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## Cerebral white matter lesions – associations with A $\beta$ isoforms and amyloid PET

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Small vessel disease (SVD) and amyloid deposition may promote each other, with a potential association between SVD and altered production or clearance of  $\beta$ -amyloid (A $\beta$ ) affecting its cleavage products. We investigated the relationship between SVD, multiple isoforms of A $\beta$  in cerebrospinal fluid (CSF) and cortical A $\beta$  in 831 subjects with cognitive performance ranging from normal to Alzheimer's disease (AD) (the Swedish BioFINDER study). SVD was estimated as white matter lesions (WML) and lacunes. 18F-flutemetamol PET was performed in 321 subjects. Lower CSF levels of A $\beta$ 338 and A $\beta$ 40 were consistently associated with increased WML in all subgroups, while lower levels of CSF A $\beta$ 42 were associated with WML mainly in AD. CSF A $\beta$ 338 and A $\beta$ 40 were associated with regional WML in all regions, while CSF A $\beta$ 42 was associated with temporal WML only. A composite measure of 18F-flutemetamol uptake was not associated with WML, and regional 18F-flutemetamol uptake only with temporal WML. Lacunes were not associated with A $\beta$  isoforms nor 18F-flutemetamol uptake. Our results suggest that WML may be associated with alterations in the production or clearance of A $\beta$  species, particularly of A $\beta$ 338 and A $\beta$ 40. However, in AD cases, A $\beta$ 42 pathology might be associated with WML, especially in the temporal lobe.

While cerebral small vessel disease (SVD) affects perforating cerebral arterioles, capillaries and venules, the term SVD is also used to describe the resulting brain damage, comprising mainly subcortical lesions such as small infarcts, lacunes, white matter lesions (WML), enlarged perivascular spaces and microbleeds<sup>1</sup>. WML are the most common manifestation of SVD and especially abundant in elderly, but the suggested prevalence has varied substantially between different studies<sup>2–4</sup>. Cerebral microbleeds (MB) are markers of vascular pathology including cerebral amyloid angiopathy (CAA) and potentially have direct effects on brain function<sup>5</sup>. SVD will lead to a number of downstream effects including fibrosis impairing drainage of fluid and proteins from the extravascular space along the blood vessels<sup>6</sup>, with accumulation of proteins, including amyloid beta (A $\beta$ )<sup>7,8</sup>.

Cleavage of the amyloid precursor protein (APP) by  $\beta$ - and  $\gamma$ -secretases yields A $\beta$  peptides with A $\beta$ 338, A $\beta$ 40 and A $\beta$ 42 as the most common variants<sup>9</sup>. In most cells, A $\beta$ 338 and A $\beta$ 42 are produced at relatively low levels, typically 5–20% of the total A $\beta$  detected, and the major species generated is A $\beta$ 40 that typically constitutes over 50% of total A $\beta$ <sup>10,11</sup>. A $\beta$ 338, A $\beta$ 40 and A $\beta$ 42 differ in aggregation propensity: A $\beta$ 338 is soluble, present in the vasculature of in sporadic and familial AD patients and never found in senile plaques, A $\beta$ 40 is somewhat aggregation-prone, it is the main component for vascular amyloid and is often found in CAA, whereas A $\beta$ 42 is the most aggregation-prone, forming cortical fibrillar A $\beta$  that will be deposited as amyloid plaques associated with Alzheimer's disease<sup>12,13</sup>.

SVD and AD share common risk factors, such as hypertension during midlife, diabetes mellitus, smoking, the apolipoprotein E (APOE)  $\epsilon$ 4 allele and particularly age<sup>14,15</sup>. A two hit, potentially self-reinforcing mechanism has been proposed, suggesting that SVD may initiate amyloid deposition on the one hand, and amyloid deposition

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	Cognitively healthy elderly (n = 267)	Subjective cognitive deficit (n = 165)	Mild cognitive impairment (n = 195)	PD patients (n = 89)	AD patients (n = 110)
Age (mean + SD)	72.9 + 5.0	70.0 + 5.8 <sup>a***</sup>	71.2 + 5.6 <sup>a***</sup>	65.4 + 11.2 <sup>ab,c***</sup>	74.5 + 7.3 <sup>a,b,c,d***</sup>
Proportion female (%)	61.4	57.6	44.6 <sup>***,b*</sup>	38.2 <sup>ab***</sup>	62.7 <sup>c,d***</sup>
APOE $\epsilon$ 4 carriers (%)	30.7	38.5	47.9 <sup>a***</sup>	N/A	67.3 <sup>ab***c***</sup>
WML volume					
(median, IQR)	5.3, 2.0–14.2	6.5, 1.7–18.9	12.5, 4.5–34.0 <sup>ab***</sup>	5.0, 1.5–16.4 <sup>c***</sup>	N/A
Fazekas total score (median, IQR)	4.0, 2.0–6.0	5.0, 3.0–7.0	6.0, 3.0–10.0 <sup>ab***</sup>	4.0, 1.0–7.5 <sup>b*,c***</sup>	4.0, 2.0–7.0 <sup>c***</sup>
AWMRC total (median, IQR)	4.0, 1.0–8.0	5.0, 2.0–8.0	6.0, 2.0–10.0 <sup>***,b*</sup>	4.0, 0.0–7.50 <sup>c*</sup>	3.5, 0.8–8.0 <sup>c***</sup>
Lacunes (%)	5.7	8.1	11.3 <sup>a*</sup>	2.2 <sup>c*</sup>	3.6 <sup>c*</sup>
CSF A $\beta$ 38 (median, IQR, pg/mL)	1727.5, 1492.3–2011.0	1797.0, 1432.3–2065.3	1646.5, 1357.0–1889.3 <sup>a***,b*</sup>	1626.0, 1310.0–1841.0 <sup>ab***</sup>	1649.0, 1360.8–1935.8 <sup>a,b***</sup>
CSF A $\beta$ 40 (median, IQR, pg/mL)	4549.0, 3515.5–5483.5	4837.5, 3514.3–6298.8	4339.0, 3427.8–5546.0 <sup>b*</sup>	3757.0, 2996.5–4784.0 <sup>a,b,c***</sup>	3826.5, 3120.5–5159.8 <sup>a,b***,c**</sup>
CSF A $\beta$ 42 (median, IQR, pg/mL)	665.0, 509.8–778.0	657.0, 440.9–802.8	468.0, 351.0–711.0 <sup>ab***</sup>	644.0, 481.5–766.5 <sup>c***</sup>	365.0, 297.8–428.8 <sup>a,b,c,d***</sup>
Composite PET SUVR (median, IQR)	1.24, 1.12–1.34 (n = 122)	1.23, 1.13–1.68 (n = 101) <sup>a*</sup>	1.55, 1.20–2.09 (n = 98) <sup>a,b***</sup>	N/A	N/A

**Table 1. Baseline characteristics by clinical diagnosis.** <sup>a</sup>vs cognitively healthy elderly. <sup>b</sup>vs subjective cognitive deficit. <sup>c</sup>vs mild cognitive impairment. <sup>d</sup>vs PD. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.005$  Only p-values  $< 0.005$  should be considered significant according to Bonferroni-adjustment.

may increase the susceptibility to develop SVD on the other hand<sup>16,17</sup>. Increased knowledge about the role of A $\beta$  species in SVD may improve treatment and prevention strategies for individuals with SVD, since accumulation of multiple vascular risk factors, especially in midlife, can substantially increase the risk for dementia<sup>18</sup>. This has been confirmed by the suggested declining trend in dementia risk, occurring in parallel with the decreasing incidence of cardiovascular events in high-income countries<sup>19,20</sup>. A recent study on the association between SVD and amyloid accumulation found higher WML to be associated with lower levels of CSF A $\beta$ 42 in vascular dementia and in subjective cognitive decline but not in AD, which was interpreted as a connection between SVD and AD pathology<sup>21</sup>. However, these findings may alternatively suggest that SVD is related to a general decrease in beta amyloid, reflecting a diminished secretion of amyloid precursor protein (APP), rather than the AD specific phenomenon of A $\beta$ 42 aggregation. If this were the case, WML would be associated with lower CSF levels of all amyloid species, including A $\beta$ 38 and A $\beta$ 40 in addition to A $\beta$ 42. Therefore, we readdressed the association between SVD and amyloid deposition including multiple markers of amyloidogenic APP-processing and A $\beta$  deposition. Specifically, we studied the relationship between WML and lacunes assessed with neuroimaging and CSF levels of A $\beta$ 38, A $\beta$ 40 and A $\beta$ 42. In addition we measured amyloid uptake using 18F-flutemetamol PET, that reflects cortical levels of fibrillar A $\beta$  associated with amyloid plaques. The study population consisted of 831 individuals from four diagnostic groups that differed in their cognitive performance, cognitively healthy elderly (CHE), and subjects with subjective cognitive decline (SCD), mild cognitive impairment (MCI) and AD; in order to confirm results in a separate cohort of subjects with a neurodegenerative disease, other than AD albeit associated with amyloid accumulation, a fifth diagnostic group, consisting of subjects with Parkinson's disease (PD) without dementia, was also included.

## Results

**Demographics.** Demographic data are given in Table 1. WML volume, total Fazekas score and total Age Related White Matter Changes (ARWMC) score were highly correlated, average partial correlation coefficient 0.806,  $p < 0.001$ , pooled data from the CHE, SCD, MCI and PD cohorts. CSF levels of A $\beta$ 38 and A $\beta$ 40 did not correlate with the composite SUVR for amyloid PET, partial correlation coefficient 0.027 and 0.088, respectively,  $p > 0.05$ . In contrast, the CSF level of A $\beta$ 42 showed a high inverse correlation with the composite SUVR for amyloid PET, partial correlation coefficient  $-0.668$ ,  $p < 0.001$ , pooled data from the CHE, SCD and MCI cohorts. All correlation analyses were corrected for age, sex and hippocampal volume.

**Associations between total WML and CSF A $\beta$ 38, A $\beta$ 40 and A $\beta$ 42.** In the CHE, SCD, MCI, and PD groups, lower CSF levels of A $\beta$ 38 and A $\beta$ 40 were significantly associated with increased WML volume (Table 2). However, there were no significant associations between CSF levels of A $\beta$ 42 and WML volume in any of the groups (Table 2). To confirm these results, visual assessment of WML using the Fazekas scale was performed, now including the AD group. Again, higher Fazekas scores (indicating more severe WML) was associated with lower CSF levels of A $\beta$ 38 and A $\beta$ 40 in the SCD and the MCI groups, and lower levels of A $\beta$ 38 in the PD group (Table 2). CSF A $\beta$ 42 was associated with WML only in the AD group, in addition to CSF A $\beta$ 38 and A $\beta$ 40 (Table 2).

Next, the subjects where <sup>18</sup>F-flutemetamol PET was performed, namely the CHE, SCD and MCI cases, were pooled to perform subgroup analyses. Lower CSF levels of A $\beta$ 38 and A $\beta$ 40 were significantly and consistently associated with increased WML volume in all participants (Table 3). The relationship between WML volume and CSF levels of A $\beta$ 42 was stronger in APOE  $\epsilon$ 4 negative than APOE  $\epsilon$ 4 positive participants (Table 3). Lower levels

Group	Parameter	WML volume $\pm$ 95% CI			Fazekas score $\pm$ 95% CI			Lacunes $\pm$ 95% CI		
Cognitively healthy elderly	CSF A $\beta$ 38	-0.151**	-0.260	-0.042	-0.071	-0.191	0.048	-0.254	-1.369	0.861
	CSF A $\beta$ 40	-0.118*	-0.228	-0.008	-0.058	-0.178	0.061	-0.363	-1.940	1.213
	CSF A $\beta$ 42	-0.075	-0.185	0.036	0.017	-0.103	0.137	-0.795	-2.406	0.815
	Composite SUVR	0.037	-0.137	0.210	-0.057	-0.241	0.127	0.570	-2.911	4.052
Subjective cognitive deficit	CSF A $\beta$ 38	-0.211**	-0.341	-0.081	-0.170*	-0.312	-0.028	2.306	-0.317	-0.030
	CSF A $\beta$ 40	-0.243***	-0.371	-0.115	-0.197**	-0.337	-0.057	1.144	-0.309	-0.020
	CSF A $\beta$ 42	-0.079	-0.213	0.055	-0.066	-0.211	0.079	0.976	-0.154	0.142
	Composite SUVR	0.099	-0.075	0.273	0.070	-0.118	0.257	-0.686	-0.187	0.231
Mild cognitive impairment	CSF A $\beta$ 38	-0.264***	-0.396	-0.133	-0.174*	-0.317	-0.030	-1.947*	-3.655	-0.239
	CSF A $\beta$ 40	-0.286***	-0.417	-0.155	-0.164*	-0.309	-0.020	-1.018	-2.312	0.276
	CSF A $\beta$ 42	0.000	-0.138	0.138	-0.006	-0.154	0.142	0.715	-0.501	1.931
	Composite SUVR	-0.028	-0.226	0.170	0.022	-0.187	0.231	0.149	-2.755	3.052
PD-patients	CSF A $\beta$ 38	-0.181**	-0.310	-0.053	-0.188*	-0.365	-0.011	NA		
	CSF A $\beta$ 40	-0.185**	-0.314	-0.056	-0.175	-0.225	-0.125	NA		
	CSF A $\beta$ 42	-0.066	-0.204	0.072	0.010	0.386	-0.366	NA		
AD-patients	CSF A $\beta$ 38	NA			-0.258*	-0.447	-0.070	NA		
	CSF A $\beta$ 40	NA			-0.299**	-0.485	-0.113	NA		
	CSF A $\beta$ 42	NA			-0.225*	-0.413	-0.038	NA		

**Table 2. Associations between cerebrovascular disease, A $\beta$  isoforms and amyloid PET per diagnostic group.** Values represent standardized beta (linear regression, WML volume and total Fazekas score) and Beta (logistic regression, lacunes) with 95% confidence intervals; prior to analysis, the natural logarithm of parameters was calculated where appropriate in order to ensure normal distribution. Values are corrected for age, gender and hippocampal volume; the latter was determined using Adaboost, the mean value of the left and right hippocampus was normalized using the scaling factor provided by this software to account for head size differences. In the PD and AD groups, 2.3% and 3.6% of the subjects had lacunes and the association between CSF A $\beta$  species and lacunes was not analyzed. Data on WML volume were not available for the AD group, where imaging mainly was performed using CT. \* $p < 0.05$ , \*\* $p < 0.0125$ , \*\*\* $p > 0.001$ . Only  $p$ -values  $< 0.0125$  should be considered significant according to Bonferroni-adjustment.

Group	Parameter	WML volume $\pm$ 95% CI			Fazekas $\pm$ 95% CI			Lacunes $\pm$ 95% CI		
All CHE, SCD and MCI	CSF A $\beta$ 38	-0.219***	-0.288	-0.15	-0.150***	-0.227	-0.073	-0.841	-1.966	0.284
	CSF A $\beta$ 40	-0.205***	-0.274	-0.136	-0.128***	-0.205	-0.051	-0.418	-1.253	0.417
	CSF A $\beta$ 42	-0.086*	-0.158	-0.014	-0.055	-0.135	0.025	0.298	-0.513	1.109
	Composite SUVR	0.074	-0.031	0.179	0.071	-0.044	0.186	-0.12	-1.723	1.483
APOE $\epsilon$ 4 - positive	CSF A $\beta$ 38	-0.282***	-0.394	-0.17	-0.203**	-0.328	-0.078	-2.354**	-4.104	-0.604
	CSF A $\beta$ 40	-0.266***	-0.381	-0.071	-0.154*	-0.277	-0.031	-1.544*	-2.879	-0.209
CHE, SCD and MCI	CSF A $\beta$ 42	-0.127*	-0.247	-0.007	-0.09	-0.221	0.041	0.971	-0.278	2.22
	Composite SUVR	0.153	-0.031	0.337	0.103	-0.105	0.311	-1.128	-3.842	1.598
APOE $\epsilon$ 4 - negative	CSF A $\beta$ 38	-0.187***	-0.276	-0.098	-0.125*	-0.224	-0.026	0.229	-1.292	1.75
	CSF A $\beta$ 40	-0.174***	-0.263	-0.085	-0.117*	-0.216	-0.018	0.314	-0.776	1.404
CHE, SCD and MCI	CSF A $\beta$ 42	-0.128**	-0.22	-0.036	-0.086	-0.188	0.016	0.054	-1.275	1.383
	Composite SUVR	0.08	-0.052	0.213	0.06	-0.084	0.204	0.432	-1.969	2.833

**Table 3. Associations between SVD, estimated as the volume of white matter lesions (WML), the total score of WML according to Fazekas, and lacunes, and amyloid isoforms in CSF and amyloid PET, in the pooled CHE, SCD and MCI subgroups.** Values represent standardized beta (linear regression, WML volume and total Fazekas score) and Beta (logistic regression, lacunes) with 95% confidence intervals; prior to analysis, the natural logarithm of parameters was calculated where appropriate in order to ensure normal distribution. Values are corrected for age, gender and hippocampal volume; the latter was determined using Adaboost, the mean value of the left and right hippocampus was normalized using the scaling factor provided by this software to account for head size differences. \* $p < 0.05$ , \*\* $p < 0.0125$ , \*\*\* $p > 0.001$ . Only  $p$ -values  $< 0.0125$  should be considered significant according to Bonferroni-adjustment.

Group	Parameter	ARWMC total	ARWMC frontal	ARWMC parieto-occipital	ARWMC temporal	ARWMC infratentorial
CHE, SCD, MCI	CSF A $\beta$ 38	-0.142***	-0.130***	-0.142***	-0.155***	-0.013
	CSF A $\beta$ 40	-0.121**	-0.111**	-0.122**	-0.137**	0.019
	CSF A $\beta$ 42	-0.040	-0.026	-0.073	-0.088*	0.031
	Composite SUVR	0.094	0.088	0.084	0.136*	0.015

**Table 4. Associations between regional cerebrovascular disease, A $\beta$  isoforms and amyloid PET, pooled data from the CHE, SCD and MCI groups.** SVD was estimated as the total and regional white matter lesion score according to the ARWMC scale. Values represent standardized beta (linear regression, ARWMC score) with 95% confidence intervals; prior to analysis, the natural logarithm of the CSF and PET parameters was calculated to ensure normal distribution. Values are corrected for age, sex and hippocampal volume; the latter was determined using Adaboost, the mean value of the left and right hippocampus was normalized using the scaling factor provided by this software to account for head size differences. \* $p < 0.05$ , \*\* $p < 0.0125$ , \*\*\* $p > 0.001$ . Only  $p$ -values  $< 0.0125$  should be considered significant according to Bonferroni-adjustment.

Group	Parameter	ARWMC frontal			ARWMC parito-occipital			ARWMC temporal		
CHE, SCD, MCI	Frontal SUVR	0.088	-0.027	0.203	0.079	-0.036	0.194	0.140*	0.022	0.259
	Parietal SUVR	0.119	0.004	0.233	0.117	0.002	0.231	0.168**	0.050	0.285
	Occipital SUVR	0.097	-0.018	0.213	0.092	-0.023	0.207	0.148*	0.029	0.266
	Temporal SUVR	0.061	-0.053	0.175	0.055	-0.059	0.168	0.104	-0.014	0.221

**Table 5. Associations between regional cerebrovascular disease and regional amyloid uptake measured as PET SUVR for CHE, SCD and MCI subjects.** Values represent standardized beta from linear regression with regional ARWMC as dependent and regional amyloid uptake as independent variable as well as the confidence intervals, and are corrected for age, sex and hippocampal volume. Cerebrovascular disease was estimated as the total score of white matter lesion rating according to the ARWMC scale, total sum, and regional, mean of left and right. PET SUVR was estimated the regional uptake, mean of left and right; the natural logarithm of PET estimates was used. \* $p < 0.05$ , \*\* $p < 0.0125$ , \*\*\* $p > 0.001$ . Only  $p$ -values  $< 0.0125$  should be considered significant according to Bonferroni-adjustment.

of CSF A $\beta$ 42 were also associated with increased WML volume, but at a lower magnitude than A $\beta$ 38 and A $\beta$ 40 (Table 3).

**Associations between total WML and amyloid PET.** We also used amyloid PET imaging with the  $^{18}\text{F}$ -flutemetamol ligand to study the cortical levels of A $\beta$  fibrils in CHE, SCD and MCI. The  $^{18}\text{F}$ -flutemetamol composite score was not significantly associated with WML volume or Fazekas score in any of the studied groups (Table 3).

**Associations between regional distribution of WML and CSF A $\beta$  species or amyloid PET.** The potential regional impact of SVD was assessed from WML in various brain regions using the ARWMC scale. Lower levels of CSF A $\beta$ 38 and A $\beta$ 40 were significantly associated with increased WML in the frontal, parieto-occipital and temporal lobes, but not in infratentorial regions (Table 4). Lower levels of CSF A $\beta$ 42 and higher levels of the composite  $^{18}\text{F}$ -flutemetamol uptake were significantly associated with WML in the temporal lobe only (Table 4). Since regional ARWMC scores correlated significantly with the total ARWMC, analysis was redone with the total ARWMC included as a predictor. Again, only the association between CSF A $\beta$ 42 and temporal ARWMC was significant.

When examining the relationship between regional WML and regional  $^{18}\text{F}$ -flutemetamol uptake, increased  $^{18}\text{F}$ -flutemetamol uptake in frontal, parietal, temporal, occipital regions were all associated with a higher temporal ARWMC score (Table 5).

**Lack of association between lacunes and CSF A $\beta$  species or amyloid PET.** Neither CSF A $\beta$ 42 nor the composite  $^{18}\text{F}$ -flutemetamol uptake were associated with the presence of lacunes in any group (Table 3, results from CHE, SCD, MCI groups).

## Discussion

Our main findings are firstly that more pronounced WML is strongly and consistently associated with lower levels of CSF A $\beta$ 38 and A $\beta$ 40, while the association with CSF A $\beta$ 42 levels is weak or absent. This pattern was found in all diagnostic groups, except for the AD group where WML were associated with all three A $\beta$  isoforms. Secondly, the composite  $^{18}\text{F}$ -flutemetamol SUVR, that reflects cortical fibrillar A $\beta$  deposition and strongly correlated with CSF A $\beta$ 42, was not associated with WML in any of the CHE, SCD and MCI groups (PET data unavailable for the PD and AD groups).

Two hypotheses might explain this consistent, negative relationship between WML and A $\beta$ 38 and A $\beta$ 40. Firstly, reduced clearance of A $\beta$ 38 and A $\beta$ 40 from the brain parenchyma may enhance deposition of these A $\beta$  species in the cerebral vessel walls, as has been reported for A $\beta$ 40<sup>11,22</sup>. Evidence favors the hypothesis that amyloid

fibrils deposited in the vessel wall and senile plaques differ from each other, as the major species in CAA is A $\beta$ 39 or A $\beta$ 40, and in senile plaque A $\beta$ 42, although A $\beta$ 40 may also be present<sup>23,24</sup>. Similarly, A $\beta$ 38 seems to be predominantly located within the vasculature in postmortem brains of sporadic and familial AD patients<sup>11,25</sup>. Impaired vascular clearance of A $\beta$  across the BBB and increased A $\beta$  brain capillary deposition has been reported in transgenic mice producing low levels of the poorly cleared Dutch/Iowa mutant forms of A $\beta$ , which are vasculotropic and rich in  $\beta$ -sheets<sup>26</sup>.  $\beta$ -amyloid deposition, that is directly toxic to smooth muscle cells, leads to constriction of cerebral blood vessels, and thus to perturbation of cerebral perfusion, loss of homeostasis of the neuronal environment, ultimately leading to ischemia and consequently WML<sup>27,28</sup>. Also the previously reported positive association between the A $\beta$ 40 level in plasma and WMH in subjects with MCI, AD and CAA, suggests that circulating A $\beta$ 40 is a potential contributor to microvascular dysfunction<sup>29</sup>. Reduced clearance may play also a role in CAA that is associated with vascular deposition of A $\beta$ 40 and A $\beta$ 42 with A $\beta$ 40 as the major isoform. Decreased levels of CSF A $\beta$ 40 as well as A $\beta$ 42 have been reported in CAA patients, suggesting that A $\beta$ 40 and A $\beta$ 42 may be trapped in the cerebral vasculature and escape the drainage pathways that otherwise transport amyloid  $\beta$  proteins toward the cerebrospinal fluid<sup>30</sup>. It has been hypothesized that SVD may exacerbate CAA by promoting cerebral edema that needs to compete with A $\beta$  for clearance via perivascular drainage pathways<sup>31</sup>. A larger prevalence of WML in CAA compared to AD and MCI has been reported with WMH correlating with plasma levels of A $\beta$ 40<sup>29</sup>.

Secondly, SVD, and especially WML, may be associated with reduced production of A $\beta$  in the brain. An inverse relationship between the volume of WML and CSF APP metabolites (including A $\beta$ 38, A $\beta$ 40 and A $\beta$ 42) in both stroke patients and SCD/MCI patients has been reported<sup>32</sup>. In that study, lower levels of CSF APP metabolites in the stroke group compared to the SCD/MCI-group suggested that ischemia influences APP metabolism, probably through inhibition of fast axonal transport of APP. This is consistent with evidence from a combined animal and neuropathological study that acute ischemic lesions can trigger accelerated amyloid deposition, most likely through interference with amyloid clearance pathways<sup>33</sup>. White matter pathology underlying WML has been suggested to affect neuronal activity by a body of clinical work reporting a direct relationship between white matter integrity and cognitive performance<sup>34</sup>. In addition, a negative correlation has been reported between WML volume and connectivity strength in regions with decreased connectivity in MCI patients as compared to controls<sup>35</sup>. Notably, the production of A $\beta$  and its secretion into the extracellular space are tightly regulated by neuronal activity *in vitro* and *in vivo*; increased neuronal activity enhances A $\beta$  production, and blocking neuronal activity has the opposite effect<sup>36</sup>.

An inverse association between WML and AD specific CSF A $\beta$ 42 has recently been reported in subjects with MCI<sup>21</sup>. Even though we reproduced this association in the pooled CHE, SCD and MCI data (Table 3), our results showed that the association with WML was much stronger for A $\beta$ 38 and A $\beta$ 40, that were not investigated in the study by Kester *et al.*<sup>21</sup> We confirm this by the lack of association between WML and <sup>18</sup>F-flutemetamol PET imaging, that reflects fibrillar A $\beta$  deposition. We found that only in cases with AD dementia, A $\beta$ 42 pathology might be associated with WML, especially in the temporal lobes. Previous smaller scale studies in subjects with cognitive status ranging from normal to mild dementia have reported a similar lack of association between WML and amyloid PET<sup>37–40</sup>. Thus, non-AD-specific subcortical changes may affect global levels of A $\beta$  isoforms in the central nervous system<sup>32</sup>. It should be noted that amyloid ligands such as <sup>18</sup>F-flutemetamol were developed to bind to aggregated A $\beta$ . For example, the Pittsburgh compound B (PiB) ligand may bind to vascular amyloid<sup>41,42</sup>. Higher PiB uptake has been consistently reported in non-demented CAA patients, at a level intermediate between healthy controls and AD<sup>43,44</sup>.

Parenchymal deposition of A $\beta$  protein and lower CSF levels of A $\beta$ 42 are associated with increased risk for AD development. In addition, vascular deposition of A $\beta$  may also be a primary driver of AD and affect the production and clearance of APP. Thus increased WML burden among patients with AD might reflect accumulation of vascular A $\beta$  to some degree. Interestingly, we found a significant association between WML and CSF A $\beta$ 42 in the AD dementia group, indicating that A $\beta$ 42-related plaque pathology might be associated with SVD in individuals with dementia due to AD.

Amyloid PET-uptake and WML volume have been shown to independently predict AD diagnosis and thus WML may be involved in the clinical manifestation of AD<sup>38</sup>. WML are more prevalent and severe in AD patients compared with nondemented adults matched for demographic characteristics and brain regions where WML are most severe in AD, reportedly occur in the same location as AD pathology and areas showing the greatest metabolic dysfunction in AD<sup>45</sup>. It cannot be ruled out that some of the subjects with clinically diagnosed AD may actually suffer from mixed dementia, with both AD and vascular (i.e. WML) contributions<sup>46</sup>. While this may affect the associations with amyloid species presently found in this group, the total Fazekas and ARWMC scores in the AD group were comparable to those in the CHE, SCD and PD groups and lower than in the MCI group.

Our main finding that the much stronger association between WML and CSF A $\beta$ 38 and A $\beta$ 40, than CSF A $\beta$ 42 was confirmed in the PD group. This strengthens previous evidence suggesting that also PD should be targeted by treatment for vascular risk factors since comorbid WML are common in PD and affect motor function and cognition<sup>47,48</sup>. We did not assess the impact of the interplay between amyloid and SVD on cognition. According to a recent study in non-demented elderly amyloid and vascular pathologies seemed to be at least partly independent processes that both affect longitudinal cognitive trajectories adversely and are major drivers of cognitive decline in the elderly<sup>49</sup>. This further stresses the need for treatment of risk factors for SVD in neurodegenerative disease.

We acknowledge limitations in the present study. Firstly, some data are lacking, as for example <sup>18</sup>F-flutemetamol PET for the PD and AD cases and quantitative WML analysis for AD cases; in the CHE, SCD and MCI groups, <sup>18</sup>F-flutemetamol PET was available for 321 subjects (Table 1). Also, there is some heterogeneity in the imaging data since mainly CT images were available for the AD group. Data on cerebral microbleeds as marker of cerebral amyloid angiopathy are not included, since susceptibility weighted images (SWI) to assess their presence were available for the PD group. Secondly, the use of linear regression for ordinal scales as the Fazekas and ARWMC scales, may be questioned since the number of categories is limited, and the underlying model assumptions (linear



change with each unit change in category) may not be valid. Data from these scales were included since the WML volume could not be determined in the AD group; in addition, these scales have been used in other studies, for example the study by Kester *et al.*<sup>14</sup> Instead, the WML volume provides a continuous variable and the lack of significant association between the Fazekas score and CSF A $\beta$  species in the CHE and PD groups, where WML were less abundant, should be explained by the inherent differences in these two variables. Thirdly, lacunes, also markers of SVD, showed a significant association with CSF A $\beta$ 338 and CSF A $\beta$ 40 only in the MCI group. Lacunes and WML differ regarding their pathogenesis, with lacunes being due to sudden, total occlusion of an end artery, whereas white matter lesions are associated with longstanding, less severe general hypoperfusion of brain tissue. The consequences of lacunes and WML with regard to amyloid production and clearance are also likely to differ, with the former resulting in severe, but focal damage to the brain parenchyma in a smaller area, and the latter in less severe, but more widespread damage. In addition, the low prevalence of lacunes in the CHE and SCD groups may have reduced statistical power.

In conclusion, our findings suggest that WML are associated with lower CSF A $\beta$ 338 and A $\beta$ 40 levels, probably reflecting lower overall A $\beta$  production due to either decreased expression or secretase processing of APP. This is confirmed by the lack of association between WML and <sup>18</sup>F-flutemetamol PET, that reflects fibrillar A $\beta$  deposition. However, in cases with AD dementia A $\beta$ 42 pathology might be associated with WML, especially in the temporal lobes.

## Methods

**Ethical approval and patient consent.** The study protocol was designed in accordance with guidelines outlined in the Declaration of Helsinki and approved by the Research Ethics Committee at Lund University, Lund, Sweden. Written informed consent was obtained from all participants.

**Study population.** The first sample, including cognitively healthy elderly (CHE) subjects and subjects with mild cognitive symptoms (MCS), i.e. patients with either subjective cognitive decline (SCD) or objective impairment (MCI), was part of the prospective and longitudinal Swedish BioFINDER (Biomarkers For Identifying Neurodegenerative Disorders Early and Reliably) study (<http://www.biofinder.se>). The 267 CHE participants, recruited from the population-based Malmö Diet Cancer study<sup>50</sup>, were eligible for inclusion in the cognitively healthy elderly cohort of the Swedish BioFinder study if they 1) were aged  $\geq 60$  years old, 2) scored 28–30 points on Mini-Mental State Examination (MMSE), 3) did not suffer from any subjective cognitive impairment and 4) were fluent in Swedish<sup>51</sup>. Exclusion criteria included presence of significant neurologic disease, severe psychiatric disease, dementia or MCI. The 360 MCS cases complaints consisted of patients enrolled consecutively at three memory outpatient clinics in Sweden. They were thoroughly assessed by physicians with special interest in dementia disorders. The inclusion criteria were: 1) referred to the memory clinics because of cognitive impairment; 2) not fulfilling the criteria for dementia; 3) a MMSE score of 24–30 points; 4) age 60–80 years and; 5) fluent in Swedish. Exclusion criteria were: 1) cognitive impairment that without doubt could be explained by a condition other than prodromal dementia; 2) severe somatic disease, and; 3) refusing lumbar puncture or neuropsychological investigation. MCS patients were assessed based on a neuropsychological battery assessing four broad cognitive domains including verbal ability, visuospatial construction, episodic memory, and executive functions. A senior neuropsychologist then stratified all patients into those with SCD (no measurable cognitive deficits) or MCI according to the consensus criteria for MCI suggested by Petersen<sup>52</sup>. In total 165 patients (46%) were classified as SCD and 195 (54%) patients as MCI.

The second sample here referred to as the ‘AD cohort’, comprised 110 subjects with AD. Subjects were recruited from a retrospective study performed between 2008 and 2014. All patients met the dementia criteria and were diagnosed as probable AD according to NINCDS-ADRDA<sup>53</sup> and to have CSF A $\beta$ 42 below 550 ng/L to confirm the presence of amyloid pathology.

The third sample, comprising 89 PD cases without dementia, here referred to as the “PD cohort”, is also part of the prospective Swedish BioFINDER study. PD diagnosis was set according to the NINDS Diagnostic Criteria<sup>54</sup>.

Demographic characteristics of the participants are presented in Table 1. More information is given in the Supplementary information online and at [www.biofinder.se](http://www.biofinder.se).

**Image acquisition and assessment of cerebrovascular disease.** In CHE, SCD, MCI and PD subjects, MR imaging was performed at 3 T systems and included transversal T2 FLAIR and high resolution isotropic MPRAGE. In AD subjects, imaging data included axial CT images in 96 cases and axial FLAIR images acquired at 1.5 T MR systems in 14 cases.

WML and lacunes assessed from MRI, and WML from CT were used as markers of SVD. For MR data in the CHE, SCD, MCI and PD subjects, automated segmentation of WML using the LST toolbox implemented in SPM8, generated a total lesion volume [mL], here named ‘WML volume’, for each individual<sup>55</sup>. Visual rating of WML on FLAIR images according to the Fazekas scale<sup>56</sup>, resulted in a total Fazekas score, and according to the ARWMC scale<sup>57</sup>, resulting in regional as well as total scores. CT images in the AD cohort were assessed for WML according to the Fazekas scale. Scores from the left and right hemispheres were summarized for statistical analysis. The presence of lacunes was assessed on FLAIR and MPRAGE images according to Wardlaw<sup>58</sup>. This variable was dichotomized as lacunes, present or absent. More information is given in the Supplementary Methods online.

**CSF collection and analysis.** The collection procedure and analysis of CSF followed the Alzheimer’s Association Flow Chart for CSF biomarkers<sup>59</sup>. Lumbar CSF samples were collected and stored in polypropylene tubes at  $-80^{\circ}\text{C}$  and analyzed in one batch. CSF levels of A $\beta$ 338 and A $\beta$ 40 were measured using EUROIMMUN ELISAs (EUROIMMUN AG, Lübeck, Germany), and CSF A $\beta$ 42 and CSF P-tau were measured using INNOTEST ELISAs (Fujirebio Europe, Gent, Belgium).

**<sup>18</sup>F-Flutemetamol PET imaging and analysis.** In 122 CHE, 101 SCD and 98 MCI subjects, the cerebral A $\beta$  burden was measured using [<sup>18</sup>F]-flutemetamol PET. Subjects received a single dose of [<sup>18</sup>F]flutemetamol and its average uptake was estimated from sum images acquired 90–110 min post injection<sup>60</sup>. Image processing was performed as previously described<sup>61</sup>. The standardized uptake value ratio (SUVR) was determined as the regional and composite tracer uptake normalized for the mean uptake in the cerebellar cortex, which is free of fibrillar plaques. More information is given in the Supplementary Methods online.

**Statistics.** Statistics were computed using SPSS version 22 (IBM). Non-normally distributed variables were transformed into normality using log transformation. Group-wise comparisons of baseline characteristics were performed using the Pearson Chi-square test for categorical variables and ANOVA for continuous variables. Since WML rating in the AD group mainly was performed on CT images, WML scores were not compared between the AD group and the other groups.

Regression analyses were performed to test the associations between estimates of SVD and amyloid deposition, with WML volume, Fazekas and ARWMC scores and lacunes as dependent variables and CSF-biomarkers and PET composite scores as independent variables. Regressions were conducted separately for each independent variable. Linear regression was used for continuous (WML volume, Fazekas and ARWMC scores) and logistic regression for binary variables (lacunes). All regression analyses were adjusted for age, sex, and hippocampal volume. Hippocampal volume was included as a co-variate in the regression analyses to adjust for AD disease stage as suggested by Kester *et al.*<sup>21</sup> The values in Table 2, 3, 4 and 5 represent standardized betas from linear regression in each group as specified in the first column. Since four markers of amyloid deposition were studied, only p-values < 0.0125 should be considered significant according to Bonferroni-adjustment. Comparison of the demographic data in Table 1 involved comparison of each diagnostic group to each of the other groups; application of Bonferroni correction would result in a significance level of p-values < 0.005 as indicated.

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## Author Contributions

D.V.W. co-designed the study, collected, analyzed and interpreted the data, conducted literature searches, prepared figures, cowrote the manuscript. D.L. co-designed the study, analyzed and interpreted the data,



conducted literature searches, cowrote the manuscript. K.B., L.M., K.N., E.S. and H.Z. collected and analyzed the data and reviewed the manuscript for intellectual content. O.H. was the principal designer and coordinator of the study, overviewed collection, analysis and interpretation of the study data, cowrote the manuscript and obtained funding.

### Additional Information

**Supplementary information** accompanies this paper at <http://www.nature.com/srep>

**Competing financial interests:** Doses of  $^{18}\text{F}$ -flutemetamol injection were sponsored by GE Healthcare. Dr Blennow has served at advisory boards for Koyowa Kirin Pharma, Eli Lilly, Pfizer, and Roche. Dr Hansson has served at advisory boards for GE Healthcare. Drs van Westen, Lindqvist, Minthon, Nägga, Stomrud and Zetterberg report no disclosures.

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