**snRNAseq Methods Section**

**High-throughput generation of single-nucleus transcriptomes from frozen human brain using cellular barcodes and droplet capture.** Nuclei will be isolated from frozen postmortem brain tissue using a procedure adapted in our laboratory for frozen human brain (see **Preliminary Studies**) from Ref.1-5. Postmortem middle temporal gyrus will be mechanically dissociated, filtered, and resuspended. DAPI-positive nuclei will be sorted using Fluorescence Activated Cell Sorting. Single nuclei will be captured using the  Chromium Controller from 10x Genomics2,3,6. cDNA libraries will be generated with the Single Cell 3′ Library & Gel Bead kit version 3 and sequenced on an Illumina NextSeq500 using 150-bp paired-end sequencing. Based on our Preliminary Studies, at least 5,000 cells will be captured per sample, for a total of more than 495,000 cells over the 99 temporal cortex samples, and sequenced with at least 150 million reads per sample (see **Preliminary Studies**). To minimize bias, for all RNA processing and RNA-Seq steps, case and control samples were processed in parallel by researchers blinded to the diagnosis.

**Single-nucleus bioinformatics analysis.** Gene counts will be obtained by aligning reads to the human genome (GRCh38.p12) using CellRanger software (version 3.0.0)4,5. Transcriptome annotation will be based on ENSEMBL v93 GTF from the CellRanger. Libraries will be aggregated from all samples using the cellranger aggr pipeline to equalize the read depth before data merging to generate a gene count matrix. Batch effects will be corrected using the mutual nearest neighbors algorithm7 that does not rely on predefined or equal batch compositions and is superior to other batch-correction methods7.

**Quality control for cells and expressed genes.** The initial dataset will be projected into two-dimensional space using t-distributed stochastic neighbor embedding (t-SNE) based on the top 10 principal components. The t-SNE coordinates will be used to visualize potential biases in apparent cell similarity due to differential cell quality. For each cell, the following quality measures will be quantified: ***1***, the number of genes for which at least one read is mapped, which is indicative of library complexity; ***2***, the total number of unique molecular identifier (UMI) counts; ***3***, the percentage of counts mapping to the top 50 genes. ***4***, Cells with a high proportion of reads mapping to the mitochondrial genome relative to the total number of detected genes will be removed as this ratio is useful in identifying low-quality cells because in a broken cell cytoplasmic RNA will be lost8, while RNAs that are enclosed in the mitochondria will be retained8. ***5***, Based on previous studies and our pilot analyses, we will remove cells with less than 200 detected genes4,9. ***6***, Mitochondrially encoded genes and genes detected in less than two cells will be excluded. After applying these filtering steps, the resulting dataset will be used for downstream analysis.

**Cell type annotation and sub-clustering.** We will further normalize and cluster the final UMI matrix with the SCANPY package10. Principal component analysis will be performed, and tSNE will be run over the top ten principal components using the MulticoreTSNE package (<https://github.com/DmitryUlyanov/Multicore-TSNE>). The Louvain graph clustering algorithm will be applied to identify cell clusters10. The top 500 ranking genes will be extracted for each cluster and tested for overlap with known marker genes11. For each cell-type cluster, we will assign a cell-type label using statistical enrichment for sets of marker genes11,12. Enrichment will be statistically defined by the hypergeometric distribution with the false discovery rate (FDR) correction. Sub-clustering analysis will be performed independently over each broad cell-type cluster. Subclusters having abnormally high number of total counts and mixed expression of markers from different cell-types will be tagged as potential doublets and not considered for downstream analyses11. To overcome the **zero-inflated expression**, the computational method MAGIC2 will be used as in Ref.3 and validated through comparison to co-expression of typical cell-type-specific marker genes3.

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