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Angiotensin-Converting Enzyme in Cerebrospinal Fluid and Risk of Brain Atrophy

Hadassa M. Jochemsen[a,b], Wiesje M. van der Flier[a,c], Emma L. Ashby[d], Charlotte E. Teunissen[e], Ruth E. Jones[d], Mike P. Wattjes[f], Philip Scheltens[a], Mirjam I. Geerlings[b], Patrick G. Kehoe[d] and Majon Muller[g,*]

[a]*Alzheimer Center & Department of Neurology, Neuroscience Campus Amsterdam, VU University Medical Center, Amsterdam, The Netherlands*
[b]*Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands*
[c]*Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, The Netherlands*
[d]*Dementia Research Group, Institute of Clinical Neurosciences, School of Clinical Sciences, University of Bristol, Frenchay Hospital, Bristol, UK*
[e]*Department of Clinical Chemistry, Neurochemistry Laboratory, VU University Medical Center, Amsterdam, The Netherlands*
[f]*Department of Radiology, VU University Medical Center, Amsterdam, The Netherlands*
[g]*Department of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, The Netherlands*

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Abstract.
Background: Higher angiotensin-converting enzyme (ACE) activity might increase the risk of Alzheimer's disease by increasing blood pressure, and subsequent development of cerebral small vessel disease (CSVD). Yet, it may also decrease this risk, as it functions to degrade amyloid-β, thereby reducing brain atrophy.
Objective: To examine the cross-sectional associations of serum and cerebrospinal fluid (CSF) ACE protein levels and activity with brain atrophy and CSVD in a memory clinic cohort.
Methods: In 118 subjects from the memory clinic based Amsterdam Dementia Cohort (mean age 66 ± 8 years), ACE protein levels (ng/ml) and activity in CSF and serum were investigated. Poisson regression analyses were used to associate ACE measurements with rated global cortical atrophy, medial temporal lobe atrophy, lacunar infarcts, white matter hyperintensities, and microbleeds on brain MRI.
Results: Higher CSF ACE activity was associated with a reduced risk of global brain atrophy. The relative risk (95% CI) of having global cortical atrophy ≥ 2 per SD increase in CSF ACE activity was 0.67 (0.49; 0.93). ACE levels were not significantly related to measures of CSVD.
Conclusions: These results show that high ACE might have protective effects on the brain. This could suggest that ACE inhibitors, which may lower CSF ACE levels, are not preferred as antihypertensive treatment in patients at risk for Alzheimer's disease.

Keywords: Alzheimer's disease, angiotensin-converting enzyme, brain atrophy, cerebral small vessel disease, hypertension

*Correspondence to: Dr. M. Muller, Department of Gerontology and Geriatrics, Leiden University Medical Center, C7Q44, Albinusdreef 2, 2333 ZA Leiden, The Netherlands. Tel.: +31 71 526 6640; E-mail: m.muller@lumc.nl.

INTRODUCTION

Hypertension is increasingly seen as an important contributor to dementia, including Alzheimer's disease (AD) [1]. Angiotensin-converting enzyme (ACE) is a key enzyme in the renin-angiotensin system (RAS), which is one of the mechanisms that regulate blood pressure. Renin converts angiotensinogen in angiotensin (ANG), and ACE in turn converts ANGI in ANGII. ANGII is a potent vasoconstrictor mediating its action via two major receptors, namely, the ANGII type 1 receptor and the type 2 receptor. Therefore, ACE may have an important role in the relation between blood pressure and AD, as its main function is to maintain fluid homeostasis and regulate blood pressure (BP) [2]. Yet, two conflicting hypotheses on the relation between ACE and AD exist [3].

The amyloid hypothesis suggests that high ACE-activity *decreases* the risk of AD by reducing accumulation of amyloid-β (Aβ). This is supported by numerous laboratory-based findings (*in vitro* and in animal models) [3]. In addition, some studies have shown that patients with genotypes or haplotypes containing the so-called I-allele of the ACE gene, associated with lower serum ACE levels, have an increased risk of the development of AD and brain atrophy [4–7].

By contrast, the vascular hypothesis suggests that high ACE-activity *increases* the risk of AD by increasing BP, subsequently leading to cerebral small-vessel disease (CSVD). The evidence supporting this hypothesis mainly comes from secondary outcomes of clinical trials showing that ACE-inhibitors reduced the risk of stroke, CSVD, cognitive decline, and dementia [8–10].

Most available evidence is based on indirect measures of ACE (such as ACE-inhibitors or ACE-genotype), rather than direct measures of the protein levels and enzyme activity of ACE in cerebrospinal fluid (CSF) or serum. Recently, we showed that higher serum ACE levels were associated with more cerebrovascular lesions, but with less brain atrophy in a population with manifest arterial disease [11, 12]. These results lend support for both the vascular and amyloid hypotheses of ACE in dementia. However serum ACE may only reflect the peripheral ACE activity, whereas CSF ACE may reflect locally regulated brain ACE activity [2, 13]. Therefore, the relationship between CSF ACE activity and structural and vascular brain measures is of particular interest, and for the first time studied in the current work.

The aim of the current study was to investigate the association of paired serum and CSF ACE protein levels and activity with brain atrophy and CSVD in a memory clinic cohort.

METHODS

Study population

Patients were included from the memory clinic based Amsterdam Dementia Cohort. They underwent a standard dementia screening including physical and neurological examination, as well as laboratory tests, electroencephalography, brain magnetic resonance imaging (MRI), and comprehensive neuropsychological testing. The diagnosis of probable AD was made according to the National Institute of Neurological Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [14] by consensus of a multidisciplinary team, without knowledge of CSF results and *APOE* genotype. When the results of all examinations were normal, patients were considered to have subjective memory complaints (i.e., criteria for mild cognitive impairment not fulfilled). For the current study, patients using antihypertensive drugs were excluded, because some of these drugs strongly influence ACE levels [12]. Of the patients aged 50 to 80 years old with available CSF and MRI, we selected 40 persons with subjective memory complaints (control group), 40 persons with AD without vascular brain lesions on MRI (no infarcts or white matter hyperintensities (WMHs)); AD group) and 38 persons with AD and severe vascular brain lesions on MRI ($\geq$2 lacunar infarcts or WMHs: Fazekas grade $\geq$2; mixed AD group). These extremes of pathology were chosen to have sufficient power to examine both the amyloid and vascular hypothesis. The institutional review board of the VUMC approved the study and all subjects gave written informed consent.

Sampling of blood and CSF

A blood sample was taken, and after half an hour of clotting the samples were spun at 1800 g for 10 min at 4°C, next aliquoted into Sarstedt polypropylene cryovials and immediately stored at −80°C until further analyses. CSF was obtained by lumbar puncture between the L3/L4 or L4/L5 intervertebral space, using a 25-gauge needle, and collected in 10 mL polypropylene tubes. Within 2 h, 2 ml of CSF samples was centrifuged at 1800 g for 10 min at 4°C and stored at −20°C for analysis of CSF biomarkers within two months (see below). The remainder of CSF was

aliquoted in polypropylene tubes of 0.5 or 1 mL, and stored at –80°C until further analysis.

ACE protein assays

A commercially available sandwich ELISA (R&D systems, Abingdon, UK) was used according to the manufacturer's guidelines to measure ACE concentration in CSF and serum, as previously described [13]. Absorbance was read at 450 nm on a FLUOstar OPTIMA plate reader (BMG Labtech, UK). The assays were repeated in duplicate and ACE concentrations were interpolated from the standard curves of known concentrations of recombinant human ACE (929-ZN) (R&D systems, Abingdon, UK). The inter-assay coefficient of variation (CV) (mean ± SD) was 8.7 ± 8.9% for CSF ACE and 13.0 ± 7.0% for serum ACE. The intra-assay CV was 7.9 ± 6.5% for CSF ACE and 7.6 ± 5.6% for serum ACE.

ACE activity assays

Monoclonal anti-human ACE antibody (MAB929), recombinant human ACE (929-ZN), and the internally quenched fluorogenic peptide substrate (Mca-RPPGFSAFK(Dnp)-OH)(ES005) were purchased from R&D systems (Abingdon, UK) and used to optimize a new immuno-capture based fluorogenic assay for measurement of ACE activity in CSF and serum (see also Supplementary Material) [15]. Fluorescence was measured by excitation at 320 nm and emission at 405 nm, in a FLUOstar OPTIMA plate reader. The mean fluorescence of the negative controls was subtracted from standard and sample readings, as was done for the fluorescence of the ACE-specific captopril-inhibited well. Captopril inhibited ACE-activity mediated fluorescence by more than 90%. Each sample was measured in duplicate and the mean fluorescence of ACE activity was calculated, based on the standard curve of recombinant ACE. The inter-assay CV (mean ± SD) was 8.5 ± 8.4% for CSF ACE and 8.1 ± 8.7% for serum ACE. The intra-assay CV was 8.7 ± 5.9% for CSF ACE and 9.1 ± 8.5% for serum ACE.

MRI protocol and image analysis

The majority of scans (20 in the control group, 22 in the AD group, and 18 in the mixed AD group) were performed on a 1.0-T whole-body MR system (Magnetom Impact; Siemens, Erlangen, Germany). Twenty-two scans (8 in the control group, 5 in the AD group, and 9 in the mixed AD group) were obtained on a 1.5-T whole-body MR system (Siemens Sonata Syngo [$n = 15$], Siemens Avanto [$n = 5$], or SignaHDxt [$n = 2$]). Thirty-six scans were made on an MR system operating at 3-T (SignaHDxt, General Electric, Milwaukee, WI, USA; 12 in the control group, 13 in the AD group, and 11 in the mixed AD group). The MRI protocol included 3 dimensional (D) T1-weighted (including multiplanar reconstructions (MPR) in the oblique coronal orientation perpendicular to the axis of this hippocampus), axial 2D T2-weighted, T2*-weighted and fluid-attenuated inversion recovery (FLAIR) sequences [16]. We performed visual rating of medial temporal lobe atrophy (MTA) on oblique coronal MPR of the T1-weighted images according to the 5-point (0–4) Scheltens scale [17]. Global cortical atrophy (GCA) (range 0–3) [18] and WMH severity (Fazekas, range 0–3) [19] were rated visually on axial FLAIR images. The highest scores represent maximal pathology. Large-vessel infarcts were rated as present or absent, based on both fluid-attenuated inversion-recovery and T2-weighted sequences. Lacunar infarcts were defined as well-demarcated lesions from 3 to 15 mm, with a CSF-like signal on all sequences. Microbleeds were defined as rounded hypo intense homogeneous foci up to 10 mm in size in the brain parenchyma on T2*-weighted images. Lesions in sulci possibly representing flow voids from pial vessels and symmetrical lesions in the basal ganglia, supposedly representing iron or calcium deposits, were not considered. Hypointense T2-signal intensities inside a lesion compatible with an infarct were not counted as microbleeds, but regarded to be probable hemorrhagic transformations. Cavernous angiomas were not taken into account. The rating was performed by three observers, blinded to the patients' clinical data. The observers were trained by an experienced neuroradiologist (>10 years) using our standard training set (19 brains) to meet consistency requirements according to our standard operating procedure. The interrater-weighted Cohen scores were 0.90 for microbleeds, 0.80 for MTA and WMH scores, and 0.60 for GCA (against internally established gold standard). Intrarater-weighted Cohen scores were 0.90 for microbleeds, 0.80 for MTA, and 0.70 for GCA and WMH.

Other patient variables

DNA was isolated from 10 mL EDTA blood and *APOE* genotype was determined using the Light Cycler

APOE mutation detection method (Roche diagnostics GmbH, Mannheim, Germany). Diabetes mellitus and hypercholesterolemia were defined based on self-reported medical history and medication use. BP was measured manually in a standardized manner using a sphygmomanometer with the patient in sitting position after 5 min of rest. Hypertension was defined as systolic BP (SBP) >140 mmHg or diastolic BP (DBP) >90 mmHg and includes only untreated hypertension, since patients using antihypertensive treatment were excluded. The level of education was classified using the 7-point rating scale of Verhage ranging from 1 (low, elementary school not completed) to 7 (high, university).

Data analyses

We used multiple imputation (10 datasets) to address missing values, and data were analyzed using SPSS version 20.0 (Chicago, IL, USA), by pooling the 10 imputed datasets [20].

First, patient characteristics were calculated for the total study population and the separate study groups (control group, AD group, and mixed AD group) and differences were evaluated by ANOVA for continuous variables, or Fisher's exact test for categorical variables. Second, Pearson's correlation coefficients between ACE protein and activity levels for each of CSF and serum in turn were examined.

Third, we used Poisson regression analyses with robust standard error to investigate the associations of CSF and serum ACE protein and activity levels (continuous) with CSVD (WMH (Fazekas $\geq$2); presence of lacunar infarcts (yes versus no); or presence of microbleeds (yes versus no)), or with brain atrophy (GCA $\geq$2; or MTA $\geq$1.5). Relative risks (RR) rather than odds ratios were estimated, since the latter are likely to overestimate the RR in cohort studies, particularly for outcomes that are common [21]. Analyses were given unadjusted (Model 1), and adjusted for age, gender, and study groups (Model 2). Additional adjustments were made for number of medications (median split: $\leq$1 and >1), for *APOE*-ε4 (carrier versus non-carrier) and cardiovascular risk factors (SBP, DBP, body mass index, smoking, history of hypercholesterolemia and diabetes mellitus) (Model 3). Finally, we adjusted for concomitant CSVD in the analyses with brain atrophy measures. Fourth, as we previously observed that a high BP together with high serum ACE levels had a significant additive effect on the risk of stroke [12], we examined whether presence of hypertension was an effect modifier in the association between ACE and CSVD or brain atrophy by stratifying by hypertension status.

RESULTS

In the total population, mean (SD) age was 66 (8) years and 52% were female. In addition, 52% of the population was heterozygous and 10% were homozygous APOE-ε4 carriers.

Table 1 shows that patients in the AD and mixed AD group were more often *APOE*-ε4 carrier and had more global cortical atrophy and MTA than patients in the control group. Further, patients in the mixed AD group were older, had higher SBP and DBP levels, and more MTA than patients in the AD and control group. Also, the mixed AD group had less *APOE*-ε4 carriers than patients in the AD group. CSF and serum ACE protein and activity levels were lower in patients with AD/mixed AD compared to the control group (Table 1).

There was moderate correlation (ρ) between CSF ACE protein and CSF ACE activity ($\rho = 0.26$, p-value = 0.005), and serum ACE protein and serum ACE activity ($\rho = 0.28$, p-value = 0.002). CSF and serum ACE protein levels correlated strongly ($\rho = 0.54$, p-value <0.001), but CSF and serum ACE activity levels were not correlated ($\rho = 0.07$, p-value = 0.494).

ACE measures and brain atrophy

One SD increase in CSF ACE activity (59 RFU) was significantly associated with a 33% reduced risk of severe GCA (Table 2 and Fig. 1A). Additional adjustment for number of medications did not materially change the associations; RR (95% CI) was 0.72 (0.51; 1.00). Further, additional adjustments for *APOE*-ε4 and cardiovascular risk factors did not substantially change the association; RR (95% CI) was 0.63 (0.44; 0.88). Also, further adjustments for the presence of WMH, microbleeds, or brain infarcts did not change the result; RR (95% CI) was 0.62 (0.43; 0.88).

When we stratified the analyses by the presence or absence of hypertension, we found that the relation (RR (95% CI)) between CSF ACE activity and GCA was 0.52 (0.34; 0.79) in patients without hypertension ($n = 63$) versus 0.90 (0.57; 1.41) in patients with hypertension ($n = 55$).

CSF ACE activity was not significantly associated with MTA, nor were CSF ACE protein level, serum ACE protein level and activity with GCA or MTA (Tables 2 and 3). Yet, the effect estimates were in a

Table 1
Baseline characteristics for the separate study groups and the total study population

	Control group ($n=40$)	AD group ($n=40$)	Mixed AD group ($n=38$)	Total study population ($N=118$)
Demographics				
Female gender, *n* (%)	19 (47%)	21 (52%)	21 (55%)	61 (52%)
Age (years)†	63 (8)	65 (6)	71 (7)*	66 (8)
Education (range 1–7)‡	6 (3–7)	5 (3–6)	5 (3–7)	5 (3–7)
APOE-ε4 carrier, *n* (%)	9 (23%)	30 (75%)*	22 (57%)*	61 (52%)
MMSE (range 0–30)‡	28 (26–30)	22 (16–27)*	23 (16–27)*,**	25 (17–29)
Vascular risk factors/disease				
Smoking, *n* (% current)	9 (23%)	9 (24%)	9 (23%)	27 (23%)
Body mass index (kg/m^2)†	25 (5)	24 (4)	25 (7)	25 (5)
Diabetes mellitus, *n* (%)	6 (14%)	5 (13%)	5 (14%)	16 (14%)
Hypercholesterolemia, *n* (%)	11 (28%)	7 (18%)	10 (26%)	28 (24%)
Blood pressure measures				
Systolic BP (mmHg)†	133 (14)	139 (20)	155 (22)*,**	142 (21)
Diastolic BP (mmHg)†	83 (9)	82 (13)	91 (13)*,**	85 (12)
Hypertension, *n* (%)	12 (30%)	15 (38%)	28 (74%)*,**	55 (47%)
ACE measures				
CSF ACE protein level (ng/ml)	3.78 (1.30)	3.69 (1.33)	3.42 (0.94)	3.63 (1.21)
CSF ACE activity (RFU)	155 (61)	148 (62)	147 (54)	150 (59)
Serum ACE protein level (ng/ml)	4.22 (1.50)	3.74 (1.26)	3.72 (1.07)	3.90 (1.30)
Serum ACE activity (RFU)	255 (152)	201 (122)	207 (110)	221 (131)
Vascular brain lesions				
WMHs, *n* (% Fazekas ≥2)	5 (13%)	0 (0%)*	36 (95%)*,**	41 (35%)
Presence of lacunar infarcts, *n* (%)	0.8 (2%)	0 (0%)	12 (32%)*,**	13 (11%)
Presence of large vessel infarct, *n* (%)	0.4 (1%)	0 (0%)	1 (3%)	1 (1%)
Presence of microbleeds, *n* (%)	7 (18%)	4 (10%)	17 (45%)*,**	28 (33%)
Brain atrophy				
GCA, *n* (%≥2)	3 (8%)	15 (38%)*	9 (24%)*	27 (23%)
MTA, *n* (%≥1.5)	3 (8%)	13 (33%)*	28 (74%)*,**	44 (37%)

*p-value <0.05 compared to controls; **P-value <0.05 compared to AD; †mean (SD), ‡median (10th–90th percentile); % of missing values in the total population: lacunar and non-lacunar infarcts 0.8%, microbleeds 4.2%, education 0.8%, *APOE* genotyping 1.7%, smoking 7.6%, diabetes mellitus and hypercholesterolemia 14%, all other variables 0%. MMSE, Mini-Mental State Examination; AD, Alzheimer's disease; mixed AD, AD with severe vascular brain lesions; BP, blood pressure; WMH, white matter hyperintensities; GCA, global cortical atrophy; MTA, medial temporal lobe atrophy.

Table 2
Regression analyses between CSF ACE measures, brain atrophy, and vascular brain measures

		Brain atrophy	
		GCA ≥2 ($n=27$)	MTA ≥1.5 ($n=44$)
	Model	RR (95% CI)	RR (95% CI)
CSF ACE activity[a]	1	0.66 (0.48; 0.90)**	0.83 (0.66; 1.04)
	2	0.67 (0.49; 0.93)*	0.89 (0.72; 1.10)
CSF ACE protein level[b]	1	0.82 (0.53; 1.27)	0.80 (0.61; 1.05)
	2	0.77 (0.51; 1.17)	0.83 (0.64; 1.10)

		Cerebral small vessel disease		
		WML, Fazekas ≥2 ($n=41$)	Presence of LI ($n=13$)	Presence of MB ($n=28$)
	Model	RR (95% CI)	RR (95% CI)	RR (95% CI)
CSF ACE activity[a]	1	0.94 (0.74; 1.19)	1.02 (0.65; 1.61)	1.08 (0.76; 1.54)
	2	1.01 (0.89; 1.15)	1.16 (0.72; 1.88)	1.16 (0.83; 1.60)
CSF ACE protein level[b]	1	0.91 (0.72; 1.14)	0.96 (0.56; 1.65)	1.16 (0.89; 1.51)
	2	1.09 (0.92; 1.30)	1.28 (0.66; 2.50)	1.28 (0.97; 1.67)

*$p<0.05$; **$p<0.01$. [a]per SD increase (59 RFU), [b]per SD increase (1.21 ng/ml). Model 1: unadjusted. Model 2: adjusted for age, gender, and study groups.

similar direction as for CSF ACE activity and GCA (Fig. 1A, B).

ACE measures and cerebral small vessel disease

In the total population, CSF ACE activity and protein level were not significantly associated with measures of CSVD, although a positive trend could be observed, particularly for higher ACE protein level and the presence of microbleeds; RR (95% CI) was 1.28 (0.97; 1.67) (Table 2, Model 2).

Stratification by hypertension status showed that the relations (RRs (95% CI)) between higher CSF ACE protein level were 1.21 (0.97; 1.51) for WMH and 1.44 (0.96; 2.16) for microbleeds in patients with hypertension ($n = 55$) versus 0.82 (0.51; 1.31) for WMH and 1.02 (0.56; 1.86) for microbleeds in patients without hypertension ($n = 63$).

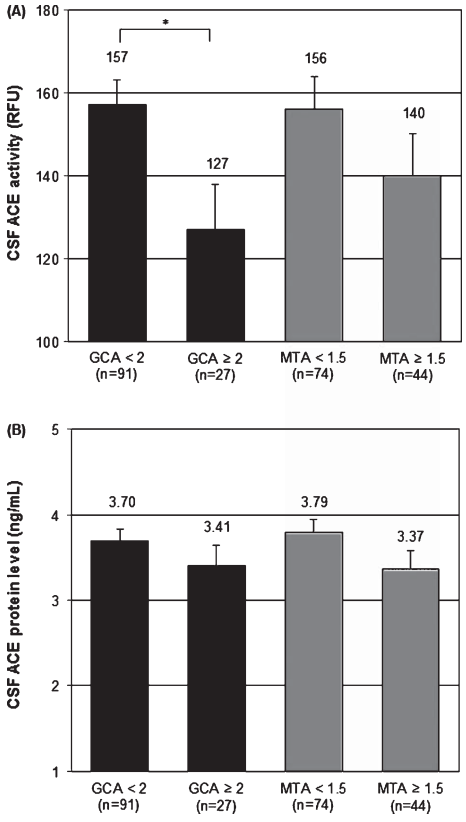

Fig. 1. CSF ACE activity or protein level and brain atrophy. Means (standard error) of CSF ACE activity (A) and CSF ACE protein level (B) across strata of brain atrophy severity. Analyses were adjusted for age and gender. GCA, global cortical atrophy, highest score represent maximal pathology; MTA, medial temporal lobe atrophy, highest score represent maximal pathology. $^{*}p < 0.05$.

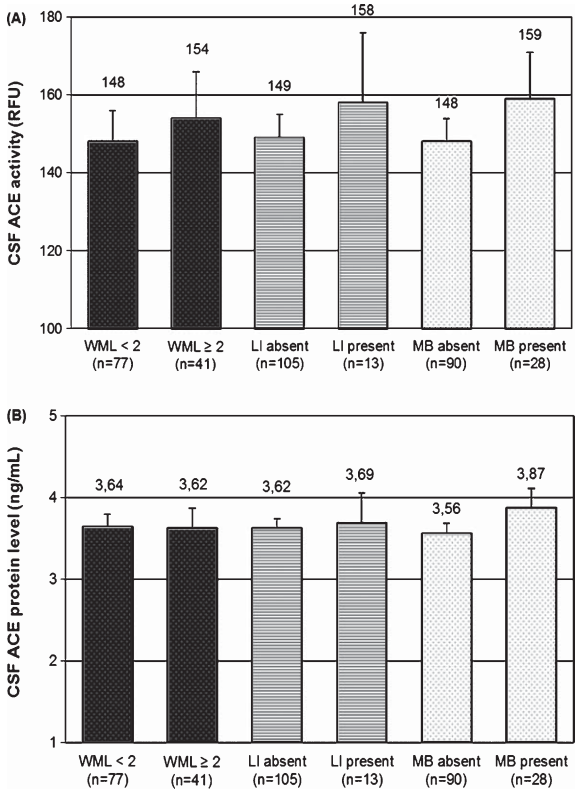

Fig. 2. CSF ACE activity or protein level and cerebral small vessel disease. Means (standard error) of CSF ACE activity (A) and CSF ACE protein level (B) across strata of WMH severity, presence or absence of lacunar infarcts and microbleeds. Analyses were adjusted for age and gender. WMHs, white matter hyperintensities, highest Fazekas score represent maximal pathology; LI, lacunar infarcts; MB, microbleeds.

When we restricted the analyses to the mixed AD group, the relation (RRs (95% CI)) between CSF ACE protein level and CSVD became 1.13 (0.97; 1.32) for WMH, 1.52 (0.77; 3.00) for lacunar infarcts and 1.26 (0.79; 2.02) for microbleeds (Model 2).

Serum ACE protein level and activity were not related to any of the measures of CSVD (Table 3).

DISCUSSION

In a memory clinic population, higher ACE levels—particularly higher CSF ACE levels—were associated with less global cortical brain atrophy (GCA). These findings were independent of demographics, *APOE* genotype, and cardiovascular risk factors. Thereby, our findings support the amyloid hypothesis, since the amyloid hypothesis suggests higher ACE activity leads to increased Aβ degradation, decreased Aβ mediated neuronal damage and brain

Table 3
Regression analyses between serum ACE measures and brain atrophy

		Brain atrophy		
		GCA ($n=27$)	MTA ($n=44$)	
	Model	RR (95% CI)	RR (95% CI)	
Serum ACE activity[a]	1	0.84 (0.58; 1.21)	0.82 (0.64; 1.04)	
	2	0.96 (0.67; 1.39)	0.96 (0.77; 1.21)	
Serum ACE protein level[b]	1	0.75 (0.53; 1.05)	0.84 (0.58; 1.21)	
	2	0.82 (0.59; 1.15)	0.85 (0.67; 1.06)	
		Cerebral small vessel disease		
		WML ($n=41$)	LI ($n=13$)	MB ($n=28$)
	Model	RR (95% CI)	RR (95% CI)	RR (95% CI)
Serum ACE activity[a]	1	0.88 (0.69; 1.13)	0.76 (0.54; 1.27)	0.96 (0.71; 1.30)
	2	0.95 (0.81; 1.12)	0.85 (0.50; 1.44)	1.03 (0.77; 1.37)
Serum ACE protein level[b]	1	0.87 (0.68; 1.10)	0.81 (0.49; 1.30)	1.20 (0.89; 1.63)
	2	0.96 (0.81; 1.13)	0.95 (0.54; 1.67)	1.34 (0.97; 1.84)

[a]per SD increase (131 RFU), [b]per SD increase (1.30 ng/ml). Model 1: unadjusted. Model 2: adjusted for age, gender, and study groups.

atrophy, and *reduced* risk of AD. In addition, our findings were not in support of the vascular hypothesis since only a weak, non-significant relation was found between higher CSF ACE levels and more CSVD.

To our knowledge, we are the first that examined CSF ACE activity and protein levels, which can be seen as direct markers of brain ACE activity, in relation to structural brain markers of AD. These findings are in agreement with our previous findings that higher serum ACE levels were associated with less progression of cortical brain atrophy in a population with manifest arterial disease [11]. Further, one study found that patients with the I/I genotype of the *ACE* gene (associated with lower ACE levels) had an increased risk of hippocampal and amygdalar atrophy [7]. We currently did not show a significant association between higher CSF ACE activity and less MTA, although a trend could be observed. Taken together, the results suggest that higher CSF ACE activity could have a beneficial effect on cortical brain atrophy. It is possible that higher ACE activity, through its degradation of Aβ [22, 23], contributes to lower Aβ burden in the brain and consequently reduce the extent of brain atrophy. In line with this, we just published a paper using the same study population which showed that lower CSF ACE protein levels, and to a lesser extent serum ACE protein and CSF ACE activity levels, were associated with lower CSF Aβ levels, indicating increased Aβ accumulation in the brain. This may indicate that higher ACE levels, through its degradation of Aβ, may contribute to less accumulation of Aβ into senile plaques in the brain and subsequently be beneficial for the occurrence of brain atrophy [24]. However, brain atrophy could also be the result of other factors such as metabolic or vascular risk factors, and cerebrovascular pathology [25, 26], although adjustments for factors related to these only slightly attenuated the association.

In our study we only found a weak and non-significant association between higher ACE and more CSVD, which was particularly present in those with hypertension. Until now, data on the association between serum ACE and WMH or lacunar infarcts have been inconsistent; studies report either no or a positive relation [11, 27, 28], and no studies have investigated the association of ACE with microbleeds. A possible explanation of the apparent synergistic effect of hypertension and ACE on CVSD could be that a high BP leads to endothelial dysfunction, which subsequently can cause an upregulation of local tissue ACE [29]. At the same time, the enzymatic actions of vascular and tissue ACE, through formation of ANGII, result in further BP-increasing effects which promotes further endothelial dysfunction and atherosclerosis, thereby increasing the risk for microbleeds and WMH [30, 31]. Conversely, we observed that the inverse association between CSF ACE activity and GCA was particularly present in patients without hypertension. A possible explanation could be that in patients with hypertension, ACE levels become lower due to a negative feedback loop of ANGII, suppressing kidney renin release, or that there are some disease-specific modifications of ACE that affect its activity [32]. On the contrary, one could imagine that the RAS is overactivated or altered in hypertension, such that ACE is mostly sensitized to the conversion of ANGI to ANGII, but not degrading Aβ.

CSF ACE levels were more strongly associated with structural brain measures than serum levels. This indicates that CSF ACE, as might be expected, better

reflects the activity of the brain RAS than serum ACE. In our previous papers, we did find associations between serum ACE levels and structural brain measures [11, 12], however the sample size of these studies was larger which enabled us to detect less powerful associations. Also, differences in characteristics between the study populations (patients with manifest arterial disease versus patients in a memory clinic) could in part explain the different results. Further, we found ACE activity in CSF to be more strongly related to GCA than ACE protein levels, whereas ACE protein levels were stronger related to WMH and microbleeds. Although these differences could be due to the small sample size of this study, it could also be hypothesized that protein levels indicate the concentration of ACE in body fluid and that activity levels are arguably more biologically meaningful because they produce ANGII. Thus we predicted that ACE activity would be more indicative and relevant to Aβ degradation and brain atrophy, as was indeed found to be the case. It is not yet definite how well CSF ACE levels and activity reflect the biological processes within the brain parenchyma. In this study we found that ACE protein levels in serum and CSF correlated strongly, suggesting the synthesis of the enzyme appears to be well correlated in different physiological compartments. Yet, levels of ACE activity did not correlate within serum and CSF. This suggests that the eventual resultant catalytic activity of the enzyme could be modified by different 'environmental' conditions within these compartments [33]. ACE activity, and its proposed conversion of Aβ, has been shown previously to be modulated by post-translational modification. In both CSF and serum, there may therefore be differential levels of glycosylation that can explain the lower than expected correlations between serum and CSF ACE activity. Other factors that could have differentially influenced serum and CSF ACE activity are tissue storage characteristics (storage duration, temperature) and Braak stage [13].

Our study has potentially important clinical implications. Our data shows that high ACE may have protective as well as detrimental effects on the brain. Thus, the use of ACE-inhibitors in hypertensive patients at risk for AD could have both favorable and unfavorable effects. ACE-inhibitors would lower ACE activity, in turn leading to less ANGII effects, thereby preventing vascular brain lesions [8]. Yet conversely, taking ACE-inhibitors, particularly those that can cross the blood-brain barrier, might also compromise Aβ degradation, increase brain atrophy, and ultimately contribute to higher AD risk or rates of mortality [30, 34]. Therefore, angiotensin-receptor blockers, which solely target ANGII effects while not interfering directly with ACE, could provide an immediate and readily available potential alternative anti-hypertensive therapy for patients at risk for AD [30, 35, 36].

Strengths of the study include the use of direct measures of ACE, such as CSF ACE protein and activity level, which may be more biologically meaningful than extrapolating from measures of *ACE* genotypes [37]. Other strengths include the inclusion of carefully characterized patients with paired CSF and serum. Further, one might argue we used crude MRI measures as outcome variables. However, the rating scales of brain atrophy and WMH were assessed in a standardized way and are clinically relevant.

One of the main limitations of our study is the relatively small sample. This limited the power to observe significant associations, especially for vascular brain lesions, as these were only present in a subset of the population. Yet some trends could be observed making replication in a larger study sample of potential importance. A second limitation is the use of different MRI scanners and scan protocols, which might have influenced the precision of the data; however, since we used robust rating scales by experienced raters we do not think this has any significant confounding influence on our results. Further, this increases the generalizability of the results. Third, we did not have information on the distinction between periventricular white matter lesions and deep white matter lesions. However, it is increasingly recognized that there seems to be a continuum between deep and periventricular WMH, and it is very difficult to make a valid distinction. Further, most associations are validly captured using a generic scale such as the Fazekas rating scale used in our study [38]. Fourth, we did not have information on CSF or serum angiotensin II levels, which is the pharmacologically active component of the RAS, and therefore of potential interest in the relation to brain atrophy and CSVD. Future research should focus on this relation. Fifth, we excluded all patients using antihypertensive drugs, to avoid the influence of those on ACE levels. Therefore, the study population is not generalizable to the general population. Finally, the cross-sectional design limits conclusions regarding direction of causality.

In conclusion, in a memory clinic cohort, higher CSF ACE activity was associated with less brain atrophy. Since ACE-inhibitors might lower ACE levels, this could suggest that angiotensin-receptor blockers, and not ACE-inhibitors, are preferred as antihypertensive treatment in patients at risk for AD.

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SUPPLEMENTARY MATERIAL

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