal atrophy in this vascular population may be independent to AD-specific neuronal loss and solely of vascular origin.

Additionally, GFAP was associated with cortical infarcts. This is in line with a previous study on serum GFAP that found an association of GFAP with infarcts, but not with subcortical vascular pathology such as WMHs (46). The specificity to cortical infarcts was also reflected in a previous study, highlighting that the site of injury may determine if GFAP is released into the blood (55). However, as this is the first study to our knowledge assessing GFAP in plasma with brain infarcts, future research should validate these findings. Aβ and p-tau181 were not associated with infarcts, which is in line with a previous study (5). Infarcts may not have a direct relationship to AD pathology, possibly due to the anatomical location of infarcts compared to WMH (56).

The current study had important strengths. We assessed multiple plasma markers using an ultrasensitive Simoa assay. Further, we used multiple imputation to account for missing data, corrected for multiple covariates, and also used a strict correction for multiple comparisons to prevent any Type I errors. However, the study also had limitations. The study population was relatively young and healthy, as the included participants were a subsample of the SMART-MR study that participated in the first follow-up assessment. Further, participants were predominantly White and male; thus, the generalizability to other populations is low. Other studies have highlighted that plasma p-tau181 and NfL may not accurately represent brain amyloidosis in African American adults compared to White individuals (57). Future studies need to be done on marginally underrepresented individuals to assess any differences regarding plasma AD biomarkers and vascular pathology in these populations. Additionally, we did not have information on microbleeds, so we unfortunately could not assess the relation between the plasma AD markers on cerebral microbleeds.

Plasma biomarkers that have been associated with AD pathophysiology are related to MRI markers of vascular pathology and neurodegeneration in patients with manifest vascular disease. The current study suggests a relationship between AD and vascular pathology in individuals with manifest vascular disease, highlighting the role of mixed pathology in these individuals. Future longitudinal studies should explore if individuals with both of these pathologies are at an increased risk of dementia.

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The authors have nothing to disclose.

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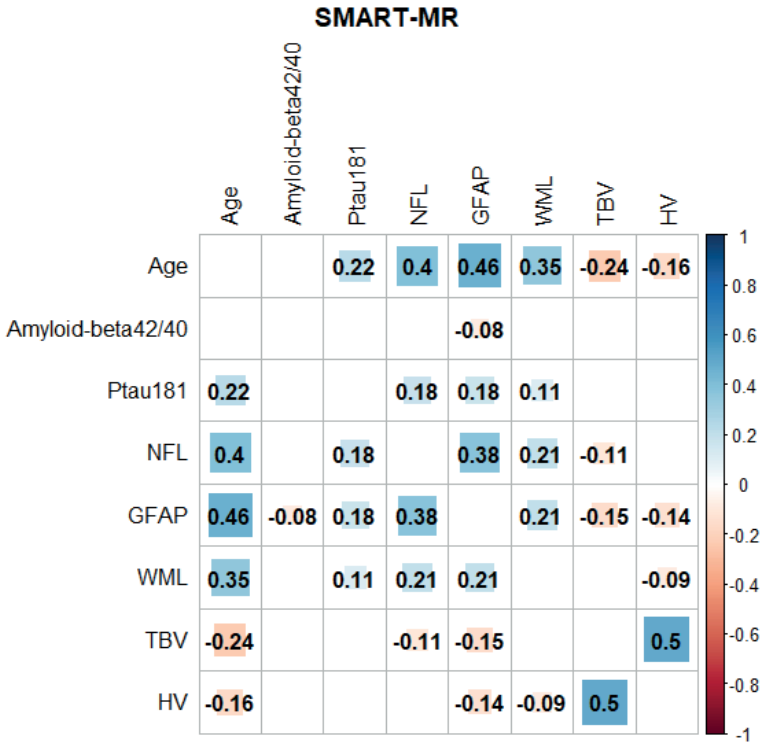
Supplemental Info

Supplemental Figure 1. Correlation matrix between age, AD plasma markers, and MRI markers of vascular pathology.



Note: Pearson correlation coefficients are shown. Bolded coefficients are significant at $p < 0.05$.

Supplemental Table 1. Associations between Aβ40 and Aβ42 and MRI markers of CSVD.

Plasma levels (per SD increase)	White matter hyperintensity volume, B (95% CI), p-value	Total brain volume, B (95% CI), p-value	Hippocampal volume, B (95% CI), p-value
Aβ40	0.12 (0.02; 0.022), $p = 0.018$	-1.88 (-4.71; 0.96), $p = 0.194$	**-0.11 (-0.17; -0.05), $p = 0.001$**
Aβ42	0.09 (-0.01; 0.18), $p = 0.071$	-3.61 (-6.26; -0.96), $p = 0.008$	-0.06 (-0.12; 0.00), $p = 0.051$
Aβ42/Aβ40*	-0.06 (-0.16; 0.03), p = 0.194	-0.45 (-3.16; 2.27), p = 0.747	0.03 (-0.03; 0.09), p =0.271
Model 2			
Aβ40	0.12 (0.01; 0.22), $p = 0.025$	-1.49 (-4.31; 1.33), $p = 0.300$	**-0.11 (-0.18; -0.05), $p = 0.001$**
Aβ42	0.08 (-0.02; 0.17), $p = 0.102$	-3.46 (-6.08; -0.83), $p = 0.010$	-0.06 (-0.12; 0.00), $p = 0.048$
Aβ42/Aβ40*	-0.07 (-0.17; 0.02), p = 0.144	-0.36 (-3.01; 2.29), p = 0.791	0.03 (-0.03; 0.09), p = 0.310

Note: Model 1 is adjusted for age, sex, education, and intracranial volume. Model 2 adds diabetes mellitus, hypertension, smoking status, and alcohol use. White matter lesion volume is log-transformed. * = including 1/Aβ40 and Aβ42 as main effects in the model.

Supplemental Table 2. Associations between Aβ40 and Aβ42 and infarcts.

Plasma levels (per SD increase)	Number of infarcts,OR (95% CI), p-value	Lacunar infarcts, OR (95% CI), p-value	Cortical infarcts, OR (95% CI), p-value
Model 1			
Aβ40	1.00 (0.83; 1.20), $p = 0.958$	0.93 (0.76; 1.14), $p = 0.492$	1.16 (0.91; 1.47), $p = 0.228$
Aβ42	1.05 (0.88; 1.25), $p = 0.620$	1.05 (0.87; 1.28), $p = 0.602$	1.23 (0.98; 1.54), $p = 0.080$
Aβ42/Aβ40*	1.18 (0.84; 1.67), p = 0.344	1.32 (0.80; 2.20), p = 0.279	0.99 (0.77; 1.26), p = 0.909
Model 2			
Aβ40	0.96 (0.79; 1.16), $p = 0.644$	0.87 (0.70; 1.07), $p = 0.185$	1.12 (0.88; 1.44), $p = 0.356$
Aβ42	1.01 (0.84; 1.21), $p = 0.933$	0.99 (0.81; 1.21), $p = 0.896$	1.21 (0.95; 1.53), $p = 0.126$
Aβ42/Aβ40*	1.20 (0.79; 1.83), p = 0.384	1.39 (0.80; 2.41), p = 0.242	0.97 (0.77; 1.23), p = 0.814

Note: Model 1 is adjusted for age, sex, and education. Model 2 adds diabetes mellitus, hypertension, smoking status, and alcohol use. White matter lesion volume is log-transformed. * = including 1/Aβ40 and Aβ42 as main effects in the model.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/ rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4-5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	*Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*)*Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-7
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		I Explain how missing data were addressed	8
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	8
		I Describe any sensitivity analyses	8

	Item No	Recommendation	Page No
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	5
		I Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9
		(b) Indicate number of participants with missing data for each variable of interest	18
		I *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*NA*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*NA*
		Cross-sectional study—Report numbers of outcome events or summary measures	*8-9, 18*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-10
		(*b*) Report category boundaries when continuous variables were categorized	NA
		I If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-10
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11-12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12-13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

PART 2

MAPPING THE BIOMARKERS TO THE PSYCHOSOCIAL

Chapter 4

Psychosocial factors and hippocampal subfields: the Medea-7T Study

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Human Brain Mapping 2023;44(5):1964-1984

Abstract

Specific subfields within the hippocampus have shown vulnerability to chronic stress, highlighting the importance of looking regionally within the hippocampus to understand the role of psychosocial factors in the development of neurodegenerative diseases. A systematic review on psychosocial factors and hippocampal subfield volumes was performed and showed inconsistent results, highlighting the need for future studies to explore this relationship. The current study aimed to explore the association of psychosocial factors with hippocampal (subfield) volumes, using high-field 7T MRI. Data were from the Memory Depression and Aging (Medea)-7T study, which included 333 participants without dementia. Hippocampal subfields were automatically segmented from T2-weighted images using ASHS software. Generalized linear models accounting for correlated outcomes were used to assess the association between subfields (i.e., entorhinal cortex, subiculum, Cornu Ammonis (CA)1, CA2, CA3, dentate gyrus, and tail) and each psychosocial factor (i.e., depressive symptoms, anxiety symptoms, childhood maltreatment, recent stressful life events, and social support), adjusted for age, sex, and intracranial volume. Neither depression nor anxiety was associated with specific hippocampal (subfield) volumes. A trend for lower total hippocampal volume was found in those reporting childhood maltreatment, and a trend for higher total hippocampal volume was found in those who experienced a recent stressful life event. Among subfields, low social support was associated with lower volume in the CA3 (B = -0.43, 95% CI: -0.72; -0.15). This study suggests possible differential effects among hippocampal (subfield) volumes and psychosocial factors.

Introduction

The hippocampus is implicated in many neuropsychiatric diseases, such as depression, schizophrenia, and dementia, where frequently a smaller hippocampal volume has been observed in comparing cases to controls. Based on animal studies, it is thought that the hippocampus is sensitive to stress and that the hippocampus mediates the stress response and release of glucocorticoids from the hypothalamic-pituitary-adrenal (HPA) axis (1). Chronic activation of the HPA axis due to stress or anxiety (2) may lead to volume loss in the hippocampus, which has been demonstrated in studies assessing stressful events (3, 4) and post-traumatic stress disorder (5, 6).

However, the hippocampus is not a homogeneous structure. It is composed of multiple subfields that have shown differential responses to psychosocial factors. In previous animals studies, chronic stress has been shown to suppress neuronal development in the dentate gyrus (DG) and remodel dendrites in the cornu ammonis (CA), specifically in the CA3 (6, 7). Further, neurogenesis inhibition in the DG has been related to psychosocial stress (8). This stress-specificity in hippocampal subfields has also been recently replicated in human studies as well (9-11). However, regarding some psychosocial factors, such as social support, studies have mostly been limited to child or adolescent samples (9, 12-16) and focused on total hippocampal volume rather than exploring the differential effect within subfields (9, 12-15, 17-20). Further, these psychosocial factors, such as low social support (21, 22), depression (23, 24), anxiety (25, 26), and childhood maltreatment (27), have been associated with an increased risk for incident dementia, which could possibly be mediated by hippocampal volumes (28-30).

Therefore, by understanding the role psychosocial factors have on regions of the hippocampus in an adult population, we can better understand how these factors may contribute to the development of neurodegenerative diseases. Early-life stress has shown specific decline in the hippocampus (31), as well as stunted hippocampal growth during adolescence (32, 33), possibly due to programming effects in childhood resulting from an interplay of immune factors and hippocampal neurogenesis (34). This highlights a possible importance of timing of stressful exposure in its influence on brain structure. Further, two reviews have highlighted that type of stressful exposure (e.g., emotional vs. physical abuse) may also have a differential effect on neurobiological alterations (35, 36). However, exploring possible differences of timing (e.g., early- versus late-life trauma) and type of exposure has yet to be assessed with hippocampal subfields.

To get a current overview of the literature, the first aim of the current study is to perform a systematic review of previous studies assessing psychosocial factors on hippocampal subfield volume in adults. The second aim is to examine the association between psychosocial factors and hippocampal (subfield) atrophy using high-field 7T MRI in a large sample. We hypothesized that psychosocial factors such as depression, childhood maltreatment, and anxiety would be associated with total hippocampal volume based on previous reviews (37, 38). We further hypothesized specific associations in the stress-sensitive DG and CA3 areas. Moreover, we hypothesized that lower social support would be negatively associated with hippocampal subfield volumes with no a-priori hypothesis on a specific subfield due to lack of previous research in adults.

Methods

Participants

The Memory Depression and Aging (Medea)-7T study (39) is a cohort study at the University Medical Center (UMC) Utrecht with the aim to investigate risk factors and structural brain changes using 7T MRI in middle-aged and older adults with and without dementia. It is explained in-depth elsewhere (39). In brief, participants were recruited from the following settings: participants from the SMART-MR study (n = 213) (40), participants from the PREDICT-MR study (n = 50) (41), participants 60 years or older without dementia from general practices (n = 70) (39), and patients with mild cognitive impairment or early Alzheimer's disease from memory clinics at the UMC Utrecht (n = 35) through the Utrecht Vascular Cognitive Impairment (VCI) Study group (see Acknowledgements) (39). Between January 2010 and October 2017, 368 participants underwent cognitive testing and MRI measurements. The 35 participants with mild cognitive impairment or dementia from the memory clinics were excluded. This left 333 individuals for the following analyses.

Psychosocial factors

The following psychosocial factors were focused on in this study: depressive symptoms, anxiety symptoms, childhood maltreatment, recent stressful life events, and social support.

Depressive symptoms were assessed with the Patient Health Questionnaire-9 (PHQ-9) (42) in the SMART-MR and PREDICT-MR cohorts and the Geriatric Depression Scale-15 (GDS-15) (43) in the general practices and memory clinics. Elevated depressive symptoms (yes/no) were defined as scoring 6 or above on the PHQ-9 (44) or on the

GDS-15 (45, 46). We chose a cut-off score of 6 or higher on the GDS-15 as it has been highlighted to have a higher sensitivity and specificity in community-based settings, as well as an overall higher specificity (45).

Anxiety was measured by the total score on the Beck Anxiety Inventory (BAI) (range: 0-63) (47) and dichotomized using population cut-offs (48) of 11 and higher being classified as elevated anxiety symptomology.

Childhood maltreatment was measured with a selection of items from the NEMESIS Trauma Interview (49) by a sum score of types of childhood maltreatment (i.e., emotional neglect, psychological abuse, physical abuse, and/or sexual abuse) that occurred before 16 years of age. Emotional neglect was described as not listened to, ignored, or unsupported. Psychological abuse was described as yelled at, insulted, unjustly punished/treated, threatened, belittled, or blackmailed. Physical abuse was defined as being kicked, hit, bitten, or hurt with an object or hot water. Sexual abuse was defined as any unwanted sexual experience. Childhood maltreatment was dichotomized as experiencing no childhood abuse or one or more type of abuse.

Recent stressful life events within the last 12 months were assessed via a questionnaire, including events such as serious illness to oneself or a close relative, job loss, and relational difficulties (50). Stressful events were dichotomized as no recent event or one or more.

Social support was assessed via seven questions regarding perceived current social support (e.g. "There are people in my family and circle of friends who cheer me up"), on a scale of "incorrect", "partially correct", or "totally correct" (51). Scores ranged from 0-14, with high scores representing more support. Social support was categorized into low, medium, and high using a median cut-off. High social support was used as the reference.

For the PREDICT-MR and general practices, all psychosocial questionnaires were completed at the same time point as MRI collection. For the SMART-MR cohort, depression, anxiety, and recent stressful life events were all assessed at the same time point as MRI. However, social support and childhood maltreatment were assessed at an earlier time point, between 7 and 9 years before MRI collection.

Demographics

Age and sex were self-reported through questionnaires.

MRI assessment

Using a 7T MRI system (Philips Healthcare, Cleveland, OH, USA) with a 32-channel receive head coil (Nova Medical, Wilmington, MA, USA), 3D T1-weighted (3D T1-weighted (TI/TR/TE=1225/4.8/2.2, acquired voxel size = 1.0x1.0x1.0 mm^3, reconstructed to 0.66x0.66x0.66 mm^3) and 3D T2-weighted (TR/TE=3158/301, acquired voxel size = 0.70x0.70x0.70 mm^3, reconstructed to 0.35x0.35x0.35 mm^3) images were acquired. T1 and T2 images were reconstructed for nominal spatial resolution. The scanning duration was 10:15 minutes long per acquisition. To partly compensate inhomogeneity in the radio frequency field, a flip angle of 120 degrees was performed. To reduce specific absorption rate and to optimize image contrast, a 12 to 90 degree tissue-specific refocusing pulse angle sweep was done (52). A field of view of 250 x 250 x 190 mm for foot-to-head x anterior-to-posterior x right-to-left was used. For more information regarding 7T sequence, please refer to (53).

Conventional MR images were obtained using 1.5T (Gyroscan ACS-NT, Philips Medical System, Best, the Netherlands) in both the SMART-MR and PREDICT-MR studies. A sagittal 3D T1-weighted sequence (SMART-MR: TR/TE: 7.0/3.2ms, voxel size = 0.94x0.94x1.00 mm^3 isotropic; PREDICT-MR: TR/TE: 6.9/1.3 ms, voxel size = 0.98x0.98x1.10 mm^3 isotropic) was acquired for segmentation of intracranial volume (ICV). MR images were collected using 3T MRI (Philips Medical Systems, Best, the Netherlands) for the participants from the general practices. This protocol included a sagittal 3D T1-weighted sequence (TR/TE=8.0/4.5, voxel size = 1.00x1.00x1.00 mm^3 isotropic). Automatic brain segmentation was performed on the 3D T1-weighted sequence of the 1.5 or 3T images by CAT12 (version 1155), SPM12 (version 6906), and MATLAB (version 8.6). CAT12 segments gray matter, white matter, and cerebrospinal fluid. Total ICV was calculated as a sum of white and gray matter and CSF volumes. As segmentation on ICV has not yet been validated in the Automatic Segmentation of Hippocampal Subfields (ASHS, see next paragraph) on 7T, 1.5 or 3T images were used for ICV segmentation. Therefore, all participants underwent both a 7T MRI as well as a 1.5 or 3T MRI scan.

For hippocampal subfield segmentation, the ASHS software was used on the 3D T2-weighted images (Upenn, PA, USA). ASHS differentiates between the CA1-3, CA4 and DG, subiculum, entorhinal cortex (ERC), and the hippocampal tail (Figure 1). The 'UMC Utrecht 7T ASHS Atlas, compatible with original (slow) ASHS' was used from the ASHS atlases validated for 7T (54). Using frequencies and histograms, segmentations were inspected for outliers. Manual, visual inspection was performed on outlier segmentations and then removed from the analysis if due to a segmentation error. Additionally, a random sample of 5% of all the segmentations were manually inspected for segmentation errors.

Systematic review

On December 13th, 2021, a PubMed search for psychosocial factors and hippocampal subfield volumes was performed (see Supplementary Info S1). A total of 1,554 articles were screened based on title/abstract. Seventy-eight articles were selected for full-text screening based on the inclusion criteria of assessing hippocampal subfield volume and assessing one or more of the relevant psychosocial factors. Systematic reviews or meta-analyses were not included. Articles were then selected for this review if 1) participants were 25 years or older (based on brain maturation in early adulthood (55)), 2) participants were not cognitively impaired or diagnosed with any illness that was not major depressive disorder, an anxiety disorder, or post-traumatic stress disorder, 3) involved relevant psychosocial factors (i.e., depression, anxiety, childhood maltreatment or trauma, recent stressful life events, or social support), and 4) reported a cross-sectional association with hippocampal subfield volume. A total of 47 articles were included in this review.

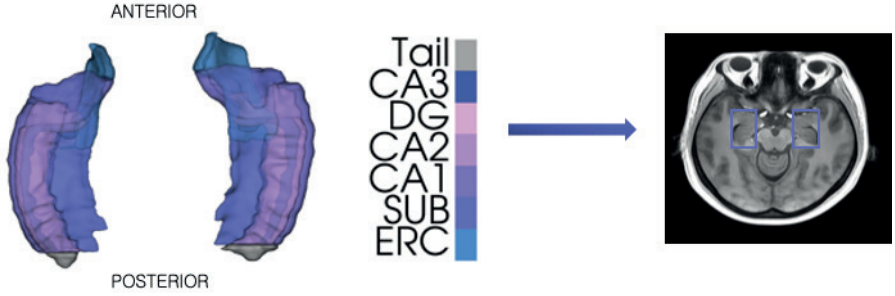


Figure 1. 3D segmentation of hippocampal subfields using ASHS on a random participant for visualization, alongside an axial view of a template brain MRI. Tail = hippocampal tail, CA = Cornu ammonis, DG = dentate gyrus, SUB = subiculum, ERC = entorhinal cortex. For segmentation display, please see https://www.nitrc.org/projects/ashs.

Data analysis

Multiple imputation was performed using the *mice* package in R (version 4.0.3) to address missing values (ranged from: 2.1% for BAI and 12.6% for the volumes of the hippocampal subfields) with 25 imputed datasets. The number of imputed datasets was chosen based on the percentage of non-complete cases (56) (e.g., if the complete case analysis is on 77% of the original N, then at least 23 imputed datasets are needed). Therefore, we chose 25 imputed datasets. Missing data on hippocampal subfield volume was due to the following: 11 individuals had no T1 or T2 available, 18 individuals had movement or signal interference, and 13 had a segmentation error. Predictive mean matching was used for continuous variables,

polytomous logistic regression for unordered categorical variables, and logistic regression imputation for dichotomous variables. Left and right hemispheres of the hippocampal subfields were summed and converted into z-scores after imputation. The outcomes (i.e., hippocampal subfields) were also used in the prediction process for imputation as well as being imputed themselves. See Supplementary Table 1 for descriptive statistics of both the complete case and imputed data.

Multiple linear regressions were fit for each psychosocial factor (i.e., depressive symptoms, anxiety symptoms, childhood maltreatment, recent stressful life events, and social support), adjusted for age, sex, and intracranial volume, on total hippocampal volume. Generalized linear models were fit for each psychosocial factor, also adjusted for age, sex, and intracranial volume, which included the unstructured correlation of each hippocampal subfield per individual (i.e., 'a multivariate approach'), to assess differential effects between subfields. In these models, all hippocampal subfields are entered as one outcome, resulting in a single model per each psychosocial factor (see Supplementary Code S1). Previous literature has shown that multivariate approaches increase the power of the model as well as reduce type I error compared to univariate approaches that ignore the correlation between outcomes (57). While in univariate analyses, one can adjust the p-value, the assumption of independence between outcomes is violated when they are correlated. Additionally, an exploratory analysis on types of childhood maltreatment was also performed for both outcomes: total hippocampal volume and hippocampal subfield volumes. The *nlme* package in R (version 4.0.3) was used for all multivariate models using the gls() function. Estimated marginal means from the multivariate models on subfield outcomes were computed using the *emmeans* package in R (see Supplementary Code S1). Pooled results are shown. To correct for multiple testing, we defined statistical significance as $p < 0.005$ to account for the ten tests performed (i.e., based on five separate predictors on two outcomes [i.e., total hippocampal volume and multivariate hippocampal subfields]). Lastly, sensitivity analyses were performed to explore possible differences when assessing type of childhood maltreatment, when using continuous data (i.e., BAI sum score, sum score on the stressful events questionnaire, and sum score on the social support questionnaire), when stratifying by cohort, when using a stricter cut-off of 10 (versus six) or higher on the PHQ-9, when including all psychosocial factors in a joint model, and when excluding missing data (i.e., a complete case analysis).

Results

Systematic review results

An overview of the literature review for psychosocial factors and their associations with hippocampal subfield volumes are displayed in Table 1. Of the 47 articles, 27 studies (57%) reported lower hippocampal subfield volumes in the presence of a psychosocial factor, specifically depression (11, 41, 58-70), anxiety (71), or childhood maltreatment or post-traumatic stress disorder (PTSD) (58, 66, 72-82). The most often affected subfields were the CA3 and DG. Most of the studies used 1.5T or 3T MRI, with four studies (9%) using high-field 7T MRI (41, 83-85). Twenty-four studies (51%) reported no significant differences in volume (61, 64, 66, 68, 74, 78, 81, 83, 85-100), and four studies (9%) found increased volumes, specifically in the left hippocampal amygdala transition area (HATA) for sexual abuse (73), the hippocampal tail in those with major depressive disorder (MDD) (101), the CA1, CA3, and molecular layer in those with childhood maltreatment (11), and in the right subiculum in those with MDD (84). No studies assessed recent stressful life events or social support. Most studies assessed differences between a clinical population and healthy controls. However, six studies (59, 78, 83, 85, 87, 99) explored associations between symptomology and subfield volumes in MDD patients only. One study found no association between anxiety symptomology in those with panic disorder. Additionally, five studies (58, 66, 74, 76, 100) studied symptomology in trauma survivors. Only one study (2%) assessed symptomology in community-dwelling adults (97), with no association found between subfield volume and depressive symptomology.

Descriptive results from the Medea-7T Study

Of the 333 participants in the current study, 3**0**% were female with an average age of 68 years (Table 2). Seventeen percent experienced elevated symptoms of depression, 1**5**% had elevated symptoms of anxiety, 2**4**% experienced any kind of childhood maltreatment, 5**1**% had experienced a recent stressful life event, and 24% had low social support. All subfields were significantly correlated with one another (Supplementary Figure 1). Chi-square tests between each psychosocial factor showed significant associations between all psychosocial factors as well (Supplementary Info 2).

Depression and anxiety

Regarding depressive and anxiety symptomology, no significant associations were found for total hippocampal volume or within a specific subfield. However, a trend of lower volume in the total hippocampus was seen in those with depressive symptoms, and a trend of greater volume in the total hippocampus was seen in those with anxiety symptoms. Further, these trends were also seen in specific subfields.

Table 1. Overview of literature researching the association between psychosocial factors and hippocampal subfield volumes.

Author	Psychosocial factor	Design and study population	Age (years)	Sex, female (%)
Abbott, Jones (86)	MDD	19 MDD + 20 HC	MDD: 65 (8) HC: 65 (9)	64%
Aghamohammadi-Sereshki, Coupland (72)	MDD + childhood maltreatment	35 MDD + 35 HC	HC: 32 (10) MDD: 35 (9)	66%
Ahmed-Leitao, Rosenstein (73)	Childhood maltreatment + PTSD + social anxiety disorder	26 SAD with trauma + 22 SAD without trauma + 17 PTSD + 25 HC	PTSD: 36 (10) SAD w/ trauma: 36 (9) SAD w/o trauma: 33 (10) HC: 31 (7)	47%
Averill, Satodiya (58)	PTSD, BDI	36 PTSD, 32 combat control veterans	21-60	0%
Brown, Rutland (83)	MDD + depressive symptoms	24 MDD + 20 HC	MDD: 40 (10) HC: 40 (13)	58%
Burhanoglu, Dinçer (87)	MDD + depressive symptoms + anxiety symptoms	59 females high-risk for depression	23 (2)	100%
Cao, Passos (88)	MDD	152 HC + 86 MDD	HC: 35 (12) MDD: 41 (12)	67%
Chalavi, Vissia (74)	PTSD + childhood maltreatment	16 PTSD + 28 HC	HC: 42 (12) PTSD: 41 (12)	100%
Chen, Sun (75)	PTSD	140 HC and 142 PTSD	HC: 39 (10); PTSD: 40 (10)	23%
Choi, Jung (59)	MDD, depressive symptoms	50 MDD + 50 HC	HC: 68 (4) MDD: 69 (7)	62%
Doolin, Allers (60)	MDD	74 MDD + 37 HC	HC: 31 (11) MDD: 33 (13)	60%

MRI field strength	Subfields	Segmentation method	ICV/TBV covariate	Results
3T	CA1, CA2/3, DG, subiculum	Van Leemput et al., *Hippocampus* 2009	NA	No significant difference between MDD and HC.
4.7T	CA1-3, subiculum, DG	Manual	ICV	CA1-3 had a negative correlation with childhood maltreatment in those with MDD.
3T	All	Freesurfer	ICV	Negative correlation was found between physical neglect and left fimbria. A positive correlation was found with sexual abuse and the left HATA. Lower left HATA and right parasubiculum in PTSD group compared to the SAD and control groups.
3T	Parasubiculum, presubiculum, subiculum, CA1, CA2/3, CA4, GC/DG, HATA, fimbria, molecular layer, hippocampal tail	Freesurfer	ICV	Total hippocampal volume negatively correlated with PTSD symptoms and BDI. PTSD negatively correlated with the HATA. BDI negatively correlated with the DG, CA4, HATA, CA2/3, molecular layer, and CA1.
7T	Subiculum, presubiculum, parasubiculum, CA1, CA3, CA4, GC of DG, ML DG, HATA, fimbria	Freesurfer	ICV	No differences in subfield volumes between groups. Positive associations were found for MDD severity and right CA1 and right CA3/4, but it did not survive multiple comparisons adjustment.
3T	Fissure, tail, subiciulm, presubiculum, parasubiculum, CA1, CA3, CA4, ML, GC ML, fimbria, HATA	Freesurfer	ICV	No difference in subfields between those with MDD and those without MDD. No association with depressive or anxiety symptomology.
1.5T	CA1, CA2/3, CA4, GCL, ML, presubiculum, subiculum, and tail	Freesurfer	ICV	No significant difference between MDD + HC.
3T	CA1, CA2-3, CA4-DG, subiculum, presubiculum, fimbria	Freesurfer	TBV	No difference between PTSD and HC subfield volumes. Left CA1, CA2-3, CA4-DG, and presubiculum were negatively correlation with severity of childhood traumatizing events.
3T	CA1, CA3, CA4, DG, fimbria, fissure, HTA, molecular layer, parasubiculum, presubiculum, subiculum + tail	Freesurfer	HV	Lower subfield volumes associated with PTSD in left CA1 and bilateral CA3, only if hippocampal volume was included as a covariate.
3T	CA1, CA2, CA3, CA4, DG, subiculum	ASHS	ICV	Bilateral CA1 and DG and right CA3 were smaller in the MDD group. Depressive symptoms were negatively correlated with left DG.
3T	CA1-4, subiculum	Freesurfer	ICV	Hippocampal subfield volumes were smaller in MDD patients than HC for CA1 (left only), CA2/3 (left and right) and CA4 (right only).

Table 1. Continued

Author	Psychosocial factor	Design and study population	Age (years)	Sex, female (%)
Frodl, Carballedo (61)	MDD + childhood maltreatment	43 MDD + 43 HC	MDD: 41 (10) HC: 37 (13)	61%
Frodl, Skokauskas (62)	MDD	38 MDD + 44 HC	MDD: 41 (11) HC: 36 (13)	63%
Han, Won (119)	MDD	105 MDD + 85 HC	MDD: 43 (11) HC: 40 (14)	77%
Han, Kim (63)	MDD	102 MDD + 135 HC	MDD: 36 (11) HC: 36 (13)	58%
Han, Won (64)	MDD	20 MDD + 21 HC	MDD: 42 (14) HC: 42 (10)	100%
Hansen, Singh (89)	MDD	30 MDD + 67 HC	MDD: 38 (16) HC: 54 (17)	43%
Hayes, Hayes (76)	PTSD	97 recent war veterans	30 (7)	6%
Hu, Zhang (90)	MDD	38 MDD + 55 HC	HC: 36 (15) MDD: 36 (12)	54%
Huang, Coupland (65)	MDD	20 MDD and 27 HC	HC: 33 (10) MDD: 35 (11)	62%
Janiri, Sani (77)	Childhood trauma	81 controls	No trauma: 45 (16) Trauma: 46 (12)	57%
Kakeda, Watanabe (91)	MDD	40 MDD + 47 HC	HC: 41 (11) MDD: 47 (14)	38%

MRI field strength	Subfields	Segmentation method	ICV/TBV covariate	Results
3T	CA1, CA2/3, CA4/DG, subiculum, presubiculum	Freesurfer	ICV	Patients with MDD had significantly smaller volumes of CA1, CA2/3, CA4/DG, and subiculum compared to healthy controls. Childhood maltreatment was not associated with any volumes.
3T	CA1, CA2/3, CA4/DG	Freesurfer	TBV	Patients with MDD had significantly smaller CA4/DG and CA2/3 volumes compared to healthy controls.
3T	CA1, CA2/3, CA4, granule-cell molecular layer of the DG, subiculum, presubiculum, fimbria, hippocampal fissure	Freesurfer	ICV	No differences between MDD and HC.
3T	CA1, CA2/3, CA4, GCL, ML, presubiculum, subiculum, tail	From Iglesias et al.	ICV	MDD had lower volumes in the bilateral CA1, CA4, the granule cell layer, the molecular layer, the left CA2/3, and right presubiculum and subiculum compared to HC.
1.5T	CA1, CA2-3, CA4/DG, subiculum, presubiculum, fimbria, fissure	Freesurfer	ICV	Bilateral subiculum, left CA2-3, and left CA4/DG were smaller in MDD than in HC.
3T	Hippocampal tail, subiculum, CA1, fissure, presubiculum, parasubiculum, molecular layer, DG, CA3, CA4, fimbria, HATA	Freesurfer	ICV	No significant difference between MDD + HC.
3T	CA4/DG, CA1, CA2/3, presubiculum, and subiculum	Freesurfer	ICV	CA4/DG was significantly smaller in veterans with PTSD compared to those without and scaled with PTSD symptom severity.
3T	Subiculum, presubiculum, CA1, CA2/3, CA4/DG, fimbria, hippocampal fissure	Freesurfer	ICV	No difference between MDD + HC.
4.7T	CA1-3, DG, subiculum	Manual	ICV	Total hippocampal volumes were smaller in unmedicated MDD participants than in controls or medicated MDD. Medicated MDD + controls did not differ from one another. CA1-3 was smaller in unmedicated MDD compared to controls. DG volume was also smaller in unmedicated MDD compared to controls + medicated MDD.
3T	CA1, CA2/3, CA4/DG, presubiculum, subiculum	From Van Leemput et al. 2009 *Hippocampus*	ICV	Childhood trauma was associated with bilaterally smaller CA1, presubiculum, and subiculum volumes.
3T	CA1, CA3, CA4, GC of DG, fimbria, subiculum, presubiculum, parasubiculum, ML, HATA, tail	Freesurfer	ICV	No difference between MDD + HC.

Table 1. Continued

Author	Psychosocial factor	Design and study population	Age (years)	Sex, female (%)
Kraus, Seiger (84)	MDD	22 HC + 28 remitted MDD + 20 acute MDD	HC: 26 (7) rMDD: 27 (6) aMDD: 31 (10)	60%
Lim, Hong (78)	MDD, depressive symptoms	30 MDD + 30 HC	HC: 72 (5) MD: 74 (6)	52%
Lindqvist, Mueller (92)	MDD	16 MDD + 19 HC	HC: 37 (12) MDD: 34 (7)	63%
Liu, Pantouw (93)	MDD	35 MDD + 35 HC	HC: 43 (12) MDD: 43 (11)	69%
Luo, Liu (79)	PTSD	57 PTSD+ + 11 PTSD- + 39 HC	PTSD+: 57 (6) PTSD-: 58 (7) HC: 56 (6)	58%
Maller, Broadhouse (101)	MDD	202 MDD + 68 HC	HC: 30 (13) MDD: 33 (13)	52%
Mikolas, Tozzi (11)	Childhood maltreatment and MDD	85 MDD and 67 HC at two sites	HC, CAMI = 37 (13); MDD, CAMI = 40 (9); HC, TCIN = 34 (11); MDD, TCIN = 38 (13)	74%
Na, Won (94)	MDD	47 MDD + 30 HC	MDD: 45 (11) HC: 44 (13)	100%
Na, Chang (95)	MDD	45 MDD + 72 HC	MDD: 42 (12) HC: 41 (14)	73%
Ota, Sato (96)	MDD	36 MDD + 35 HC	MDD: 38 (11) HC: 39 (13)	47%

MRI field strength	Subfields	Segmentation method	ICV/TBV covariate	Results
7T	CA1, CA3, CA4, fimbria, fissure, granule cell layer of the dentate gyrus, hippocampus–amygdala transition area, molecular layer, parasubiculum, presubiculum, subiculum, and tail	Freesurfer	TBV + GM	Right hippocampal fissure and right HATA were larger in remitted MDD compared to HC. Larger right subiculum values in both MDD groups compared to HC.
3G	CA1 CA2-3, CA4-DG, subiculum, presubiculum, fimbria, fissure	Freesurfer	ICV	Bilateral presubiculum, bilateral subiculum, left CA1, bilateral CA2-3, left CA4-DG, and bilateral fimbria smaller in MDD. No significant correlations between subfield volumes and depressive symptoms in those with MDD.
4T	CA1, CA1/2, CA3/DG, subiculum	From Mueller et al., 2007 *Human Brain Mapping*	ICV	No significant differences between MDD and control.
1.5T	Presubiculum, subiculum, CA1, CA2-3, CA4/DG, fimbria, hippocampal fissure	Freesurfer	ICV	MDD patients had smaller volumes in left CA2/3 and CA4/DG. However, these did not remain significant after correction for multiple comparisons.
3T	CA1, CA2/3, CA4/DG, subiculum, presubiculum, and fimbria	Freesurfer	ICV	PTSD+and PTSD- group had smaller CA2-3, CA4/DG, subiculum volumes than HC.
3T	CA1, CA2/3, CA4, DG, HATA, fimbria, alveus	Freesurfer	TBV + THV	Larger hippocampal tail in those with MDD. Uncorrected, associations were also found for the molecular layer, the granule cells of the molecular layer, the CA2/3 + CA4, and the combine alveolus/fimbria, with lower volumes in MDD except for higher volumes in the fimbria/alveolus.
3T	CA1, CA3, CA4, fimbria, sum of granular layer and dentate gyrus, hippocampus-amygdala-transition-area, hippocampal fissure, molecular layer, hippocampal tail, parasubiculum, presubiculum, subiculum	Freesurfer	TBV	Those with MDD had smaller CA1, CA3, CA4, granular layer + dentate gyrus, and molecular layer. The whole hippocampus was also smaller in those with MDD compared to HC. In patients with ELA, larger volumes were found in the CA1, CA3, and ML compared to MDD patients without ELA.
3T	CA1, CA3, CA4, molecular layer, granule cells, subiculum, presubiculum, parasubiculum, HATA	Freesurfer	ICV	No differences between MDD and HC in subfield volume.
3T	CA1, CA2/3, CA4/DG, subiculum, presubiculum, fimbria, fissure	Freesurfer	ICV	No differences between MDD and HC in subfield volume.
3T	CA, DG, subicul	ASHS	ICV	No difference between MDD + HC.

Table 1. Continued

Author	Psychosocial factor	Design and study population	Age (years)	Sex, female (%)
Postel, Mary (66)	PTSD + trauma exposure + depressive symptoms	53 trauma-exposed with PTSD + 39 trauma-exposed without PTSD + 80 HC	PTSD+ = 37 (9) PTSD- = 36 (7) Non-exposed = 32 (12)	53%
Szymkowicz, McLaren (97)	Depressive symptoms	48 community-dwelling adults	69 (7)	70%
Su, Faluyi (67)	MDD	5 MDD+ 13 HC	MDD: 73 (5 HC: 68 (6)	61%
Takaishi, Asami (71)	Panic disorder + symptoms	38 PD + 38 HC	PD: 39 (10) HC: 38 (10)	66%
Tannous, Godlewska (85)	CTQ, BDI, HAM-D, STAI	46 HC + 71 MDD	HC = 32 (11), MDD = 32 (10)	55%
Taylor, Deng (98)	MDD	59 MDD + 21 HC	66 (6)	62%
Travis, Coupland (68)	MDD	15 MDD and 15 HC	HC = 33 (10); MDD = 36 (9)	63%
Travis, Coupland (99)	MDD	14 MDD + 14 HC	HC: 33 (10) MDD: 36 (9)	73%
Treadway, Waskom (69)	MDD	51 HC + 52 MDD	HC: 37 (13) MDD: 41 (13)	52%
Wang, Neylan (80)	PTSD	17 PTSD + 19 HC	41 (12)	0%
Weis, Webb (100)	PTSD	215 trauma survivors	33.1 (10.8)	55%

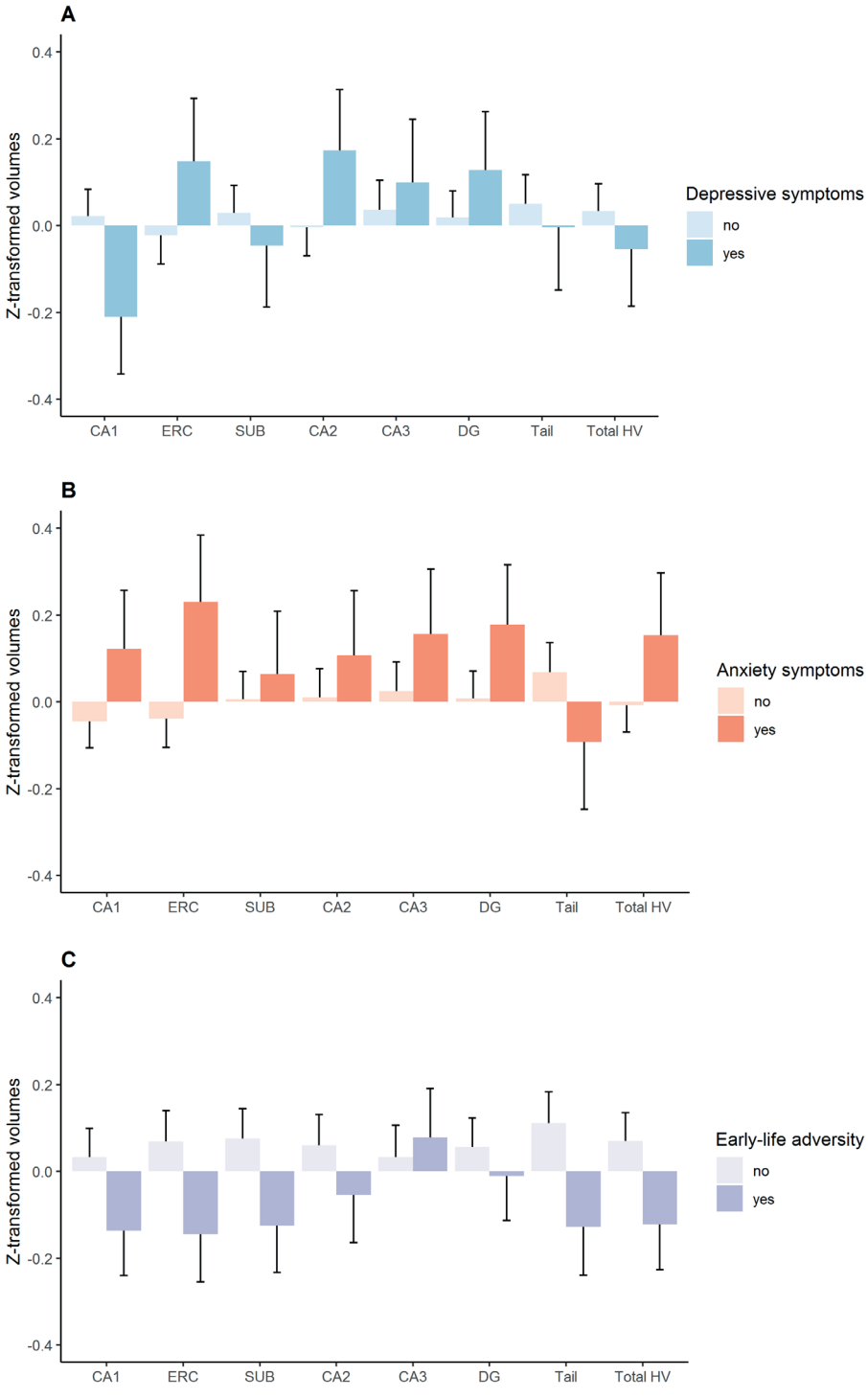
MRI field strength	Subfields	Segmentation method	ICV/TBV covariate	Results
3T	CA1, CA2-3/DG, subiculum, tail	ASHS	ICV	Smaller volumes of the CA1 and the CA2-3/DG were found in the PTSD group compared to those without PTSD but trauma-exposed. There were no differences between those exposed to trauma and those unexposed. CA2-3/DG region was negatively associated with depressive symptoms.
3T	CA1, CA2-3, subiculum	Freesurfer	ICV	No main effects of depressive symptoms of hippocampal subfield volume.
3T	CA1, CA2, CA3/DG, subiculum	Manual	N/A	MDD had smaller volumes in the CA1 and subiculum.
1.5T	Presubiculum, CA1, CA2/3, fimbria, subiculum, CA4/DG	Freesurfer	ICV	PD had smaller right CA2/3 than HC. No association between subfields and symptom severity.
7T	All	Freesurfer	ICV	No group differences in any subfields. No association between any subfield and CTQ score, illness duration, or mood rating scale.
3T	CA1-3,, CA4/DG, subiculum	ASHS	ICV	No differences between MDD + HC.
4.7T	CA1-3, DG	Manual	ICV	No difference between MDD and HC in hippocampal volume. MDD patients showed smaller DG volumes compared to HC. Duration of depression negatively correlated with total HV and CA1-3 and DG subfields.
4.7T	CA1-3, DG, subiculum	From Malykhin et al., 2010 *Neuroimage*	ICV	No significant differences between MDD and controls. No significant correlations between depressive symptoms and hippocampal subfield volume.
1.5T	CA1, CA2/3, CA4/DG, stratum, subiculum	Multiple Automatically Generated Templates for different Brains (MAGeT Brain)	ICV	DG was associated with a significant reduction in volume as the number of episodes increased in all subjects. In MDD, significant reductions were seen across all subfields.
4T	Entorhinal cotex, subiculum, CA1, CA3/DG	From Mueller et al., 2007 *Neurobiol Aging*	ICV	CA3/DG was smaller in PTSD than in the controls.
3T	Hippocampal tail, subiculum, CA1, hippocampal fissure, presubiculum, parasubiculum, molecular layer, granule cell layer of the dentate gyrus, CA3, CA4, fimbria, hippocampal-amygdaloid trainsition area, and whole hippocampus	Freesurfer	TBV	There was no relationship found cross-sectionally or longitudinally on PTSD symptoms and subfield volumes.

Table 1. Continued

Author	Psychosocial factor	Design and study population	Age (years)	Sex, female (%)
Wisse, Biessels (41)	Major depressive episodes	47 participants from GP attendees, no MDE = 34, ever MDE = 13.	60 (10)	62%
Yuan, Rubin-Falcone (81)	MDD + childhood maltreatment	44 HC + 17 abused MDD + 24 non-abused MDD	HC: 33 (12) MDD: 35 (11)	59%
Zhang, Lu (82)	PTSD	145 survivors of a major earthquake and 56 HC	PTSD: 43 (10); TC: 44 (9); HC 40 (12)	67%
Zhou, Wu (70)	MDD	44 MDD + 45 HC	MDD: 35 (12) HC: 33 (11)	59%

MRI field strength	Subfields	Segmentation method	ICV/TBV covariate	Results
7T	Subiculum, CA1, CA2, CA,3, DG+CA4, total hippocampus, ERC	Manual	ICV	All subfields except the CA3 were significantly smaller in the ever MDE group.
3T	CA1, CA3, DG, subiculum, parasubiculum	Freesurfer	ICV	No differences in subfields between MDD + HC. Smaller volumes of the left CA1 were found in those abused with MDD compared to those without abuse.
3T	CA1, CA2/3, CA4, molecular + granule layers of the DG, molecular layer, subiculum, presubiculum, parasubiculum, fimbria, fissure, and HATA	Freesurfer	ICV	The total hippocampus was smaller in both PTSD and trauma-exposed groups compared to HC. Smaller volumes were also found in the CA3, CA4, DG, subiculum, and presubiculum.
3T	CA1, CA3, CA4, fimbria, GC + ML DG, HATA, fissure, tail, ML, parasubiculum, presubiculum, and subiculum	Freesurfer	ICV	MDD had smaller left CA1, CA4, GC ML DG, HATA, and ML, and right GC ML DG, and subiculum.

Note: MDD = Major Depressive Disorder, HC = healthy control, CA = Cornu Ammonis, PTSD = post-traumatic stress disorder, SAD = social anxiety disorder, ICV = intracranial volume, HATA = hippocampal amygdala transition area, BDI = Beck Depression Inventory, GC = granule cell, DG = dentate gyrus, ML = molecular layer, TBV = total brain volume, HV = hippocampal volume, ASHS = Automatic Segmentation of Hippocampal Subfields, GM = gray matter, CTQ = Childhood Trauma Questionnaire, HAM-D = Hamiltor Depression Rating Scale, STAI = State Trait Anxiety Inventory, MDE = mild depressive episode, ERC = entorhinal cortex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Depressive symptoms
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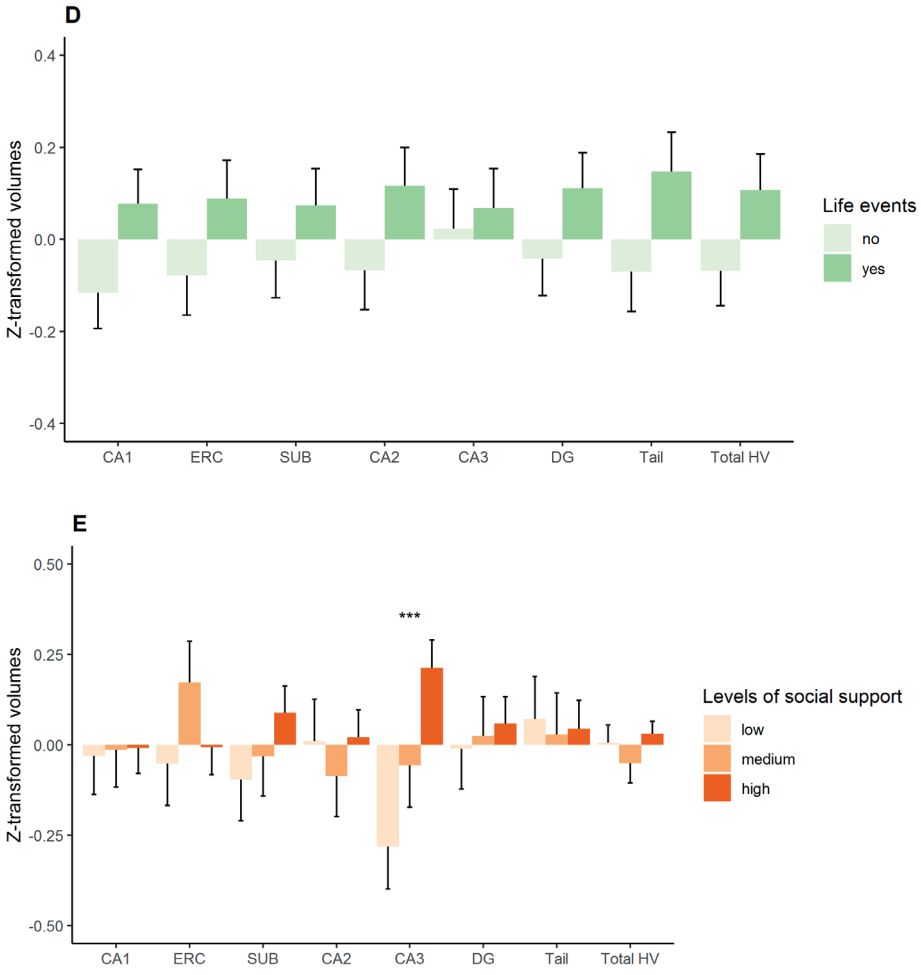

Figure 2. Age-, sex-, and intracranial volume-adjusted means (z-transformed) for each hippocampal subfield and total hippocampal volume per psychosocial factor.

One-sided standard error bars are shown. P-values less than < 0.05 are indicated with two asterisks (**), and p-values less than < 0.001 are indicated with three asterisks (***).

CA = Cornu ammonis, ERC = entorhinal cortex, SUB = subiculum, DG = dentage gyrus, HV = hippocampal volume.

Table 2. Baseline characteristics (n = 333).

	Mean ± SD or n (%)	% missing
Demographics		
Age, mean ± SD, years	68 ±9	0%
Sex, female, n (%)	101 (30%)	0%
College/university education, n (%)	129 (39%)	1%
Psychosocial factors		
Elevated levels of depressive symptoms, n (%)	55 (17%)	0%
Elevated levels of anxiety symptoms, n (%)	51 (15%)	2%
Any childhood maltreatment, n (%)	80 (24%)	3%
Any emotional abuse	55 (17%)	3%
Any physical abuse	32 (10%)	3%
Any psychological abuse	44 (13%)	3%
Any sexual abuse	34 (10%)	3%
One or more recent life events, n (%)	171 (51%)	2%
Social support, n (%)		4%
Low social support	80 (24%)	4%
Moderate social support	76 (23%)	4%
High social support	177 (53%)	4%
Brain volumes		
Intracranial volume, cm^3,mean ± SD	1511 ± 144	4%
Entorhinal cortex, mm^3, mean ± SD	840 ± 166	13%
Subiculum, mm^3, mean ± SD	1171 ± 177	13%
Cornu ammonis 1, mm^3, mean ± SD	2986 ± 353	13%
Cornu ammonis 2, mm^3, mean ± SD	120 ± 21	13%
Cornu ammonis 3, mm^3, mean ± SD	198 ± 47	13%
Dentate gyrus, mm^3, mean ± SD	1591 ± 224	13%
Hippocampal tail, mm^3, mean ± SD	291 ± 67	13%
Total hippocampus, mm^3, mean ± SD	6353 ± 730	13%

Lower volumes in the CA1 were observed in those with depressive symptomology, and higher volumes in the almost all subfields but the hippocampal tail were seen in those with anxiety symptoms (Figure 2, Table 3, Supplementary Table 2, Supplementary Table 3).

Any type of childhood maltreatment

For those who experienced any childhood maltreatment, a trend of lower volumes was seen in the total hippocampus and in almost all subfields but the CA3 (Figure 2, Table 3, Supplementary Table 2).

Recent stressful event

For those who experienced a recent stressful event, a trend of greater volumes in the total hippocampus and all subfields was observed, but it did not reach statistical significance (Figure 2, Table 3, Supplementary Table 2).

Social support

There were no associations with moderate vs. low social support or high vs. low social support with the total hippocampus. However, lower volumes were seen in the CA3 in those with low social support compared to those with high social support (B per standard deviation = -0.4**3**; 95% CI: -0.**72**; -0.**15**, p = 0.003) (Figure 2, Table 3, Supplementary Table 2).

Sensitivity analyses

When we explored specific types of childhood maltreatment, no significant associations were found with hippocampal (subfield) volume and any type of childhood maltreatment (Table 3, Supplementary Figure 2). There were trends of higher hippocampal (subfield) volumes in those who reported physical abuse and lower (subfield) volumes in those who reported sexual abuse. Additionally, a trend was also observed in those who reported sexual abuse and higher volumes in the CA3 (Table 3, Supplementary Figure 2). However, the observations within type of adversity should be interpreted with caution due to small sample size.

Due to differences in timing of the social support and childhood maltreatment questionnaires in the SMART-MR cohort as well as differences in 1.5 or 3T used for ICV segmentation between cohorts, analyses were repeated in a sensitivity analysis stratifying by cohort. Similar results were found for all subfields and total hippocampus in all three cohorts.

Sensitivity analyses on continuous psychosocial variables (i.e., BAI sum score, sum score of the recent stressful events questionnaire, and sum score of the social support questionnaire) were in line with the dichotomous results.

Sensitivity analyses when using a cut-off of 10 or higher on the PHQ-9 resulted in similar results for both hippocampal subfield volume as well as total hippocampal

Table 3. Associations of each psychosocial factor on standardized volumes of each hippocampal subfield.

	CA 1	ERC	SUB	CA 2
	Estimate (95% CI), *Cohen's d*	Estimate (95% CI), *Cohen's d*	Estimate (95% CI), *Cohen's d*	Estimate (95% CI), *Cohen's d*
Depressive symptoms	-0.23 [-0.52; 0.05]	0.17 [-0.14; 0.48]	-0.07 [-0.38; 0.23]	0.18 [-0.13; 0.48]
	-0.23	*0.16*	*-0.07*	*0.16*
Anxiety symptoms	0.17 [-0.12; 0.46]	0.27 [-0.06; 0.60]	0.06 [-0.25; 0.37]	0.10 [-0.22; 0.42]
	0.17	*0.24*	*0.05*	*0.09*
Childhood maltreatment	-0.17 [-0.41; 0.07]	-0.21 [-0.47; 0.04]	-0.20 [-0.45; 0.05]	-0.11 [-0.37; 0.14]
	-0.17	*-0.20*	*-0.19*	*-0.10*
Emotional abuse	-0.12 [-0.41; 0.16]	-0.12 [-0.42; 0.19]	-0.05 [-0.35; 0.25]	0.00 [-0.32; 0.31]
	-0.12	*-0.11*	*-0.05*	*-0.00*
Physical abuse	0.28 [-0.10; 0.65]	-0.16 [-0.60; 0.27]	0.25 [-0.15; 0.66]	0.32 [-0.12; 0.77]
	0.27	*-0.15*	*0.24*	*0.29*
Psychological abuse	-0.15 [-0.47; 0.18]	-0.06 [-0.42; 0.30]	0.01 [-0.35; 0.36]	0.18 [-0.18; 0.54]
	-0.14	*-0.06*	*0.01*	*0.16*
Sexual abuse	-0.23 [-0.59; 0.12]	-0.12 [-0.53; 0.29]	-0.27 [-0.65; 0.10]	-0.21 [-0.63; 0.20]
	-0.23	*-0.11*	*-0.26*	*-0.19*
Recent life events	0.19 [-0.01; 0.40]	0.17 [-0.07; 0.40]	0.12 [-0.09; 0.33]	0.18 [-0.05; 0.42]
	0.20	*0.15*	*0.12*	*0.17*
Moderate vs. high social support	-0.07 [-0.33; 0.18]	0.15 [-0.14; 0.45]	-0.02 [-0.30; 0.25]	0.10 [-0.19; 0.40]
	-0.08	*0.15*	*-0.02*	*0.10*
Low vs. high social support	0.05 [-0.21; 0.31]	0.03 [-0.26; 0.31]	0.00 [-0.27; 0.27]	0.09 [-0.20; 0.37]
	0.05	*0.02*	*-0.00*	*0.08*

Note: Generalized linear models, adjusting for age, sex, and intracranial volume. CA = Cornu Ammonis, ERC = entorhinal cortex, SUB = subiculum, DG = dentate gyrus, HV = hippocampal volume.

volume compared to using the cut-off of 6 or higher. A stronger association was found for total hippocampal volume and high depressive symptomology; however, it was still not significant.

When putting all psychosocial factors into a joint model, an association was found in the CA1 for depressive symptoms (B=-0.34, 95% CI: -0.65; -0.03, p=0.03). The negative association of low vs. high social support remained with the CA3 (B=-0.44, 95% CI: -0.73; -0.16, p=0.003) when controlling for all other psychosocial factors (Supplementary Table 3).

	CA 3	DG	Tail	Total HV
	Estimate (95% CI), *Cohen's d*	Estimate (95% CI), *Cohen's d*	Estimate (95% CI), *Cohen's d*	Estimate (95% CI), *Cohen's d*
	0.06 [-0.25; 0.37]	0.11 [-0.18; 0.40]	-0.05 [-0.37; 0.26]	-0.09 [-0.37; 0.19]
	0.06	*0.11*	*-0.05*	*-0.09*
	0.13 [-0.19; 0.45]	0.17 [-0.13; 0.47]	-0.16 [-0.50; 0.18]	0.16 [-0.15; 0.47]
	0.12	*0.16*	*-0.14*	*0.16*
	0.04 [-0.22; 0.31]	-0.07 [-0.30; 0.17]	-0.24 [-0.50; 0.02]	-0.19 [-0.43; 0.04]
	0.04	*-0.07*	*-0.22*	*-0.19*
	-0.02 [-0.33; 0.30]	-0.03 [-0.31; 0.26]	-0.23 [-0.55; 0.08]	-0.10 [-0.39; 0.18]
	-0.01	*-0.02*	*-0.21*	*-0.10*
	0.18 [-0.22; 0.59]	0.30 [-0.08; 0.67]	0.44 [-0.01; 0.88]	0.35 [-0.03; 0.72]
	0.16	*0.29*	*0.39*	*0.33*
	0.01 [-0.35; 0.36]	-0.13 [-0.46; 0.20]	0.09 [-0.28; 0.46]	-0.10 [-0.43; 0.24]
	0.01	*-0.12*	*0.08*	*-0.09*
	0.37 [-0.04; 0.79]	-0.11 [-0.47; 0.25]	-0.38 [-0.77; 0.00]	-0.23 [-0.58; 0.12]
	0.33	*-0.11*	*-0.35*	*-0.22*
	0.04 [-0.18; 0.27]	0.15 [-0.06; 0.36]	0.22 [-0.02; 0.45]	0.18 [-0.03; 0.38]
	0.04	*0.15*	*0.20*	*0.18*
	-0.23 [-0.52; 0.06]	-0.05 [-0.31; 0.21]	0.00 [-0.29; 0.29]	-0.05 [-0.31; 0.20]
	-0.22	*-0.05*	*0.00*	*-0.05*
	-0.43[-0.72; -0.15]	0.00 [-0.27; 0.27]	0.07 [-0.22; 0.37]	0.01 [-0.25; 0.27]
	-0.40	*0.00*	*0.07*	*0.01*

Lastly, when performing a complete case analysis, all associations found in the imputed analysis remained (Supplementary Table 4).

Discussion

In our review, we found that most studies found lower volumes in association with the presence of a psychosocial factor, specifically depression, anxiety, and childhood maltreatment. Regarding hippocampal subfields, the most affected regions were the

CA3 and DG. However, some studies found no association or increased association. No found studies assessed recent stressful life events or social support. This highlighted a gap in the literature assessing social support as well as differences in timing of exposure (early-life versus late-life) in adults. In our original study using 7T brain MRI, specific psychosocial factors were associated with total hippocampal (subfield) volume. There was no association between specific hippocampal (subfield) volumes and depression or anxiety. There was a trend towards lower hippocampal (subfield) volumes in those reporting childhood maltreatment and a trend towards higher hippocampal volumes in those who experienced recent stressful life events. Psychosocial factors were generally not associated with volumetric differences within hippocampal subfields, except for low social support which was associated with lower volumes in the CA3 compared to high social support.

No association between hippocampal (subfield) volumes were found for depression or anxiety. These null findings are in line with a previous study observing null effects for depressive symptomology (17). However, in those with MDD diagnosis, a recent meta-analysis has highlighted lower global hippocampal volume (102). Possibly, subclinical depression may not be severe enough for hippocampal atrophy. This is in line with our sensitivity analysis on a stricter cut-off on the PHQ-9 (i.e., 10 or higher), which found a stronger association with lower total hippocampal volume and high depressive symptomology compared to using a lower cut-off of six. Further, no association was found for anxiety symptomology and total hippocampal volume, which is in line with other studies as well (17, 19, 103). Although, there was a trend towards higher hippocampal volume in those with anxiety symptoms, which is in agreement with a previous study that also found a nominal positive association (104). To note, this trend was driven by the entorhinal cortex, which is the major input and output structure to the hippocampus.

The current study found a trend towards a difference in early- versus late-life stressful events and total hippocampal volume. A trend towards lower hippocampal volume was observed in those who reported childhood maltreatment. This is in line with previous literature on clinical PTSD (82), as well as on previous childhood maltreatment (19). Further, this highlights a possible role of programming effects. Epigenetic programming (i.e., when an environmental stimulus that occurs during development has an impact on DNA methylation and other epigenetic markers) has been hypothesized to explain the link between childhood maltreatment and risk for adult pathophysiology (105). Programming effects can also occur via the HPA axis (106), as studies have shown that stress in early life can impair the neuroendocrine homeostasis in the HPA axis in the long-term (107). Please see (108) for a review

on early-life stress and programming effects. In contrast, a trend towards higher volumes in the hippocampus were seen in those who experienced a recent stressful event, which is in line with a previous study (109). However, other studies found a negative association (110) or no association (110, 111). Discrepancy in the literature could be due to the severity of the life event or timing of the life event, as one study (110) did not find an association with midlife events or total life events, only with increasing severity. Some studies have postulated that stress exposure may have a biphasic effect on the hippocampus, with acute increases in volume due to metabolic activity followed by later atrophy (112). These studies highlight a possible timing effect, as well as a possible difference in the severity of stress exposure, with hippocampal volume and should be investigated further.

Previous literature, specifically in animal models, has shown that the hippocampus is heterogeneous regarding stress sensitivity. The CA3 and DG show specific sensitivity to stress through dendrite remodeling and neurogenesis inhibition as a response to chronic stress. The current study highlights that social support may play a protective role of these sensitive regions as higher volumes were found in the CA3 in association with high social support, even when correcting for other psychosocial factors. This finding in the CA3 could reflect possible protective effects of social support on episodic memory (113), which the CA3 is responsible for. While little research has been conducted on specific subfield volume, some studies have explored total hippocampal volume with social support. Previous studies have been mixed, with some studies reporting no association (114) and one study also finding a positive association with total volume (115). However, no other differences in subfields were found for other psychosocial factors. This is in line with a previous study looking at symptomology rather than specific clinical diagnosis, with finding no differences associated with depressive symptomology in community-dwelling adults (97). This could highlight that hippocampal subfields are not sensitive enough to differential volumetric associations when looking at symptomology only. However, volumetric differences could be visualized with trends based on psychosocial factor.

To assess differences regarding type of childhood maltreatment, we performed a sensitivity analysis based on maltreatment type. Trends regarding specific differences were found in those who experienced physical abuse as well as in those who experienced sexual abuse. A previous meta-analysis (116) on childhood maltreatment and adulthood inflammation also found significant increases in inflammation specifically in physical and sexual abuse. A trend towards higher volumes were found in almost all hippocampal subfields in those who reported physical abuse. This trend of increased volume may reflect signatures of resiliency in later life. A trend towards

lower volumes in the total hippocampus is in line with previous research on atrophy associated with childhood sexual abuse (117). Surprisingly, we also observed a trend between reporting sexual abuse and higher CA3 volume. A previous study found increased volumes in those reporting sexual abuse, specifically in the HATA (73). Reporting sexual abuse may lead to a resiliency later in life in subfields related to emotional processing, reflected by increased volumes in these specific subfields. These types of maltreatment may have specific biological consequences and require further investigation.

Strengths of the current study include using high-field 7T MRI, as well as using the validated and readily available ASHS software for segmentation of subfields in the hippocampus. Previous studies have mostly used 1.5 or 3T MRI (Table 1), which may make differentiation between subfields more difficult for assessment and more prone to noise. Missing data was handled using multiple imputation to avoid loss of power, and multivariate models were used to account for correlation between the subfields and to reduce the possibility for false positives when performing multiple tests. The current study consisted of 333 participants, larger than previous studies assessing psychosocial factors and subfield volumes (Table 1). However, our standard errors were large, with many volumes showing trends towards significance. Future studies with larger sample sizes should be performed to increase power and validate findings within subfields.

A limitation Is that the current study is cross-sectional; thus, we were unable to look longitudinally on the effect of psychosocial factors on hippocampal subfield volumes. Future studies should consider longitudinal assessment of psychosocial factors and hippocampal volumes during the aging process to explore their effect in detail on neurodegeneration. Additionally, we only correct for a minimal number of confounders (i.e., age, sex, and ICV) for consistency due to studying multiple psychosocial factors that have varying confounders. However, we did perform a sensitivity analysis of a joint model using all psychosocial factors to assess their impact on one another. There could be residual confounding in the current study and future studies should include possible confounders per psychosocial factors for validation. Most participants originated from the SMART-MR study, where all individuals have a history of vascular disease; therefore, these results may not be generalizable to other populations. It is also critical to note that these participants mostly came from a White, Western background. Studies have shown that marginally underrepresented populations also experience a disproportionately larger amount of maltreatment (118). Future studies need to be done to assess the effect of psychosocial factors on hippocampal subfields in these populations. Further, there were some differences between cohorts regarding study protocol. Specifically, social

support and childhood maltreatment were assessed at an earlier time point in the SMART-MR cohort, as well as differences in MRI strength between studies for ICV segmentation, which could have affected the current findings. However, sensitivity analyses when stratifying by cohort led to similar results. Lastly, our finding in the CA3 subfield should be interpreted with caution, as the CA3 is one of the smallest subfields within the hippocampus and therefore prone to measurement error, possibly including portions of the CA2, CA3, or DG. More studies assessing social support and hippocampal subfield volume are warranted for validation of our finding on CA3 volume.

Conclusively, the current study highlights that hippocampal (subfield) volumes may differ based on the psychosocial factor. Consistency between subfield volumes or differential effects also may depend on the psychosocial factor. As the hippocampus is involved in both emotional and memory processing, understanding the effects of psychosocial factors on hippocampal decline is crucial in the prevention of neurodegenerative diseases.

Conflict of Interest

The authors declare no conflicts of interest.

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Supplementary Info S1.

("hippocamp*"[Title/Abstract] OR "Parahippocampal Gyrus"[MeSH Terms] OR "ca2 region, hippocampal"[MeSH Terms] OR "ca1 region, hippocampal"[MeSH Terms] OR "ca3 region, hippocampal"[MeSH Terms] OR "Hippocampus"[MeSH Terms]) AND ("subfield*"[Title/Abstract] OR "subregion*"[Title/Abstract]) AND ("depressive disorder"[MeSH Terms] OR ("depressive disorder"[MeSH Terms] OR "depression"[MeSH Terms]) OR "depression"[Title/Abstract] OR "depressive"[Title/Abstract] OR "depressed"[Title/Abstract] OR ("anxiety"[Title/Abstract] OR "anxious"[Title/Abstract] OR "Anxiety Disorders"[MeSH Terms]) OR ("stress"[Title/Abstract] OR "adversity"[Title/Abstract] OR "maltreatment"[Title/Abstract] OR "trauma"[Title/Abstract] OR "abuse"[Title/Abstract] OR "psychosocial"[Title/Abstract] OR "social support"[Title/Abstract] OR "support"[Title/Abstract] OR "social network"[Title/Abstract] OR "events"[Title/Abstract]))

Supplementary Info 2. Chi-square tests between each psychosocial factor.

Depressive symptoms were associated with anxiety symptoms ($F(1)=4091$; $p<0.001$), social support ($F(2)=265$; $p<0.001$), childhood maltreatment ($F(1)=548$; $p<0.001$), and recent stressful experiences ($F(1)=80$; $p<0.001$). Anxiety symptoms were associated with social support ($F(2)=100$; $p<0.001$), childhood maltreatment ($F(1)=531$; $p<0.001$), and recent stressful experiences ($F(1)=186$; $p<-0.001$).

Social support was associated with childhood maltreatment ($F(2)=260$; $p<0.001$) and recent stressful experiences ($F(2)=83$; $p<0.001$). Childhood maltreatment was associated with recent stressful experiences ($F(1)=39$; $p<0.001$).

Supplementary Code S1.

```
### Generalized linear model for depressive symptoms (depsymptoms) in one imputed dataset (dataset_imp1), adjusting for age (age), sex (sex), and intracranial volume (ICV), allowing for unstructured correlation between the hippocampal subfields (variable) per individual (id)

dep_imp1 <- gls(value ~  -1 + variable + variable:factor(depsymptoms) + variable:age + variable:sex + variable:ICV, data = dataset_imp1, method="ML", correlation=corSymm(form = ~ 1 | id), weights = varIdent(form= ~1 | variable))

### Compute estimated marginal means based on the model (dep_imp1) for each subfield, for those with and without high depressive symptomology (variable:factor(depsymptoms)), adjusted for age, sex, and intracranial volume
```

em.dep <- emmeans(dep_imp1, ~variable:factor(depsymptoms), mode = "df.error")

See also the following GitHub tutorial: https://etwait.github.io/correlated-outcomes/.

Supplementary Table 1. Baseline characteristics between the complete-case analysis and the ten pooled, imputed datasets (n = 333).

	Complete-case	Imputed	% missing
Demographics			
Age, mean ± SD, years	68 ±9	-	0%
Sex, female, %	30%	-	0%
College/university education, %	39%	39%	1%
Psychosocial factors			
Elevated levels of depressive symptoms, %	17%	17%	0%
Elevated levels of anxiety symptoms, %	15%	15%	2%
Any childhood maltreatment, %	24%	24%	3%
Any emotional abuse	15%	17%	3%
Any physical abuse	8%	10%	3%
Any psychological abuse	12%	13%	3%
Any sexual abuse	9%	10%	3%
One or more recent life events, %	53%	51%	2%
Social support, %			4%
Low social support	24%	24%	4%
Medium social support	23%	23%	4%
High social support	54%	53%	4%
Brain volumes			
Intracranial volume, cm^3,mean ± SD	1513 ± 141	1511 ± 144	4%
Entorhinal cortex, mm^3, mean ± SD	842 ± 152	840 ± 166	14%
Subiculum, mm^3, mean ± SD	1170 ± 167	1171 ± 177	14%
Cornu ammonis 1, mm^3, mean ± SD	2985 ± 349	2986 ± 353	14%
Cornu ammonis 2, mm^3, mean ± SD	120 ± 22	120 ± 21	14%
Cornu ammonis 3, mm^3, mean ± SD	198 ± 46	198 ± 47	14%
Dentate gyrus, mm^3, mean ± SD	1589 ± 218	1591 ± 224	14%
Hippocampal tail, mm^3, mean ± SD	291 ± 68	291 ± 67	14%
Total hippocampus, mm^3, mean ± SD	6350 ± 714	6353 ± 730	14%

Supplementary Table 2. Standardized means and standard errors per psychosocial factor (exposed and unexposed).

	CA 1	ERC	SUB	CA 2	CA 3	DG	Tail	Total HV
Depressive symptoms								
Yes	-0.210 (0.132)	0.148 (0.145)	-0.046 (0.142)	0.173 (0.140)	0.099 (0.146)	0.128 (0.134)	-0.004 (0.145)	-0.054 (0.132)
No	0.022 (0.062)	-0.023 (0.066)	0.029 (0.064)	-0.004 (0.066)	0.036 (0.068)	0.018 (0.062)	0.050 (0.067)	0.034 (0.062)
Anxiety symptoms								
Yes	0.122 (0.135)	0.231 (0.153)	0.064 (0.145)	0.107 (0.149)	0.156 (0.150)	0.178 (0.138)	-0.092 (0.156)	0.153 (0.144)
No	-0.045 (0.061)	-0.039 (0.066)	0.006 (0.064)	0.010 (0.066)	0.025 (0.067)	0.008 (0.063)	0.068 (0.068)	-0.008 (0.062)
Childhood maltreatment								
Yes	-0.137 (0.103)	-0.145 (0.110)	-0.125 (0.108)	-0.054 (0.110)	0.078 (0.113)	-0.011 (0.102)	-0.128 (0.111)	-0.122 (0.105)
No	0.033 (0.066)	0.069 (0.071)	0.075 (0.069)	0.060 (0.071)	0.033 (0.073)	0.056 (0.067)	0.111 (0.072)	0.070 (0.065)
Emotional abuse								
Yes	-0.118 (0.132)	-0.090 (0.141)	-0.024 (0.137)	0.023 (0.144)	0.032 (0.145)	0.016 (0.133)	-0.151 (0.146)	-0.066 (0.133)
No	0.005 (0.062)	0.028 (0.067)	0.025 (0.065)	0.027 (0.067)	0.049 (0.069)	0.041 (0.063)	0.084 (0.068)	0.038 (0.063)
Physical abuse								
Yes	0.230 (0.182)	-0.141 (0.209)	0.244 (0.196)	0.317 (0.215)	0.210 (0.196)	0.303 (0.181)	0.431 (0.214)	0.329 (0.181)
No	-0.046 (0.059)	0.023 (0.064)	-0.010 (0.062)	-0.007 (0.063)	0.028 (0.065)	0.006 (0.060)	-0.004 (0.064)	-0.017 (0.060)
Psychological abuse								
Yes	-0.143 (0.152)	-0.047 (0.171)	0.021 (0.166)	0.180 (0.171)	0.052 (0.167)	-0.073 (0.155)	0.118 (0.174)	-0.065 (0.158)
No	0.004 (0.061)	0.015 (0.065)	0.015 (0.064)	0.000 (0.065)	0.045 (0.067)	0.055 (0.062)	0.028 (0.067)	0.033 (0.062)
Sexual abuse								
Yes	-0.225 (0.169)	-0.102 (0.197)	-0.228 (0.179)	-0.164 (0.198)	0.377 (0.197)	-0.061 (0.171)	-0.299 (0.186)	-0.183 (0.167)
No	0.009 (0.059)	0.020 (0.064)	0.047 (0.062)	0.050 (0.064)	0.004 (0.066)	0.049 (0.060)	0.084 (0.064)	0.044 (0.061)
Recent life-threatening event								
Yes	0.077 (0.075)	0.088 (0.084)	0.074 (0.079)	0.116 (0.084)	0.068 (0.085)	0.111 (0.077)	0.147 (0.085)	0.107 (0.078)
No	-0.116 (0.078)	-0.079 (0.086)	-0.046 (0.081)	-0.068 (0.085)	0.023 (0.086)	-0.042 (0.080)	-0.071 (0.086)	-0.069 (0.075)
Social support								
High	-0.012 (0.073)	-0.033 (0.078)	0.022 (0.077)	-0.014 (0.079)	0.189 (0.080)	0.047 (0.074)	0.025 (0.081)	0.027 (0.074)

Supplementary Table 2. Continued

	CA 1	ERC	SUB	CA 2	CA 3	DG	Tail	Total HV
Moderate	-0.086 (0.112)	0.122 (0.127)	-0.003 (0.120)	0.088 (0.127)	-0.042 (0.126)	-0.003 (0.114)	0.026 (0.126)	-0.025 (0.111)
Low	0.039 (0.112)	-0.007 (0.124)	0.019 (0.117)	0.071 (0.122)	-0.243 (0.123)	0.049 (0.117)	0.100 (0.128)	0.040 (0.114)

Note: The estimates are age, sex, and ICV-adjusted means for those with the given psychosocial factor and those without (z-standardized volumes of the hippocampus). CA = Cornu Ammonis; ERC = entorhinal cortex; SUB = subiculum; DG = dentate gyrus; HV = hippocampal volume.

Supplementary Table 3. Joint model associations for each psychosocial factor on standardized volumes of each hippocampal subfield.

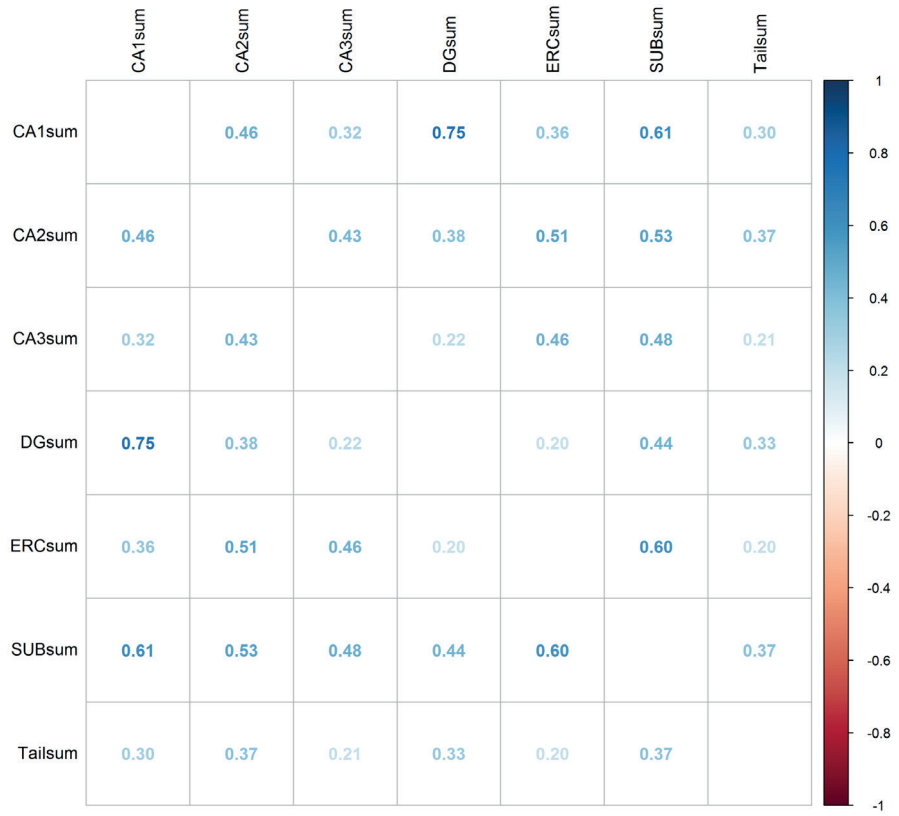
	CA 1	ERC	SUB	CA 2	CA 3	DG	Tail	Total HV
	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)
Depressive symptoms	**-0.34(-0.65; -0.03)**	0.11 (-0.23; 0.46)	-0.10 (-0.43; 0.24)	0.19 (-0.14; 0.53)	-0.04 (-0.40; 0.31)	0.06 (-0.25; 0.37)	0.04 (-0.30; 0.39)	-0.16 (-0.47; 0.15)
Anxiety symptoms	0.30 (-0.01; 0.62)	0.25 (-0.12; 0.62)	0.11 (-0.23; 0.46)	0.02 (-0.34; 0.38)	0.15 (-0.21; 0.51)	0.14 (-0.18; 0.47)	-0.17 (-0.55; 0.20)	0.23 (-0.11; 0.57)
Childhood maltreatment	-0.18 (-0.43; 0.06)	-0.25 (-0.51; 0.00)	-0.21 (-0.47; 0.04)	-0.15 (-0.41; 0.11)	0.06 (-0.21; 0.33)	-0.10 (-0.34; 0.14)	-0.25 (-0.51; 0.01)	-0.21 (-0.45; 0.03)
Recent life events	0.19 (-0.01; 0.40)	0.17 (-0.07; 0.40)	0.12 (-0.09; 0.34)	0.19 (-0.05; 0.42)	0.03 (-0.20; 0.26)	0.14 (-0.07; 0.36)	0.24 (0.00; 0.47)	0.17 (-0.03; 0.38)
Moderate vs. high social support	-0.08 (-0.34; 0.17)	0.17 (-0.12; 0.46)	-0.03 (-0.31; 0.25)	0.12 (-0.17; 0.41)	-0.23 (-0.52; 0.07)	-0.04 (-0.30; 0.23)	0.00 (-0.29; 0.29)	-0.06 (-0.31; 0.20)
Low vs. high social support	0.04 (-0.22; 0.29)	0.05 (-0.23; 0.33)	0.01 (-0.27; 0.28)	0.11 (-0.18; 0.40)	**-0.44(-0.73; -0.16)**	0.01 (-0.26; 0.28)	0.11 (-0.19; 0.40)	0.01 (-0.25; 0.27)

Note: Generalized linear models, adjusting for all psychosocial factors, age, sex, and intracranial volume. CA = Cornu Ammonis, ERC = entorhinal cortex, SUB = subiculum, DG = dentate gyrus, HV = hippocampal volume. P-value threshold is set to $p<0.01$.

Supplementary Table 4. Complete case analysis on the associations between psychosocial factors and standardized hippocampal (subfield) volumes.

	CA 1	**ERC**	**SUB**	**CA 2**
	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)
Depressive symptoms	-0.19 (-0.50; 0.11)	0.17 (-0.16; 0.50)	-0.05 (-0.37; 0.27)	0.25 (-0.07; 0.57)
Anxiety symptoms	0.20 (-0.11; 0.52)	0.33 (-0.01; 0.67)	0.09 (-0.24; 0.42)	0.21 (-0.12; 0.53)
Childhood maltreatment	-0.19 (-0.44; 0.06)	-0.17 (-0.44; 0.10)	-0.20 (-0.46; 0.06)	-0.04 (-0.30; 0.23)
Emotional abuse	-0.10 (-0.41; 0.21)	-0.12 (-0.46; 0.21)	-0.05 (-0.38; 0.28)	0.06 (-0.27; 0.38)
Physical abuse	0.28 (-0.12; 0.68)	-0.09 (-0.52; 0.34)	0.33 (-0.09; 0.75)	0.37 (-0.04; 0.78)
Psychological abuse	-0.08 (-0.45; 0.28)	-0.01 (-0.41; 0.38)	0.02 (-0.37; 0.40)	0.27 (-0.10; 0.65)
Sexual abuse	-0.34 (-0.70; 0.03)	-0.11 (-0.51; 0.29)	-0.30 (-0.68; 0.09)	-0.16 (-0.54; 0.23)
Recent life events	0.19 (-0.02; 0.40)	0.14 (-0.09; 0.37)	0.13 (-0.10; 0.35)	0.20 (-0.03; 0.42)
Moderate vs. high social support	-0.11 (-0.38; 0.17)	0.13 (-0.16; 0.42)	-0.10 (-0.38; 0.19)	-0.04 (-0.32; 0.24)
Low vs. high social support	0.06 (-0.21; 0.33)	0.05 (-0.24; 0.33)	-0.01 (-0.30; 0.27)	0.09 (-0.19; 0.37)

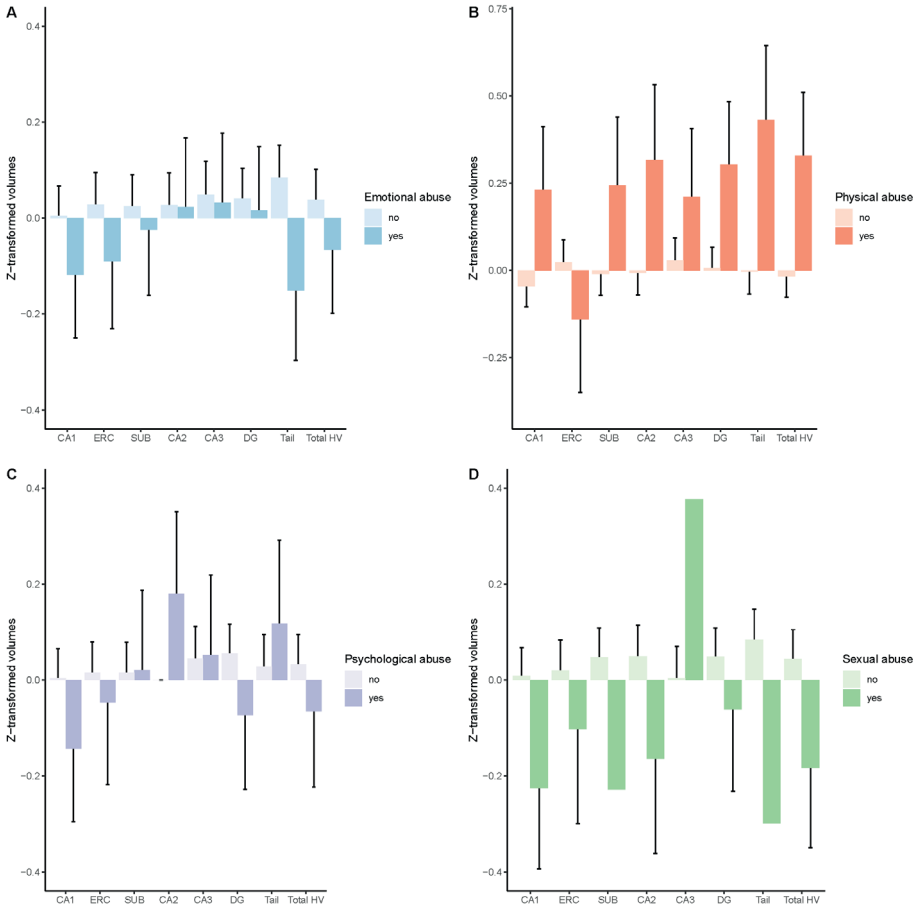
	CA 3	DG	Tail	Total HV
	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)
	0.09 (-0.25; 0.42)	0.16 (-0.15; 0.47)	-0.06 (-0.39; 0.27)	-0.05 (-0.36; 0.26)
	0.14 (-0.21; 0.49)	0.25 (-0.07; 0.57)	-0.14 (-0.48; 0.20)	0.20 (-0.11; 0.51)
	0.05 (-0.23; 0.33)	-0.04 (-0.29; 0.22)	-0.23 (-0.51; 0.04)	-0.17 (-0.42; 0.08)
	0.04 (-0.30; 0.39)	0.03 (-0.29; 0.35)	-0.24 (-0.57; 0.09)	-0.07 (-0.38; 0.25)
	0.13 (-0.31; 0.57)	0.39 (-0.01; 0.80)	0.46 (0.04; 0.88)	0.40 (-0.01; 0.80)
	0.08 (-0.32; 0.48)	-0.07 (-0.44; 0.30)	0.14 (-0.25; 0.52)	-0.03 (-0.40; 0.34)
	0.36 (-0.04; 0.77)	-0.15 (-0.53; 0.23)	-0.34 (-0.73; 0.05)	-0.29 (-0.66; 0.08)
	0.02 (-0.22; 0.26)	0.14 (-0.08; 0.35)	0.24 (0.01; 0.47)	0.19 (-0.02; 0.41)
	-0.34 (-0.64; -0.05)	-0.10 (-0.38; 0.18)	-0.06 (-0.35; 0.23)	-0.13 (-0.41; 0.14)
	-0.43(-0.72; -0.14)	-0.02 (-0.30; 0.26)	0.06 (-0.23; 0.35)	0.00 (-0.27; 0.27)



Supplementary Figure 1. A correlation matrix of the hippocampal subfields.

Note: All correlations are significant, $p < 0.001$.

CA = Cornu Ammonis; ERC = entorhinal cortex; SUB = subiculum; DG = dentate gyrus; HV = hippocampal volume.



Supplementary Figure 2. Age-, sex-, and intracranial volume-adjusted means (z-transformed) for each hippocampal subfield and total hippocampal volume per type of childhood maltreatment.

CA1 = Cornu ammonis 1, ERC = entorhinal cortex, SUB = subiculum, CA2 = cornu ammonis 2, CA3 = cornu ammonis 3, DG = dentage gyrus, HV = hippocampal volume.

Chapter 5

Association of amyloid-beta with depression or depressive symptoms in older adults without dementia: A systematic review and meta-analysis

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Abstract

Several lines of evidence have indicated that depression might be a prodromal symptom of Alzheimer's disease (AD). This systematic review and meta-analysis investigated the cross-sectional association between amyloid-beta, one of the key pathologies defining AD, and depression or depressive symptoms in older adults without cognitive impairment. A systematic search in PubMed yielded 612 peer-reviewed articles. After full-text screening, eight PET studies, eight CSF studies, and four plasma studies were included. No association between amyloid-beta and depression or depressive symptoms were found using positron emission topography (PET) (Cohen's *d:* 0.23; 95% CI: -0.07; 0.52), cerebrospinal fluid (CSF) (0.20; 95% CI: -0.03; 0.43), or plasma (-0.08; 95% CI: -0.37; 0.21). This meta-analysis showed no conclusive evidence of a cross-sectional association between amyloid-beta burden and depression. Amyloid burden and late-life depression may be independent characteristics of the extended preclinical stage of AD.

Introduction

Depression is one of the leading mental disorders seen in older individuals, which can lead to decreased quality of life, disability, and higher comorbidity from other medical conditions (1). A recent meta-analysis found a pooled prevalence of 7% of major depression in later life (2). Further, late-life depression is associated with an increased risk of all-cause dementia and Alzheimer's disease (3-5). The main pathological hallmark of AD is amyloid-β (Aβ) peptide aggregation which forms amyloid plaques (6, 7). In clinical practice, Aβ positron-emission tomography (PET) scans and measurement of Aβ in CSF are validated methods for identifying AD pathophysiology (8, 9). Plasma Aβ level has also demonstrated potential clinical importance in detecting brain Aβ burden (9). Alongside being attributed to AD, plasma and CSF Aβ levels have also been highlighted in individuals with depression in several studies, defining the amyloid hypothesis for depression. However, the results have been inconsistent.

A previous systematic review and meta-analysis by Nascimento, Silva (10) on 12 studies reported significantly lower Aβ42 levels and higher Aβ40/Aβ42 ratio (i.e., higher Aβ burden) in plasma, but no significant differences in CSF Aβ42 were found. However, some studies not included in the review reported contradictory results. For example, a cross-sectional study assessing the correlation of plasma Aβ42 and depressive symptoms in the Korean elderly found higher plasma Aβ42 levels in the elderly with depressive symptoms compared to those without depressive symptoms (11). Additionally, a longitudinal study comparing individuals diagnosed with depression and healthy controls found significantly lower CSF Aβ42 levels at baseline in the individuals with depression (12). Further, this study did not assess how cognitive impairment may have impacted results, as several included studies included individuals with mild cognitive impairment (MCI).

Further, the previous meta-analysis (10) did not include PET studies. Compared to the previous review, more recent studies that measured Aβ burden are expected to be included with an updated search to be able to assess the relationship between Aβ burden and depression, as assessed by PET, CSF, or plasma. By including more studies, it allows for more statistical power to capture the association between Aβ and depression or depressive symptoms.

In this systematic review and meta-analysis, we aim to examine the cross-sectional association of Aβ burden (measured by PET, CSF, or plasma) with depression or depressive symptoms in cognitively unimpaired older adults to assess possible biological mechanisms of depression in the extended preclinical period of AD.

Methods

This systematic review and meta-analysis was conducted and reported following the PRISMA guidelines (13). The review was not registered on PROSPERO as data collection had already been performed.

Search and study selection

A search string including the terms depression, amyloid, method of amyloid measurement (i.e., PET, CSF, or plasma), and their synonyms (Supplementary Info 1) was developed for PubMed, focusing on older adults without cognitive impairment. The original search was performed on May 14, 2021, and duplicate results from our search were removed with EndNote (v. 20.2) (The EndNote Team, 2013) reference management software. Subsequently, two reviewers (E.T. and M.K.) independently screened titles and abstracts using the Rayyan app (14) to assess eligibility. On May 18, 2022, an updated search was performed by two reviewers (E.T. and J.W.) using the same screening strategies listed above. Full texts of the remaining articles were retrieved and screened against eligibility criteria. Any disagreements were resolved by discussion between the two reviewers (E.T. and J.W.). Snowballing and reverse snowballing were performed by scanning the reference lists of the included articles for any other publications of interest as well as searching Scopus for other works that cited the included articles.

Eligibility criteria

Studies reporting an association between Aβ burden (measured by either PET, CSF, or plasma) and depression diagnosis (determined by a clinical depression diagnosis from medical history or based on established depression evaluation criteria) or depressive symptoms (assessed with a depressive symptom questionnaire) were eligible for inclusion. Eligible studies i) presented observational cross-sectional associations or ii) were longitudinal in design but reported baseline characteristics and associations. Only articles reporting associations in non-demented older adults (i.e., mean age of study population ≥ 50 years old) were included. There were no criteria for the language or publication date of the study. In addition, studies with insufficient information for calculating an effect size were excluded. If multiple articles used the same cohort to investigate the association, the study containing the largest number of study participants was included.

Data extraction and risk of bias assessment

Information extracted from the selected articles was the cohort, size of the study sample, baseline characteristics, Aβ measurement (PET, CSF, or plasma), Aβ burden

classification (continuous or categorical), depression assessment criteria (clinical diagnosis or depressive symptoms), covariate adjustment (whether the study controlled for age, gender, education, or other factors), and the effect size between Aβ and depression or depressive symptoms.

The risk of bias was assessed using an adjusted version of the Newcastle-Ottawa Quality Assessment Scale for Cohort Studies (Supplementary Info 2), in which the included studies were rated with stars based on nine criteria divided into three sections: the quality of the study population selection, the comparability of cohorts based on the study design or analysis, and the quality of outcome assessment.

Statistical analyses

Statistical analyses were performed using R version 4.0.5 (Rstudio, 2022). Based on means and standard deviations between those with and without depression or depressive symptoms or through correlations between Aβ burden and depression or depressive symptoms, these metrics from each study were transformed into standardized mean differences (i.e., Cohen's d) using the *esc* package in R (15). Notably, lower CSF or plasma amyloid levels indicate a higher brain amyloid burden (16, 17); therefore, effect sizes were reversed if studies measured Aβ via CSF or plasma. By reversing the effect size in such cases, a positive Cohen's d would represent an association between higher Aβ burden and depression or depressive symptoms. Considering the possible heterogeneity between studies, such as depression assessment criteria, it might not be reasonable to assume a common effect across included studies. Therefore, the pooled estimate was calculated using a random-effects model (18).

Several studies reported multiple Aβ metrics from the same subjects (e.g., reporting both Aβ40, Aβ42, and their ratio, continuous and categorical scales of Aβ burden, depression assessed based on clinical diagnosis and depressive symptoms, and both adjusted and unadjusted associations). To prevent including one study multiple times in the meta-analysis, a prioritization was made to include only one effect size from each study. We chose a continuous scale of Aβ, depression assessment based on clinical diagnosis, Aβ42/40 ratio, and analyses adjusted for covariates as our prioritization criteria for the meta-analysis, to produce a more clinically relevant result and reduce possible heterogeneity. Therefore, no studies were included twice.

Cochran's Q test and I^2 statistics were used to test heterogeneity. Based on the Cochrane Handbook (19), 30-60%, 50-90%, and more than 75% were rated, respectively, as moderate, substantial, or considerable heterogeneity. To assess the

risk of publication bias, visual inspection of funnel plots and Egger's t-test were performed. Subgroup analyses were done to explore biological and methodological heterogeneity. Subgroups were stratified according to: adjusted/unadjusted for covariates, depression assessment (based on clinical diagnosis/depressive symptoms), PET tracer (^{18}F or ^{11}C), and cohort origin (general population/clinical settings). Meta-regression was performed to assess if sex/gender distribution or prevalence of APOE e4 allele genotype affected the results. For all tests, a p-value < 0.05 was considered statistically significant.

Results

Search results

Following the removal of duplicates, 612 articles were retrieved, of which 57 articles were assessed full-text for eligibility (Figure 1). After the full-text screening, our meta-analysis included 18 studies (11, 20-36) (Figure 1).

The demographics of the participants from each study are presented in Table 1. There was a total of 8614 study participants from the 18 included studies, with a sample size varying from 28 to 4492, a mean age ranging from 67 to 76 years, the percentage of female participants ranging from 26 to 100%, a mean education ranging from three to 17 years, a prevalence of an APOE e4 allele ranging from 12 to 34%, if reported. Prevalence of a clinical diagnosis or high depressive symptomology ranged from three to 17% in the studies. All of the studies used depression as the outcome. The origin of the study cohort varied from general population to clinical settings, such as hospitals or memory clinics. Eight (44%) studies measured amyloid in the brain with amyloid PET, eight (44%) studies measured amyloid in CSF, and four (22%) studies measured amyloid in plasma. Two studies (11%) reported multiple amyloid measurement methods (i.e., one reported both CSF and PET, one reported both plasma and PET). Of all the 18 studies, 11 studies (61%) used a clinical diagnosis of depression, six studies (33%) used a depressive symptom questionnaire, and one study (6%) assessed both (Table 2). Only one (6%) of the 18 studies assessed Aβ categorically. Nine (50%) studies controlled for one or more covariates, such as age, sex/gender, and education. Of the eight PET studies, six studies (75%) used a ^{18}F tracer and two (25%) studies used a ^{11}C tracer. Of the eight CSF studies, five (63%) studies reported only Aβ42, two (25%) studies reported both Aβ42 and Aβ40, and one (13%) study reported the Aβ42/40 ratio. Of the four plasma studies, two (50%) studies reported both Aβ42 and Aβ40, one study (25%) reported Aβ42, Aβ40, and the ratio, and one study (25%) reported only Aβ42.

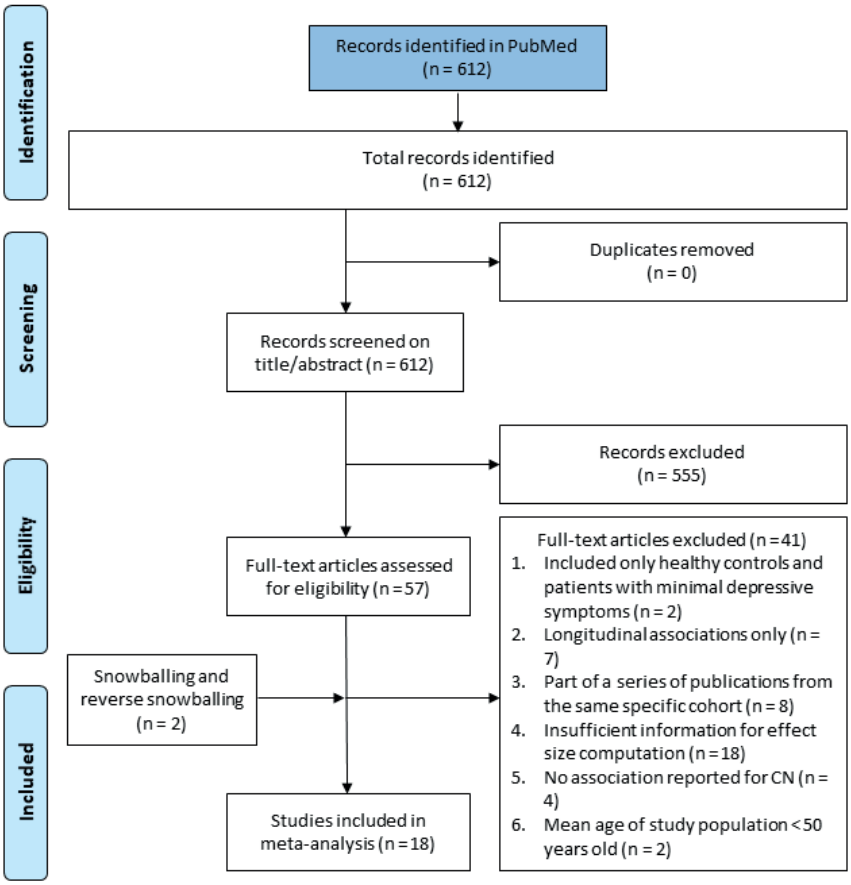


Figure 1. PRISMA flow chart of the original literature search.

The adjusted Newcastle-Ottawa Quality Assessment Scale for cohort studies was used to evaluate the risk of bias, and the included studies scored between four and nine stars on the assessment (Table 3). Regarding selection criteria, six (33%) studies lost stars as their sample was not representative of community-dwelling older adults without cognitive impairment. Eight (50%) studies did not adjust for any covariates. All studies ascertained Aβ burden and depression continuously or categorically based on validated cut-off values; the same method to ascertain Aβ burden and depression or depressive symptoms was implemented for depressed cases and healthy controls in each study. Thus, risk of bias based on the ascertainment of outcome was assumed low. Four studies (22%) scored all nine stars. The Egger's t statistic for the PET studies (bias = 1.44, SE = 1.45, t(6) = 1.00, p = 0.36), CSF studies (bias = -1.12, SE = 1.36, t(6), = -0.82, p = 0.44), and plasma studies (bias = -2.88, SE = 2.46, t(2), p = 0.36) suggested that significant publication bias was unlikely (19).

Table 1. Characteristics of the participants of the included studies in the meta-analysis.

Study (Year, Country)	Cohort origin	Sample size	Age (Mean ± SD in years)	Sex/gender (% women)
PET studies				
Almdahl, Agartz (20) (2022, USA)	Memory clinic	241	74 ± 6	47%
Babulal, Roe (31) (2020, USA)	Research center	301	70 ± 8	57%
Byun, Choe (32) (2016, South Korea)	Hospital	42	70 ± 6	52%
Kumar, Kepe (22) (2011, USA)	Population	39	67 ± 7	56%
Lewis, Bernstein (23) (2022, USA)	Hospital	4492	71 ± 5	59%
Moriguchi, Takahata (24) (2021, Japan)	Hospital	40	72 ± 7	75%
Wang, Kim (29) (2021, South Korea)	Hospital	235	70 ± 9	71%
Wu, Hsiao (30) (2014, Taiwan)	Population	36	69 ± 6	81%
CSF studies				
Almdahl, Agartz (20) (2022, USA)	Hospital	241	74 ± 6	47%
Diniz, Teixeira (33) (2014, Brazil)	Hospital	41	70 ± 4	35%
Gudmundsson, Skoog (35) (2007, Sweden)	Populations	84	73 ± 3	100%
Hertze, Minthon (36) (2010, Sweden)	Memory clinic	66	69 ± 13	62%
Krell-Roesch, Rakusa (21) (2022, USA)	Population	699	72 ± 7	43%
Pomara, Bruno (25) (2012, USA)	Population	47	67 ± 6	47%
Reis, Brandão (26) (2012, Brazil)	Population	28	71 ± 6	89%
Siafarikas, Kirsebom (27) (2021, Norway)	Hospital	60	67 ± 7	83%
Plasma studies				
Byun, Choe (32) (2016, South Korea)	Hospital	42	70 ± 6	52%
Direk, Schrijvers (34) (2013, Netherlands)	Population	980	72 ± 7	59%
Moon, Kang (11) (2011, South Korea)	Population	123	76 ± 7	26%
Sun, Chiu (28) (2009, USA)	Population	1060	75 ± 9	76%

Note: GDS = Geriatric Depression Scale, BDI = Beck Depression Inventory, CES-D = Center for Epidemiologic Studies Depression Scale

	Education (Mean ± SD in years)	APOE e4 allele presence (%)	Prevalence of depression diagnosis or high depressive symptoms (%)
	17 ± 3	27%	40%
	16 ± 2	33%	13%
	11 ± 5	12%	36%
	16 ± 3	-	51%
	-	-	3% GDS > 5
	13 ± 2	-	50%
	13 ± 4	23%	50%
	8 ± 4	19%	69%
	17 ± 3	27%	40%
	13 ± 5	-	39%
	-	-	17%
	-	27%	42%
	14 ± 2	26%	7% BDI > 13
	17 ± 3	34%	60%
	5 ± 4	-	71%
	13 ± 3	-	32%
	11 ± 5	12%	36%
	4 ± 2	-	7%, CES-D > 16
	3 ± 3	-	47%
	-	24%	34%

Table 2. Extracted data used in the meta-analyses from the included studies.

Study (Year)	N	Amyloid-beta scale	Measurement method	Clinical diagnosis/ depressive symptoms (criteria)
PET studies				
Almdahl, Agartz (20) (2022)	241	Continuous	^{18}F-Florbetapir	Clinical diagnosis (Medical history + NPI)
Babulal, Roe (31) (2020)	301	Categorical	^{18}F-Florbetapir	Clinical diagnosis (NACC Form D1)
Byun, Choe (32) (2016)	42	Continuous	^{11}C-PiB	Clinical diagnosis (DSM-IV)
Kumar, Kepe (22) (2011)	39	Continuous	^{18}F-FDDNP	Clinical diagnosis (DSM-IV)
Lewis, Bernstein (23) (2022)	4492	Continuous	^{18}F-Florbetapir	Depressive symptoms (GDS)
Moriguchi, Takahata (24) (2021)	40	Continuous	^{11}C-PiB	Clinical diagnosis (DSM-IV)
Wang, Kim (29) (2021)	235	Continuous	^{18}F-Flutemetamol	Depressive symptoms (HAMD)
Wu, Hsiao (30) (2014)	36	Continuous	^{18}F-Florbetapir	Clinical diagnosis (DSM-IV)
CSF studies				
Almdahl, Agartz (20) (2022)	241	Continuous	ELISA, Roche	Clinical diagnosis (Medical history + NPI)
Diniz, Teixeira (33) (2014)	41	Continuous	INNO-BIA	Clinical diagnosis (DSM-IV)
Gudmundsson, Skoog (35) (2007)	84	Continuous	ELISA, Innotest	Clinical diagnosis (DSM-III) Depressive symptoms (MADRS)
Hertze, Minthon (36) (2010)	66	Continuous	xMAP	Clinical diagnosis (DSM-IV)
Krell-Roesch, Rakusa (21) (2022)	699	Continuous	Elecsys	Depressive symptoms (BDI-II)
Pomara, Bruno (25) (2012)	47	Continuous	Meso Scale Discovery	Clinical diagnosis (DSM-IV)
Reis, Brandão (26) (2012)	28	Continuous	ELISA, Innotest	Clinical diagnosis (DSM-IV)
Siafarikas, Kirsebom (27) (2021)	60	Continuous	Meso Scale Discovery	Clinical diagnosis (ICD-10)
Plasma studies				
Byun, Choe (32) (2016)	42	Continuous	INNO-BIA	Clinical diagnosis (DSM-IV)
Direk, Schrijvers (34) (2013)	980	Continuous	ELISA, EUROIMMUN	Depressive symptoms (CES-D)
Moon, Kang (11) (2011)	123	Continuous	ELISA, Biosource	Depressive symptoms (GDS)
Sun, Chiu (28) (2009)	1060	Continuous	ELISA	Depressive symptoms (CES-D)

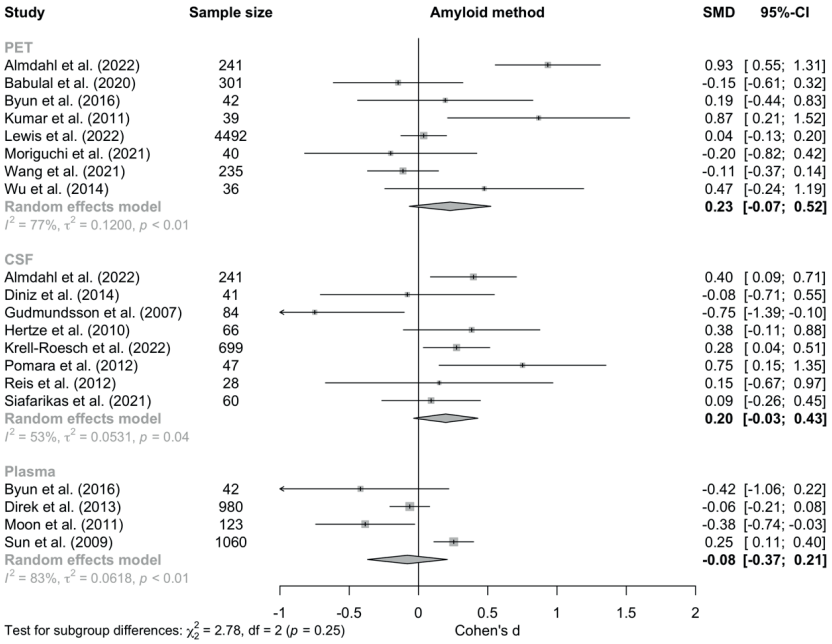
	Covariate-adjustment	Cohen's d ± SE
	No	0.93 ± 0.19 (SUVR)
	Age, gender, race, education, APOE e4	-0.15 ± 0.24 (SUVR)
	No	0.19 ± 0.32 (SUVR)
	No	0.87 ± 0.34 (DVR)
	Race, ethnicity, gender, age, employment, housing situation, marital status, education level, heavy alcohol use, any smoking use, medical morbidity score, hours of exercise per week, hours of sleep per night, history of neurological disease	0.04 ± 0.08 (SUVR)
	Age	-0.20 ± 0.32 (SUVR)
	Age, handedness, education	-0.11 ± 0.13 (SUVR)
	No	0.48 ± 0.37 (SUVR)
	No	0.40 ± 0.16 (Aβ42)
	No	-0.08 ± 0.32 (Aβ42)
	Age	-0.75 ± 0.33 (Aβ42)
		-0.39 ± 0.22 (Aβ42)
	No	0.38 ± 0.25 (Aβ42)
		1.10 ± 0.27 (Aβ40)
	Age, sex, education, APOE e4	0.28 ± 0.12 (Aβ42)
	No	0.75 ± 0.31 (Aβ42)
		0.56 ± 0.30 (Aβ40)
	No	0.15 ± 0.42 (Aβ42)
	No	0.09 ± 0.18 (Aβ42/Aβ40)
	No	-0.42 ± 0.33 (Aβ42)
		-0.14 ± 0.32 (Aβ40)
		-0.25 ± 0.32 (Aβ40/Aβ42)
	Age, gender, education, MMSE score, plasma creatinine levels, antidepressant use	-0.06 ± 0.07 (Aβ42)
		-0.16 ± 0.09 (Aβ40)
	No	-0.37 ± 0.18 (Aβ42)
	Age, sex, education	-0.38 ± 0.18 (Aβ42)
	Age, race, gender, education, creatinine, cardiovascular disease, APOE e4	0.25 ± 0.07 (Aβ42)
		-0.13 ± 0.21 (Aβ40)

The characteristics and effect sizes (Cohen's d ± standard error) of each included study are shown in Table 3. The meta-analysis of the eight PET studies resulted in an insignificant effect size of 0.23 (95% CI: -0.07; 0.52, p = 0.13) (Figure 2). For the eight CSF studies, no association was found between Aβ and depression or depressive symptoms (0.20, 95% CI: -0.03; 0.43, p = 0.10). Lastly, for the four plasma studies, no association was found between Aβ burden and depression or depressive symptoms (-0.08, 95% CI: -0.37; 0.21, p = 0.59). There was no statistically significant difference between the effect sizes based on how Aβ was assessed (Q(2) = 2.78, p = 0.25). However, there was substantial heterogeneity in the PET (I^2 = 77%), CSF (I^2 = 53%), and plasma subgroups (I^2 = 83%).

There was a statistically significant subgroup difference between the PET studies that controlled for covariates and the ones that did not (4 vs. 4 study groups, Q(1) = 13.46, p < 0.001). In the studies that did not adjust for covariates, an association was found between Aβ and depression or depressive symptoms (0.68; 95% CI: 0.32; 1.03, p < 0.001). Whereas in the covariate-adjusted studies, a null association was found between Aβ and depression or depressive symptoms (Supplementary Figure 1). When assessing differences between PET tracer, clinical diagnosis or depressive symptom questionnaire, or population-based study versus clinical settings, no subgroup differences were found in the PET studies. There was only one study that reported Aβ burden categorically based on an established cut-off value. Leaving out this study did not substantially change the effect size. The meta-regression did not reveal that sex/gender distribution or prevalence of APOE e4 allele genotype influenced the meta-analysis results for the PET studies.

There were no significant differences between the CSF studies based on covariate adjustment, clinical depression diagnosis versus depressive symptom questionnaire, or population-based versus clinical settings. When removing the one study that only assessed women, a significant association between Aβ burden in CSF and depression or depressive symptoms was found (0.29; 95% CI: 0.13; 0.43, p < 0.001). Further, the heterogeneity lessened (I^2 = 0%) (Supplementary Figure 2). The meta-regression on prevalence of APOE e4 allele did not show an effect on the meta-analysis of CSF studies. However, meta-regression reveled that sex/gender did influence the effect size (QM(1) = 3.98, p = 0.05). Further, the R^2 was 45%, meaning that 45% of the heterogeneity of the meta-analysis on CSF studies could be explained by differences in the sex/gender distribution of the participants. The expected effect size for men was 0.80 (95% CI: 0.19; 1.41), whereas for women it was -1.00 (95% CI: -1.98; -0.02).

Figure 2. Meta-analyses on the association between amyloid-beta and depression or depressive symptoms using PET, CSF, and plasma.



Note: The effect sizes of the individual studies are represented by the squares, of which the size is proportional to the weight of the study. The diamond represents the pooled estimate. The horizontal lines represent the 95% confidence intervals of the individual effect sizes. A positive Cohen's d represents an association between higher Aβ burden and depression or depressive symptoms. The effect sizes of studies assessing Aβ via cerebrospinal fluid or plasma were flipped.

No significant subgroup differences were found between the plasma studies that controlled for covariates or did not, assessed depression by clinical diagnosis or symptom questionnaire, or was performed in a clinical setting or in the general population. Meta-regression on APOE e4 allele could not be performed in the plasma studies as only two plasma studies reported the prevalence of APOE e4 allele in the study. Meta-regression revealed that sex/gender also influenced the effect size of the plasma studies (QM(1) = 16.26, p < 0.001) and accounted for the majority of the heterogeneity. However, the opposite was found in plasma; where the expected effect size for men was -0.89 (95% CI: -1.35; -0.42) and the expected effect size for women was 1.46 (95% CI: 0.75; 2.17).

Table 3. Risk of bias assessment using the adjusted Newcastle-Ottawa Quality Assessment Scale Cohort Studies.

	Selection		
Study	Representative	Selection	Exposure
Almdahl, Agartz (20)	*	*	*
Babulal, Roe (31)	*	*	*
Byun, Choe (32)	*	*	*
Diniz, Teixeira (33)	*	*	*
Direk, Schrijvers (34)	*	*	*
Gudmundsson, Skoog (35)	*	*	*
Hertze, Minthon (36)	*	*	*
Krell-Roesch, Rakusa (21)	*	*	*
Kumar, Kepe (22)	-	*	*
Lewis, Bernstein (23)	-	*	*
Moon, Kang (11)	*	*	*
Moriguchi, Takahata (24)	*	*	*
Pomara, Bruno (25)	-	*	*
Reis, Brandão (26)	-	*	*
Siafarikas, Kirsebom (27)	*	*	*
Sun, Chiu (28)	-	*	*
Wang, Kim (29)	*	*	*
Wu, Hsiao (30)	-	*	*

Comparability				Outcome		Overall score (max. 9)
Age	Sex/gender	Education	Other factors	Outcome	Same method	
-	-	-	-	*	*	5
*	*	*	*	*	*	9
-	-	-	-	*	*	5
-	-	-	-	*	*	5
*	*	*	*	*	*	9
*	*	-	-	*	*	7
-	-	-	-	*	*	5
*	*	*	*	*	*	9
*	*	*	-	*	*	7
*	*	*	*	*	*	8
*	*	*	-	*	*	8
*	-	-	-	*	*	6
-	-	-	-	*	*	4
-	-	-	-	*	*	4
-	-	-	-	*	*	5
*	*	*	*	*	*	8
*	*	*	*	*	*	9
-	-	-	-	*	*	4

Discussion

This systematic review and meta-analysis aimed to explore if depression or depressive symptoms are associated with Aβ burden assessed via PET, CSF, or plasma in older adults without cognitive impairment. No association was found between Aβ and depression or depressive symptoms in the PET, CSF, or plasma studies. The Egger's t-test suggested there was no publication bias. However, there was substantial heterogeneity in the PET, CSF, and plasma studies (19). Meta-regression revealed that sex/gender distribution in the included studies influenced the effect size in both the CSF and plasma studies and contributed to the heterogeneity.

Two previous systematic reviews have been conducted on Aβ and depression (10, 37), with one including a meta-analysis on CSF and plasma studies (10). While Nascimento, Silva (10) also did not find an association between CSF levels of Aβ and depression, there was an association between plasma levels of Aβ and depression. However, the included

studies in the meta-analysis of Nascimento, Silva (10) included studies assessing serum levels of Aβ, rather than plasma levels. Plasma Aβ levels have been found to be more stable under storage conditions than in serum (38), which was also the reason the current study focused on only plasma assessment of Aβ. The only study that was included both in the current meta-analysis and in the meta-analysis of Nascimento, Silva (10) is Sun, Chiu (28) which was the only included plasma study that found a significant association between Aβ and depressive symptoms. To note, this study was the oldest study of the included plasma studies, and as plasma assays have improved exponentially in the last years for Aβ assessment, it will be of interest to elucidate the possible role of plasma Aβ and depression using the newer, more sensitive plasma assays.

Due to the low number of studies assessing a longitudinal relationship between Aβ and depression and depressive symptoms, these studies were not included in the systematic review and meta-analysis. While there was a trend towards higher levels of Aβ deposition on PET and depression and depressive symptoms, the current meta-analysis did not find a significant relationship. One longitudinal study did find an association between an increase in depressive symptoms and a higher rate of increase in Aβ deposition on PET (39). A similar pattern was seen in a longitudinal study on plasma Aβ40/42, where no baseline association was found with depressive symptoms, but a longitudinal association was found with plasma Aβ40/42 and depressive symptoms nine years later (40). There is a possibility that early in the preclinical phase of dementia, Aβ burden as well as prodromal depressive symptoms may not be high enough for a relationship to be established. Future studies should include both repeated measures of Aβ and depressive symptoms to assess their temporal trajectory during the extended preclinical phase of AD.

Further, some articles could not be included due to insufficient information to calculate an effect size. These studies also did not find an association between Aβ and depression or depressive symptoms (41-43). However, two studies that looked regionally found higher levels of amyloid deposition based on PET imaging in either the temporal, parietal, and occipital areas in those who have a late-life depression diagnosis compared to non-depressed controls (44) or in just the medial temporal region in those with depressive symptoms (45). The current meta-analysis focused only on total rather than regional levels of amyloid in PET. It is possible that depression or depressive symptoms is associated first with amyloid deposition in temporal regions, which is why our current meta-analysis on PET studies found a null result. Future studies should elucidate this possible region-specific association between depression or depressive symptoms and amyloid accumulation.

This systematic review and meta-analysis had some limitations. Of note, only four studies reported the ethnicity of study participants, and participants were mostly Caucasian. This is of importance as the limited ethnicities could restrict the generalizability of our findings. However, this study also had many strengths. We assessed multiple methods to assess Aβ burden, used a random-effects meta-analysis, and performed multiple subgroup analyses to elucidate the heterogeneity in the meta-analyses.

In conclusion, this meta-analysis demonstrated no evidence of an association between depression or depressive symptoms and Aβ in PET, CSF, or plasma in older adults without cognitive impairment. It is possible that late-life depressive symptoms are independent to amyloid accumulation or that they may interact with one another later in the disease progression. More longitudinal studies with repeated measurements are needed to discover if depression is a reaction to the development of cognitive decline symptoms in late-life or driven by biological mechanisms shared by both depression and AD, such as inflammation.

Acknowledgements

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Conflict of interest

The authors have no conflict of interest to report.

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Supplemental Info

Supplementary Info 1

("Depression"[MeSH Terms] OR "Depressive Disorder"[MeSH Terms] OR "depress*"[Title/Abstract] OR "Depression"[Title/Abstract] OR "Depressions"[Title/Abstract] OR "Depressive"[Title/Abstract] OR "Depressed"[Title/Abstract] OR "affective disorder"[Title/Abstract] OR "Dysphoria"[Title/Abstract] OR "Dysthymia"[Title/Abstract] OR "depressed mood"[Title/Abstract] OR "mood disorder"[Title/Abstract] OR "depressive symptoms"[Title/Abstract]) AND (("Amyloid"[MeSH Terms] OR "plaque, amyloid"[MeSH Terms] OR "amyloid*"[Title/Abstract] OR "AB"[Title/Abstract] OR "Abeta40"[Title/Abstract] OR "Abeta42"[Title/Abstract] OR "abeta 40"[Title/Abstract] OR "abeta 42"[Title/Abstract] OR "ath"[Title/Abstract] OR "betaA40"[Title/Abstract] OR "betaA42"[Title/Abstract] OR "ath 40"[Title/Abstract] OR "ath 42"[Title/Abstract] OR "beta amyloid"[Title/Abstract] OR "Abeta"[Title/Abstract] OR "Abeta40"[Title/Abstract] OR "Abeta42"[Title/Abstract] OR "abeta 40"[Title/Abstract] OR "abeta 42"[Title/Abstract] OR "PIB"[Title/Abstract] OR "athology compound b"[Title/Abstract] OR "flutemetamol"[Title/Abstract] OR "florbetapir"[Title/Abstract] OR "florbetaben"[Title/Abstract] OR "senile plaque*"[Title/Abstract]) AND ("Positron-Emission Tomography"[MeSH Terms] OR "PET"[Title/Abstract] OR "positron emission tomograph*"[Title/Abstract] OR "Cerebrospinal Fluid"[MeSH Terms] OR "CSF"[Title/Abstract] OR "cerebrospinal fluid*"[Title/Abstract] OR "cerebro spinal fluid*"[Title/Abstract] OR "plasma"[Title/Abstract] OR "plasmas"[Title/Abstract] OR "athologylogy*"[Title/Abstract] OR "amyloid athology*"[Title/Abstract] OR "Neuropathology"[MeSH Terms]))

Supplementary Info 2

Adjusted version of the Newcastle-Ottawa Quality Assessment Scale for Cohort Studies Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability. Total maximum number of stars is nine.

Selection criteria

1) Representativeness of the exposed cohort (depressed, cognitively unimpaired)

a) truly representative of the average older adult without dementia in the community (i.e., community-based cohort, can include individuals with MCI or subjective cognitive decline as well) *

b) somewhat representative of the average older adult without dementia in the community (e.g., inclusion criteria regarding only individuals without MCI) *

c) selected group of users, e.g., volunteers, memory clinic visitors, only individuals at higher risk (e.g., only subjective complaints, only APOE e4 carriers)
d) no description of the derivation of the cohort

2) Selection of the non-exposed cohort (non-depressed)
a) drawn from the same community as the exposed cohort *
b) drawn from a different source
c) no description of the derivation of the non-exposed cohort

3) Ascertainment of exposure
a) clinical interview *
b) established depressive symptom questionnaire *
c) categorized based on established or published cut-offs for a symptom questionnaire *
d) categorized based on non-established cut-offs (e.g., z-score cut-off, mean split, median split)
e) no description

Comparability

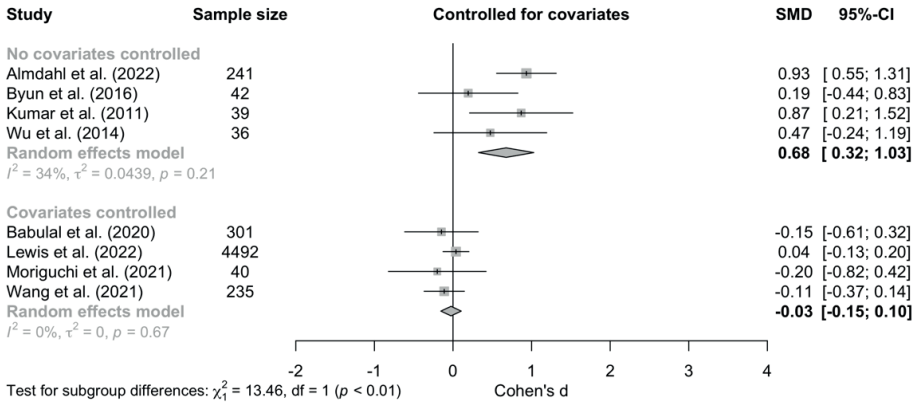
1) Comparability of cohorts regarding the design or analysis
a) study controls for age *
b) study controls for sex/gender *
c) study controls for education *
d) study controls for any additional factor *

Outcome (Amyloid)

1) Ascertainment of outcome
a) via PET scan *
b) via CSF *
c) via plasma *
d) no description

2)Same method of assessment for cases (depressed) and controls (non-depressed)
a) yes *
b) no

Figure 1. Subgroup analysis on PET studies that either adjusted for covariates or did not.



Supplementary Figure 2. Sensitivity analysis on CSF studies when removing one cohort that consisted only of women (Gudmundsson et al.).

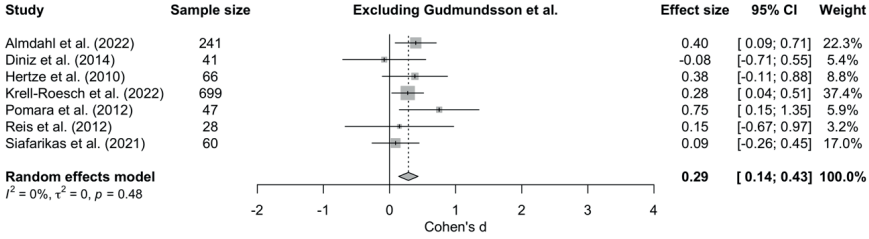


Table 1. Continued

Chapter 6

Depressive symptoms and plasma markers of Alzheimer's disease and neurodegeneration: a coordinated meta-analysis of 8 cohort studies

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Abstract

Background: Depressive symptoms are associated with an increased risk of Alzheimer's disease (AD). There has been a recent emergence in plasma biomarkers for AD pathophysiology, such as amyloid-beta (Aβ) and phosphorylated tau (p-tau), as well as for axonal damage (neurofilament light, NfL) and astrocytic activation (glial fibrillary acidic protein, GFAP). Hypothesizing that depressive symptoms may occur along the AD process, we investigated associations between plasma biomarkers of AD with depressive symptoms in individuals without dementia.

Methods: A two-stage meta-analysis was performed on 2 clinic-based and 6 population-based cohorts (N=7210) as part of the Netherlands Consortium of Dementia Cohorts. Plasma markers (Aβ42/40, p-tau181, NfL, and GFAP) were measured using Single Molecular Array (Simoa; Quanterix) assays. Depressive symptoms were measured with validated questionnaires. We estimated the cross-sectional association of each standardized plasma marker (determinants) with standardized depressive symptoms (outcome) using linear regressions, correcting for age, sex, education, and APOE ε4 allele presence, as well as subgrouping by sex and APOE ε4 allele. Effect estimates were entered into a random-effects meta-analysis.

Results: Mean age of participants was 71 years. The prevalence of clinically-relevant depressive symptoms ranged from 1% to 22%. None of the plasma markers were associated with depressive symptoms in the meta-analyses. However, NfL was associated with depressive symptoms only in APOE ε4 carriers (β 0.11; 95% CI: 0.05-0.17).

Conclusions: Late-life depressive symptoms did not show an association to plasma biomarkers of AD pathology. However, in APOE ε4 allele carriers, a more profound role of neurodegeneration was suggested with depressive symptoms.

Introduction

Depression has been coined as one of the main risk factors for Alzheimer's disease (AD) dementia (1-4). One longitudinal study in older adults found that with every increasing point on a depressive symptom scale, the risk of AD increased by 19% (5). Another study found that high levels of depressive symptoms were associated with a 50% increased risk of dementia (6). However, the mechanistic relationship between the two is not yet fully understood. Some studies have highlighted depression as a possible risk factor while others suggest that depression may in fact be an early manifestation of underlying AD (7, 8). These hypotheses may coexist and are not mutually exclusive. Late-life depressive symptoms and AD may have a common biological cause (9, 10), where depressive symptoms may indicate preclinical AD pathology (7, 11, 12). Studying AD-related biomarkers associated with depressive symptoms may help entangle the pathophysiology of depressive symptoms in late-life.

Biomarkers related to AD pathology are amyloid-beta (Aβ) plaques and phosphorylated tau (p-tau) (13). These pathologies have thus far mostly been detected using position emission tomography (PET) scans (14) or in cerebrospinal fluid (CSF) obtained through a lumbar puncture (15). These methods are costly and invasive, hampering their wider application to the general population. Two other developing biomarkers, neurofilament light (NfL) (reflecting axonal damage) and glial fibrillary acidic protein (GFAP) (reflecting astrocyte activation), have also been suggested as biomarkers for AD-related pathological changes and can be assessed in CSF (16-18).

However, studies exploring the association of these biomarkers in plasma rather than PET or CSF with late-life depressive symptoms are scarce. Regarding Aβ, studies have consistently found an association between Aβ and depressive symptoms (19-21). Thus far, no studies on plasma p-tau181 and depression have been done, only in tau PET (22, 23) and CSF p-tau181 (24, 25). Regarding the non-specific biomarkers, studies thus far have focused on the clinical stage of depression, with high levels of serum GFAP (26) and plasma NfL (27) found in those with major depressive disorder. Previous studies have highlighted the need for assessing the relationship between plasma AD pathophysiology and depression using ultrasensitive immunoassays (28), such as Simoa, that have higher reliability of measuring both Aβ and tau levels, which occur in exponentially smaller quantities in plasma (29).

Given the lack of current studies assessing depressive symptoms with AD-related pathophysiology in plasma, as well as in population-based settings, we aimed to

assess the cross-sectional relationship between plasma Aβ42/40, p-tau181, NfL, and GFAP with depressive symptomology in the Netherlands Consortium for Dementia Cohorts (NCDC) including eight cohort studies of individuals without dementia.

Methods and materials

Study design

This study incorporated a two-stage meta-analysis within the NCDC consortium. The NCDC consortium consists of nine prospective, Dutch cohort studies. Cohorts from the NCDC consortium were selected for the current study based on a number of criteria, including 1) availability of plasma markers, 2) assessment of depressive symptoms, and 3) no diagnosis of dementia.

The current study excluded two cohorts (i.e., Lifelines and the Maastricht Study), because those did not have data on plasma biomarkers at the time of the study. One cohort (i.e., EPAD+) included two subcohorts (i.e., EMIF-Twins and EMIF-90+). Therefore, a total of eight cohorts were included in the meta-analysis: the Amsterdam Dementia Cohort (ADC) (30), Doetinchem Cohort Study (DCS) (31), EMIF-Twins (32), EMIF-90+ (33), Longitudinal Aging Study Amsterdam (LASA) (34), Leiden Longevity Study (LLS) (35), Rotterdam Study (36), and the SMART-MR Study (37, 38). The ADC came from a memory clinic, but only participants with subjective cognitive decline were included in the current study. The SMART-MR Study originated from a hospital setting where individuals all have a history of vascular disease. The other six cohorts were population-based studies. Only a small subsample from each cohort had plasma markers determined (total N = 7210). More information per cohort regarding participant selection for plasma assessment can be found in Supplementary Info 1.

Plasma markers

The plasma markers (Aβ40, Aβ42, p-tau181, NfL, and GFAP) were measured using novel immunoassays in all cohorts except for EMIF-Twins and the Rotterdam Study. In brief, participants underwent venipuncture under non-fasting or fasting conditions (depending on the cohort) for EDTA-plasma sample assessment after storage. The Neurology 4-Plex E kit (Quanterix) was used for $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$, NfL, and GFAP (39). P-tau181 was assessed using the P-tau181 Advantage V2 kit (Quanterix). Assays were run on-board of the Simoa HDx analyzer following manufacturer's instructions, using automated on-board sample dilution. Neurology 4-Plex kits were run in monoplo, and the p-tau181 kits were run in duplicates.

For EMIF-Twins, Aβ40 and Aβ42 were measured with in-house developed immunoassays ("Amyblood"; prototype of the Neurology 4-Plex E kit) (40), p-tau181 with a prototype immunoassay employing AT270 (Thermo Fisher Specific) and LRL (Eli Lilly and Company) antibodies and GFAP was assessed with the commercially-available Simoa GFAP Discovery kit (Quanterix), using the Simoa HD-x analyzer, as described elsewhere (32). NfL was not assessed in EMIF-Twins.

For the Rotterdam Study, the Simoa Neurology 3-Plex A assay was used to assess $A\beta_{x-40}$ and $A\beta_{x-42}$ (41) and the Simoa NF-light advantage kit (42, 43) to assess NfL on the Simoa HD-1 analyzer platform. P-tau181 and GFAP were not assessed in the Rotterdam Study.

All plasma measurements were performed at Amsterdam UMC, except for the Rotterdam Study. AD plasma biomarker assessment for ADC, DCS, LASA, LLS, and SMART-MR were all performed using the same kit lot number. EMIF-90+ was assessed with the same assays, but using different kit lot numbers. Further information regarding plasma storage and collection per cohort can be found in Supplementary Info 1.

Depressive symptoms

Depressive symptoms were collected from validated questionnaires and included continuous sum scores. Depressive symptoms were collected at the same time-point as plasma measurements for all cohorts. For three cohorts (i.e., ADC, EMIF-90+, EMIF-Twins), the Geriatric Depression Scale-15 (44) (GDS-15) was used. The total sum score on the GDS-15 is 15, with a higher score representing more depressive symptoms. LASA, LLS, and the Rotterdam Study used the Center for Epidemiologic Studies Depression Scale (CES-D) (45). The CES-D has a sum score up to 60 based on 20 items with a possible score of 0-3 per item. The SMART-MR study used the Patient Health Questionnaire-9 (PHQ-9) (46). The PHQ-9 has a total score of 27, based on nine items with scores ranging from 0-3 per item. The DCS used the Mental Health Inventory questionnaire (MHI-5) (47). The total score on the MHI-5 is up to 100, transformed based on a five-item questionnaire with a five-point scale regarding the frequency of positive and negative feelings. As higher scores on the MHI-5 reflect lower depressive symptoms, this questionnaire was reverse-coded. As questionnaires differed across cohorts, sum scores were standardized into z-scores. Additionally, we dichotomized these sum scores based on published cut-offs reflecting clinically-relevant depressive symptoms. The cut-off for the GDS-15 was six or higher in the short form(44, 48). For the CES-D, a cut-off of 16 or higher was used (49). For the PHQ-9, a cut-off of six or higher is used (50). Lastly, for the MHI-5, we used a cut-off of 35 or lower (51).

Covariates

Age and sex were assessed via self-report. Education was assessed categorically into less than high school education, at least some high school education, or college/university degree based on the Dutch education system. *APOE* ε4 allele (rs429358 C allele) presence was assessed via blood sample according to methods described previously (32, 43, 52-56) and defined dichotomously as the presence of at least one ε4 allele or not.

Statistical analysis

For the first stage, standardized analyses were performed using Rstudio (v.4.0.3) by E.T. locally per cohort. Multiple imputation of 10 imputed datasets was performed separately in each cohort with missing data using the *mice* package in R to address missing data. Both outcomes and predictors were imputed if needed (57), using covariate information (i.e., age, sex, education, and APOE ε4 allele) as predictors in the imputation (see Table 1 for missing data per cohort). The same variables for imputation were used in each cohort. Linear regressions were performed to estimate the association between the plasma markers (determinant) and depressive symptoms (outcome). Logistic regressions were performed for the dichotomous outcome of high vs. low depressive symptomology. As assumptions were not met for linear regression based on non-normality, rank inverse normal transformation was done on the depressive symptom data (58). Thereafter, models met all assumptions. Models were adjusted for age, sex, education, and presence of APOE ε4 allele. Sensitivity analyses were performed stratifying by sex and APOE ε4 allele status, and when excluding missing data (i.e., a complete case analysis). Further, analyses were performed assessing the interaction between sex and plasma marker and APOE ε4 allele status and plasma marker. Another sensitivity analysis was done on the models of Aβ42/40 by adding 1/Aβ40 and Aβ42 as main effects, as suggested by previous work on using a ratio in regression analyses (59). Lastly, we also assessed as sensitivity analyses Aβ40 and Aβ42 as separate determinants for Aβ pathology.

For stage two, effect estimates for each analysis were pooled using random-effects meta-analyses. A random-effects meta-analysis was chosen over a fixed-effects meta-analysis as a random-effects meta-analysis relatively weights the studies more equally when there is heterogeneity between studies (60). Between-cohort heterogeneity was assessed via the I^2 and Cochrane's Q. We also performed meta-regressions based on the mean age of the studies and the proportion of individuals with an APOE ε4 allele to see if these factors influenced meta-analysis results.

Statistical significance was set to $p < 0.01$ for Bonferroni correction as we assessed four plasma markers: Aβ42/40, p-tau181, NfL, and GFAP.

Results

Cohort characteristics

Table 1 shows the demographic characteristics of each of the eight cohorts. Table 2 presents the distributions of the plasma biomarkers and depressive symptoms per cohort. In total, 7210 individuals were included in the meta-analysis. The total mean age was 71, and mean age per cohort ranged from 60 to 92 years. Percentage women per cohort ranged from 17% to 59%. Median values of the plasma biomarkers are provided in Table 2; prevalence of high depressive symptomology based on clinical cut-offs ranged from 1% to 22% per cohort.

Table 1. Cohort demographic characteristics

Cohorts	N	Setting	AgeM (SD) [Range]	SexN (%) female	EducationN (%) high	APOE ε4 alleleleN (%)
ADC	307	Memory clinic, subjective cognitive decline	60 (9) [38-82]	130 (42%)	174 (57%)	123 (40%)
DCS	365	General population	68 (3) [64-75]	177 (48%)	85 (23%)	108 (30%)
EMIF-90+	129	General population	92 (3) [85-102]	74 (57%)	23 (18%)	29 (22%)
EMIF-Twins	220	General population	71 (8) [60-94]	129 (59%)	22 (10%)	74 (34%)
LASA	370	General population	69 (7) [61-90]	203 (55%)	26 (7%)	161 (44%)
LLS	370	General population	67 (5) [55-85]	189 (51%)	121 (33%)	105 (28%)
Rotterdam Study	4855	General population	72 (8) [58-99]	2783 (57%)	675 (14%)	1327 (27%)
SMART-MR	594	Patients with vascular disease, hospital-based	64 (8) [50-83]	101 (17%)	146 (25%)	184 (31%)
Total	7210					

Note: Imputed data is shown for cohorts with missing data.
For ADC, n = 1 missing for education and n = 15 missing for APOE ε4 allele genotype.
For DCS, n = 8 missing for APOE ε4 genotype.
For EMIF-90+, n = 26 missing on APOE ε4 allele genotype.
For EMIF-Twins, n = 4 missing on age, n = 3 missing on sex, n = 3 missing on education, and n = 6 missing on APOE ε4 allele genotype.
For LLS, n = 4 missing on education, n = 5 missing on APOE ε4 allele.
For Rotterdam Study, n = 2 missing on age, n = 70 missing on education, n = 242 missing on APOE ε4 allele genotype.
For SMART-MR, n = 5 on education and n = 27 missing for APOE ε4 genotype.

Correlation matrices per cohort for age, depressive symptoms, and AD biomarker levels are shown in Supplementary Figure 1. A meta-analysis on the correlations between the plasma markers and age showed a positive correlation between p-tau181, NfL, and GFAP, and a negative correlation between Aβ42/40 and age (Supplementary Figure 2). Men had lower levels of Aβ42/40 and GFAP (Supplementary Figure 3). There was no difference in p-tau181 and NfL levels between men and women. APOE ε4 allele carriers had lower levels of Aβ42/40 and higher levels of GFAP (Supplementary Figure 4), but no difference in levels of p-tau181 and NfL.

Meta-analysis findings

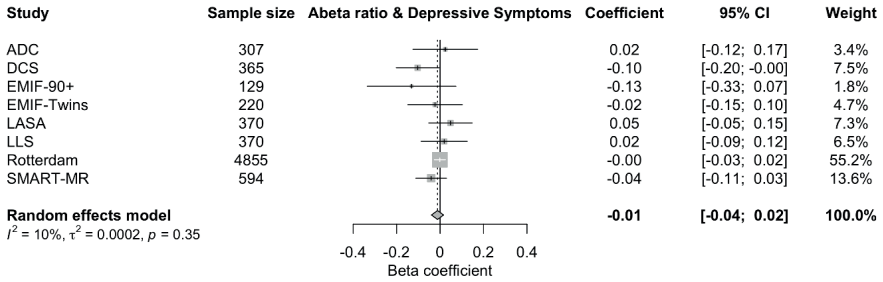
There was no evidence for an association between any of the AD plasma markers and depressive symptoms in the random-effects meta-analysis (standardized regression coefficient β: -0.03 – 0.04, all $p > 0.05$; Figure 1).

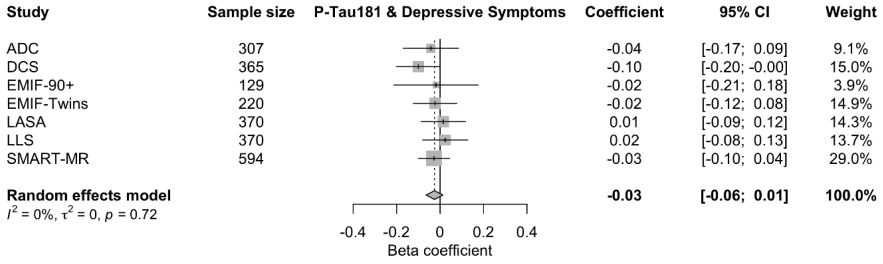
Due to a low number of individuals with high depressive symptomology (n=3), logistic regression could not be performed in the EMIF-Twins study and therefore not included in the meta-analyses. Random-effects meta-analyses also revealed no association with any plasma biomarker and high depressive symptomology (Supplementary Figure 5). Per cohort, results were similar to the linear regressions.

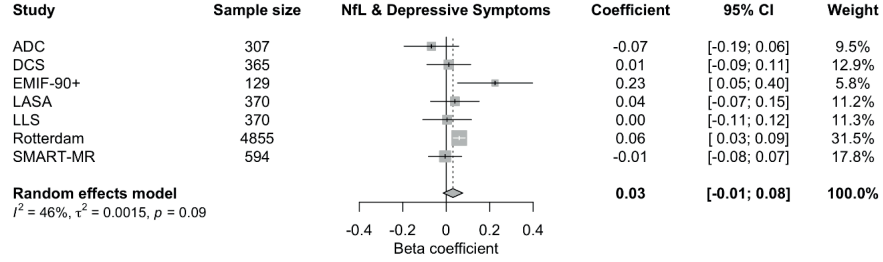
Subsequently, we performed a number of sensitivity analyses. When assessing women and men separately, higher levels of NfL were associated with higher depressive symptoms in women (β: 0.07, 95% CI: 0.03; 0.10), but not men (β: 0.01, 95% CI: -0.04; 0.05). However, there was not a significant interaction with sex (p-interaction: 0.95, Figure 2). There were no other sex differences in the other plasma markers with depressive symptoms (see Supplementary Table 1 for per cohort data).

When stratifying by APOE ε4 allele presence, higher levels of NfL were associated with higher depressive symptoms in individuals with an APOE ε4 allele in the stratified meta-analyses (β: 0.11, 95% CI: 0.05; 0.17, $p < 0.001$), while effects in APOE ε4 non-carriers were much smaller (β: 0.01, 95% CI: -0.06; 0.07). When testing the interaction of NfL and APOE, there was a significant interaction (β: 0.08, 95% CI: 0.02; 0.13, $p = 0.009$, Figure 2). No other subgroup differences were found in other AD plasma markers for APOE ε4 carrier status (see Supplementary Table 1 for per cohort data).

Complete case analysis is shown in Supplementary Table 2. Main findings remained largely similar. When adding the main effects of Aβ42 and 1/Aβ40 to the Aβ42/40 ratio model, results remained insignificant (Supplementary Figure 6). When assessing Aβ40







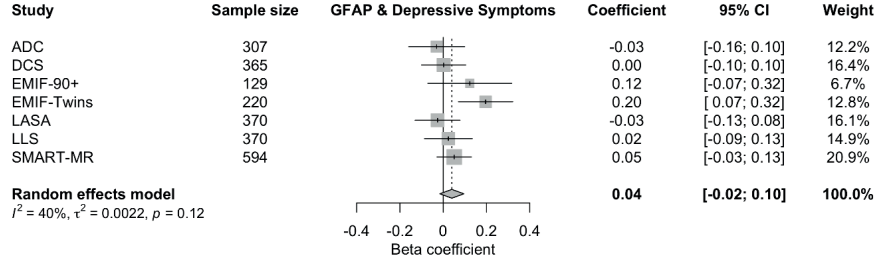


Figure 1. Meta-analysis on linear regressions of AD plasma markers and depressive symptoms.

Table 2. Descriptives of plasma biomarker and depressive symptoms per cohort

Cohorts	Aβ40 (pg/mL)	Aβ42 (pg/mL)	Aβ42/40 (pg/mL)	Ptau-181 (pg/mL)
ADC[a]	119 (107-135)	6.9 (6.1-7.6)	0.06 (0.05-0.06)	1.36 (1.06-1.99)
DCS	119 (100-137)	6.7 (5.6-7.8)	0.06 (0.05-0.07)	1.51 (1.15-1.93)
EMIF-90+[a]	110 (100-129)	7.7 (6.6-9.0)	0.07 (0.06-0.08)	3.02 (2.13-4.40)
EMIF-Twins[a]	138 (124-154)	32.6 (28.4-37.8)	0.24 (0.21-0.26)	5.93 (4.97-7.49)
LASA	122 (108-138)	6.9 (6.1-8.0)	0.06 (0.05-0.06)	1.58 (1.24-2.11)
LLS	126 (114-140)	7.3 (6.3-8.3)	0.06 (0.05-0.06)	1.43 (1.17-1.81)
Rotterdam Study[a]	259 (229-293)	10.3 (8.9-12.1)	0.04 (0.04-0.05)	NA
SMART-MR	113 (99-130)	6.8 (5.8-7.7)	0.06 (0.05-0.07)	1.37 (1.05-1.84)

Note: [a] EMIF-Twins and the Rotterdam Study use a different plasma assay. ADC and EMIF-90+ have been assessed at prior time points with a different batch.
Median and interquartile range. Imputed data is shown for cohorts with missing data.
For ADC, n = 1 missing on both Abeta1-40, 1-42, GFAP, and NFL. N = 2 missing for Ptau-181 and n = 21 missing for depressive symptoms.
For DCS, n = 1 missing for depressive symptoms.
For EMIF-90+, n = 20 missing on all plasma marker data.
For EMIF-Twins, n = 14 missing on Abeta1-40, n = 33 missing on Abeta1-42, n = 26 missing on Ptau-181, n = 14 missing for GFAP, and n = 1 missing on depressive symptoms. NFL was not assessed.
For LASA, n = 1 missing on depressive symptoms.
For LLS, n = 2 missing on depressive symptoms.
For Rotterdam Study, n = 99 missing on depressive symptoms.
For SMART-MR, n = 5 missing for depressive symptoms. Aβ = amyloid-beta; Ptau = phosphorylated tau; GFAP = glial fibrillary acidic protein; NfL = neurofilament light.

and Aβ42 separately, both biomarkers showed higher levels associated with increased depressive symptoms (Supplementary Figure 7).

Meta-regressions on mean age per study showed that age influenced the results of the meta-analysis on levels of NfL and depressive symptoms ($QM(1) = 10.78$, $p = 0.001$, Supplementary Figure 8). For each increase of mean age per year per study, there was an increase of 0.01 in the z-score levels of NfL and depressive symptoms. For the meta-regression on proportion of APOE ε4 carriers per study, there was no evidence of an influence of levels of AD biomarkers and depressive symptoms.

Discussion

In this meta-analysis on eight Dutch cohorts, where depressive symptoms are not severe or highly prevalent, we found no association between Aβ42/40, p-tau181, NfL, or GFAP and depressive symptoms. In the subgroup analyses, we found higher levels of NfL associated with depressive symptoms in individuals with an APOE ε4 allele,

NfL (pg/mL)	GFAP (pg/mL)	Depressive symptomsN (%) high
11.1 (8.5-15.4)	67 (48-98)	45 (15%)
15.6 (12.6-19.6)	106 (84-144)	78 (21%)
44.4 (32.2-56.1)	186 (147-267)	10 (8%)
NA	134 (105-179)	3 (1%)
14.9 (11.7-20.1)	94 (73-131)	24 (6%)
15.4 (12.3-19.1)	99 (76-129)	81 (22%)
13.3 (10.0-18.3)	NA	543 (11%)
13.8 (10.1-19.8)	86 (61-115)	100 (17%)

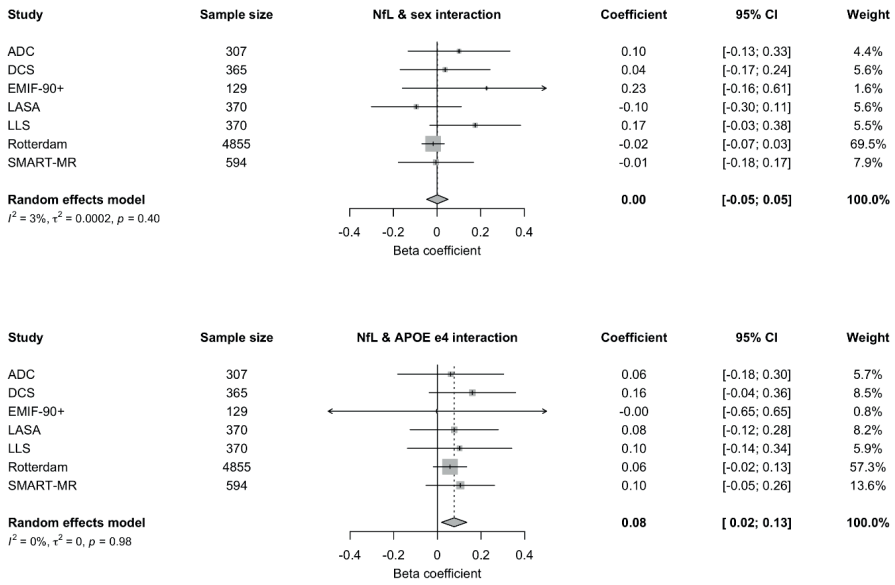


Figure 2. Meta-analyses on the interaction of NfL with sex and APOE ε4 carrier status.
Note: A positive interaction would signify higher levels of NfL associated with depressive symptoms in women or in APOE ε4 carriers.

suggesting that in those individuals with a genetic risk for AD, neurodegenerative changes may underlie the occurrence of depressive symptoms.

We did not find an association between Aβ42/40 and depressive symptoms, which is not in line with a previous meta-analysis that found a relationship with lower levels of Aβ42/40 in those with depression compared to healthy controls (61). However, all of those studies focused on a clinical population with depression. Further, one recent study found a difference between Aβ42/40 between those with clinical depression compared to controls, but there was no cross-sectional association between depressive symptom severity and Aβ levels (28). Therefore, subclinical levels of depressive symptoms may not be associated with accumulating Aβ levels. Regarding tau pathology, a systematic review and meta-analysis on tau pathology and depression also did not find a relationship (62). Our null finding in GFAP is not in line with previous literature that found an association with major depressive disorder in serum GFAP (26) and in CSF (63). To our knowledge, no studies yet have been performed assessment of GFAP with depressive symptoms. Similar to Aβ, there is a possibility that GFAP levels may be altered only when clinical depression is present.

Our results suggest an association between NfL and depressive symptoms in individuals with an APOE ε4 allele. This suggests that in individuals with high genetic risk for AD, neurodegenerative changes may underlie the occurrence of depressive symptoms, while in APOE ε4 noncarriers, depressive symptoms may have another origin. This may be explained by increased rates of neurodegeneration seen in those with an APOE ε4 allele (64). Further, the presence of an APOE ε4 allele has been associated with late-life depression (65, 66). NfL has also been related to cerebral vascular damage (67), perhaps explaining a vascular pathway to depressive symptoms. As literature on NfL in depression is limited (68), particularly in population-based studies, more research is needed to validate these findings.

This meta-analysis has multiple strengths. By using a meta-analytical approach, we were able to gain power to assess the relationship between plasma AD biomarkers and depressive symptoms. Additionally, the ultrasensitive Simoa platform was used for biomarker assessments. Further, we harmonized methods across cohort studies, employed the same statistical procedure, and controlled for the same confounders.

The study also comes with some limitations. Depressive symptoms, as well as biomarker assessment, were collected at only one time-point. We also could not assess a temporal relationship between biomarker levels and depressive symptoms. Therefore, we could not infer causal directionality between neuropathology and

depressive symptoms. Different depressive symptom questionnaires were also used amongst the cohorts, which could have increased heterogeneity and dampened replicability of our findings between the cohorts (69). However, the lack of an association was mostly consistent across cohorts. Importantly, cohort participants were almost exclusively White individuals. As biomarker levels have shown to differ across ethnicities (70, 71), future studies should also assess the relationship between AD plasma biomarkers and depressive symptoms in other ethnicities.

Our study did not provide evidence for a direct link between plasma markers of the AD pathophysiological process and depressive symptoms. Subgroup analyses did suggest a more profound role of neurodegeneration and depressive symptomology in those with an APOE ε4 allele, but further replication and longitudinal studies are needed to elucidate the temporal role of depressive symptoms with neurodegeneration.

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Competing interests

The authors declare none.

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Supplemental Info

Supplementary Info 1. Plasma collection and selection criteria per cohort

Within the Amsterdam Dementia Cohort (ADC), we only included individuals with subjective cognitive decline. Samples were stored at -80°C between 2000 and 2016 until analysis in 2021.

The Doetinchem Cohort Study (DCS) selected individuals with at least 4 repeated cognitive measurements, aged 65+ during their second cognitive measurement and with blood available were selected (n=348), supplemented with a random selection of participants with at least 4 repeated cognitive measurements, aged 64 years during their second cognitive measurement and with blood available (n=17). Plasma was collected between 2000-2007 and first stored at -20°C. If a full batch is collected (within about 6 weeks), the plasma is stored at -86°C. Plasma markers were analyzed in 2021.

In the EMIF-Twins study, participants were selected from the EMIF-AD PreclinAD study based on the following criteria: being monozygotic twins, aged 60 years or older, and with normal cognition. Blood was sampled between 2014 and 2017 at baseline. Plasma is originally stored at -80°C until analysis. The EMIF-90+ Study sampled blood between 2016 and 2018. Plasma was stored at -80°C until analysis. For both the EMIF-Twins and EMIF-90+ Study, blood was analyzed in 2021.

For the Longitudinal Aging Study Amsterdam (LASA), individuals were selected based on the following data: 60 years or older and available MMSE, APOE e4 allele status, and GWAS. Blood was collected in 2008 and 2009 and analyzed in 2021. The samples were centrifuged and stored at -80°C.

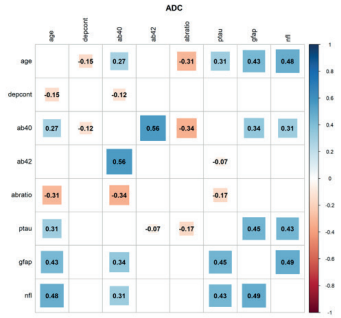
The Leiden Longevity Study (LLS) recruited between 2002 and 2006 1671 members of long-lived families (mean age 60 years) and their 744 partners (mean age 60 years) as population controls [1]. In 2009 and 2010, 503 participants took part in follow up measurements. In accordance with the Declaration of Helsinki, we obtained informed consent from all participants prior to their entering the study. Good clinical practice guidelines were maintained. The study protocol was approved by the ethical committee of the Leiden University Medical Center before the start of the study (P01.113). From these 503 participants, the EDTA plasma samples of a subset of 357 participants were selected for the current study by selection those individuals that 1) were still alive in 2020, 2) had an APOE genotyping available, 3) were 60 years or

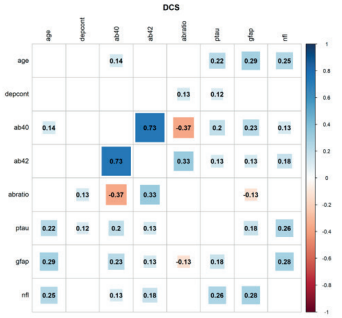
older in age. There were N = 13 APOE e4 allele carriers with an age >54.8 years added to the main selection, resulting in a total group of N = 370 individuals. EDTA plasma was collected between 2009 and 2010, and since then stored at -80°C in aliquot of 500µl. AD plasma markers were assessed in 2021.

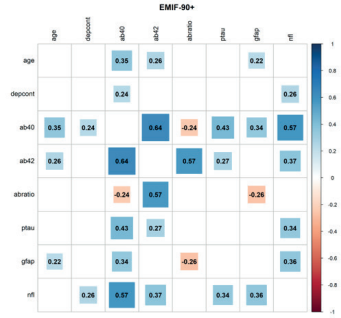
The Rotterdam Study Iled all participants who had available data during the fourth measurement. Blood was collected between 2002 and 2005 and assessed in 2018. The samples were stored at -80°C with no additional freeze/thaw cycles.

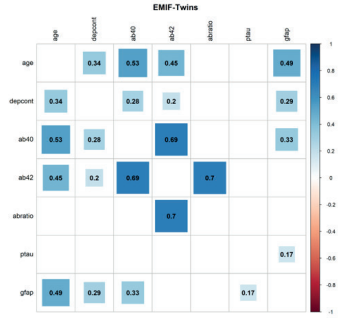
The SMART-MR Study selected individuals 50 years or older, having a brain MRI scan, and available cognitive measurements for AD plasma marker assessment. Plasma measurements were performed between 2006 and 2009, during the first follow-up moment, under overnight-fasting conditions. Plasma measurements were also analyzed in 2021.

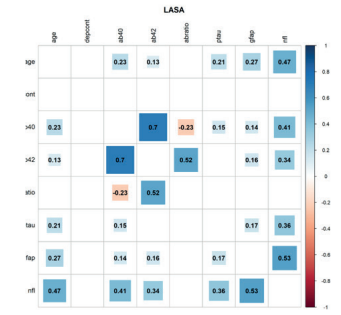
Supplementary Figure 1. Correlation matrices for each AD plasma marker, depressive symptoms, and age.

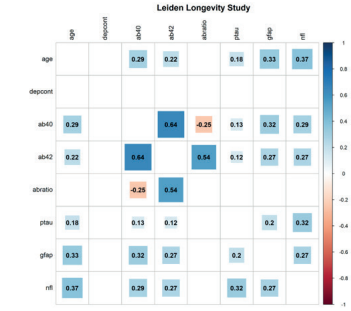


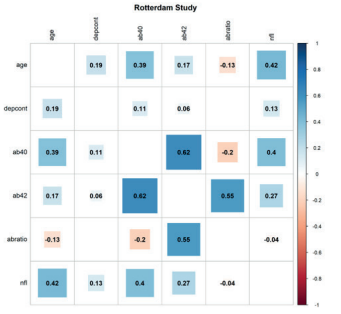


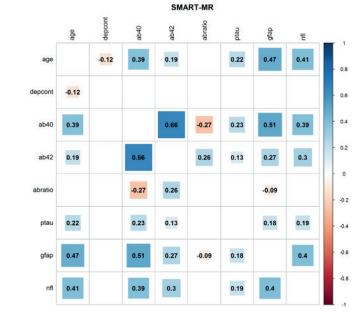




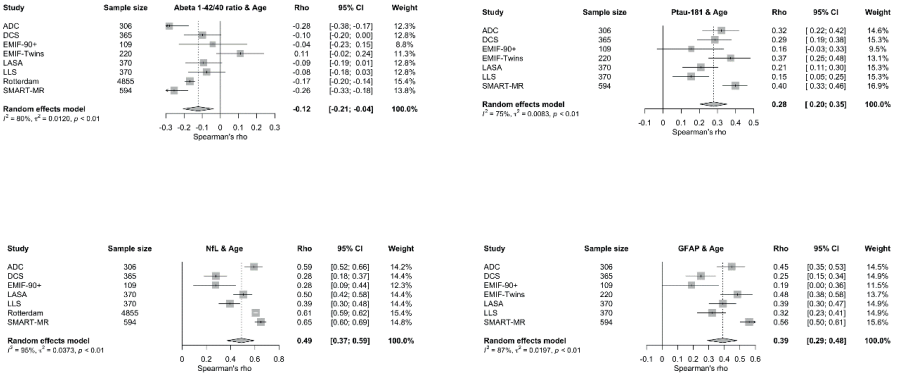




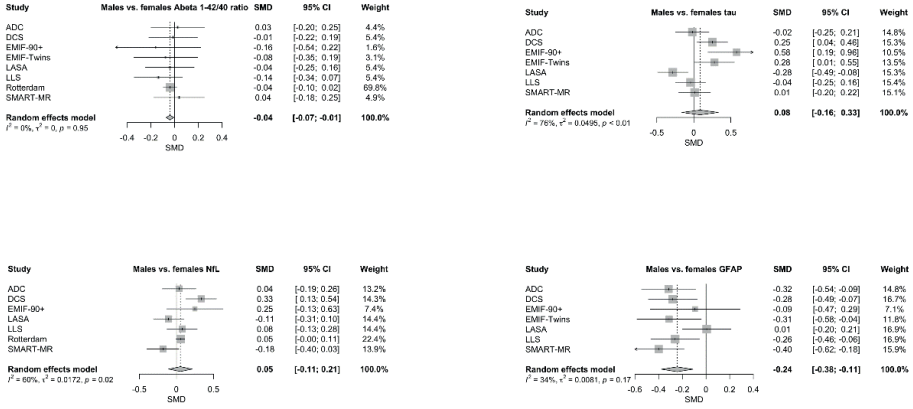




Supplementary Figure 2. Age correlations between the AD plasma markers and age per cohort.

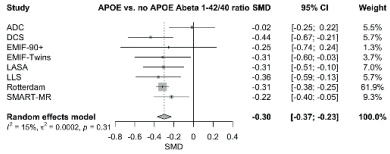


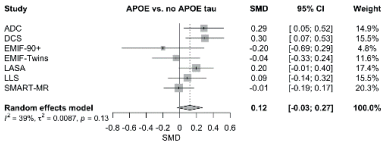
Supplementary Figure 3. Sex differences between AD plasma markers per cohort.

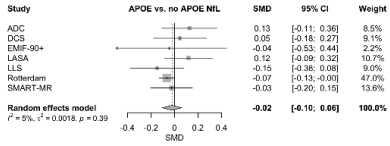


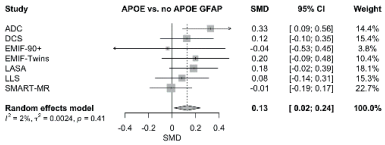
Note: SMD below 1 signify lower levels in men.

Supplementary Figure 4. APOE e4 allele differences per plasma marker and cohort.



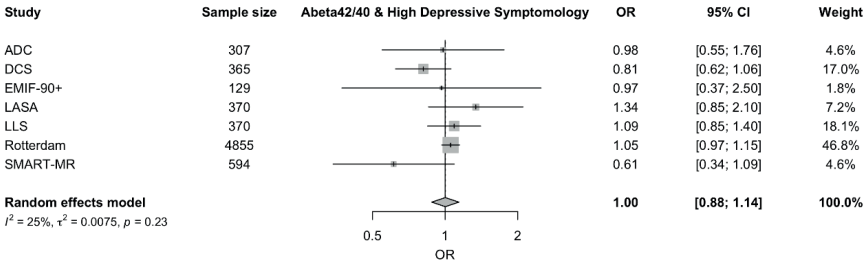


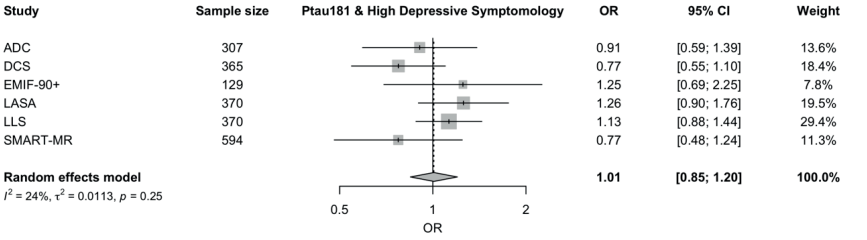


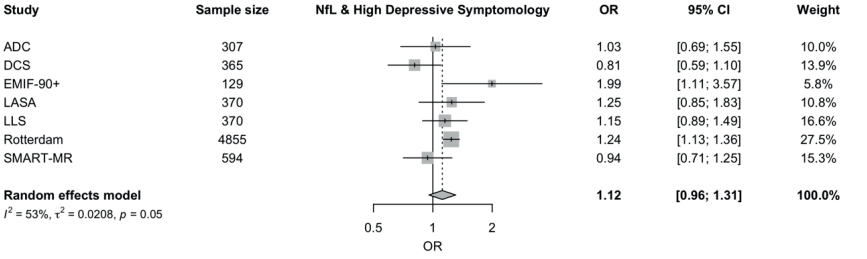


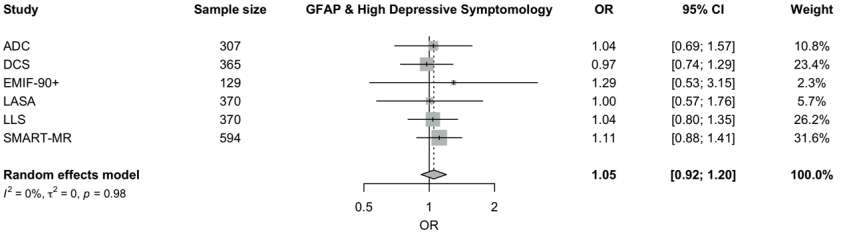
Note: SMD below 0 signify lower levels in APOE e4 carriers. SMD above 0 signify higher levels in APOE e4 carriers.

Supplementary Figure 5. Meta-analyses on logistic regressions of plasma markers and high depressive symptomology.

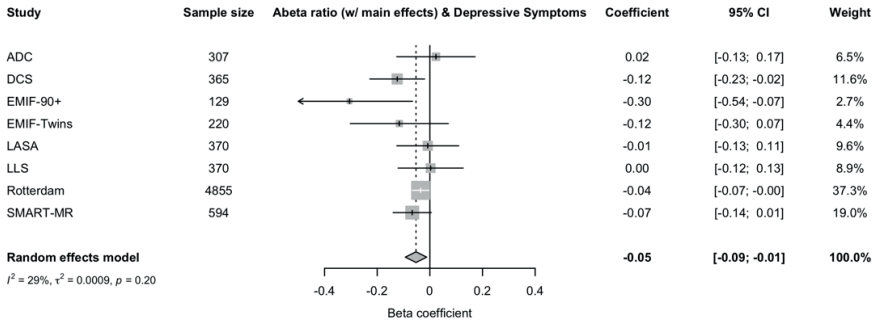






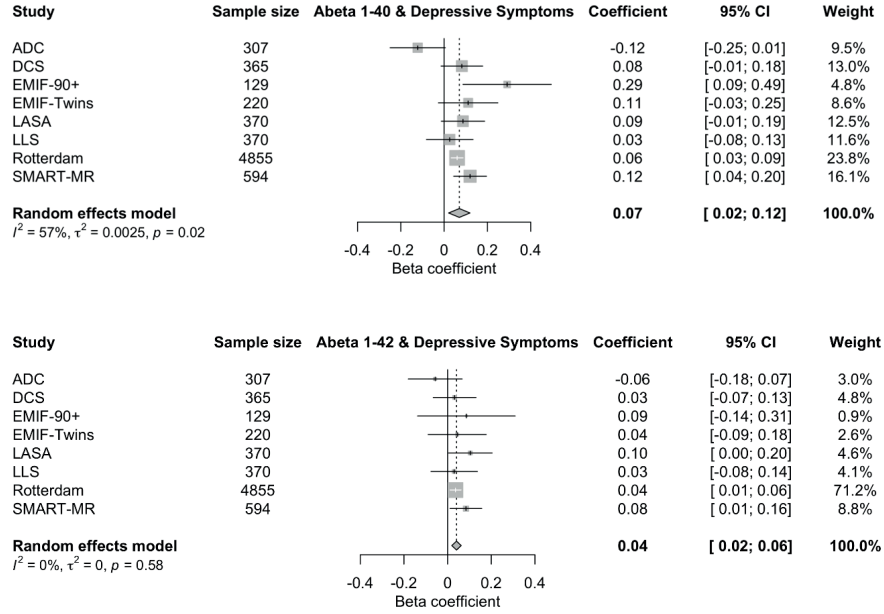


Supplementary Figure 6. Meta-analyses on Aβ42/40 ratio and depressive symptoms when adding Aβ42 and 1/Aβ40 as main effects.

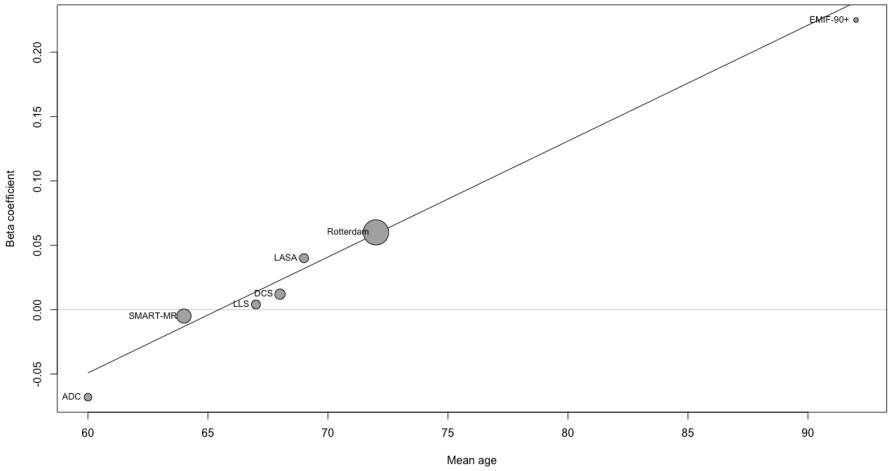


Note: After correction for multiple comparisons, $p > 0.05$.

Supplementary Figure 7. Meta-analyses on Aβ40 and Aβ42 separately.



Supplementary Figure 8. A bubble plot representation of the mean age and beta coefficient per study.



Supplementary Table 1. Sex differences in the associations between plasma AD biomarkers and depressive symptoms.

	Depressive symptoms (continuous)		**Depressive symptoms (continuous)**	
	Estimate (95% CI)		**Estimate (95% CI)**	
	Males	**Females**	**No APOE e4 allele**	**APOE e4 allele**
ADC	**(n = 177)**	**(n = 130)**	**(n = 184)**	**(n = 123)**
Amyloid 42/40	0.02 (-0.15; 0.19), *p* = 0.82	0.01 (-0.44; 0.47), p = 0.96	0.09 (-0.30; 0.47), p = 0.65	0.00 (-0.18; 0.18), p = 0.99
P-tau181	-0.04 (-0.21; 0.14), *p* = 0.68	-0.03 (-0.22; 0.17), p = 0.78	-0.10 (-0.27; 0.07), p = 0.23	-0.00 (-0.19; 0.19), p = 0.99
Neurofilament light	-0.10 (-0.24; 0.05), *p* = 0.19	0.06 (-0.20; 0.33), p = 0.64	-0.12 (-0.27; 0.04), p = 0.13	0.04 (-0.20; 0.27), p = 0.77
Glial fibrillary acidic protein	-0.07 (-0.24; 0.11), *p* = 0.44	0.05 (-0.15; 0.25), p = 0.59	-0.02 (-0.24; 0.20), p = 0.86	-0.03 (-0.21; 0.16), p = 0.78
DCS	**(n = 188)**	**(n = 177)**	**(n = 257)**	**(n = 108)**
Amyloid 42/40	-0.09 (-0.24; 0.06), *p* = 0.22	-0.11 (-0.25; 0.02), p = 0.10	-0.07 (-0.19; 0.05), p = 0.26	-0.20 (-0.40; -0.01), p = 0.04
P-tau181	-0.13 (-0.25; -0.01), *p* = 0.03	-0.04 (-0.22; 0.15), p = 0.69	-0.13 (-0.25; -0.01), p = 0.03	-0.07 (-0.27; 0.14), p = 0.52
Neurofilament light	-0.00 (-0.13; 0.12), *p* = 0.97	0.04 (-0.14; 0.22), p = 0.67	-0.06 (-0.19; 0.07), p = 0.34	0.14 (-0.04; 0.32), p = 0.13
Glial fibrillary acidic protein	0.03 (-0.12; 0.19), *p* = 0.65	-0.03 (-0.18; 0.11), p = 0.64	-0.04 (-0.16; 0.08), p = 0.50	-0.04 (-0.16; 0.08), p = 0.33
EMIF-90+	**(n = 55)**	**(n = 74)**	**(n = 100)**	**(n = 29)**
Amyloid 42/40	-0.18 (-0.47; 0.10), *p* = 0.20	-0.11 (-0.41; 0.19), p = 0.45	-0.13 (-0.31; 0.06), p = 0.19	0.07 (-0.59; 0.73), p = 0.82
P-tau181	0.01 (-0.32; 0.35), *p* = 0.94	-0.05 (-0.30; 0.20), p = 0.69	-0.05 (-0.24; 0.14), p = 0.60	0.13 (-0.68; 0.93), p = 0.74
Neurofilament light	0.09 (-0.25; 0.42), p = 0.61	**0.30 (0.09; 0.51), p = 0.01**	**0.24 (0.07; 0.42), p = 0.01**	0.49 (-0.27; 1.24), p = 0.19
Glial fibrillary acidic protein	-0.00 (-0.34; 0.34), p = 0.99	0.19 (-0.07; 0.45), p = 0.15	0.24 (0.06; 0.43), p = 0.01	-0.46 (-1.04; 0.12), p = 0.11
EMIF-Twins	**(n = 91)**	**(n = 126)**	**(n = 142)**	**(n = 72)**
Amyloid 42/40	0.07 (-0.08; 0.23), p = 0.35	-0.08 (-0.27; 0.12), p = 0.43	0.04 (-0.14; 0.21), p = 0.69	0.00 (-0.14; 0.15), p = 0.96
P-tau181	-0.02 (-0.10; 0.07), p = 0.69	-0.19 (-0.72; 0.34), p = 0.48	-0.03 (-0.14; 0.08), p = 0.57	0.07 (-0.22; 0.36), p = 0.64
Neurofilament light	NA	NA	NA	NA
Glial fibrillary acidic protein	0.05 (-0.11; 0.21), p = 0.51	**0.28 (0.10; 0.46), p < 0.01**	0.27 (0.07; 0.46), p < 0.01	0.10 (-0.05; 0.24), p = 0.19
LASA	**(n = 167)**	**(n = 203)**	**(n = 209)**	**(n = 161)**
Amyloid 42/40	0.06 (-0.10; 0.22), *p* = 0.48	0.06 (-0.07; 0.19), *p* = 0.40	0.03 (-0.10; 0.16), p = 0.67	0.09 (-0.08; 0.25), p = 0.29
P-tau181	-0.06 (-0.18; 0.067), *p* = 0.36	0.16 (-0.01; 0.34), *p* = 0.07	-0.00 (-0.14; 0.13), p = 0.96	0.06 (-0.10; 0.21), p = 0.47

Supplementary Table 1. Continued

	Depressive symptoms (continuous)		**Depressive symptoms (continuous)**	
	Estimate (95% CI)		**Estimate (95% CI)**	
	Males	**Females**	**No APOE e4 allele**	**APOE e4 allele**
ADC	**(n = 177)**	**(n = 130)**	**(n = 184)**	**(n = 123)**
Neurofilament light	-0.02 (-0.15; 0.118), *p* = 0.80	0.12 (-0.08; 0.32), *p* = 0.22	-0.03 (-0.21; 0.16), p = 0.78	0.08 (-0.06; 0.22), p = 0.27
Glial fibrillary acidic protein	-0.04 (-0.15; 0.07), *p* = 0.43	0.06 (-0.28; 0.39), *p* = 0.75	0.11 (-0.26; 0.48), p = 0.57	-0.02 (-0.13; 0.09), p = 0.74
Leiden Longevity Study	**(n = 181)**	**(n = 189)**	**(n = 266)**	**(n = 104)**
Amyloid 42/40	0.11 (-0.06; 0.28), p = 0.20	-0.03 (-0.17; 0.11), p = 0.70	0.02 (-0.10; 0.14), p = 0.80	0.05 (-0.19; 0.28), p = 0.70
P-tau181	0.00 (-0.13; 0.13), p = 0.98	0.06 (-0.10; 0.23), p = 0.44	0.04 (-0.09; 0.16), p = 0.57	-0.01 (-0.21; 0.20), p = 0.96
Neurofilament light	-0.05 (-0.18; 0.08), p = 0.43	0.13 (-0.07; 0.32), p = 0.19	-0.02 (-0.14; 0.11), p = 0.81	0.10 (-0.17; 0.36), p = 0.47
Glial fibrillary acidic protein	-0.03 (-0.18; 0.12), p = 0.71	0.08 (-0.08; 0.24), p = 0.34	-0.00 (-0.13; 0.12), p = 0.96	0.10 (-0.13; 0.32), p = 0.39
Rotterdam Study	**(n = 2072)**	**(n = 2783)**	**(n = 3528)**	**(n = 1327)**
Amyloid 42/40	-0.02 (-0.06; 0.02), *p* = 0.38	0.01 (-0.03; 0.05), *p* = 0.57	-0.01 (-0.04; 0.02), *p* = 0.65	0.03 (-0.03; 0.10), *p* = 0.29
P-tau181	NA	NA	NA	NA
Neurofilament light	**0.06 (0.02; 0.10), *p* < 0.01**	**0.06 (0.02; 0.10), *p* < 0.01**	**0.05 (0.02; 0.08), *p* < 0.01**	**0.14 (0.06; 0.23), *p* < 0.01**
Glial fibrillary acidic protein	NA	NA	NA	NA
SMART	**(n = 493)**	**(n = 101)**	**(n = 410)**	**(n = 184)**
Amyloid 42/40	-0.04 (-0.11; 0.03), *p* = 0.23	0.06 (-0.44; 0.56), *p* = 0.80	-0.04 (-0.11; 0.04), p = 0.35	-0.26 (-0.60; 0.08), p = 0.14
P-tau181	-0.02 (-0.09; 0.05), *p* = 0.62	-0.22 (-0.56; 0.12), *p* = 0.21	-0.02 (-0.10; 0.06), p = 0.65	-0.07 (-0.27; 0.12), p = 0.46
Neurofilament light	-0.01 (-0.10; 0.07), *p* = 0.77	0.01 (-0.17; 0.18), *p* = 0.95	-0.02 (-0.12; 0.07), p = 0.64	0.04 (-0.10; 0.18), p = 0.58
Glial fibrillary acidic protein	0.08 (-0.01; 0.17), *p* = 0.08	-0.08 (-0.28; 0.13), *p* = 0.48	0.05 (-0.05; 0.14), p = 0.32	0.05 (-0.13; 0.23), p = 0.59

Note: In sex stratified models, models are adjusted for only age, education, and APOE e4 allele. For analyses stratified by APOE e4 allele, models are adjusted for age, sex, and education.

Supplementary Table 2. Sensitivity analyses on complete case data for cohorts with missing data.

	Depressive symptoms (continuous)	**Depressive symptoms (dichotomized)**
	Estimate (95% CI)	**Estimate (95% CI)**
ADC		
Amyloid 42/40	0.11 (-0.17; 0.39), $p = 0.44$	1.01 (0.43; 2.49), $p = 0.98$
P-tau181	-0.06 (-0.19; 0.07), $p = 0.38$	0.85 (0.51; 1.30), $p = 0.50$
Neurofilament light	-0.08 (-0.21; 0.04), $p = 0.20$	1.05 (0.65; 1.52), $p = 0.81$
Glial fibrillary acidic protein	-0.03 (-0.17; 0.11), $p = 0.66$	1.09 (0.70; 1.62), $p = 0.68$
DCS		
Amyloid 42/40	-0.11 (-0.21; -0.01), $p = 0.03$	0.80 (0.62; 1.05), $p = 0.11$
P-tau181	-0.11 (-0.22; -0.01), $p = 0.03$	0.77 (0.53; 1.08), $p = 0.15$
Neurofilament light	0.01 (-0.09; 0.11), $p = 0.86$	0.82 (0.59; 1.10), $p = 0.19$
Glial fibrillary acidic protein	-0.00 (-0.10; 0.10), $p = 0.99$	0.98 (0.74; 1.29), $p = 0.88$
EMIF-90+		
Amyloid 42/40	-0.13 (-0.30; 0.05), $p = 0.15$	1.04 (0.41; 2.90), $p = 0.94$
P-tau181	-0.04 (-0.22; 0.14), $p = 0.64$	1.21 (0.58; 2.23), $p = 0.53$
Neurofilament light	**0.23 (0.06; 0.40), $p < 0.01$**	2.12 (1.20; 4.79), $p = 0.02$
Glial fibrillary acidic protein	0.14 (-0.04; 0.32), $p = 0.12$	1.56 (0.62; 4.10), $p = 0.34$
EMIF-Twins		
Amyloid 42/40	-0.04 (-0.14; 0.07), $p = 0.50$	NA
P-tau181	-0.03 (-0.13; 0.08), $p = 0.61$	NA
Neurofilament light	NA	NA
Glial fibrillary acidic protein	**0.19 (0.07; 0.30), $p < 0.01$**	NA
LASA		
Amyloid 42/40	0.05 (-0.05; 0.15), $p = 0.35$	1.34 (0.87; 2.13), $p = 0.20$
P-tau181	0.01 (-0.09; 0.12), $p = 0.78$	1.26 (0.84; 1.72), $p = 0.18$
Neurofilament light	0.04 (-0.07; 0.15), $p = 0.48$	1.25 (0.80; 1.79), $p = 0.26$
Glial fibrillary acidic protein	-0.03 (-0.13; 0.08), $p = 0.62$	1.00 (0.39; 1.42), $p = 0.99$
Leiden Longevity Study		
Amyloid 42/40	0.02 (-0.09; 0.13), $p = 0.73$	1.09 (0.84; 1.41), $p = 0.53$
P-tau181	0.02 (-0.08; 0.13), $p = 0.65$	1.12 (0.87; 1.43), $p = 0.36$
Neurofilament light	0.00 (-0.11; 0.12), $p = 0.94$	1.11 (0.83; 1.44), $p = 0.43$
Glial fibrillary acidic protein	0.02 (-0.09; 0.14), $p = 0.66$	1.02 (0.78; 1.33), $p = 0.87$
Rotterdam Study		
Amyloid 42/40	0.00 (-0.02; 0.03), $p = 0.75$	1.07 (0.979; 1.168), $p = 0.12$
P-tau181	NA	NA
Neurofilament light	**0.06 (0.03; 0.09), $p < 0.01$**	**1.23 (1.12; 1.35), $p < 0.01$**
Glial fibrillary acidic protein	NA	NA

Supplementary Table 2. Continued

	Depressive symptoms (continuous)	Depressive symptoms (dichotomized)
	Estimate (95% CI)	**Estimate (95% CI)**
SMART-MR		
Amyloid 42/40	-0.05 (-0.12; 0.03), $p = 0.21$	0.55 (0.30; 0.99), $p = 0.06$
P-tau181	-0.03 (-0.10; 0.05), $p = 0.49$	0.78 (0.45; 1.08), $p = 0.31$
Neurofilament light	-0.00 (-0.08; 0.07), $p = 0.96$	0.96 (0.68; 1.20), $p = 0.76$
Glial fibrillary acidic protein	0.05 (-0.04; 0.13), $p = 0.28$	1.11 (0.84; 1.40), $p = 0.44$

Adjusted for age, sex, education, and APOE e4 allele. Plasma markers are standardized.

1. Westendorp RG, van Heemst D, Rozing MP, et al. Nonagenarian siblings and their offspring display lower risk of mortality and morbidity than sporadic nonagenarians: The Leiden Longevity Study. *J Am Geriatr Soc*. Sep 2009;57(9):1634-7. doi:10.1111/j.1532-5415.2009.02381.x

Chapter 7

Depression and dementia: the role of cortisol and vascular brain legions. The AGES-Reykjavik Study

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Abstract

Background: Late-life depression (LLD) is related to an increased risk of developing dementia; however, the biological mechanisms explaining this relationship remain unclear.

Objective: To determine whether the relationship between LLD and dementia can be best explained by the glucocorticoid cascade or vascular hypothesis.

Methods: Data are from 4,354 persons (mean age 76±5 years) without dementia at baseline from the AGES-Reykjavik Study. LLD was assessed with the MINI diagnostic interview (current and remitted Major Depressive Disorder [MDD]) and the Geriatric Depression Scale-15. Salivary cortisol measures were obtained after waking and at night (glucocorticoid cascade hypothesis). White matter hyperintensities (WMH; vascular hypothesis) volume was assessed using 1.5T brain MRI. Using Cox proportional hazard models, we estimated the associations of LLD, cortisol levels, and WMH volume with incident all-cause dementia, AD and non-AD dementia.

Results: During 8.8±3.2 years of follow-up, 843 persons developed dementia, including 397 with AD. Current MDD was associated with an increased risk of developing all-cause dementia (HR=2.17; 95% CI 1.66-2.67), with risks similar for AD and non-AD, while remitted MDD was not (HR=1.02; 95% CI 0.55-1.49). Depressive symptoms were also associated with increased risk of dementia, in particular non-AD dementias. Higher levels of evening cortisol increased risk of dementia, but this was independent of MDD. WMH partially explained the relation between current MDD and dementia risk but remained increased (HR=1.71; 95% CI 1.34-2.08).

Conclusion: The current study highlights the importance of LLD in developing dementia. However, neither the glucocorticoid cascade nor the vascular hypotheses fully explained the relation between depression and dementia.

Introduction

Prospective studies have shown that late-life depression (LLD) increases the risk of dementia, including Alzheimer's disease (AD) and vascular dementia (1-3). Notably, a recent review demonstrated that depression shows the most consistent evidence as a risk factor for dementia (4). However, little is known about the neurobiological mechanisms underlying the relation between LLD and dementia and to what extent LLD is a risk factor or a prodromal stage of dementia (2). There are two main hypotheses for this connection; the glucocorticoid cascade hypothesis (5), which stipulates that depression (6) leads to AD through age-associated hippocampal atrophy and increased levels of cortisol due to dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis (1, 7-13); and the vascular hypothesis, stating that depression precedes dementia through small vessel changes in mood-regulating areas resulting from or contributing to depression (2, 14-16). One study showed that cerebrovascular disease explained cognitive deficits in LLD better than salivary cortisol levels (17), but there is a lack of longitudinal studies that jointly investigate these hypotheses with dementia (18). This is important, as better understanding of the role of depression in the etiology of dementia, may help develop strategies to prevent, delay, or treat the disease.

We aimed to investigate the relationships between LLD, cortisol levels, white matter hyperintensity (WMH) volume and incident dementia in a large community-based prospective cohort study of older persons. We hypothesized that LLD increased the risk of dementia (4); that higher evening cortisol levels interact with LLD to increase dementia risk, particularly AD, reflecting the glucocorticoid cascade; and that larger WMH volume partially explained the relationship between current LLD and dementia, reflecting the underlying contributing factor of WMH in both LLD and dementia.

Methods

Study population

The Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study is a population-based prospective cohort study of the National Institute on Aging and the Icelandic Heart Association, initiated to investigate the genetic and environmental factors contributing to clinical and subclinical disease at older age (19). It is a continuation of the Reykjavik Study, which was initiated in 1967 by the Icelandic Heart Association, and included men and women born in 1907 to 1935 and living in the Reykjavik area

(19). The original cohort of the Reykjavik Study was examined 1 to 6 times according to a schedule that allowed longitudinal and cross-sectional analyses over the 30-year follow-up period.

From 2002 through 2006, 5,764 individuals (mean age: 76 years) randomly chosen from the survivors of the Reykjavik Study were examined for the AGES-Reykjavik Study. They underwent comprehensive assessments at the Reykjavik research center including comprehensive questionnaires, blood tests, biometry, 1.5 Tesla brain MRI, and depression, cognitive and dementia assessments.

From 2007 to 2011, 3,316 persons received one follow-up examination. Monitoring of incident dementia diagnoses continued until a maximum of 12 years follow-up time. Reasons for loss to follow-up have been described elsewhere (20).

Standard protocol approvals, registrations, and patient consents The AGES-Reykjavik Study was approved by the Icelandic National Bioethics Committee (VSN: 00-063), the Icelandic Data Protection Authority, and by the Institutional Review Board for the National Institute on Aging, NIH. Written informed consent was obtained from all participants.

Dementia diagnosis

The procedure for dementia assessment at baseline and follow-up has been described elsewhere (20-22). In brief, dementia ascertainment and classification of subtypes was performed using a 3-step protocol following international criteria. A cognitive screening of the total sample was performed, with a detailed neuropsychological exam in screen positives and a further neurologic and proxy exam performed in step 2 persons who screened positive on test results. A consensus diagnosis according to international guidelines was made by a multidisciplinary panel including a neurologist, geriatrician, neuroradiologist, and neuropsychologist. Additional cases were identified through medical and nursing home records, as well as death certificates. When an individual moved into a nursing home, all-cause dementia and Alzheimer's disease diagnosis was based on the intake exam into the nursing home. Additional cases were identified in the nursing home following a standardized protocol followed by all Icelandic nursing homes (23). For the present study, we defined all-cause dementia, Alzheimer dementia, and other dementias.

Depression assessment

Depression measures have been described in detail elsewhere (24). Briefly, remitted and current (i.e. in the past two weeks) diagnosis of major depressive disorder (MDD) was assessed with the Mini-International Psychiatric Interview (MINI) diagnostic

interview (25) by trained health professionals at baseline. For the purpose of this study, we categorized participants into 3 groups: never MDD, remitted (past) MDD, and current MDD.

The Geriatric Depression Scale-15 (26) was administered and categorized using the cutoff of 6 or higher to indicate elevated depressive symptoms.

Cortisol measures

Measures of cortisol have been described in more detail elsewhere (13). Using Salivette® devices (Sarstedt, Rommelsdorf, Germany), saliva samples were collected at night the day before visiting the clinic and the next morning 45 minutes after awaking. Instructions were given not to eat, drink, or brush teeth before sampling. Salivary cortisol was analyzed with a time-resolved immunoassay with fluorescence detection (Delfia; PerkinElmer, Waltham, MA) (27). Inter-assay variability was below 12% and intra-assay variability was below 10%. The lower detection limit was 0.43 nmol/L. We excluded 0.7% of the morning samples and 0.5% of the evening samples as they had values of >100 nmol/L which were considered unreliable. Morning and evening levels of cortisol were natural log-transformed due to skewed distribution and z-scores were calculated.

Brain MRI and brain segmentation

The MRI protocol and segmentation procedure have been described elsewhere (13, 28). In short, eligible participants underwent MRI on a 1.5T Signa Twinspeed system (General Electric Medical Systems, Waukesha, WI) including a 3-dimensional axial T1-weighted spoiled gradient echo sequence, a fluid attenuated inversion recovery (FLAIR) sequence, a proton density/T2-weighted (PD/T2) fast spin echo sequence, and a T2*-weighted gradient echo type echoplanar sequence. The FLAIR, PD/T2 and T2* sequences were acquired with 3mm thick interleaved slices. Regional gray and white matter, CSF and WMH were segmented automatically with an AGES-Reykjavik Study modified algorithm described elsewhere (28). In brief, an artificial neural network classifier categorized each voxel as belonging to either gray matter, white matter, CSF or WMH. The automatic classification was further validated by having a trained radiographer assess a sample of MRI scans (28). Visibile hyperintense lesions on both T2-weighted and FLAIR images were classified as WMHs (29). WMHs were classified by trained radiographers using the Achten Scale, which takes into account both lesion size and number (30).

Covariates

Covariates assessed with questionnaires included age, sex, educational level (categorized into three categories [primary, secondary, college/university] from four categories [primary, secondary, college, university]), smoking history (current vs. non-smoker), alcohol intake (gram/week), and physical activity (never, rarely, occasionally, moderate, or high in the past 12 months). Body mass index (BMI) was calculated from height and weight and expressed as kg/m^2. Systolic and diastolic blood pressure was measured with a standard mercury sphygmomanometer. Hypertension was defined as systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg, use of antihypertensives or self-reported physician's diagnosis of hypertension. Diabetes mellitus was defined as use of blood glucose-lowering drugs or fasting blood glucose level ≥7.0 mmol/L or self-reported history of diabetes. APOE genotyping was carried out using the microplate array diagonal gel electrophoresis (MADGE) system (31).

Analytical sample

Of the 5,764 members of the cohort, a total of 4,349 had no dementia and brain MRI segmentation available. The majority of people not having a MRI had a home visit, scheduling conflicts, contraindications, or refused. A small proportion of those with MRI did not have brain segmentation (missing sequences or movement artefacts (20, 24, 29)). The average age of those who had an MRI was 76 years compared to 80 years in those who did not receive an MRI. Of those who received a MRI, 58% were women compared to 55% in those who did not receive an MRI. Average score on the GDS-15 was 2 in those who received an MRI and 3 in those who did not. Of those with a brain MRI, 4% had ever MDD diagnosis compared to 3% in those without brain MRI. Average morning cortisol levels were 19.8 nmol/L in those who had a brain MRI compared to 17.8 nmol/L in those who did not. Evening cortisol in those with a brain MRI was 3.9 nmol/L and 4.4 nmol/L in those without.

Data analysis

Participants were followed from date of inclusion until diagnosis of dementia, death, loss to follow-up, or end of follow-up (October 2015), whichever came first. Censoring date for participants who received a diagnosis of dementia during follow-up was set halfway between date of inclusion and follow-up visit. For incident cases identified through nursing homes, date of diagnosis was based on the nursing home intake exam or the date when the nursing home staff diagnosed dementia based on a standardized protocol followed by all Icelandic nursing homes. For those who died, the censoring date was date of death. Those lost to follow-up were assumed not to have dementia, and the censoring date was set halfway between date of inclusion and end of follow-up.

Multiple imputation (AregImpute in R version 2.13.1) was used to address missing values at baseline (0-9.3% [medication use]). Incident dementia was not used for imputation. R (epiR, survival, and survminer in version 3.6.1) was used for the data analyses. Pooled results of 10 datasets are presented. Proportional hazards assumption (i.e, Schoenfeld's residuals), influential observations (i.e., dfbetas), and nonlinearity (i.e., martingale residuals) were checked. Cox regression models used age as the timescale. Sex and level of education were added as covariates. Lastly, stratified results by sex are additionally presented to explore possible sex differences.

Depression and risk for dementia

We calculated baseline characteristics for the total study sample and according to depression diagnosis (no history of MDD, remitted MDD, and current MDD). Cox proportional hazard models were fit to estimate the hazard ratio (HR) of the associations of lifetime MDD, current and remitted MDD compared with never MDD with incident all-cause dementia, AD, and other (non-AD) dementias. To further assess the aspect of time from baseline depression to dementia diagnosis, we fit models stratified by time from depression assessment at baseline to dementia diagnosis with a cut-off of 7 years follow-up time. Models were fit using depressive symptoms (i.e., the GDS-15) because dementia cases were too few when analyzing current MDD.

Glucocorticoid cascade hypothesis

To investigate the role of glucocorticoids, the relationship between morning and evening cortisol (i.e., the z-score of natural log-transformed values) with incident dementia was estimated. Next, morning and evening cortisol levels were added to the model with depressive symptoms. To estimate additive interaction (32) and the relative excess risk due to interaction, four groups with or without depression (defined by GDS-15 scores <6 or 6 or higher, as there were too few cases with current MDD) and low/normal or high levels of evening cortisol (defined as the highest tertile of evening cortisol >3.3 nmol/L versus the lower two tertiles) were created and their relationship with later dementia outcome was explored. Dummy variables were used for ease of interpretation of the interaction (33) with confidence intervals from Hosmer and Lemeshow (34). We additionally estimated additive interaction using continuous measurements (32), standardizing with z-scores both GDS-15 scores and log-transformed cortisol levels.

Vascular hypothesis

Similarly, depressive symptoms and total WMH volume (i.e., the z-score of natural log-transformed values) were entered together into the Cox regression model to

explore the vascular hypothesis, with standardized intracranial volume (ICV) added as a covariate. Next, the relative excess risk due to interaction of high depressive symptoms and larger WMH volumes on dementia risk was estimated by calculating four dummy variables where large WMH volume was defined as the highest tertile of ICV-corrected natural log-transformed WMH volume (>0.28 % ICV) and GDS-15 scores of 6 or higher were used to indicate presence of depression. We also assessed additive interaction using continuous measurements, of standardized GDS-15 score and log-transformed WMH volume.

Glucocorticoid cascade and vascular hypotheses

Additionally, to explore to what extent the glucocorticoid cascade and vascular pathways are independent contributors, cortisol levels and WMH volume were entered together in a model with depression diagnosis. To correct for vascular risk factors, additional adjustments were made for APOE genotype (e4 positive vs. e4 negative), current smoking (vs. never or former), alcohol intake (gram/week), physical activity (never, rarely, occasionally, moderate, or high in the past 12 months), BMI, hypertension and diabetes mellitus. ICV was also added as a covariate in the model.

Results

Of the 4,349 participants without dementia at baseline, the mean age was 76±5 years and 59% were women; 194 had a lifetime diagnosis of MDD, 130 of whom had a past diagnosis, and 64 a current diagnosis of MDD (Table 1). Of those with a current diagnosis, 75% also had a history of MDD. Median (10-90%) morning cortisol level in the study sample was 17.3 (5.6-36.2) nmol/L, and median evening cortisol level 2.3 (0.9-6.9) nmol/L. During a total of 38,221 person-years of follow-up (mean per person 8.8 ± 3.2 years, range 0.11 – 13.4 years), 843 persons developed dementia, 397 of whom had a diagnosis AD, and 446 were diagnosed with dementias other than AD.

Depression and risk for dementia

Of the 843 persons with incident dementia, 35 had a lifetime diagnosis of MDD. Cox regression analysis adjusted for age (timescale), sex and education showed that the risk of dementia for lifetime MDD was increased (HR 1.37; 95% CI 1.03-1.72). Current MDD increased the risk of dementia more than two-fold (HR 2.17; 95% CI 1.66-2.67), whereas remitted MDD was not associated with incident dementia (HR 1.02; 95% CI 0.55-1.49). Similar risks were observed for AD and non-AD dementias, although 95% confidence intervals were wider (Table 2). Depressive symptoms were also associated with increased risk of dementia, in particular non-AD dementias (Table 2).

To assess time between depression assessment and dementia onset, those who had a less than 7 year gap between baseline depressive symptoms and dementia diagnosis had a greater risk of developing dementia during that time, although the estimate did not reach statistical significance (HR 1.30; 95% CI 0.97-1.63), whereas no association was observed between high depressive symptomology and dementia in those who had a 7 year or greater interval (HR 0.97; 95% CI 0.56-1.39) (Table 3). Number of incident dementia cases in those with current MDD were too small to stratify by time interval.

Table 1. Baseline characteristics of study sample (N=4,349) according to depression diagnosis.

	Never MDD	Past MDD	Current MDD	Total
	n=4155	n=130	n=64	N=4349
Age, mean (SD), years	76 (5)	74 (5)	75 (5)	**76 (5)**
Women, no. (%)	2417 (58)	88 (68)	42 (66)	**2547 (59)**
Primary education, no. (%)	1373 (33)	43 (33)	20 (31)	**1435 (33)**
Current smoker, no. (%)	490 (12)	21 (16)	12 (19)	**523 (12)**
Alcohol use, mean (SD), gr/week	15 (33)	9 (19)	19 (49)	**15 (33)**
Physical activity, moderate/high, no. (%)	1352 (33)	41 (32)	10 (16)	**1403 (32)**
Body mass index, mean (SD)	27 (4)	28 (4)	28 (5)	**27 (4)**
Blood pressure, systolic, mean (SD), mmHg	142 (20)	138 (18)	138 (20)	**142 (20)**
Blood pressure, diastolic, mean (SD), mmHg	74 (10)	74 (10)	74 (9)	**74 (10)**
Hypertension, no. (%)	3342 (80)	100 (77)	47 (73)	**3489 (80)**
Diabetes, no. (%)	451 (11)	21 (16)	12 (19)	**484 (11)**
APOE e4 positive, no. (%)	1131 (27)	33 (25)	21 (33)	**1185 (27)**
History of MDD, no. (%)	0 (0)	130 (100)	48 (75)	**194 (4)**
GDS-15, 6+, no. (%)	221 (5)	25 (19)	39 (61)	**285 (7)**
Morning cortisol, median (IQR), nmol/L	17.3 (15.7)	14.1 (15.9)	15.0 (19.0)	**17.3 (15.8)**
Evening cortisol, median (IQR), nmol/L	2.3 (2.4)	2.0 (2.6)	2.5 (4.0)	**2.3 (2.4)**
WMH, median (IQR), ml	13.5 (18.1)	11.6 (16.3)	14.6 (20.2)	**13.5 (18.1)**

Abbreviation: MDD, Major Depressive Disorder

Results were similar for both men and women, except for depressive symptoms, which showed an increased risk for all-cause dementia only in men (Supplementary Table 1).

Glucocorticoid cascade hypothesis

When depression diagnosis and cortisol levels were entered together in the Cox regression analyses, the risk of all-cause dementia for current MDD remained increased (HR 2.09; 95% CI 1.59-2.59) (Figure 1, model 2a). Similar patterns in associations were seen for AD and non-AD dementias. The relative excess risk due to interaction to calculate additive interaction (32) did not suggest interaction (Table 5, Supplementary Table 3). Since we found no association between morning cortisol and dementia, we did not further examine the interaction of depressive symptoms and morning cortisol levels on dementia.

Table 2. Hazard ratios for the relation between LLD and risk of dementia (N=4,349).

	No. of cases	All-cause dementia (n=843) HR (95% CI)	No. of cases	Alzheimer's disease (n=397) HR (95% CI)	No. of cases	Other dementias (n=446) HR (95% CI)
Never MDD (n=4155)	808	1 (reference)	379	1 (reference)	429	1 (reference)
Ever MDD (n=194)	35	1.37 (1.03-1.72)	18	1.38 (0.91-1.86)	17	1.43 (0.94-1.92)
Never MDD (n=4155)	808	1 (reference)	379	1 (reference)	429	1 (reference)
Remitted MDD (n=130)	18	1.02 (0.55-1.49)	9	0.98 (0.31-1.65)	9	1.06 (0.40-1.72)
Current MDD (including past) (n=64)	17	2.17 (1.66-2.67)	9	2.32 (1.63-3.02)	8	2.35 (1.61-3.09)
	No. of cases	**All-cause dementia (n=843) HR (95% CI)**	**No. of cases**	**Alzheimer's disease (n=397) HR (95% CI)**	**No. of cases**	**Other dementias (n=446) HR (95% CI)**
GDS-15 score <6 (n=4078)	777	1 (reference)	370	1 (reference)	408	1 (reference)
GDS-15 score 6 or higher (n=271)	66	1.31 (1.05-1.57)	27	1.16 (0.77-1.55)	38	1.49 (1.14-1.85)

Models are adjusted for age (timescale), sex and level of education.

When analyzed for men and women separately, the increased risk of current MDD with all-cause dementia and dementias other than AD was stronger in men, while evening cortisol was slightly stronger associated with all types of dementia in women and lost statistical significance in men (Supplementary Table 2).

Vascular hypothesis

When WMH volume was added to the model (Figure 1, model 2b), the association of current MDD and incident all-cause dementia (HR 2.00; 95% CI 1.50-2.49), AD, and non-AD dementia attenuated; however, it remained statistically significant. Table 5 shows the joint association of high levels of depressive symptoms with large WMH volume on dementia risk. HRs were strongest for the combination of depressive symptoms and large WMH volume, although the relative excess risk due to interaction to calculate additive interaction (32) did not suggest interaction. Results were similar for men and women (Supplementary Table 2).

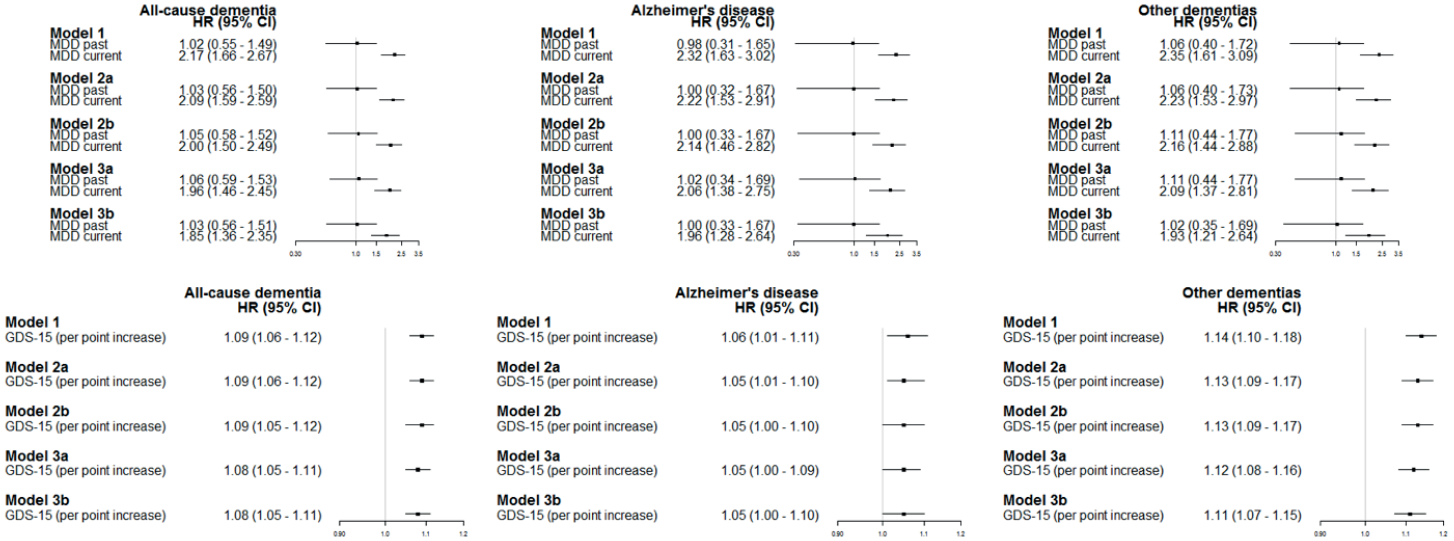

Figure 1. Hazard ratios for the association of depression with dementia

model 1: adjusted for sex and education

model 2a: model 1 + morning cortisol and evening cortisol

model 2b: model 1 + white matter hyperintensity volume (WMH) and intracranial volume (ICV)

model 3a: model 1 + morning cortisol, evening cortisol, WMH, and ICV

model 3b: model 3a + current smoking, alcohol intake, physical activity, body mass index, hypertension, diabetes mellitus, and APOE-e4 allele

Table 3. Hazard ratios for the relation between baseline depressive symptoms and risk of dementia, stratified for follow-up time (N=4,349).

	No. of cases	All-cause dementia (n=843) HR (95% CI)	No. of cases	Alzheimer's disease (n=397) HR (95% CI)	No. of cases	Other dementias (n=446) HR (95% CI)
Less than 7 years between baseline depression and time of dementia diagnosis (N=1330)						
GDS-15 score <6 (n=1213)	367	1 (reference)	191	1 (reference)	176	1 (reference)
GDS-15 score 6 or higher (n=117)	42	1.30 (0.97-1.63)	17	1.00 (0.49-1.52)	25	1.77 (1.33-2.21)
7 years or more between baseline depression and time of dementia diagnosis (N=3019)						
GDS-15 score <6 (n=2851)	410	1 (reference)	178	1 (reference)	232	1 (reference)
GDS-15 score 6 or higher (n=168)	24	0.97 (0.56-1.39)	11	1.01 (0.40-1.62)	13	0.96 (0.38-1.53)

Models are adjusted for age (as timescale), sex and level of education.

Table 4. Results of the Cox regression model with MDD, cortisol levels, and WMH volume entered together (model 3a).

	All-cause dementia (n=843) HR (95% CI)	Alzheimer's disease (n=397) HR (95% CI)	Other dementias (n=446) HR (95% CI)
Remitted MDD	1.06 (0.59-1.53)	1.02 (0.34-1.69)	1.11 (0.44-1.77)
Current MDD (including past)	1.96 (1.46-2.45)	2.06 (1.38-2.75)	2.09 (1.37-2.81)
Morning cortisol	0.99 (0.92-1.07)	1.03 (0.92-1.14)	0.97 (0.87-1.07)
Evening cortisol	1.13 (1.06-1.20)	1.16 (1.06-1.26)	1.15 (1.04-1.25)
WMH volume	1.30 (1.22-1.37)	1.25 (1.15-1.36)	1.43 (1.33-1.53)

Adjusted for sex, education, and intracranial volume
WMH: white matter hyperintensities
MDD: major depressive disorder
Cortisol levels and WMH volume per SD increase

Table 5. Hazard ratios (HR) for the independent and combined association of depressive symptoms, evening cortisol, and white matter hyperintensities volume with incident dementia.

	All-cause dementia	Alzheimer's disease	Other dementias
	(n=843)	(n=397)	(n=446)
	HR (95% CI)	HR (95% CI)	HR (95% CI)
Neither (n=2804)	1	1	1
Depressive symptoms (n=165)	1.37 (1.02-1.72)	1.13 (0.58-1.68)	1.66 (1.19-2.12)
High evening cortisol (n=1261)	1.29 (1.14-1.44)	1.30 (1.08-1.52)	1.41 (1.20-1.62)
Both (n=120)	1.51 (1.11-1.91)	1.44 (0.88-2.00)	1.74 (1.14-2.34)
Additive RERI (95% CI) (32)	**-0.15 (-1.74-1.44)**	**0.01 (-2.49-2.50)**	**-0.33 (-2.66-2.00)**
Neither (n=2732)	1	1	1
Depressive symptoms (n=168)	1.43 (1.07-1.80)	1.40 (0.90-1.90)	1.54 (1.01-2.07)
Larger WMH (n=1333)	1.51 (1.37-1.65)	1.35 (1.14-1.56)	1.83 (1.63-2.02)
Both (n=117)	1.71 (1.34-2.08)	1.16 (0.52-1.80)	2.47 (1.99-2.95)
Additive RERI (95% CI) (32)	**-0.23 (-1.85-1.38)**	**-0.59 (-2.25-1.08)**	**0.10 (-3.28-3.48)**

Adjusted for sex and education.

RERI: relative excess risk due to interaction.

WMH: white matter hyperintensities

The additive RERI is calculated with the epi.interaction() function in the epiR package in Rstudio with Hosmer and Lemeshow confidence intervals. The equation for calculating the additive RERI is (HR_{A+B+} - 1) – (HR_{A+B-} - 1) – (HR_{A-B+} - 1) (32). Therefore, RERI = (1.51 – 1) – (1.37 – 1) – (1.29 – 1) = -0.15.

Glucocorticoid cascade and vascular hypotheses

When cortisol levels and WMH volume were entered together in a model with depression diagnosis, higher evening cortisol, and higher WMH volume were each independently associated with increased risk of dementia (Figure 1, model 3a; Table 4). After adjusting for APOE genotype, current smoking, alcohol intake, physical activity, BMI, hypertension and diabetes mellitus (Figure 1, model 3b), associations attenuated further, but remained statistically significant. When all analyses were repeated with GDS-15 score, the association with incident dementia was weaker (HR 1.09; 95% CI 1.06-1.12), yet increased. HRs barely changed after further adjustment for cortisol levels, WMH volume, and other covariates (Figure 1).

Due to using age as timescale, proportional hazards for sex was not met due to differences in risk for dementia between men and women in age in the models for Alzheimer's disease. As noted in Supplementary Figure 1, risk for dementia was higher for women than men during older age, which has also been seen previously in population-based cohorts (35).

Discussion

The aim of our study was to investigate two leading hypotheses explaining the relation between LLD and risk of dementia. We found that a lifetime diagnosis of MDD increased the risk of dementia 1.4 fold, and that a current – not remitted – diagnosis of MDD in older persons without dementia was associated with a two-fold risk for incident dementia over 13 years follow-up, including AD and non-AD dementia, confirming estimates found in meta-analyses (3, 36). Similarly, more depressive symptoms were also associated with increased risk of dementia, particularly non-AD dementia.

One of the prevailing hypotheses to explain the increased risk of dementia with depression has been the glucocorticoid cascade hypothesis (5). In our study, we observed that higher evening, but not morning, cortisol increased the risk of developing dementia, including AD and non-AD dementia, but cortisol was an independent contributor and did not explain the relation between LLD and dementia. Thus, we found no evidence to support this hypothesis. Increased cortisol levels have been previously found in patients with dementia (37) and have been shown to predict dementia (38). However, results are inconclusive regarding the timing of cortisol measure, as morning, evening and diurnal variation in cortisol have been shown to predict dementia onset (39, 40). In our study, we found a relation with evening cortisol only, which is consistent with a previous study where we showed that evening cortisol more so than morning cortisol was related with lower brain volumes (13). Other studies also found that evening cortisol levels are associated with increased age (41), hypertension (42), diabetes (43) and neurodegeneration (13, 44).

Another proposed hypothesis linking LLD with dementia is the vascular depression hypothesis (2, 14-16), as vascular brain pathology has often been associated with LLD (45). In another cohort, we previously showed that lacunar infarcts are related to higher and more fluctuating depressive symptoms at follow-up (46), as well as in those with cerebrovascular disease (47). Our current results confirmed other studies that more WMH increased the risk of dementia (47, 48), but it only partially explained the relationship between MDD and dementia. Also, while the additive association of high depressive symptoms and more WMH was strongest for non-AD dementias, it did not suggest moderation or mediation.

While other studies have looked at either dementia or depression with WMH or cortisol, studies exploring the simultaneous influence of glucocorticoid and vascular

pathways on depression and dementia are scarce. Comparable findings were reported in a small preceding study with 18 months of follow-up where cortisol levels rather than white matter lesions were related to cognitive decline in older depressed persons (17).

The association between current depression and dementia prevailed after correcting for additional cardiovascular risk factors, with WMH having a greater relationship with risk than evening cortisol. This is in line with a recent study that found WMHs were independently associated with cognitive decline and GDS-15 scores (49) and highlights the role of cerebrovascular disease in dementia and depression (50).

Our findings of that current but not past depression increased dementia risk could be interpreted as LLD being a prodromal stage of dementia. Indeed, a study with 28 years of follow-up (48) found that depressive symptoms increased prior to dementia diagnosis, suggesting that depression increases dementia risk closer to the time of dementia diagnosis. Consistent with this, in our study, those with a shorter time period between depression assessment and dementia diagnosis had a higher risk of developing dementia, particularly dementia other than AD, whereas those with a longer time period between depression and dementia diagnoses were not at higher risk. It should be noted that we were only able to stratify the sample for depressive symptoms (i.e., GDS-15 score) and not current MDD because of small numbers, and we therefore do not know who had previous episodes of depressive symptoms. Indeed, in our sample of participants with current MDD, the vast majority also had a history of MDD, and the association may partly reflect previous episodes of depression. Potentially, different mechanisms underlie different subtypes of LLD depending on history of MDD, age of onset, and number of previous episodes. To further unravel the direction of causation, similar studies with multiple measures of depression and longer follow-up are needed.

Strengths include the community-based population, the prospective design with long follow-up, the large sample size, and the complete ascertainment of incident dementia. Further, LLD was assessed with a structured diagnostic interview in addition to a depressive symptom questionnaire.

A limitation of this study is that current MDD was assessed with a two-week time window rather than 6 or 12 months. Therefore, the number of participants with current MDD was low and risk estimates had wide confidence intervals. We could not examine the combined association of current MDD and high cortisol or WMH volume and instead relied on depressive symptoms (although, results were similar for high

WMH volume with current MDD compared to using depressive symptoms). Further, it is preferred to include samples of cortisol collected over multiple days (13, 51), to decrease possible within-participant variability (52, 53); however, due to the large sample size of our cohort, we were only able to include one-day measurements. By investigating cortisol in such a large sample, the large variation on group level may have reduced the possibility of intra-subject variation.

The current study highlights the importance of LLD in developing dementia. While higher basal cortisol levels and WMH were also associated with increased risk of dementia, they were not a major mechanism underlying the relation between depression and risk of dementia. Future studies with long follow-up and repeated measures of LLD should investigate other explanatory factors and subtypes of LLD to further elucidate the pathophysiology behind depression and dementia and investigate to what extent LLD is a susceptibility feature of dementia rather than a causal risk factor.

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Conflict of interest

The authors have no conflict of interest to report.

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Supplementary Table 1. Hazard ratios for the relation between depression and risk of dementia (n=4,349), stratified by sex.

	No. of cases	All-cause dementia (N=843) HR (95% CI)	No. of cases	Alzheimer's disease (N=397) HR (95% CI)	No. of cases	Other dementias (N=446) HR (95% CI)
Never MDD (N=1739) *Males*	316	1 (reference)	142	1 (reference)	174	1 (reference)
Ever MDD (N=63) *Males*	12	1.49 (0.91-2.07)	6	1.54 (0.72-2.36)	6	1.56 (0.74-2.38)
Never MDD (N=2417) *Females*	492	1 (reference)	237	1 (reference)	255	1 (reference)
Ever MDD (N=130) *Females*	23	1.32 (0.90-1.74)	12	1.30 (0.72-1.89)	11	1.37 (0.76-1.98)
Never MDD (N=1739) *Males*	316	1 (reference)	142	1 (reference)	174	1 (reference)
Remitted MDD (N=42) *Males*	6	1.01 (0.19-1.83)	4	1.31 (0.29-2.34)	2	0.77 (0.66-2.14)
Current MDD (N=21) *Males*	6	2.79 (1.98-3.60)	2	2.25 (0.83-3.66)	4	3.55 (2.55-4.54)
Never MDD (N=2417) *Females*	492	1 (reference)	237	1 (reference)	255	1 (reference)
Remitted MDD (N=87) *Females*	12	1.02 (0.44-1.59)	5	0.80 (0.09-1.68)	7	1.22 (0.46-1.98)
Current MDD (N=43) *Females*	11	1.95 (1.32-2.58)	7	2.39 (1.60-3.18)	4	1.75 (0.72-2.77)
GDS-15 score <6 (N=1705) *Males*	303	1 (reference)	136	1 (reference)	167	1 (reference)
	No. of cases	**All-cause dementia (N=843) HR (95% CI)**	**No. of cases**	**Alzheimer's disease (N=397) HR (95% CI)**	**No. of cases**	**Other dementias (N=446) HR (95% CI)**
GDS-15 score 6 or higher (N=97) *Males*	25	1.57 (1.12-2.01)	12	1.57 (0.95-2.19)	13	1.66 (1.01-2.31)
GDS-15 score <6 (N=2359) *Females*	474	1 (reference)	233	1 (reference)	241	1 (reference)
GDS-15 score 6+ (N=188) *Females*	41	1.19 (0.86-1.52)	16	0.97 (0.46-1.48)	25	1.44 (1.00-1.88)

Models are adjusted for level of education, and age as timescale.

Supplementary Table 2. Results of the Cox regression model with MDD, cortisol levels, and WMH volume entered together (model 3a), stratified by sex.

	All-cause dementia	Alzheimer's disease	Other dementias
	(n_{males}=328, $n_{females}$=515)	(n_{males}=148, $n_{females}$=249)	(n_{males}=180, $n_{females}$=266)
	HR (95% CI)	HR (95% CI)	HR (95% CI)
Males			
Remitted MDD	1.05 (0.23-1.87)	1.36 (0.32-2.39)	0.80 (0.60-2.20)
Current MDD (including past)	2.61 (1.79-3.43)	2.12 (0.71-3.52)	3.26 (2.25-4.27)
Morning cortisol	0.92 (0.80-1.04)	0.96 (0.78-1.15)	0.88 (0.73-1.04)
Evening cortisol	1.08 (0.96-1.21)	1.07 (0.89-1.25)	1.12 (0.95-1.29)
WMH volume	1.37 (1.25-1.49)	1.29 (1.11-1.47)	1.55 (1.38-1.71)
Females			
Remitted MDD	1.03 (0.46-1.61)	0.82 (0.07-1.71)	1.23 (0.48-1.99)
Current MDD (including past)	1.78 (1.17-2.39)	2.12 (1.35-2.90)	1.60 (0.59-2.60)
Morning cortisol	1.04 (0.95-1.13)	1.07 (0.94-1.20)	1.03 (0.89-1.16)
Evening cortisol	1.15 (1.06-1.24)	1.19 (1.07-1.31)	1.16 (1.03-1.28)
WMH volume	1.26 (1.17-1.36)	1.24 (1.11-1.38)	1.36 (1.23-1.49)

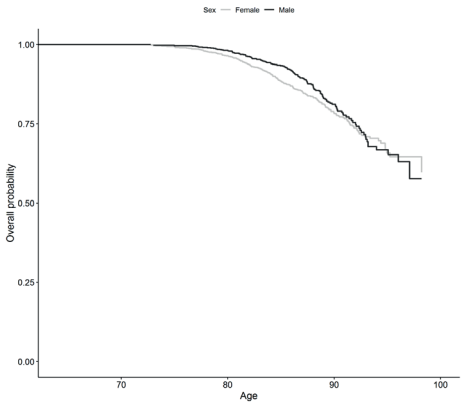
Models are adjusted for education and intracranial volume. WMH: white matter hyperintensities. MDD: major depressive disorder. Cortisol levels and WMH volume are shown per SD increase.

Supplementary Table 3. Regression coefficients for the independent and combined association of depressive symptoms, evening cortisol, and white matter hyperintensities volume with incident dementia.

	All-cause dementia	Alzheimer's disease	Other dementias
	(n=843)	(n=397)	(n=446)
	Coefficient (SE)	Coefficient (SE)	Coefficient (SE)
Depressive symptoms (β1)	0.17 (0.03)	0.10 (0.05)	0.25 (0.04)
Evening cortisol (β2)	0.12 (0.04)	0.16 (0.05)	0.13 (0.05)
Interaction term (β3)	-0.01 (0.03)	0.00 (0.05)	-0.01 (0.05)
Additive RERI (95% CI) (32)	**0.02 (-0.20-0.24)**	**0.02 (-0.30-0.34)**	**0.02 (-0.29-0.34)**
Depressive symptoms (β1)	0.18 (0.03)	0.11 (0.05)	0.27 (0.04)
WMH (β2)	0.28 (0.04)	0.24 (0.05)	0.39 (0.05)
Interaction term (β3)	-0.05 (0.03)	-0.03 (0.05)	-0.08 (0.04)
Additive RERI (95% CI) (32)	**-0.01 (-0.22-0.20)**	**-0.01 (-0.32-0.30)**	**0.01 (-0.32-0.34)**

Adjusted for sex and education.
RERI: relative excess risk due to interaction.
WMH: white matter hyperintensities
The additive RERI is calculated with the epi.interaction() function in the epiR package in Rstudio. The equation is as follows: $e^{\beta1+\beta2+\beta3}$- $e^{\beta1}$-$e^{\beta2}$+1 (32). Confidence intervals come from Hosmer and Lemeshow.

Supplementary Figure 1. Adjusted survival curves for model 3a stratified by sex and Alzheimer's disease.



Model 3a: adjusted for past/current depression diagnosis, education, morning and evening cortisol, white matter hyperintensities, and intracranial volume.

PART 3

MOVING TOWARDS PRECISION MEDICINE FOR DEMENTIA

Chapter 8

Late-life depression, allostatic load, and risk of dementia: The AGES-Reykjavik Study

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Abstract

Background: The current study aimed to assess if the relation between depression and dementia could be explained by allostatic load (AL) profiles, as well as assessing their risk on incident all-cause dementia, Alzheimer's disease (AD), and non-AD dementias.

Methods: The study included individuals without dementia at baseline from the population-based AGES-Reykjavik Study. Depressive symptoms assessed with the Geriatric Depression Scale-15 and AL markers were collected at baseline. Latent profile analysis (LPA) was performed on the AL markers. Incident dementia was measured during 12-years of follow-up.Cox regressions adjusted for AL profiles were performed to evaluate if AL could explain the relation between depressive symptoms and incident dementia. Additional Cox regressions exploring the interaction with depressive symptoms and AL profiles were also performed.

Results: LPA revealed four profiles based on AL factors: 'Low cardiovascular dysregulation' (43%), 'Average' (42% prevalence), 'High cardiovascular dysregulation' (11%), and 'Multisystem dysregulation' (4%). Cox regression analyses found an increased risk for dementia in the 'Multisystem dysregulation' group (HR 1.72; 95% CI 1.26-2.33), as well as for AD (HR 1.75; 95% CI: 1.12-2.71) and non-AD dementias (HR 1.87; 95% CI: 1.23-2.84). AL profiles did not mediate the risk of all-cause dementia with depressive symptoms; however, there was evidence of additive interaction with depressive symptoms and the 'Multisystem dysregulation' profile and all-cause dementia (RERI 0.15; 95% CI 0.03-0.26).

Conclusion: AL profiles and depressive symptoms were independently related to dementia. Individuals with multisystem dysregulation could be more susceptible to the negative effects of depressive symptomology on incident dementia.

Introduction

Dementia is characterized by debilitating cognitive impairment that increases the risk of mortality (1). Today, 50 million people in the world have dementia, which is expected to triple by 2050 (2, 3). The etiology is still not completely known, and no effective treatment is available (4). Further research on modifiable risk factors is crucial to better understand the biological underpinnings of dementia, allowing for the development of new interventions and prevention strategies, which would better the outcome for those at risk.

One of the most consistent determinants for dementia is depression (5), yet the mechanistic relationship between the two is still not fully understood (6). Two main hypotheses regarding the relation between depression and dementia are the neurotoxicity and the vascular hypotheses. The neurotoxicity hypothesis stipulates that depression is related to dementia through increased cortisol due to dysregulation of the hypothalamic-pituitary-adrenal axis (7-9), whereas the vascular hypothesis states that depression may precede dementia through small vessel changes in mood-regulating areas (10). In a recent study, we found that the neurotoxicity hypothesis did not explain the relation, while the vascular hypothesis did in part (11). However, many risk factors overlap both depression and dementia, not only vascular and glucocorticoid factors, but also metabolic and inflammatory factors. We hypothesize that using a multisystem approach may better explain the relation between depression and dementia. An umbrella term encompassing all these biological risk factors is allostatic load (AL), which refers to the long-term, damaging physiological actions the body performs in response to stressful stimuli. While these biological factors are adaptive in response to acute stress (i.e., 'allostasis'), chronic stress over time leads to wear and tear on the body (12), which can be measured by dysregulation in multiple physiological systems.

Depression has been linked to AL factors through two depressive subtypes (13): atypical and melancholic depression. Atypical depression is characterized by altered energy intake, increased weight, female sex, and immune-metabolic physiological factors (e.g., high c-reactive protein [CRP], triglycerides, and blood pressure) (14, 15). Whereas melancholic depression, characterized by symptoms of decreased appetite, lower body mass index, and smoking, is associated with increased cortisol levels (16, 17). Further, hyperactivity in the hypothalamic-pituitary-adrenal (HPA) axis due to excess cortisol has been linked to depression (17).

Dementia has also been attributed to AL factors. Cardiovascular factors, such as hypertension and Framingham vascular risk factors (e.g., total cholesterol, high-density lipoprotein [HDL] cholesterol), increase the risk for dementia (18, 19). A recent systematic review also highlighted type 2 diabetes as one of the top modifiable risk factors for dementia, emphasizing the role of metabolic factors as well (5). Additionally, chronically-raised high-sensitivity CRP, an inflammatory marker, has been associated with an increased risk of vascular dementia (20). Lastly, regarding glucocorticoids, a recent review has outlined the relationship between higher levels of cortisol and increased risk for cognitive decline and dementia (21). While these markers have been linked individually to both dementia and depression, there has been increased need to explore multisystem etiological models.

While many studies have used sum scores to assess AL, there has been an increasing need to look at possible subsystems of biomarkers to account for the complex interactions that may exist between them (22, 23) and to assess if one subsystem (e.g., immune-metabolic) may be more of a driving factor for disease risk than another system (e.g., cardiovascular). Additionally, by utilizing latent profile analysis (LPA) over latent class analysis (LCA), using continuous data rather than dichotomizing, we allow for more variation within and between the profiles. By using a profile-based technique that can unravel these subsystems, one can link the use of studying individual biomarkers and cumulative scores by looking at possible AL subsystems. Previous research has explored profiling individuals based on AL biomarkers, highlighting increased risk for mortality based on AL profiles (24-26). Further research has also found associations between higher AL and lower cognitive functioning (23, 24, 27-29), as well as with increased depressive symptoms (24, 29-31). However, to our knowledge, assessing if AL profiles may explain the relationship between depression and dementia has yet to be done.

The current study aimed to explore the role of AL in the known relation between depression and dementia by assessing: 1) the relationship between AL profiles and risk of dementia; 2) the relationship between depressive symptoms and these AL profiles; 3) whether AL mediates the relationship between depression and dementia; and 4) if there is additive or multiplicative interaction between depression and AL profiles on dementia risk. Based on previous research, we hypothesized to find at least one AL profile characterized by metabolic and inflammatory criteria, one by cardiovascular factors, and one without any increased AL qualities (32). We further hypothesized that the metabolic-inflammatory and cardiovascular profiles will be associated with depressive symptoms as well as an increased risk for dementia. We had no a-priori hypothesis regarding possible mediation or interaction of AL on the relation between depression and dementia.

Methods

Participants

The Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study is a population-based cohort study comprised of individuals aged 65 years and older living in the Reykjavik area. It is explained in-depth elsewhere (33). Briefly, the AGES-Reykjavik Study stems from the Reykjavik Study, which was initiated in 1967 by the Icelandic Heart Association. Between 2002 and 2006, 5,764 participants were included in the study, randomly selected from survivors from the Reykjavik Study. All participants underwent baseline cognitive and biometric assessments at the Reykjavik research center. Participants were followed up until 2014 to identify incident dementia diagnoses.

Standard protocol approvals, registrations, and patient consents

The Icelandic National Bioethics Committee (VSN: 00-063), the Icelandic Data Protection Authority, and the Institutional Review Board for the National Institute on Aging, NIH approved this study. Written informed consent was obtained from all participants.

Depression assessment

The Geriatric Depression Scale-15 (GDS-15) (34) was used to assess depressive symptoms at baseline. The GDS-15 consists of items such as apathy (e.g., 'Have you dropped many of your activities or interests?'), feelings of helplessness and hopelessness, and life satisfaction. The answer categories are binary (i.e., either present or absent), and the internal consistency has been shown to be high, with a Cronbach's alpha of 0.80 (35). For sensitivity analyses, a cut-off of 6 or higher was also explored to define high depressive symptomology. We chose a cut-off of 6 or higher as it has been highlighted to have a higher sensitivity and specificity in community-based settings (36). A diagnosis of major depressive disorder (MDD) was also assessed using the Mini-International Psychiatric Interview. For more information on depression assessment, refer to (37).

Dementia assessment

Ascertainment of dementia was done using a three-step procedure based on international criteria and is described in detail elsewhere (38-40). The total sample underwent cognitive assessment, and further neuropsychological testing was done in screen positives. In step 2, persons who were positive on test results, received further neurologic and proxy examinations. Next, a multidisciplinary panel consisting of a neurologist, geriatrician, neuroradiologist, and neuropsychologist diagnosed dementia according to international guidelines (33) at baseline for exclusion and at follow-up (between 2007 to 2011) for incident dementia. All participants were also

continuously followed up for incident dementia using medical and nursing home records and death certificates for less misclassification bias of cases as controls. When an individual moved into a nursing home, all-cause dementia and Alzheimer's disease (AD) diagnoses were based on an intake exam. Additional cases within a nursing home were done by a standardized procedure done by all Icelandic nursing homes (41). For the current study, all-cause dementia, AD, and non-AD dementias were defined.

AL measures

Based on previous research (42), we included the following *cardiovascular factors* as indicative of AL: systolic blood pressure and pulse pressure (43, 44); *lipids* as HDL, low-density lipoprotein (LDL) (32), and triglycerides (42); *metabolic factors* as abdominal circumference (45) and fasting glucose (32); an *inflammatory factor* as high-sensitivity CRP (46), and *stress factors* as morning and evening salivary cortisol (32). Two consecutive measurements of blood pressure were taken with a mercury sphygmomanometer, with the mean systolic blood pressure value being used. Pulse pressure was defined as diastolic blood pressure subtracted from systolic blood pressure. Fasting glucose, HDL cholesterol, triglycerides, and CRP were measured on a Hitachi 912, using reagents from Roche Diagnostics. Salivary cortisol samples were collected the night before visiting the research center and the next morning 45 minutes after waking with Salivette® devices (Sarstedt, Rommelsdorf, Germany) and analyzed with a time-resolved immunoassay with fluorescence detection (Delfia; PerkinElmer, Waltham, MA) (47). Inter-assay and intra-assay variabilities were below 12% and 10%, respectively. The lower detection limit was 0.43 nmol/L (47). Salivary cortisol, CRP, triglycerides, and fasting glucose were natural log-transformed due to skewed distribution.

Other measures

At baseline, age, sex, education, and lifestyle variables were assessed via questionnaires. Education was categorized as primary, secondary, college, or university degree. Smoking was characterized as current, former, or never smoker. Alcohol use was quantified as grams per week. Physical activity (moderate-vigorous intensity) was classified by a self-reported questionnaire as never, rarely, occasionally (weekly but <1 h), moderate (1-3 h per week), or high (>4 h per week) (48) and included in the model as a nominal variable. Antihypertensive or antidepressant medication was classified as none or any. Mild cognitive impairment was defined by scoring less than 1.5 standard deviations below a cut-point determined from the cohort on memory or two other domains (e.g., language, visuoperceptual/visuoconstructional, psychomotor speed, executive functions, fine motor control)

(49) and was diagnosed by a multidisciplinary panel of specialists (see above with dementia diagnosis). Metabolic syndrome was defined based on WHO criteria (50, 51) as having insulin resistance (i.e., type 2 diabetes or impaired fasting glucose or tolerance), as well as any two of the following: 1) hypertension or taking antihypertensive medications, 2) dyslipidemia, or 3) obesity accompanied by a high albumin excretion rate. Prevalent stroke was defined through self-assessment or from hospital registries. Presence of APOE ε4 genotype was assessed via microplate array diagonal gel electrophoresis (MADGE) (52). APOE ε4 was characterized as dichotomous, classifying those with ε2/4, ε3/4, and ε4/4 genotypes as APOE ε4 positive and those with ε2/2, ε2/3, and ε3/3 genotypes as APOE ε4 negative.

Data analysis

Excluding those with dementia at baseline, 5,343 individuals were included in the current analysis. To address missing values (max: 12%) at baseline, multiple imputation (10 datasets) was performed in Mplus (v. 6.12, Muthen & Muthen, 2004). Multiple imputation in Mplus is based on Bayesian Markov chain Monte-Carlo estimation. The outcome, incident dementia, was also used as a predictor in the imputation process, but it was not imputed itself. Results from the 10 datasets were then pooled for the rest of the analyses. Chi-square tests and ANOVAs were performed to assess differences in demographic and AL variables in those with high and low depressive symptomology.

First, we created profiles based on AL variables using LPA. LPA was performed using Mplus (v. 6.12, Muthen & Muthen, 2004) with AL items as indicators. LPA uses covariance across the indicator variables to find relationships amongst individuals (53). All AL factors were treated as continuous in the model. To determine the number of profiles, we used the Bayesian information criterion (BIC) and Akaike information criterion (AIC) with lower values indicating a better fitting model, the Vuong-Lo-Mendell-Rubin Likelihood Ratio test (VLMR), entropy with higher values indicating a better fit, and that at least 1% of the cohort fitting into one profile. We estimated 2-6 profiles to assess best model fit. Participants were classified based on their most likely latent profile membership for further analyses. ANOVAs were performed to assess differences between profiles on AL markers and depressive symptomology.

Next, we determined the risk of these AL profiles on developing all-cause dementia, AD, and non-AD. Univariate Cox regression analyses were performed in IBM SPSS Statistics (version 25) to estimate the hazard ratio (HR) of the association between AL profiles and all-cause dementia, AD and non-AD with follow-up years on the time scale. Model 1 corrected for age, sex, and education, and model 2 added history

of stroke, smoking, alcohol use, antihypertensive and antidepressant medication, physical activity, and APOE e4 genotype as covariates. The Cox proportional hazards, influential observations, and nonlinearity assumptions were tested and met.

Finally, we estimated the risk of depression with developing all-cause dementia, AD, and non-AD, with the AL profiles as covariates. Cox regression analyses first were done with depressive symptoms as main predictor and compared to joint models adding the AL profiles to assess their individual and joint contributions to dementia risk. Next, we also assessed multiplicative interaction with depressive symptoms and AL profiles by adding product terms between depressive symptoms and AL profiles into the model. We also calculated the relative excess risk due to interaction (RERI) to assess additive interaction (54) and used the delta method to calculate the confidence interval (55). Model 1 correcting for age, sex, and education, and model 2 for additional correction (see above) were also performed. Sensitivity analyses were done to explore differences in models 1 and 2 when using a clinical cut-off of the GDS-15 (6 or higher) or using a clinical diagnosis of MDD. To explore the robustness of the RERI, a sensitivity analysis exploring interaction using standardized depressive symptom scores was also performed. Lastly, a competing risk model was performed with all-cause mortality and dementia-free mortality as separate outcomes in Cox regression models.

Results

Of the 5343 participants (mean age at baseline: 77 years), 58% were women (Table 1). During a 12-year follow-up (M = 8.43 years; SD = 3.43 years), 1099 individuals developed dementia with 492 cases having AD diagnosis. Most individuals (n = 900) were diagnosed via assessment in nursing homes, and an additional (n = 160) were diagnosed by the Icelandic Heart Association, and 39 by death certificates. Internal consistency of the GDS-15 was quite high with a Cronbach's alpha of 0.71.

The LPA on AL variables showed that four profiles were determined as the best-fitting model (see Supplemental Table 1). According to BIC and AIC criteria, more profiles resulted in a better fitting model. Additionally, based on the VLMR, four profiles compared to five profiles resulted in a better model fit ($p = 0.047$). Lastly, entropy was higher in the four profile model (0.829 v. 0.777). Therefore, we chose a four profile model. A figure of the five profile model is shown in Supplemental Figure 1.

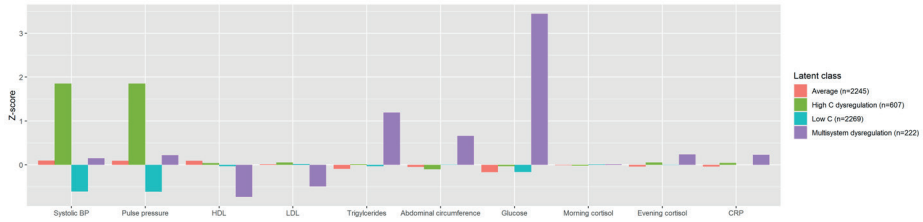

Figure 1. Average allostatic load factor value per profile.

Note: z-scores are represented here for visualization purposes. However, the variables are used in their non-standardized format in the latent profile analysis.

Table 1. Baseline characteristics in the study sample and stratified by high depressive symptomology (n=5343).

	Total population (n=5343)	**GDS <6 (n=4933)**	**GDS 6+ (n=410)**
Age, years	77 ± 6	77 ± 6	78 ± 6
Women	58%	57%	65%
Education, college + university	27%	28%	19%
Current smoker	12%	12%	16%
Alcohol use, gr/week	15 ± 32	15 ± 32	11 ± 24
Physical activity, moderate/high	32%	33%	21%
Stroke/blood clot in brain	7%	6%	12%
MCI at baseline	10%	10%	18%
Metabolic syndrome	32%	31%	33%
Diabetes	13%	12%	16%
Antihypertensive medication	48%	48%	47%
Antidepressant medication	14%	13%	34%
APOE e4 genotype	28%	28%	28%
Depression (M ± SD)			
GDS-15, total	2 ± 2	2 ± 1	8 ± 2
Allostatic load indicators (M ± SD)			
Systolic blood pressure (mmHg)	143 ± 21	143 ± 21	142 ± 23
Diastolic blood pressure (mmHg)	74 ± 10	74 ± 10	73 ± 11
Pulse pressure (mmHg)	69 ± 18	69 ± 18	68 ± 20
High-density lipoprotein (mmol/L)	1.6 ± 0.5	1.6 ± 0.5	1.6 ± 0.4
Low-density lipoprotein (mmol/L)	3.5 ± 1.0	3.5 ± 1.0	3.4 ± 1.0
Abdominal circumference (cm)	101 ± 12	101 ± 12	101 ± 12
Fasting glucose (mg/dL)	5.8 ± 1.2	5.8 ± 1.2	5.8 ± 1.4

Table 1. Continued

	Total population (n=5343)	GDS <6 (n=4933)	GDS 6+ (n=410)
Triglycerides (mg/dL)	1.2 ± 0.7	1.2 ± 0.7	1.3 ± 0.6
C-reactive protein (mg/L)	3.8 ± 6.8	3.7 ± 6.4	5.2 ± 10.7
Morning cortisol (nmol/L)	20 ± 13	20 ± 14	18 ± 15
Evening cortisol (nmol/L)	4 ± 7	4 ± 7	5 ± 6

NOTE: Diastolic blood pressure was not used in the latent profile analysis, only systolic blood pressure and pulse pressure. Missings were less than 1% for all indicators except: 9% for evening cortisol, 10% for morning cortisol, and 12% for GDS-15 sum score.
GDS = Geriatric Depression Scale-15; MCI = mild cognitive impairment.

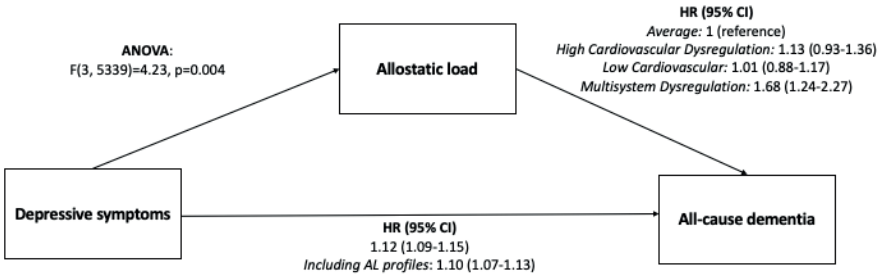

Figure 2. Schematic diagram of the relations between depressive symptoms, allostatic load profiles, and all-cause dementia. Hazard ratios (HRs) and 95% confidence intervals are shown for the relationships between allostatic load and all-cause dementia, as well as between depressive symptoms and all-cause dementia (also adjusted for allostatic load profiles), adjusted for age, sex, education, smoking, alcohol, physical activity, stroke at baseline, antihypertensive medication, antidepressant medication, and APOE e4 genotype. AL = allostatic load.

Description of AL profiles

The profile with the highest prevalence (i.e., 43%) was named the 'Low cardiovascular' profile due to lower blood pressure compared to the total sample (mean systolic blood pressure: 130 mmHg vs. in the total sample: 143 mmHg) and generally average levels on all other AL markers (Figure 1). The profile with the second highest prevalence (i.e., 42%) was distinguished by average values across all AL domains and therefore called 'Average'. This profile was defined as the reference group for all remaining analyses. A third profile was described by high pulse pressure (mean pulse pressure: 103 mmHg vs. in the total sample: 69 mmHg) with a prevalence of 11%, and therefore termed the 'High cardiovascular dysregulation' profile due to high levels on only cardiovascular AL markers. Lastly, a profile containing 4% of the sample, was characterized by higher values across multiple AL domains, with higher triglycerides (2.0 mg/dL vs. 1.2 mg/dL), higher abdominal circumference (109 cm vs. 101 cm),

higher glucose (10 mg/dL vs. 5.8 mg/dL), higher evening cortisol (6 nmol/L vs. 4 nmol/L), and higher CRP (5.4 mg/L vs. 3.8 mg/L). Average levels were seen regarding cardiovascular AL markers. Therefore, it was named 'Multisystem dysregulation' (Table 2, Figure 1). ANOVAs on the AL markers reported significant differences amongst all AL markers between the profiles. Briefly, the 'High cardiovascular dysregulation' profile had the highest mean age (79 years), highest proportion of women (62%), and lowest proportion of individuals with high education (26%). Whereas the 'Multisystem dysregulation' profile had the lowest proportion of women (44%) and the highest proportion of individuals with high education (30%). Demographic and covariate information per AL profile is shown in Supplemental Table 2.

Table 2. Baseline characteristics of the indicators in the latent profile analysis with four profiles.

	Average n=2245 (42%)	**High Cardiovascular Dysregulation n=607 (11%)**	**Low Cardiovascular n=2269 (43%)**	**Multisystem Dysregulation n=222 (4%)**
Allostatic load indicators (M ± SD)				
Systolic blood pressure (mmHg)	145 ± 10	181 ± 14	130 ± 10	146 ± 17
Diastolic blood pressure (mmHg)	74 ± 9	78 ± 12	73 ± 10	73 ± 11
Pulse pressure (mmHg)	71 ± 9	103 ± 13	58 ± 9	73 ± 14
High-density lipoprotein (mmol/L)	1.6 ± 0.4	1.6 ± 0.4	1.6 ± 0.5	1.3 ± 0.4
Low-density lipoprotein (mmol/L)	3.5 ± 1.0	3.6 ± 1.1	3.5 ± 1.0	3.0 ± 1.1
Abdominal circumference (cm)	100 ± 12	100 ± 12	101 ± 12	109 ± 13
Fasting glucose (mg/dL)	5.6 ± 0.7	5.8 ± 0.8	5.6 ± 0.6	10.0 ± 2.5
Triglycerides (mg/dL)	1.2 ± 0.6	1.2 ± 0.6	1.2 ± 0.6	2.0 ± 1.2
C-reactive protein (mg/L)	3.6 ± 7.0	4.1 ± 7.4	3.8 ± 6.1	5.4 ± 9.2
Morning cortisol (nmol/L)	20 ± 16	19 ± 15	20 ± 14	20 ± 14
Evening cortisol (nmol/L)	4 ± 7	4 ± 6	4 ± 5	6 ± 10
Depressive symptoms (GDS-15 sum score)	2 ± 2	3 ± 2	2 ± 2	3 ± 2

Note: GDS-15 = Geriatric Depression Scale-15

Table 3. HRs and 95% CIs from the Cox regression on all-cause dementia, AD, and non-AD with allostatic load profiles.

	No. Of cases	All-cause dementia (n=1099) HR (95% CI)	No. Of cases	Alzheimer's disease (n=492) HR (95% CI)	No. Of cases	Other dementias (n=607) HR (95% CI)
Model 1						
Average	459	1 (reference)	216	1 (reference)	242	1 (reference)
High Cardiovascular Dysregulation	153	1.09 (0.89; 1.32)	50	0.83 (0.61; 1.13)	102	1.34 (1.04; 1.72)
Low Cardiovascular	438	1.04 (0.86; 1.26)	203	1.01 (0.83; 1.23)	236	1.06 (0.80; 1.40)
Multisystem Dysregulation	49	1.59 (1.17; 2.15)	23	1.49 (0.96; 2.31)	27	1.81 (1.19; 2.76)
Model 2						
Average	459	1 (reference)	216	1 (reference)	242	1 (reference)
High Cardiovascular Dysregulation	153	1.19 (0.97; 1.44)	50	0.87 (0.64; 1.19)	102	1.40 (1.09; 1.79)
Low Cardiovascular	438	1.03 (0.86; 1.24)	203	1.00 (0.82; 1.22)	236	1.03 (0.82; 1.31)
Multisystem Dysregulation	49	1.72 (1.26; 2.33)	23	1.67 (1.08; 2.59)	27	1.85 (1.21; 2.81)

Model 1 is adjusted for age, sex, and education.
Model 2 is adjusted for age, sex, education, smoking, alcohol, physical activity, stroke at baseline, antihypertensive medication, antidepressant medication, and APOE e4 genotype.

Depression and AL profiles

When comparing those with high vs. low depressive symptomology, a one-way ANOVA showed that CRP was higher in those with high depressive symptoms ($F(1, 5341)=16.33$, $p<0.001$), as well as higher evening cortisol ($F(1, 5341)=14.18$, $p<0.001$). No other AL variables differed between those with low or high depressive symptomology. When comparing the AL profiles, depressive symptoms were slightly higher in the 'High cardiovascular dysregulation' and 'Multisystem dysregulation' profiles (Supplemental Info 1).

AL profiles and dementia risk

Cox regression analyses for the first model, adjusting for age, sex, and education, showed no association between the 'High cardiovascular dysregulation' profile (HR 1.09; 95% CI 0.89-1.32) or the 'Low cardiovascular' profile (HR 1.04; 95% CI 0.86-1.26) compared to the 'Average' profile with all-cause dementia. There was a 59% increased risk for all-cause dementia in the 'Multisystem dysregulation' profile (HR 1.59; 95% CI 1.17-2.15) compared to the 'Average' profile (Table 3, model 1). Estimates and confidence intervals slightly changed in the second model after further correction for additional lifestyle factors (Table 3, model 2).

Table 4. Additive and multiplicative interaction between depressive symptoms and AL profiles on all-cause dementia, AD, and non-AD.

	No. Of cases	All-cause dementia	No. Of cases	Alzheimer's disease	No. Of cases	Other dementias
		(n=1099)		(n=492)		(n=607)
Model 1		**HR (95% CI)**		**HR (95% CI)**		**HR (95% CI)**
Multiplicative interaction						
Depressive symptoms x High cardiovascular dysregulation	153	0.99 (0.91; 1.08)	50	0.97 (0.84; 1.13)	102	0.99 (0.89; 1.10)
Depressive symptoms x Low cardiovascular	438	1.01 (0.94; 1.09)	203	1.00 (0.92; 1.10)	236	1.01 (0.93; 1.10)
Depressive symptoms x Multisystem dysregulation	49	1.10 (0.97; 1.24)	23	1.09 (0.88; 1.35)	27	1.06 (0.92; 1.23)
Additive interaction		**RERI (95% CI)**		**RERI (95% CI)**		**RERI (95% CI)**
Depressive symptoms x High cardiovascular	153	0.00 (-0.07; 0.08)	50	-0.03 (-0.16; 0.09)	102	0.04 (-0.07; 0.14)
Depressive symptoms x Low cardiovascular	438	0.01 (-0.05; 0.07)	203	0.00 (-0.08; 0.08)	236	0.01 (-0.05; 0.08)
Depressive symptoms x Multisystem dysregulation	49	**0.15 (0.04; 0.26)**	23	0.13 (-0.07; 0.33)	27	**0.17 (0.01; 0.33)**
Model 2		**HR (95% CI)**		**HR (95% CI)**		**HR (95% CI)**
Multiplicative interaction						
Depressive symptoms x High cardiovascular dysregulation	153	0.97 (0.89; 1.05)	50	0.95 (0.82; 1.10)	102	0.95 (0.85; 1.05)
Depressive symptoms x Low cardiovascular	438	1.08 (0.96; 1.21)	203	0.99 (0.91; 1.08)	236	1.04 (0.90; 1.20)
Depressive symptoms x Multisystem dysregulation	49	1.01 (0.95; 1.07)	23	1.09 (0.89; 1.35)	27	1.00 (0.93; 1.08)
Additive interaction		**RERI (95% CI)**		**RERI (95% CI)**		**RERI (95% CI)**
Depressive symptoms x High cardiovascular	153	-0.02 (-0.10; 0.06)	50	-0.05 (-0.19; 0.08)	102	-0.01 (-0.14; 0.11)
Depressive symptoms x Low cardiovascular	438	0.01 (-0.05; 0.06)	203	-0.01 (-0.09; 0.07)	236	0.00 (-0.06; 0.06)
Depressive symptoms x Multisystem dysregulation	49	**0.15 (0.03; 0.26)**	23	0.16 (-0.05; 0.37)	27	0.15 (-0.01; 0.32)

Model 1 is adjusted for age, sex, and education.

Model 2 is adjusted for age, sex, education, smoking, alcohol, physical activity, stroke at baseline, antihypertensive medication, antidepressant medication, and APOE e4 genotype.

For information on calculation of the additive RERI by using a product term in a regression model, please see (54).

AL = allostatic load; AD = Alzheimer's disease.

For AD dementias, there was an increased risk in the 'Multisystem dysregulation' group with full adjustment for covariates (HR 1.75; 95% CI 1.12-2.71). For non-AD dementias, an increased risk was found in the 'High cardiovascular dysregulation' group (HR 1.34; 95% CI 1.04-1.72) and in the 'Multisystem dysregulation' group (HR 1.81; 95% CI 1.19-2.76) in model 1 and remained with further adjustment for covariates in model 2 (Table 3).

Depression and incident dementia and the role of AL profiles

Cox regression analyses found an increased risk for incident dementia in relation to the sum-score on the GDS-15 (HR per point increase 1.12; 95% CI 1.09-1.15) which remained in model 2. An increased risk for AD dementia (HR 1.07; 95% CI 1.03-1.12) and non-AD dementias (HR 1.16; 95% CI 1.12-1.21) was also found in relation to depressive symptoms, which also remained in model 2. However, when adding the AL profiles into the Cox regression to assess mediation, the effect estimates of depressive symptoms on incident dementia remained increased (Figure 2). Further, HRs and confidence intervals were similar in the AL profiles for all-cause dementia, AD, and non-AD dementias in the joint model with depressive symptoms compared to a model with AL profiles alone (Supplemental Table 3, Table 3). Sensitivity analyses based on the GDS-15 cut-off of 6 or higher or using current MDD diagnosis showed similar results as well (Supplemental Table 4). Evidence for possible additive interaction with depressive symptoms and the 'Multisystem dysregulation' profile was found for all-cause dementia (RERI 0.15; 95% CI 0.04-0.26), as well as non-AD dementia (RERI 0.17; 95% CI 0.01-0.33) in model 1 (Table 4). That is, the combined effect of depressive symptoms and the 'Multisystem dysresgulation' profile on all-cause dementia and non-AD dementia was larger than the sum of the individual effects. Results stayed similar for all-cause dementia after further correction for covariates (Table 4) and when standardizing depressive symptoms (Supplemental Table 5). No evidence for interaction on the multiplicative scale was found with depressive symptoms and any AL profile (Table 4; Supplemental Table 5).

The competing risk model did not find a difference in risk when looking at dementia-free mortality compared to all-cause dementia (Supplemental Table 6).

Discussion

The current study aimed to explore the role of AL in the relation between depressive symptoms and incident dementia. Using LPA, we identified four profiles: 'Low cardiovascular', 'Average', 'High cardiovascular dysregulation', and 'Multisystem

dysregulation'. A 72% increased risk of all-cause dementia was found in the 'Multisystem Dysregulation' group, and a 41% increased risk of non-AD dementias was found for the 'High cardiovascular dysregulation' group. Depressive symptoms were associated with a 10% higher risk of all-cause dementia with each point increase on the GDS-15, which remained after further correction of the AL profiles. Therefore, no evidence for mediation was found. Evidence for additive interaction was found between depressive symptoms and the 'Multisystem dysregulation' profile for all-cause dementia, specifically for non-AD related dementias. No multiplicative interaction was found with depressive symptoms and any AL profile.

While AL profiles and dementia have yet to be assessed previously, the results of the AL profiles with incident dementia are in line with previous studies on depression and AL profiles. This suggests that AL profiles may show similar associations with both dementia and depression. The highest risk for incident dementia was found in the 'Multisystem Dysregulation' group, which was characterized by metabolic and inflammatory factors. Previous studies on AL and depression also found an association between depression and AL profiles characterized by dysregulation in metabolic and inflammatory subsystems (56-59). Further, this profile was associated specifically with AD dementia as well, whereas both the 'Multisystem Dysregulation' and the 'High cardiovascular dysregulation' were associated with AD and non-AD dementias. This could be due to vascular dementia cases in the non-AD dementia subgroup. This distinction in AL profiles between subtypes of dementia should be assessed further for more precise and individualized intervention implementation.

Previous research has highlighted the most consistent evidence for risk of dementia being depression (5). Hypotheses regarding this association have included inflammatory, stress, and vascular mechanisms that all cumulatively represent AL (11, 60-62). We did find that there was an indication that the joint effect of the 'Multisystem dysregulation' profile and depressive symptoms was greater than the sum of the effects of the 'Multisystem dysregulation' profile alone and depressive symptoms alone. This implies those in the 'Multisystem dysregulation' profile could be more susceptible to the negative effects of late-life depressive symptoms on incident dementia. As this is the first study assessing the role of AL profiles and depressive symptoms on incident dementia, and we had no a-priori hypothesis regarding this finding, future studies need to replicate this finding.

Strengths of this study include a large, community-based population, extensive follow-up time to determine incident dementia and the monitoring of dementia diagnosis with virtually no loss to follow-up for dementia outcome. Multiple imputation was done to address missing data and HRs were corrected for potential

confounders. Further, using LPA as the analytical method allowed for using empirically-based classification instead of arbitrary cut-offs.

One limitation of the current study was that a wide range of AL markers were not available for inflammatory and stress processes, such as interleukin-6 or D-HEAS. Additionally, subtyping of dementias other than AD was not done reliably in those diagnosed in nursing homes. Therefore, we were unable to examine vascular dementia as an outcome and infer with categorical certainty our results regarding AD and non-AD individuals. It is critical to note that the population of the AGES-Reykjavik study is ethnically homogeneous. These findings need to be replicated in other populations, especially in those who are marginally underrepresented. Further, we did not have the power to distinguish between those who had remitted or prior depressive symptoms and those who only experienced late-life depressive symptoms. Thus, these results need to be validated in those who also experience high depressive symptoms in early- to midlife. Lastly, our findings regarding the interaction between depressive symptoms and the 'Multisystem dysregulation' profile needs to be replicated, as this profile was less prevalent (i.e., 4% of the study sample).

The current study found that both a profile specifically associated with metabolic and inflammatory dysregulation, as well as increased depressive symptoms, were independently associated with an increased risk of all-cause dementia. Further, this profile showed specific susceptibility to the effects of depressive symptoms on dementia risk. Future studies on dementia should take a multifaceted approach to guide awareness for subsequent individualized prevention and treatment efforts.

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Supplemental Table 1. Model fit criteria for 2-6 classes.

Model	LL	AIC	BIC	aBIC	Entropy
2 classes	-99488	199038	199242	199144	0.811
3 classes	-98492	197068	197345	197211	0.867
4 classes	-97582	195269	195618	195450	0.829
5 classes	-96997	194122	194544	194340	0.777
6 classes	-96541	193232	193726	193488	0.785

Supplemental Info 1.

High vs. low depressive symptomology based on GDS-15 cut-off score (<6 and 6+)

A one-way ANOVA revealed that those with high depressive symptoms were significantly older compared to those with low depressive symptoms ($F(1, 5341)=13.05$; $p<0.001$). Current alcohol consumption was lower in those with high depressive symptomology ($F(1, 5341)=7.63$; $p=0.01$). C-reactive protein was also higher in those with high depressive symptoms ($F(1, 5341)=16.33$; $p<0.001$). Lastly, greater evening cortisol was also observed in those with high depressive symptoms ($F(1, 5341)=14.18$; $p<0.001$). No other variables significantly differed between the groups. Chi-square tests revealed a significant difference in sex ($F(1)=9.26$; $p=0.002$), education ($F(3)=8.28$; $p<0.001$), physical activity ($F(4)=7.87$; $p<0.001$), history of stroke ($F(1)=14.43$; $p<0.001$), mild cognitive impairment ($F(1)=26.48$; $p<0.001$), and use of antidepressants ($F(1)=122.89$; $p<0.001$). More women, a lower proportion of those with high education, a lower proportion of those with high physical activity level, a higher number of individuals with prevalent stroke and mild cognitive impairment, and more individuals on antidepressant medication were in the group with high depressive symptomology. Marginal significance was found in smoking status ($F(2)=3.01$; $p=0.05$) and type 2 diabetes ($F(1)=3.75$; $p=0.05$), with more current smokers and individuals with diabetes in those with high depressive symptomology.

Allostatic load risk subgroups

A one-way ANOVA revealed significant differences between the allostatic load subgroups on age ($F(3, 5339)=61.16$; $p<0.001$), pulse pressure ($F(3, 5339)=5087.39$; $p<0.001$), high-density lipoprotein ($F(3, 5339)=59.70$; $p<0.001$), low-density lipoprotein ($F(3, 5339)=19.09$; $p<0.001$), abdominal circumference ($F(3, 5339)=41.90$; $p<0.001$), glucose ($F(3, 5339)=1893.11$; $p<0.001$), triglycerides ($F(3, 5339)=123.94$; $p<0.001$), c-reactive protein ($F(3, 5339)=6.74$; $p<0.001$), evening cortisol ($F(3, 5339)=6.35$; $p<0.001$), and depressive symptoms ($F(3, 5339)=4.23$; $p=0.004$).

Supplemental Table 2. Baseline characteristics of demographic information and covariates in the four profiles.

	Average n=2245 (42%)	High Cardiovascular Dysregulation n=607 (11%)	Low Cardiovascular n=2269 (43%)	Multisystem Dysregulation n=222 (4%)
Age, years	77 ± 6	79 ± 6	76 ± 6	76 ± 6
Women	59%	62%	57%	44%
Education, college + university	27%	26%	28%	30%
Current smoker	11%	8%	14%	13%
Alcohol use, gr/week	15 ± 30	16 ± 35	14 ± 31	15 ± 35
Physical activity, moderate/high	32%	28%	32%	31%
Stroke/blood clot in brain	7%	7%	6%	9%
MCI at baseline	10%	12%	10%	12%
Metabolic syndrome	29%	35%	28%	87%
Diabetes	8%	15%	8%	99%
Antihypertensive medication	48%	67%	41%	61%
Antidepressant medication	14%	11%	15%	16%
APOE e4 genotype	28%	27%	27%	25%
GDS-15 sum score	2 ± 2	3 ± 2	2 ± 2	3 ± 2

Supplemental Table 3. HRs and 95% CIs from the Cox regression on all-cause dementia, AD, and non-AD with depressive symptoms, correcting for allostatic load profiles.

	No. of cases	All-cause dementia (n=1099) HR (95% CI)	No. of cases	Alzheimer's disease (n=492) HR (95% CI)	No. of cases	Non-AD dementias (n=607) HR (95% CI)
Model 1						
GDS-sum score	1099	1.12 (1.09-1.15)	492	1.07 (1.03-1.12)	607	1.16 (1.12-1.20)
Average	459	1 (reference)	216	1 (reference)	242	1 (reference)
High Cardiovascular Dysregulation	153	1.07 (0.89-1.30)	50	0.82 (0.60-1.11)	102	1.32 (1.04-1.68)
Low Cardiovascular	438	1.02 (0.87-1.21)	203	1.00 (0.82-1.21)	236	1.04 (0.82-1.31)
Multisystem Dysregulation	49	1.58 (1.17-2.13)	23	1.49 (0.96-2.30)	27	1.77 (1.17-2.67)
Model 2						
GDS-sum score	1099	1.10 (1.07-1.13)	492	1.07 (1.03-1.12)	607	1.14 (1.09-1.18)
Average	459	1 (reference)	216	1 (reference)	242	1 (reference)
High Cardiovascular Dysregulation	153	1.13 (0.93-1.36)	50	0.85 (0.62-1.16)	102	1.34 (1.06-1.71)
Low Cardiovascular	438	1.01 (0.88-1.17)	203	0.99 (0.81-1.21)	236	1.02 (0.83-1.24)
Multisystem Dysregulation	49	1.68 (1.24-2.27)	23	1.67 (1.08-2.60)	27	1.80 (1.19-2.73)

Model 1: Adjusted for age, sex, and education.

Model 2: Adjusted for age, sex, education, smoking, alcohol, physical activity, stroke at baseline, antihypertensive medication, antidepressant medication, and APOE e4 genotype.

AD = Alzheimer's disease.

Supplemental Table 4. HRs and 95% CIs from the Cox regression on all-cause dementia, AD, and non-AD with depressive symptoms (dichotomized) as well as for current diagnosis for major depressive disorder.

	No. of cases	All-cause dementia (n=1099) HR (95% CI)	No. of cases	Alzheimer's disease (n=492) HR (95% CI)	No. of cases	Non-AD dementias (n=607) HR (95% CI)
Model 1						
GDS-score 6+	109	1.43 (1.14-1.79)	40	1.18 (0.83-1.67)	69	1.69 (1.26-2.26)
GDS-score 6+ (correcting for AL)	109	1.43 (1.14-1.78)	40	1.18 (0.84-1.68)	69	1.67 (1.25-2.25)
Model 2						
GDS-score 6+	109	1.32 (1.06-1.66)	40	1.16 (0.82-1.65)	69	1.52 (1.12-2.06)
GDS-score 6+ (correcting for AL)	109	1.32 (1.05-1.65)	40	1.17 (0.82-1.66)	69	1.50 (1.10-2.03)
Model 1						
Current MDD	25	2.13 (1.32-3.46)	11	1.95 (1.01-3.77)	14	2.87 (1.38-5.99)
Current MDD (correcting for AL)	25	2.12 (1.32-3.42)	11	1.95 (1.01-3.76)	14	2.80 (1.38-5.67)
Model 2						
Current MDD	25	1.63 (1.03-2.59)	11	1.64 (0.85-3.18)	14	1.93 (1.00-3.75)
Current MDD (correcting for AL)	25	1.62 (1.03-2.55)	11	1.65 (0.85-3.21)	14	1.83 (0.95-3.52)

Model 1: Adjusted for age, sex, and education.

Model 2: Adjusted for age, sex, education, smoking, alcohol, physical activity, stroke at baseline, antihypertensive medication, antidepressant medication, and APOE e4 genotype.

Note: n=410 for those with GDS-sum score of 6 or higher and n = 76 for those with a current MDD diagnosis. GDS = Geriatric Depression Scale 15; AD = Alzheimer's disease.

Supplemental Table 5. Additive & multiplicative interaction between standardized depressive symptoms & AL profiles on all-cause dementia, AD, and non-AD.

	No. of cases	All-cause dementia	No. of cases	Alzheimer's disease	No. of cases	Other dementias
		(n=1099)		(n=492)		(n=607)
Model 1		**HR (95% CI)**		**HR (95% CI)**		**HR (95% CI)**
Multiplicative interaction						
Depressive symptoms x High cardiovascular dysregulation	153	0.98 (0.82; 1.17)	50	0.94 (0.69; 1.29)	102	0.97 (0.78; 1.22)
Depressive symptoms x Low cardiovascular	438	1.03 (0.88; 1.20)	203	1.01 (0.83; 1.22)	236	1.03 (0.86; 1.23)
Depressive symptoms x Multisystem dysregulation	49	1.21 (0.94; 1.57)	23	1.20 (0.76; 1.88)	27	1.14 (0.84; 1.54)
Additive interaction		**RERI (95% CI)**		**RERI (95% CI)**		**RERI (95% CI)**
Depressive symptoms x High cardiovascular	153	0.00 (-0.23; 0.22)	50	-0.08 (-0.38; 0.21)	102	0.07 (-0.29; 0.43)
Depressive symptoms x Low cardiovascular	438	0.04 (-0.15; 0.23)	203	0.01 (-0.21; 0.23)	236	0.05 (-0.20; 0.29)
Depressive symptoms x Multisystem dysregulation	49	**0.52 (0.01; 1.03)**	23	0.41 (-0.48; 1.30)	27	0.56 (-0.17; 1.29)
Model 2		**HR (95% CI)**		**HR (95% CI)**		**HR (95% CI)**
Multiplicative interaction						
Depressive symptoms x High cardiovascular dysregulation	153	0.93 (0.78; 1.11)	50	0.90 (0.66; 1.22)	102	0.89 (0.71; 1.11)
Depressive symptoms x Low cardiovascular	438	1.01 (0.89; 1.15)	203	0.98 (0.81; 1.18)	236	1.01 (0.86; 1.19)
Depressive symptoms x Multisystem dysregulation	49	1.17 (0.91; 1.49)	23	1.21 (0.78; 1.88)	27	1.09 (0.80; 1.47)
Additive interaction		**RERI (95% CI)**		**RERI (95% CI)**		**RERI (95% CI)**
Depressive symptoms x High cardiovascular	153	-0.06 (-0.28; 0.16)	50	-0.13 (-0.42; 0.17)	102	-0.08 (-0.41; 0.26)
Depressive symptoms x Low cardiovascular	438	0.01 (-0.14; 0.17)	203	-0.03 (-0.24; 0.19)	236	0.01 (-0.20; 0.23)
Depressive symptoms x Multisystem dysregulation	49	0.46 (-0.07; 1.00)	23	0.52 (-0.48; 1.52)	27	0.44 (-0.26; 1.14)

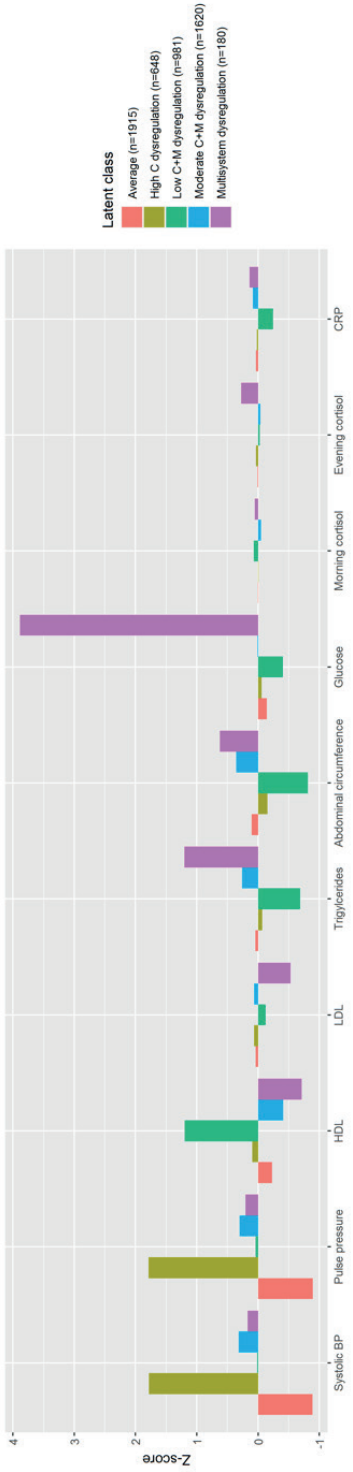
Model 1 is adjusted for age, sex, and education. Model 2 is adjusted for age, sex, education, smoking, alcohol, physical activity, stroke at baseline, antihypertensive medication, antidepressant medication, and APOE e4 genotype. For information on calculation of the additive RERI by using a product term in a regression model, please see (53). AL = allostatic load; AD = Alzheimer's disease.

Supplemental Table 6. Hazard ratios and 95% CIs from Cox regression models of subgroups on all-cause dementia, all-cause mortality, dementia-free mortality, and dementia and/or mortality.

	No. of cases	All-cause dementia (n=1099) HR (95% CI)	No. of cases	All-Cause Mortality (n=2525) HR (95% CI)
GDS-sum score	1099	1.12 (1.09-1.15)	2525	1.11 (1.09-1.13)
Average	459	1 (reference)	1035	1 (reference)
High C Dysregulation	153	1.07 (0.89-1.30)	354	1.10 (0.97-1.24)
Low C	438	1.02 (0.87-1.21)	1000	1.02 (0.91-1.13)
Multisystem Dysregulation	49	1.58 (1.17-2.13)	136	1.77 (1.47-2.12)
GDS-sum score	1099	1.10 (1.07-1.13)	2525	1.09 (1.07-1.11)
Average	459	1 (reference)	1035	1 (reference)
High C Dysregulation	153	1.13 (0.93-1.36)	354	1.14 (1.01-1.29)
Low C	438	1.01 (0.88-1.17)	1000	1.00 (0.92-1.10)
Multisystem Dysregulation	49	1.68 (1.24-2.27)	136	1.77 (1.48-2.13)

Models are adjusted for age, sex, and level of education in the first panel and for age, sex, level of education, smoking, alcohol, physical activity, stroke at baseline, anti-hypertension medication, anti-depressant medication, and APOE e4 genotype in the second panel.

	No. of cases	Dementia-Free Mortality (n=1746)	No. of cases	Dementia and/or Mortality (n=2845)
	1746	1.11 (1.09-1.14)	2845	1.11 (1.09-1.13)
	705	1 (reference)	1164	1 (reference)
	237	1.11 (0.95-1.29)	389	1.09 (0.96-1.24)
	702	1.03 (0.90-1.18)	1141	1.03 (0.91-1.16)
	102	1.87 (1.51-2.32)	151	1.76 (1.48-2.10)
	1746	1.09 (1.06-1.11)	2845	1.09 (1.07-1.11)
	705	1 (reference)	1164	1 (reference)
	237	1.14 (0.98-1.34)	389	1.14 (1.01-1.28)
	702	1.02 (0.90-1.15)	1141	1.01 (0.92-1.12)
	102	1.85 (1.49-2.29)	151	1.79 (1.50-2.12)



Supplemental Figure 1. The five class model with z-scores on the allostatic load criteria.

Chapter 9

Dementia prediction in the general population using clinically accessible variables: a proof-of-concept study using machine learning. The AGES-Reykjavik Study

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Abstract

Background: Early identification of dementia is crucial for prompt intervention for high-risk individuals in the general population. External validation studies on prognostic models for dementia have highlighted the need for updated models. The use of machine learning in dementia prediction is in its infancy and may improve predictive performance. The current study aimed to explore the difference in performance of machine learning algorithms compared to traditional statistical techniques, such as logistic and Cox regression, for prediction of all-cause dementia. Our secondary aim was to assess the feasibility of only using clinically accessible predictors rather than MRI predictors.

Methods: Data are from 4,793 participants in the population-based AGES-Reykjavik Study without dementia or mild cognitive impairment at baseline (mean age: 76 years, % female: 59%). Cognitive, biometric, and MRI assessments (total: 59 variables) were collected at baseline, with follow-up of incident dementia diagnoses for a maximum of 12 years. Machine learning algorithms included elastic net regression, random forest, support vector machine, and elastic net Cox regression. Traditional statistical methods for comparison were logistic and Cox regression. Model 1 was fit using all variables and model 2 was after feature selection using the Boruta package. A third model explored performance when leaving out neuroimaging markers (clinically accessible model). Ten-fold cross-validation, repeated ten times, was implemented during training. Upsampling was used to account for imbalanced data. Tuning parameters were optimized for recalibration automatically using the caret package in R.

Results: Nineteen percent of participants developed all-cause dementia. Machine learning algorithms were comparable in performance to logistic regression in all three models. However, a slight added performance was observed in the elastic net Cox regression in the third model (c = 0.78, 95% CI: 0.78-0.78) compared to the traditional Cox regression (c = 0.75, 95% CI: 0.74-0.77).

Conclusions: Supervised machine learning only showed added benefit when using survival techniques. Removing MRI markers did not significantly worsen our model's performance. Further, we presented the use of a nomogram using machine learning methods, showing transportability for the use of machine learning models in clinical practice. External validation is needed to assess the use of this model in other populations. Identifying high-risk individuals will amplify prevention efforts and selection for clinical trials.

Introduction

Dementia is characterized by debilitating cognitive impairment that increases the risk of mortality, while quality of life decreases for both the patient and his or her caregivers. Currently, 50 million people in the world have dementia, which is expected to triple by 2050 (1). While much research has been done on the risk factors for dementia, no effective treatment is available (2). Further, by the time of diagnosis, the brain has already substantially declined in function. Thus, early classification is crucial for prompt intervention and better outcomes for high-risk individuals. Many prognostic models for incident dementia have been developed using 'traditional' statistical techniques, such as logistic or Cox regression (3-6). However, external validation of these models showed poor calibration and performance (7, 8), highlighting the need for updated models for prognostication of dementia. The recent increased application of machine learning for disease prediction offers the possibility to improve dementia prognostic models. Machine learning can aid in unraveling complex relationships between predictors, taking into account nonlinear relationships and interactions, while additionally using that information to increase a model's predictive performance (9).

Research thus far using machine learning for dementia prediction is in its infancy and current models primarily focus on magnetic resonance imaging (MRI) for prediction (10, 11). Some studies have explored demographic factors (12, 13) and plasma proteomic data (14-16), but no studies have yet also explored some commonly assessed biomarkers (e.g., glucose, cholesterol, blood pressure) along with demographic and lifestyle information in dementia prediction using machine learning classifiers (10). A recent review also highlighted the need for the development of new prognostic models for dementia that focus on clinical variables over imaging variables (10). An emphasis on predictors that are more clinically accessible than MRI is crucial for the potential future use of prognostic models for dementia in clinical practice. Focusing on accessible predictors will allow for wider generalizability of the assessment of high-risk individuals for dementia into the general population.

Previous studies using machine learning methods have mostly used the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort for algorithm testing (10), with relatively limited sample sizes (i.e., less than 1,000 participants). Discrimination has focused on differentiating mild cognitive impairment (12) from Alzheimer's disease (10), the leading cause of dementia. Further, most studies that implemented machine learning methods did not take class imbalance into account (10), which focuses on

negative predictive value over positive predictive value and introduces possible bias. As previous studies have also focused on cohorts that have more cases than controls, the possible generalizability of the prognostic model decreases (17). Therefore, there is a current gap in developing a dementia risk model using machine learning for the general population, using a large sample size.

We aimed to assesses if machine learning algorithms (e.g., elastic net regression, support vector machine) aid in the performance of dementia prognosis compared to traditional statistical techniques (e.g., logistic and Cox regression) in a large, population-based cohort from Reykjavik, Iceland of almost 5,000 individuals without dementia or mild cognitive impairment. Further, we wanted to assess if performance remained high when focusing only on clinically accessible predictors. Lastly, we wanted to assess if performance differed when stratifying by sex.

Methods

This study was reported following the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) Statement (18).

Study sample

Data originated from the Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study, a community-based cohort study of individuals 65 years or older living in the Reykjavik area. More details are provided elsewhere (19). In brief, participants from the AGES-Reykjavik Study stem from the Reykjavik study, initiated in 1967 by the Icelandic Heart Association. Between 2002 and 2006, 5,764 individuals randomly selected from survivors of the Reykjavik Study were included. Baseline cognitive, biometric, and MRI assessments were done at the Reykjavik research center. Individuals with dementia or mild cognitive impairment at baseline were excluded from the current analysis, leaving 4,793 individuals in the analytical sample. Cognitive, biometric, and MRI assessments were done at baseline between 2002 and 2006, with follow-up of incident dementia diagnoses for a maximum of 12 years. Written informed consent was obtained from all participants. The Icelandic National Bioethics Committee (VSN: 00-0063), the Icelandic Data Protection Authority, and the Institutional Review Board for the National Institute on Aging, NIH approved this study.

Dementia assessment

Details regarding the procedure for dementia ascertainment can be found elsewhere (20-22). In brief, a three-step procedure based on international guidelines (19) was used. First, all participants underwent neuropsychological testing of cognition using the Mini-Mental State Examination (MMSE) and the Digit Symbol Substitution Test (21), with the next step in those who screened positive undergoing further neuropsychological examination. In the third step, in those who screened positive on the neuropsychological examinations, further proxy and diagnostic assessments were performed regarding the Activities of Daily Living (ADL), as well as social and cognitive functioning. Then, a multidisciplinary panel including a neurologist, geriatrician, neuroradiologist, and neuropsychologist performed a consensus diagnosis that included exam measures and brain MRI (22). Additional dementia cases were also obtained through medical and nursing home records as well as in death certificates. Dementia cases obtained through nursing homes were collected following a standardized protocol in Icelandic nursing homes (23). The current study focused on all-cause dementia only.

Demographics

Age (continuous), sex (dichotomous), education (categorical; categorized as primary school, secondary school, college, or university), and current marital status (married/living together, widowed, divorced, single) were collected by questionnaire at baseline.

Clinical variables

A wide range of clinical variables were used, including metabolic, lipid, and inflammatory levels, as well as medical diagnoses (more information in Supplementary Info 1).

Medication use

Medication use was treated as dichotomous (yes/no) for benzodiazepines, beta-adrenergic blockers, glucocorticoids, psycholeptics, or anti-depressants.

Lifestyle variables

We included the following continuous variables: alcohol consumption, mental leisure activity (days per month), social leisure activity (days per month), number of close friends, and number of living close relatives. The categorical variables we included are as follows: smoking status (current, former, never), physical activity within the last 12 months (never, rarely, occasionally, moderate, high), difficulty in walking 2 kilometers (very easy, somewhat easy, not that easy), difficulty in walking 500 meters (very easy, somewhat easy, not that easy), and how often fish is consumed as the

main meal (never, less than once a week, 1-2 times a week, 3-4 times a week, 5-6 times a week, daily, more than once a day).

Cognitive assessment

The raw total score of the test of global cognitive function, the MMSE, was the only variable used to assess cognition.

Neuroimaging variables

MR images were collected using 1.5T brain MRI (Signa TwinSpeed; General Electric Medical Systems). For more information on the MRI protocol, refer to (24-26). Log-transformed white matter lesion volume and hippocampal volume, as well as the ratio of gray matter/intracranial volume (to account for correlation), and the number of cerebral microbleeds were entered as continuous predictors. The presence of infarcts (yes/no) was entered as a dichotomous variable.

Statistical analyses

All analyses were performed in R (v 4.0.3). Before beginning the analyses, data were split into a two-thirds (proportion: 0.66) training set and a one-third test set, ensuring for balanced incident dementia cases in the train/test sets by using the split_df() function in R.

Sample size calculations

We performed a post-hoc sample size calculation using *pmsampsize* package in R to calculate the number of events/cases required using logistic regression as best-case-scenario (27). If all predictors are included, the required sample size is at least 1,691, which is less than the current sample of 4,793.

Missing data

Half of the individuals (55%) had at least one missing value on predictors (max: 27% missing on ability to walk 2km or 500m). There were no missing values on the outcome (i.e., dementia). Missing data were handled with multiple imputation using the *mice* package in R separately in the training and test sets using ten imputed datasets. The predictor matrix for the training set was used for imputation in the test set. All predictors as well as the outcome were used in the imputation process. A random imputed dataset from a total of ten was selected for further analyses for both the training and test sets as pooling methods for machine learning prognostic models have yet to be validated. See Supplementary Table 1 for an overview of predictors and outcome in both training and test sets.

Model building

The *caret* package in R (28) was used for all prediction models, i.e. elastic net regression, random forest, support vector machine, and logistic regression. To take time-to-event and censoring into account, we also performed a regular Cox regression using the *glmnet* package (29) and elastic net Cox regression using the *hdnom* package (30) in R. For the support vector machine classifier, a radial kernel was used to allow for nonlinear separations of the data. Hyperparameter tuning was performed automatically by caret. Pseudocode can be found in Supplementary Code 1. The models were first fitted with all features (model 1). Then, models were fit after feature selection using the *Boruta* package in R (31) for more parsimonious models (model 2). In short, Boruta uses a random forest classifier and applies mean decrease accuracy to evaluate each feature's importance based on 99 iterations. Tentative features were not included. Lastly, to evaluate a clinically accessible model (i.e., one that does not include MRI features), models were fit only with features selected from Boruta that were not MRI (model 3). Tuning parameters were optimized for recalibration and varied across all three models (Supplementary Table 2).

Internal validation

Using cross-validation, more variability is introduced into the training of each classifier. Ten-fold cross-validation, repeated ten times, for a total of 100 times, was used in training each machine learning algorithm. The training data are divided into ten folds, with the given classifier trained on nine folds, using the tenth for testing. This is repeated until each of the ten folds is held back for testing. The performance metrics are then averaged across all repetitions. Further, upsampling was performed to handle imbalanced data and was implemented during cross-validation. This is done by resampling with replacement our class with incident dementia (i.e., the minority class) to be the same size as those who do not develop dementia (i.e., the majority class). If models failed to converge with upsampling, downsampling was used, which deletes samples from the majority class (i.e., those who do not develop dementia). Additionally, we tested different thresholds for classification other than 0.5, ranging from 0.10 to 0.90 by steps of 0.02.

Performance metrics

The following performance measures were used to assess the models: area under the receiver operating characteristic (ROC) curve (AUC), sensitivity, specificity, positive predictive value, and negative predictive value. The model with the highest AUC was then used for the test set. For the survival models, the *c*-statistic was used. *C*-statistics and AUC values are comparable to assess performance. The *Mleval* package in R was used to calculate 95% confidence intervals. Bootstrapping using the *hdnom* package

was done to calculate 95% confidence intervals in the elastic net Cox regression models. The *hdnom* package was used to create calibration plots for the elastic net Cox regression as well as to create a clinically relevant nomogram.

Sensitivity analysis

To assess if the prognostic model has similar performance in men and women, the trained model in both sexes was tested on men and women separately.

Results

During an average of 9 ± 3 years of follow-up, 892 (n = 750 from nursing homes) individuals developed dementia. Mean (SD) age at baseline for all participants was 76 (6) years and 59% were female. Demographic and clinical information for the full study sample on all predictor variables and the outcome are shown in Table 1.

Model performance

Logistic regression (AUC = 0.73, 95% CI: 0.71-0.75) had a similar AUC to the elastic net regression (AUC = 0.74, 95% CI: 0.72-0.76) and random forest classifiers (AUC = 0.74, 95% CI: 0.72-0.76) in model 1 (i.e., the full model), as well as in the model after feature selection and after removal of neuroimaging variables (Table 2). Support vector machine showed lower performance compared to all other machine learning classifiers and the logistic regression. Both logistic regression and the elastic net regression had the same performance in model 3 without neuroimaging variables (AUC = 0.71, 95% CI: 0.68-0.74) (Table 2).

When taking time-to-event into account with the elastic net Cox model, the *c*-statistic was high (c = 0.80, 95% CI: 0.79-0.80) in model 1 and higher than the traditional Cox model (c = 0.78, 95% CI: 0.77-0.79). The same *c*-statistics and confidence intervals were seen in model 2. Performance slightly lowered in model 3, but the elastic net Cox regression still showed higher *c*-statistics (c = 0.78, 95% CI: 0.78-0.78, model 3) compared to the traditional Cox model (c = 0.75, 95% CI: 0.74-0.77). The results of the elastic net Cox regression for model 3 are presented as a nomogram in Figure 1 for 12-year overall risk. To predict the patient's risk for dementia, one can draw a vertical line to the top given each variable to get the number of points per that variable. The points from each variable are then summed and the total number of points is used to give a patient's overall 12-year risk.

Table 1. Characteristics of the predictors in the study sample (n = 4793).

	Mean (SD) or n (%)	% missing per variable
Demographics		
Age (years)*+	76 (6)	0%
Sex (female)*+	2822 (59%)	0%
Education (college + university)	1392 (29%)	6%
Neuroimaging variables		
Log-transformed white matter lesion volume (ml)*	13.5 (2.5)	18%
Hippocampal volume (ml)*	5.6 (0.7)	17%
Number of microbleeds*	0.3 (1.6)	17%
Presence of infarcts	1491 (31%)	16%
Gray matter volume (ml)*	676 (63)	18%
Intracranial volume (ml)*	1501 (148)	18%
Clinical variables		
Abdominal circumference (cm)	101 (12)	1%
Carotid intima-media thickness test (CIMT)	1 (0.1)	10%
High-density lipoprotein (mmol/L)	1.6 (0.5)	<1%
Low-density lipoprotein (mmol/L)	3.5 (1.0)	<1%
Triglycerides (mmol/L)	1.2 (0.7)	<1%
Fasting glucose (mmol/L)	5.8 (1.2)	<1%
B-hemoglobin A1c (g/dl)	0.5 (0.1)	8%
High-sensitive c-reactive protein (mg/L)	3.8 (6.7)	<1%
Systolic blood pressure (mmHg)	142 (21)	1%
Diastolic blood pressure (mmHg)	74 (10)	1%
Hypertension	3855 (80%)	1%
Coronary artery disease	842 (18%)	0%
Diabetes mellitus	591 (12%)	0%
Metabolic syndrome	1499 (31%)	1%
Stroke/blood clot in the brain	297 (6%)	2%
History of cancer	753 (16%)	1%
Experienced a head trauma or lost consciousness	416 (9%)	5%
Subjective memory decline*+	1431 (30%)	3%
Often forget the names of a friend	1522 (32%)	5%
Often forget where items are*+	2083 (44%)	5%
Difficulty finding the right words	1517 (32%)	5%
Difficulty finding the way to familiar places*+	385 (8%)	5%
Inability in managing money*+	132 (3%)	4%
Inability in dressing oneself*+	29 (1%)	6%
Intermit claudication in legs	227 (5%)	5%

Table 1. Continued

	Mean (SD) or n (%)	% missing per variable
Insomnia	1438 (30%)	3%
Poor health status	276 (6%)	1%
ADL score, full dependence on all items*+	52 (1%)	6%
Morning salivary cortisol (nmol/L)	19.8 (13.3)	9%
Evening salivary cortisol (nmol/L)	3.8 (6.6)	9%
GDS-15 sum score*+	2.3 (2.1)	6%
All anxiety questions 'yes'	40 (1%)	1%
Diagnosis of current GAD, social phobia, panic disorder, or agoraphobia	98 (2%)	5%
Current/past diagnosis of major depressive disorder	248 (5%)	5%
Medication use		
Benzodiazepines	396 (8%)	0%
Beta-adrenergic blockers	1660 (35%)	0%
Glucocorticoids	171 (4%)	0%
Psycholeptics	818 (17%)	0%
Anti-depressants	662 (14%)	0%
Lifestyle variables		
Current smoker, %	582 (12%)	4%
Alcohol consumption (g/week)	16 (33)	4%
Moderate/high physical activity	1509 (31%)	7%
Mental leisure activity (days per month)	7 (6)	6%
Social leisure activity (days per month)	4 (4)	6%
Single marital status, %	288 (6%)	6%
Number of close friends	4 (4)	6%
Not that easy to walk 2 km*+	960 (20%)	27%
Not that easy to walk 500 m*+	233 (5%)	27%
Number of living close relatives	7 (4)	6%
Never fish consumption, %	26 (1%)	6%
Cognitive assessment		
MMSE total score*+	27 (3)	1%
Outcome		
Incident dementia	892 (19%)	0%
Follow-up time (years)	9 (3)	0%

Note: * marks variables entered in model 2. + marks variables entered in model 3. GAD = generalized anxiety disorder. GDS-15 = Geriatric Depression Scale-15. CVLT = California Verbal Learning Test.

When testing different thresholds, all classifiers demonstrated optimal sensitivity and specificity at 0.50.

Regarding resampling, up-sampling was used for all models except for all support vector machine models. Down-sampling was used instead for model convergence.

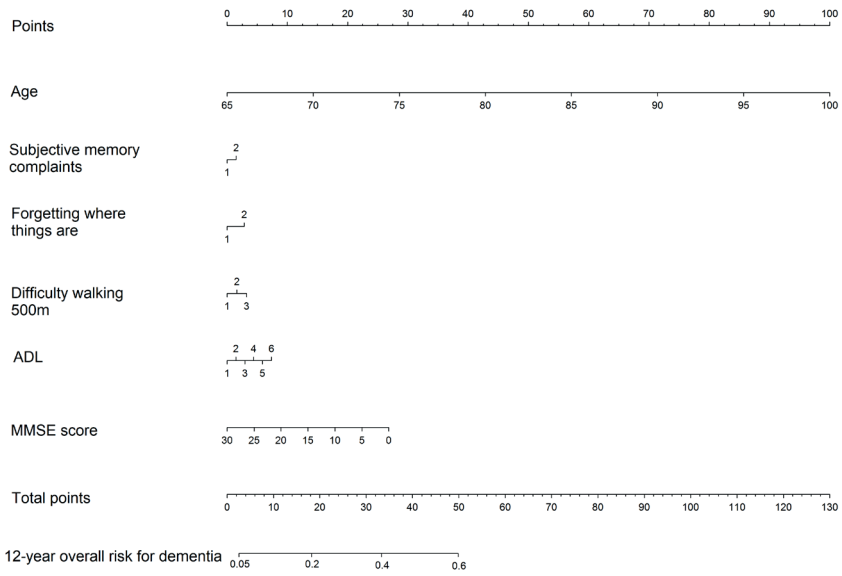

Figure 1. Predictive nomogram for 12-year overall risk for incident dementia in the elastic net Cox regression for model 3. To predict the patient's risk for dementia, one can draw a vertical line to the top given each variable to get the number of points per that variable. The points from each variable are then summed and the total number of points represents a patient's overall 12-year risk.

Feature selection

For feature selection, Boruta ranked the following variables as most important: age, hippocampal volume, log-transformed white matter lesion volume, gray matter/intracranial volume ratio, MMSE score, difficulty finding the way to familiar places, difficulty in dressing oneself, subjective memory decline, the ADL score, forgetting where items are, number of microbleeds, the sum score of the Geriatric Depression Scale-15, how difficult it is to walk 500m, sex, inability to manage money, and how difficult it is to walk 2km (Supplementary Figure 1). These variables were then used as the predictors in the parsimonious model (model 2), and then the MRI variables were removed for the clinically accessible model (model 3).

Variable importance slightly differed per algorithm in model 3. The least amount of variables used were in the elastic net regression (Supplementary Figure 2). As there

is no built-in variable importance for support vector machine, the AUC is shown instead on the x-axis.

Internal validation

As the elastic net model performed the best regarding AUC, sensitivity, and specificity, it was chosen as the classifier to be used on the test data. The AUC was the same for both models 1 and 2 (AUC = 0.73; 95% CI: 0.70-0.76) and slightly decreased in model 3 when MRI variables were removed (AUC = 0.72; 95% CI: 0.69-0.75) (Table 3). Sensitivity was the same in all models (Sensitivity = 61%; 95% CI: 56-66%), and specificity was highest in model 2 (Specificity = 71%; 95% CI: 69-74%) (Table 3). For the elastic net Cox model, *c*-statistics were comparable for all three models (model 3: *c* = 0.77; 95% CI: 0.77-0.78).

Table 2. Summary of cross-validated prediction models on trained data (n = 3473).

Model	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Model 1					
Logistic regression	0.73 [0.71-0.75]	64 [60-68]	70 [68-71]	32 [30-35]	89 [88-91]
Elastic net	0.74 [0.72-0.76]	68 [64-71]	69 [67-71]	33 [31-36]	90 [89-92]
Random forest	0.74 [0.72-0.76]	6 [4-8]	99 [99-99]	60 [47-71]	82 [81-83]
SVM	0.65 [0.62-0.68]	49 [45-53]	73 [71-74]	29 [27-32]	86 [85-88]
Model 2					
Logistic regression	0.74 [0.72-0.76]	67 [63-70]	70 [68-72]	34 [31-36]	90 [89-91]
Elastic net	0.74 [0.72-0.76]	67 [63-70]	69 [67-71]	33 [30-36]	90 [89-91]
Random forest	0.74 [0.72-0.76]	47 [43-51]	84 [82-85]	40 [36-44]	88 [86-89]
SVM	0.73 [0.71-0.75]	72 [69-76]	63 [61-65]	31 [28-33]	91 [89-92]
Model 3					
Logistic regression	0.71 [0.68-0.74]	64 [60-68]	68 [66-70]	31 [29-34]	89 [88-91]
Elastic net	0.71 [0.68-0.74]	64 [60-67]	67 [65-69]	31 [28-33]	89 [88-90]
Random forest	0.71 [0.68-0.74]	55 [51-59]	75 [73-77]	34 [31-37]	88 [87-89]
SVM	0.70 [0.67-0.73]	69 [65-73]	61 [59-63]	29 [27-31]	90 [88-91]

Note: AUC = area under the ROC curve. SVM = support vector machine.

Table 3. Summary of the elastic net models on test data (n = 1870), as well as stratified by sex.

	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Model 1	0.73 [0.70-0.76]	61 [55-66]	71 [69-73]	33 [29-37]	89 [87-91]
Women	0.74 [0.70-0.78]	60 [53-67]	70 [67-73]	35 [30-40]	87 [84-89]
Men	0.73 [0.67-0.79]	64 [55-73]	71 [67-75]	29 [23-35]	92 [89-94]
Model 2	0.73 [0.70-0.76]	61 [56-66]	71 [69-74]	33 [29-37]	89 [87-91]
Women	0.73 [0.69-0.77]	59 [52-66]	71 [67-74]	35 [30-40]	87 [84-89]
Men	0.73 [0.67-0.79]	63 [54-72]	72 [68-76]	29 [24-36]	92 [89-94]
Model 3	0.72 [0.69-0.75]	61 [56-66]	69 [66-71]	31 [28-35]	89 [86-90]
Women	0.71 [0.67-0.75]	59 [52-65]	69 [66-72]	33 [29-38]	86 [84-89]
Men	0.72 [0.66-0.78]	66 [57-75]	67 [63-71]	27 [22-33]	92 [89-94]

Note: AUC = area under the ROC curve; PPV = positive predictive value; NPV = negative predictive value.

Calibration

Calibration was assessed for all models. All models showed overfitting, which was resolved after re-calibration (Figure 2). Re-calibration was performed by training a logistic regression using the uncalibrated probabilities as a predictor. In the elastic net Cox regression, calibration was optimal in both our training (internal calibration) and testing sets (external calibration) (Figure 3).

Sex stratification

Models were also tested on women only and men only to assess possible differences in predictive accuracy when stratified by sex. Across all models using elastic net regression, men and women had similar AUCs. Sensitivity was slightly higher in men, whereas specificity was slightly higher in women (Table 3). However, confidence intervals overlapped. In the elastic net Cox regression model, men (c = 0.86, 95% CI: 0.85-0.87, model 3) had higher c-statistics than women (c = 0.73, 95% CI: 0.72-0.74, model 3) in all three models.

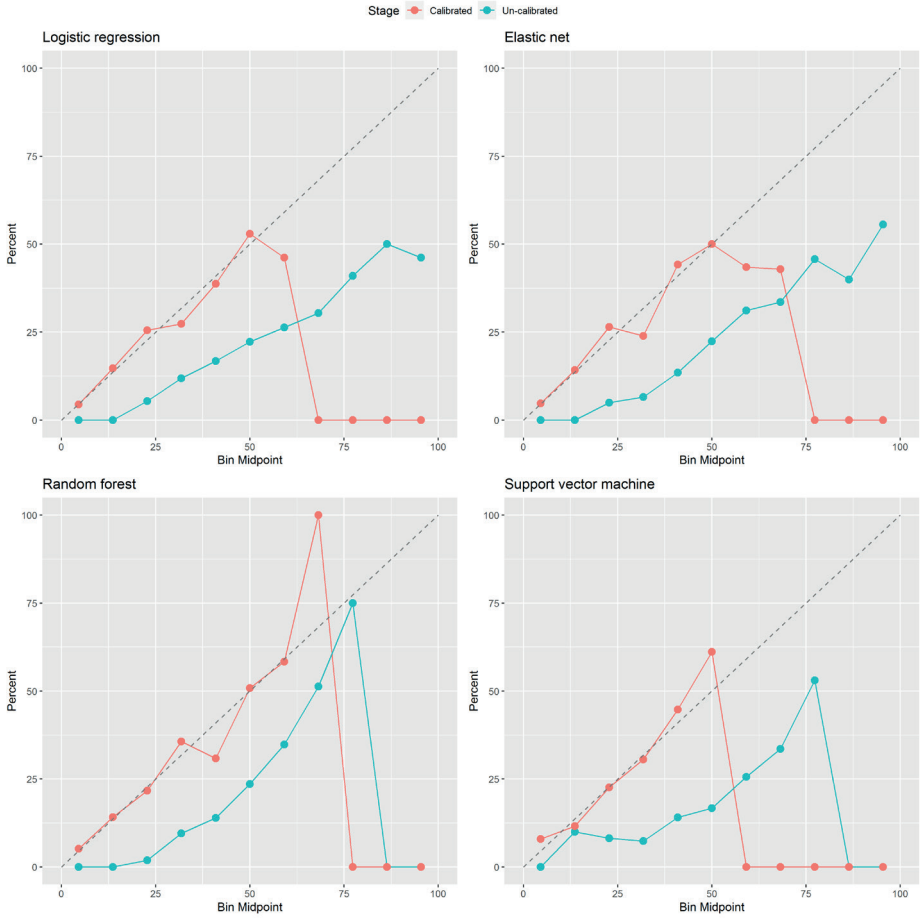

Figure 2. Calibration plots for logistic regression, elastic net regression, random forest, and support vector machine in model 3 (clinically accessible model) both before and after recalibration. Performance above the diagonal represents under-forecasting and performance below the diagonal represent over-forecasting. There were no individuals in the bins after 77.

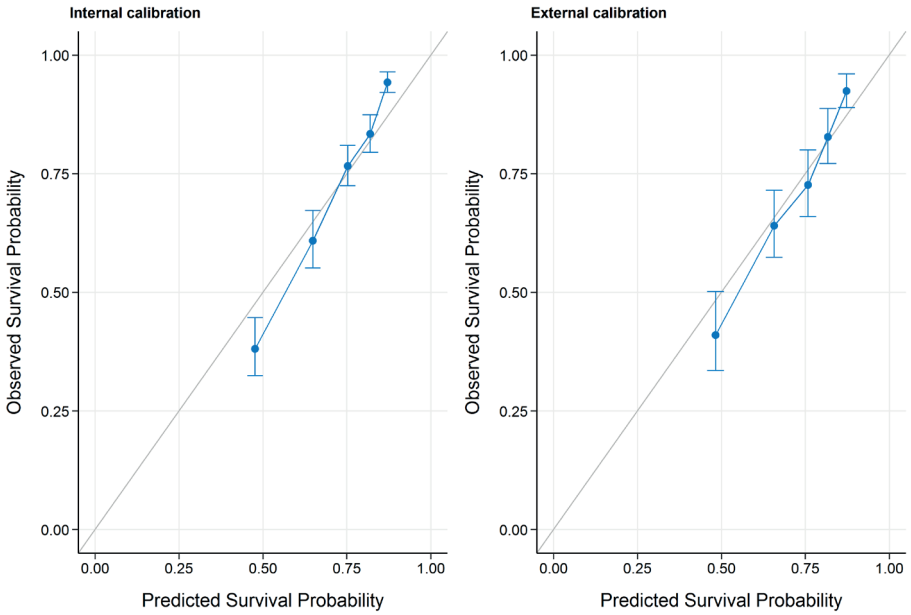

Figure 3. Calibration plots for the elastic net Cox regression in both the training set (internal calibration) and in the test set ('external' calibration). Performance above the diagonal represents under-forecasting and performance below the diagonal represents over-forecasting.

Discussion

The current study aimed to explore the difference in performance between machine learning algorithms and traditional statistical methods for a prognostic model for dementia. We further aimed to assess the feasibility of only using clinically accessible predictors compared to including structural brain MRI, as well as exploring model performance when stratifying by sex. Machine learning only showed benefit over traditional statistical methods when using survival methods. When removing imaging variables from the prediction model, AUC and *c*-statistic values slightly lowered but remained high. Models performed similarly in men and women in the elastic net regression; however, in the elastic net Cox regression, men had higher *c*-statistics compared to women.

The current study explored the difference in performance when using machine learning methods compared to traditional statistical techniques. Previous prediction models using machine learning yielded high performance accuracy when using only MRI variables (32), yet systematic reviews have highlighted the lack of exploration on other, more clinically accessible variables for dementia prediction (10, 33). Machine learning showed added benefit only when using survival techniques, as our elastic

net Cox regression outperformed the regular Cox regression. A recent comparative study on various machine learning survival models and Cox regression for dementia prediction also found similar accuracy across techniques (34), which is also in line with previous studies assessing possible performance differences between conventional regression techniques and machine learning (35, 36). Further, a study predicting two-year incident dementia also found similar performance across traditional techniques (i.e., logistic regression) and machine learning algorithms, with a slight added benefit of machine learning models regarding positive predictive value (37). The current study found a slight advantage over elastic net regression, which was also found in a simulation study (36). To note, elastic net reduces the risk of overfitting by penalizing the estimates. This also increases comprehensibility of the prognostic model by decreasing the number of required variables. We were also able to build a nomogram from our elastic net Cox regression, highlighting the feasibility and explainability of using machine learning in clinical settings (38). This study highlights the importance of censoring in risk prediction as well as the use of algorithms that can capture interactions and high-dimensional relationships within predictors, such as with machine learning (39). Further, when removing neuroimaging markers, the performance of all models, including those using traditional statistical techniques, lowered, but remained high overall.

The most Important variables for prediction in our final elastic net Cox regression included age, subjective memory complaints, and MMSE score. Subjective memory decline has been shown to be present years before mild cognitive impairment and later dementia (40), highlighting its possible use in early prediction. Further, variables such as 'forgetting where things are' or 'difficulty dressing oneself' were also present in our final model, which are items similar to those being used to create a telephonic interview for dementia prediction (41). Functional limitations were also found in previous studies to be highly predictive of later developing dementia (42, 43). Previous studies have explored the use of neuropsychological assessments for prognostic models of dementia (7, 44), however the current study only used the MMSE and still showed high performance. To note, the variables with most predictive power in our model were used in the three-step procedure to diagnose dementia during follow-up at the clinic, i.e., the MMSE and the ADL score, which may have induced overfitting into our model. However, our study focused on the feasibility of using machine learning methods for dementia prediction.

One recent study using population-based data from the UK Biobank also explored the use of machine learning for dementia prediction, with five and ten-year predictions (45). However, one of the top predictors was APOE e4 genotype, making this model

less clinically accessible due to the need for genotyping. APOE e4 genotype was also used in some previous prediction models, focusing on individuals already at risk (i.e., those with amnestic mild cognitive impairment) (46), and it is also included in the well-known Disease State Index (DSI) model (47). The current study focused on the feasibility of using clinically accessible variables; therefore, we aimed to assess if performance can remain high for prediction even without genotyping.

While performing sex-stratified validation of prediction models is still quite novel and explorative, our study found differences in the elastic net Cox regression when testing our prediction model in women and men separately. As sex differences in dementia have been highlighted previously with the push for sex-based prognostic models (48, 49), future studies should further explore the possible benefit of creating sex-stratified prognostic models.

Strengths of the current study include using multiple imputation to address missing data and cross-validation to increase variability in training of the prediction models. We additionally address differences between novel machine learning classifiers, classical logistic and Cox regression, and using a survival-based machine learning method (i.e., the elastic net Cox regression). The current study also had a large sample size from a well-phenotyped, community-based population. We also report calibration, which has been highlighted as lacking in previous prognostic studies (35, 50). Further, tuning of the machine learning classifiers was done for recalibration. We also were able to extract a clinically relevant nomogram from our elastic net Cox regression that makes our machine learning methods translatable to clinical practice. Lastly, we performed resampling and threshold adjustment which further helps address imbalanced classification.

The current study also had limitations. The models presented first need to be externally validated to assess its transportability to other populations. Further, the ascertainment of dementia was done with a three-step procedure that consisted of the ADL and MMSE, which were also used as predictors. Further, the AGES-Reykjavik cohort is predominantly White; therefore, it is crucial for the validation of this model in marginally underrepresented populations. Further, development of prognostic models in systemically minoritized groups should also be prioritized for future research. Lastly, we did not assess different time-windows for our survival models as we solely aimed to assess the comparability of techniques. Future studies should assess which models suit best for shorter- or longer-term prediction of dementia.

Our results showed that prediction models developed using supervised machine learning classifiers are feasible and add to the model's performance, only when

using survival methods. We also exemplify ways to implement machine learning in a classical point-based method using a nomogram. Additionally, model performance remained high after the removal of MRI variables. As dementia becomes a leading problem in developing countries, focusing on clinically accessible variables for the prognostication of dementia is crucial.

Acknowledgements

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Supplementary Info 1. Details on clinical variables used.

The following variables were continuous: abdominal circumference, high-density lipoprotein, low-density lipoprotein, triglycerides, fasting glucose, b-hemoglobin A1c, high-sensitive c-reactive protein, systolic and diastolic blood pressure, log-transformed morning salivary cortisol, log-transformed evening salivary cortisol, total score on the Geriatric Depression Scale-15 (GDS-15), and the carotid intima-media thickness test (CIMT).

The following variables were dichotomous: hypertension, coronary artery disease, diabetes mellitus (type 2), metabolic syndrome, stroke/blood clot in the brain, history of cancer, ever experienced a head trauma or lost consciousness, subjective cognitive decline, often forget the names of a friend, often forget where left things, difficulty finding the right words, difficulty finding the way to familiar places, ever felt intermittent claudication in legs, insomnia, Mini-International Neuropsychiatric Interview (MINI) diagnosis of current generalized anxiety disorder, social phobia, panic disorder, or agoraphobia, and the MINI diagnosis of history/current of major depressive disorder.

Categorical predictors were as follows: difficulty in managing money (no difficulty, some difficulty, much difficulty, I am unable to do it), difficulty dressing (e.g., tie, zippers, or buttons) (no difficulty, some difficulty, much difficulty, I am unable to do it), health status (excellent, very good, good, fair, poor), Activities of Daily Living (ADL) total score (dressing, bathing, transferring, eating, walking), and the total score of following anxiety questions (experienced anxiety/fright in the last 30 days, lately felt anxious/not well, or that special situations may you anxious).

Supplemental Info 2. Definition of all acronyms used.

ADL	**Activities of Daily Living**
ADNI	**Alzheimer's Disease Neuroimaging Initiative**
AGES-Reykjavik Study	**Age, Gene/Environment Susceptibility-Reykjavik Study**
AUC	**Area under the receiver operating characteristic curve**
DSI	**Disease State Index**
MMSE	**Mini Mental State Examination**
MRI	**Magnetic resonance imaging**
TRIPOD	**Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD)**

Supplementary Code 1. Pseudocode of algorithms used.

Upsample <- trainControl(method = "repeatedcv", number = 10, repeats = 10, classProbs = TRUE, summaryFunction = twoClassSummary, savePredictions = TRUE, sampling = "up")

downsample <- trainControl(method = "repeatedcv", number = 10, repeats = 10, classProbs = TRUE, summaryFunction = twoClassSummary, savePredictions = TRUE, sampling = "down")

Logistic regression

Logfit <- train(outcome ~ ., training.data, metric = "ROC", method = "glm", trControl = upsample)

Elastic net regression

Elastic_net <- train(outcome ~ ., train, metric = "ROC", method = "glmnet", trControl = upsample, importance = TRUE)

Random forest

Rf <- train(outcome ~ ., train, metric = "ROC", method = "ranger", tuneGrid = expand.grid(mtry = c(2, 5, 10, 19), splitrule = c("gini", "extratrees"), min.node.size = 1), trcontrol = upsample, importance = "permutation")

Support vector machine

Svm <- train(outcome ~ ., train, metric = "ROC", method = "svmRadial", trControl = downsample, scale = FALSE)

Cox regression

Coxreg <- cv.glmnet(x = variables, y = outcome, family = "cox", nfolds = 10, type.measure = "C")

Elastic net Cox regression

Enet.cox <- fit_enet(x = variables, y = outcome, rule = "lambda.1se", seed = c(5, 7), parallel = TRUE)

Supplementary Table 1. Characteristics of the predictors in the study sample (n = 4793).

	Train data (n = 3138)	**Test data (n = 1655)**
	Mean (SD) or n (%)	**Mean (SD) or n (%)**
Demographics		
Age (years)*+	76 (6)	76 (6)
Sex (female)*+	1823 (58%)	999 (60%)
Education (college + university)	911 (29%)	481 (29%)
Neuroimaging variables		
Log-transformed white matter lesion volume (ml)*	2.6 (0.9)	2.6 (0.9)
Hippocampal volume (ml)*	5.6 (0.7)	5.6 (0.7)
Number of microbleeds*	0.3 (1.8)	0.3 (1.4)
Presence of infarcts	961 (31%)	530 (32%)
Gray matter/intracranial volume ratio*	0.5 (0.04)	0.5 (0.04)
Clinical variables		
Abdominal circumference (cm)	101 (12)	101 (12)
Carotid intima-media thickness test (CIMT)	1 (0.1)	1 (0.1)
High-density lipoprotein (mmol/L)	1.6 (0.5)	1.6 (0.5)
Low-density lipoprotein (mmol/L)	3.5 (1)	3.5 (1)
Triglycerides (mmol/L)	1.2 (0.6)	1.2 (0.7)
Fasting glucose (mmol/L)	5.8 (1.1)	5.8 (1.3)
B-hemoglobin A1c (g/dl)	0.5 (0.1)	0.5 (0.1)
High-sensitive c-reactive protein (mg/L)	3.8 (7)	3.7 (6.3)
Systolic blood pressure (mmHg)	143 (21)	142 (20)
Diastolic blood pressure (mmHg)	74 (10)	74 (10)
Hypertension	2548 (81%)	1307 (79%)
Coronary artery disease	560 (18%)	282 (17%)
Diabetes mellitus	368 (12%)	223 (14%)
Metabolic syndrome	967 (31%)	532 (32%)
Stroke/blood clot in the brain	195 (6%)	102 (6%)
History of cancer	482 (15%)	271 (16%)
Experienced a head trauma or lost consciousness	245 (8%)	171 (10%)
Subjective memory decline*+	952 (30%)	479 (29%)
Often forget the names of a friend	982 (31%)	540 (33%)
Often forget where items are*+	1374 (44%)	709 (43%)
Difficulty finding the right words	983 (31%)	534 (32%)
Difficulty finding the way to familiar places*+	238 (8%)	147 (9%)
Inability in managing money*+	74 (2%)	58 (4%)
Inability in dressing oneself*+	13 (<1%)	16 (1%)
Intermit claudication in legs	148 (5%)	79 (5%)
Insomnia	963 (31%)	527 (32%)

Supplementary Table 1. Continued

	Train data (n = 3138)	Test data (n = 1655)
	Mean (SD) or n (%)	**Mean (SD) or n (%)**
Poor health status	181 (6%)	95 (6%)
ADL score, full dependence on all items*+	28 (1%)	24 (2%)
Morning salivary cortisol (nmol/L)	19.8 (13.2)	19.6 (13.3)
Evening salivary cortisol (nmol/L)	3.9 (6.4)	4.0 (7.4)
GDS-15 sum score*+	2 (2)	2 (2)
All anxiety questions 'yes'	23 (1%)	17 (1%)
Diagnosis of current GAD, social phobia, panic disorder, or agoraphobia	68 (2%)	30 (2%)
Current/past diagnosis of major depressive disorder	167 (5%)	81 (5%)
Medication use		
Benzodiazepines	258 (8%)	138 (8%)
Beta-adrenergic blockers	1090 (35%)	570 (34%)
Glucocorticoids	108 (3%)	63 (4%)
Psycholeptics	539 (17%)	279 (17%)
Anti-depressants	427 (14%)	235 (14%)
Lifestyle variables		
Current smoker, %	377 (12%)	205 (12%)
Alcohol consumption (g/week)	16 (35)	14 (28)
Moderate/high physical activity	964 (31%)	545 (33%)
Mental leisure activity (days per month)	7 (6)	7 (6)
Social leisure activity (days per month)	4 (4)	4 (4)
Single marital status, %	187 (6%)	101 (6%)
Number of close friends	3 (4)	3 (3)
Not that easy to walk 2 km*+	615 (20%)	345 (21%)
Not that easy to walk 500 m*+	157 (5%)	76 (5%)
Number of living close relatives	7 (5)	7 (5)
Never fish consumption, %	19 (1%)	7 (<1%)
Cognitive assessment		
MMSE total score*+	27 (3)	27 (3)
Outcome		
Incident dementia	583 (19%)	309 (19%)
Follow-up time (years)	9 (3)	9 (3)

Note: * marks variables entered in model 2. + marks variables entered in model 3. A significant difference was found between train and test data for experiencing a head trauma or losing consciousness ($\chi2 = 8.4$, $p = 0.004$), inability to dress oneself ($\chi2 = 9.0$, $p = 0.03$), and ability to walk 2 kilometers ($\chi2 = 6.7$, $p = 0.03$).

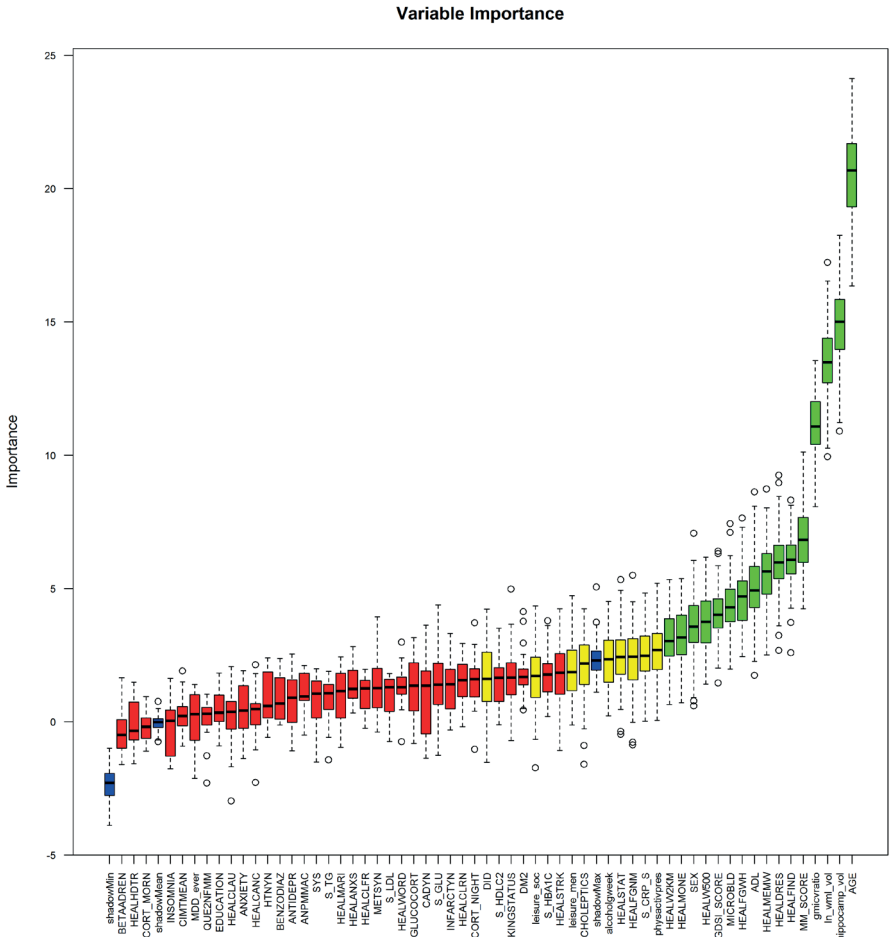
Supplementary Table 2. Tuning parameters for each machine learning classifier.

Classifier	Tuning parameter
Model 1	
Elastic net	Alpha = 1, lambda = 0.02
Random forest	Mtry = 5, splitrule = gini, minimum node size = 1
Support vector machine	Sigma = 0.01, cost = 0.25
Model 2	
Elastic net	Alpha = 0.55, lambda = 0.02
Random forest	Mtry = 2, splitrule = gini, minimum node size = 1
Support vector machine	Sigma = 0.05, cost = 0.25
Model 3	
Elastic net	Alpha = 0.55, lambda = 0.02
Random forest	Mtry = 2, splitrule = gini, minimum node size = 1
Support vector machine	Sigma = 0.07, cost = 0.25

Supplementary Table 3. Elastic net and logistic regression coefficients in models 2 and 3.

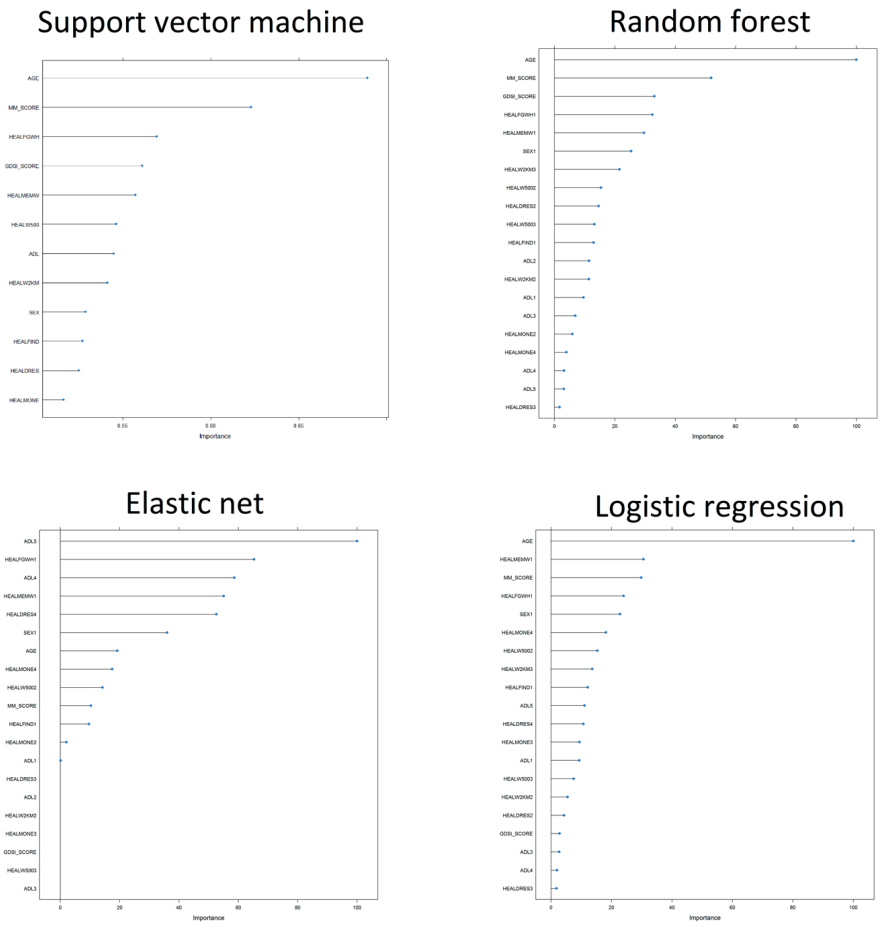
	Elastic net regression		Logistic regression	
	Model 2	**Model 3**	**Model 2**	**Model 3**
Intercept	-1.621	-6.583	-0.732	-7.927
Age	0.070	0.098	0.082	0.124
Sex	0.034	0.184	0.288	0.296
Subjective cognitive decline	0.349	0.282	0.459	0.445
Difficulty in remembering where things are	0.221	0.334	0.300	0.326
Difficulty finding familiar places	0	0.050	0.080	0.278
Difficulty in managing money (some difficulty)	0	0.011	-0.178	-0.049
Difficulty in managing money (much difficulty)	0	0	-0.854	-0.741
Difficulty in managing money (unable)	-0.120	-0.089	-0.722	-0.845
Difficulty in dressing oneself (some difficulty)	0	0	0.205	0.159
Difficulty in dressing oneself (much difficulty)	0	0	0.199	0.141
Difficulty in dressing oneself (unable)	0.092	0.269	0.961	1.155
Difficulty in walking 2 km (somewhat easy)	0	0	-0.072	-0.093
Difficulty in walking 2 km (not very easy)	-0.098	0	-0.312	-0.268
Difficulty in walking 500 m (somewhat easy)	0.132	0.073	0.317	0.351
Difficulty in walking 500 m (not very easy)	0	0	0.084	0.259
1 ADL item dependent	0	-0.001	-0.165	-0.181
2 ADL items dependent	0	0	0.043	0.009
3 ADL items dependent	0	0	-0.012	0.128
4 ADL items dependent	0	0.300	-0.082	0.137
5 ADL items dependent	0.839	0.512	0.723	0.832
GDS-15 score	0	0	-0.023	-0.010
MMSE score	-0.043	-0.053	-0.071	-0.078
Log-transformed WML volume	0.360	*Not included*	0.468	*Not included*
Hippocampal volume	-0.432	*Not included*	-0.553	*Not included*
Microbleeds	0.009	*Not included*	0.015	*Not included*
Gray matter/ICV ratio	-3.419	*Not included*	-5.220	*Not included*

Note: 0s represent variables that were penalized to 0 during model fit. GDS= Geriatric Depression Scale; ICV = intracranial volume; WML = white matter lesion.



Supplementary Figure 1. Boruta feature selection.

Green variables are selected for feature selection, yellow variables are rated as tentative, and red variables are ranked as unimportant. Ninety-nine iterations were performed.



Supplementary Figure 2. Variable importance per trained prognostic model in model 3, the clinically accessible model.

Note: As there is no built-in variable importance for support vector machine, the AUC is shown instead on the x-axis.

Chapter 10

General discussion

The present thesis filled several gaps in the etiology of dementia by looking at the associations between biological and psychosocial factors. This thesis further combined both biological and environmental risk factors using data-driven methodology to aim for precision medicine for dementia.

Associations between amyloid-beta and vascular pathology during the preclinical stage of dementia

The first portion of this thesis focused on the association between the two main biomarkers for dementia: amyloid-beta and vascular burden. To first get an overview of previous literature, we performed a systematic review and meta-analysis on the association between amyloid-beta burden and white matter hyperintensities (WMH), which was described in the general introduction of this thesis as one of the main pathologies related to vascular burden (1). Previous systematic reviews and meta-analyses on the association between amyloid-beta and vascular burden did not explicitly look at the preclinical stage of dementia (2-4). By focusing on the extended preclinical stage of dementia, we can reduce possible heterogeneity of results as well as increase the timeliness of detection of high-risk individuals and possible routes for prevention. Therefore, in **chapter 2**, the primary focus was on cognitively unimpaired individuals, to elucidate if an association is present before cognitive symptoms begin. Our meta-analysis found a small- to medium-sized effect size between amyloid-beta burden and WMH. However, no association was found in the studies assessing amyloid-beta burden in blood plasma.

As only two studies on blood plasma were included in our systematic review and meta-analysis and did not utilize the innovative, highly sensitive blood assays for amyloid-beta detection in plasma, we decided to assess this relationship in **chapter 3.** This chapter further aimed to assess other aspects of Alzheimer's disease (AD) pathophysiology in blood plasma alongside amyloid-beta, such as phosphorylated tau (p-tau181), neurofilament light (NfL), and glial fibrillary acidic protein (GFAP). In this study, we also aimed to assess infarcts as well as WMH for aspects of vascular pathology. We also wanted to examine associations between these novel AD plasma biomarkers with neurodegeneration on MRI, specifically in the hippocampus as well as on total brain volume. Higher levels of p-tau181 were associated with more WMH burden. NfL, a blood biomarker for neurodegeneration non-specific to AD, was associated with total brain volume as well as cortical infarcts. Plasma p-tau181 and NfL may be noninvasive markers for monitoring vascular pathology and neurodegeneration.

Mapping the biological to the psychosocial

The second part of this thesis extended on the biomarkers for AD by assessing possible relations with psychosocial factors. Many psychosocial factors have shown to be associated with incident dementia, such as depression (5-7), anxiety (8), early-life adversity (9), and social support (10). **Chapter 4** focused on assessing if depressive symptoms, anxiety symptoms, early- or late-life adversity, or social support show an association with hippocampal (subfield) atrophy. We observed trends across hippocampal subfields with lower volumes related to early-life adversity and higher volumes related to late-life adversity. We found a protective effect of higher social support associated with higher volumes in the cornu ammonis (CA) 3 subfield of the hippocampus, which is a subfield associated with specific sensitivity to stress (11). However, we did not find an association between depressive symptoms or anxiety symptoms and neurodegeneration in the hippocampus. Therefore, in the next chapters, we assessed if the relation between depression and dementia could be explained through other biological mechanisms.

In **chapter 5**, we performed a systematic review and meta-analysis to assess if amyloid-beta is associated with depression diagnosis or depressive symptoms in cognitively unimpaired older adults. We found no association between amyloid-beta using PET, CSF, or plasma and depression or depressive symptoms, but there was a trend towards a positive association in the PET and CSF studies. However, all included plasma studies were performed in 2016 or before and none utilized highly-sensitive plasma assays. As amyloid-beta exists in ten-fold lower concentrations in blood plasma compared to in CSF (12), using highly-sensitive techniques is of importance. Therefore, in **chapter 6**, we assessed the association between AD plasma biomarkers (amyloid-beta, p-tau181, NfL, and GFAP), analyzed with highly-sensitive plasma assays, with depressive symptoms in eight cohort studies conducted throughout the Netherlands. AD pathology in plasma was not associated with depressive symptoms, using a meta-analytical technique on the cohort studies of varying settings. Therefore, in **chapter 7,** we explored the neurotoxicity and vascular hypotheses in the relation between depression and dementia.

As mentioned in the general introduction, the neurotoxicity hypothesis stipulated that increased cortisol and activation of the HPA axis could explain the relation between depression and dementia (13). Whereas the vascular hypothesis specified that the relation may lie in vascular changes occurring in mood-regulating areas of the brain (14). We explored both of these hypotheses in **chapter 7** by assessing depression diagnosis, WMH volume, and salivary cortisol at baseline with incident dementia with up to 12 years of follow-up time. Both current depression diagnosis

and high levels of salivary cortisol were independently associated with increased risk of dementia. However, WMH volume partially explained the relationship between current depression and dementia, but not fully. Therefore, the final section of this thesis assessed if utilizing data-driven techniques and combining both biomarkers and psychosocial factors may help further untangle risk for dementia.

Towards precision medicine: the use of high-risk clusters and machine learning prognostic model

Complex interactions between dementia biomarkers and psychosocial risk factors exist (15). Instead of studying these factors in isolation, we can make use of these interactions and use a biopsychosocial framework to assess risk for dementia. With the development of recent data-driven statistical techniques, this becomes increasingly possible. In **chapter 8**, we first assessed a multimodal biomarker framework and if clusters of various biomarkers explained the association between depression and dementia. In **chapter 7,** we saw vascular pathology played a partial role In this relationship. We hypothesized that this could be further explained by other biomarker pathways, such as through inflammatory or metabolic systems. This study found four biomarker profiles: low cardiovascular dysregulation, average, high cardiovascular dysregulation, and multisystem dysregulation. We found additive interaction between depressive symptoms and the multisystem dysregulation profile on incident all-cause dementia. Individuals that have dysregulation across multiple biomarker systems, specifically inflammatory, metabolic, and stress systems, may be more susceptible of the negative impact of depressive symptoms on incident dementia.

Lastly, **chapter 9** assessed if utilizing machine learning techniques improved performance and calibration of prediction models for dementia. Previous external validation studies found poor calibration of prediction models for dementia (16, 17), highlighting the need for the development of better dementia prediction models. We further assessed if an accurate prediction model could be developed without the use of expensive biomarkers such as MRI markers. Machine learning, through an elastic net Cox regression, provided a benefit to performance only when using survival techniques (e.g., a traditional Cox regression model). However, we found that when removing MRI markers as predictors in our model, performance did not decrease significantly.

Future research

Personalized interventions that include biopsychosocial factors have been suggested to be the most promising to prevent Alzheimer's disease (AD) and other dementias (18, 19) and pave the way towards precision medicine. Clinical precision medicine

has been adapted as an extended clinical history of a patient, not only focusing on past medical history and physical examination, but also including psychosocial factors (e.g., educational history, social support, life course events, physical activity) (18, 20, 21). By focusing on biopsychosocial factors, a clinician can more definitely assess a patient's risk as well as create a more personalized prevention and/or treatment plan (20). One of the current proposed personalized prevention methods for AD are the "ABCs", i.e. anthropometrics, blood biomarkers, and cognition (18, 20). I hypothesize the future use of the "ABCDs" for AD prevention, extending this also to include demographic factors, such as life course events, education, socioeconomic status, and other psychosocial factors. Personalized treatment for AD has also shown promising results, highlighting that dementia is a multifactorial disease and should be prevented and treated as such (22).

The translation of a biopsychosocial framework from research to clinical practice has shown disadvantages alongside the aforementioned advantages. In a recent qualitative study, healthcare professionals were asked to explain their experience of implementing a biopsychosocial approach in clinical practice (23). While the healthcare professionals valued the implementation of a biopsychosocial framework, many barriers remained regarding how time-consuming it was to incorporate. However, studies have shown that incorporating biopsychosocial approaches in practice can save time and resources in the long-term (23, 24). In line with the time costs of implementing more individualized clinical medicine approaches, future studies should explore the use of AI in the clinic with its purpose to minimize time costs (25).

Further, the implementation of prevention comes along with ethical considerations when a clinician is met with a high-risk patient. While studies did not show increased psychosocial stress after disclosing APOE ε4 allele status (26), ethical considerations remain crucial moving forward and understanding the cost/benefit ratio of disclosing high-risk status to a patient. The implementation of cognitive therapy for those who are assigned high-risk for disease, such as eye-movement desensitization and reprocessing (EMDR) therapy, may help increase quality of life and reduce any psychological impact post-disclosure (27).

The Importance of precision medicine remains for the understanding of differential impact of risk factors on dementia risk. Zahodne (28) defined differential impact where subgroups show different strengths of association between risk factor and dementia risk. This could be based on sex/gender, ethnicity, or genetics. For example, APOE ε4 allele carriers show differential impact on physical activity, smoking, and alcohol consumption for dementia risk compared to APOE e4 allele non-carriers

(18, 29). Previous studies have highlighted differences in dementia risk based on ethnicity and sex/gender and that this may be mediated by differences in risk factors (30). Unfortunately, the studies included in this thesis did not include ethnic or sex/gender minorities. To successfully achieve an individualized approach for dementia prevention and treatment, future studies need to incorporate diverse samples in their studies and then stratify their findings based on sex/gender and ethnicity to explore if differential impact based on sex/gender identity or orientation or ethnicity exists with specific risk factors. Future research on sex/gender and ethnicity will also allow the clinician to then make further individualized considerations per patient.

Specifically, studies assessing transgender or nonbinary individuals are unfortunately scarce, some recent studies have found that transgender and nonbinary individuals have higher rates of subjective cognitive decline (31) and a higher prevalence of dementia (32). As assessing sex differences in risk factors for dementia is of priority for precision medicine for dementia (33), the inclusion of sex and gender minorities (SGM) in these studies will be crucial. The inclusion of systemically underrepresented populations can be done at the governmental level by facilitating clinical research in high-risk groups, diversifying research groups, or through international collaborations with research groups with data on marginally underrepresented populations (34). By prioritizing the creation of cohort studies and consortiums that ensure the inclusion of minority groups across all domains – ethnic, socioeconomic, and SGM – we will unravel the true etiology of dementia and the interplay between biopsychosocial factors.

Future research should not only ensure to include diverse samples but also a wide variety of psychosocial factors. As many psychosocial factors are not routinely collected in cohort studies (28), the prioritization of the inclusion of psychosocial factors will be crucial to elucidate the multifactorial etiology of neurological diseases such as dementia. Further, many psychosocial factors may be racially patterned, such as discrimination, and therefore understanding these psychosocial factors when including marginally underrepresented populations will be crucial to understand the underpinnings of disease in these groups. Lastly, it will be vital to not only prioritize the inclusion of psychosocial risk factors in future cohort studies, but psychosocial *protective* factors as well. By focusing equally on disease prevention as well as health promotion, we can steer public policy to implement not only prevention but healthy ageing practices as well.

Biological factors not explored in this thesis should also be considered for future studies. Our findings did not solidify a specific biological mechanism linking psychosocial factors to dementia. Recent evidence has found associations between

gut microbiota and depression (35) and AD pathology (36). It is possible that disruption in the gut microbiota, which also has a role in one's inflammatory system (37), may explain the relation between depression and dementia. There is also a possibility that psychotropic medication (38) or diet (39) play a role in the associations found and should be included as possible confounders in future research. Further, recent evidence has shown air pollution may also be a risk factor for dementia (40-42), as well as depression (43). Future studies should comprehensively include a wide range of biological and psychosocial factors as we used data that was already collected from previous cohort studies and were restricted to the availability of the factors included. Future cohort studies should prioritize repeated assessments of both biomarkers as well as psychosocial factors to elucidate the key factors – and their temporal occurrence – during the preclinical stage of dementia.

We may achieve this comprehensive goal through collaboration with primary care researchers and general practice databases. By using a 'real-world' data approach to epidemiological studies, we have the ability to gain a life course perspective on dementia etiology (44). This can be done using a 'longitudinal exposome-wide association study' (LEWAS) approach (45). In this approach, it is also possible to explore further protective factors, to discover possible reasons why high-risk individuals do not develop dementia, or how in some individuals with mild cognitive impairment, they transition back to normal cognition (46). As having a biopsychosocial background on a patient is becoming more prioritized in clinical practice, future primary care clinics can focus on intakes that gain a more holistic background of the patient. As mentioned previously, incorporating AI techniques in the clinic can make this goal more achievable, more cost-effective, and without creating an added burden for clinicians (25).

Lastly, the current study highlighted the added benefit of using data-driven analytical approaches to apply precision medicine to the etiology of dementia. Previous neuroimaging studies have highlighted the benefits of using data-driven methods to unravel heterogeneity and discover possible subgroups (47). Specifically, data-driven methods can validate previous hypothesis-driven subtypes as well as elucidate subgroups that may go beyond previous characterizations (47). However, this methodology will require the implementation of international consortia that include diverse cohorts to externally validate if subgroups may exist within specific or broad samples. As a previous systematic review has highlighted that methodological implementations on addressing the complex systems behind disease are lacking (48), future studies should make methodological prioritizations as well as focus on guidelines on when to use which methodology.

Conclusions

To conclude, the current thesis first assessed the individual relation between biomarkers and psychosocial factors in risk for dementia. However, the use of multimodal and data-driven techniques that encapsulate biopsychosocial factors all together showed advantageous in understanding the etiology of dementia.

Further, the current study was able to implement the recent advancements in reliable blood biomarker assessment for dementia by performing large-scale assessments of AD pathophysiology with other biomarkers (e.g., vascular pathology and neurodegeneration) as well as with psychosocial factors (e.g., depressive symptoms) through a nationwide Dutch consortium on dementia cohort studies. This study, to our knowledge, is the first to look at blood biomarkers for dementia in a non-demented, 50+ population. Further, we aimed to focus primarily on individuals without dementia, to assess the role of both biomarkers as well as psychosocial factors during the prolonged preclinical stage of dementia. We also aimed to focus on individuals at higher risk for dementia, by using the hospital-based SMART population of individuals with a history of vascular disease. Focusing on the earliest disease stage, as well as a high-risk population, allows for the future utilization of modes of prevention and treatment monitoring.

The path towards precision medicine for dementia is still a long way to go. Through the creation of consortia and cohort studies that prioritize the ability to assess risk factor trajectories through repeated measurements as well as differences between groups through the inclusion of diverse individuals, the future for individualized prevention, prediction, and treatment will be attainable.

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English summary

Dementia is a neurodegenerative disease, the prevalence of which is expected to triple by 2050 (1). It is characterized by decline in cognitive functioning as well as a decrease in quality of life. There are many risk factors associated with dementia, both biological as well as psychosocial. The first part of this thesis aimed to assess the relation between the two main causes of dementia, amyloid-beta and vascular pathology, in the stage before dementia diagnosis. The second aim of this thesis was then to assess if there is a relation between these biomarkers for dementia and psychosocial risk factors. Lastly, the third aim of this thesis was to combine both biomarkers and psychosocial factors into a biopsychosocial framework, through clustering and creating a machine learning prediction model.

In **part I** of this thesis, we assessed the relationship between amyloid-beta and vascular pathology. **Chapter 2** systematically reviewed the literature on amyloid-beta burden and white matter hyperintensities (WMH) in cognitively unimpaired older adults and performed a meta-analysis on PET, CSF, and plasma studies. We found a small-to-medium- effect size between amyloid-beta burden on PET and CSF with WMH burden. However, no association was found in the plasma studies.

Chapter 3 explored the association not only with plasma biomarkers for AD pathophysiology (i.e., amyloid-beta and phosphorylated tau), but also with biomarkers for neurodegeneration (i.e., neurofilament light; NfL) and astrocytic activation (i.e., glial fibrillary acidic protein; GFAP) with vascular pathology (i.e., WMH and infarcts) as well as neurodegeneration (i.e., total brain and hippocampal atrophy). We used a sample of 594 individuals all with comorbid vascular disease from the SMART-MR study. P-tau181 was associated with WMH, whereas NfL was associated with total brain atrophy and infarct presence. As this was the first study to our knowledge assessing plasma AD biomarkers with total brain atrophy, this should be validated in other studies.

Part II of this thesis aimed to assess the biological mechanisms behind the psychosocial factors associated with incident dementia. Using high-field 7T MRI, **chapter 4** assessed if depressive symptoms, anxiety symptoms, early- or late-life adversity, or social support were associated with hippocampal (subfield) volume. Early- and late-life events showed a trend of association with hippocampal (subfield) volume. Early-life adversity was associated with lower hippocampal (subfield) volumes, whereas late-life adversity was associated with higher hippocampal (subfield) volumes. Lower levels of social support was associated with lower volumes in the cornu ammonis (CA) CA3, a region of the hippocampus specifically sensitive to stress. Previous studies on social support and hippocampal volume have been scarce; thus, further studies on social support and hippocampal volume are warranted.

Chapter 5 assessed the role of amyloid-beta burden on depression and depressive symptoms in cognitively unimpaired older adults through a systematic review and meta-analysis. PET, CSF, and plasma studies on amyloid-beta and depression or depressive symptoms did not show a relationship between the two. However, a trend towards an association was found for the PET and CSF studies. Regarding plasma studies on amyloid-beta, those included in the meta-analysis were quite old and did not implement the newer, more sensitive techniques for amyloid-beta ascertainment in plasma. Therefore, **chapter 6** aimed to assess multiple plasma AD biomarkers, including amyloid-beta, with depressive symptoms in eight Dutch cohort studies. In this meta-analysis on a total of 7210 participants, no association was found between depressive symptoms and AD plasma markers (amyloid-beta42/40, p-tau181, NfL, or GFAP). Late-life depressive symptoms during this stage may be explained by other mechanisms independent of those related to AD, neurodegeneration, and astrocytic activation.

Chapter 7 aimed to assess if the relation between depression and dementia may be explained by either the neurotoxicity hypothesis (e.g., through elevated salivary cortisol levels) or the vascular hypothesis (e.g., through WMH). In this study using the population-based AGES-Reykjavik study, we assessed baseline depression diagnosis, morning and evening salivary cortisol levels, and WMH volume and their relation with incident dementia with up to 12-years of follow-up time. Current depression diagnosis and evening salivary cortisol levels were independently associated with incident dementia. However, WMH volume partially explained the relationship between current depression diagnosis and incident dementia.

Part III of this thesis explored the use of data-driven techniques on biomarkers and psychosocial factors together to move towards precision medicine for dementia. As interactions exist between and within biomarkers and psychosocial factors, using advanced statistical techniques is of interest to take into account and take advantage of those interactions when assessing dementia etiology. **Chapter 8** of this thesis used a clustering technique of different biomarkers (i.e., cardiovascular, metabolic, inflammatory, and stress) to assess if these biomarker profiles explained the relationship between depression and dementia. Using the AGES-Reykjavik study, we found four profiles: low cardiovascular dysregulation, average, high cardiovascular dysregulation, and multisystem dysregulation. We found additive interaction between the multisystem dysregulation group and late-life depressive symptoms on incident all-cause dementia. Those with dysregulation across multiple biomarker domains (i.e., metabolic, inflammatory, and stress) showed specific vulnerability to the negative effects of late-life depressive symptoms on incident dementia.

Lastly, **chapter 9** assessed the use of machine learning in the prognostication of dementia risk. We also assessed the feasibility of a prediction model for dementia that did not include MRI markers for a more clinically-accessible model. We found that machine learning only added a benefit to predictive performance when we took time-to-event into account. However, when removing MRI markers from our prediction model, performance remained high, highlighting the utility of readily accessible markers in predicting dementia.

In conclusion, we assessed multiple biomarkers and psychosocial factors and their relation to one another before diagnosis of dementia. We found that some biomarkers in plasma may be of use for monitoring of neurodegeneration and vascular pathology. Further, we found a specific time effect of adverse life events on hippocampal neurodegeneration, with early-life adverse events showing detrimental effects on hippocampal volume that remained in late-life. We also found a protective effect of high social support on hippocampal volume in stress-sensitive regions. Finally, we found that using a multimodal approach was the most advantageous for clustering and prediction. Future studies should prioritize combining both the internal experience (i.e., biomarkers) and the external experience (i.e., psychosocial factors) when assessing disease etiology, prevention, and treatment.

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Nederlandse samenvatting

Dementie is een neurodegeneratieve ziekte waarvan het aantal patiënten dat lijdt aan deze ziekte naar verwachting tegen 2050 zal verdrievoudigen. Dementie wordt gekenmerkt door achteruitgang van cognitief functioneren en een afname van kwaliteit van leven. Er zijn veel risicofactoren verbonden aan dementie, zowel biologisch als psychosociaal. Het **eerste deel** van dit proefschrift was gericht op het beoordelen van de relatie tussen de twee hoofdoorzaken van de ziekte van Alzheimer, amyloïd-bèta en vasculaire pathologie, in het stadium vóór de diagnose van dementie. In het **tweede deel** van dit proefschrift lag de focus op het onderzoeken van een mogelijk verband tussen deze biomarkers voor dementie en psychosociale risicofactoren. Ten slotte was de focus van het **derde deel** van dit proefschrift om zowel biomarkers als psychosociale factoren te combineren in een psychosociaal raamwerk, door middel van clustering en het creëren van een voorspellingsmodel met behulp van machine learning.

In **deel 1** van dit proefschrift hebben we de relatie tussen amyloïd-bèta en vasculaire pathologie onderzocht. **Hoofdstuk 2** besprak systematisch de literatuur over amyloïde-bèta-belasting en hyperintensiteiten van de witte stof (WMH) bij cognitief gezonde ouderen en voerde een meta-analyse uit van PET-, CSF-, en plasma-onderzoeken. We vonden een klein tot middelgroot effect van amyloïde-bèta gemeten met PET scans en gemeten in CSF met WMH gemeten met MRI. Er werd echter geen verband gevonden tussen amyloïde-bèta gemeten in bloed plasma en WMH.

In **Hoofdstuk 3** werd de associatie met bloedplasma biomarkers voor AD pathofysiologie (d.w.z. amyloïde-bèta en gefosforyleerd tau) en biomarkers voor neurodegeneratie (d.w.z. neurofilament licht; NfL) en astrocyte activatie (d.w.z. gliaal fibrillair zuur eiwit; GFAP) met vasculaire pathologie (d.w.z. WMH en infarcten) evenals neurodegeneratie (d.w.z. totale hersen- en hippocampale atrofie) onderzocht. We gebruikten daarvoor een steekproef van 594 personen, allen met een manifeste vaatziekte uit de SMART-MR-studie. P-tau181 was geassocieerd met WMH, terwijl NfL geassocieerd was met totale hersenatrofie en de aanwezigheid van een infarct. Aangezien dit voor zover ons bekend de eerste studie was waarin plasma-AD-biomarkers met totale hersenatrofie werden beoordeeld, zou dit in andere studies moeten worden gevalideerd.

Deel 2 van dit proefschrift was gericht op het onderzoeken van de biologische mechanismen achter de psychosociale factoren die samenhangen met incidentele dementie. in **hoofdstuk 4** beoordeeld of depressieve symptomen, angstsymptomen, levensgebeurtenissen in het vroege of late leven of sociale steun geassocieerd waren met hippocampus volume gemeten op high-field 7T MRI. Levensgebeurtenissen in het vroege en late leven vertoonden een trend van een associatie met het volume

van de hippocampus. Levensgebeurtenissen in het vroege leven waren geassocieerd met kleinere hippocampus volumes, terwijl levensgebeurtenissen op latere leeftijd geassocieerd waren met grotere hippocampus) volumes. Minder sociale steun was geassocieerd met kleinere volumes in de cornu ammonis (CA) CA3, een regio van de hippocampus die specifiek gevoelig is voor stress. Eerdere studies over sociale steun en hippocampusvolume waren schaars; daarom zijn verdere studies over sociale steun en hippocampusvolume gerechtvaardigd.

Hoofdstuk 5 onderzocht de rol van amyloïd-bèta-belasting op depressie en depressieve symptomen bij cognitief gezonde ouderen door middel van een systematische review en meta-analyse. Plasma-onderzoeken naar amyloïd-bèta en depressie of depressieve symptomen lieten geen verband tussen beide zien. Er werd echter een trend in de richting van een associatie gevonden voor de PET- en CSF-onderzoeken. Wat betreft plasma-onderzoeken naar amyloïd-bèta, waren de onderzoeken die in de meta-analyse waren opgenomen vrij oud en implementeerden ze niet de nieuwere, meer gevoelige technieken voor het vaststellen van amyloïd-bèta in plasma. Daarom was **hoofdstuk 6** gericht op het beoordelen van meerdere plasma AD biomarkers, waaronder amyloïd-bèta, met depressieve symptomen in acht Nederlandse cohortstudies. In deze meta-analyse van in totaal 7210 deelnemers werd geen verband gevonden tussen depressieve symptomen en AD-plasmamarkers (amyloid-beta42/40, p-tau181, NfL of GFAP). Depressieve symptomen op latere leeftijd tijdens deze fase kunnen worden verklaard door andere mechanismen die onafhankelijk zijn van de processen welke verband houden met biomarkers van AD, neurodegeneratie en astrocytische activering.

Hoofdstuk 7 had als doelstelling om te beoordelen of de relatie tussen depressie en dementie kan worden verklaard door ofwel de neurotoxiciteitshypothese (bijv. door verhoogde cortisolspiegels) of de vasculaire hypothese (bijv. door WMH). In deze studie met behulp van de populatie-gebaseerde AGES-Reykjavik-studie, hebben we de basisdiagnose van depressie, de speekselcortisolspiegels in de ochtend en de avond en het WMH-volume en hun relatie met incidentele dementie onderzocht met een follow-uptijd tot 12 jaar. De huidige diagnose van depressie en cortisolspiegels in het avondspeeksel waren onafhankelijk geassocieerd met incidentele dementie. Het WMH-volume verklaarde echter gedeeltelijk de relatie tussen de huidige diagnose van depressie en incidentele dementie.

Deel 3 van dit proefschrift onderzocht het gecombineerde gebruik van data-gestuurde technieken op biomarkers en psychosociale factoren om tot precisiegeneeskunde voor dementie te kunnen komen. Aangezien er interacties

bestaan tussen en binnen biomarkers en psychosociale factoren, is het gebruik van geavanceerde statistische technieken van belang om rekening te houden met en te profiteren van die interacties bij het beoordelen van de etiologie van dementie. **Hoofdstuk 8** van dit proefschrift gebruikte een clustertechniek van verschillende biomarkers (d.w.z. cardiovasculair, metabolisch, inflammatoir en stress) om te beoordelen of deze biomarkerprofielen de relatie tussen depressie en dementie verklaren. Met behulp van de AGES-Reykjavik-studie vonden we vier profielen: lage cardiovasculaire ontregeling, gemiddelde, hoge cardiovasculaire ontregeling en multisysteemontregeling. We vonden een additieve interactie tussen de multisysteemdisregulatiegroep en depressieve symptomen op latere leeftijd bij incidentele dementie. Mensen met ontregeling over meerdere biomarkerdomeinen (d.w.z. metabolisch, inflammatoir en stress) vertoonden een verhoogde kwetsbaarheid voor de negatieve effecten van depressieve symptomen op latere leeftijd op incidentele dementie.

Ten slotte beoordeelde **hoofdstuk 9** het gebruik van machine learning bij het voorspellen van het risico op dementie. We hebben ook de geschiktheid beoordeeld van een voorspellingsmodel voor dementie zonder MRI-markers voor een meer klinisch toegankelijk model. We vonden dat machine learning alleen voordelig was voor de voorspellende prestaties als we rekening hielden met de tijd totdat de dementie zich ontwikkelde. Bij het verwijderen van MRI-markers uit ons voorspellingsmodel bleven de prestaties echter hoog, wat het nut van gemakkelijk toegankelijke markers in de klinische praktijk bij het voorspellen van dementie benadrukte.

Concluderend hebben we meerdere biomarkers en psychosociale factoren en hun relatie tot elkaar beoordeeld op het risico voor het ontwikkelen van dementie. We ontdekten dat sommige biomarkers in plasma nuttig kunnen zijn voor het monitoren van neurodegeneratie en vasculaire pathologie. Verder vonden we een specifiek tijdseffect van ongunstige levensgebeurtenissen op neurodegeneratie van de hippocampus, waarbij stressvolle levensgebeurtenissen in het vroege leven nadelige effecten vertoonden op het volume van de hippocampus en deze effecten hielden op latere leeftijd aan. We vonden ook een beschermend effect van hoge sociale steun op het volume van de hippocampus in stressgevoelige regio's. We ontdekten dat het gebruik van een multimodale aanpak het meest voordelig was voor clustering en voorspelling van dementie. Toekomstige studies zouden prioriteit moeten geven aan het combineren van zowel de biomedische factoren (d.w.z. biomarkers) als de psychologische factoren (d.w.z. psychosociale factoren) bij het beoordelen van ziekte-etiologie, preventie en behandeling.

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Twait EL, Gerritsen L, Moonen J, Verberk IMW, Teunissen CE, Visser PJ, van der Flier WM, Geerlings MI. Plasma Alzheimer's disease markers and MRI load of vascular pathology and neurodegeneration: the SMART-MR Study. *Submitted to JAHA.*

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About the author

Emma Lindsay Twait was born on July 28th, 1995 in Charlotte, North Carolina in the United States. With the guidance of her high school psychology and chemistry teachers, she decided to study a psychology and neuroscience bachelor at the University of North Carolina at Wilmington in 2013. She graduated in three years summa cum laude, and during her time at a summer school in Italy, she decided that she wanted to continue her studies in Europe. Moving to Maastricht in 2016, she completed a master's in neuropsychology, with a thesis on a fMRI brain-computer interface in locked-in syndrome. Afterwards, she moved to Haifa, where she received a second master's in cognitive science at the Technion in 2019 with a thesis on neuroimaging in neurodevelopmental and psychiatric disorders. In 2020, she moved back to the Netherlands to start a PhD in Epidemiology at the Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht within the Netherlands Consortium of Dementia Cohorts (NCDC), supervised by dr. Mirjam Geerlings, prof.dr. Wiesje van der Flier, and dr. Lotte Gerritsen. During her PhD trajectory, Emma completed her third master's in Epidemiology with a specialization in Medical Statistics, participated as a chair for the JOB group of PhD students within the Julius Center, supervised several students, and presented work at international and Dutch conferences.

The results of her PhD research, titled "Towards precision medicine for dementia: a biopsychosocial approach", are presented in this thesis. Since June 2023, Emma works as a postdoctoral researcher at the Department of General Practice at the Amsterdam UMC. She continues her work on neurological diseases, with a focus on multi-factorial etiology for prevention of disease.

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