Age-dependent study of pathological progression of Alzheimer's disease in hippocampal and cortical tissue of human and an AD mouse model

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ABSTRACT

Degeneration of synaptic plasticity plays a critical role in Alzheimer's disease (AD) pathogenesis. Recent studies have suggested that neuroinflammation may contribute to this degeneration by disrupting the amyloid metabolism and by microglial overgrowth. This study aims to investigate the pathological progression of AD hippocampal and cortical tissues of human and an AD mouse model. To examine the amyloid and microglial activity in the human brain, we acquired brain tissues from clinically diagnosed AD and non-dementia (ND) patients. To examine this activity in the mouse model, we acquired brain tissues from age-matched wild type (WT) and AD affected (3xTg-AD) mice in 4, 6, 8 and 12 month age groups. Immunohistochemical (IHC) analysis was utilized to determine the characteristics of pathological AD hallmarks. Analysis of the human brain tissue showed an alteration in amyloid precursor protein (APP) in the hippocampus of AD patients as compared to the ND patients. The activation of microglial cells in the AD patients was increased, which indicates neuroinflammation. Results of mouse brain tissue analysis indicated a heightened proliferation of microglial cells in the CA3 region (*p<0.05) of the hippocampus in the 6 month old 3xTg-AD male mice. Additionally, results indicated hyper-proliferation of microglial cells in multiple regions of the hippocampus in 8 month old 3xTg-AD female mice, (*p<0.05). Moreover, increased presence of amyloid burdened neurons was observed in both the cortical and amygdala regions of 4, 6, 8, and 12 month age groups of 3xTg-AD mice as compared to their age-matched WT. Phosphorylated tau protein (pTau) was additionally found to be increased in the 3xTg-AD male mice as compared to their 3xTg-AD female counterparts in multiple age groups. Neuroinflammation and aberrant activity of microglial proliferation contribute to the progression of AD. The ability to understand, and therefore modulate, neuroinflammation may be a promising approach for prevention of progression in AD.

INTRODUCTION

Alzheimer's Disease (AD)

[2, 10]**Impact:** Approximately 1 in 10 people over 65 suffer from AD, in 2019 is expected to cost the US $290 billion, AD is the 6th leading cause of death in the US, causes progressive atrophy of the hippocampus resulting in detriment to integral human functions like memory and learning
[2,10]**History:** Identified in 1906 by Dr. Alois Alzheimer after examining brain Tissue of a patient who had died with an unknown mental illness
Neuroinflammation: plaques and neuronal death activate microglial cells which release cytokines, can lead to neuroinflammation
[3]**Amyloid-beta (Aβ) plaques:** form from APP, sequential cleavage by β-secretase and γ-secretase enzymes can play a role in the misfolding of Aβ forming clumps of oligomers, these can aggregate into insoluble plaques, weakens synaptic plasticity and communication ability
Neurofibrillary tangles: form from aggregates of hyperphosphorylated tau protein

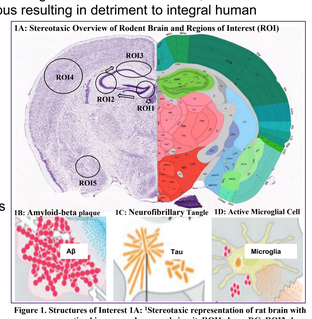


Figure 1. Structures of Interest 1A: [5]Stereotaxic representation of rat brain with arrows representing hippocampal neuronal circuit, ROI1 shows DG, ROI2 shows CA3, ROI3 shows CA1, ROI4 shows cortex, ROI5 shows amygdala 1B: [5]Representation of amyloid-beta plaque 1C: [5]Representation of tau neurofibrillary tangle 1D: [5]Representation of active microglial cell

Hippocampal Complex and Amygdala

Limbic system: brain area involved in emotional and behavior responses, particularly as it relates to survival, significant structures include hippocampus and amygdala
Hippocampus: significant structure in the limbic system, involved in long-term memory, memory consolidation, encoding, retrieving, information relating to time and space, interconnectivity allows for association of memories with various senses and modalities, made of subregions, significantly impacted in AD through atrophy of the structure, leads to significant and early impairment of aforementioned processes

- [7, 11]**Dentate gyrus (DG):** significant subregion of the hippocampus proper, one of the few sites in adult brains capable of neurogenesis, consolidation of multiple sensory inputs, interconnectivity allows for association between stimuli, thought to distinguish incoming stimuli from previous experiences and tag it as novel
- [7]**CA3:** significant subregion of the hippocampus proper, interconnectivity allows for association between stimuli, thought to be involved in pattern completion, ability of whole memory retrieval through matching of incoming stimuli to familiar experience, even when only a part of originally encoded stimuli is presented, as suggested by intrinsic network of recurrent collaterals
- [7]**CA1:** significant subregion of the hippocampus proper, end-point processing of hippocampus proper, thought to be a mismatch detector through simultaneous receipt of information about current events and previous experiences, may play a role in temporal context

Amygdala: significant structure in the limbic system, involved in emotional responses and emotional memories
Cortex: brain area generally responsible for higher order processing such as planning and decision making

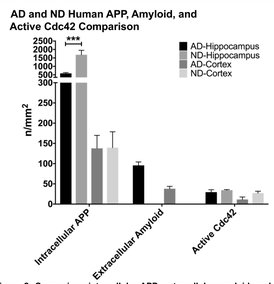
Key Targets:

[4]**Microglial cells:** type of glial cell, primary form of immune system defense in the central nervous system (CNS), phagocytose infectious agents, damaged neurons, and foreign substances, clean out and prune synapses, cytokines, which have a role in inflammatory response, are indicative of higher activity level
Tau protein: involved in stabilizing microtubules
[3]**Amyloid precursor protein (APP):** generally thought to relate to protecting body from infections, maintenance of the blood brain barrier, promoting injury recovery, and regulation of synaptic function

Project Overview

Significance: Adding to the understanding of the relative time-dependence of AD progression, this study allows for more targeted investigations as to the underlying mechanisms behind AD and potential interventions in the future.
Goal: The goal of this study is to investigate the pathological progression of Alzheimer's disease (AD) in hippocampal and cortical tissues in human and triple-transgenic (3xTg-AD) mouse model.
Purpose: By showing the progression of AD hallmarks in varying ages of 3xTG mice, this study will investigate the potential timeline of pathological progression in this model. This allows this investigation to serve as a benchmark to further evaluate ongoing RhoGTPase studies. Additionally, it potentially illuminates an optimal timeframe for therapeutic target of various pathologies of AD in the 3xTg-AD model.
Process: 3xTg-AD mice have an age-matched control, mice were sacrificed at 4 months, 6 months, 8 months, and 12 months brain tissue was collected, stained with IHC, and quantified, added stains and quantification of AD and non-AD human tissue

RESULTS



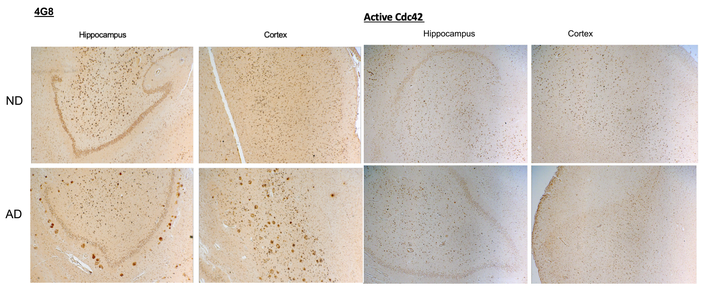


Figure 2. Comparison intracellular APP, extracellular amyloid, and active cdc42 in AD and ND patients, ND patient showed significantly higher (***p<0.0001) presence of intracellular APP in the hippocampus that AD patient, indicative or a healthily functioning protein, extracellular amyloid only presented in AD patient, results significant in the hippocampus, one of the first areas impacted by AD, active cdc42 is indicative of RhoGTPase activity in related pathways, value represents mean ± SEM, ***p<0.0001

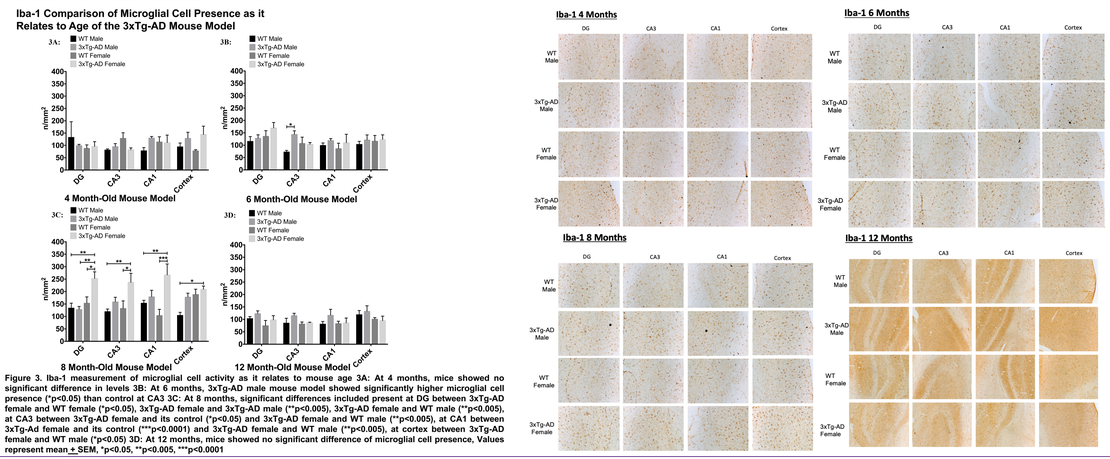


Figure 3. Iba-1 measurement of microglial cell activity as it relates to mouse age 3A: At 4 months, mice showed no significant difference in levels 3B: At 6 months, 3xTg-AD male mouse model showed significantly higher microglial cell presence (*p<0.05) than control at CA3 3C: At 8 months, significant differences included present at DG between 3xTg-AD female and WT female (*p<0.05), 3xTg-AD female and 3xTg-AD male (**p<0.005), 3xTg-AD female and WT male (**p<0.005), at CA3 between 3xTg-AD female and its control (*p<0.05) and 3xTg-AD female and WT male (**p<0.005), at CA1 between 3xTg-Ad female and its control (***p<0.0001) and 3xTg-AD female and WT male (**p<0.005), at cortex between 3xTg-AD female and WT male (*p<0.05) 3D: At 12 months, mice showed no significant difference of microglial cell presence, Values represent mean ± SEM, *p<0.05, **p<0.005, ***p<0.0001

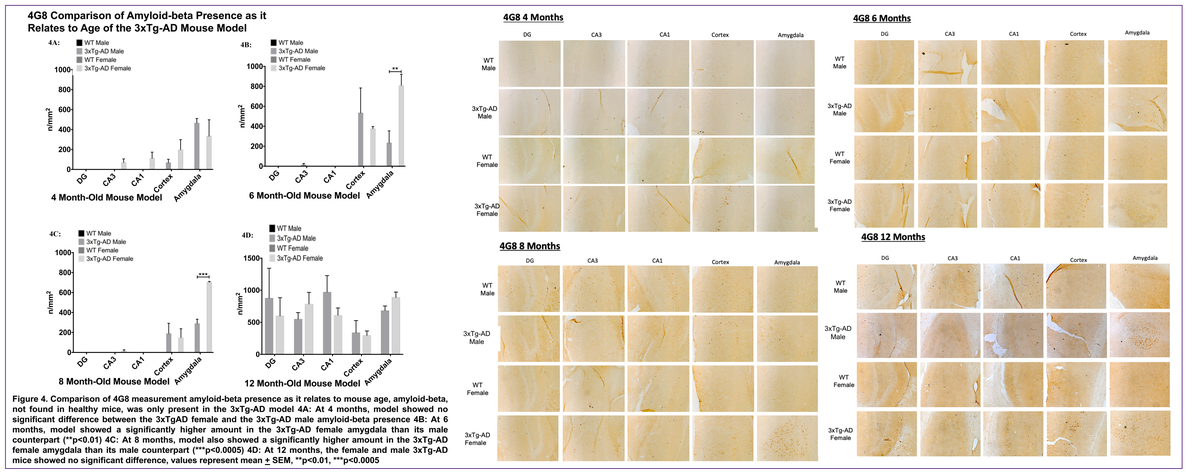


Figure 4. Comparison of 4G8 measurement amyloid-beta presence as it relates to mouse age, amyloid-beta, not found in healthy mice, was only present in the 3xTg-AD model 4A: At 4 months, model showed no significant difference between the 3xTgAD female and the 3xTg-AD male amyloid-beta presence 4B: At 6 months, model showed a significantly higher amount in the 3xTg-AD female amygdala than its male counterpart (**p<0.01) 4C: At 8 months, model also showed a significantly higher amount in the 3xTg-AD female amygdala than its male counterpart (***p<0.0005) 4D: At 12 months, the female and male 3xTg-AD mice showed no significant difference, values represent mean ± SEM, **p<0.01, ***p<0.0005

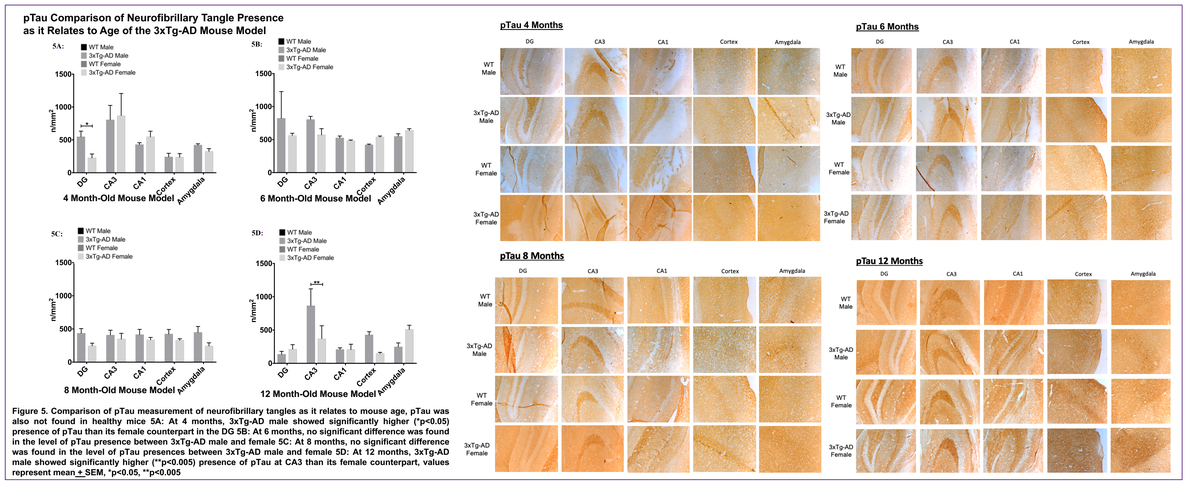
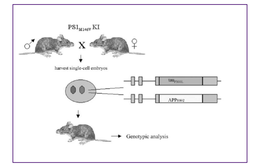


Figure 5. Comparison of pTau measurement of neurofibrillary tangles as it relates to mouse age, pTau was also not found in healthy mice 5A: At 4 months, 3xTg-AD male showed significantly higher (*p<0.05) presence of pTau than its female counterpart in the DG 5B: At 6 months, no significant difference was found in the level of pTau presence between 3xTg-AD male and female 5C: At 8 months, no significant difference was found in the level of pTau presences between 3xTg-AD male and female 5D: At 12 months, 3xTg-AD male showed significantly higher (**p<0.005) presence of pTau at CA3 than its female counterpart, values represent mean ± SEM, *p<0.05, **p<0.005

MATERIALS & METHODS

[12]3xTg-AD Mouse Model:

The 3xTg-AD mouse model contains three genetic mutations that contribute to the development of AD: $PS1_{M146V}$, APP_{swe}, and tau_{P301L}. As compared to other AD mouse models, 3xTg-AD offers the benefit of combining mutations for both Aβ, $PS1_{M146V}$ and APP_{swe}, as well as tau, tau_{P301L}. This allows for investigation as it relates to potential interactions between portions of the pathology. This model was developed by obtaining a $PS1_{M146V}$ line and microinjecting the remaining two genes of interest into embryos (Figure 6). The group confirmed the effectiveness of the model by documenting sequential pathology progression as follows: loss of synaptic density and synapse number, Aβ deposits, and neurofibrillary tangles, respectively.



[12]Figure 6. Development of the 3xTg-AD Mouse Model, original line and embryonic gene injection to process to develop the 3xTg-AD mouse model

Study Groups:

Utilizing the 3xTg-AD model as the experimental group, we obtained hippocampal tissue from both one male and one female of each of the following age groups: 4 months, 6 months, 8 months, and 12 months. Each mouse of the experimental group had an age and gender matched WT counterpart. These age groups were chosen because of their comparability to the early to late stage progression of AD presentation in humans, relative to the average lifespan of a mouse model. For human tissue analysis we utilized donated tissue from individuals that were of similar age to each other, one AD patient and one ND patient

IHC Staining and Quantification:

Brain tissue of the aforementioned study groups was sectioned. These tissues were then stained via IHC with use of a sequential chamber or pep pen for hallmarks of interest. Mouse on mouse (M.O.M.) kit was used for staining of the 4G8 mouse antibody. A rabbit kit was used for the IBA-1 and pTau antibodies. All antibodies were at 1:500. Images were taken of the areas of interest, DG, CA3, CA1, cortex, and amygdala, and quantified using an average value of presentation of each hallmark from three randomly placed boxes in each respective area.

Hallmarks of Interest:

- **Aβ :** visualized with 4G8 antibody measurements
- **Neurofibrillary tangles:** visualized with phosphorylated tau (pTau) antibody measurements
- **Neuroinflammation:** microglial cells visualized with IBA-1 antibody measurements

CONCLUSION

- Neuroinflammation, as shown through microglial cell presence, was indicated at 6 months and further indicated at 8 months. Interestingly, there was no significant difference in microglial cell levels in the 12 month age group. This could potentially indicate an eventual breakdown of the body's attempted immune response and desensitization as AD pathology progresses.
- A higher prevalence of microglial activity and Aβ in the results of many of the 3xTg-AD female mice, as compared to the 3xTg-AD male mice of the same age group could be indicative of a parallel between the gender differences in the mouse model as compared to gender differences seen in clinical patients, as females show higher rate of presentation of AD as compared to their male counterparts. It could be of interest to investigate to apparent reverse response in the pTau presented of male 3xTg-AD mice. Further investigation of pathology as it relates to gender differences would be of additional interest.
- Overall, this investigation sheds light on trends in presentation and activity of AD pathological hallmarks as the disease progression, potentially increasing the effectiveness of studies of mechanisms of disease progression and potential intervention.

DISCUSSION

Analysis of tissue from human patients found significantly higher intracellular APP in the ND patient hippocampus as compared to the AD patient hippocampus. Intracellular APP has not been incorrectly cleaved and is indicative of a healthily functioning protein. Thus, these findings were in line with expectations. Significant difference in the hippocampus of the two patients, and not the cortex, is in line with memory-related symptoms that present in AD patients. Extracellular APP, protein that has been cleaved and is susceptible to forming Aβ plaques, is symptomatic of AD pathology and, as expected, was found only in the AD patient.

Quantification of microglial cells showed significantly higher presentation in varying groups of the 3xTg-AD model after 6 months of development. Additionally, there was significantly higher presence in some of the 3xTg-AD female models than their male counterparts. Aβ is not present healthy WT mouse model. It did present at a significantly higher rate in the amygdala of the 6 month 3xTg-AD females and 8 month 3xTg-AD females as compared to their 3xTg-AD male counterparts of similar age. Likely neurofibrillary tangles, as indicated by the present of pTau, were also not expected to present in the WT mice. These were found at a significantly higher rate in the 3xTg-AD male as compared to the 3xTg-AD female of similar age as early as 4 and as late as 12 months of age.

Interesting observations include the increased pathology present in the amygdala of the 3xTg-AD affected mice. Upon this observation, amygdala pictures and quantifications were added to the investigation. Future studies could benefit by adding to the data from the amygdala in the areas assess before this observation was made. 3xTg-AD mice show increased aggression and trends in agitated behavior, which could be a likely result from this damage in the amygdala. In addition to quantification, there were interesting observations in the difference of morphology, size, and distribution in presentation of the hallmarks of interest in respective groups.

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