

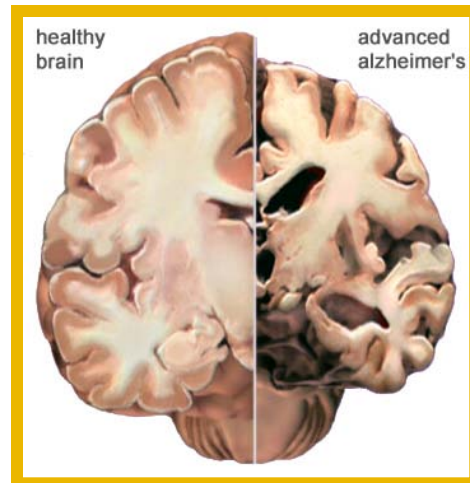
The Role of APP Intracellular Domain (AICD) in Alzheimer's Disease

Adaeze Okafor, Sanjay Pimplikar, Ph. D., Kaushik Ghosal, Ph. D.
Cleveland Clinic



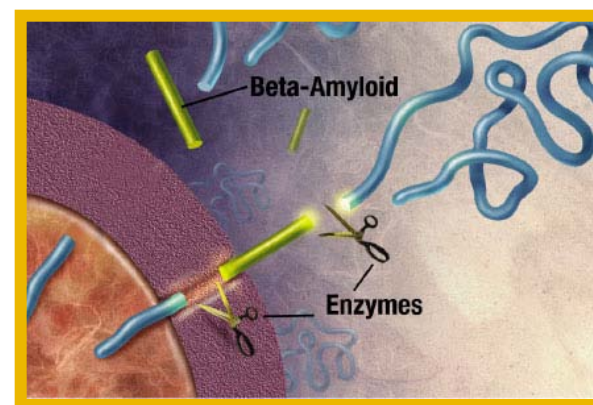
Introduction

Alzheimer's disease (AD) is a fatal degenerative disease of nerve cells in the cerebral cortex that leads to diminution in the size of the brain and senile dementia. As the disease progresses, a variety of symptoms may arise, including memory loss, confusion, anxiety, restlessness, and irritability, as well as disorientation, and impaired judgment.



In AD brains, the cortex shrivels up and damages areas involved with thinking, planning, and remembering. The hippocampus, an area that plays a key role in the formation of memories shrinks to a severe extent. The cause of AD is unknown. There is also no known current cure. □

AD is characterized by malformed nerve cells called tangles and the abnormal accumulation of plaques. The plaques result from the release and accumulation of excessive amounts of Aβ proteins. The tangles destroy a vital cell transport system made of proteins. Nutrients and other essential supplies can no longer move through the cells, which eventually die.

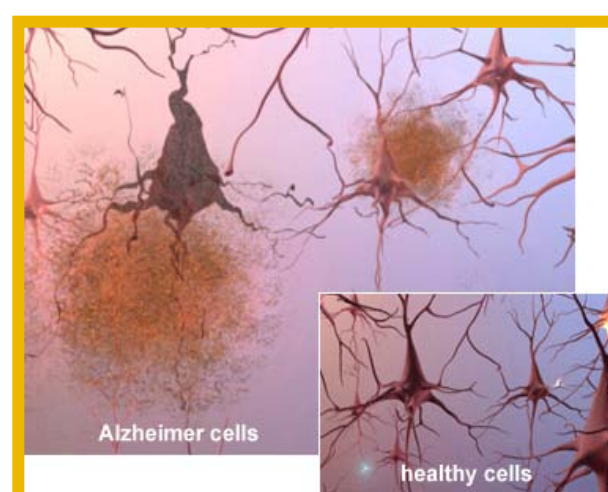


The amyloid precursor protein (APP) molecule is a transmembrane protein, with portions existing both inside and outside the cell. Aβ is clipped from the APP molecule and remains outside the cell. The fragment that remains

inside the cell's cytoplasm after APP is cleaved is called APP Intracellular Domain (AICD). AICD appears to affect cell functions in various ways and may contribute to AD. □

Hypothesis

It is widely believed that AICD plays an unappreciated role in AD pathology. Specifically, there is speculation that AICD contributes to Alzheimer's disease by enhancing both neuroinflammation and the hyper-phosphorylation of tau proteins.



Methodology

Mice:

- Transgenic (Tg) mice were generated to express AICD in the hippocampal regions of the brain; some were fed a special NSAID diet, an anti-inflammatory drug that may reduce the risk of AD
- Wild type (WT) mice were also participants in the experiment as the control group

Tissue Preparation and Immunostaining:

Hemi brains were placed in PBS containing 4% paraformaldehyde overnight, fixed in 30% sucrose and embedded in OCT. 30µm sections were sliced and sunk in cryostorage solution.

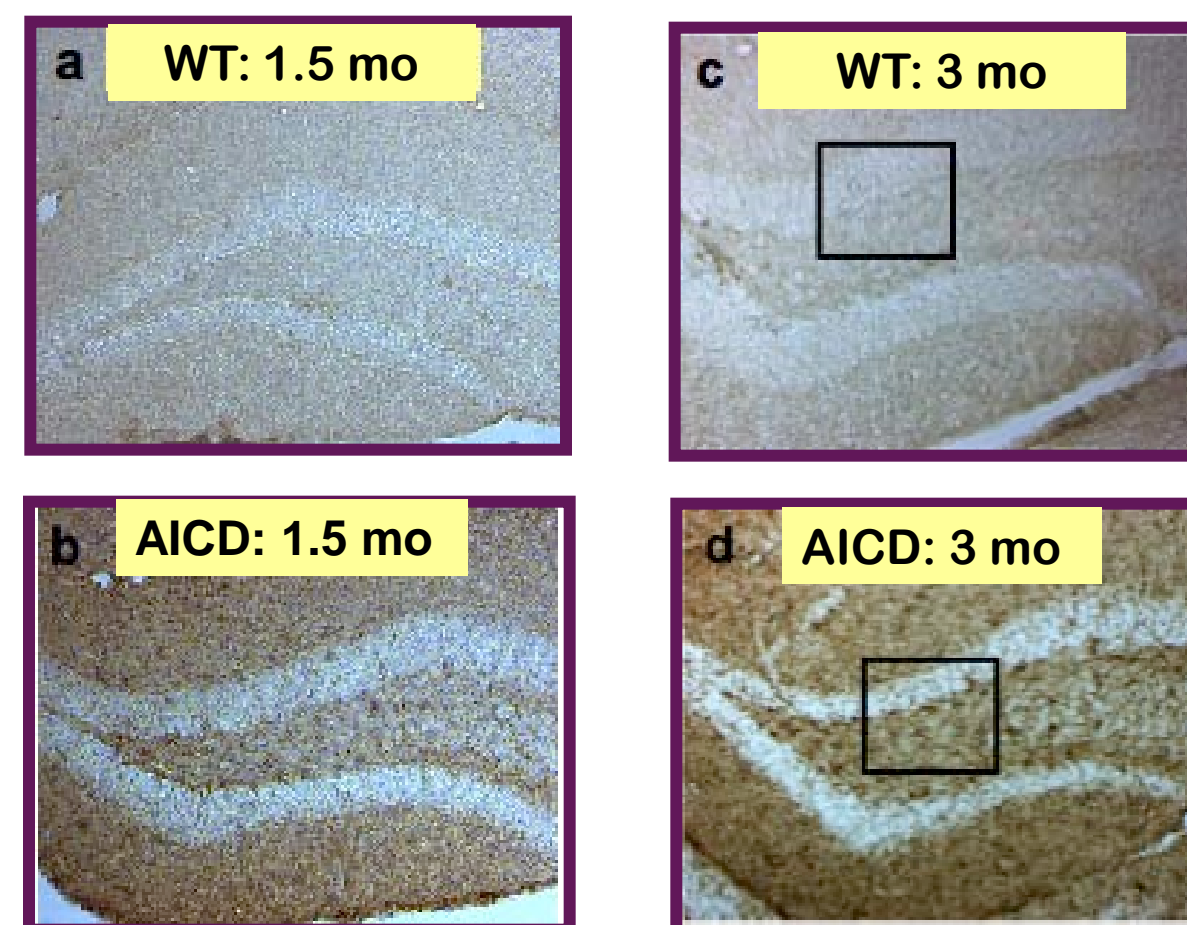
Sections were washed in PBS and incubated in 3% hydrogen peroxide for 30 minutes. After washing, sections were incubated in blocking solution for 1 hour at room temperature. Following blocking, sections were incubated with primary antibodies (AT8 or AT180) overnight at 4°C.

On Day 2, sections were incubated in secondary antibodies for 2 hours at room temperature. Sections were then mounted onto slides with glycerol. Microscopy was performed using a Leica DMR microscope.

Several outcomes were monitored: AICD's effect on neuroinflammation and tau phosphorylation, and how an NSAID diet influences the risk of developing AD.

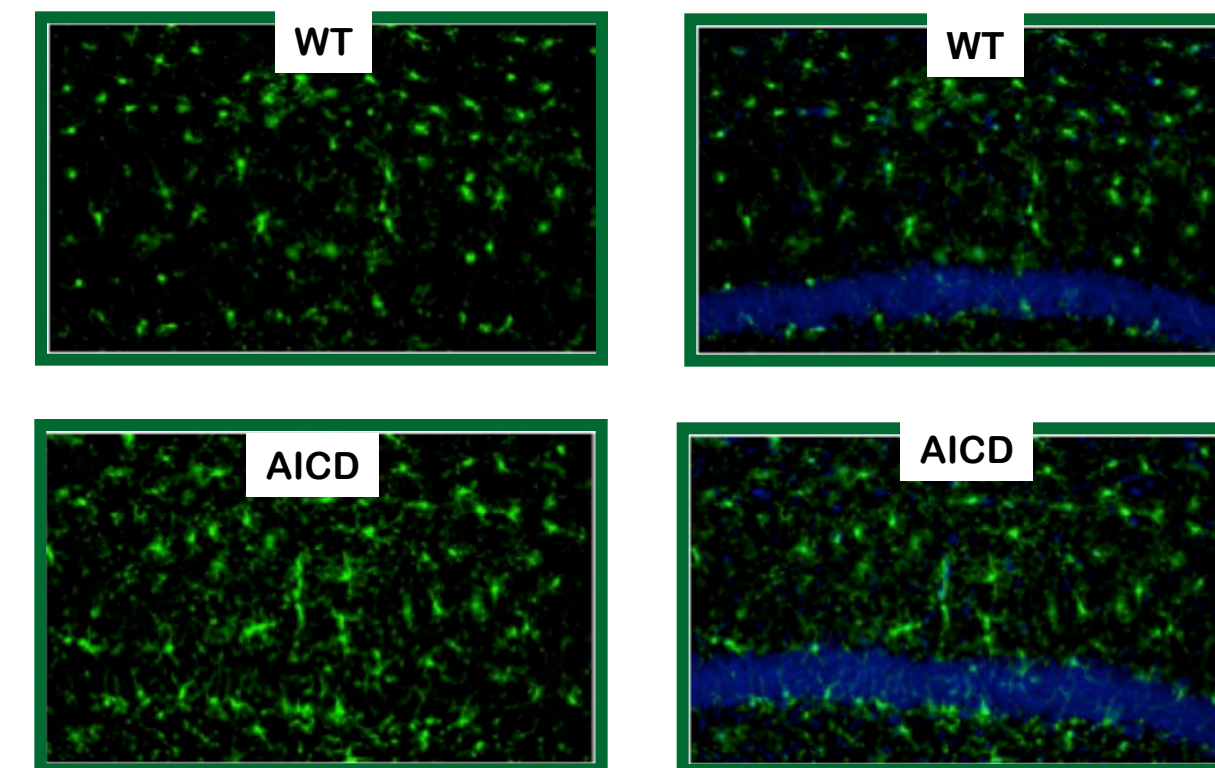
Data

Fig. 1 - Increased Inflammation in AICD mice brains



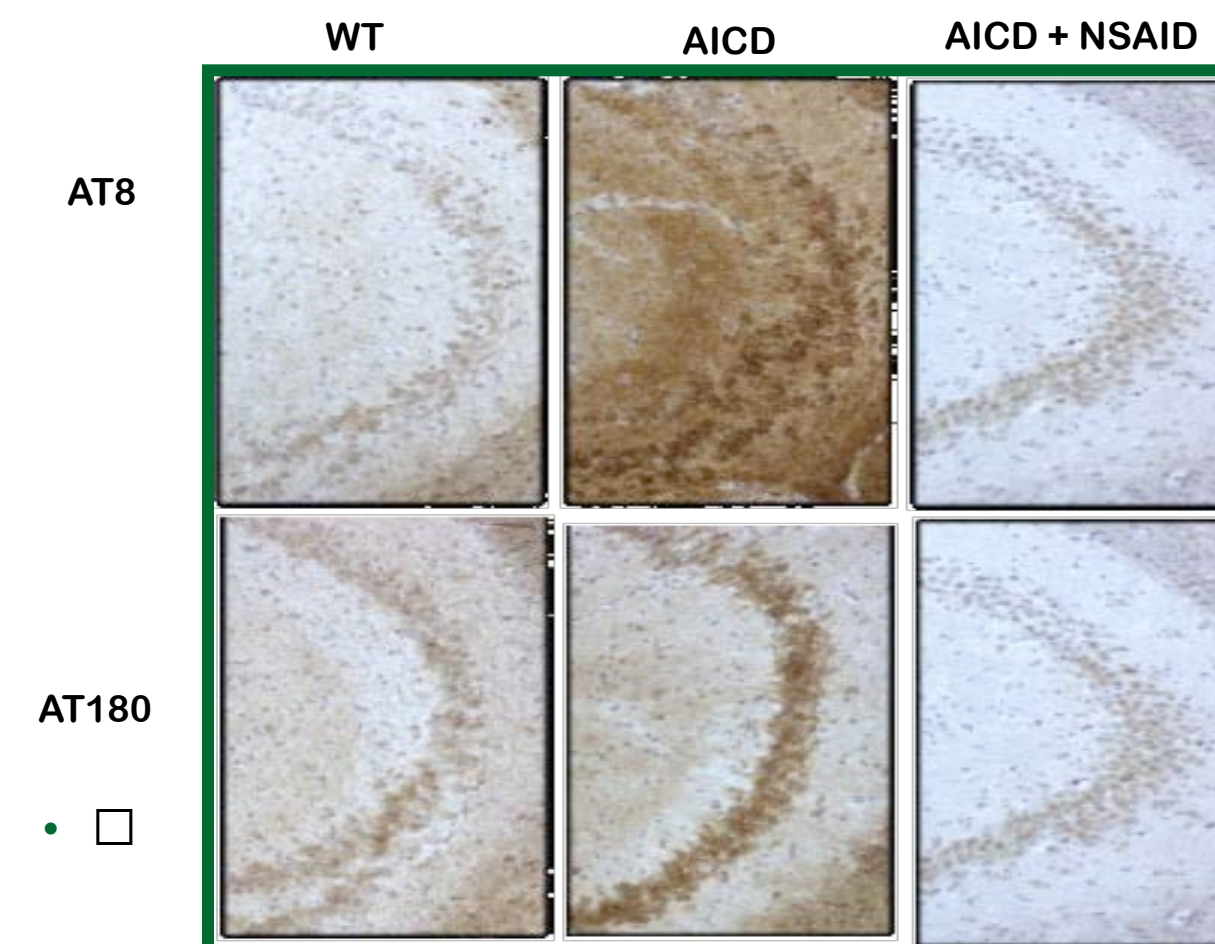
CD-45: Inflammation marker

Fig. 2 - Increased Inflammation in AICD mice brains



Iba1: Microglia and Inflammation marker

Fig. 3 - Increased Phospho Tau reversed by NSAIDs



Results

Fig.1: There was an increase in neuroinflammation in AICD mice brains when stained with a CD-45 antibody from 1.5 months to 3 months, supporting the hypothesis.

Fig. 2: The areas where inflammation occurred were marked with a blue inflammation marker. Undoubtedly inflammation was present in both the wild type and AICD transgenic mice

Fig. 3: The AICD mice were fed an NSAID (anti-inflammatory drug) diet help curb neuroinflammation. The image with AICD and NSAID depicts the idea that neuroinflammation decreases with the presence of NSAIDs

Neuroinflammation and tau phosphorylation were both enhanced by AICD in the brains of AD-like mice, supporting the hypothesis. In addition, feeding the mice an NSAID diet did in fact reduce inflammation in the brain.

Conclusions

Although there is a lot of evidence suggesting that Aβ plays a central role in Alzheimer's disease pathology, there is increasing affirmation that other factors contribute to AD pathology. Up until recently, the pathophysiological effects of AICD have not been examined thoroughly. One important finding from this study is that AICD transgenic mice display pathological features found in AD. The AICD transgenic mice showed an increase in phosphorylation of tau and the presence of neuroinflammation. In addition, the utilization of NSAIDs as an anti-inflammatory drug proved successful. These neurodegenerative changes have been detected in both human and mice brains with AD. Hence these findings are important because they suggest that in addition to Aβ, AICD causes pathological features of AD. In sum, it appears as though AICD has a significant effect on neuroinflammation and the hyper-phosphorylation of tau proteins.

Recommendations

- Obtain a larger sample size for more accurate data
- Test the hypothesis on a human population
- Look at a molecular mechanism

References

- <http://www.alz.org/index.asp>
- Gau, Y. & Pimplikar, S.W. The gamma-secretase-cleaved C-terminal fragment of amyloid precursor protein mediates signaling to the nucleus. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 14979-14984 (2001).
- Hardy, J., Duff, K., Hardy, K.G., Perez-Tur, J. & Hutton, M. Genetic dissection of Alzheimer's disease and related dementias: amyloid and its relationship to tau
- Price, D.L., Tanzi, R.E. Borchelt, D.R. & Sisodia, S.S. Alzheimer's disease: genetic studies and transgenic models. *Annual review of genetics* **32**, 461-493 (1998)
- Tesco, G., et al. APP substitutions V715F and L720P alter PS1 conformation and differentially affect Aβ and AICD generation. *J Neurochemistry* **95**, 446-456 (2005).