The Relationship Between Secretagogin and Neurogenesis with Increasing AD Pathology

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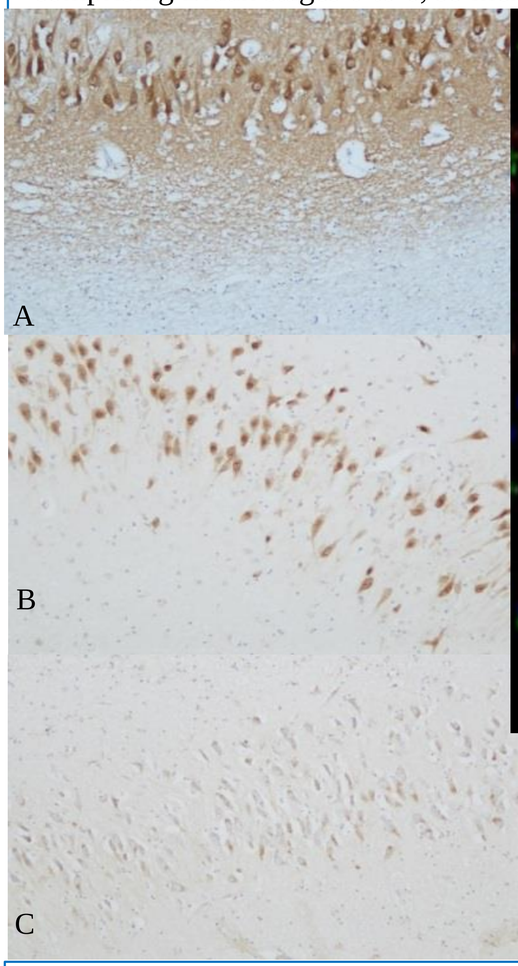
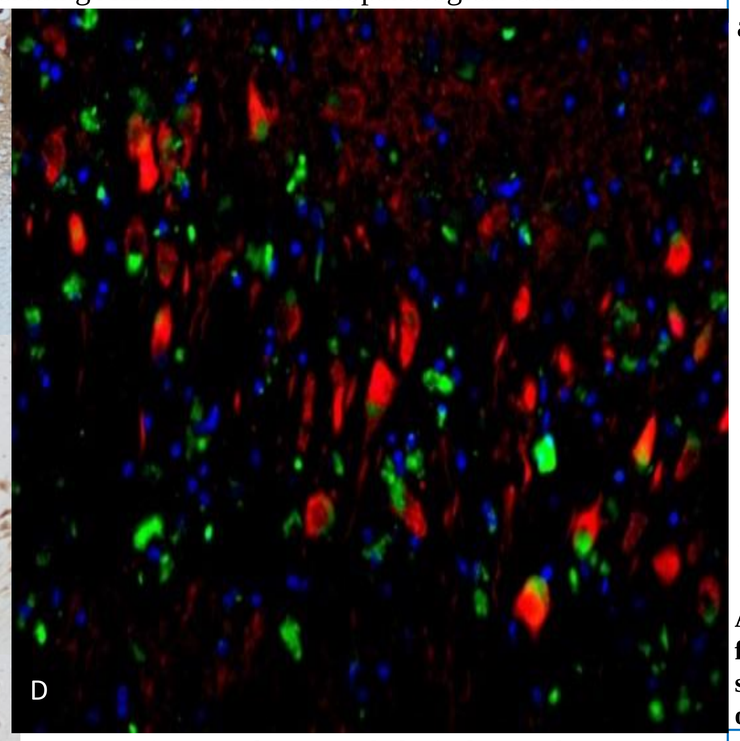
Introduction

Alzheimer's disease (AD) is characterised by amyloid plaques and tau protein deposits in the brain. Amyloid beta peptides and tau proteins have been found to occur in non-AD brains, suggesting that they are a product of healthy ageing.
Calcium binding proteins (CBPs) such as secretagogin rarely colocalise with tau, suggesting that secretagogin positive neurons may be resistant to neurodegeneration . This project will examine both neurogenic regions and secretagogin-positive pyramidal neurons in cases comprising Braak stages 0 to 6, so including Alzheimer and neuropahological control cases.





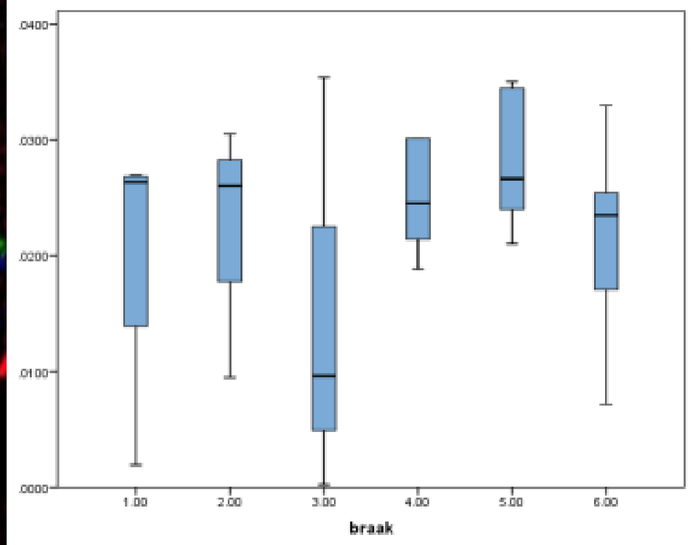
CA2 x 20 images of neuropathological control cases stained with secretagogin (A) HuC/D ((B) and doublecortin (C). Fluorescent staining (D) captured by EVOS FL Cell Imaging System. 488nm (red) indicating secretagogin and 594nm (green) indicating doublecortin. Nuclei stained blue with DAPI. Co-localised staining shown yellow.

Methods

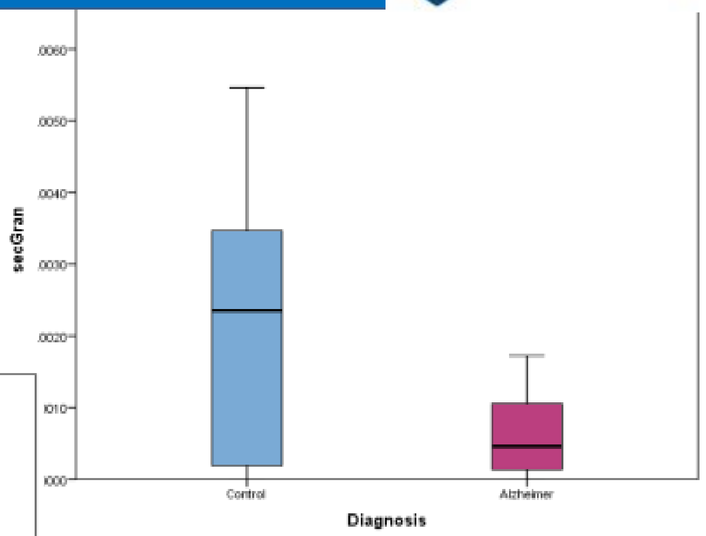
- IHC - sections dewaxed in xylene and re-hydrated through alcohol into water. Heat induced epitope retrieval by microwaving sections in 0.1M citrate buffer
- Single antibody staining - 3% hydrogen peroxide then TBS wash with 0.5% Tween. Primary antibodies applied, secretagogin 1:3000, HuC/D 1:1500 and doublecortin 1:800. Visualised with DAB, nuclei counterstained with haematoxylin.
- Immunofluorescent double labelling – sections incubated with polyclonal (secretagogin and doublecortin) and monoclonal (HuC/D) primary antibodies. Sections were stained with Alexa Fluor 594 (polyclonal) and 488 (monoclonal).

Results

Highest density of antibody staining in CA2 and CA3, lowest in SGL. Mann Whitney U test showed significance for doublecortin in CA1, 2 and 3. Secretagogin significant in SGL and Granule layers. No significance between control and disease groups. The Spearman test showed a correlation in CA2 between each antibody for % area. IOD values showed no correlations.



Above: Graph showing a decrease in secretagogin staining from Braak 1-3 and an increase from 4-6. As pathology starts, neurogenesis is upregulated., as it worsens cell loss occurs



Above Graph showing AD group (pink) has less secretagogin staining than controls (blue), loss of cells is associated with pathology.

Secet Vs HuC/D				
% Area				
CA1	CA2	CA3	SGL	Gran
.016	.000	.066	-.159	-.801
IOD				
CA1	CA2	CA3	SGL	Gran
.163	.078	.172	-.202	-.975

Above: Spearman test. Significant values shown in red.

Discussion

A previous study has shown that neurons migrate from the SVZ towards the olfactory bulb where they integrate as bipolar neurons, these have been shown to express secretagogin. The density of secretagogin-positive neurons was found to decrease in AD, however their neurochemical integrity was unchanged, suggesting that neurons may express neurogenic markers and show no AD pathology because they are newly integrated from neurogenesis.

Conclusion

In the hippocampus, new neurons migrate from the SGL to the GL. We have found that there is no significant difference between secretagogin staining in these areas, suggesting that it is not a neurogenic protein. As the pathology first develops, neurogenesis is upregulated to compensate for the cell loss caused by AD, however as the pathology worsens the cells are unable to cope resulting in dramatic cell loss and AD symptoms.

References
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