White Matter Hyperintensities Potentiate Hippocampal Volume Reduction in Non-Demented Older Individuals with Abnormal Amyloid-beta

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White Matter Hyperintensities Potentiate Hippocampal Volume Reduction in Non-Demented Older Individuals with Abnormal Amyloid-β

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Abstract. Cerebral small vessel disease (cSVD) and amyloid-β (Aβ) deposition often co-exist in (prodromal) dementia, and both types of pathology have been associated with neurodegeneration. We examined whether cSVD and Aβ have independent or interactive effects on hippocampal volume (HV) in a memory clinic population. We included 87 individuals with clinical diagnoses of Alzheimer's disease (AD) ($n = 24$), mild cognitive impairment (MCI) ($n = 26$), and subjective cognitive complaints (SCC) ($n = 37$). cSVD magnetic resonance imaging markers included white matter hyperintensity (WMH) volume, lacunar infarct presence, and microbleed presence. Aβ pathology was assessed as cerebrospinal fluid-derived $A\beta_{1\text{-}42}$ levels and dichotomized into normal or abnormal, and HV was determined by manual volumetric measurements. A linear hierarchical regression approach was applied for the detection of additive or interaction effects between cSVD and Aβ on HV in the total participant group ($n = 87$) and in the non-demented group (including SCC and MCI individuals only, $n = 63$). The results revealed that abnormal Aβ and lacunar infarct presence were independently associated with lower HV in the non-demented individuals. Interestingly, Aβ and WMH pathology interacted in the non-demented individuals, such that

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WMH had a negative effect on HV in individuals with abnormal CSF $A\beta_{42}$ levels, but not in individuals with normal CSF $A\beta_{42}$ levels. These associations were not present when individuals with AD were included in the analyses. Our observations suggest that relatively early on in the disease process older individuals with abnormal Aβ levels are at an increased risk of accelerated disease progression when concomitant cSVD is present.

Keywords: Amyloid-beta, cerebral small vessel disease, dementia, neurodegeneration

INTRODUCTION

Alzheimer's disease (AD) and vascular dementia (VaD), the two most prevalent subtypes of dementia, are both characterized by progressive cognitive decline and neurodegeneration. Important pathophysiological processes underlying AD and VaD are cerebral amyloid-beta (Aβ) protein deposition and cerebral small vessel disease (cSVD) [1, 2]. Even though both types of dementia are often considered as separate entities, they share many risk factors and Aβ and cSVD pathology frequently co-occur [3–6]. These observations suggest that the differentiation between AD and VaD can be difficult, and that the pure forms may rather represent two extremes of a pathologic continuum [7]. Moreover, recent studies report positive associations between cerebral Aβ deposition and cSVD [8–12], and the presence of mixed Aβ and cSVD pathology has been shown to increase the risk of developing dementia [13]. These findings indicate that the underlying pathophysiological processes may be interdependent.

Hippocampal volume (HV) reduction is an important neurodegenerative marker for disease progression in dementia [14]. Hippocampal atrophy has been independently associated with both Aβ pathology and cSVD in (prodromal) dementia [15, 16]. Interestingly, a recent animal study has pointed out that comorbid cerebrovascular damage and abnormal cerebral Aβ levels may interact in causing hippocampal cell degeneration [17]. As such, presence of both elevated Aβ and cerebrovascular pathology may predispose individuals to being more vulnerable to neurodegeneration compared to individuals with only a single type of pathology. To date, it remains unclear whether Aβ deposition and cSVD interact in their detrimental effects on HV in individuals across the spectrum of dementia.

The aim of this study was to assess whether Aβ and cSVD pathologies interact in their effects on HV. We investigated cerebrospinal fluid $A\beta_{42}$ levels as well as markers of cSVD pathology (white matter hyperintensities, lacunar infarcts, and microbleeds) and HV in a representative sample of aging memory clinic patients without large stroke. We hypothesized that Aβ and cSVD pathologies interacted on HV, such that the negative effect of either type of pathology on HV would be larger in the presence of the other type of pathology. We expected that these interactions would be present during the early stages of the disease, when dynamic and most pronounced changes in Aβ accumulation occur before reaching a plateau [2]. *Post-hoc*, we examined whether there was an interaction on cognition, a topic that is still debated in the current literature [18–21]. Our results are important for the understanding of the cascade of pathological events in AD.

MATERIALS AND METHODS

Participants

Participants were recruited via three academic memory clinics (Maastricht University Medical Center, Radboud University Medical Center, and VU University Medical Center) enrolled in the Dutch multi-center LeARN project. The process of recruitment and diagnostic classification has been described in detail elsewhere [22]. In short, patients with subjective and/or objective memory complaints who were suspected of having a primary neurodegenerative disease were included. The present study selected all participants with available cross-sectional data on magnetic resonance imaging (MRI), lumbar puncture, and neuropsychological assessment. Individuals with macrovascular abnormalities including large cerebral infarction (diameter > 15 mm) or hemorrhage (diameter > 10 mm) were excluded ($n = 1$). This resulted in a selection of 87 memory clinic patients, among which 37 individuals were diagnosed as having subjective cognitive complaints (SCC), 26 as having mild cognitive impairment (MCI), and 24 as having possible or probable AD. Diagnoses were made by experienced physicians based on core clinical criteria for MCI [23] and AD [24]. Criteria for SCC diagnosis included presence of subjective cognitive complaints, but absence of objective cognitive impairment in any of the cognitive domains, and absence of dementia. For all participants, educational

level and medical history were recorded. Hypertension was defined as current use of antihypertensive medication. Diabetes was defined as known history of diabetes type 1 or 2 and cardiovascular disease as known history of myocardial infarction, angina pectoris, carotid stenosis, dotter/stent placement, and/or coronary bypass operation. The study was carried out in accordance with the rules and regulations of institutional research and ethics committees and written informed consent was obtained from all participants prior to participation [22].

MRI data acquisition and processing

At each medical center, MRI data were acquired on a 3.0 Tesla scanner. The scan protocol included a T1-weighted gradient-echo sequence, a fluid attenuated inversion recovery (FLAIR) sequence, as well as T2-weighted and susceptibility weighted imaging (SWI) sequences. The complete details of the acquisition and scan parameters per center can be found in the supplementary material (Supplementary Table 1). cSVD imaging markers were scored by expert raters who were trained to reach excellent intra-rater agreement compared to a gold standard (weighted kappa ≥ 0.85), unless otherwise specified. Raters were always blinded for clinical data when scoring neuroimaging cSVD markers and structural changes.

White matter hyperintensities (WMH)

WMH volumes were quantified using a semi-automated method (in-house developed software package GIANT) [25]. For each medical center, an algorithm to classify WMH was trained separately, including the following steps. First, the FLAIR and T2 scans were corrected for intensity non-uniformities [26], followed by a co-registration of the T2 scans with the corresponding FLAIR scans using FLIRT from the FMRIB's Software Library (FSL) version 5.0.4 (http://www.fmrib.ox.ac.uk/fsl). Next, the signal intensities of the FLAIR and T2 scans were normalized [27]. Then we selected five scans with a substantial amount of WMH. The axial FLAIR and T2-weighted images were displayed side by side allowing visual inspection and easy identification of WMH while they were traced manually. From these manual tracings, parameters were derived for automated classification of WMH. Finally, the actual segmentation was performed semi-automatically by clicking on each identified WMH region in each slice of the simultaneously displayed axial FLAIR and T2-weighted images, providing a seed point to initiate a region growing algorithm. Manual corrections were performed where necessary by a single rater (W.M.F.). Excellent inter- and intra-rater variability were computed for a subset ($n = 9$) of scans that were also semi-automatically segmented by a second rater (S.B.) (intra class correlation both >0.98). Total WMH load was measured in cm^3 and log-transformed to better approximate a normal distribution.

Lacunar infarcts

The number of lacunar infarcts was counted by characterizing lesions of minimal 3 to maximal 15 mm in diameter with a signal intensity identical to CSF on FLAIR and T2-weighted images, possibly surrounded by a hyperintense rim on FLAIR images. Lacunar infarct count was dichotomized as 0 (absent) versus 1 (present).

Microbleeds

Cerebral microbleeds (MBs) were defined as small, homogeneous, round foci of low signal intensity with associated blooming on SWI images of 2 to 10 mm in diameter [28]. Hypointensities at the site of the nucleus lentiformis, likely representing iron or calcium deposits, were excluded. MB scores were dichotomized as 0 (absent) versus 1 (present).

Hippocampal volume

Coronal T1-weighted images were reformatted in a plane perpendicular to the long axis of the (left) hippocampus, and HV was measured bilaterally by manual delineation as described previously [29, 30]. In short, the most anterior slice measured was the slice where the hippocampal formation was first visible. The ventral border was formed by the white matter of the parahippocampal gyrus, while the dorsal border was formed by the amygdala on anterior slices and by CSF and choroid plexus on posterior slices. The HV included the dentate gyrus, cornu ammonis, subiculum, fimbria, and alveus. HV was computed by summing the delineated area of the region of interest on each slice and multiplying by the slice thickness. Delineation was done by expert raters with mean intrarater variability below 5% and mean interrater variability below 8% (calculated from the ratio of the absolute difference in two measurements to the mean

on a test set of scans, $n = 10$). HV was measured in cm^3 and expressed as the sum volume of the left and right hippocampus.

Intracranial volume

Intracranial volume (ICV) was derived from the T1 scans by using the SIENAX tool from FSL. ICV was measured in cm^3.

CSF analysis

CSF was obtained by lumbar puncture between the L3/L4 or L4/L5 intervertebral space and stored at –20°C in polypropylene tubes at each center. Samples of Maastricht and Nijmegen were transported to Amsterdam on dry ice for analysis. CSF $A\beta_{42}$ levels were quantified with commercially available INNOTEST sandwich enzyme-linked immunosorbent assays (Fujirebio, formerly Innogenetics, Ghent, Belgium) as previously described [31]. To classify individuals according to CSF $A\beta_{42}$ status (normal versus abnormal), routinely used validated cut-offs were applied, which could differentiate between subjects with SCC and AD-type dementia with 85% sensitivity (abnormal CSF $A\beta_{42} \leq 550$ pg/ml) [32].

Cognition

The Mini-Mental State Examination (MMSE) [33] was used to assess cognitive impairment and the Clinical Dementia Rating scale (CDR) was used to measure cognitive status. Neuropsychological testing for specific cognitive domains included quantitative tests to measure delayed memory recall (verbal 15 word learning test delayed word recall [34] [number of correctly reproduced words 20 minutes after the last learning trial]), language (Verbal Fluency Test [35] [number of correctly named animals within 1 minute]), and executive functioning (Stroop Color-Word Test [36] [interference score taken as outcome measure [37]]). Raw test scores were converted to z-scores, adjusted for age, sex and education based on normative data [34, 35, 37].

Statistical analysis

All analyses were conducted using SPSS statistical software (version 22.0; IBM Corp., Armonk, NY). A threshold of $\alpha < 0.05$ was used to determine statistical significance. Participant characteristics were described for each diagnostic group (SCC, MCI, AD). One-way analyses of variance (ANOVA) were performed to assess group differences for continuous variables, and chi-squared tests or Fisher's exact tests (where applicable) to test for differences in proportions between groups for categorical variables.

Interaction and additive effects of Aβ status and cSVD markers on HV

We conducted linear hierarchical regression analyses to investigate our aims. First a baseline step 1 model was built, with covariates age, sex, ICV, center, and MMSE score (to correct for the degree of cognitive impairment) included. In step 2, $A\beta_{42}$ status was added to the baseline model together with WMH, lacunar infarcts, or MBs, and their interaction was added in the third step. Step 2 and 3 were performed for each cSVD marker separately. We applied these analyses to the total participant group, and to the non-demented group (including only SCC and MCI participants). We applied false discovery rate control to the *p*-values to correct for multiple comparisons of different predictor variables (three different cSVD markers as predictor of HV) [38] and checked whether possible outliers influenced the results.

Post-hoc interaction and additive effects of Aβ and cSVD markers on cognition

We performed the same analyses with z-transformed cognitive measures of delayed memory recall, language, and executive functioning as dependent variables. Because z-scores were already corrected for age, sex, and education, only MMSE score and center were included as covariates in step 1. Because MMSE score is associated with the cognitive outcome measures, and may as such explain a substantial amount of the variance, we also did the analyses with only center as a covariate in step 1 to see whether this changed the results. We applied false discovery rate control to the *p*-values to correct for multiple comparisons of the three different variables of interest [38].

RESULTS

Participant characteristics stratified by clinical status are summarized in Table 1. Individuals with AD were less often recruited via the MUMC and were more likely to have abnormal $A\beta_{42}$ levels compared with individuals with SCC. Individuals with AD or MCI were older, had smaller HVs, and had larger WMH volumes compared with participants

with SCC. Individuals with AD had lower $A\beta_{42}$ levels and less often lacunar infarction compared with all other individuals. CDR scores were higher in individuals with AD as compared with all other participants, whereas MMSE, delayed memory recall, language, and executive functioning scores were lower. Furthermore, CDR scores were higher, and MMSE and delayed memory recall scores were lower in participants with MCI as compared with participants with SCC.

Interaction and additive effects of Aβ and cSVD markers on HV

Table 2 shows the additive and interaction effects of CSF $A\beta_{42}$ status and different cSVD markers on HV. There were no independent or additive effects in the whole group analyses. In the non-demented group, abnormal CSF $A\beta_{42}$ and lacunar infarct presence were both independently and additively associated with lower HV volume, but the effect

Table 1
Participant characteristics

	SCC	MCI	AD
	($n = 37$)	($n = 26$)	($n = 24$)
Demographics			
Age, mean (SD), y	63.7 (9.3)	69.2 (8.4)[a]	70.0 (8.7)[a]
Female sex, n (%)	11(30)	7 (27)	9 (38)
Education, mean (SD), y	12.0 (3.8)	11.1 (3.1)	10.7 (3.2)
MUMC, n (%)	20 (54)	11 (42)	5 (21)[a]
NUMC, n (%)	10 (27)	9 (35)	9 (38)
VUMC, n (%)	7 (19)	6 (23)	10 (42)
Vascular risk factors, n (%)			
Hypertension	13 (35)	9 (35)	7 (29)
Diabetes mellitus	1 (3)	4 (15)	4 (17)
Cardiovascular disease	2 (5)	2 (8)	5 (21)
MRI characteristics			
WMH, mean (SD), cm^3	5.8 (5.5)	10.7 (10.1)[a]	10.1 (9.2)[a]
Lacunes present, n (%)	8 (22)	7 (27)	0 (0)[ab]
Microbleeds present, n (%)	10 (27)	7 (27)	6 (25)
HV, mean (SD), cm^3	6.7 (0.8)	5.8 (1.0)[a]	6.1 (1.0)[a]
ICV, mean (SD), cm^3	1951 (171)	1933 (209)	1901 (135)
CSF biomarker level			
$A\beta_{42}$, mean (SD), pg/mL	776 (249)	737 (284)	540 (173)[ab]
Abnormal $A\beta_{42}$, n (%)	8 (22)	10 (38)	13 (54)[a]
Cognition			
MEM z-score, mean (SD)	–0.5 (1.1)	–2.0 (1.0)[a]	–2.6 (1.1)[ab]
LA z-score, mean (SD)	–0.59 (0.70)	–0.92 (0.90)	–1.50 (0.72)[ab]
EF z-score, mean (SD)	–0.50 (1.69)	–0.10 (1.35)	–2.5 (3.7)[ab]
MMSE, mean (SD)	28.2 (1.4)	27.2 (2.2)[a]	23.6 (2.7)[ab]
CDR, mean (SD)	0.35 (0.26)	0.50 (0.00)[a]	0.84 (0.24)[ab]

SCC, subjective cognitive complaints; MCI, mild cognitive impairment; AD, Alzheimer's disease; MUMC, Maastricht University Medical Center; NUMC, Nijmegen University Medical Center; VUMC, Vrije Universiteit Medical Center; WMH, white matter hyperintensities; HV, hippocampal volume; Aβ, amyloid-beta; MEM, delayed memory recall; LA, language; EF, executive functioning; MMSE, Mini-Mental State Examination; CDR, clinical dementia rating scale; SD, standard deviation. [a]$p < 0.05$ versus SCC; [b]$p < 0.05$ versus MCI; Data were missing, LA z-score, $n = 86$; EF z-score, $n = 80$; CDR, $n = 81$.

Table 2
Additive and interactive effects of cSVD markers and CSF $A\beta_{42}$ on HV

	Whole group			Non-demented individuals only		
	Additive impact		Interaction	Additive impact		Interaction
	cSVD	$A\beta_{42}$	cSVDx$A\beta_{42}$	cSVD	$A\beta_{42}$	cSVDx$A\beta_{42}$
WMH	–0.01	–0.11	–1.98	–0.19	–0.31**	–3.63*
Lacunes	–0.20	–0.16	0.13	–0.30§	–0.37*	0.25
MB	0.11	–0.12	0.08	0.16	–0.30*	0.20

Values represent standardized beta coefficients. Aβ, amyloid-beta; WMH, white matter hyperintensities (log transformed); MB, microbleeds; HV, hippocampal volume; cSVD, cerebral small vessel disease. §$p = 0.054$; *$p < 0.05$; **$p < 0.01$ (after correction for multiple comparisons).

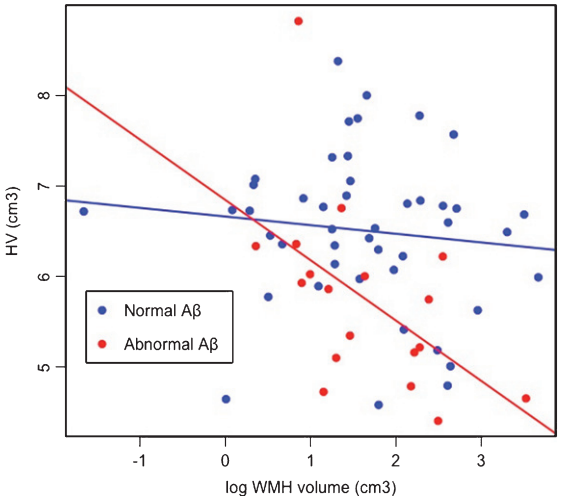

Fig. 1. The association between HV and WMH volume varies according to $A\beta_{42}$ status (dichotomized). Higher WMH volumes are associated with lower HV in individuals with abnormally low CSF$A\beta_{42}$ levels ($n = 18$; $r = –0.53$, $p < 0.05$), but not in individuals with normal CSF$A\beta_{42}$ levels ($n = 45$; $r = –0.12$, n.s.). Aβ, amyloid-beta; HV, hippocampal volume; WMH, white matter hyperintensities; n.s., not significant. Unadjusted Pearson's correlations are reported.

of lacunar infarct presence remained only marginally significant after correction for multiple comparisons (i.e., $p = 0.054$). Moreover, an interaction effect was present between CSF $A\beta_{42}$ and WMH volume in the non-demented group, such that larger WMH volumes were associated with lower HV volumes in individuals with abnormal CSF $A\beta_{42}$, but not in individuals with normal CSF $A\beta_{42}$ (Fig. 1). No interactions were found in the whole group analyses. Because exclusion of influential data points did not change the results, we used all data points in our analyses.

Interaction and additive effects of Aβ and cSVD markers on cognition

In the whole group and in the non-demented group, WMH volume was negatively associated with delayed memory recall performance (Supplementary Table 2). No other independent effects of cSVD markers or $A\beta_{42}$ were observed. There were no interaction effects between CSF $A\beta_{42}$ and the cSVD imaging markers on any cognitive measure in the whole group or in the non-demented group (Supplementary Table 2A-C). No changes occurred in the results when MMSE score was excluded from the model as a covariate.

DISCUSSION

The main objective of this study was to assess whether Aβ and cSVD interact on HV in older individuals ranging from cognitively normal to demented. Our results indicated that abnormal CSF $A\beta_{42}$ levels were independently associated with smaller HV in non-demented older individuals. In addition, cSVD burden in the form of lacunar infarct presence was associated with HV in this group (this effect remained marginally significant after correction for multiple comparisons). Although independent effects of WMH volume or MB presence on HV were absent in all analyses, the results did reveal an interaction effect between $A\beta_{42}$ pathology and WMH volume in the non-demented group. Interestingly, WMH had a negative relationship with HV in individuals with abnormal $A\beta_{42}$ levels, but such associations were absent in individuals with normal $A\beta_{42}$ levels. This interaction effect disappeared when individuals with dementia were included in the analyses. Importantly, these findings extend the current literature by showing that especially in the early stages of the disease process, individuals with Aβ pathology may be at increased risk for accelerated disease progression in the presence of comorbid cSVD pathology.

Our results are in line with recent work showing that the effects of cerebrovascular risk factors on cortical atrophy are influenced by the amount of Aβ pathology [39]. The authors found that the negative effects of vascular risk on cortical thickness, especially in AD vulnerable regions, were increased when higher amounts of cerebral Aβ deposition were present. This interaction effect was found in a similar population sample of non-demented individuals with normal and mildly impaired cognition. Our findings add new insights by showing that Aβ pathology facilitates the impact of actual small vessel damage on neurodegeneration within the hippocampus, a crucial structure that is also highly vulnerable to AD.

However, the existing literature remains inconclusive. Hohman et al. investigated the interplay between Aβ levels and cerebrovascular risk, and indicated a reversed interaction effect, such that cerebrovascular risk was strongest related to hippocampal atrophy in individuals without Aβ pathology [40]. This reversed interaction effect might have been influenced by inclusion of clinical-stage dementia. In our analyses, inclusion of individuals with dementia made the interaction effect between cSVD and Aβ disappear. It could be that at a later disease stage, Aβ

pathology related processes take over and the role of vascular factors becomes smaller [41]. Another recent study found no interaction between Aβ levels and cerebrovascular pathology in cognitively normal individuals with hippocampal atrophy as outcome measure [42]. Although this was a well-designed longitudinal study, it could be that such interactions are not detectable at the earliest stages preceding subjective or objective cognitive problems. Additional work in population-based samples is needed to clarify the dynamic interplay between vascular damage and Aβ from normal aging to dementia.

It remains unknown whether vascular and Aβ pathology directly influence each other in their rates of pathologic progression, or whether these are simply distinct processes occurring in parallel. In case of the first scenario, cSVD pathology may lead to reduced parenchymal Aβ clearance or increased deposition at the level of the neurovascular unit, while Aβ accumulation in and around the cerebral vessels may in turn lead to small vessel damage. Such interactions have been reported in hypertensive rats [12]. These possible mechanisms need further exploration since they might have important implications for the diagnostic classification of AD and VaD, suggesting that a continuous approach might be more appropriate than separate classification into AD and VaD. In case of the independent processes scenario, it can be hypothesized that Aβ related processes reduce compensatory brain reserve [43, 44] and lower the threshold for (further) neurodegeneration due to cerebrovascular damage. Importantly, the interaction effect between Aβ and WMH found in the present study demonstrates that WMH pathology impacts hippocampal neurodegeneration only when abnormal Aβ levels are already present. This suggests that individuals with both types of pathology are at increased risk of accelerated disease progression, which is in line with earlier findings that individuals with cerebrovascular damage are at increased risk of developing AD [13, 45, 46].

The fact that Aβ pathology was independently associated with smaller HV in the non-demented sample is in accordance with previous studies consistently showing negative associations between cerebral Aβ deposition and HV at early disease stages [47, 48]. Besides Aβ pathology, lacunar infarct presence was independently and negatively associated with HV, although this effect became marginally significant after adjusting for multiple comparisons. This finding suggests that hippocampal neurodegeneration may also be independently driven by vascular factors [16]. This effect was absent in the whole group analyses because none of the AD individuals displayed signs of lacunar infarction. We did not find any associations between HV and cerebral MBs or WMH volume. Although MBs have previously been associated with HV reduction [49], our sample included only a limited number (around 25%) of participants that displayed these lesions. As such, our analyses may lack statistical power and sensitivity to detect associations between MBs and HV.

Post-hoc, we examined a possible interplay between Aβ and cSVD on cognition. In line with previous studies, we found no interaction effects between Aβ and cSVD on various cognitive domains [19, 50, 51]. Although emerging evidence suggests that dementia is more likely to occur when vascular and Aβ pathology coexist [13, 45], it may be the case that the effects on cognition are mediated by neurodegenerative factors. For example, previous work indicated that the association between Aβ and cognitive decline is mediated by gray matter atrophy and glucose metabolism [52]. Others found that brain atrophy interacts with cSVD in affecting cognitive decline [53]. Thus, when not taking other factors into account, Aβ and cSVD pathology may have independent or additive effects on cognition. Recent work supports the additive effect hypothesis by showing that cognitively normal individuals with both cerebrovascular and amyloid pathology displayed faster cognitive decline that seemed additive compared to the rate in individuals with only one type of pathology [21]. However, a recent study in individuals with subcortical vascular dementia reported interactions between cSVD and Aβ pathology on longitudinal cognitive outcomes [20]. As such, the subtle interactive effects of cSVD and Aβ may only become apparent at advanced disease stage and may only be detectable when cognition is measured longitudinally. Further studies are needed to clarify this matter.

Our results showed a relationship between WMH volume and delayed memory recall in the whole sample and in the non-demented individuals, which confirms the well-established association between cerebrovascular injury and cognition [21]. However, no associations between cognition and the other cSVD imaging markers were found. Although the prevalence of these markers was relatively high in the nondemented group ($\sim$25%), the sample size may have been too small to reveal any associations. While the association of Aβ pathology with cognition is less pronounced [54, 55], previous studies do indicate subtle independent associations with cognitive

performance [18, 19]. It is therefore important not to overlook the possible effects of both Aβ and cerebrovascular disease when studying cognition in dementia. However, the effects of neurodegeneration, glucose metabolism, and other possible influencing factors should be taken into account.

Strengths of this study are the multi-center nature and representativeness of our study sample for general memory-clinic visiting individuals. Furthermore, the exclusion of large strokes enabled us to study the effects of mild cSVD in interaction with Aβ, although this may be a limitation to the generalizability of our results to populations with large vessel disease. The range in cognitive impairment allowed us to investigate our aims across the whole spectrum of dementia, from cognitively normal to mild dementia. Pitfalls of this study include its restricted sample size, the relatively low diversity of cSVD severity (The absence of lacunar infarcts in the AD group may form a potential sample bias in this study), and the cross-sectional nature of the data, which makes it impossible to make direct causal inferences. The multi-center nature may also be a confounding factor, because the scans are acquired on three different MR systems with different scan parameters. As such, the WMH volume calculation might have been influenced by center. We tried to correct for this by calculating separate algorithms for each center and by adding center as a covariate in the statistical models.

Taken together, the findings in this study suggest that Aβ pathology and cerebrovascular damage can both independently and synergistically facilitate hippocampal neurodegeneration in non-demented older individuals. Our results showed that cSVD in the form of lacunar infarcts was associated with smaller HVs in individuals with subjective to mild cognitive problems. Likewise, these individuals were more likely to have lower HV when abnormal cerebral Aβ deposition was present. These associations were independent, meaning that their negative effects of Aβ pathology and lacunar infarcts on HV were additive. In contrast, an interaction effect on HV was present between abnormal Aβ levels and WMH in the non-demented individuals. That is, WMH volume had no effect on HV in individuals with normal Aβ levels, while HV reduction was (even further) amplified in individuals with abnormal cerebral Aβ deposition when comorbid cerebrovascular disease in the form of WMH was present. We found no associations or interactions when individuals with AD were included in the analyses. Importantly, our results suggest that associations and interactions between Aβ and cSVD pathologies in relation to HV take place at the earlier stages of the disease process when cognitive impairment is only mild or not yet detectable. At this early stage, brain reserve may play a protective role, while during clinical disease stage other Aβ-related processes such as advanced neurodegeneration and/or tau pathology may have impacted this reserve. The observations in this study underline the importance of taking both cerebrovascular disease and Aβ deposition into account when studying cerebral atrophy in dementia. Vascular care in early memory clinic patients may form an important opportunity to slow down disease progression.

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SUPPLEMENTARY MATERIAL

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