Gut microbiota analysis of an Alzheimer's Disease transgenic rat model

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Background

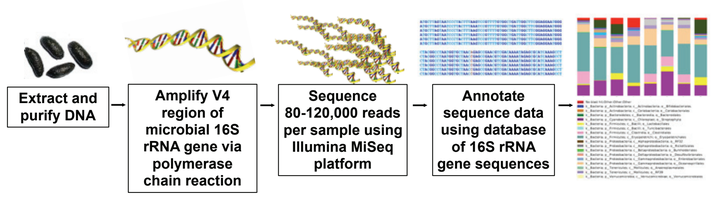
- Alzheimer's Disease (AD) is the leading cause of dementia in the growing elderly population.[1]
- A new transgenic rat model, F344-Tg(Prp-APP,Prp-PS1)19/Rrrc (TgF344-AD), carries two mutations, amyloid beta (A4) precursor protein (*APP*) and human Presenilin 1 (*PS1*), both associated with familial AD.[2]
- The TgF344-AD rat strain has been previously shown to have a phenotype more representative of human disease than existing rodent models.[2]
- There is growing evidence for the role and interaction of the gut microbiota in neurodegenerative disease progression.[3]

Hypothesis

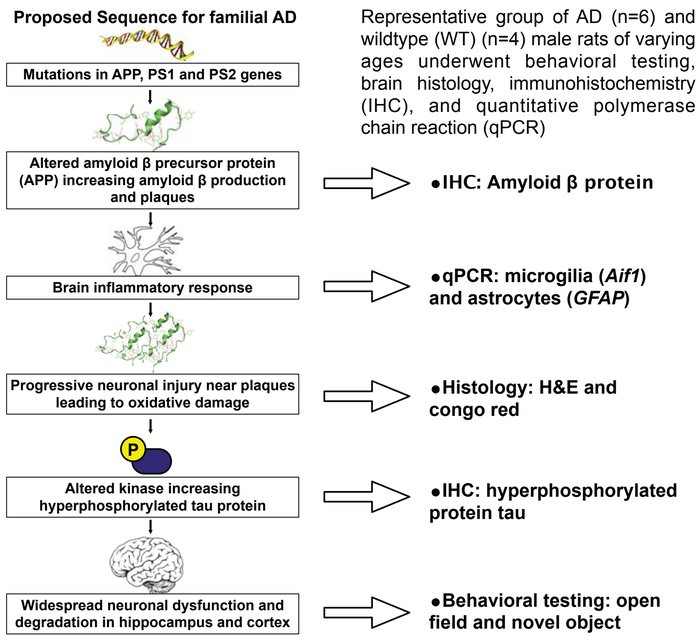
Neuropathological and behavioral changes in TgF344-AD rats will have accompanying microbiota alterations with increasing disease severity and age.

Methods

Microbiota Analysis



Characterization of AD Phenotype



Representative group of AD (n=6) and wildtype (WT) (n=4) male rats of varying ages underwent behavioral testing, brain histology, immunohistochemistry (IHC), and quantitative polymerase chain reaction (qPCR)

- **IHC: Amyloid β protein**
- **qPCR: microglia (*Aif1*) and astrocytes (*GFAP*)**
- **Histology: H&E and congo red**
- **IHC: hyperphosphorylated protein tau**
- **Behavioral testing: open field and novel object**

Figure 1. Modified from Selkoe (2001).

Microbiota Analysis

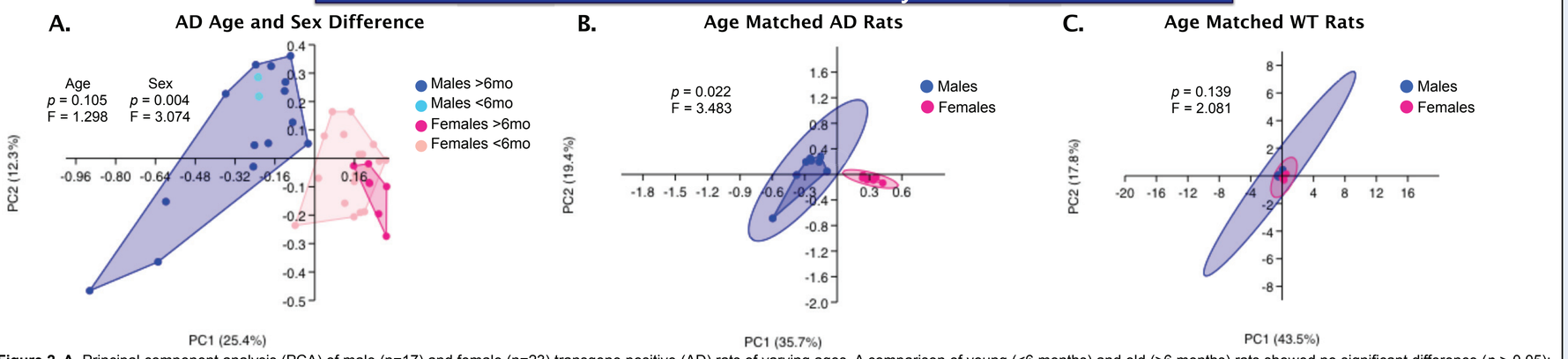


Figure 2. A. Principal component analysis (PCA) of male (n=17) and female (n=23) transgene positive (AD) rats of varying ages. A comparison of young (<6 months) and old (>6 months) rats showed no significant difference ($p > 0.05$); however, there was a significant difference between the sexes ($p < 0.05$). **B.** PCA for age matched AD rats showing a significant difference ($p < 0.05$) between males (n=8) and females (n=7). Outer circles around each group represent 95% confidence intervals. Bacteria contributing to the greatest disparity between the sexes are *Bacteroides, Bifidobacterium, Desulfovibrio, Granulicatella, Ruminococcus, Proteus,* and *Turicibacter*. **C**. PCA for age matched WT rats (n=7) of the same background Fisher 344 strain as F344-AD. There is no significant difference ($p > 0.05$) between males (n=3) and females (n=4). Outer circles around each group represent 95% confidence intervals.

Alzheimer's Disease Phenotype

Behavioral Testing

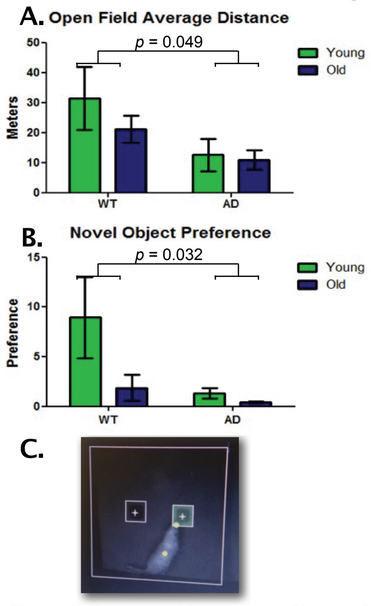


Figure 3. 7 months WT (young, n=2) and 13 months WT (old, n=2) rats compared to 6 and 8 months AD (young, n=3) and 12 months AD (old, n=3) rats. **A.** Open field average distance over 30 minutes measuring activity level. Significant ($p < 0.05$) between genotypes. **B.** Preference for novel object assessing learning and memory. Significant ($p < 0.05$) between genotypes. **C.** Novel object set up tracking rat head 2 cm from object.

Immunohistochemistry and Histology

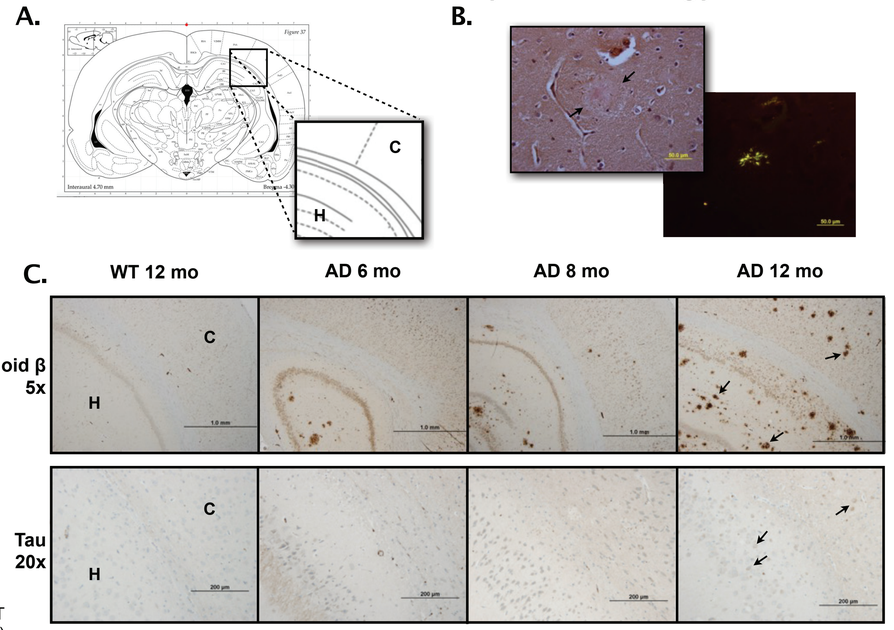


Figure 4. A. Rat Brain Atlas[5] at bregma -4.30 for hippocampus (H) and cortex (C). **B.** Congo red stain showing an amyloid β plaque (between arrows) at 40x standard (left) and polarized (right) light microscopy. **C.** Immunohistochemistry for amyloid β protein and hyperphosphorylated tau protein in WT rats at 12 months and AD rats at 6, 8, and 12 months. There is progressing accumulation of both amyloid β plaques around sites of neuronal degeneration and neuronal intracellular tau protein with increasing age in AD rats. Arrows indicate IHC staining for either amyloid β or tau protein.

qPCR

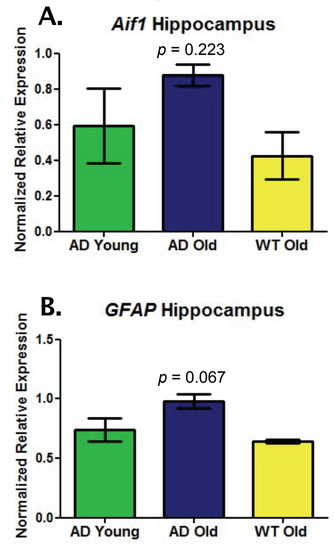


Figure 5. Average microglia (*Aif1*) **(A)** and astrocyte (*GFAP*) **(B)** gene expression in the hippocampus of 6 and 8 months AD (young, n=3),12 months AD (old, n=3), and 13 months WT (old, n=2) rats. Although there is a trend of increased expression in the AD rats, the results were not statistically significant ($p > 0.05$). This trend also occurs in the cortex of AD rats (data not shown).

Summary and Future Directions

- Although there was no difference in the microbiota among age groups, a significant difference was seen between male and female TgF344-AD rats of all ages.
- The AD phenotype was consistent with previous studies. We noted a unexpected decreased activity level in the AD rats in the open field behavioral test.[2]
- Future studies with comparisons to unaffected litter mates are needed to confirm the microbiota results.

References and Acknowledgements

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