

# CSF amyloid is a consistent predictor of white matter hyperintensities across the disease course from ageing to Alzheimer's Disease

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This study investigated the relationship between white matter hyperintensities (WMH) and cerebrospinal fluid (CSF) Alzheimer's disease (AD) biomarkers. Subjects included 180 controls, 107 individuals with a significant memory concern (SMC), 320 individuals with early mild cognitive impairment (EMCI), 171 individuals with late mild cognitive impairment (LMCI) and 151 individuals with AD, with 3T MRI and CSF A $\beta$ 1-42, total tau (t-tau) and phosphorylated tau (p-tau) data. Multiple linear regression models assessed the relationship between WMH and CSF A $\beta$ 1-42, t-tau and p-tau. Directionally, a higher WMH burden was associated with lower CSF A $\beta$ 1-42 within each diagnostic group, with no evidence for a difference in the slope of the association across diagnostic groups ( $p=0.4$ ). Pooling all participants, this association was statistically significant after adjustment for t-tau, p-tau, age, diagnostic group and APOE- $\epsilon$ 4 status ( $p<0.001$ ). Age was the strongest predictor of WMH (partial  $R^2\sim 16\%$ ) compared with CSF A $\beta$ 1-42 (partial  $R^2\sim 5\%$ ). There was no evidence for an association with WMH and either t-tau or p-tau. This data is supportive of a link between amyloid burden and presumed vascular pathology.

## Keywords

Alzheimer's disease, vascular disease, white matter hyperintensities, amyloid, tau, cerebrospinal fluid

## 1. Introduction

The co-existence of cerebrovascular disease (CVD) and Alzheimer's disease (AD) is increasingly well recognised; AD patients often display both AD pathology and underlying vascular pathologies at autopsy such as small vessel disease (SVD), microvascular injury and cerebrovascular lesions (Brayne et al., 2009, Esiri et al., 2014, Jellinger and Attems, 2006, Kapasi et al., 2017, Schneider et al., 2007, Schneider et al., 2009). Cerebrovascular pathology is well established as an important contributor to cognitive decline, acting as the primary cause of at least 20% of dementias (Gorelick et al., 2011). However, the relationship between CVD and the hallmark Alzheimer's pathologies including tau and amyloid beta ( $A\beta$ ) is yet to be fully characterised.

$A\beta$  deposition in the brain can be globally measured *in vivo* by analysing cerebrospinal fluid (CSF) levels of  $A\beta$ 1-42, whereby an increase in parenchymal  $A\beta$  is signified by a decrease in CSF  $A\beta$ 1-42 (Blennow et al., 2010). Cerebral tau levels can also be assessed using CSF analysis, with the levels of total tau (t-tau) and phosphorylated tau (p-tau) thought to indicate neuronal damage and pathological tau deposition respectively (Jack et al., 2018). Markers of CVD are visible and quantifiable on magnetic resonance imaging (MRI), with perhaps the best characterised being white matter hyperintensities (WMH) that appear hyperintense on T2-weighted or FLAIR imaging and hypointense on T1-weighted imaging (Wardlaw et al., 2013). WMH have numerous histopathological correlates, including ependymal loss, cerebral ischemia and demyelination (Gouw et al., 2011). However, it may be that WMH are not only a marker of CVD, but also occur as a result of Alzheimer's pathologies (McAleese et al., 2015, McAleese et al., 2017).

Research studies are now moving towards a more biomarker led approach to AD (Jack et al., 2018), making it important to understand how possible biomarkers are interrelated at different stages of disease. Although no cerebrovascular biomarkers have been included in the research framework proposed by Jack et al. (2018), WMH are a good candidate marker to enhance our understanding of the associations between AD and vascular pathology.

WMH have shown associations with amyloid accumulation, as measured by positron emission tomography (Kandel et al., 2016, Marnane et al., 2016) and CSF biomarkers (Marnane et al., 2016, Pietroboni et al., 2018). Tau aggregation has also been linked to WMH burden (McAleese et al., 2015, Tosto et al., 2015), although results are somewhat less consistent (Kester et al., 2014, Osborn et al., 2018). The aim of this study was to add evidence to further characterise these complex relationships between AD and vascular biomarkers, and to extend the current literature by examining these relationships across the full spectrum from normal ageing to AD. We used continuous as opposed to binary measures of AD biomarkers, to more sensitively examine relationships with WMH. To the best of our knowledge, this study is the first to look at relationships between traditional biomarkers of AD (CSF  $A\beta$ 1-42, t-tau and p-tau) and WMH in controls, individuals with a significant memory concern (SMC), individuals with early MCI (EMCI), individuals with late MCI (LMCI) and individuals with AD. We hypothesise that WMH will show associations with CSF  $A\beta$ 1-42, t-tau and p-tau biomarkers, reflecting the potential relationships between vascular dysfunction, amyloid deposition, neurodegeneration and tau deposition. Since WMH are likely to represent different admixtures of pathology at different disease stages, with recent evidence suggesting in later disease stages WMH associations with tau may be stronger (McAleese et al., 2017) and  $A\beta$ 1-42 may be weaker (Zhou et al., 2009), we also hypothesise that the relationships between  $A\beta$  and tau biomarkers and WMH may change

throughout the disease course with stronger associations between tau markers and WMH at later disease stages, and weaker associations between A $\beta$ 1-42 and WMH at the more advanced stages.

## 2. Materials and methods

### 2.1 Cohort:

The demographic, imaging and biological data used for this study were downloaded from the ADNI database (<http://adni.loni.usc.edu/>). Both ADNIGO and ADNI2 data were used. ADNI is a multicentre, longitudinal public-private funded partnership, with the primary goal of using demographic, biomarker, neuropsychological and MRI data to monitor progression of AD. Since 2003, Principle Investigator Michael W. Weiner, MD, has overseen recruitment of healthy controls, MCI and AD subjects from over 60 sites across the United States and Canada. (For up-to-date information, see [www.adni-info.org](http://www.adni-info.org)).

Ethical approval was obtained by the Institutional Review Board at each participating centre. All study participants provided written informed consent. Participants took part in baseline and follow-up clinical, neuropsychometric and MRI assessments. CSF data were collected from all subjects where possible.

Five diagnostic groups were used for this study: Control, SMC, early MCI, late MCI and AD. A diagnosis of MCI was based on a significant memory concern (by participant or informant) and a clinical dementia rating of 0.5, but a Mini-Mental State Exam (MMSE) no lower than 24. Level of MCI (early or late) was determined by the Wechsler Memory Scale Logical Memory II. The SMC group was developed by ADNI to bridge the gap between controls and MCI participants, and is characterised by individuals who have self-reported a memory complaint but score within a normal range for cognition.

### 2.2 CSF measurements

CSF A $\beta$ 1-42, t-tau and p-tau were measured at the ADNI biomarker core (University of Pennsylvania) using the micro-bead-based multiplex immunoassay, the INNO-BIA AlzBio3 RUO test (Fujirebio, Ghent, Belgium), on the Luminex platform (Luminex Corp, Austin, Tx, USA). Raw, untransformed CSF values were used for all analyses.

Additional analyses were carried out on CSF data obtained using the Roche Elecsys cobas e 601 fully automated immunoassay platform at the ADNI biomarker core (University of Pennsylvania).

### 2.3 Images and image processing:

MRI (3T) were acquired across sites using a standardised protocol that was validated across platforms and described in Jack (2015). Images underwent quality control at the Mayo Clinic (Rochester, MN) which included assessments for protocol compliance and image quality, as well as checks for any significant neurological/radiological abnormalities. Baseline T1-weighted and FLAIR images from newly recruited individuals (i.e. with a first visit in ADNI2 or GO) were downloaded from the ADNI database.

Bayesian Model Selection (BaMoS), a fully automated lesion segmentation pipeline, was applied to the co-registered image pairs (T1 and FLAIR) (Sudre et al., 2015). Briefly, BaMoS uses a

Gaussian mixture model to jointly model healthy tissue and unexpected intensity observations for the different anatomical brain tissue. The number of Gaussian components required to appropriately model each tissue type is dynamically optimised using a split and merge strategy. After fitting the model, candidate hyperintense WMH voxels are selected and the formed connected components classified as lesion or artefacts in a post-processing step based on anatomy rules. The resultant probability map of WMH was integrated to calculate the WMH volume. Only supratentorial WMH were included which were located in the white matter and subcortical grey matter. Total intracranial volumes (TIV) were calculated using the Geodesic information flows (GIF) label fusion framework, using T1-weighted images only (Cardoso et al., 2015).

All automated WMH segmentations were visually inspected by a single rater who had been trained to segment WMH manually. The post-processing stage of the lesion classification was iteratively improved so as to limit the number of unusable segmentations and improve segmentations where large sections of lesion had been excluded. 22 cases were improved in this way and re-checked by a single rater, 21 of which were useable. Altogether, 12 regions were considered unusable out of 944 regions in separate individuals. 3 further individuals were excluded due to lack of demographic/diagnostic information.

To calculate the parietal and non-parietal WMH, Euclidean distance maps from the cortical lobar parcellation obtained from GIF were used to assign each WM voxel to the closest associated lobe.

#### 2.4 Statistical analyses:

All statistical analysis was performed using STATA v13 (Stata Corp.). For WMH, most analyses were performed on  $\log_e$  transformed values to reduce skewness. To estimate differences in means across groups for demographic, imaging and CSF variables, linear regression models were used for continuous variables. For  $\log_e$ WMH, linear regression models were used with group as the predictor of interest and adjustment for TIV. We also present untransformed WMH volumes and again test for differences in these across diagnostic groups, using linear regression with adjustment for TIV.

For analyses involving associations between WMH volume and CSF biomarkers, CSF biomarker values were standardised using the pooled within-group standard deviations (SDs) calculated from a linear regression model for each biomarker that allowed for differences in mean levels by group. Since WMH volumes were analysed on a logarithmic scale the resulting coefficients were back-transformed so that they expressed percentage increases or decreases in WMH for each 1SD increase in CSF biomarkers.

In order to explore whether CSF A $\beta$ 1-42 was associated with WMH across diagnostic groups, separate diagnostic group-specific linear regression models were fitted with  $\log_e$ WMH as the dependent variable and standardised A $\beta$ 1-42 and TIV as predictors. Between-group differences in slope were assessed by fitting a further model in the combined diagnostic groups, with interactions between diagnostic group and A $\beta$ 1-42, and carrying out a joint interaction test. All models were also refitted with age at baseline as an additional covariate. Analyses including age were secondary, since age at baseline may be on the causal pathway (theoretically a proxy for the number of vascular events that may have happened to an individual and the ability to clear amyloid). Analogous procedures were used with A $\beta$ 1-42 values being replaced by t-tau and then by p-tau.

Scatter plots of  $\log_e$ WMH volume against CSF A $\beta$ 1-42, t-tau or p-tau in each group were also generated to show unadjusted associations.

We decided that should the joint interaction terms between participant group and A $\beta$ 1-42 in the above models not be statistically significant then any further analysis would be carried out in the cohort as a whole, adjusting for participant group but without including the interaction terms. In a first set of linear regression models we explored the A $\beta$ 1-42-WMH relationship adjusting for: i) nothing ii) TIV; iii) TIV and diagnostic group; iv) TIV, group and age; v) TIV, group age and APOE- $\epsilon$ 4 status. This was repeated in a second set of models adding t-tau as a covariate to all models in the set i) to v) and in a third set of models by adding p-tau to all models in the set i) to v). Partial R<sup>2</sup> values for these potential confounders were also calculated for each covariate from model v) in each set.

Supplementary analyses were also performed assessing the relationship between age and WMH using linear regression models with  $\log_e$ WMH being the dependent variable and age and TIV being predictors. This was initially performed in each diagnostic group separately, with one further model fitted that allowed for an interaction between diagnostic group and age. To assess the impact of vascular risk factors on the WMH-age relationship, smoking, hypertension, BMI and diabetes were added as covariates into separate diagnostic group-specific models and then in a combined model. To examine relationships between age and CSF variables, linear regression models were initially performed with A $\beta$ 1-42 as the dependent variable and age as the predictor, before replacing A $\beta$ 1-42 with t-tau and then p-tau. We also assessed associations between CSF biomarkers and parietal lobe WMH volumes vs. non-parietal WMH. These analyses used CSF A $\beta$ 1-42, t-tau or p-tau as the dependent variable and  $\log_e$  transformed parietal and non-parietal WMH volumes as predictor variables. The main analyses were repeated using the Elecsys biomarker data. These included the group-specific linear regression models with  $\log_e$ WMH as the dependent variable and standardised biomarker and TIV as predictors, and the analysis of CSF A $\beta$ 1-42 and WMH in the cohort as a whole.

### 3. Results

#### 3.1 Group demographics

**Table 1:** Demographic, imaging and CSF summary statistics

	Controls	SMC	EMCI	LMCI	AD	<i>p</i> value across groups
N (total = 929)	180	107	320	171	151	
Age	73.4 (6.2)	72.3 (5.5)	71.0 (7.5)	72.4 (7.7)	75.0 (8.0)	<0.001
Male (%)	46	43	54	56	56	0.08
MMSE /30	29.0 (1.3)	29.0 (1.3)	28.3 (1.6)	27.6 (1.8)	23.1 (2.1)	<0.001
APOE-ε4 positive (%)	33	36	47	60	71	<0.001
TIV (ml)	1403 (134)	1414 (125)	1423 (136)	1436 (132)	1416 (151)	0.2
WMH volume (ml)    Median (IQR)	3.4 (4.8)	3.4 (4.4)	3.8 (6.1)	3.7 (8.1)	5.8 (9.0)	<b>0.005<sub>a</sub></b>
Log <sub>e</sub> WMH (ml)	1.3 (0.9)	1.3 (0.9)	1.4 (1.0)	1.4 (1.0)	1.7 (1.0)	<b>0.002<sub>a</sub></b>
CSF Aβ1-42 (pg/ml) <sub>b</sub> Median (IQR)	270.5 (80.2) 287.5 (120.5)	308.3 (91.2) 318.0 (157.0)	251.0 (79.0) 255.0 (136.0)	212.0 (77.3) 190.5 (103.0)	181.1 (64.3) 169.0 (53.0)	<0.001
CSF t-tau (pg/ml) <sub>b</sub> Median (IQR)	62.5 (29.0) 53.0 (33.6)	59.6 (24.7) 53.6 (27.3)	70.9 (42.3) 59.2 (39.1)	93.3 (50.2) 81.3 (72.6)	120.1 (56.3) 108.0 (71.2)	<0.001
CSF p-tau (pg/ml) <sub>b</sub> Median (IQR)	21.8 (10.0) 19.6 (11.1)	21.6 (10.6) 18.3 (10.6)	23.0 (11.0) 20.4 (12.9)	28.6 (14.3) 25.5 (17.0)	34.2 (17.8) 31.3 (17.9)	<0.001
History of hypertension (%)	49	47	50	45	52	0.8
Smoking: Never (%)	58	53	59	65	61	0.4
Previous (%)	37	45	39	33	34	
Current (%)	4	2	2	2	4	
BMI	27.4 (4.6)	28.4 (6.3)	28.2 (5.3)	27.4 (4.9)	26.2 (5.3)	<0.001
History of diabetes (%)	7	10	11	9	15	0.2

Values are mean (SD) unless reported.

Key: SMC, significant memory concern; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; AD, Alzheimer’s disease; MMSE, Mini Mental State Examination; TIV, total intracranial volume; WMH, white matter hyperintensities; BMI, body mass index

<sub>a</sub> Adjusted for TIV

<sub>b</sub> Available in 138 controls, 92 SMC, 252 EMCI, 144 LMCI, 119 AD

There were 929 subjects with useable WMH values and available demographic. Table 1 shows demographic, imaging and CSF biomarker summary statistics for each diagnostic group. Between-group differences were seen in age, with the AD group being the oldest and the early mild cognitive impairment (EMCI) group being the youngest. Participants differed as expected in terms of MMSE, APOE-ε4 status, WMH volume, CSF Aβ1-42, CSF t-tau and CSF p-tau with poorer scores, greater ε4 carriage and CSF biomarkers indicative of more pathology from controls to EMCI to late mild cognitive impairment (LMCI) to AD. In terms of vascular risk factors,

differences were only observed between groups in body mass index (BMI), with a lower BMI observed in the AD group. Summary CSF marker statistics for the subgroup with Elecsys CSF A $\beta$ 1-42 measures are shown in Supplementary Table 1.

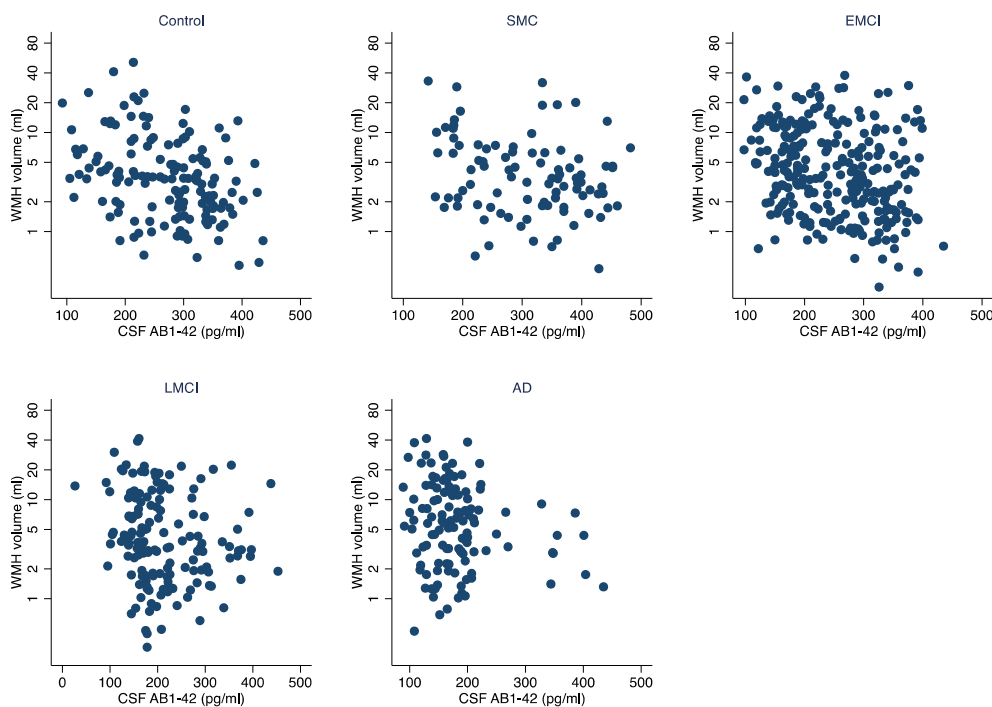
### 3.2 CSF biomarkers and WMH

**Table 2:** Regression model results for the relationship between WMH and CSF A $\beta$ 1-42, by group

WMH and A $\beta$ 1-42	Controls	SMC	EMCI	LMCI	AD
<b>TIV adjusted</b>					
% reduction in WMH (ml) per 1SD change in A $\beta$ 1-42	30.4	20.0	22.0	14.9	18.0
95% confidence intervals	(20.3 to 39.3)	(6.4 to 31.6)	(12.5 to 30.5)	(-0.5 to 28.0)	(-0.2 to 32.8)
<i>p</i> value	<0.001	0.006	<0.001	0.06	0.05
<b>TIV and age adjusted</b>					
% reduction in WMH (ml) per 1SD change in A $\beta$ 1-42	26.7	18.7	12.3	9.4	21.7
95% confidence intervals	(15.7 to 36.2)	(5.2 to 30.2)	(2.5 to 21.0)	(-5.5 to 22.2)	(6.8 to 34.2)
<i>p</i> value	<0.001	0.009	0.02	0.2	0.006
<b>Between group differences in % reductions per 1SD change in A<math>\beta</math>1-42</b>			<i>p</i> value = 0.4 (0.4 with age adjustment)		

Estimates are percentage decrease in WMH volume per one SD change (1 SD = 78.3pg/ml) in A $\beta$ 1-42. The SD is the pooled within-group SD, calculated from a linear regression model that allowed for differences in mean levels of A $\beta$ 1-42 by group.

Key: SMC, significant memory concern; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; AD, Alzheimer's disease; TIV, total intracranial volume; WMH, white matter hyperintensities.



**Figure 1:** Scatter plots showing the relationship between WMH and CSF A $\beta$ 1-42 in controls, significant memory concern (SMC), early mild cognitive impairment (EMCI), late mild cognitive impairment (LMCI), Alzheimer's disease (AD) groups. WMH volume is shown on a log scale and is unadjusted for any covariates.

Table 2 shows the estimated percentage decreases in WMH for increases in CSF A $\beta$ 1-42 after adjustment for TIV and Figure 1 shows the corresponding scatter plots. Although the estimated slope of the association in the five diagnostic groups varied somewhat (and was not formally statistically significant in the LMCI group), 95% confidence intervals were relatively wide and the variability in estimates was consistent with chance (joint tests for differences in slopes among



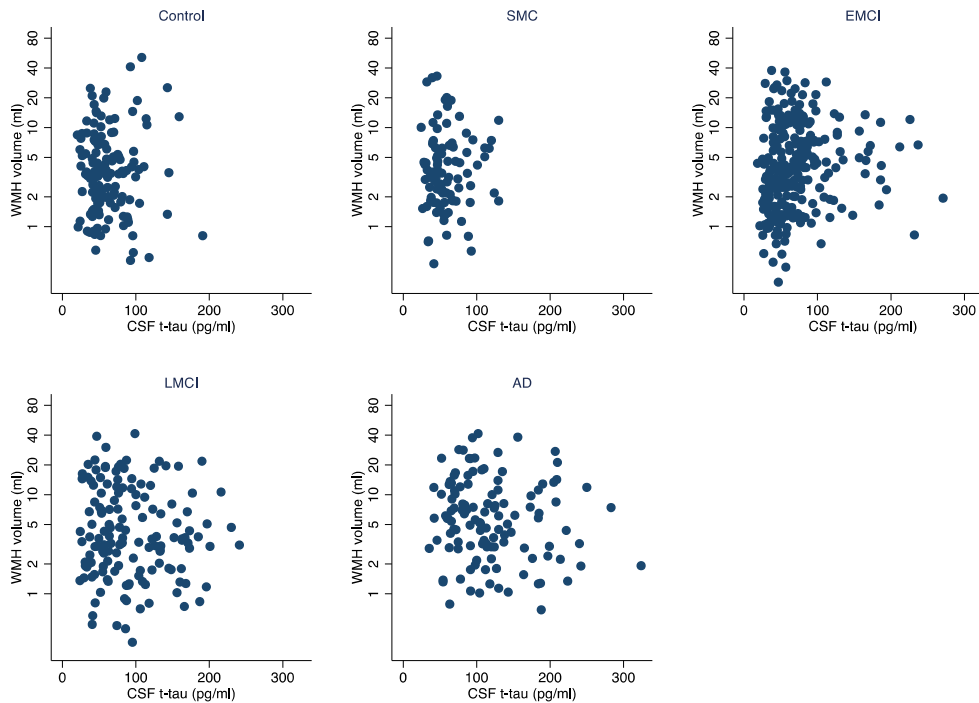
groups were not statistically significant). The effect of CSF A $\beta$ 1-42 on WMH was typically reduced after adjustment for age, but we did not find any material changes in effects after adjustment for gender (Supplementary Table 2). Supplementary Table 3 shows equivalent results to Table 2 using the Elecsys CSF A $\beta$ 1-42 assay. These results demonstrate similar findings using this alternative assay.

**Table 3:** Regression model results for the relationship between WMH and CSF t-tau, by group

WMH and t-tau	Controls	SMC	EMCI	LMCI	AD
<b>TIV adjusted</b>					
% increase in WMH (ml) per 1SD change in t-tau	5.1	3.8	13.2	-4.0	-3.3
95% confidence intervals	(-16.4 to 32.0)	(-24.9 to 43.5)	(0.2 to 27.7)	(-16.9 to 11.1)	(-15.2 to 10.2)
<i>p</i> value	0.7	0.8	<b>0.05</b>	0.6	0.6
<b>TIV and age adjusted</b>					
% increase in WMH (ml) per 1SD change in t-tau	-7.3	-5.1	2.4	-3.9	3.6
95% confidence intervals	(-26.2 to 16.3)	(-31.2 to 30.9)	(-8.0 to 14.0)	(-15.7 to 9.5)	(-7.9 to 16.5)
<i>p</i> value	0.5	0.8	0.7	0.6	0.6
<b>Between group differences in % increases per 1SD change in t-tau</b>			<i>p</i> value = 0.4 (0.7 with age adjustment)		

Estimates are percentage increase in WMH volume per one SD change (1 SD = 42.8pg/ml) in t-tau. The SD is the pooled within-group SD, calculated from a linear regression model that allowed for differences in mean levels of t-tau by group.

Key: SMC, significant memory concern; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; AD, Alzheimer's disease; TIV, total intracranial volume; WMH, white matter hyperintensities.



**Figure 2:** Scatter plots showing the relationship between WMH and CSF t-tau in controls, significant memory concern (SMC), early mild cognitive impairment (EMCI), late mild cognitive impairment (LMCI) and Alzheimer's disease (AD) groups. WMH volume is shown on a log scale and is unadjusted for any covariates.

Table 3 shows the percentage increases in WMH for increases in CSF t-tau and Figure 2 shows the corresponding scatter plots. With adjustment for TIV, WMH burden was borderline positively



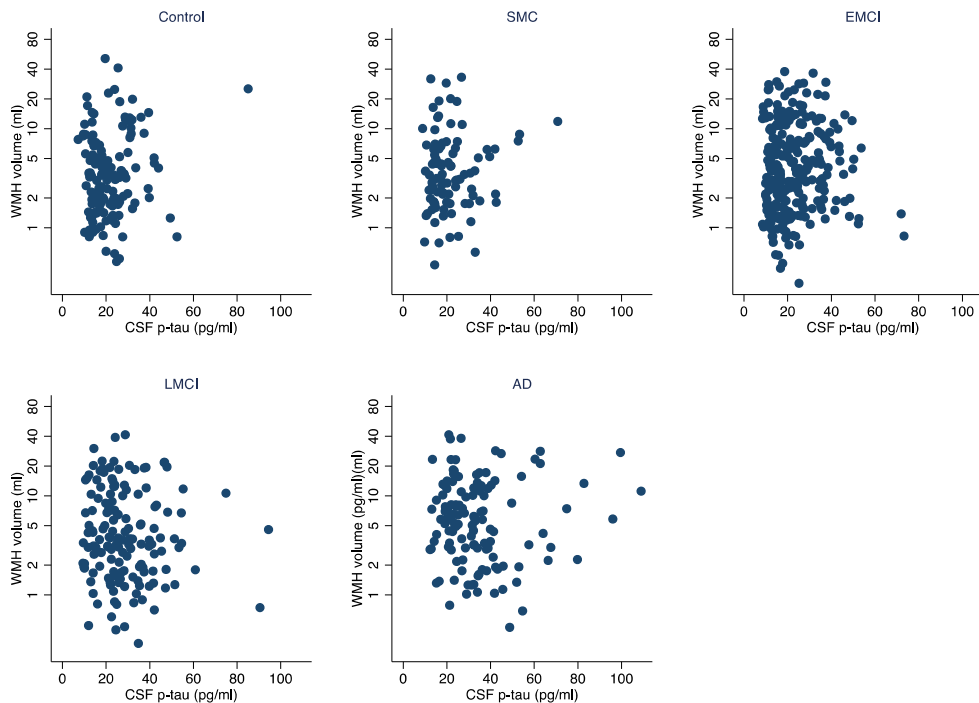
associated with t-tau only in the EMCI group. This association was no longer significant after adjusting for age. There was no evidence for an interaction between t-tau and group.

**Table 4:** Regression model results for the relationship between WMH and CSF p-tau, by group

WMH and p-tau	Controls	SMC	EMCI	LMCI	AD
<b>TIV adjusted</b>					
% increase in WMH (ml) per 1SD change in p-tau	13.6	14.1	3.5	-8.7	0.5
95% confidence intervals	(-6.6 to 38.3)	(-8.8 to 42.8)	(-10.0 to 18.9)	(-21.3 to 5.9)	(-10.8 to 13.3)
<i>p</i> value	0.2	0.3	0.6	0.2	0.9
<b>TIV and age adjusted</b>					
% increase in WMH (ml) per 1SD change in p-tau	7.3	13.1	-3.9	-3.0	8.0
95% confidence intervals	(-11.3 to 29.9)	(-9.0 to 40.5)	(-14.8 to 8.4)	(-15.3 to 11.1)	(-3.0 to 20.3)
<i>p</i> value	0.5	0.3	0.5	0.7	0.1
<b>Between group differences in % increases per 1SD change in p-tau</b>			<i>p</i> value = 0.4 (0.5 with age adjustment)		

Estimates are percentage increase in WMH volume per one SD (1SD = 12.8pg/ml) change in p-tau. The SD is the pooled within-group SD, calculated from a linear regression model that allowed for differences in mean levels of p-tau by group.

Key: SMC, significant memory concern; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; AD, Alzheimer's disease; TIV, total intracranial volume; WMH, white matter hyperintensities.



**Figure 3:** Scatter plots showing the relationship between WMH and CSF p-tau in controls, significant memory concern (SMC), early mild cognitive impairment (EMCI), late mild cognitive impairment (LMCI), Alzheimer's disease (AD) groups. WMH volume is shown on a log scale and is unadjusted for any covariates.

Table 4 shows the percentage increases in WMH for increases in CSF p-tau and Figure 3 shows the corresponding scatter plots. There was no evidence for an association between WMH and p-tau in any diagnostic group. There no evidence for an interaction between group and p-tau.

### 3.3 Relationships of CSF A $\beta$ 1-42 with WMH

**Table 5:** Regression model results examining the association between CSF A $\beta$ 1-42 and WMH with various covariates

a)

Model set	Covariates	% reduction in WMH per 1SD change in A $\beta$ 1-42	95% confidence interval	p value
1	i) None	21.9	17.1 to 26.5	<0.001
	ii) TIV	21.5	16.7 to 26.0	<0.001
	iii) TIV, group	21.6	16.2 to 26.6	<0.001
	iv) TIV, group, age	16.4	11.2 to 21.3	<0.001
	v) TIV, group, age, APOE- $\epsilon$ 4 status	17.9	12.2 to 23.2	<0.001
2	i) T-tau	24.5	19.2 to 29.5	<0.001
	ii) T-tau, TIV	23.2	17.9 to 28.1	<0.001
	iii) T-tau, TIV, group	23.1	17.5 to 28.3	<0.001
	iv) T-tau, TIV, group, age	18.0	12.6 to 23.1	<0.001
	v) T-tau, TIV, group, age, APOE- $\epsilon$ 4 status	18.8	13.0 to 24.2	<0.001
3	i) P-tau	24.1	18.7 to 29.1	<0.001
	ii) P-tau, TIV	23.8	18.6 to 28.7	<0.001
	iii) P-tau, TIV, group	23.8	18.2 to 29.0	<0.001
	iv) P-tau, TIV, group, age	17.7	12.2 to 23.0	<0.001
	v) P-Tau, TIV, group, age, APOE- $\epsilon$ 4 status	18.9	12.9 to 24.4	<0.001

b)

Model set	Covariates from v)	Partial R <sup>2</sup>
1	A $\beta$ 1-42	0.05
	TIV	0.05
	Group	0.003
	Age	0.16
	APOE- $\epsilon$ 4 status	0.002
2	A $\beta$ 1-42	0.05
	t-tau	0.005
	TIV	0.06
	Group	0.005
	Age	0.16
	APOE- $\epsilon$ 4 status	0.0007
3	A $\beta$ 1-42	0.05
	p-tau	0.003
	TIV	0.06
	Group	0.004
	Age	0.15
	APOE- $\epsilon$ 4 status	0.001

All models assess associations between WMH and A $\beta$ 1-42 with various covariates, with model 2 additionally adjusting for t-tau and model 3 additionally adjusting for p-tau. In a) estimates are percentage changes in WMH volume per one SD change (1 SD = 78.3pg/ml) in A $\beta$ 1-42. The SD is the pooled within-group SD, calculated from a linear regression model that allowed for differences in mean levels of A $\beta$ 1-42 by group. In b) partial R<sup>2</sup> are shown for each covariate in the fully-adjusted model v) from each of the three model sets. R<sup>2</sup> values are shown to 2 significant figures for values >0.1 and 1 significant figure for values <0.1.

Key: TIV, total intracranial volume; WMH, white matter hyperintensities

Table 5a shows the percentage reductions in WMH volume for a 1 SD increase in CSF A $\beta$ 1-42 adjusting for group, age, APOE- $\epsilon$ 4 status, t-tau and p-tau. The relationship between WMH and A $\beta$ 1-42 remains significant and materially unchanged (between a 16 and 22% reduction in WMH per 1SD change in A $\beta$ 1-42) after progressive adjustment for covariates. This association between CSF A $\beta$ 1-42 and WMH was also shown to be independent of the effects of CSF t-tau and p-tau.

Table 5b shows partial R<sup>2</sup> values for all the covariates in each model set. A $\beta$ 1-42 consistently uniquely explained approximately 5% of the variance in WMH across all models. Age explained the largest variance in WMH at ~15%, compared with tau biomarkers, group and APOE- $\epsilon$ 4 status, with each only explaining only around 1%.

Supplementary Table 4 shows analogous results to Table 5 using the Elecsys assay. The results are not materially changed by the use of this alternative CSF A $\beta$ 1-42 measurement.

## 4. Discussion

In this study, we found strong evidence for a consistent association between CSF A $\beta$ 1-42 and WMH in all diagnostic groups, whereby a decrease in CSF A $\beta$ 1-42 was associated with an increase in WMH, that was independent of CSF t-tau and independent of CSF p-tau. Furthermore, this relationship remained significant in the pooled cohort when adjusting for age, diagnostic group, TIV and APOE- $\epsilon$ 4 status. There was very little evidence for a relationship between t-tau pathology and WMH, with a borderline positive association only in the EMCI group. No association of p-tau with WMH was observed. To our knowledge, this is the first study to look for a direct association between A $\beta$ 1-42 and tau CSF biomarkers and WMH volumes across the disease spectrum, from normal ageing to AD, including patients with EMCI and SMC.

Our findings provide evidence for a link between WMH and amyloid pathology, from normal ageing through to clinical AD. Notably, evidence from autosomal dominantly inherited familial AD suggests that changes in WMH could occur in the presymptomatic stage of disease, deviating from normal controls around 6 years before symptom onset (Lee et al., 2016). Our observations from groups at higher risk of developing sporadic AD dementia add further weight to the notion that WMH are a core feature of AD.

We did not find any evidence for differing relationships in WMH and A $\beta$ 1-42, p-tau or t-tau between diagnostic groups. WMH are heterogeneous and are likely to represent multiple pathologies that could potentially vary throughout the disease course. Given the recent evidence that WMH may be more related to neurodegenerative and tau pathologies in clinical AD (McAleese et al., 2017), it would have been reasonable to propose stronger relationships with tau at later disease stages. Since A $\beta$ 1-42 levels are thought to be relatively stable in clinical AD stages (Zhou et al., 2009), it could also be expected that there might be less of an association with WMH in later disease stages. The fact that we did not find any differences between disease stages suggests that WMH relationships with AD CSF biomarkers actually remain consistent across the disease course.

Age at baseline was a stronger independent predictor of WMH than A $\beta$ 1-42, which likely reflects the contributions of vascular risk factors, vascular events, and co-morbidities that accrue with age. Taken together, although our study is purely correlative, our results are compatible with the theory that some WMH are part of the AD pathological process, and some are the result of an

ageing/vascular process (McAleese et al., 2015, McAleese et al., 2017). Although we did not find any evidence for contributions from smoking, hypertension, BMI and diabetes to the relationship between WMH and age (Supplementary Table 5), a more comprehensive examination of the effects of vascular risk factors, vascular disease and vascular related events on the age-WMH relationship may provide greater insight into the potential causes of WMH associated with age.

In studies of A $\beta$  and WMH there have been mixed findings; some have reported a lack of any association (Kalheim et al., 2017, Kester et al., 2014), whilst several studies confirm our findings that lower CSF A $\beta$ 1-42 is associated with higher WMH in both controls and disease groups (Marnane et al., 2016, Pietroboni et al., 2018). Some have reported that lower levels of CSF A $\beta$ 1-42 were particularly associated with WMH in AD subjects, as opposed to controls or MCI subjects (Shams et al., 2016, Van Westen et al., 2016). Results from amyloid PET studies have also demonstrated significant associations with WMH in controls (Kandel et al., 2016, Marnane et al., 2016), MCI and AD groups (Zhou et al., 2015). Although a recent systematic review concluded that most studies demonstrate a lack of significant relationship between amyloid PET and WMH burden (Roseborough et al., 2017).

This study adds to the expanding body of literature attempting to characterise the complex relationship between AD and presumed vascular pathology at different disease stages. As well as evidence that both A $\beta$  deposition and WMH independently predict cognitive impairment or brain atrophy (Barnes et al., 2013, Gordon et al., 2015, Haight et al., 2013, Provenzano et al., 2013), a growing number of studies are suggestive of an additive effect of combined pathology on neurodegeneration (Bos et al., 2018) and/or cognitive decline (Lopez et al., 2014, Vemuri and Knopman, 2016, Vemuri et al., 2015). Several studies have investigated the possibility of an interactive effect between the two pathologies, whereby there is a direct influence of one pathology on the other. One study found that WMH predicted hippocampal atrophy in preclinical AD subjects with abnormal CSF A $\beta$  (Freeze et al., 2016), and another found that low CSF A $\beta$  predicted greater WMH accrual over time (Scott et al., 2016).

Hypotheses into mechanistic links between A $\beta$  and WMH have mainly focused on the proposed vascular origin of WMH and reflect the idea that there may be positive feedback between the two pathologies (Smith and Greenberg, 2009, Zlokovic, 2011). Cerebral amyloid angiopathy (CAA) may be a potential mediator of the relationship between WMH and A $\beta$ , whereby SVD could lead to reduced clearance of A $\beta$  from brain parenchyma, enhancing its deposition in cerebral vessel walls. This in turn may result in cerebrovascular dysfunction, leading to eventual hypoperfusion and ischaemia and subsequent white matter lesions that appear as WMH (Grinberg and Thal, 2010, Hawkes et al., 2014, Weller et al., 2008). Previous research has demonstrated associations of CAA with WMH (Ryan et al., 2015) and with CSF A $\beta$  (Renard et al., 2012, Verbeek et al., 2009). A recent paper has shown that WMH related to A $\beta$  are associated with cerebral microbleeds (a surrogate of CAA pathology) (Graff-Radford et al., 2019). In addition to the vascular origin of WMH, recent evidence suggests that parietal WMH could be a result of degenerative axonal loss caused by AD pathology (McAleese et al., 2015, McAleese et al., 2017). In this study though, we did not find any evidence for a stronger association between CSF biomarkers and WMH in the parietal lobe (Supplementary Table 6).

There are comparatively fewer studies regarding the association with tau and WMH and results are often conflicting. A positive association has been demonstrated neuropathologically (McAleese et al., 2015) and with PET (Vemuri et al., 2017); more direct measures of brain tau load than CSF measures. A positive association has been demonstrated over time in CSF, but the relationship is less clear with baseline measures of tau (Tosto et al., 2015). Our study did not find

much evidence of a relationship between tau pathology and WMH, which is in line with several other studies (Guzman et al., 2013, Kester et al., 2014, Osborn et al., 2018).

The fact that age was the strongest predictor of WMH is consistent with previous studies (de Leeuw et al., 2001, Raz et al., 2012, Schneider et al., 2009). We also found that the association between biomarkers and age was not uniform between diagnostic groups (Supplementary Table 7). In controls and EMCI groups, older ages were associated with lower A $\beta$ 1-42 and higher tau, but in the AD group, younger ages were associated with higher tau. This differential effect of age on CSF biomarkers between AD groups and controls has been previously reported (Mattsson et al., 2012).

Investigations of the associations between AD CSF biomarkers and WMH in previous studies have either been performed on small samples or in studies with variable acquisition and analysis protocols. (Bos et al., 2018, Freeze et al., 2016, Kalheim et al., 2017, Pietroboni et al., 2018, Tosto et al., 2015). Using the ADNI cohort therefore provides the benefits of a multi-centre standardised data acquisition protocol, as well as participants who have been systematically evaluated. Our study adds to previous findings using the ADNI cohort (Marnane et al., 2016, Scott et al., 2016, Haight et al., 2013, Guzman et al., 2013), in that data from all diagnostic groups are used and biomarkers for both tau and A $\beta$  are compared with quantitative WMH. We also treated both our biomarker and WMH data as continuous variables; most previous studies have dichotomised variables.

There are several limitations to this work, which include the selection criteria for the ADNI cohort meaning any generalisations to the wider population must be made cautiously. ADNI participants tend to be of high socioeconomic status, well-educated and, importantly for this study, free of large amounts of cerebrovascular disease (a score of 4 or lower on the Hachinski Ischemic Scale)(<https://adni.loni.usc.edu/wp-content/uploads/2008/07/adni2-procedures-manual.pdf>). This study was also only carried out using baseline data; longitudinal studies are necessary to characterise the relationship between WMH and AD biomarkers over time, an approach which will be facilitated by the increasing use of volumetric FLAIR imaging providing more precise measures of change.

There are limitations surrounding the use of CSF biomarkers in this study to investigate AD pathology. CSF biomarkers can now be measured by several different assays, introducing the possibility that these methodological differences may influence study findings. However, Supplementary Tables 3 and 4 demonstrate that analyses carried out using another assay platform, Elecsys, confirm our findings of the associations between CSF biomarkers and WMH. Additionally, despite the benefits of using continuous biomarker variables, it would be interesting to look at associations within biomarker-negative and biomarker-positive individuals to explore whether associations may be driven by those on the amyloid pathway (Jack et al., 2018) or those who are not. CSF biomarkers are increasingly used to provide information about underlying biochemical changes associated with disease, with A $\beta$ 1-42 and p-tau well established as markers of AD pathology and t-tau as an established marker of neurodegeneration (Blennow et al., 2010, Jack et al., 2018). However, measures of actual brain A $\beta$  and tau burden and their local distribution, through PET imaging or pathological investigations, were either not available or not used.

This study only examined associations between WMH and the well-established AD biomarkers of CSF A $\beta$ 1-42, t-tau and p-tau. The existence of additional co-pathologies in AD suggest that further biomarkers are needed to complement these classical measurements. One biomarker of particular relevance is neurofilament light (NFL), a marker of large calibre axonal injury (Skillback et al., 2014). Previous studies of associations between WMH and NFL have reported mixed results

(Mattsson et al., 2019, Osborn et al., 2018, Zetterberg et al., 2016), and therefore future analysis across the full spectrum of the disease course using our well-characterised WMH segmentation tool would be highly pertinent.

A lack of pathological confirmation of cases in the ADNI cohort may, in part, explain our findings. Diagnostic groups may be bimodal in nature; control groups may contain atypical or early stage AD subjects along with the cognitively normal, and a significant proportion of MCI subjects supposedly at high risk for conversion to AD will not have any underlying AD pathology (Jicha et al., 2006, Petersen et al., 2006). Indeed in our study, this may have been a contributing factor to the lack of observed differences in biomarker and WMH relationships between diagnostic groups.

Additionally, although the four measures of vascular risk used in this study are unlikely to fully characterise all aspects of SVD, they are among the most notable risk factors, as well as being the most consistently documented for the ADNI cohort.

## 5. Conclusion

In summary, our study suggests that CSF A $\beta$ 1-42 is a consistent predictor of WMH across all diagnostic groups, in a manner that is not dependent on CSF tau. As WMH are thought to be largely vascular in origin, this study adds to the literature exploring the complex relationship between amyloid and presumed vascular pathologies. Understanding the different relationships between disease markers are important to build more realistic models of biomarkers changes across the full spectrum of normal ageing to AD.

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## 7. Disclosure statement

The authors have no actual or potential conflicts of interest.

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