What determines cognitive functioning in the oldest-old? The EMIF-AD 90+ Study

Nienke Legdeur[a,*], MD, PhD; Maryam Badissi[a], MSc; Maqsood Yaqub[b], PhD; Nina Beker[a], MSc; Carole H. Sudre[c,d,e], PhD; Mara ten Kate[a,b], MD, PhD; Mark Forrest Gordon[f], MD; Gerald Novak[g], MD; Frederik Barkhof[b,h], MD, PhD; Bart N. M. van Berckel[b], MD, PhD; Henne Holstege[a,i], PhD; Majon Muller[j], MD, PhD; Philip Scheltens[a], MD, PhD; Andrea B. Maier[k,l], MD, PhD; Pieter Jelle Visser[a,m,n], MD, PhD

a. Alzheimer Center Amsterdam, Department of Neurology, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, Amsterdam UMC, Amsterdam, The Netherlands

b. Department of Radiology & Nuclear Medicine, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, Amsterdam UMC, Amsterdam, The Netherlands

c. School of Biomedical Engineering and Imaging Sciences, King's College London, London, United Kingdom

d. Dementia Research Centre, Institute of Neurology, University College London, London, United Kingdom

e. Department of Medical Physics and Biomedical Engineering, University College London, London, United Kingdom

f. Teva Pharmaceuticals, Frazer, Pennsylvania, USA

g. Janssen Pharmaceutical Research and Development, Titusville, NJ, USA

h. Institutes of Neurology and Healthcare Engineering, University College London, London, UK

i. Department of Clinical Genetics, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, Amsterdam UMC, Amsterdam, The Netherlands

j. Department of Internal Medicine, Amsterdam UMC, Amsterdam, The Netherlands

k. Department of Medicine and Aged Care, @AgeMelbourne, Royal Melbourne Hospital, University of Melbourne, Melbourne, Australia

l. Department of Human Movement Sciences, @AgeAmsterdam, Vrije Universiteit Amsterdam, Research Institute Amsterdam Movement Sciences, Amsterdam, The Netherlands

m. Department of Psychiatry & Neuropsychology, School for Mental Health and Neuroscience, Maastricht University, Maastricht, The Netherlands

n. Department of Neurobiology, Care Sciences Division of Neurogeriatrics, Karolinska Institutet, Stockholm, Sweden

*Corresponding author:

Nienke Legdeur, MD

Alzheimer Center Amsterdam, Department of Neurology, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, Amsterdam UMC, PO Box 7057, 1007 MB Amsterdam, the Netherlands

E-Mail: n.legdeur@amsterdamumc.nl, tel: + 31 (0) 20 444 8023, fax: +20 444 8529

2

ABSTRACT

Objectives Determinants of cognitive functioning in individuals aged 90 years and older, the oldest-old, remain poorly understood. We aimed to establish the association of risk factors, white matter hyperintensities (WMH), hippocampal atrophy and amyloid aggregation with cognition in the oldest-old.

Methods We included 84 individuals without cognitive impairment and 38 individuals with cognitive impairment from the EMIF-AD 90+ Study (mean age 92.4 years) and tested cross-sectional associations between risk factors (cognitive activity, physical parameters, nutritional status, inflammatory markers and cardiovascular risk factors), brain pathology biomarkers (WMH and hippocampal volume on MRI, and amyloid binding measured with PET) and cognition. Additionally, we tested whether the brain pathology biomarkers were independently associated with cognition. When applicable, we tested whether the effect of risk factors on cognition was mediated by brain pathology.

Results Lower values for handgrip strength, Short Physical Performance Battery (SPPB), nutritional status, HbA1c and hippocampal volume, and higher values for WMH volume and amyloid binding were associated with worse cognition. Higher past cognitive activity and lower BMI were associated with increased amyloid binding, lower muscle mass with more WMH, and lower SPPB scores with more WMH and hippocampal atrophy. The brain pathology markers were independently associated with cognition. The association of SPPB with cognition was partially mediated by hippocampal volume.

3

Discussion In the oldest-old, physical parameters, nutritional status, HbA1c, WMH, hippocampal atrophy and amyloid binding are associated with cognitive impairment. Physical performance may affect cognition through hippocampal atrophy. This study highlights the importance to consider multiple factors when assessing cognition in the oldest-old.

Key words: cognitive aging; oldest-old; brain pathology biomarkers; risk factors; Alzheimer's disease

4

1. Introduction

The number of individuals aged 90 years and older, the oldest-old, is estimated to increase five-fold, resulting in 77 million oldest-old individuals worldwide by 2050 (*World Population Prospects: The 2017 Revision. Population by Broad Age Groups - Both Sexes*, 2017). The prevalence of dementia in the oldest-old is 40% (Corrada et al., 2008). Despite this high prevalence, studies focusing on the underlying pathophysiology of cognitive impairment in the oldest-old are scarce (Nienke Legdeur et al., 2018). Worldwide there are two cohort studies specifically focusing on cognition in nonagenarians: *The 90+ Study* in the USA and the Danish Birth Cohort Studies. Besides these two, there are eight more studies that have a broader focus on successful aging or start inclusion from age 85 years: the H85 Gothenburg study, Leiden 85-plus Study, New-castle 85+ Study, NonaSantfeliu study, Octabaix study, Project of Longevity and Aging in Dujangyan (PLAD), Umeå study and Vantaa 85+ Study. These earlier studies have identified physical performance (Bullain et al., 2016) and nutritional status (Hai et al., 2017) as potential protective factors for cognitive impairment, while findings were conflicting for premorbid cognitive activity, inflammatory markers and cardiovascular risk factors (Nienke Legdeur et al., 2018). Cognitive impairment is associated with white matter hyperintensity (WMH) volume, hippocampal atrophy and amyloid aggregation in younger elderly (Petersen et al., 2000; Vemuri et al., 2015). It has been suggested that the association of brain pathologies with cognition weakens with older age (Savva et al., 2009), although WMH volume, hippocampal atrophy and amyloid aggregation have also been related to lower cognitive performance in the oldest-old (Kawas et al., 2013; Nienke Legdeur et al., 2019).

In younger elderly, vascular risk factors for cognitive decline have also been identified as risk factors for brain pathologies (Debette et al., 2011; Rodrigue et al., 2013). Therefore, it has been suggested that brain pathologies might mediate the association between vascular risk

5

factors and cognitive decline (Wang et al., 2017). Whether risk factors of cognitive impairment are also risk factors for brain pathology biomarkers in the oldest-old remains unknown and it is unclear whether the effect of risk factors on cognition is mediated by brain pathology biomarkers in this age group.

We aimed to determine the association of risk factors, WMH volume, hippocampal atrophy and amyloid aggregation with cognition in the oldest-old using data from the EMIF-AD 90+ Study. Additionally, we determined the association of these risk factors with WMH volume, hippocampal atrophy and amyloid aggregation and explored possible mediation between risk factors, brain pathology biomarkers and cognition.

2. Methods

2.1. <u>Study sample</u>

Individuals were included from the European Medical Information Framework for Alzheimer's disease (EMIF-AD) 90+ Study, a case-control study including cognitively normal and cognitively impaired individuals (Nienke Legdeur et al., 2018). The inclusion and exclusion criteria and recruitment strategy described below were used in the EMIF-AD 90+ Study so also applied to the present study. Inclusion criteria for cognitively normal individuals were a global Clinical Dementia Rating (CDR) score of 0 and a score ≥ 26 points on the Mini-Mental State Examination (MMSE) (Melikyan et al., 2019). We also included 3 individuals with an MMSE <26 points, who were determined to be cognitively normal after extensive cognitive testing. Inclusion criteria for individuals with cognitive impairment were a diagnosis of amnestic mild cognitive impairment (aMCI) (Petersen, 2004) or a diagnosis of probable or possible Alzheimer's disease (AD) (McKhann et al., 1984); a global CDR score ≥ 0.5 and a MMSE score of 20–28 points (inclusive). The overlap in MMSE score between the cognitively normal and impaired individuals is justified as CDR score and an aMCI or AD

6

diagnosis was also used to make the distinction. Given a lower than expected enrolment rate, we also included six cognitively impaired individuals aged 85-89 and two cognitively normal individuals aged 88-89 years. Analyses without these individuals did not substantially change the findings. Exclusion criteria were physical inability to undergo the procedures, visual or hearing impairment interfering with neuropsychological testing, severe depression and comorbidities or medications that could impair cognition, as judged by the investigator. We recruited individuals from June 2016 to July 2018 via advertisement, outreach to general practitioners (GPs), and the 100-plus Study (Holstege et al., 2018). The Medical Ethical Committee of the Amsterdam UMC approved this study. All individuals provided written informed consent.

2.2. Clinical characteristics

Data about the medical history, medication use and education were collected through structured interview, in combination with information provided by the study partner (if available, this can either be a husband, wife, family member, friend or caregiver), GP and/or medical specialist. The presence of hypertension, diabetes mellitus (DM) and atrial fibrillation were based on a positive medical history or, in case of hypertension and DM, the use of antihypertensive or antidiabetic medication.

2.3. Cognitive functioning

Cognitive tests were administered by a trained neuropsychologist. To reduce the number of outcome measures and thereby the chance of a type 1 error, we computed three cognitive composite scores by calculating z-scores and averaging them per cognitive domain. For memory we included the immediate recall (range: 0-30 words) and 10-minutes delayed recall (range: 0-10) over three trials of the CERAD 10-words test (Morris et al., 1989), immediate recall (range: 0-23) and delayed recall after 20-30 minutes (range: 0-23) of the logical

7

memory test (Abikoff et al., 1987), Rey Complex Figure Test delayed copy after 3 minutes (range: 0-36) corrected for the immediate copy (Meyers et al., 1996), Visual Association Test (range: 0-12) (Lindeboom et al., 2002) and the total adjusted errors on the Paired Associate Learning (PAL) test of the computerized Cambridge Neuropsychological Test Automated Battery (CANTAB) (range: 0-70) (Robbins et al., 1994). For executive functioning we included Letter fluency (1 minute per letter, letters D-A-T) (Tombaugh, 1999), Trail Making Test (TMT) B corrected for A (Reitan, 1958) and the Clock Drawing Test (range: 0-14) (Royall et al., 1998). For processing speed we included the Digit Symbol Substitution Test (DSST, range: 0-93 points) from the Wechsler Adult Intelligence Scale-Revised (Wechsler, 1981), TMT A and the median five-choice reaction time of the CANTAB. There was no time limit for the assessment of the TMT A and B. However, some individuals could not perform the TMT B due to cognitive problems. To minimize the number of missing values on the TMT B/A ratio, we assigned maximum scores to the TMT A and B if scores were missing due to cognitive problems. Maximum scores were based on the time 2SD above the study sample mean. All scores higher than the maximum score were limited to the maximum score in order to avoid outliers influencing associations.

2.4. <u>Risk factors</u>

2.4.1. Cognitive activity

Past cognitive activity was assessed with the cognitive activities questionnaire (CAQ) (Wilson et al., 2003). Individuals were asked to rate how often they participated in cognitive activities, such as reading, writing and playing board games (in total 8 different activities), at age 6, 12, 18 and 40 years. Frequency of participation in each activity was rated on a five-point scale. The mean score over the different activities and ages was calculated to determine past CAQ.

8

2.4.2. Physical parameters

Handgrip strength of the dominant hand was measured twice with a hand dynamometer (Jamar hand dynamometer; Sammons Preston, Inc., Bolingbrook, IL., USA) and the highest score in kilograms was used in the analyses (Reijnierse et al., 2017). Skeletal muscle mass was measured in kilograms using a Bioelectrical Impedance Analyzer (BIA; InBody 770 or S10; Biospace Co., Ltd, Seoul, Korea) (Ling et al., 2011). We calculated the skeletal muscle mass index (further described as muscle mass) by dividing skeletal muscle mass by height (kg/m^2). The Short Physical Performance Battery (SPPB) included balance tests, a 4m walk to measure walking speed and the chair stand test (range 0-12 points) (Guralnik et al., 1995).

2.4.3. Nutritional status

We used the Mini Nutritional Assessment (MNA) and the Body Mass Index (BMI) to measure nutritional status. The MNA consists of a screening and assessment score (Isautier et al., 2019). For the present analyses, we used the assessment score (range 0-16 points).

2.4.4. Inflammatory markers

We included the acute-phase protein C-reactive protein (CRP) level in mg/L and number of leukocytes in $10^{\land 9}$/L measured in serum as inflammatory markers. Blood samples were collected after approximately two hours of fasting (Reijs et al., 2015). CRP was measured with an immunoturbidimetric assay on a Roche/Hitachi cobas c system. The lower detection limit of the assay is 2.5 mg/L. The number of leukocytes was determined using the Fluorocell WPC channel on a Sysmex XN hematology analyzer (Sysmex, Kobe, Japan). Individuals with a CRP >15 mg/L were excluded from the analyses, as such CRP levels primarily reflect acute infection. All CRP levels < 2.5 mg/L were set at 1.0 mg/L in the analyses and CRP levels were log transformed because of a skewed distribution.

2.4.5.

9

2.4.6.   Cardiovascular risk factors

Mean systolic and diastolic blood pressure (BP) were calculated over three measurements

performed in lying position (eight individuals had only one BP measurement and one

individual had two BP measurements due to logistic reasons, we did not exclude these

individuals). Total cholesterol in mmol/L was determined in serum by an enzymatic test on a

Roche/Hitachi cobas c system. Levels of hemoglobin A1c (HbA1c), as a measure of average

glucose levels over the past weeks, were determined with the HA-8160 (Menarini).

2.5. <u>Brain pathology biomarkers</u>

2.5.1.   White matter hyperintensity and hippocampal volume

Brain MRI-scans were performed on a Philips 3T Achieva scanner and structural three-

dimensional (3D) T1-weighted images and 3D sagittal fluid-attenuated inversion recovery

(FLAIR) sequences were acquired with isotropic 1 mm resolution (Konijnenberg et al.,

2018). A neuroradiologist visually inspected the MRI-scans for incidental findings. White

matter hyperintensity (WMH) segmentation was performed by using an algorithm based on a

three-level Gaussian mixture model to model healthy tissues and lesions (Sudre et al., 2015).

Volumetric segmentation of the 3D T1 images was performed with the FreeSurfer image

analysis suite (http://surfer.nmr.mgh.harvard.edu/). The automated procedure includes motion

correction, removal of non-brain tissue, automated Talairach transformation and

segmentation of the white matter and deep gray matter volumetric structures (including

hippocampus, amygdala, caudate, putamen, ventricles) (Fischl et al., 2002). Hippocampal

volume was computed from the segmented images in native space and mean hippocampal

volume was calculated by averaging the left and right side. Both the WMH and FreeSurfer

segmentations were visually inspected. In the present analyses, we computed the percentage

of WMH and hippocampal volume relative to total intracranial volume (TIV, which is the

10

sum of grey matter, white matter and cerebrospinal fluid), in order to correct for head size. Because of a skewed distribution, WMH volume was log transformed in the analyses.

2.5.2. *Amyloid binding*

Individuals underwent amyloid positron emission tomography (PET) imaging using a specific fibrillary amyloid β radiotracer, [18F]flutemetamol, which was produced by General Electric (GE) Healthcare at the Cyclotron Research Centre of the University of Liège (Liège, Belgium). PET scans were performed on a Philips Ingenuity TF PET-MRI. The emission scan was performed in two parts starting with a 30 minute dynamic scan simultaneously with the bolus intravenous injection of 185 MBq [18F]flutemetamol, followed by a 20 minute scan performed 90-110 minutes after the injection. A T1-weighted gradient echo pulse MRI-scan was obtained for attenuation correction. The dynamic data were analyzed on a voxel-by-voxel level using the basis function approach of the Simplified Reference Tissue Model (SRTM) with cerebellar grey matter as reference tissue to determine amyloid non-displaceable binding potential (BP_{ND}) (Wu & Carson, 2002). Global amyloid BP_{ND} was determined by determining the volume-weighted average BP_{ND} of the frontal (superior, middle, and inferior frontal gyrus), parietal (posterior cingulate, superior parietal gyrus, postcentral gyrus, and inferolateral remainder of parietal lobe) and temporal regions (parahippocampal gyrus, hippocampus, medial temporal lobe, superior, middle, and inferior temporal gyrus). The distribution volume ratio (DVR) was calculated using global amyloid BP_{ND} + 1. For the analyses, DVR was log transformed because of a skewed distribution.

2.6. Statistical analyses

Differences between the cognitively normal and cognitively impaired individuals were investigated using *t*-tests, chi-square tests or Wilcoxon tests. First, we performed linear regression analyses in the total sample to investigate associations between the cognitive composite scores as dependent variables and the risk factors and brain pathology biomarkers

11

as independent variables. To test the independent effect of the brain pathology biomarkers on the cognitive composite scores, we performed multivariate linear regression analyses including the significant brain pathology biomarkers in the same model and we tested whether the brain pathology biomarkers were mutually associated. Secondly, we performed linear regression analyses to investigate associations between the brain pathology biomarkers as dependent variables and the risk factors as independent variables in the total sample. To test whether results were driven by the cognitively impaired individuals, we repeated the linear regression analyses separately in the cognitively normal and impaired individuals for the variables that showed a significant association in the total sample. All linear regression analyses were adjusted for age, sex and years of education. If a risk factor was both significantly associated with a cognitive composite score and a brain pathology biomarker, mediation analyses were performed to test whether the brain pathology biomarker mediated the effect of the risk factors on the cognitive composite. For these mediation analyses, we first tested whether the brain pathology biomarker was significantly associated with the cognitive composite score in the presence of the risk factor. If so, we calculated the direct, indirect and total effect (direct + indirect effect) of the risk factor on the cognitive composite score by using structural equation modeling of the Lavaan package in R (Rosseel, 2002). The proportion mediated was determined as the indirect effect divided by the total effect and all mediation analyses were adjusted for age, sex and years of education. The p-value threshold for significance was set at 0.05 and additionally we indicated significance after Bonferroni correction (p-value < 0.05/3). Analyses were performed in R-Studio version 1.1.414 with R version 3.4.3.

3. Results

Characteristics of the 122 individuals included in the EMIF-AD 90+ Study are shown in
Table 1. Individuals were on average 92.4 years (SD 2.8, IQR: 90.5-93.5 years), 70 (57.4%)
were female and their mean years of education was 11.4 (SD 3.4). We included 84
cognitively normal and 38 cognitively impaired individuals. The individuals with cognitive
impairment scored lower on the three cognitive composite scores (see eTable 1 for scores on
individual tests). The three cognitive composite scores were significantly correlated with each
other in the total sample (p-values < 0.05). Individuals with cognitive impairment had a lower
handgrip strength, a lower score on the SPPB, a lower score on the MNA, a lower level of
leukocytes, more hippocampal atrophy and a higher amyloid binding (Table 1).

3.1. Associations of risk factors and brain pathology biomarkers with cognition

Lower values for handgrip strength, SPPB, MNA, HbA1c and hippocampal volume, and
higher values for WMH volume and amyloid binding were associated with worse scores on
all three cognitive composites (Table 2).

In order to test the independent effect of brain pathology biomarkers on memory and
processing speed, we analyzed hippocampal volume, WMH and amyloid binding together in
a multivariate model. These analyses showed that WMH volume (memory: β = -0.25, p-value
< 0.01; processing speed: β = -0.21, p-value = 0.02), hippocampal volume (memory: β =
0.47, p-value < 0.01; processing speed: β = 0.22, p-value = 0.03) and amyloid binding
(memory: β = -0.28, p-value < 0.01; processing speed: β = -0.31, p-value < 0.01) were all
independently associated with memory and processing speed. In line with this, WMH,
hippocampal volume and amyloid binding did not correlate with each other.

Analyses of the significant associations in the total sample were repeated separately for the
cognitively normal and impaired individuals (Figure 1, eTable 2 and eTable 3). In the

13

cognitively normal individuals 12 out of 17 associations of risk factors and brain pathology biomarkers with cognition were statistically significant (n=8) or tended to be significant (n=4) while in the cognitively impaired individuals, there was only one association with cognition statistically significant.

3.2. <u>Associations of risk factors with brain pathology biomarkers</u>

Lower muscle mass and a lower score on the SPPB were associated with higher WMH volume (Table 3). A lower score on the SPPB was also associated with hippocampal atrophy. Higher past cognitive activity and lower BMI were associated with higher amyloid binding. Analyses of the significant associations were repeated separately for the cognitively normal and impaired individuals and showed that associations were present in the cognitively normal individuals, except for muscle mass with WMH volume, but not in the cognitively impaired individuals (Figure 2, eTable 4 and eTable 5 in the Supplement).

3.3. <u>Mediation analyses</u>

The SPPB score was the only correlate of cognition that was also associated with brain pathology biomarkers. Criteria for mediation analyses were met for the association between hippocampal volume with memory and executive functioning. Mediation analyses showed that hippocampal volume mediated 38.5% of the effect of SPPB on memory and 33.3% of the effect of SPPB on executive functioning (Figure 3).

14

4. Discussion

In this unique, extensively tested population of oldest-old individuals, physical parameters and brain pathology biomarkers were the most important factors to be associated with cognition. The three brain pathology biomarkers, WMH, hippocampal volume and amyloid aggregation, were all independently related to cognition. We also found an association between the physical parameters and brain pathology biomarkers. The association between cognition and physical performance was partly mediated by hippocampal volume. In the following paragraphs, we will discuss our findings more specifically per risk factor group.

4.1. Cognitive activity

Most longitudinal studies in younger elderly have associated higher cognitive activity with reduced cognitive decline (Vemuri et al., 2014; Wilson et al., 2013). We did not find an association between past cognitive activity at the ages 6, 12, 18 and 40 years, and cognitive functioning after age 90 years indicating that the protective effect of cognitive activity at these ages may not persist in the oldest-old. This is in line with another study that did not find an association between lifestyle factors, including cognitive activities, at age 70 years and incident dementia after age 90 years (Paganini-Hill et al., 2016). Surprisingly, higher past cognitive activity was related to more amyloid aggregation which is in contrast to previous studies in younger cognitively normal elderly (Landau et al., 2012; Wirth et al., 2014). A possible explanation for this association in the cognitively normal oldest-old individuals might be that high past cognitive activity leads to more cognitive reserve which may protect against the negative effect of amyloid aggregation on cognition. In line with this explanation, we did not find a significant association between past cognitive activity and amyloid aggregation in the cognitively impaired individuals.

15

4.2. <u>Physical parameters</u>

Of all risk factors tested, handgrip strength and physical performance showed the strongest association with cognition. The associations of higher handgrip strength and physical performance with better cognition have been shown before in populations over the age of 40 years (Clouston et al., 2013) and also in populations aged 85 years and older (Bullain et al., 2016; Taekema et al., 2010). Different mechanisms may explain the association between physical parameters and cognition. Physical activity has been shown to increase hippocampal volume, potentially by increasing the secretion of myokines and subsequently the level of brain-derived neurotrophic factor (BDNF), and thereby improving cognitive performance (Delezie & Handschin, 2018). Our study showed that the association between physical performance and cognition was partially mediated by hippocampal volume. This indicates that the mechanism described for physical activity, might also apply to physical performance. Another explanation for the association between physical parameters and cognition includes the possibility of a common driving factor, for example WMH or other vascular brain pathologies (Guttmann et al., 2000). Furthermore, poor physical function can also be a consequence of cognitive impairment rather than a cause (Taekema et al., 2012). However, due to the cross-sectional nature of this study we can only speculate about possible mechanisms without making any statements about potential causalities.

4.3. <u>Nutritional status</u>

In line with our findings, malnutrition has been associated with lower cognitive performance in the oldest-old (Hai et al., 2017) and with brain pathology in individuals who were on average 80 years old (de van der Schueren et al., 2016). Most likely, malnutrition is a consequence of worse cognitive functioning, which also explains the lack of an association in cognitively normal individuals. The association between lower BMI and more amyloid

16

aggregation has been shown before in younger elderly with normal cognition or MCI (Vidoni et al., 2011). This association might be explained by neuropathological changes in brain areas that are important for metabolism regulation or by lifestyle changes in individuals with AD pathology (Vidoni et al., 2011).

4.4. Inflammatory markers

We found a lower number of leukocytes in the cognitively impaired compared to the cognitively normal individuals but no association between leukocyte count and cognition. In AD, decreased levels of lymphocytes and basophils have been shown before, which may indicate bone marrow suppression in the presence of neurodegeneration (Chen et al., 2017). More specific white blood cell counts, for example by studying peripheral blood mononuclear cells (PBMC's), are necessary to further elucidate this finding (Arosio et al., 2014). In addition, we did not find an association between CRP level and cognitive functioning, which is in line with a previous study performed in individuals aged 85 years (Schram et al., 2007). An association between CRP and cognitive functioning has, however, been found in younger elderly (Schram et al., 2007). The lack of an association in our study might be explained by selective survival as high levels of CRP are related to increased mortality risk leaving only the individuals who are less susceptible for the negative consequences of high CRP levels in the study sample (Kravitz et al., 2009). Alternatively, this finding might be due to the low sensitivity assay used to measure CRP.

17

4.5. <u>Cardiovascular risk factors</u>

The lack of an association between blood pressure and cholesterol with cognition is in line with most previous literature who found that cardiovascular risk factors measured in late-life are not associated with cognitive impairment (N. Legdeur et al., 2018), unlike cardiovascular risk factors measured in mid-life (Knopman et al., 2018).

The positive association of glucose levels with memory and executive functioning may reflect a better nutritional status in those with better cognitive functioning (Abdelhafiz & Sinclair, 2015). There was no association between the cardiovascular risk factors and brain pathology biomarkers. Especially for WMH and blood pressure this is remarkable as previous studies in younger elderly showed that hypertension is an important risk factor for WMH (Skoog, 1998). A possible explanation is the selective survival of individuals who are less susceptible for the negative effects of cardiovascular risk factors on brain pathologies (de Leeuw et al., 2002). In addition, we did not have information about the duration of the high blood pressure and especially long-standing high blood pressure might be associated with WMH (de Leeuw et al., 2002).

4.6. <u>Brain pathology biomarkers</u>

Neuropathological studies suggested that the association between brain pathologies and cognitive status weakens in the oldest-old (Savva et al., 2009). However, we found that WMH, hippocampal atrophy and amyloid aggregation were still independently associated with memory, executive functioning or processing speed. These biomarkers did not correlate with each other, which is for WMH and amyloid aggregation in line with most previous literature (Roseborough et al., 2017). However, in younger cognitively normal elderly, amyloid aggregation and WMH are associated with hippocampal atrophy (Bourgeat et al., 2010; Fiford et al., 2017). This could indicate that other pathologies than AD pathology or

18

vascular damage underlie hippocampal atrophy in the oldest-old, such as hippocampal sclerosis and argyrophilic grain disease (Barkhof et al., 2007). The finding that brain pathologies are associated with cognitive functioning at higher age, is in line with another study conducted in a population aged 90 years and older (Nienke Legdeur et al., 2019).

4.7. Strengths and limitations

The strength of this study is that we tested the association between a broad range of risk factors with three important brain pathology biomarkers in the oldest-old. We applied Bonferroni correction (after which four of the 22 significant associations became non-significant) and reported these in Table 2 and 3, but given the explorative nature of this study we described the uncorrected results. A limitation of the study is the small sample size which may have limited statistical power. The cross-sectional design precluded conclusions on the causality of associations observed. Muscle strength was measured by use of handgrip strength, which cannot be seen as proxy for overall muscle strength. Furthermore, CRP and leukocyte count are relatively insensitive markers to measure inflammation and also for cognitive activity a more comprehensive measurement than the CAQ would potentially be more sensitive.

4.8. Conclusions and future directions

Cognitive impairment in the oldest-old does not occur in isolation and this study showed that a broad range of risk and protective factors needs to be considered. This study is unique in its extensive phenotyping of oldest-old individuals. We found that especially higher physical performance and strength seem to be important aspects in preserving cognitive functioning in

19

the oldest-old, which indicates that physical parameters are potential targets for interventions to prevent cognitive deterioration in cognitively normal individuals at high age. Furthermore, this study adds important findings regarding the role of brain pathology biomarkers in the oldest-old as it shows that, although some post-mortem studies have suggested otherwise, brain pathology biomarkers are also in the oldest-old associated with cognitive functioning. Longitudinal studies are needed to further elucidate the effect of these factors on cognition in the oldest-old.

Acknowledgments

The authors thank all study participants.

Anonymized data, analytic methods and study materials will be shared on request.

This study was not preregistered.

Conflict of Interest

None reported

References

Abdelhafiz, A. H., & Sinclair, A. J. (2015). Low HbA1c and increased mortality risk-is frailty a confounding factor? *Aging and Disease*, *6*(4), 262. https://doi.org/10.14336/ad.2014.1022

Abikoff, H., Alvir, J., Hong, G., Sukoff, R., Orazio, J., Solomon, S., & Saravay, S. (1987). Logical memory subtest of the Wechsler Memory Scale: age and education norms and alternate-form reliability of two scoring systems. *J Clin Exp Neuropsychol*, *9*(4), 435–448. https://doi.org/10.1080/01688638708405063

Arosio, B., D'Addario, C., Gussago, C., Casati, M., Tedone, E., Ferri, E., Nicolini, P., Rossi, P. D., Maccarrone, M., & Mari, D. (2014). Peripheral blood mononuclear cells as a laboratory to study dementia in the elderly. *BioMed Research International*, *2014*. https://doi.org/10.1155/2014/169203

Barkhof, F., Polvikoski, T. M., Straaten, E. C. W. van, Kalaria, R. N., Sulkava, R., Aronen, H. J., Niinistö, L., Rastas, S., Oinas, M., Scheltens, P., & Erkinjuntti, T. (2007). The significance of medial temporal lobe atrophy: a postmortem MRI study in the very old. *Neurology*, *69*(15), 1521–1527. https://doi.org/10.1212/01.wnl.0000277459.83543.99

Bourgeat, P., Chételat, G., Villemagne, V. L., Fripp, J., Raniga, P., Pike, K., Acosta, O., Szoeke, C., Ourselin, S., Ames, D., Ellis, K. A., Martins, R. N., Masters, C. L., Rowe, C. C., & Salvado, O. (2010). β-Amyloid burden in the temporal neocortex is related to hippocampal atrophy in elderly subjects without dementia. *Neurology*, *74*(2), 121–127. https://doi.org/10.1212/WNL.0b013e3181c918b5

Bullain, S. S., Corrada, M. M., Perry, S. M., & Kawas, C. H. (2016). Sound Body Sound Mind? Physical Performance and the Risk of Dementia in the Oldest-Old: The 90+ Study. *Journal of the American Geriatrics Society*, *64*(7), 1408–1415. https://doi.org/10.1111/jgs.14224

Chen, S. H., Bu, X. L., Jin, W. S., Shen, L. L., Wang, J., Zhuang, Z. Q., Zhang, T., Zeng, F., Yao, X. Q., Zhou, H. D., & Wang, Y. J. (2017). Altered peripheral profile of blood cells in Alzheimer disease: A hospital-based case-control study. *Medicine (Baltimore)*, *96*, e6843. https://doi.org/10.1097/MD.0000000000006843

Clouston, S. A. P., Brewster, P., Kuh, D., Richards, M., Cooper, R., Hardy, R., Rubin, M. S., & Hofer, S. M. (2013). The dynamic relationship between physical function and cognition in longitudinal aging cohorts. *Epidemiologic Reviews*, *35*(1), 33–50. https://doi.org/10.1093/epirev/mxs004

Corrada, M. M., Brookmeyer, R., Berlau, D., Paganini-Hill, A., & Kawas, C. H. (2008). Prevalence of dementia after age 90: results from the 90+ study. *Neurology*, *71*(5), 337–343. https://doi.org/10.1212/01.wnl.0000310773.65918.cd

de Leeuw, F.-E., de Groot, J. C., Oudkerk, M., Witteman, J. C. M., Hofman, A., van Gijn, J., & Breteler, M. M. B. (2002). Hypertension and cerebral white matter lesions in a prospective cohort study. *Brain*, *125*(Pt 4), 765–772. https://doi.org/10.1093/brain/awf077

de van der Schueren, M. A. E., Lonterman-Monasch, S., van der Flier, W. M., Kramer, M. H., Maier, A. B., & Muller, M. (2016). Malnutrition and Risk of Structural Brain Changes Seen on Magnetic Resonance Imaging in Older Adults. *Journal of the American Geriatrics Society*, *64*(12), 2457–2463. https://doi.org/10.1111/jgs.14385

Debette, S., Seshadri, S., Beiser, A., Au, R., Himali, J. J., Palumbo, C., Wolf, P. A., & DeCarli, C. (2011). Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. *Neurology*, *77*(5), 461–468. https://doi.org/10.1212/WNL.0b013e318227b227

Delezie, J., & Handschin, C. (2018). Endocrine crosstalk between Skeletal muscle and the brain. *Frontiers in Neurology*, *9*(AUG). https://doi.org/10.3389/fneur.2018.00698

Fiford, C. M., Manning, E. N., Bartlett, J. W., Cash, D. M., Malone, I. B., Ridgway, G. R., Lehmann, M., Leung, K. K., Sudre, C. H., Ourselin, S., Biessels, G. J., Carmichael, O. T., Fox, N. C., Cardoso, M. J., & Barnes, J. (2017). White matter hyperintensities are associated with disproportionate progressive hippocampal atrophy. *Hippocampus*, *27*(3), 249–262. https://doi.org/10.1002/hipo.22690

Fischl, B., Salat, D. H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., van der Kouwe, A., Killiany, R., Kennedy, D., Klaveness, S., Montillo, A., Makris, N., Rosen, B., & Dale, A. M. (2002). Whole Brain Segmentation: Neurotechnique Automated Labeling of NeuroanatomicalStructures in the Human Brain. *Neuron*, *33*(3), 341–355. https://doi.org/10.1016/S0896-6273(02)00569-X

Guralnik, J. M., Ferrucci, L., Simonsick, E. M., Salive, M. E., & Wallace, R. B. (1995). Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. *New England Journal of Medicine*, *332*(9), 556–562.

Guttmann, C. R. G., Benson, R., Warfield, S. K., Wei, X., Anderson, M. C., Hall, C. B., Abu-Hasaballah, K., J.P. Mugler III, & Wolfson, L. (2000). White matter abnormalities in mobility-impaired older persons. *Neurology*, *54*(6), 1277–1283. https://doi.org/10.1212/wnl.54.6.1277

Hai, S., Cao, L., Yang, X., Wang, H., Liu, P., Hao, Q., & Dong, B. (2017). Association Between Nutrition Status and Cognitive Impairment Among Chinese Nonagenarians and Centenarians. *International Journal of Gerontology*, *11*, 215–219. https://doi.org/10.1111/ggi.12410

Holstege, H., Beker, N., Dijkstra, T., Pieterse, K., Wemmenhove, E., Schouten, K., Thiessens, L., Horsten, D., Rechtuijt, S., Sikkes, S., van Poppel, F. W. A., Meijers-Heijboer, H., Hulsman, M., & Scheltens, P. (2018). The 100-plus Study of cognitively healthy centenarians: rationale, design and cohort description. *European Journal of Epidemiology*, *3*, 1229–1249. https://doi.org/10.1007/s10654-018-0451-3

Isautier, J., Bosnić, M., Yeung, S., Trappenburg, M., Meskers, C., Whittaker, A., & Maier, A. (2019). Validity of Nutritional Screening Tools available for Community-Dwelling Older Adults: A Systematic Review and Meta-Analysis. Under review. *J Am Med Dir Assoc*, *20*(10), 1351.e13-1351.e25. https://doi.org/10.1016/j.jamda.2019.06.024

Kawas, C. H., Greenia, D. E., Bullain, S. S., Clark, C. M., Pontecorvo, M. J., Joshi, A. D., & Corrada, M. M. (2013). Amyloid imaging and cognitive decline in nondemented oldest-old: the 90+ Study. *Alzheimer's & Dementia : The Journal of the Alzheimer's Association*, *9*(2), 199–203. https://doi.org/10.1016/j.jalz.2012.06.005

Knopman, D. S., Gottesman, R. F., Sharrett, A. R., Tapia, A. L., DavisThomas, S., Windham, B. G., Coker, L., Schneider, A. L. C., Alonso, A., Coresh, J., Albert, M. S., & Mosley, T. H. (2018). Midlife vascular risk factors and midlife cognitive status in relation to prevalence of mild cognitive impairment and dementia in later life: The Atherosclerosis Risk in Communities Study. *Alzheimer's and Dementia*, *14*, 1406–1415. https://doi.org/http://dx.doi.org/10.1016/j.jalz.2018.03.011

Konijnenberg, E., Carter, S. F., Ten Kate, M., Den Braber, A., Tomassen, J., Amadi, C., Wesselman, L., Nguyen, H. T., Van De Kreeke, J. A., Yaqub, M., Demuru, M., Mulder, S. D., Hillebrand, A., Bouwman, F. H., Teunissen, C. E., Serné, E. H., Moll, A. C., Verbraak, F. D., Hinz, R., … Visser, P. J. (2018). The EMIF-AD PreclinAD study: Study design and baseline cohort overview. *Alzheimer's Research and Therapy*, *10*(1), 1–12. https://doi.org/10.1186/s13195-018-0406-7

Kravitz, B. A., Corrada, M. M., & Kawas, C. H. (2009). High levels of serum C-reactive protein are associated with greater risk of all-cause mortality, but not dementia, in the oldest-old: results from The 90+ Study. *J Am Geriatr Soc*, *57*(4), 641–646. https://doi.org/10.1111/j.1532-5415.2009.02169.x

Landau, S. M., Marks, S. M., Mormino, E. C., Rabinovici, G. D., Oh, H., O'Neil, J. P., Wilson, R. S., & Jagust, W. J. (2012). Association of lifetime cognitive engagement and low β-amyloid deposition. *Archives of Neurology*, *69*(5), 623–629. https://doi.org/10.1001/archneurol.2011.2748

Legdeur, N., Heymans, M. W., Comijs, H. C., Huisman, M., Maier, A. B., & Visser, P. J. (2018). Age dependency of risk factors for cognitive decline. *BMC Geriatrics*, *18*(1), 1–10. https://doi.org/10.1186/s12877-018-0876-2

Legdeur, Nienke, Badissi, M., Carter, S. F., Crom, S. De, Kreeke, A. Van De, Vreeswijk, R., Trappenburg, M. C., Oudega, M. L., Koek, H. L., Campen, J. P. Van, Keijsers, C. J. P. W., Amadi, C., Hinz, R., Gordon, M. F., Novak, G., Podhorna, J., Serné, E., Verbraak, F., Yaqub, M., … Kramer, S. E. (2018). Resilience to cognitive impairment in the oldest-old: design of the EMIF-AD 90 + study. *BMC Geriatrics*, *18*(289), 1–16. https://doi.org/10.1186/s12877-018-0984-z

Legdeur, Nienke, Visser, P. J., Woodworth, D. C., Muller, M., Fletcher, E., Maillard, P., Scheltens, P., DeCarli, C., Kawas, C. H., & Corrada, M. M. (2019). White matter hyperintensities and hippocampal atrophy in relation to cognition: The 90+ Study. *J Am Geriatr Soc*, 1–8. https://doi.org/10.1111/jgs.15990

Lindeboom, J., Schmand, B., Tulner, L., Walstra, G., & Jonker, C. (2002). Visual association test to detect early dementia of the Alzheimer type. *Journal of Neurology Neurosurgery and Psychiatry*, *73*(2), 126–133. https://doi.org/10.1136/jnnp.73.2.126

Ling, C. H. Y., Craen, A. J. M. De, Slagboom, P. E., Gunn, D. A., Stokkel, M. P. M., Westendorp, R. G. J., & Maier, A. B. (2011). Accuracy of direct segmental multi-frequency bioimpedance analysis in the assessment of total body and segmental body composition in middle-aged adult population. *Clinical Nutrition*, *30*, 610–615. https://doi.org/10.1016/j.clnu.2011.04.001

McKhann, G., Drachman, D., Folstein, M., & Katzman, R. (1984). Clinical diagnosis of Alzheimer's disease: Report of the MINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Forse on Alzheimer's Disease. *Neurology*, *34*(7), 939. https://doi.org/10.3233/JAD-122299

Melikyan, Z. A., Corrada, M. M., Dick, M. B., Whittle, C., Paganini-Hill, A., & Kawas, C. H. (2019). Neuropsychological Test Norms in Cognitively Intact Oldest-Old. *Journal of the International Neuropsychological Society*, *25*(5), 530–545. https://doi.org/10.1017/S1355617719000122

Meyers, J. E., Bayless, J. D., & Meyers, K. R. (1996). Rey complex figure: memory error patterns and functional abilities. *Applied Neuropsychology*, *3*(2), 89–92. https://doi.org/10.1207/s15324826an0302

Morris, J., Heyman, A., Mohs, R., Hughes, J., van Belle, G., Fillenbaum, G., Mellits, E., & Clark, C. (1989). The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assesment of Alzheimer's disease. *Neurology*, *39*(9), 1159. https://doi.org/10.1212/WNL.39.9.1159

Paganini-Hill, A., Kawas, C. H., & Corrada, M. M. (2016). Lifestyle Factors and Dementia in the Oldest-old: The 90+ Study. *Alzheimer Dis Assoc Disord*, *30*(1), 21–26. https://doi.org/10.1097/WAD.0000000000000087

Petersen, R. C. (2004). Mild cognitive impairment as a diagnostic entity. *Journal of Internal Medicine*, *256*(3), 183–194. https://doi.org/10.1111/j.1365-2796.2004.01388.x

Petersen, R. C., Jack, C. R., Xu, Y.-C., Waring, S. C., O'Brien, P. C., Smith, G. E., Ivnik, R. J., Tangalos, E. G., Boeve, B. F., & Kokmen, E. (2000). Memory and MRI-based hippocampal volumes in aging and AD. *Neurology*, *54*(3), 581–587. https://doi.org/10.1212/WNL.54.3.575

Reijnierse, E. M., de Jong, N., Trappenburg, M. C., Blauw, G. J., Butler-Browne, G.,

Gapeyeva, H., Hogrel, J. Y., McPhee, J. S., Narici, M. V, Sipila, S., Stenroth, L., van Lummel, R. C., Pijnappels, M., Meskers, C. G. M., & Maier, A. B. (2017). Assessment of maximal handgrip strength: how many attempts are needed? *J Cachexia Sarcopenia Muscle*, *8*(3), 466–474. https://doi.org/10.1002/jcsm.12181

Reijs, B. L., Teunissen, C. E., Goncharenko, N., Betsou, F., Blennow, K., Baldeiras, I., Brosseron, F., Cavedo, E., Fladby, T., Froelich, L., Gabryelewicz, T., Gurvit, H., Kapaki, E., Koson, P., Kulic, L., Lehmann, S., Lewczuk, P., Lleo, A., Maetzler, W., … Visser, P. J. (2015). The Central Biobank and Virtual Biobank of BIOMARKAPD: A Resource for Studies on Neurodegenerative Diseases. *Front Neurol*, *6*, 216. https://doi.org/10.3389/fneur.2015.00216

Reitan, R. M. (1958). Validity of the Trail Making Test as an indicator of organic brain damage. *Perceptual and Motor Skills*, *8*(3), 271–276.

Robbins, T. W., James, M., Owen, A. M., Sahakian, B. J., McInnes, L., & Rabbitt, P. (1994). Cambridge Neuropsychological Test Automated Battery (CANTAB): a factor analytic study of a large sample of normal elderly volunteers. *Dementia (Basel, Switzerland)*, *5*, 266–281.

Rodrigue, K. M., Rieck, J. R., Kennedy, K. M., Devous, M. D., Diaz-Arrastia, R., & Park, D. C. (2013). Risk factors for β-amyloid deposition in healthy aging: Vascular and genetic effects. *JAMA Neurology*, *70*(5), 600–606. https://doi.org/10.1001/jamaneurol.2013.1342

Roseborough, A., Ramirez, J., Black, S. E., & Edwards, J. D. (2017). Associations between amyloid β and white matter hyperintensities: A systematic review. *Alzheimer's and Dementia*, *13*(10), 1154–1167. https://doi.org/10.1016/j.jalz.2017.01.026

Rosseel, Y. (2002). lavaan: An R Package for Structural Equation Modeling. *Journal of Statistical Software*, *48*(2), 1–36.

Royall, D. R., Cordes, J. A., & Polk, M. (1998). CLOX: an executive clock drawing task. *J Neurol Neurosurg Psychiatry*, *64*, 588–594.

Savva, G. M., Wharton, S. B., Ince, P. G., Forster, G., Matthews, F. E., & Brayne, C. (2009). Age, Neuropathology, and Dementia. *The New England Journal of Medicine*, *360*, 2302–2309. https://doi.org/10.1056/NEJMoa0806142

Schram, M. T., Euser, S. M., de Craen, A. J. M., Witteman, J. C., Frolich, M., Hofman, A., Jolles, J., Breteler, M. M. B., & Westendorp, R. G. J. (2007). Systemic markers of inflammation and cognitive decline in old age. *Journal of the American Geriatrics Society*, *55*(5), 708–716. https://doi.org/10.1111/j.1532-5415.2007.01159.x

Skoog, I. (1998). A Review on Blood Pressure and Ischaemic White Matter Lesions. *Dementia and Geriatric Cognitive Disorders*, *9*(suppl 1), 13–19. https://doi.org/10.1159/000051184

Sudre, C. H., Cardoso, M. J., Bouvy, W. H., Biessels, G. J., Barnes, J., & Ourselin, S. (2015). Bayesian Model Selection for Pathological Neuroimaging Data Applied to White Matter Lesion Segmentation. *IEEE Transactions on Medical Imaging*, *34*(10), 2079–2102. https://doi.org/10.1109/TMI.2015.2419072

Taekema, D. G., Gussekloo, J., Maier, A., Westendorp, R., & de Craen, A. (2010). Handgrip strength as a predictor of functional, psychological and social health. A prospective population-based study among the oldest old. *Age Ageing*, *39*(3), 331–337. https://doi.org/10.1093/ageing/afq022

Taekema, D. G., Ling, C. H., Kurrle, S. E., Cameron, I. D., Meskers, C. G., Blauw, G. J., Westendorp, R. G., De craen, A. J., & Maier, A. B. (2012). Temporal relationship between handgrip strength and cognitive performance in oldest old people. *Age and Ageing*, *41*(4), 506–512. https://doi.org/10.1093/ageing/afs013

Tombaugh, T. N. (1999). Normative Data Stratified by Age and Education for Two Measures

25

of Verbal Fluency: FAS and Animal Naming. *Archives of Clinical Neuropsychology*, *14*(2), 167–177.

Vemuri, P., Lesnick, T. G., Przybelski, S. A., Knopman, D. S., Preboske, G. M., Kantarci, K., Raman, M. R., Machulda, M. M., Mielke, M. M., Lowe, V. J., Senjem, M. L., Gunter, J. L., Rocca, W. A., Roberts, R. O., Petersen, R. C., & Jack, C. R. (2015). Vascular and amyloid pathologies are independent predictors of cognitive decline in normal elderly. *Brain*, *138*(3), 761–771. https://doi.org/10.1093/brain/awu393

Vemuri, P., Lesnick, T. G., Przybelski, S. A., Machulda, M., Knopman, D. S., Mielke, M. M., Roberts, R. O., Geda, Y. E., Rocca, W. A., Petersen, R. C., & Jack, C. R. J. (2014). Association of lifetime intellectual enrichment with cognitive decline in the older population. *JAMA Neurology*, *71*(8), 1017–1024. https://doi.org/10.1001/jamaneurol.2014.963

Vidoni, E. D., Townley, R. A., Honea, R. A., & Burns, J. M. (2011). Alzheimer disease biomarkers are associated with body mass index. *Neurology*, *77*(21), 1913–1920. https://doi.org/10.1212/WNL.0b013e318238eec1

Wang, R., Fratiglioni, L., Kalpouzos, G., Lövdén, M., Laukka, E. J., Bronge, L., Wahlund, L. O., Bäckman, L., & Qiu, C. (2017). Mixed brain lesions mediate the association between cardiovascular risk burden and cognitive decline in old age: A population-based study. *Alzheimer's and Dementia*, *13*(3), 247–256. https://doi.org/10.1016/j.jalz.2016.06.2363

Wechsler, D. (1981). *Wechsler adult intelligence scale. Revised manual (WAIS-R)*.

Wilson, R. S., Barnes, L. L., & Bennett, D. A. (2003). Assessment of lifetime participation in cognitively stimulating activities. *Journal of Clinical and Experimental Neuropsychology*, *25*(5), 634–642. https://doi.org/10.1076/jcen.25.5.634.14572

Wilson, R. S., Boyle, P. A., Yu, L., Barnes, L. L., Schneider, J. A., & Bennett, D. A. (2013). Life-span cognitive activity, neuropathologic burden, and cognitive aging. *Neurology*, *81*(4), 314–321. https://doi.org/10.1212/WNL.0b013e31829c5e8a

Wirth, M., Villeneuve, S., La Joie, R., Marks, S. M., & Jagust, W. J. (2014). Gene-environment interactions: lifetime cognitive activity, APOE genotype, and beta-amyloid burden. *The Journal of Neuroscience*, *34*(25), 8612–8617. https://doi.org/10.1523/JNEUROSCI.4612-13.2014

World Population Prospects: The 2017 Revision. Population by Broad Age Groups - Both Sexes. (2017). United Nations, Department of Economic and Social Affairs, Population Division. https://population.un.org/wpp/Download/Standard/Population/

Wu, Y., & Carson, R. E. (2002). Noise reduction in the simplified reference tissue model for neuroreceptor functional imaging. *Journal of Cerebral Blood Flow & Metabolism*, *22*(12), 1440–1452. https://doi.org/https://doi.org/10.1097/01.WCB.0000033967.83623.34

Table 1. Clinical characteristics and correlates of cognition in the total sample and separately for the cognitively normal (CN) and impaired (CI) individuals

	N	Total	N	CN	N	CI	p-value
				Cognitive group			
Clinical characteristics							
Age, y	122	92.4 (2.8)	84	92.8 (2.9)	38	91.6 (2.4)	**0.02**[e]
Sex, female[a]	122	70 (57.4)	84	45 (53.6)	38	25 (65.8)	0.29[f]
Education, y	122	11.4 (3.4)	84	11.2 (3.4)	38	11.9 (3.3)	0.32[g]
MMSE, points	122	27.1 (3.1)	84	28.5 (1.5)	38	23.8 (3.3)	**<0.01**[g]
CERAD memory test, words	114	15.6 (4.8)	79	17.6 (3.8)	35	11.2 (3.5)	**<0.01**[e]
Hypertension[b]	122	84 (69.0)	84	58 (69.0)	38	26 (68.4)	1.00[f]
Diabetes mellitus[b]	122	8 (7.0)	84	5 (6.0)	38	3 (7.9)	0.70[h]
Atrial fibrillation[b]	122	21 (17.0)	84	11 (13.1)	38	10 (26.3)	0.13[f]
Infarction on MRI	92	7 (8.0)	67	6 (9.0)	25	1 (4.0)	0.67[h]
Visual read amyloid, pos[a,c]	84	33 (39.3)	63	20 (31.7)	21	13 (61.9)	**0.03**[f]
Cognitive activity							
Past CAQ, points	100	2.6 (0.6)	70	2.5 (0.5)	30	2.6 (0.7)	0.53[e]
Physical parameters							
Handgrip strength males, kg	50	21.3 (7.0)	38	22.5 (6.7)	12	17.5 (6.8)	**0.04**[e]
Handgrip strength females, kg	66	11.5 (4.6)	44	12.3 (4.0)	22	9.9 (5.4)	0.08[e]
Muscle mass index, kg/m^2	95	9.0 (1.0)	66	9.2 (1.0)	29	8.8 (1.0)	0.09[e]
SPPB, points	103	7.5 (2.8)	70	8.0 (2.7)	33	6.2 (2.6)	**<0.01**[e]

27

	N	Total	N	CN	N	CI	p-value
				Cognitive group			
Nutritional status							
MNA, points	102	12.7 (1.3)	72	12.8 (1.4)	30	12.2 (1.1)	**0.02**[e]
BMI, kg/m^2	108	25.7 (3.8)	75	26.0 (3.8)	33	24.9 (3.7)	0.17[e]
Inflammatory markers							
CRP, mg/L	100	3.0 (3.1)	67	2.9 (2.9)	33	3.2 (3.5)	0.85[e,i]
Leukocytes, 10^9/L	104	7.0 (2.4)	71	7.4 (2.6)	33	6.3 (1.7)	**0.01**[e]
Cardiovascular risk factors							
Systolic BP, mmHg	109	151.9 (24.3)	76	152.0 (26.2)	33	151.6 (19.6)	0.93[e]
Diastolic BP, mmHg	109	78.0 (11.8)	76	77.9 (11.9)	33	78.4 (11.8)	0.85[e]
HbA1c, %	104	5.8 (0.4)	71	5.8 (0.4)	33	5.7 (0.4)	0.22[e]
Total cholesterol, mmol/L	104	5.0 (1.3)	71	5.0 (1.3)	33	5.1 (1.3)	0.62[e]
Brain pathology biomarkers							
WMH volume, % ICV	90	1.5 (1.0)	64	1.4 (1.0)	26	1.7 (1.1)	0.26[e,i]
Hippocampal volume, % ICV	91	0.19 (0.03)	65	0.19 (0.03)	26	0.17 (0.03)	**<0.01**[e]
Amyloid binding[d]	84	1.3 (0.3)	63	1.3 (0.3)	21	1.5 (0.3)	**0.03**[e,i]

Note: Values are presented as mean (SD), unless stated otherwise. P-values compare the cognitively normal (CN) with the cognitively impaired (CI) individuals. BMI: Body Mass Index; BP: blood pressure; CAQ: cognitive activities questionnaire; CRP: C-reactive protein; kg: kilograms; HbA1c: hemoglobin A1c; ICV: intracranial volume; MNA: Mini Nutritional Assessment; N: sample size per variable; pos: positive; WMH: white matter hyperintensity; y: years. [a]Presented as number (%); [b]Based on medical history and/or medication use; [c]Visual read based on amyloid non-displaceable binding potential (BP_{ND}) images; [d]Numbers are the distribution volume ratio (DVR); [e]Tested using t-test; [f]Tested using chi-square test; [g]Tested using Wilcoxon test; [h]Tested using Fisher's exact test; [i]These values are log transformed in the analyses.

28

Table 2. Associations of risk factors and brain pathology biomarkers with cognitive composite scores[a]

	Memory		Executive functioning		Processing speed	
	β[b]	p-value	β[b]	p-value	β[b]	p-value
Cognitive activity						
Past CAQ, points	0.05	0.67	-0.14	0.17	-0.13	0.28
Physical parameters						
Handgrip strength, kg	0.53	**<0.01**[c]	0.21	0.06	0.45	**<0.01**[c]
Muscle mass, kg/m^2	0.20	0.12	0.21	0.07	0.22	0.10
SPPB, points	0.43	**<0.01**[c]	0.24	**<0.01**[c]	0.42	**<0.01**[c]
Nutritional status						
MNA, points	0.23	**0.02**	0.20	**0.02**[c]	0.24	**0.02**[c]
BMI, kg/m^2	0.19	0.06	0.10	0.25	0.18	0.07
Inflammatory markers						
CRP, mg/L	-0.15	0.15	-0.05	0.61	-0.05	0.61
Leukocytes, 10^9/L	0.10	0.31	0.09	0.30	0.11	0.28
Cardiovascular risk factors						
Systolic BP, mmHg	0.11	0.30	-0.03	0.77	0.04	0.72
Diastolic BP, mmHg	0.00	1.00	-0.05	0.58	-0.02	0.86

29

	Memory		Executive functioning		Processing speed	
	β^b	p-value	β^b	p-value	β^b	p-value
HbA1c, %	0.25	**0.01**[c]	0.19	**0.02**[c]	0.02	0.84
Total cholesterol, mmol/L	0.06	0.56	0.05	0.62	-0.02	0.86
Brain pathology biomarkers						
WMH volume, % ICV	-0.25	**0.02**	-0.18	0.06	-0.27	**0.02**[c]
Hippocampal volume, % ICV	0.48	**<0.01**[c]	0.23	**0.02**[c]	0.30	**<0.01**[c]
Amyloid binding[d]	-0.23	**0.05**	-0.08	0.44	-0.27	**<0.01**[c]

Note: BMI: Body Mass Index; BP: blood pressure; CRP: C-Reactive Protein; HbA1c: hemoglobin A1c; MNA: Mini Nutritional Assessment; SPPB: Short Physical Performance Battery; WMH: white matter hyperintensity. [a]The cognitive composite score are z-scores; [b]Standardized regression coefficient from linear regression analysis adjusted for age, sex and years of education; [c]P-value is significant after correcting for the three cognitive outcomes using Bonferroni correction (0.05/3); [d]Determined with the distribution volume ratio (DVR).

Table 3. Associations of risk factors with brain pathology biomarkers

	WMH volume, % ICV		Hippocampal volume, % ICV		Amyloid binding[a]	
	β[b]	p-value	β[b]	p-value	β[b]	p-value
Cognitive activity						
Past CAQ, points	0.10	0.43	0.04	0.75	0.26	**0.05**
Physical parameters						
Handgrip strength, kg	-0.26	0.07	0.18	0.18	0.08	0.58
Muscle mass, kg/m^2	-0.35	**0.01**[c]	-0.04	0.75	-0.25	0.11
SPPB, points	-0.32	**0.01**[c]	0.37	**<0.01**[c]	0.10	0.44
Nutritional status						
MNA, points	-0.18	0.10	0.20	0.07	0.07	0.54
BMI, kg/m^2	-0.06	0.58	0.10	0.36	-0.29	**0.02**[c]
Inflammatory markers						
CRP, mg/L	0.17	0.17	0.13	0.27	-0.11	0.37
Leukocytes, 10^9/L	-0.00	0.99	0.11	0.29	-0.06	0.61
Cardiovascular risk factors						
Systolic BP, mmHg	-0.01	0.91	0.16	0.11	0.06	0.57
Diastolic BP, mmHg	0.08	0.46	0.12	0.26	-0.15	0.20
HbA1c, %	0.02	0.85	0.08	0.43	0.06	0.66

31

	WMH volume, % ICV		Hippocampal volume, % ICV		Amyloid binding[a]	
	β[b]	p-value	β[b]	p-value	β[b]	p-value
Total cholesterol, mmol/L	-0.01	0.96	0.10	0.36	-0.17	0.17

Note: BMI: Body Mass Index; CAQ: cognitive activities questionnaire; CRP: C-Reactive Protein; HbA1c: hemoglobin A1c; MNA: Mini Nutritional Assessment; SPPB: Short Physical Performance Battery; WMH: white matter hyperintensity. [a]Determined with the distribution volume ratio (DVR); [b]Standardized regression coefficient from linear regression analysis adjusted for age, sex and years of education; [c]P-value is significant after correcting for the three cognitive outcomes using Bonferroni correction (0.05/3).
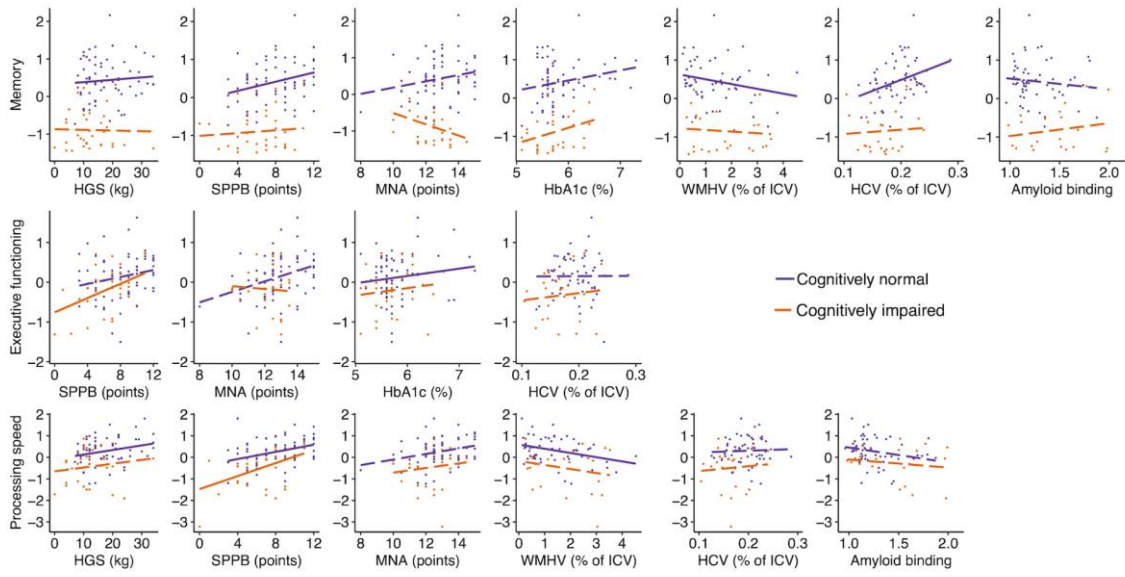
Figure titles and legends

Figure 1. Associations of risk factors and brain pathology biomarkers with cognitive composite scores separately for the cognitively normal and impaired individuals. Scatterplots depict raw values for ease of interpretation and are not corrected for age, sex and education as presented in eTable 2 and eTable 3 in the Supplement. Only the significant associations from Table 2 are visualized. Solid lines indicate a significant (p-value < 0.05) association within that specific cognitive group. Amyloid binding is represented by the distribution volume ratio. BMI: Body Mass Index; HCV: hippocampal volume; HGS: handgrip strength; ICV: intracranial volume; kg: kilograms; MNA: Mini Nutritional Assessment; SPPB: Short Physical Performance Battery; WMHV: white matter hyperintensity volume

Figure 2. Associations of risk factors with brain pathology biomarkers separately for the cognitively normal and impaired individuals. Scatterplots depict raw values for ease of interpretation and are not corrected for age, sex and education as presented in Table 3. Only the significant associations from Table 3 are visualized. Solid lines indicate a significant (p-value < 0.05) association within that specific cognitive group. Amyloid binding is represented by the distribution volume ratio. BMI: Body Mass Index; HCV: hippocampal volume; ICV: intracranial volume; kg/m^2: kilogram per square meter; SPPB: Short Physical Performance Battery; WMHV: white matter hyperintensity volume

Figure 3. Mediation analyses. β is the standardized regression coefficient from the mediation analysis adjusted for age, sex and years of education performed with Lavaan. Proportion mediated is determined as the indirect effect divided by the total effect. SPPB: Short Physical Performance Battery

Figure 1

Figure 2

Figure 3

A

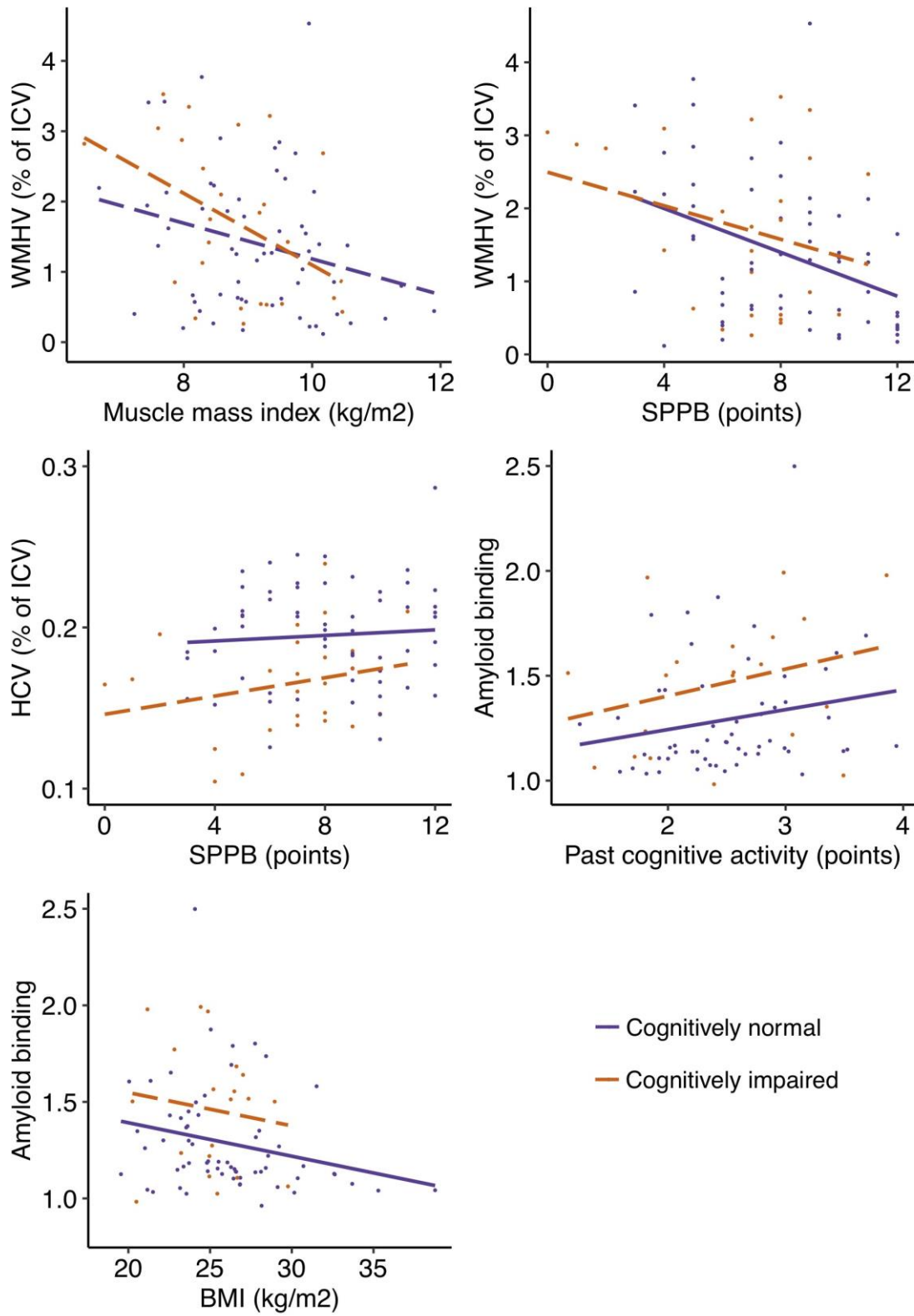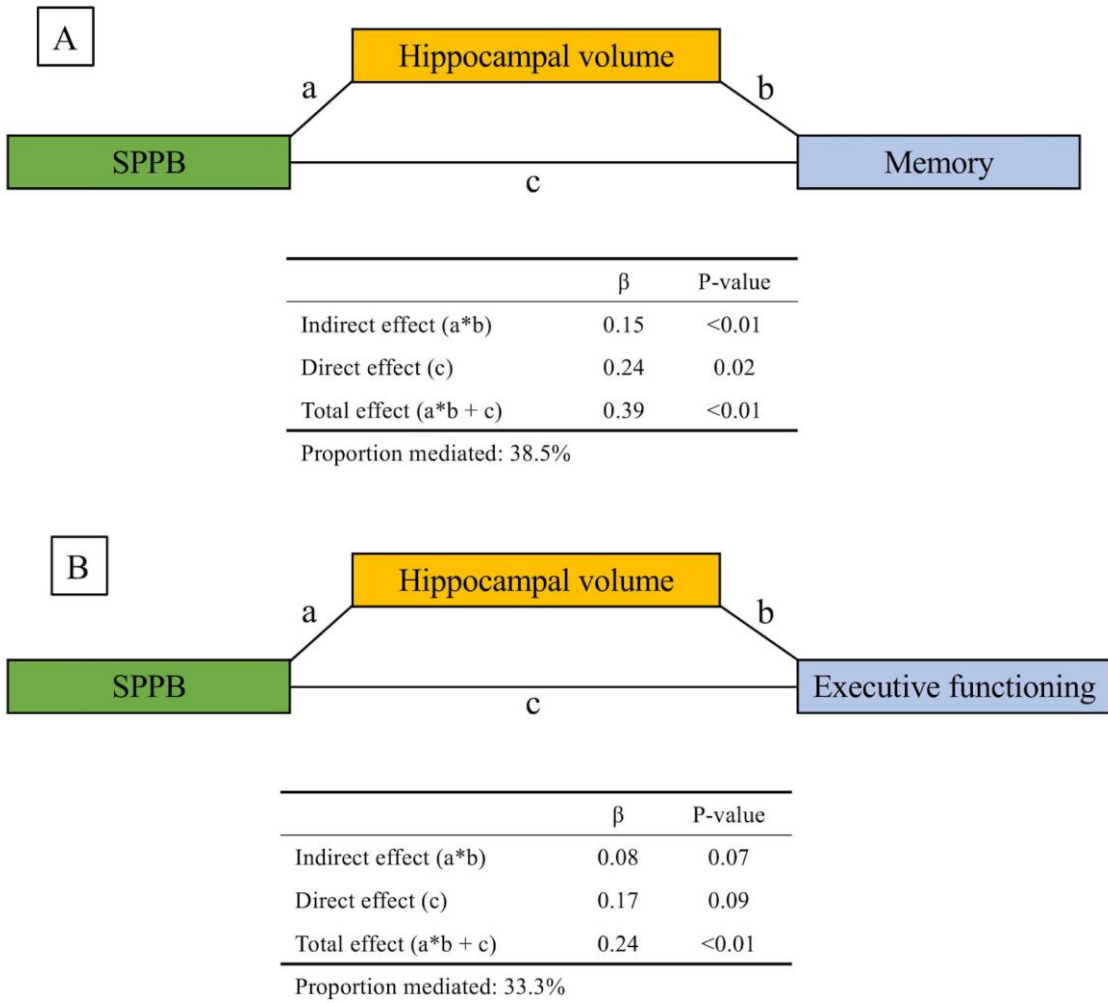Hippocampal volume

a   b

SPPB ──────── c ──────── Memory

	β	P-value
Indirect effect (a*b)	0.15	<0.01
Direct effect (c)	0.24	0.02
Total effect (a*b + c)	0.39	<0.01

Proportion mediated: 38.5%

B

Hippocampal volume

a   b

SPPB ──────── c ──────── Executive functioning

	β	P-value
Indirect effect (a*b)	0.08	0.07
Direct effect (c)	0.17	0.09
Total effect (a*b + c)	0.24	<0.01

Proportion mediated: 33.3%