

Title: Cerebrospinal fluid changes in the renin-angiotensin system in Alzheimer's disease.

Authors: Kehoe PG¹, Al Mulhim N¹, Zetterberg H^{2,3,4,5}, Blennow K^{2,3}, Miners JS¹

¹ University of Bristol, Clinical Neuroscience, Level 1, Learning and Research Building, Southmead Hospital, Bristol, UK, BS10 5NB

²Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, S-431 80 Mölndal, Sweden

³Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, S-431 80 Mölndal, Sweden

⁴Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London WC1N 3BG, United Kingdom

⁵UK Dementia Research Institute at UCL, London WC1E 6BT, United Kingdom

Corresponding author: Scott Miners, scott.miners@bristol.ac.uk

Keywords renin-angiotensin system; cerebrospinal fluid; Alzheimer's disease; angiotensin-converting enzyme-1 (ACE1); angiotensin-converting enzyme-2 (ACE2); angiotensin-II; angiotensin-(1-7).

Running title CSF RAS changes in AD

Number of figures: 5 figures and 1 table

Total word count: 4212 (including figure legends and references)

Abstract (words 230)

Aims: Overactivation of the brain classical renin-angiotensin system (RAS) is implicated in the pathogenesis of Alzheimer's disease (AD). The major aim of this study was to investigate whether brain RAS changes are mirrored in ante-mortem CSF in AD.

Methods: ACE1 and ACE2 activities were measured using fluorogenic substrates and the concentrations of angiotensin-I, III, and 1-7 were calculated using direct ELISA in ante-mortem CSF samples (40 AD and 40 controls) and a subset of mid-frontal cortex and post-mortem CSF samples (10 AD and 10 controls).

Results: ACE1 activity was elevated in mid-frontal cortex ($p < 0.05$), post-mortem CSF ($p < 0.05$) and ante-mortem CSF ($p < 0.01$). ACE1 and ACE2 enzyme activity correlated positively in post-mortem ($r = 0.682$, $p = 0.0013$) and ante-mortem CSF ($r = 0.375$, $p = 0.0007$). ACE1 activity in CSF correlated positively with CSF total tau ($r = 0.271$, $p = 0.015$) and CSF phosphorylated tau ($r = 0.345$, $p = 0.0018$) but not $A\beta_{42}$. Ang-II level ($p < 0.05$) and the ratio of Ang-II:Ang-I ($p < 0.05$) and of Ang-II:Ang-(1-7) ($p < 0.01$) increased in mid-frontal cortex but not in CSF.

Conclusions: These data show that ACE1 overactivity in brain RAS in AD is reflected in ante-mortem CSF and CSF-ACE1 is related to CSF-tau but not A β ₄₂ in AD, however, changes in brain angiotensin levels and ratios are not reflected in CSF.

Introduction

The renin-angiotensin-system (RAS) functions independently within the brain and is dysfunctional in Alzheimer's disease (reviewed in ^{1, 2}). Histological and biochemical assessment of human post-mortem brain tissue indicates that angiotensin-converting enzyme (ACE1) activity, angiotensin-II (Ang-II) and angiotensin-II type 1 receptor (AT1R), the major components of the classical RAS pathway, commonly attributed to raising blood pressure, are increased in humans in AD in relation to A β and Tau level³⁻⁵.

Cerebroventricular infused Ang-II increased A β production (via proteolytic cleavage of APP) and Tau in Sprague Dawley rats associated with reduced cognitive performance ³⁻⁷. Disease pathology associated with overactive 'classical' RAS is associated with inflammation, oxidative stress and vascular dysfunction (reviewed in ²), which are commonly observed features of early AD that predict the onset, progression and severity of disease ⁸.

Disease pathology and cognitive decline is limited in Tg-APP mice by ACE1 inhibitors (ACEIs) or AT1R-blockers (ARBs) ⁹⁻¹⁴. Their use to treat hypertension has also been associated with reduced prevalence ¹⁵⁻¹⁷, delayed cognitive decline ¹⁸ and improved

cognition in AD compared with non-RAS anti-hypertensives ¹⁹. Recent studies indicate a disease-association between RAS in Tau hyperphosphorylation in AD ²⁰ and clinical studies reveal that individuals prescribed brain RAS-targeting anti-hypertensives have lower CSF levels of Tau ²¹ and reduced CSF-A β during ageing ²². Lastly, an *ACE1* SNP was reported almost 2 decades ago to be a major risk factor for AD ²³ – more recently supported by several meta-analyses (Lehmann et al, 2005) and Genome Wide Association Studies (REF = <https://www.biorxiv.org/content/early/2018/04/05/294629>). *ACE1* has a divergent and complex role in AD, as it is not only the rate-limiting enzyme in the conversion of Ang-I to Ang-II but can also degrade and facilitate the clearance of A β (refs).

In addition to classical RAS axis, several alternative ‘downstream’ RAS pathways exist within the brain that counter-regulate the classical RAS axis ²⁴⁻²⁶. We recently found that *ACE2* activity, which is primarily responsible for converting Ang-II to Ang(1-7)^{27, 28} was reduced by almost 50% in the mid-frontal cortex in AD and that the reduction in *ACE2* was strongly related to parenchymal A β and Tau load ⁴ and inversely related to elevated *ACE1* activity. Brain Ang-(1-7) level is reportedly reduced in a senescence-accelerated mouse prone 8 (SAMP8) mice, a mouse model of sporadic AD ²⁰, in relation to disease progression and Tau pathology and reduced plasma Ang-(1-7) levels have also been reported in AD patients ²⁹. Together, these data suggest that an imbalance in brain RAS resulting in classical RAS overactivation is related to disease pathology and is caused, in part, to a loss in regulatory RAS activity.

Previous studies have reported elevated *ACE1* in CSF in AD ^{30, 31} whilst others reported either reduced ³²⁻³⁴ or unchanged *ACE1*³⁵. CSF-*ACE2* remains uncharacterised in AD, as do potential changes in CSF angiotensin level. This study aimed to identify if disease-

associated changes in markers of classical and regulatory RAS in antemortem CSF reflect established changes in human post-mortem brain tissue and thus serve as future potential biomarkers for AD.

Materials and Methods.

Brain tissue and CSF cohorts

Human post-mortem brain tissue and CSF was obtained from the South West Dementia Brain Bank, University of Bristol, UK. The study was undertaken with local research ethics committee approval under the terms of ethical approval from NRES Committee South West – Central, Bristol, U.K. Tissue was dissected from the mid-frontal cortex (Brodmann area 9) from 10 cases of AD and 10 age-matched control brains. Post-mortem CSF samples were obtained from 10 AD and 10 controls (we were unable to match with post-mortem brain tissue). All cases had previously undergone detailed neuropathological assessment and a diagnosis of AD was made according to the NIA-AA guidelines. Controls had no history of dementia with few or absent neuritic plaques, a Braak tangle stage of III or less, and no other neuropathological abnormalities. Brain tissue homogenates were prepared in 1% SDS lysis buffer, as previously described^{3, 4, 36} and stored at -80 C. Antemortem CSF samples were from patients who sought medical advice because of cognitive impairment. Patients were designated as AD or non-AD according to CSF biomarker (total tau [T-tau], tau phosphorylated at amino acid 181 [P-tau] and the 42 amino acid form of amyloid β [A β 42], measured using INNOTEST ELISAs [Fujirebio, Ghent, Belgium]) levels using cutoffs that are 90% sensitive and specific for AD (ref:

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16488378). None of the biochemically normal subjects fulfilled these criteria. The demographic data for each of the cohorts is summarised in Table 1.

ACE1 fluorogenic activity assay

ACE1 activity was measured as previously described with minor modifications^{4, 5, 36, 37} using an ACE1 specific fluorogenic peptide substrate (Abz-FRK(Dnp)-P) (Biomol International, Exeter, UK).

Recombinant ACE1 (1000-31.1 ng/ml), brain tissue samples (50 µl at 250 µg/ml diluted in assay buffer (50mM HEPES, pH 6.5)), post-mortem CSF (50 µl undiluted) and ante-mortem-CSF (50 µl undiluted) were incubated with the FRET substrate (10 µM diluted in assay buffer) for 2.5 h at 26 C. Fluorescence was measured with excitation at 320nm and emission at 405nm, in a fluorescent plate reader (FLUOstar, BMG Labtech, Aylesbury, UK). All cases were run in duplicate in the presence and absence of captopril, which was incubated for 10 m at 26 C, prior to the addition of the FRET substrate – we previously have shown that captopril blocks ACE1 activity by over 90%. ACE1 activity was determined by subtracting the fluorescence in the captopril-inhibited from the untreated wells. To minimise variation resulting from plate-to-plate variation a serial dilution of recombinant human ACE1 was used for calibration.

ACE2 activity assay

ACE2 activity was measured using the ACE2 specific fluorogenic peptide substrate (Mca-APK(Dnp) (Enzo Life Sciences, Exeter, UK) in the presence of an ACE2 specific inhibitor, MLN4760 (10 µM) (Merck, Nottingham, UK) as previously described⁴. Recombinant ACE2 (440 – 6 ng/ml) (R&D systems, Cambridge, UK), brain tissue samples (50 µl diluted 1 in 10 in assay buffer), post-mortem CSF (50 µl undiluted) and ante-mortem-CSF (50 µl undiluted) were incubated with the FRET substrate (10 µM) diluted in assay buffer (75 mM Tris, 1M NaCl, Ph7.5) for 3 h at 37 C. Standards and samples were assayed in duplicate in the presence or absence of ACE2 specific inhibitor diluted in distilled water for 10 m at 37 prior to the addition of the fluorogenic substrate. ACE2 activity was determined by subtracting the fluorescence in the inhibited from the untreated wells. Cleavage of the ACE2 FRET peptide was measured using a BMG FLUOstar OPTIMA microplate reader (BMG

Labtech, Aylesbury, UK) at excitation/emission wavelength 330/390nm. To minimise variation resulting from plate-to-plate variation a serial dilution of recombinant human ACE2 was used for calibration.

Angiotensin-I, -II and -(1-7) measurements by direct ELISA

Ang-I, II and Ang-(1-7) were measured by direct ELISA's as previously described^{3,4}. Serial dilutions of recombinant human Ang-I, Ang-II and Ang-(1-7) (5000-78.125 pg/ml) (Enzo/Abcam), brain tissue homogenates diluted 1 in 10 in PBS, or CSF samples (100ul undiluted) were incubated for 2 hours in clear high-binding capacity NUNC maxisorp plates (ThermoFisher Scientific, Waltham, MA, USA) at 26°C with shaking. The wells were washed five-times in PBS with 0.05% tween-20 and blocked for 1 hour in 1 % PBS: BSA (Sigma Aldrich, Dorset, UK). After another five washes, the wells were incubated with either biotinylated anti-human Ang-I (diluted 1 in 100 in PBS) (Cloud-Clone, Wuhan, China), anti-human Ang-II (diluted 1 in 500 in PBS) or anti-human Ang-(1-7) (diluted 1 in 100 in PBS) for 2 hours at 26°C with shaking, followed by a further wash step. The respective plates were incubated with strep:HRP (1:200) in PBS:0.01% Tween-20 for 20 minutes at room temperature in the dark and TMB substrate (R&D systems) was added after a further wash and left to develop in the dark for 20 minutes and the reaction was stopped following the addition of 2N sulphuric acid. The absorbance signal for each assay was read at 450nm using a FLUOstar OPTIMA plate reader (BMG labtech, Aylesbury, BUCKS, UK). Concentrations were interpolated from standard curve generated by serially diluting recombinant standards and were measured in duplicate. Each assay was specific for the respective target and showed minimal cross reactivity with closely-related angiotensin peptides.

Statistical analysis

Unpaired two-tailed t-tests or ANOVA with Bonferroni post-hoc analysis were used for comparisons between groups, and Pearson's or Spearman's test to assess linear or rank order correlation, as

appropriate, with the help of SPSS version 16 (SPSS, Chicago) and GraphPad Prism version 6 (GraphPad Software, La Jolla, CA). P-values < 0.05 were considered statistically significant.

Results.

ACE1 was elevated in antemortem CSF in Alzheimer's disease and correlated with tau/p-tau level and ACE2 activity

ACE1 activity was higher in the mid-frontal cortex in AD compared to age-matched controls ($p < 0.05$) (Figure 1A) confirming findings in our previous larger study⁵. ACE1 was also significantly elevated in post-mortem CSF in AD ($p < 0.05$) (Figure 1B) and was significantly higher in ante-mortem CSF in ($p = 0.008$) (Figure 1C). ACE2 activity was lower in mid-frontal cortex (Figure 1D), but not significantly as previously reported in a previous cohort⁴ likely reflecting the much smaller sample size. In contrast, ACE2 activity was significantly higher in post-mortem CSF ($p < 0.05$) but was unchanged in ante-mortem CSF ($p = 0.16$) (Figure 1F).

We previously reported that ACE2 activity was inversely correlated to ACE1 in mid-frontal cortex in AD⁴. In both post- and ante-mortem CSF, CSF-ACE1 activity correlated positively with CSF-ACE2 activity (Figure 2 A-B).

We previously reported that brain ACE1 and ACE2 activities were associated with A β and Tau load in post-mortem human brain tissue^{4, 5}. Here, ACE1 CSF activity correlated positively with both CSF t-tau ($r = 0.271$, $p = 0.015$) and CSF p-tau ($r = 0.345$, $p = 0.0018$) but not with CSF-A β_{42} level (Figure 3A-C).

Changes in brain angiotensin (-I, -II, and -(1-7)) level and ratio in mid-frontal cortex in AD are not reflected in CSF

We recently reported increased Ang-II and Ang-III level in the mid-frontal cortex in AD^{3,4}. In this study, Ang-II was again significantly increased in the smaller subset of mid-frontal cortex in AD ($p < 0.05$) (Figure 4A) whereas Ang-I (albeit much lower) and Ang-(1-7) level were unchanged (Fig 4D,G). In post-mortem CSF, Ang-I level was significantly lower in AD (Fig 4E) whilst Ang-II and Ang-(1-7) level were unchanged. No differences were observed for angiotensin levels in ante-mortem CSF (Fig 3C,F,I).

Previous studies have suggested that the ratio of Ang-I:Ang-II and Ang-II:Ang-(1-7) are proxy markers of ACE1 and ACE2 activity and are useful indicators of activity within the classical and regulatory RAS pathways. We found that the ratio of Ang-II:Ang-I was increased in the mid-frontal cortex in AD ($P < 0.05$) indicating that AD brains had relatively more Ang-II than Ang-I, as would be expected with increased ACE1 activity (Figure 5A). The Ang-I:Ang-II ratio was, however, unchanged in post-mortem and ante-mortem CSF (Figure 5B-C). The Ang-II:Ang-(1-7) ratio was significantly increased in the mid-frontal cortex in AD ($P < 0.05$) (Figure 5D) indicating that AD brains had relatively higher Ang-II level compared to Ang-(1-7), as would be expected with reduced ACE2 activity. In contrast there was no significant difference in post-mortem or ante-mortem CSF in either the Ang-I:Ang-II or Ang-II:Ang-(1-7) ratio (Figure 5E-F). Moreover, Ang-I, Ang-II and Ang-(1-7) levels and the ratio of Ang-II:Ang-I and Ang-II:Ang-(1-7) did not correlate with A β , Tau and pTau in ante-mortem CSF (data not shown).

Discussion

In this study, we have explored whether disease-associated changes in the classical and regulatory RAS pathways in brain RAS in the mid-frontal cortex in AD are reflected in post and ante-mortem CSF. Our data indicate that (i) ACE1 overactivity in brain RAS and post-

mortem CSF in AD is mirrored in ante-mortem CSF (ii) CSF-ACE1 in AD is associated higher CSF-t-tau and CSF-p-tau but not CSF-A β ₄₂ (iii) in contrast to brain tissue, ACE2 positively correlates with ACE1 activity in post and ante-mortem CSF (an inverse correlation was observed in brain tissue) and (iv) brain angiotensin changes in AD are not reflected in CSF. These findings increase our understanding of the role of RAS in AD and indicate that some markers of brain RAS are mirrored in CSF but that the relationship is complex.

Overactivation of the brain classical RAS axis in AD, indicated by increased ACE1^{5, 36} activity and elevated Ang-II level³, and a concurrent loss of regulatory RAS as a result of reduced ACE2 activity⁴, is associated with elevated A β and Tau load in AD. Here, we provide evidence that ACE1 overactivity observed in post-mortem AD brains is mirrored in antemortem CSF. Higher CSF-ACE1 activity is associated with increased level of total tau but not A β . These findings support an emerging idea that RAS-targeting anti-hypertensives associated with improved cognition or delayed onset of cognitive decline is related to hyperphosphorylation of Tau²⁰ and lower CSF-tau c²¹. CSF-ACE1 activity positively correlated with CSF-ACE2 activity whereas it is inversely correlated with ACE2 activity, which is reduced in brain tissue in AD. Elevated ACE1 and ACE2 activity in CSF may simply reflect solubilisation of both ACE1 and ACE2 that are ordinarily membrane bound, but which can be cleaved at their respective transmembrane domains and released extracellularly in an active form in disease (39, 40). In contrast to brain RAS in which ACE2 activity is reduced in AD, shedding of membrane ACE2 by ADAM-17/10 resulting in loss of membrane ACE2 has previously implicated in the pathogenesis of neurogenic hypertension (ref 35) and could also account for the differences observed between brain and CSF.

Previous studies have reported increased ACE1 in CSF in AD^{30, 31} whilst others reported reduced ACE1³²⁻³⁴ or no change³⁵. We have previously reported reduced ACE1 protein level (measured by a sandwich ELISA) in CSF but elevated ACE1 enzyme activity (and repeated here) (measured by A FRET substrate) in AD³⁶. These studies highlight that ACE1 protein is reduced, and ACE1 activity is increased, in CSF and indicates that one cannot be used as a proxy for another. This apparent inverse relationship could also account for the inconsistent reporting of ACE1 changes in CSF in previous studies. The differences between brain related and more peripheral measures of RAS are further complicated by findings by other groups that plasma ACE1 (ref) and serum ACE2³⁸ are reduced in AD. Further studies are needed to clarify the relationships between CSF protein and activity in the CSF and serum.

Less is known regarding changes in angiotensin peptides in AD. We recently reported that Ang-II and Ang-III, despite short half-lives, were measurable in post mortem brain tissue, and were elevated in AD in relation to markers of disease pathology³. Here we found that the ratio of Ang-II:Ang-I was increased in mid-frontal cortex in AD, consistent with increased ACE1 activity. Ang-(1-7) level remained unchanged but the ratio of Ang-II:Ang(1-7) was reduced in brain tissue in AD indicating that there is relatively lower level of Ang-(1-7) than Ang-II, consistent with reduced ACE2 activity in AD. These pilot data in a small subset of brains indicate that the ratios of Ang-II:Ang-I and Ang-II:Ang-(1-7) are potentially useful proxy markers of RAS activation in human brain tissue and may help to determine the extent of imbalance in RAS in future studies. Despite changes in brain, the ratios of Ang-II:Ang-I and Ang-II:Ang-(1-7) are unchanged in post-mortem and ante-mortem CSF in AD and do not correlate with disease markers. The difference between brain and CSF may reflect

the relative instability of the angiotensin peptides in CSF and seem to preclude their usefulness as potential CSF markers of disease status.

Together, these data indicate that overactivation of brain RAS, indicated by ACE1 activity, as observed in post-mortem brain tissue is also mirrored in antemortem CSF in and is related to higher CSF-tau level but not CSF-A β . These data further support a role for RAS and support an emerging link between Tau pathology and RAS in the pathogenesis of AD.

Acknowledgements

HZ is a Wallenberg Academy Fellow supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712) and Swedish State Support for Clinical Research (#ALFGBG-720931).

Ethical approval

All Original Articles should include a paragraph confirming that the research has been given ethical approval. The paragraph should appear after the Acknowledgements section.

Author contributions

Data and data citation

Neuropathology and Applied Neurobiology encourages authors to share the data and other artefacts supporting the results in the paper by archiving it in an appropriate public repository. Authors should include a data accessibility statement, including a link to the

repository they have used, in order that this statement can be published alongside their paper.

Figures

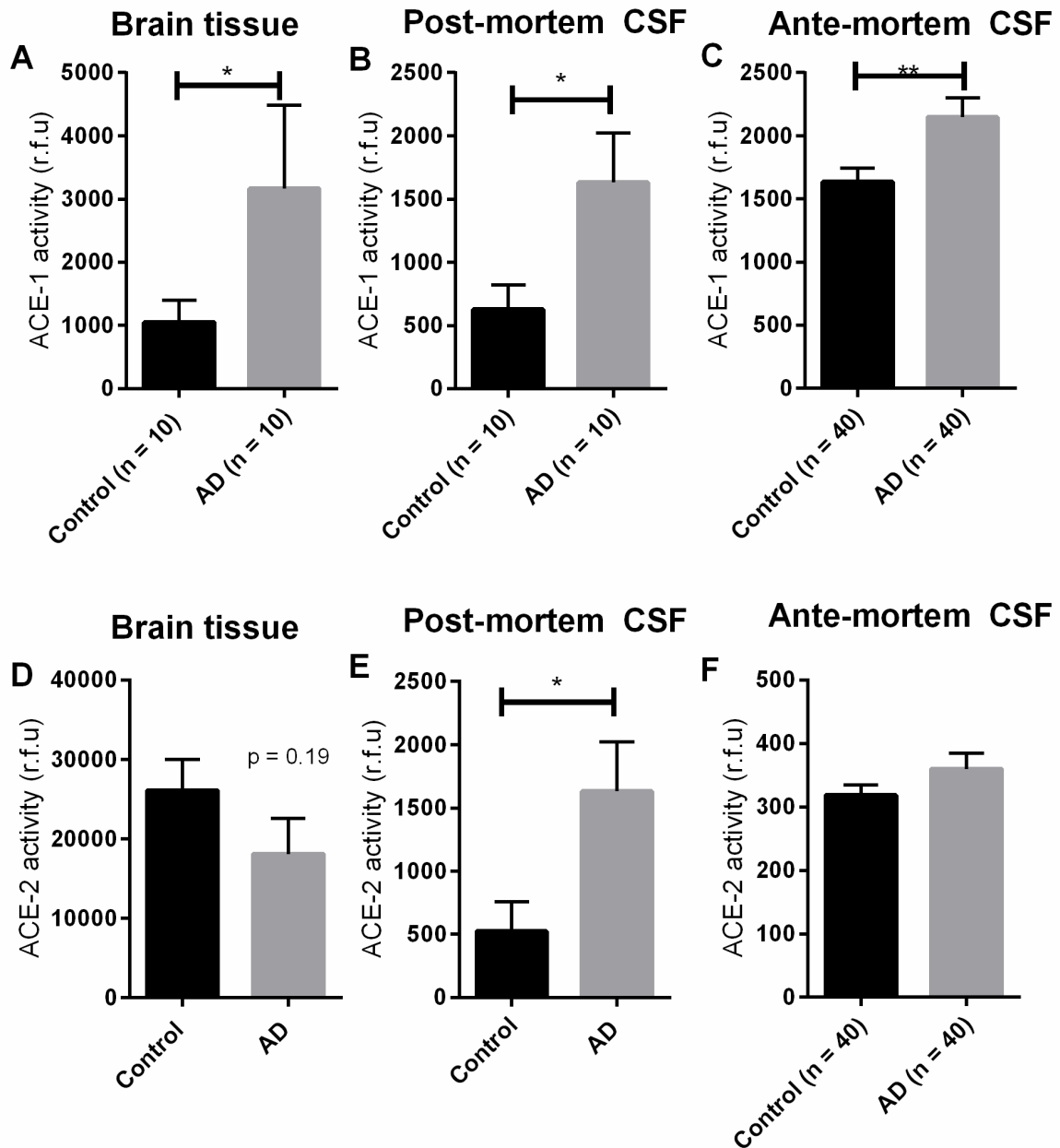


Figure 1. Bar charts showing changes in ACE1 and ACE2 enzyme activity in mid-frontal cortex and post and ante-mortem CSF in Alzheimer's disease (AD) compared to age-matched controls. (A-C) ACE1 enzyme activity was significantly increased in mid-frontal cortex (10 AD vs 10 controls ($p < 0.05$)), post-mortem CSF (10 AD Vs 10 controls ($p < 0.05$)) and ante-mortem CSF (40 AD Vs 40 controls). (D-F) ACE2 enzyme activity was unchanged in mid-frontal cortex in AD but was significantly elevated in post-mortem CSF ($p < 0.05$) and

approaching significance in ante-mortem CSF. Bars represent the mean level and standard error of the mean. * $P < 0.05$

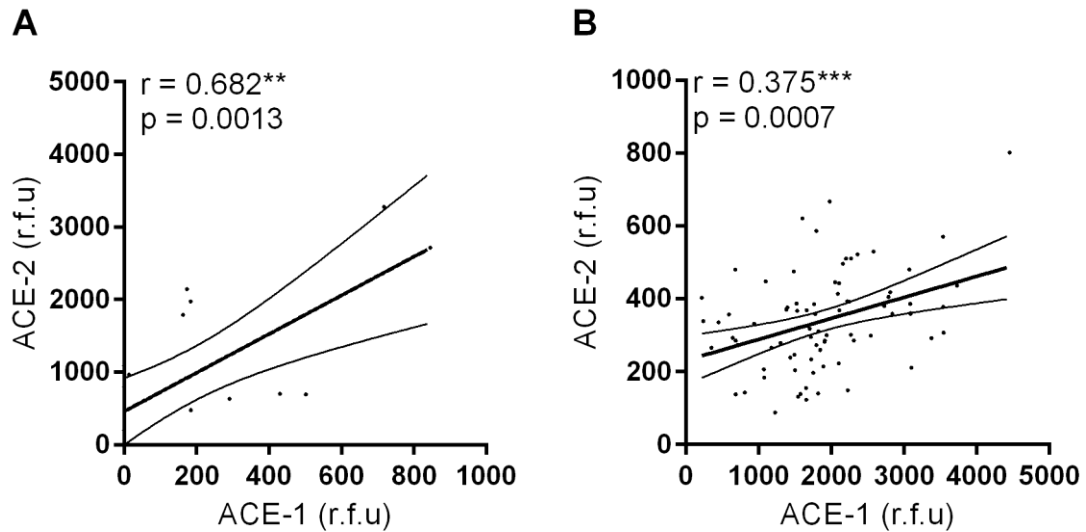


Figure 2. Scatterplots showing a positive correlation between ACE1 enzyme activity and ACE2 enzyme activity in A. post-mortem (10 Vs 10) and B. ante-mortem CSF (40 Vs 40) from a combined cohort of controls and AD. Each point in the scatterplot indicates a single brain. The best-fit linear regression lines and 95% confidence intervals are superimposed.

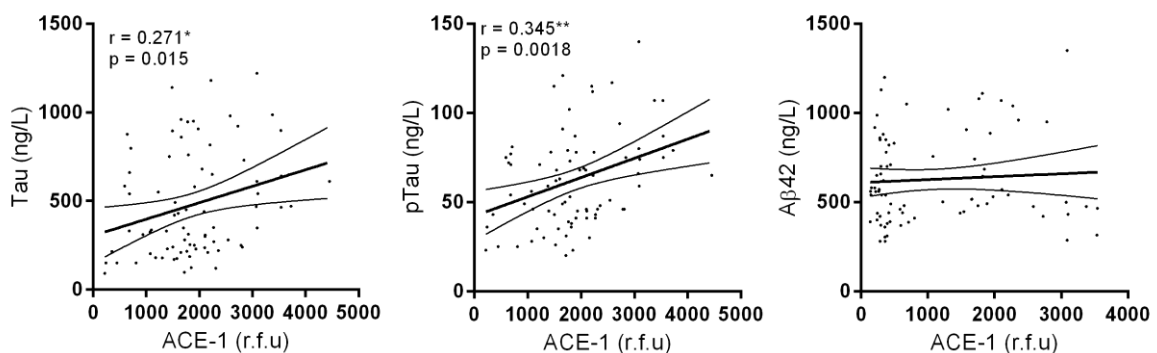


Figure 3: Scatterplots showing the relationship between ACE1 activity and CSF-Tau, pTau and A β in a combined AD and control cohort (AD = 40 and control = 40). ACE1 activity positively correlated with t-tau ($r = 0.271$, $p = 0.015$) and p-tau ($r = 0.345$, $p = 0.0018$) but

not CSF-A β 42 load. Each point in the scatterplot indicates a single brain. The best-fit linear regression lines and 95% confidence intervals are superimposed.

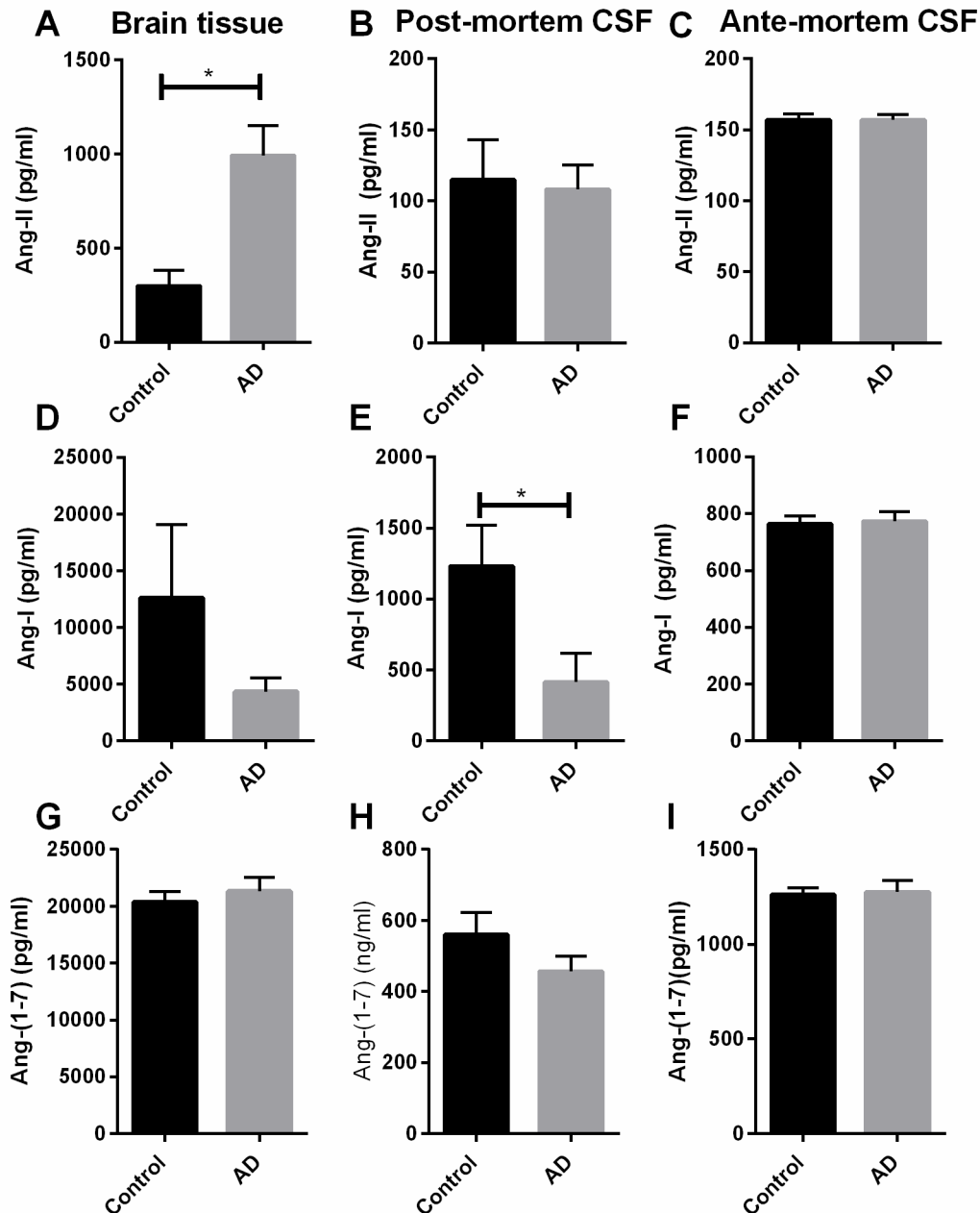


Figure 4. Bar charts showing angiotensin-I, -II and -(1-7) level in mid-frontal cortex (10 AD Vs 10 controls), post-mortem (10 AD Vs 10 controls), and in a subset of ante-mortem CSF samples (20 AD Vs 20 controls) in Alzheimer's disease. (A-C) Ang-II level was significantly increased in mid-frontal cortex in AD but was unchanged in post- and ante-mortem CSF. (D-

F) Ang-I was lower in mid-frontal cortex (approaching significance) and was reduced in post-mortem CSF but was unchanged in ante-mortem CSF. (G-I) Ang-(1-7) level was unaltered in mid-frontal cortex, post- and ante-mortem CSF in AD. Bars represent the mean level and standard error of the mean. * $P < 0.05$

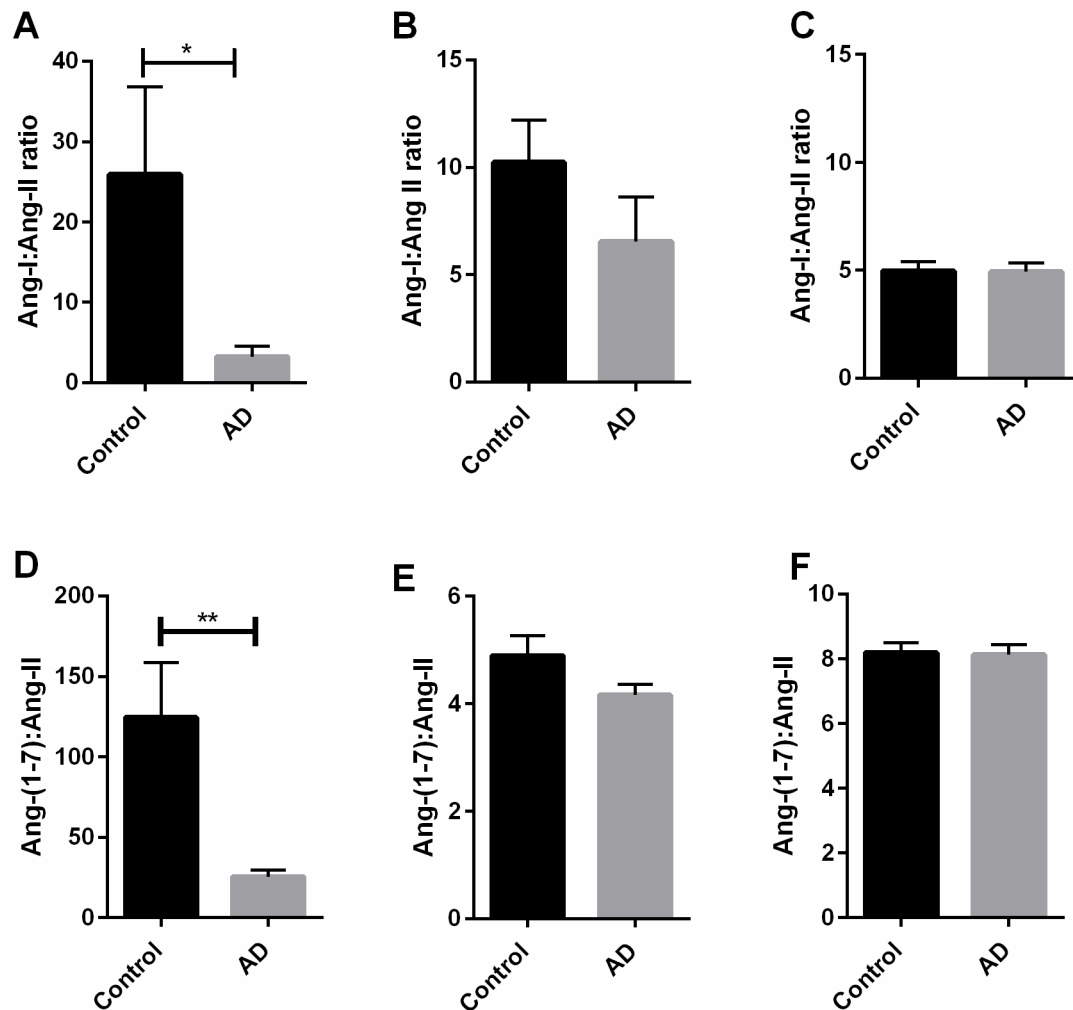


Figure 4. Bar charts showing the ratio of Ang-I:Ang-II and Ang-(1-7):Ang-II in mid-frontal cortex (10 controls Vs 10 AD), post-mortem CSF (10 controls Vs 10 AD) and ante-mortem CSF (20 controls and 20 AD) in Alzheimer's disease. (A-C) The Ang-I:Ang-II ratio was significantly lower in (A) mid-frontal cortex in AD but remained unaltered in (B) post- and (C)

ante-mortem CSF. (D-F). The Ang-(1-7):Ang-II ratio was reduced in (D) mid-frontal cortex in AD but was unaltered in (E) post- and (F) ante-mortem CSF. * P < 0.05 ** P < 0.01

References

1. Kehoe PG. The Coming of Age of the Angiotensin Hypothesis in Alzheimer's Disease: Progress Toward Disease Prevention and Treatment? *J Alzheimers Dis.* 2018; 62: 1443-66.
2. Kehoe PG, Miners S and Love S. Angiotensins in Alzheimer's disease - friend or foe? *Trends Neurosci.* 2009; 32: 619-28.
3. Kehoe PG, Hibbs E, Palmer LE and Miners JS. Angiotensin-III is Increased in Alzheimer's Disease in Association with Amyloid-beta and Tau Pathology. *J Alzheimers Dis.* 2017; 58: 203-14.
4. Kehoe PG, Wong S, Al Mulhim N, Palmer LE and Miners JS. Angiotensin-converting enzyme 2 is reduced in Alzheimer's disease in association with increasing amyloid-beta and tau pathology. *Alzheimers Res Ther.* 2016; 8: 50.
5. Miners JS, Ashby E, Van Helmond Z, et al. Angiotensin-converting enzyme (ACE) levels and activity in Alzheimer's disease, and relationship of perivascular ACE1 to cerebral amyloid angiopathy. *Neuropathol Appl Neurobiol.* 2008; 34: 181-93.
6. Tian M, Zhu D, Xie W and Shi J. Central angiotensin II-induced Alzheimer-like tau phosphorylation in normal rat brains. *FEBS Lett.* 2012; 586: 3737-45.
7. Zhu D, Shi J, Zhang Y, et al. Central angiotensin II stimulation promotes beta amyloid production in Sprague Dawley rats. *PLoS One.* 2011; 6: e16037.
8. Iturria-Medina Y, Hachinski V and Evans AC. The vascular facet of late-onset Alzheimer's disease: an essential factor in a complex multifactorial disorder. *Curr Opin Neurol.* 2017; 30: 623-9.
9. Danielyan L, Klein R, Hanson LR, et al. Protective effects of intranasal losartan in the APP/PS1 transgenic mouse model of Alzheimer disease. *Rejuvenation Res.* 2010; 13: 195-201.
10. Dong YF, Kataoka K, Tokutomi Y, et al. Perindopril, a centrally active angiotensin-converting enzyme inhibitor, prevents cognitive impairment in mouse models of Alzheimer's disease. *FASEB J.* 2011; 25: 2911-20.
11. Ongali B, Nicolakakis N, Tong XK, et al. Angiotensin II type 1 receptor blocker losartan prevents and rescues cerebrovascular, neuropathological and cognitive deficits in an Alzheimer's disease model. *Neurobiol Dis.* 2014; 68: 126-36.
12. Tsukuda K, Mogi M, Iwanami J, et al. Cognitive deficit in amyloid-beta-injected mice was improved by pretreatment with a low dose of telmisartan partly because of peroxisome proliferator-activated receptor-gamma activation. *Hypertension.* 2009; 54: 782-7.
13. Wang J, Ho L, Chen L, et al. Valsartan lowers brain beta-amyloid protein levels and improves spatial learning in a mouse model of Alzheimer disease. *J Clin Invest.* 2007; 117: 3393-402.
14. Yamada K, Uchida S, Takahashi S, et al. Effect of a centrally active angiotensin-converting enzyme inhibitor, perindopril, on cognitive performance in a mouse model of Alzheimer's disease. *Brain Res.* 2010; 1352: 176-86.
15. Davies NM, Kehoe PG, Ben-Shlomo Y and Martin RM. Associations of anti-hypertensive treatments with Alzheimer's disease, vascular dementia, and other dementias. *J Alzheimers Dis.* 2011; 26: 699-708.
16. Li NC, Lee A, Whitmer RA, et al. Use of angiotensin receptor blockers and risk of dementia in a predominantly male population: prospective cohort analysis. *BMJ.* 2010; 340: b5465.
17. Yasar S, Xia J, Yao W, et al. Antihypertensive drugs decrease risk of Alzheimer disease: Ginkgo Evaluation of Memory Study. *Neurology.* 2013; 81: 896-903.
18. Wharton W, Goldstein FC, Zhao L, Steenland K, Levey AI and Hajar I. Modulation of Renin-Angiotensin System May Slow Conversion from Mild Cognitive Impairment to Alzheimer's Disease. *J Am Geriatr Soc.* 2015; 63: 1749-56.

19. Ohrui T, Tomita N, Sato-Nakagawa T, et al. Effects of brain-penetrating ACE inhibitors on Alzheimer disease progression. *Neurology*. 2004; 63: 1324-5.
20. Jiang T, Zhang YD, Zhou JS, et al. Angiotensin-(1-7) is Reduced and Inversely Correlates with Tau Hyperphosphorylation in Animal Models of Alzheimer's Disease. *Mol Neurobiol*. 2016; 53: 2489-97.
21. Hajjar I, Goldstein FC, Martin GS and Quyyumi AA. Roles of Arterial Stiffness and Blood Pressure in Hypertension-Associated Cognitive Decline in Healthy Adults. *Hypertension*. 2016; 67: 171-5.
22. Nation DA, Ho J, Yew B and Alzheimer's Disease Neuroimaging I. Older Adults Taking AT1-Receptor Blockers Exhibit Reduced Cerebral Amyloid Retention. *J Alzheimers Dis*. 2016; 50: 779-89.
23. Kehoe PG, Russ C, McIlroy S, et al. Variation in DCP1, encoding ACE, is associated with susceptibility to Alzheimer disease. *Nat Genet*. 1999; 21: 71-2.
24. Ferrario CM, Trask AJ and Jessup JA. Advances in biochemical and functional roles of angiotensin-converting enzyme 2 and angiotensin-(1-7) in regulation of cardiovascular function. *Am J Physiol Heart Circ Physiol*. 2005; 289: H2281-90.
25. Santos RA, Campagnole-Santos MJ and Andrade SP. Angiotensin-(1-7): an update. *Regul Pept*. 2000; 91: 45-62.
26. Santos RA, Simoes e Silva AC, Maric C, et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci U S A*. 2003; 100: 8258-63.
27. Elased KM, Cunha TS, Marcondes FK and Morris M. Brain angiotensin-converting enzymes: role of angiotensin-converting enzyme 2 in processing angiotensin II in mice. *Exp Physiol*. 2008; 93: 665-75.
28. Vickers C, Hales P, Kaushik V, et al. Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. *J Biol Chem*. 2002; 277: 14838-43.
29. Jiang T, Tan L, Gao Q, et al. Plasma Angiotensin-(1-7) is a Potential Biomarker for Alzheimer's Disease. *Curr Neurovasc Res*. 2016; 13: 96-9.
30. Mateos L, Ismail MA, Gil-Bea FJ, et al. Upregulation of brain renin angiotensin system by 27-hydroxycholesterol in Alzheimer's disease. *J Alzheimers Dis*. 2011; 24: 669-79.
31. He M, Ohrui T, Maruyama M, et al. ACE activity in CSF of patients with mild cognitive impairment and Alzheimer disease. *Neurology*. 2006; 67: 1309-10.
32. Rocha NP, Toledo A, Corgosinho LTS, et al. Cerebrospinal Fluid Levels of Angiotensin-Converting Enzyme Are Associated with Amyloid-beta42 Burden in Alzheimer's Disease. *J Alzheimers Dis*. 2018; 64: 1085-90.
33. Zubenko GS, Volicer L, Drenfeld LK, Freeman M, Langlais PJ and Nixon RA. Cerebrospinal fluid levels of angiotensin-converting enzyme in Alzheimer's disease, Parkinson's disease and progressive supranuclear palsy. *Brain Res*. 1985; 328: 215-21.
34. Jochemsen HM, Teunissen CE, Ashby EL, et al. The association of angiotensin-converting enzyme with biomarkers for Alzheimer's disease. *Alzheimers Res Ther*. 2014; 6: 27.
35. Konings CH, Kuiper MA, Scheltens P, Grijpma AM, van Pelt W and Wolters EC. Re-evaluation of cerebrospinal fluid angiotensin-converting enzyme activity in patients with 'probable' Alzheimer's disease. *Eur J Clin Chem Clin Biochem*. 1993; 31: 495-7.
36. Miners S, Ashby E, Baig S, et al. Angiotensin-converting enzyme levels and activity in Alzheimer's disease: differences in brain and CSF ACE and association with ACE1 genotypes. *Am J Transl Res*. 2009; 1: 163-77.
37. Miners JS, van Helmond Z, Raiker M, Love S and Kehoe PG. ACE variants and association with brain Abeta levels in Alzheimer's disease. *Am J Transl Res*. 2010; 3: 73-80.
38. Liu S, Liu J, Miura Y, et al. Conversion of Abeta43 to Abeta40 by the successive action of angiotensin-converting enzyme 2 and angiotensin-converting enzyme. *J Neurosci Res*. 2014; 92: 1178-86.