Contribution of White Matter Tau and beta Amyloid pathology in the Spatial Progression of Alzheimer's Disease

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Introduction

Braak Neurofibrillary tangles (NFT) staging suggests that hyperphosphorylated tau (HPT) first appear in the enthorhinal cortex and hippocampus (Braak stages I-II), spreading to the temporal cortex (Braak stage III-IV) and later throughout the neocortex (Braak Stage V-VI) in Alzheimer's disease (AD). Amyloid beta (Aβ) plaques initially occur in the fronto-orbital cortex and spread through the neocortex (as per Thal phases). Thus, early stages NFT and Aβ plaques are spatially divergent, in contrast to Amyloid cascade hypothesis, which proposes a causative link between Aβ and Tau pathology.

However, prior work indicates that progressive phosphorylation of tau at specific sites may proceed NFT formation, suggesting a regions affection with tau pathology may occurs earlier than is indicated by Braak NFT staging.

Furthermore, mechanisms of prion-like spread following neuronal connection throughout brain have been suggested to account for spread of Tau pathology, which likely dictate the affection of regions in a sequential manner.

If indeed the mechanism of spread, HPT pathology should first be detected within the associated white matter of a given region prior to the development of traditional grey matter pathology.

Aims

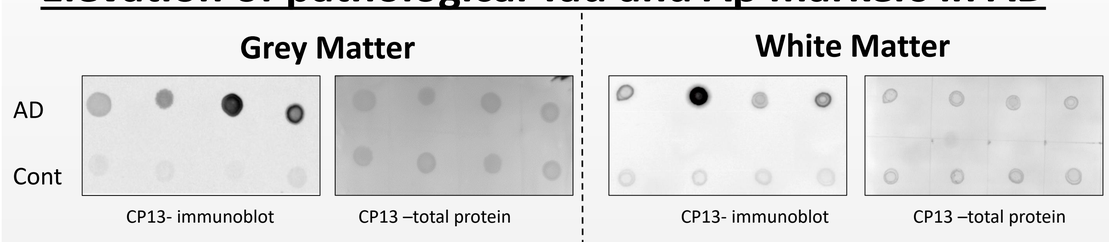
- Determine biochemical measures of HPT and Aβ within the frontal white and grey matter
- Compare these biochemical measures across a cohort of Healthy Aged Controls and AD cases
- Contextualise white and grey matter pathology with AD neuropathological scores

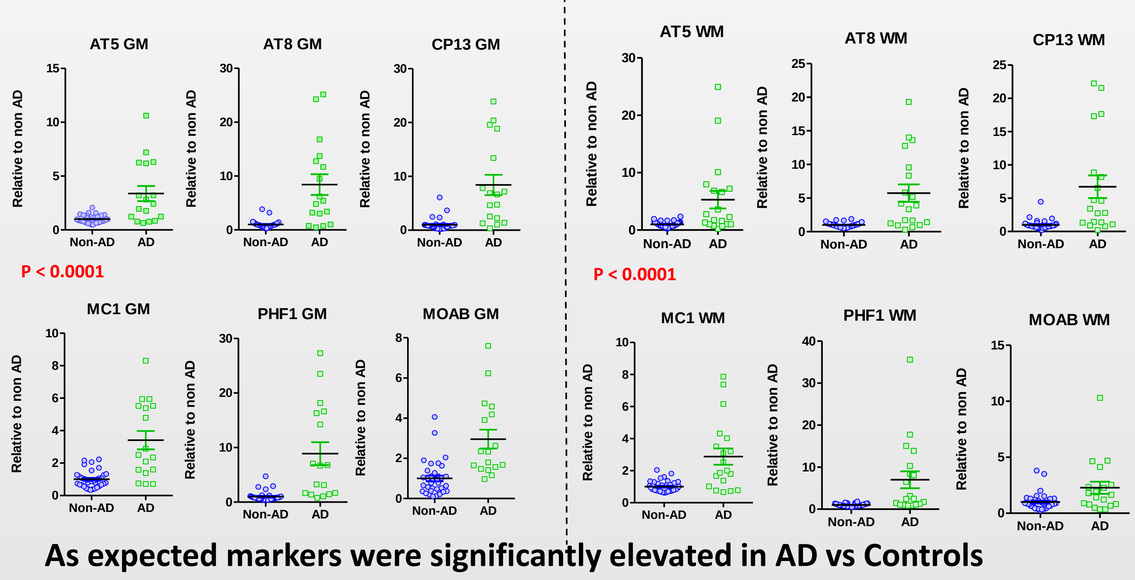
Methods

- White and grey matter frozen brain tissue (Brodmann area 8/9) from 52 cases (17 AD and 35 healthy aged Controls) were homogenised in 0.2M Tetraethylammonium bromide, dotted onto nitrocellulose membranes (5µg/dot) and treated as per standard dot-blot protocols.
- Blots comprising either white or grey matter samples were stained with primary antibodies towards HPT (CP-13, AT-8 and PHF-1), total Tau (AT5) and Aβ (MOAB-2 – does not cross reactive with APP or other metabolites) and goat anti-mouse IgG-HRP secondary antibodies conjugated to HRP (1:5000) and was visualised via standard chemiluminescence.
- For each case, immunoreactivity was quantified by means of area under curve densitometric quantification and adjusted to total protein loaded via total protein stain (Ponceau S).
- For each blot values were expressed relative to mean Controls thus normalising signals within blots and prior to be pooled.
- Significance between Controls and AD was determined via non-parametric Mann-Whittney tests and correlations between variables were calculated using Spearman's correlation test.
- In all analysis $p<0.05$ was taken as significant.

Results

Elevation of pathological Tau and Aβ markers in AD

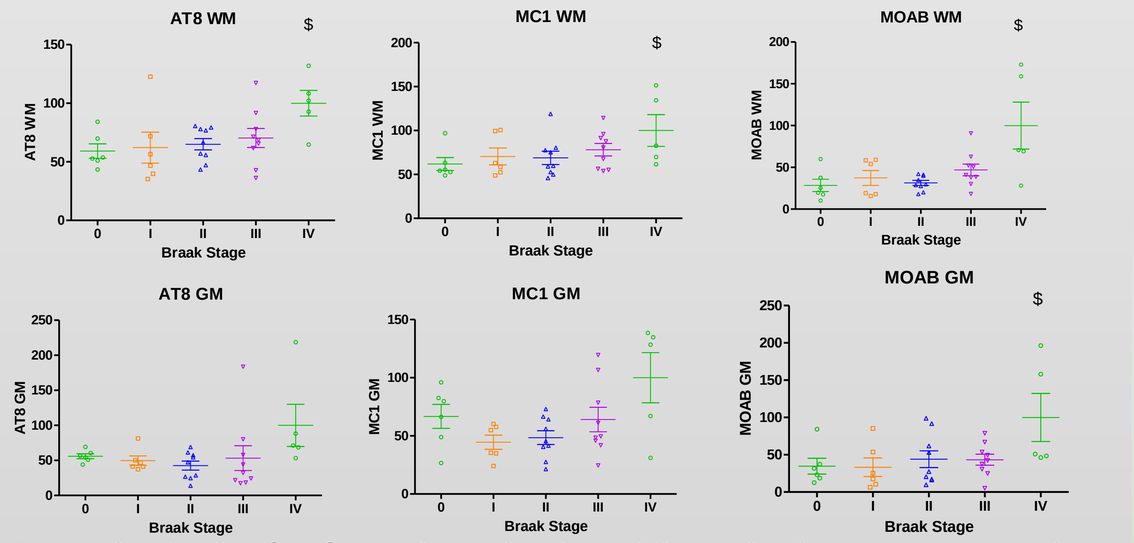




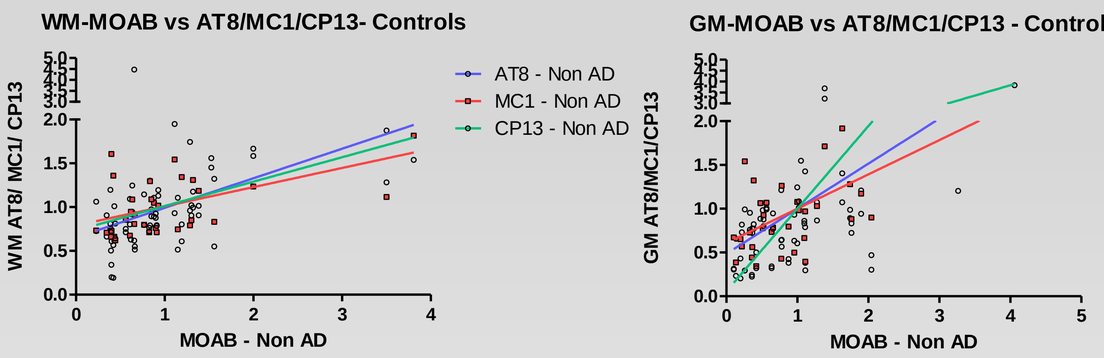
As expected markers were significantly elevated in AD vs Controls

White matter tau pathology precedes that of grey matter

$ = Significantly different from Braak 0



Correlation of Aβ and Early & Mid Pathology Tau Markers CP13, AT8 and MC1



In controls, Aβ and pathological tau markers already correlate, demonstrating their co-occurrence even in the frontal cortex which is conventionally thought to be effect with tau pathology at late Braak stages (V-VI).

	N	Sex (% M)	Age (Avg)	Braak	Thal	CERAD
Controls	35	57.1	80.5	0-4	0-4	0-2
AD	17	35.3	85.5	5-6	4-5	3

Select correlations between tau , Aβ and Braak stage in controls

A: Controls Only — Grey Matter × White Matter; Spearman's Rho Correlation Coefficient; N = 35

White Matter \ Grey Matter	Braak	Thal	AT8	CP13	MC1	MOAB	PHF1	AT5
Braak	1.000	0.261	0.099	0.188	0.207	.349*	0.066	0.079
Thal	0.261	1.000	0.321	0.259	0.130	.501**	0.136	0.060
AT8	.413*	0.276	0.246	0.282	0.320	.348*	0.206	0.052
CP13	0.083	0.058	0.257	.424*	0.171	0.182	0.241	0.149
MC1	.389*	0.185	0.196	.389*	.353*	.580**	.371*	.454**
MOAB	.508**	.383*	0.279	0.319	0.304	.588**	0.244	0.152
PHF1	0.125	0.082	0.195	.392*	.423*	0.270	.350*	0.274
AT5	0.205	-0.095	0.054	0.232	.363*	0.099	0.254	0.166

B: AD Only — Grey Matter × White Matter; N = 17

White Matter \ Grey Matter	Braak	Thal	AT8	CP13	MC1	MOAB	PHF1	AT5
Braak	1.000	0.165	.580*	.501*	0.422	-0.105	0.475	.501*
Thal	0.165	1.000	-0.373	-0.447	-.484*	-0.335	-0.354	-0.298
AT8	.553*	-0.224	.838**	.770**	.740**	0.225	.712**	.826**
CP13	.606**	-0.298	.811**	.750**	.713**	0.118	.715**	.762**
MC1	0.395	-0.261	.748**	.770**	.760**	0.025	.820**	.767**
MOAB	.580*	0.224	.485*	0.404	0.407	0.015	0.390	.500*
PHF1	.580*	-0.224	.838**	.784**	.743**	0.235	.730**	.806**
AT5	.606**	-0.037	.814**	.757**	.703**	0.294	.747**	.821**

A: (Controls) Tau pathology in white matter correlate with Braak stage. In Grey matter, Tau pathology does not correlate with Braak stage.

B: (AD) Tau markers correlate with Braak stage in Grey and White matter. Note correlation of conformational tau marker (MC1) is lost from controls to AD in white matter.

C: Controls Only — White Matter × White Matter; N = 35

White Matter	Braak	Thal	AT8	CP13	MC1	MOAB	PHF1	AT5
Braak	1.000	0.261	.413*	0.083	.389*	.508**	0.125	0.205
Thal	0.261	1.000	0.276	0.058	0.185	.383*	0.082	-0.095
AT8	.413*	0.276	1.000	.410*	.344*	.703**	.380*	0.287
CP13	0.083	0.058	.410*	1.000	0.241	.399*	.364*	0.185
MC1	.389*	0.185	.344*	0.241	1.000	.510**	.482**	0.060
MOAB	.508**	.383*	.703**	.399*	.510**	1.000	0.289	0.326
PHF1	0.125	0.082	.380*	.364*	.482**	0.289	1.000	.552**
AT5	0.205	-0.095	0.287	0.185	0.060	0.326	.552**	1.000

D: Controls Only — Grey Matter × Grey Matter; N = 35

Grey Matter	Braak	Thal	AT8	CP13	MC1	MOAB	PHF1	AT5
Braak	1.000	0.261	0.099	0.188	0.207	.349*	0.066	0.079
Thal	0.261	1.000	0.321	0.259	0.130	.501**	0.136	0.060
AT8	0.099	0.321	1.000	.713**	.638**	.478**	.695**	0.284
CP13	0.188	0.259	.713**	1.000	.682**	.565**	.818**	.480**
MC1	0.207	0.130	.638**	.682**	1.000	.522**	.680**	.534**
MOAB	.349*	.501*	.478**	.565**	.522**	1.000	.489*	.489*
PHF1	0.066	0.136	.695**	.818**	.680**	.489*	1.000	.633**
AT5	0.079	0.060	0.284	.480**	.534**	.489*	.633**	1.000

C & D: (White & Grey Matter) Tau markers correlate with Amyloid ß marker (MOAB). Amyloid ß correlate with Braak stages

Conclusions

- **Biochemical changes in tau occur earlier in frontal cortex than is indicated by histological HPT assessment via Braak stages and supports a modified amyloid cascade hypothesis.**
- **Pathological tau and Aβ co-occur early in disease development, likely independent from NFTs.**
- **Tau pathology in Frontal White matter precedes that in Frontal Grey matter.**
- **Studies assuming the Frontal cortex as unaffected by tau pathology prior to Braak stage V-VI may be confounded by the pre-existing relationship between Aβ and tau.**

Acknowledgments -Tissue for this study was provided by the Newcastle Brain Tissue Resource which is funded in part by a grant from the UK Medical Research Council (G0400074), by NIHR Newcastle Biomedical Research Centre awarded to the Newcastle upon Tyne NHS Foundation Trust and Newcastle University, and as part of the Brains for Dementia Research Programme jointly funded by Alzheimer's Research UK and Alzheimer's Society. Antibodies CP13 and PHF1 were kindly gifted to us by Prof P. Davies.