Sex-specific Microglial Responses To Amyloid-í Pathology In The Murine Brain

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Abstract

Background: Alzheimer's disease (AD) is a common neurodegenerative disorder that exhibits pronounced sex differences in prevalence, pathology, and clinical outcomes. Females comprise approximately two-thirds of the AD population and often display greater amyloid-β (Aβ) plaque and tau tangle burden, whereas males experience more rapid mortality following diagnosis. The biological mechanisms underlying these disparities remain poorly understood. Microglia, the brain's resident immune cells, play a central role in maintaining homeostasis and responding to pathology. Our prior work in the murine hippocampus demonstrates sex-specific aging trajectories in microglial reactivity. We show that females exhibit heightened inflammatory and senescent signatures, and an increased propensity to adopt the disease-associated microglia (DAM) phenotype. DAMs, which are closely linked to Aβ pathology, transition from a primed state (Stage 1) to a fully reactive state (Stage 2). We therefore hypothesized that microglial responses to Aβ pathology differ by sex and contribute to sex-biased outcomes in AD. **Methods**: We used the AppSAA amyloid mouse model of AD to examine sex differences in Aβ burden, microglial activation, and cognitive-related behaviors. Behavioral assessments were followed by analyses of brain tissue from AppSAA and wildtype mice at 10–14 mo using flow cytometry, microscopy, and single-cell transcriptomics. **Results**: Female AppSAA mice exhibited greater hyperactivity during behavioral testing, enhanced DAM activation, and a trend toward greater microglial Aβ phagocytosis compared to males. Single-cell transcriptomic analyses revealed an overall expansion of DAM populations in AppSAA mice, with females displaying an increased proportion of Stage 2 DAMs. Pathway analyses identified female-biased enrichment of chemotaxis and senescence-related pathways, implicating key regulatory drivers of chemotaxis (VEGF) and senescence (SPP1). **Conclusions:** These findings demonstrate that microglial reactivity to Aβ pathology is sexually divergent and suggests that heightened DAM responses in females may contribute to sex-specific features of AD. Ongoing studies will investigate the functional roles of the identified molecular pathways and dissect the contributions of sex chromosomes and gonadal hormones to microglial responses in AD. Together, this work provides a foundation for the development of sex-informed therapeutic strategies for Alzheimer's disease.

Experimental Design

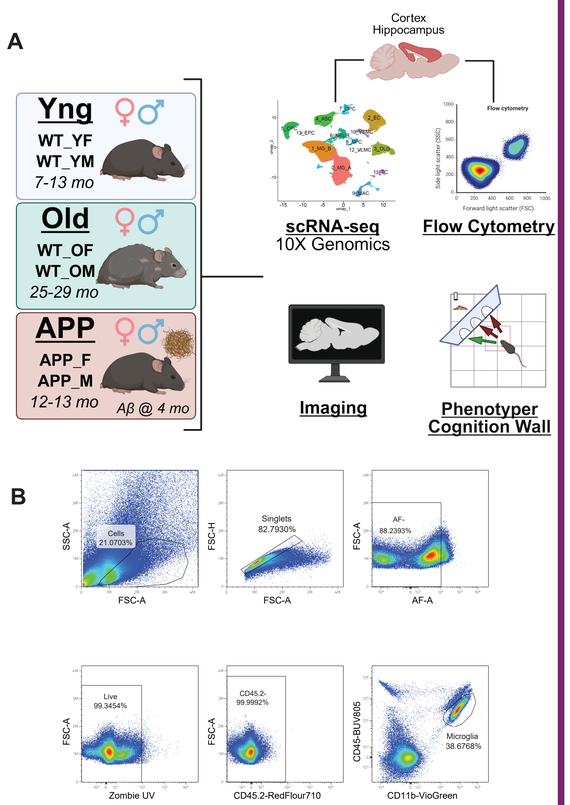


Figure 1. Experimental design to investigate sex differences in response to aging & amyloid pathology in the murine brain. A Schematic of experimental design to investigate sex differences in the APP^{SAA} model of Alzheimer's Disease. Single-cell RNA sequencing was performed on cortical and hippocampal tissue from male and female mice in wildtype (Yng [7-13 mo] & Old [24-29 mo]) and APP^{SAA} (APP [12-13 mo]). 15-17 mo wildtype and APP^{SAA} mice were intraperitoneally injected with Methoxy-X04 to label Aβ plaques. Cortices and hippocampi from were isolated for flow cytomtery analysis. Axioscan 7 imaging and Phenotyper cognition wall were also performed (DATA NOT SHOWN). **B** Gating strategy for flow cytometric analysis.

Results

Aβ pathology mirrors aging trajectories with amplified signatures

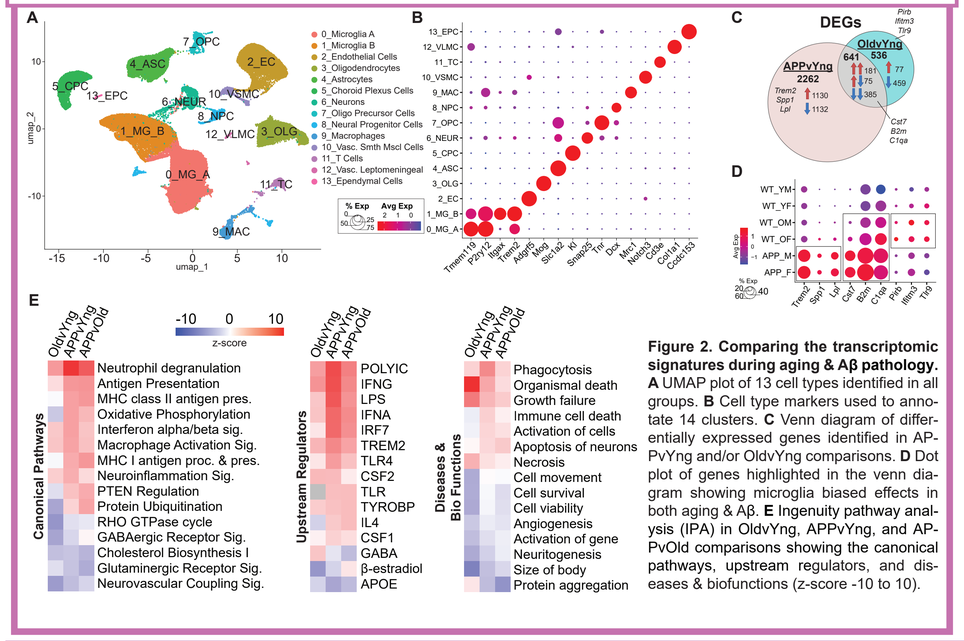


Figure 2. Comparing the transcriptomic signatures during aging & Aβ pathology. A UMAP plot of 13 cell types identified in all groups. **B** Cell type markers used to annotate 14 clusters. **C** Venn diagram of differentially expressed genes identified in APPvYng and/or OldvYng comparisons. **D** Dot plot of genes highlighted in the venn diagram showing microglia biased effects in both aging & Aβ. **E** Ingenuity pathway analysis (IPA) in OldvYng, APPvYng, and APPvOld comparisons showing the canonical pathways, upstream regulators, and diseases & biofunctions (z-score -10 to 10).

Cell-type specific responses to aging & Aβ

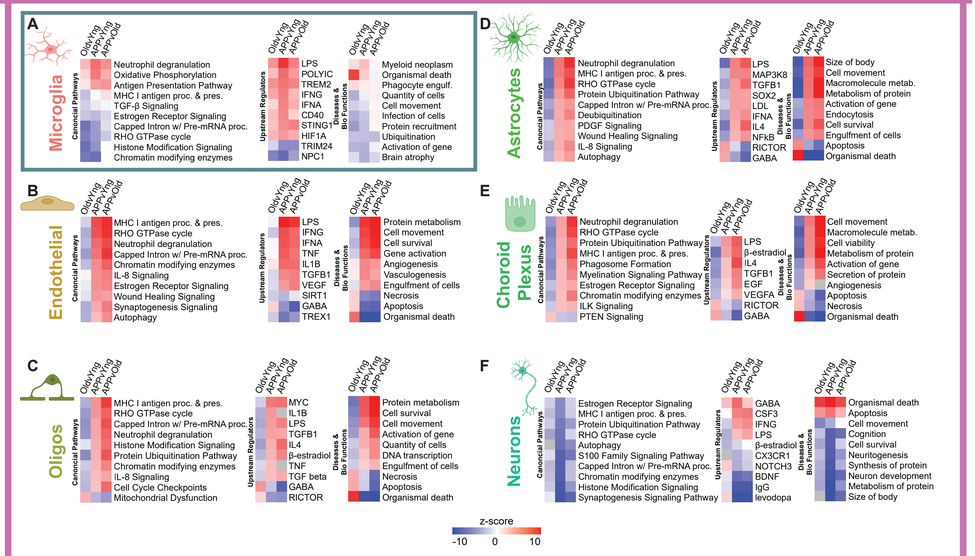


Figure 3. Cell-type specific analysis of transcriptomic responses to aging and Aβ pathology. Ingenuity pathway analysis (IPA) in OldvYng, APPvYng, and APPvOld comparisons showing the canonical pathways, upstream regulators, and diseases & biofunctions (z-score -10 to 10) for **A** Microglia, **B** Endothelial, **C** Oligodendrocytes, **D** Astrocytes, **E** Choroid Plexus, and **F** Neurons.

Microglia are the main drivers of sex-specifc responses to aging & Aβ

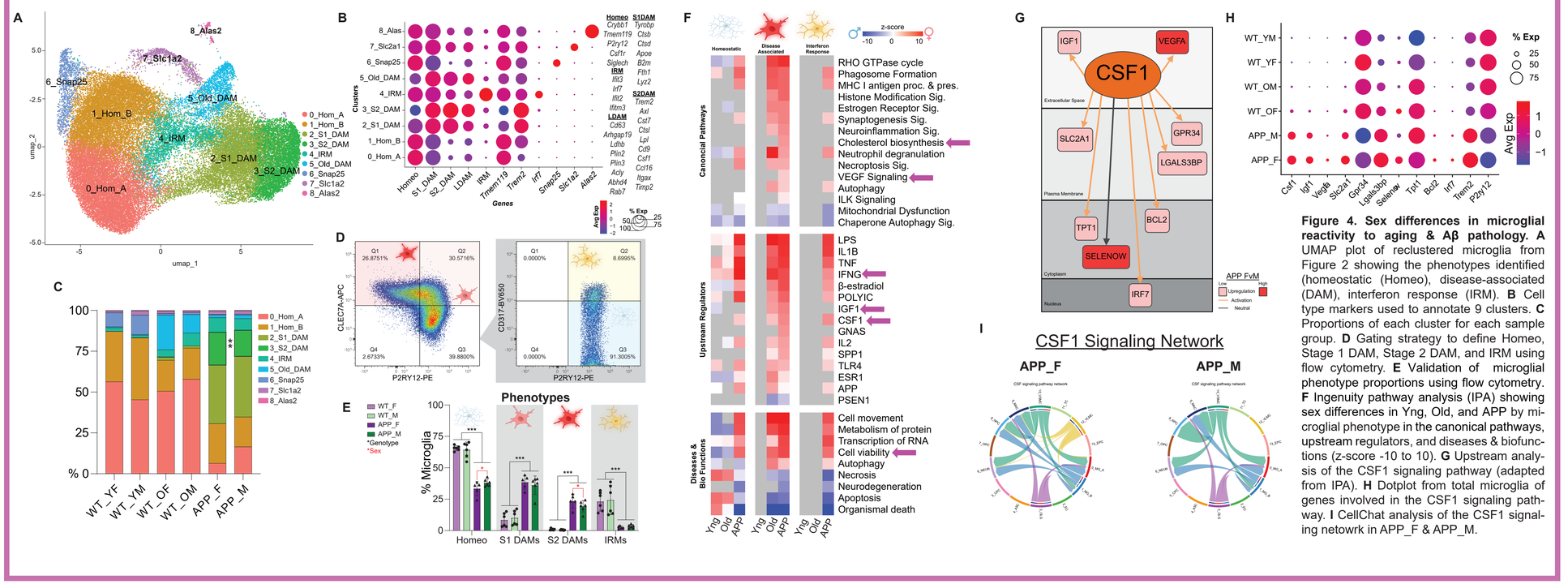
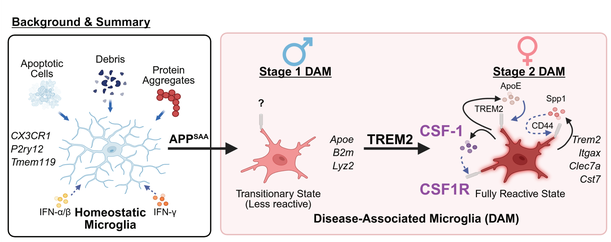


Figure 4. Sex differences in microglial reactivity to aging & Aβ pathology. A UMAP plot of reclustered microglia from Figure 2 showing the phenotypes identified (homeostatic (Homeo), disease-associated (DAM), interferon response (IRM). **B** Cell type markers used to annotate 9 clusters. **C** Proportions of each cluster for each sample group. **D** Gating strategy to define Homeo, Stage 1 DAM, Stage 2 DAM, and IRM using flow cytometry. **E** Validation of microglial phenotype proportions using flow cytometry. **F** Ingenuity pathway analysis (IPA) showing sex differences in Yng, Old, and APP by microglial phenotype in the canonical pathways, upstream regulators, and diseases & biofunctions (z-score -10 to 10). **G** Upstream analysis of the CSF1 signaling pathway (adapted from IPA). **H** Dotplot from total microglia of genes involved in the CSF1 signaling pathway. **I** CellChat analysis of the CSF1 signaling netowrk in APP_F & APP_M.

Conclusions & Future Directions



1. **The APP^{SAA} model recapitulates aging-associated transcriptomic signatures, supporting its relevance to late-onset AD.**
2. **Aβ pathology follows transcriptomic trajectories similar to normal aging, but with more robust and cell type-specific activation patterns.**
3. **Microglia are primary drivers of the sex differences observed during amyloid pathology:**
   - **A. Females exhibit greater microglial reactivity and inflammatory signaling**
   - **B. Females display a higher proportion of DAMs, particularly Stage 2 DAMs**
   - **C. Females show increased CSF1-associated signaling**
4. **These divergent neuroimmune responses may contribute to the sex biases observed in AD prevalence and pathology.**
5. **Future studies will investigate the interactive effects of sex chromosomes and sex hormones in regulating microglial reactivity and will further evaluate these signatures in human hippocampal and cortical datasets.**

Sex differences in microglial aging:
Ocañas et al. (2023)

Protocol for microglial phenotypes:
Cox et al. (2024)



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