1. 16S rRNA Next Generation Sequencing Analysis Shows Bacteria in Alzheimer's Post-Mortem Brain (https://www.frontiersin.org/journals/aging-neuroscience/articles/10.3389/fnagi.2017.00195/full#supplementary-material)
    a. In order to ensure maximum taxonomic coverage:
        i. representative 16S ribosomal gene sequences from the major phyla commonly found in the human microbiome, Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria and Proteobacteria,
        ii. NGS analysis
            1. custom quality control (QC) pipeline including the use of seqtk and trimmomatic
            2. Filtered sequence quality
            3. Filtered by OTUs
                a. OTUs - a practical grouping of microorganisms or other organisms based on sequence similarity, rather than strict species definitions. OTUs are used in fields like metagenomics to simplify diversity analysis when precise identification of individual species is not possible or necessary. They are clusters of DNA sequences, often the 16S rRNA gene in bacteria, that meet a specific similarity threshold, such as 97% identity, to represent a common organism
                b. taxonomies were assigned using the uclust method
                c. phylogeny generated using FastTree
                d. OTU tables using standard QIIME tools, generated alpha and beta diversity metrics
                    i. Calculated using UniFrac, visualized with Emperor
    b. Amplicon generation
        i. Need read depth of 20,000 for accurate OTU diversity
        ii. Age of patients 62 years to 98 years, with Braak stages of 3 or 4 (controls) and 5 or 6 (AD)
    c. Assessment of contamination
        i. In order to assess levels of peri-mortem contamination, PMD was plotted against total bacterial reads for each individual
        ii. Found no correlation between AD or control with age by linear regression analysis
        iii. PMD did not significantly correlate with bacterial reads in control or AD
        iv. PMD = post mortem delay = time elapsed after death
        v. data suggest that contamination from PMD is not a significant factor
        vi. increased levels of bacterial reads are associated strongly with AD compared to normal individuals and not with age
        vii. Possible contamination with proteobacteria for non AD brain
        viii. AD brain has actinobacteria as largest component

ix. largest Proteobacteria component is Alpha-proteobacteria; Rhizobiales; Methylobacteriaceae, which as an environmental rhizobial bacterium is listed as a common contaminant and displays a random distribution between control and AD brain

d. Comparison of bacteria population in AD vs control
    i. FPPE - paraffin embedded
    ii. AD samples had more reads than controls
    iii. Actinobacteria reads are higher in AD samples compared to controls
    iv. Proteobacteria are lower in AD samples vs controls
    v. Firmicutes has a greater percentage of reads in AD in FFPE, but not in frozen tissue, and this was due to a large staphylococcal presence in one sample.
    vi. Actinobacteria content seen here consists primarily of Propionibacteriaceae, (Figure 2B) with the largest OTUs being P. acnes
    vii. three Propionibacteriaceae OTUs against the NCBI 16S rRNA database revealed them all to be Propionibacterium acnes
    viii. P. acnes as a Possible Contributing Factor in Neuroinflammation
    ix. Our data suggests that Actinobacteria (P. acnes) increases in AD brain over and above Proteobacteria
    x.
e. bacteria most frequently described as associated with AD are those of the oral microbiome.
    i. Spirochetes such as Treponema have been linked to AD
    ii. increased levels of immunoglobulin to P. gingivalis (Sparks Stein et al., 2012), F. nucleatum and P. intermedia) have all been associated with cognitive impairment and/or AD
    iii. Evidence of increased levels of Helicobacter pylori and the spirochete B. burgdorferi in AD
f. Summary
    i. 16S NGS in terms of both PCR sensitivity and taxonomic coverage is extremely well suited to the detection and analysis of bacterial populations in both frozen and FFPE temporal cortex, despite background human genomic DNA being present in overwhelming excess.

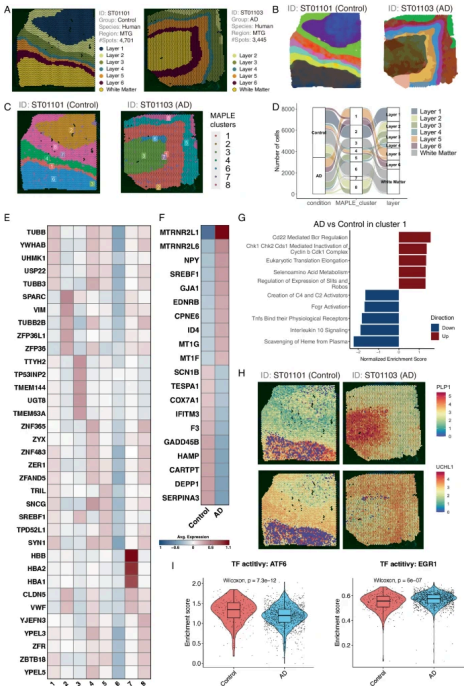
2. A single-cell and spatial RNA-seq database for Alzheimer's disease (ssREAD) (https://www.nature.com/articles/s41467-024-49133-z)
    a. single-cell and spatial RNA-seq database for Alzheimer's disease (ssREAD)
    b. Introduction
        i. Previous databases:
            1. TACA database facilitates differential expression comparisons to identify cell type-specific gene expression alterations, cell–cell interactions, and drug screening opportunities
            2. SC2Disease database
                a. comprehensive and accurate resource of gene expression profiles across various cell types for 25 diseases.

        b. AD database limited to single data set with one brain region
    3. SCAD-brain
        a. Public database dedicated to AD, focus on human and mouse brain data

ii. Lack of focus on spatial transcriptomics in AD and comprehensive differential analyses under various conditions

iii. ssREAD
    1. each dataset is meticulously annotated, providing pertinent details such as species, gender, brain region, disease/control distinction, and AD Braak stages
    2. sREAD provides functions including cell clustering, cell type annotation, marker gene expression visualization, and cell proportion analysis
    3. DEG - differentially expressed gene
    4. ST - spatial transcriptomics
    5. Database summary: 381 ST samples from 16 AD-related studies and 1,053 sc/snRNA-seq samples from 85 studies

iv. Results
    1. 144 out of 277 integrated sc/snRNA-seq datasets are from human samples, whereas 133 datasets are from mouse samples
    2. Database contains scRNA and snRNA, snRNA may be better for brain samples because samples hard to dissociate, nuclei isolated from tissue
    3. Spatially-informed subpopulation analysis
        a. Used ST01101 and ST01103
        b. deviations in layer 5 of AD cases compared to controls may also be a result of differences in their spatial organization as well as their gene expression patterns.
        c. Used MAPLE - multi-dimensional exploration of spatially informed sub-populations

d. 

4. Looked at top 10 upregulated and downregulated pathways
    a. all five downregulated pathways are associated with immune responses/functions
5. RESEPT used for detection of spatial domains
6. Spatial feature plots highlighting the variance in gene expression of PLP1 and UCHL1 from Cluster 1
7. violin plots showcasing the activity of two selected TFs between AD and Control
8. expression of DEPP1, also known as PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha), was found to be significantly lower in AD than the control
9.

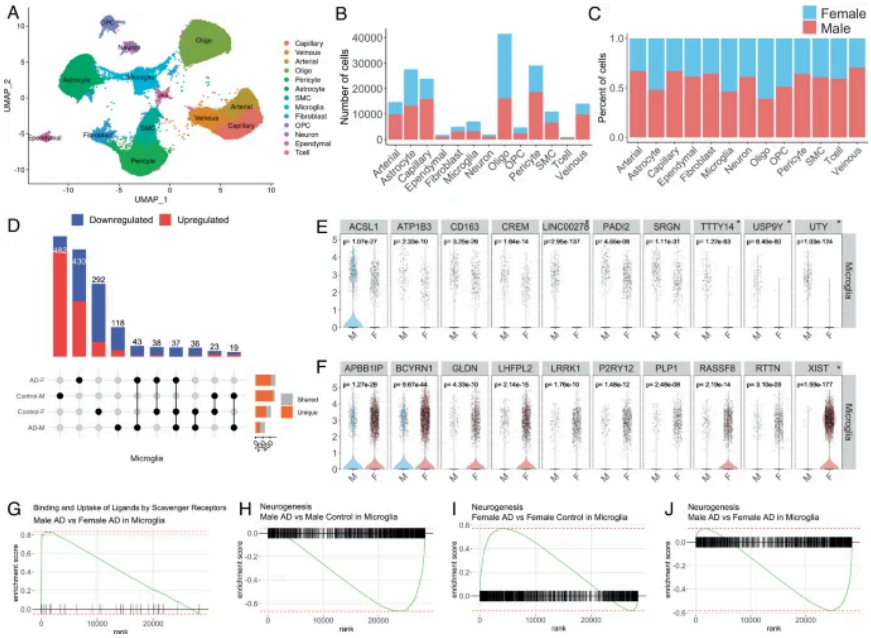
v. To further investigate regulatory mechanisms in different clusters between the Control (ST01101) and AD (ST01103) samples, we implement DeepMAPS33 in the ssREAD framework for spatial transcriptomics-guided gene regulatory network analysis.
    1. Highlighted network with 10 TFs (i.e., NR1H3, SREBF1, ATF6, TAL1, SOX10, NFYA, AR, MYC, HIF1A, and EGR
        a. Differences in AD and control in ATF6 and EGR1, related to stress response

vi. Spot deconvolution analysis
    1. a computational technique used to computationally resolve a mixture of cells in a single "spot" from spatial transcriptomics data
    2. Thal phase refers to the five stages of amyloid-beta plaque deposition in the brain, a key pathological hallmark of Alzheimer's disease (AD)

3. UMAP representation of cell types derived from the Seattle Alzheimer's Disease Brain Cell Atlas (SEA-AD). Clockwise from the top left, corner insets elucidate the Braak stage, Thal phase, ethnicity, and sex attributes.
4. b–e Bar plots visualizing the distribution of cells based on the Braak stage, Thal phase, sex, and ethnicity, categorized by the condition in the atlas.
5. f Bar plots displaying the fractional representation of cell types, contrasting AD and control within the SEA-AD atlas.
6. g Heatmap of top and bottom 25 DEGs identified between AD and control samples in the integrated AD035 MTG dataset in Microglia. h Comparison of DEGs overlap in Microglia among AD035 MTG, AD048 PFC, and their integrated datasets.
7. i–l Deconvolution analysis of cell types between ST samples (ST01101 and ST01103) and the scRNA-seq SEA-AD atlas, showing the cell type fractions for Astrocytes
8. (j) and Oligodendrocytes
9. (l). Marker gene expression indicators, GFAP for Astrocytes (i) and MOBP for Oligodendrocytes (k).
10. m–t Spatial distributions of DEGs in AD and control samples within MAPLE cluster 1, cross-validated with single-cell DEGs from the integrated AD035 datasets. Upregulated DEGs in Astrocytes GJA1 (m) and MT-ATP8 (n). Downregulated DEGs in Astrocytes IFITM3 (q) and TUBB2B (r). Upregulated DEGs in Oligodendrocyte ERBIN (o), GPRC5B (p), MID1IP1 (s), and SLC44A1 (t).

vii. Sex specific differences in AD

1. UMAP visualization of the single-cell data, color-coded by cell type



2. a UMAP visualization of the single-cell data used in the analysis, with different cell types color-coded.
3. b–c Bar plots illustrate the count and proportion of each cell type, segregated by sex. This reveals any potential differences in cellular composition between male and female samples.
4. d UpSet plot showing the unique and shared DEGs across four groups: Male AD patients, Female AD patients, Male controls, and Female controls.
5. e Violin plots for the top 10 upregulated DEGs between male and female in microglia. * Indicates sex-chromosomal genes. p-values were calculated based on a two-sided Wilcoxon Rank-Sum test and adjusted using Bonferroni correction.
    a. ATP1B3, a previously identified ARM and DAM gene is downregulated in our comparison of female microglia to male microglia
    b. LHFPL2, and RTTN are DAM genes that we find are upregulated in female microglia compared to male
    c. ACSL1, ATP1B3, CD163, CREM, and SRGN as downregulated in female microglia cell nuclei compared to male
    d. upregulation of genes APBB1IP, GLDN, LHFPL2, LRRK1, P2RY12, RASSF8, and RTTN in female microglia compared to male is also consistent with previous findings
6. f Violin plots for the top 10 downregulated DEGs between male and female in microglia. p-values were calculated based on a

two-sided Wilcoxon Rank-Sum test and adjusted using Bonferroni correction.

7. g Gene Set Enrichment Analysis (GSEA) plot showing the enrichment of genes involved in binding and uptake of ligands by scavenger receptors.
8. h–j GSEA plots showing the enrichment of genes involved in neurogenesis for three different comparisons: Male AD vs. Male Control in Microglia (h), Female AD vs. Female Control in Microglia. (i), and Male AD vs. Female AD in Microglia (j). These plots highlight the sex-specific differences in neurogenesis-related gene activity under AD conditions.
    a. dysregulation of scavenger receptor activity has been implicated in the clearance of Aβ plaques
    b. Neurogenesis: upregulation observed in female AD, and downregulation in male AD patients.

viii.