Investigating age- and disease-associated changes in the renin-angiotensin system in Alzheimer's disease

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Introduction

- The renin-angiotensin system (RAS) is a hormonal pathway that regulates systemic blood pressure and fluid homeostasis.
- RAS is expressed locally and functions independently within organs including the brain[1].
- Overactivity within the "classical" renin-angiotensin system (cRAS) is observed in Alzheimer's disease (AD) and is associated with disease pathology[2].
- Commonly prescribed anti-hypertensives that block cRAS signalling reduce disease pathology and cognitive decline in mouse models of AD[3].
- Large population-based studies indicate a lower incidence of AD with centrally-acting cRAS-targeting medications associated with slower conversion of mild cognitive impairment (MCI) to AD and lower rates of cognitive decline[4,5].
- Clinical trails, such as the RADAR and SARTAN-AD trials are investigating the therapeutic potential of cRAS-targeting anti-hypertensive medication in AD patients[6].

Aims and hypothesis

- The aim of this study is to investigate age- and disease-related changes in RAS to inform future clinical trials of RAS-targeting agents in AD.
- We wish to explore the hypothesis that age- and disease-related overactivation of brain cRAS is associated with cognitive decline and disease pathology in AD and intervention using cRAS blockers at an early stage of disease can be an effective therapeutic strategy in AD.

Renin-angiotensin system

- Brain RAS is balanced between disease-associated cRAS overactivity and protective regulatory RAS (rRAS) underactivity in AD (Figure 1).
- Angiotensin converting enzyme 1 (ACE-1) mediated production of Angiotensin II (Ang II) is a key regulator and marker of cRAS activity.
- Angiotensin converting enzyme 2 (ACE-2) mediated conversion of Ang-II to Angiotensin (1-7) (Ang (1-7)) is a key regulator and marker of rRAS activity.

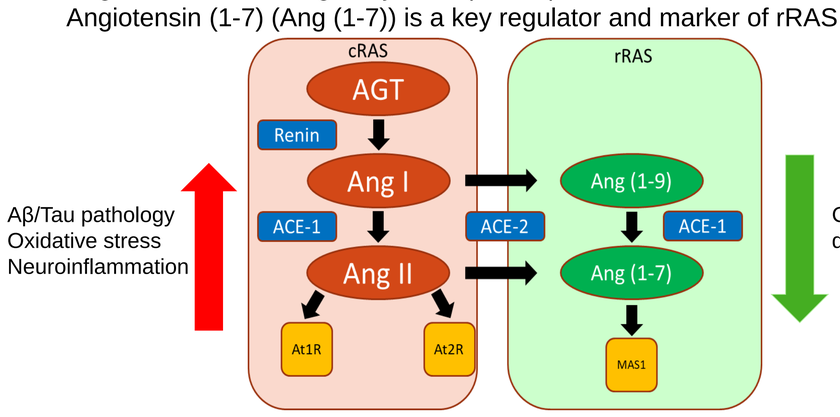

Figure 1 depicts a simplified representation of the brain renin-angiotensin system in Alzheimer's disease

Methods

Human post-mortem cohort- Will characterise cRAS and rRAS pathways in a normal ageing cohort and in AD stratified into disease stage groups (Braak tangle stage):

Brain region		Frontal cortex (BA46)		Temporal cortex (BA41/42)	
Total (n)		71		65	
Sex		Male	Female	Male	Female
Age-at-death (y)	≤ 45 (n)	11	7	13	7
	45 < x ≤ 65 (n)	32	7	26	10
	> 65 (n)	12	5	9	3
Postmortem delay (SD)		79(±25)		75(±24)	

Brain region		Frontal cortex (BA46)		Temporal cortex (BA41/42)	
Total (n)		60		60	
Sex		Male	Female	Male	Female
Braak stage	0-II (n)	10	10	10	10
	III-IV (n)	10	10	10	10
	V-VI (n)	10	10	10	10
Postmortem delay (SD)		44(±10)		44(±10)	

- ACE-1 activity assay was measured using an ACE-1 specific FRET peptide (Abz-FRK (Dnp)-P). ACE-1 specific activity is calculated by subtracting enzyme activity in the presence of captopril from total substrate cleavage.
- ACE-2 activity assay was measured using an ACE-2 specific FRET peptide (Mca-APK (Dnp)). ACE-2 specific activity is calculated by subtracting enzyme activity in the presence of an ACE-2 inhibitor (MLN-4760) from total substrate cleavage.
- ACE-1 and ACE-2 levels were measured by sandwich ELISA.
- Angiotensin II level was measured by direct ELISA[7].

Age-related changes in RAS

ACE-1

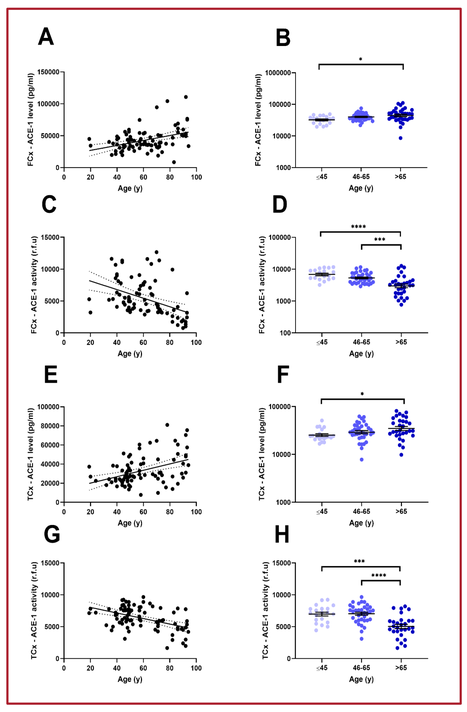

Figure 2: ACE-1 level is increased with age in both the frontal and temporal cortex (A, B, E, F). ACE-1 activity is reduced with age in both the frontal and temporal cortex (C, D, G, H).

ACE-2

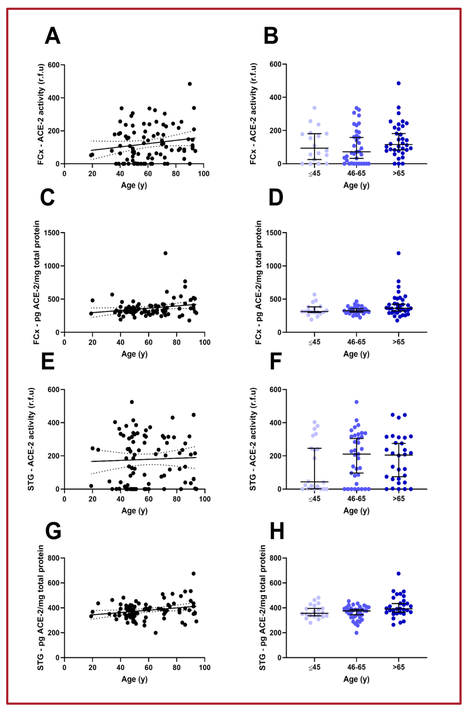

Figure 3: ACE-2 level and activity was unchanged with age in both the frontal and temporal cortex (A. B, C, D, E, F, G, H).

Angiotensin-II

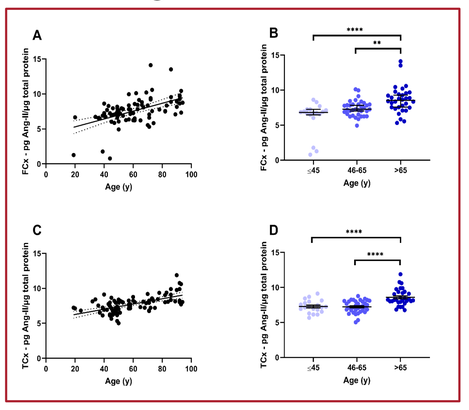

Figure 4: Angiotensin II level was increased with age in both the frontal and temporal cortex (A, B, C, D).

Disease-related changes in RAS

ACE-1

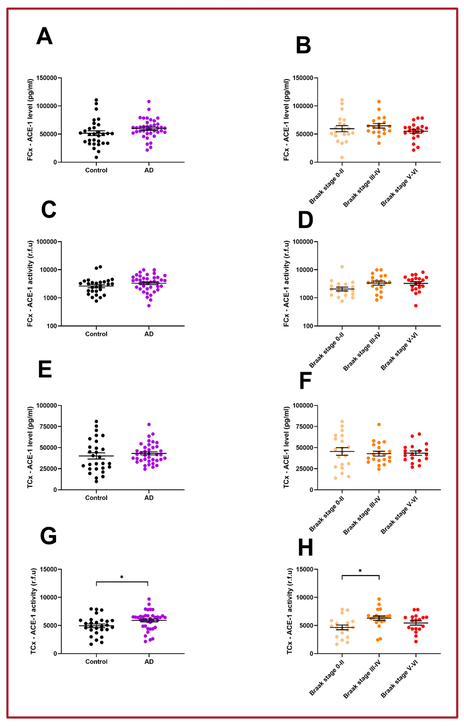

Figure 5: ACE-1 level was unchanged with disease in both the frontal and temporal cortex (A, B, E, F). ACE-1 activity was increased in the early stages of disease in the temporal cortex (G, H) but not in the frontal cortex (C, D).

ACE-2

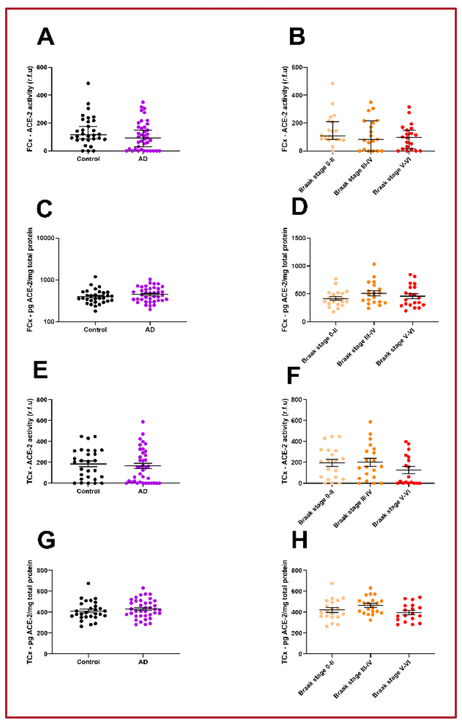

Figure 6: ACE-2 level and activity was unchanged with disease in both the frontal and temporal cortex (A, B, C, D, E, F, G, H).

Angiotensin-II

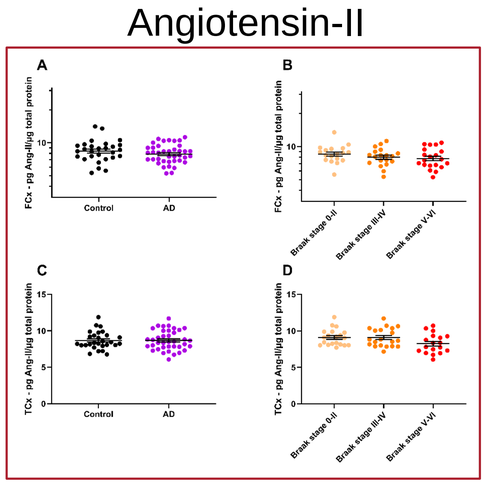

Figure 7: Angiotensin II level was unchanged in relation to disease in both the frontal and temporal cortex (A, B, C, D).

Conclusions

- ACE-1 level and activity were effected by age (although in opposing directions) whereas ACE-2 level and activity were unchanged.
- Angiotensin-II level also increased with age and positively correlated with level and negatively correlated with activity. We therefore hypothesise that Ang-II level regulates ACE-1 activity.
- ACE-1 level was unchanged in AD compared to controls but activity was increased in the temporal cortex. The increase in ACE-1 activity occurred between Braak stages 0-II and III-IV (mild-moderate AD). Understanding the timing of this dysregulation helps us identify when RAS targeting medications will be most beneficial at reducing AD progression and cognitive decline.
- ACE-2 activity and level were unchanged in AD (a reduction in activity was previously reported) and no changes in Ang-II level were observed.

References

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