OLFACTORY CLEFT BIOPSY ANALYSIS OF EARLY ALZHEIMER'S DISEASE PATHOBIOLOGY

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INTRODUCTION

Alzheimer's Disease (AD) is a neurodegenerative condition affecting millions worldwide. Defining early pathobiological events remains challenging, in part due to inaccessibility of neural tissue. Because olfactory neurons are accessible, and olfactory loss is prevalent in AD, we evaluated olfactory brush biopsies from controls, individuals with cerebrospinal fluid (CSF) biomarker-confirmed AD, and cognitively typical individuals whose positive CSF biomarkers signal a pre-clinical AD stage.

METHODS

Olfactory cleft brush biopsies were obtained from 22 living control (n = 6), pre-clinical AD (n = 9), and clinical AD (n=7) participants recruited through the Duke/UNC ADRC and Memory Disorders Clinic. AD status was defined using the 2024 Alzheimer's Association Criteria using cognitive status together with CSF Aβ42/Aβ40 biomarkers or amyloid PET. Brush biopsies were performed in the clinic in awake participants using a topical nasal anesthetic spray, then processed for single-cell RNA sequencing and flow cytometry.

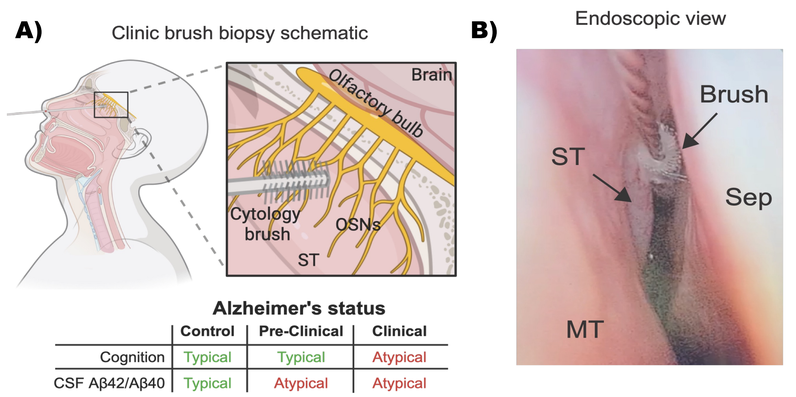


Figure 1. Brush biopsies as a practical strategy for sampling olfactory epithelium in Alzheimer's Disease. A) Schematic showing brush biopsy of the olfactory cleft in Control (typical cognition and CSF biomarkers), Pre-clinical AD (typical cognition and atypical CSF biomarkers) and Clinical AD (atypical cognition and CSF biomarkers) subjects. ST = superior turbinate. **B)** zero-degree endoscopic view of brush biopsy technique. ST = superior turbinate; MT = middle turbinate; Sep = nasal septum. **C)** (left) UMAP shows brush biopsy cell types identified by single cell RNA-sequencing. (right) same UMAP by Alzheimer's status.

RESULTS

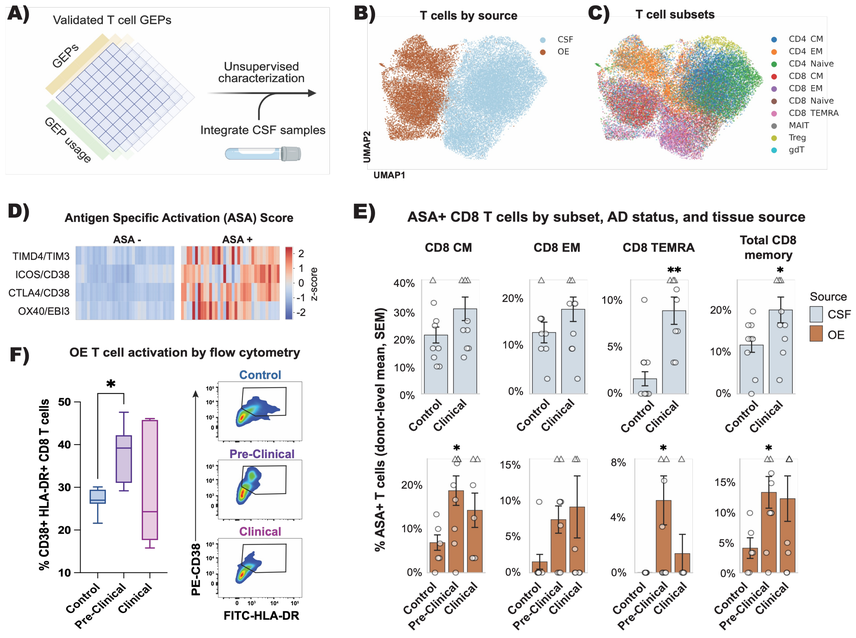


Figure 2. Olfactory epithelium parallels clinical AD CSF T-cell inflammation at the pre-clinical stage. A) Experimentally validated gene expression programs (GEPs) were projected onto T cells, CSF samples from AD subjects were integrated for comparison. **B)** UMAP of T cells by source (CSF or OE) and **C)** T cell subsets. **D)** Heatmaps display the antigen-specific activation (ASA) score. **E)** Bar graphs show the mean ± SEM of the percent ASA$^+$ CD8 T cells across donor-level pseudo-bulk replicates **F)** Box and whisker plots show flow cytometry data of percent double-positive CD38 and HLA-DR CD8 T cells from the OE.

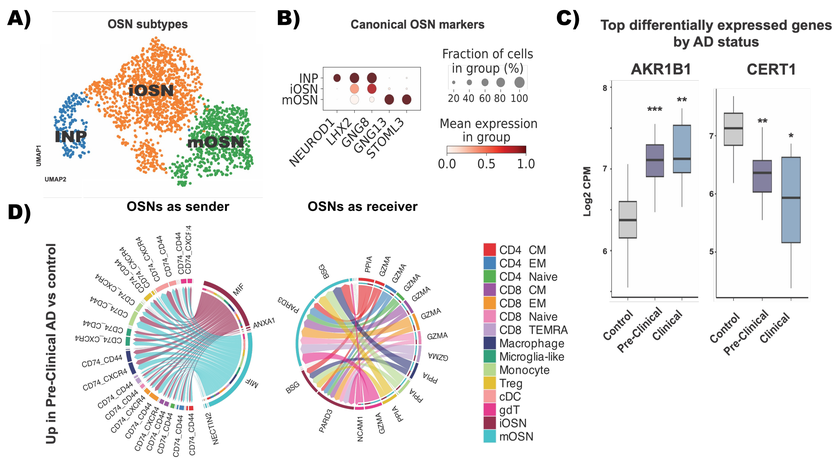


Figure 3. OSNs demonstrate AD pathology and heightened immune cell interaction potential. A) UMAP shows olfactory sensory neuron subsets. iOSN = immature olfactory sensory neuron; mOSN = mature olfactory sensory neuron; INP = immediate neuronal progenitor. **B)** Dot plot shows canonical gene profiles used to characterize each neuronal subset. **C)** Box-and whisker shows the most upregulated (AKR1B1) and downregulated (CERT1) AD OSN genes. **D)** Chord diagrams show neuroinflammatory pathways that are differentially expressed in Pre-clinical AD OSNs.

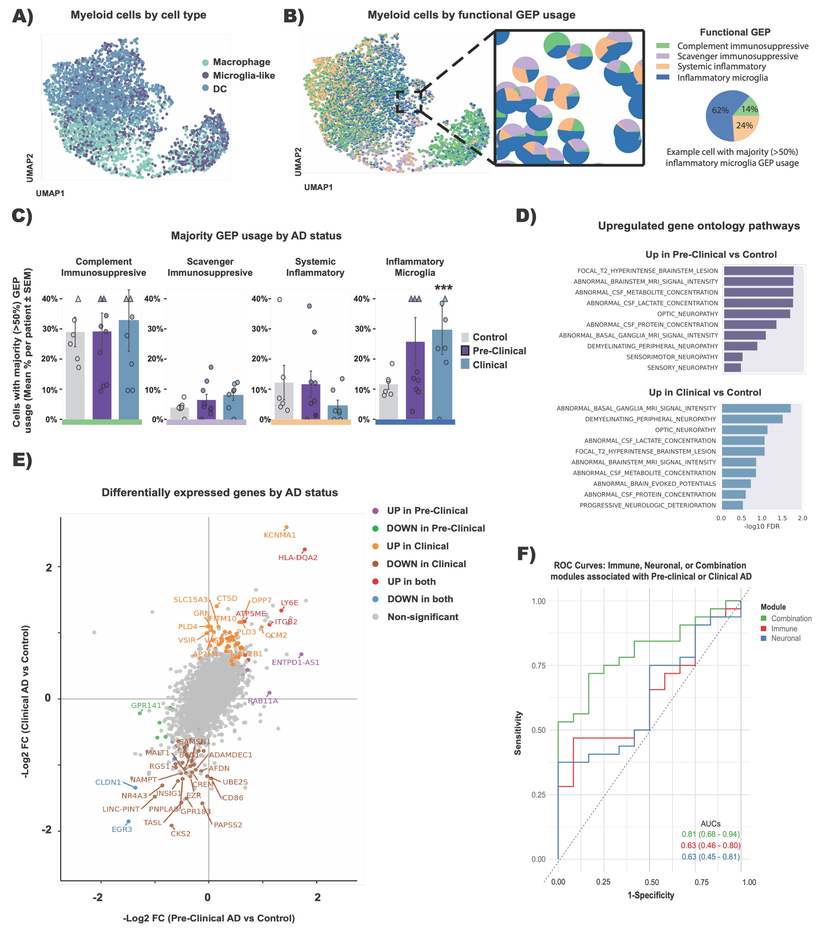


Figure 4. Myeloid cells in the OE demonstrate inflammatory changes associated with AD. A) UMAP shows the myeloid cell types in the OE. **B)** Same UMAP as A) with each cell showing the proportion of 4 functional myeloid Gene Expression Programs (GEPs). **C)** Bar charts show the percentage of myeloid cells per subject (mean, SEM) that contain a majority (>50%) of either of the four GEPs. **D)** Top up-regulated pathways in myeloid cells. **E)** Two-way volcano plot shows differentially expressed genes between conditions. **F)** shows ROC AUC plots of either the immune module, neuronal module, or combination in classifying Pre-clinical or Clinical AD compared to controls.

CONCLUSIONS

Here, using scRNA-seq and flow cytometry of OE biopsies, we show neuroinflammatory T cell, myeloid cell, and OSN changes detectable even in preclinical AD. Activated memory OE T cells parallel CSF T cell phenotypes seen in advanced disease, accompanied by microglia-like inflammatory programs and evidence of OSN inflammatory injury. Together, our findings establish a novel platform for analysis of neural tissue in early AD, with potential for biomarker development or new therapeutic targets.

REFERENCES

D'Anniballe, V.M., Kim, S., Finlay, J.B. et al. Olfactory cleft biopsy analysis of Alzheimer's disease pathobiology across disease stages. *Nature Communications* 17, 2245 (2026). https://doi.org/10.1038/s41467-026-70099-7