Title

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Journal

Alzheimer's & Dementia, 20(5)

ISSN

1552-5260

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Publication Date

2024-05-01

DOI

10.1002/alz.13790

Received: 20 October 2023 | Revised: 25 January 2024 | Accepted: 17 February 2024

DOI: 10.1002/alz.13790

Alzheimer's & Dementia®
THE JOURNAL OF THE ALZHEIMER'S ASSOCIATION

REVIEW ARTICLE

Gene networks and systems biology in Alzheimer's disease: Insights from multi-omics approaches

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Funding information
National Institutes on Aging, Grant/Award Numbers: 1RF1AG071683, U54 AG054349-06; Adelson Medical Research Foundation funds; National Institute of Health (NIH) Institutional National Research Service Award, Grant/Award Number: 1T32GM136624-01; National Institute of Health, Grant/Award Number: R01NS128523

Abstract

Despite numerous studies in the field of dementia and Alzheimer's disease (AD), a comprehensive understanding of this devastating disease remains elusive. Bulk transcriptomics have provided insights into the underlying genetic factors at a high level. Subsequent technological advancements have focused on single-cell omics, encompassing techniques such as single-cell RNA sequencing and epigenomics, enabling the capture of RNA transcripts and chromatin states at a single cell or nucleus resolution. Furthermore, the emergence of spatial omics has allowed the study of gene responses in the vicinity of amyloid beta plaques or across various brain regions. With the vast amount of data generated, utilizing gene regulatory networks to comprehensively study this disease has become essential. This review delves into some techniques employed in the field of AD, explores the discoveries made using these techniques, and provides insights into the future of the field.

KEYWORDS
Alzheimer's disease, epigenomics, genetics, multi-omics, single-nucleus RNA-seq, spatial, spatial transcriptomics, transcriptomics

1 | INTRODUCTION

Dementia is a complex condition characterized by cellular dysfunction in the brain, leading to the loss of synapses, cell death, inflammation, gliosis, and disruption of memory and cognitive processes.[1] Alzheimer's disease (AD) is the most prevalent form, accounting for 60% to 80% of cases.[2] One in three seniors pass away with Alzheimer's or another form of dementia, surpassing the combined mortality of breast and prostate cancer.[2] The economic burden attributed to AD and related dementias in 2023 amounts to a staggering $345 billion for the nation, a figure that does not even encompass the substantial value of unpaid caregiving.[2] The costs associated with healthcare and long-term care for individuals afflicted by dementia are notably high, rendering it one of the most financially burdensome conditions society faces.[2] Hence, from the perspectives of maintaining a sustained quality of life and alleviating the substantial financial encumbrances, there exists an urgent imperative to comprehensively comprehend and effectively address this debilitating disorder.

Neuronal loss and damage are the hallmarks of AD.[3] The "amyloid cascade hypothesis" introduced an amyloid beta (Aβ)-centric mechanism for AD pathology, where the accumulation of Aβ plaques in parenchyma leads to cerebral amyloid angiopathy, neurofibrillary tangles, and glial responses, alongside the absence or degradation of neurons and synaptic connections.[4] Anti-Aβ monoclonal antibodies

have been developed as major therapeutic interventions against AD. Lecanemab is one such drug recently approved by the Food and Drug Administration, a humanized monoclonal antibody that binds with high affinity to Aβ-soluble protofibrils and removes them.[5] In individuals with early AD, this drug was associated with reduced brain amyloid levels and slowed cognitive decline over 18 months but was also associated with adverse events.[5] Therefore, further research and longer trials are necessary to determine the safety and efficacy of these interventions.[6] The precise mechanisms underlying neuronal loss in AD remain incompletely understood, despite a host of mechanisms having been introduced. Among these mechanisms, the accumulation of misfolded proteins, including Aβ and tau, leading to the formation of toxic aggregates causing neuronal damage and eventual cell death, stands as a pivotal contributor.[7] Oxidative stress, arising from an imbalance between the production of reactive oxygen species and the body's antioxidant defenses, represents another significant pathway.[8] Chronic inflammation in the brain, as well as excitotoxicity, a phenomenon wherein neurons are overstimulated by neurotransmitters, further contributes to neuronal depletion in AD.[9] Moreover, studies have demonstrated reduced cerebral blood flow and glucose metabolism as well as an increase in brain's vascular resistance both in human AD patients and in mice overexpressing amyloid precursor protein (APP) and apolipoprotein E (apoE)4 protein.[10]

In all of the suggested mechanisms, there is considerable interplay between the genes involved, the transcription factors (TFs), splicing factors, chromatin state, proteins, and other metabolites. These mechanisms can also interact with each other and lead to a complex cascade of events that ultimately results in AD pathology and cognitive decline. Gene regulatory networks (GRNs) and systems biology approaches yield computational models of gene regulation in the form of networks, visually shown in topological graphs, helping to decipher the complex molecular landscape of AD.[11–15] In its simplest representation, a GRN maps the interactions between TFs and their potential target genes, with nodes representing TFs and genes, and the edges denoting the regulatory interactions between them.[12] Bulk profiling, while providing averaged signals across cell types in a tissue sample, fails to distinguish regulatory programs specific to particular cell types or states.[12] Notably, transcriptomic data alone do not capture the full scope of regulatory mechanisms, including the post-translational modifications, genomic structure and accessibility, chromatin states, and the abundance of TFs and cofactors. The incorporation of these data can enrich GRNs to more accurately represent regulatory mechanisms in vivo. For instance, integrating chromatin accessibility data refines TF-gene connections by accounting for gene "openness" and factoring in cis-regulatory elements in GRN inference.[12] Single-cell multi-omics provide genomic, transcriptomic, and chromatin accessibility information that can be leveraged to infer TF-gene interactions across various cell types.[12] Consequently, GRNs derived from single-cell data offer insights into cellular responses to both intra- and extracellular signals in AD. However, single-cell datasets present their own set of challenges, such as cellular heterogeneity, variable sequencing depth across cells, significant sparsity from dropout events, and influences from the cell cycle.[16] Despite these challenges, more than a dozen methods for inferring GRNs from single-cell datasets have been introduced.[16] Choosing the most suitable method is complex, compounded by the absence of universally accepted benchmark data for method validation and the variety of benchmarks employed to evaluate and compare these methods.[16]

To understand the phenomena of neuronal loss and damage, it is necessary to meticulously characterize cell types and their

RESEARCH IN CONTEXT

1. **Systematic review**: In conducting this review, we employed a methodology that integrated literature search and data analyses from published Alzheimer's disease (AD) datasets, incorporating contributions from esteemed institutions such as the Swarup lab, renowned for their expertise in AD single cell/nucleus research. Our approach involved a comprehensive exploration of single-cell and multi-omics experimentations, specifically focusing on transcription profiles and epigenomic changes, notably those identified through transposase-accessible chromatin with sequencing (ATAC-Seq) profiling. This allowed us to gain insights into crucial transcription factors and their interactions with target genes, examining whether these interactions lead to transcription. This process ensured a robust foundation for our interpretations.
2. **Interpretation**: This review underscores the importance of the network analysis approach as an integrated method. Conducting separate and joint network analyses of single-nucleus (sn)RNA-seq and ATAC-seq modalities provides a comprehensive understanding of dysregulated genes, genes with similar expression patterns, and integrations between target genes of specific AD-associated transcription factors. These approaches offer holistic insights, allowing us to navigate the vast volume of high-throughput data generated, and further our understanding of AD pathogenesis.
3. **Future directions**: The crucial and pivotal question that remains unanswered is whether the gene networks found in AD mouse models are similar and translatable to humans. This is of utmost importance in developing mouse models that can accurately represent the disease as model organisms, thereby ensuring the reliability of the results obtained from them. Furthermore, the multi-omics field still needs to achieve true single-cell resolution. Insights from AD studies have revealed that cell types like microglia exhibit distinct states in disease conditions. Studying these states at the single-cell level enables us to better comprehend the genes contributing to these conditions.

interactions, both intercellular and intracellular, within tissues and among the cells themselves.[17] For example, it is known that glial cell types, such as microglia, normally function to protect the brain environment.[18] However, in AD this cell type transitions into other states that exacerbate inflammation.[19–21] Excitatory and inhibitory neuronal cell types exhibit altered subpopulations in AD, accompanied by synaptic dysregulation.[22–25] Studying these changes can further elucidate the neuronal loss pathology. Additionally, cell-type specific genetic variants in AD contribute to the dysregulation of amyloid metabolism, tau protein function, synaptic activity, and inflammation.[26,27] A classic example is the *APOE* ε4 allele, known as the strongest genetic risk factor for sporadic AD, which is reported to be expressed predominantly in A1 reactive astrocytes.[28] On the other hand, the *APOE* ε2 allele is known to be the strongest genetic protective factor against AD, as determined by multiple large-scale genome-wide association studies (GWAS) and meta-analyses.[26] Since non-neuronal cells largely express the *APOE* gene, astroglia and microglia are essential cell types that warrant further transcriptomic investigation at single-cell or single-nucleus resolution.[26]

In this review, we summarize the advances made in single-cell multi-omics and how these new methods have accelerated our understanding of the disease pathogenesis and molecular programs associated with the disease at single-cell resolution. We also summarize how newer methods like spatial transcriptomics and proteomics hold promise to unravel novel disease biology by delving deeper into the disease mechanism.

2 | BACKGROUND

Since 1907, when Alois Alzheimer employed Bielschowsky's silver staining method to observe the degenerating neurons characterized by bundles of fibrils and miliary foci of silver-staining deposits scattered throughout the cortex,[29] the landscape of AD research has undergone a remarkable transformation. Subsequent biochemical assays, including electron microscopy, immunoblotting, and immunohistochemistry, identified Aβ as a major component of the plaques observed earlier. GWAS then identified several risk factors associated with late-onset AD including *APOE*, *CLU*, *PICALM*, and *BIN1*.[30,31] Majority of AD cases are sporadic, and the associated triggers are complex and can be connected to both genetics and environmental elements such as stress, sleep abnormalities, and traumatic brain injury.[32–34]

The familial AD cases, accounting for approximately 0.6% of AD cases, have been connected to the mutations in presenilin1 (*PSEN1*), presenilin 2 (*PSEN2*), *APOE* ε4, and the amyloid precursor protein (*APP*) genes.[33,34] Mutations in these genes result in aberrant amyloid beta productions and aggregation.[32,33]

Currently, AD is primarily characterized by two main pathological features: extracellular Aβ plaques and intracellular neurofibrillary tangles (NFTs) made of hyperphosphorylated tau protein in the brain along with the presence of cognitive decline as individuals age.[33,35] Essential to comprehending the initiation of Aβ pathology is understanding how Aβ monomers are generated, removed from the system, and aggregated into oligomeric Aβ.[33] Aβ pathology emerges from the improper breakdown of APP, resulting in the formation of Aβ monomers which then create oligomeric Aβ aggregates.[36,37] These oligomers eventually accumulate into Aβ fibrils and form plaques. Although the role of APP is not fully understood, it is thought to play a role in cellular health and growth.[36] APP normal processing involves nonamyloidogenic proteolysis of APP via a- and λ-secretases, generating soluble fragments. Erroneous APP processing with β-secretase and λ-secretase leads to insoluble amyloid beta peptides, aggregating to form Aβ plaques.[33,36,38] Faulty decomposition of APP results in formation of Aβ42 amino acid long fibrils elevating the $A\beta_{42}/A\beta_{40}$ ratio, exacerbating plaque formation.[4] Amyloid plaques then aggregate and cluster together in between neurons, interfering with communication between neurons, disrupting cellular functions, and triggering harmful inflammatory responses in the brain.[9] The exact role of Aβ in the progression of AD pathology remains unclear, as Aβ plaques can accumulate in the brain for up to a decade before any noticeable AD symptoms or diagnosis occur.[9] Because of this disconnect between pathology and clinical diagnosis in AD patients, understanding changes in gene expression will delve deeper into the intricate nuances of mechanisms giving rise to AD.[39]

Hyperphosphorylation of tau, a microtubule-associated protein stabilizing microtubules, leads to NFTs—another fundamental AD pathology.[1,40,41] Normal tau phosphorylation is essential for regulating microtubule-dependent axonal transport by allowing tau to detach from microtubules, facilitating intracellular trafficking.[9] Dephosphorylation then allows the return of tau to the microtubules and stabilizing their cellular structure. Conversely, abnormal phosphorylation of tau contributes to neurodegeneration in AD by causing tau to dissociate from the microtubules, leading to the collapse of microtubule structure and subsequent disruption of cellular morphology.[9,41] Hyperphosphorylated tau aggregates into tangles, giving rise to NFTs, which disrupt cellular functions, lead to neuronal disruption, and eventually result in apoptosis.[41,42] Following this line of thought, high-throughput sequencing methods provide insights into the heterogeneous nature of AD, uncovering the critical juncture at which plaques and tau processing may transition into a toxic state.[39]

In the context of AD and neurodegenerative disorders, bulk transcriptomic analysis has revealed distinct gene expression patterns across different brain regions.[38,43,44] In bulk-tissue sequencing studies, the gene expression profiles of interest are obtained by averaging signals across all cells within a sample. This technique is advantageous when assessing gene expression changes across brain regions, providing information on coherent cellular mechanisms that are associated with cognitive decline and neuropathological changes.[45] This approach offers an unsupervised, tissue-specific perspective, identifying transcriptional programs linked to disease phenotypes independent of biases arising from prior research focused on specific genes and pathways. For example, Neff et al. explored molecular heterogeneity in AD across five brain regions.[45] Their multiscale network analysis reported subtype-specific drivers, including *GABRB2* (neuronal), *LRP10* (astrocytes), *MSN* (microglia), *PLP1* (oligodendrocytic gene), and *ATP6V1A* (neuronal gene).[45] Guennewig et al. examined tau deposition

from the precuneus to the less affected primary visual cortex.[38] By combining up- and down-regulated genes for gene ontology (GO) analysis, two major themes emerged among the enriched biological pathways: exocytosis (eg, *STXBP2* and *NSF*) and immune function.[38] High-dimensional data from bulk-tissue RNA sequencing are analyzed using correlation networks to identify interconnected expression changes across cell types. This approach, termed weighted correlation network analysis (WGCNA),[14] identifies pairwise relationships among gene transcripts and reports a set of genes that show similar changes in their expression profiles, known as co-expressed modules. These modules can be assigned to a particular cell type, given the cell-type specific gene marker expression profiles.[14] The resulting co-expressed modules are then correlated with clinical pathologies, cognitive decline, and amyloid pathology. This reveals cell type-specific alterations linked to clinical phenotypes, thereby connecting clinical diagnosis and pathology with cell type expression patterns. Our lab conducted a consensus WGCNA analysis on combined single-cell and bulk RNA sequencing (RNA-seq) data from early- and late-stage AD cases, along with pathological controls with additional samples from the Religious Orders Study and Memory and Aging Project (ROSMAP).[23] This analysis investigated shared expression profiles across these brain banks, and found[46,47] four co-expressed oligodendrocyte modules (OM1, 2, 4, and 5) that were significantly correlated with AD.[23] These findings underscore the need for more targeted investigations into cell type–specific changes in AD.

Though cell type changes can be inferred using bulk RNA-seq, this approach has limitations in accurately characterizing the intricate biology of the brain, which consists of diverse neuronal and glial cell types with distinct regional specificity. Moreover, bulk profiling provides mixed measures across cell types and cannot distinguish regulatory mechanisms unique to cell types and states.[12] To overcome bulk RNA-seq limitations, single-cell RNA-seq (scRNA-seq) methods employ nucleotide barcoding strategies to trace sequencing reads back to their respective cells.[48–50] Unlike bulk RNA-seq, which generates a single data point per gene for each sample, scRNA-seq provides data at the cellular level, resulting in hundreds to tens of thousands of data points per cell, depending on the methodology.[35,51]

3 | SINGLE-NUCLEUS TRANSCRIPTOMICS ELUCIDATES CELL TYPE–SPECIFIC ALTERATIONS IN AD

Recent advances in single-cell omics technologies have resulted in a menagerie of datasets available from various modalities. Single-cell transcriptomic data typically contains the transcriptional profiles of thousands to millions of individual cells, with each cell expressing tens of thousands of genes that may reveal regulatory mechanisms in a cell type–specific manner. This substantial increase in data granularity enables us to study how specific cell types respond to diseases, especially in the context of neurological disease risk signals. However, this wealth of information has also created a confusing landscape where common or critical mechanisms are obscured among the heterogeneous nature of each dataset, specific to a disease. The volume and complexity of single-cell data require the development of robust computational methods to accurately and holistically interpret the data, extracting meaningful biological insights. These methods include strategies for cell type identification, differential gene expression (DGE) analysis, and integration of data from multiple samples and various modalities.[52–54]

3.1 | Single-nucleus RNA-seq

3.1.1 | Methods in single-nucleus RNA-seq

Currently, two primary approaches are used for measuring the quantity of mRNA transcripts per cell in single-cell and single-nucleus RNA-seq (snRNA-seq) methods. The first approach is the plate-based protocols, where cells are isolated onto plates. The other is droplet-based techniques, such as those employed by 10x Genomics, which use microfluidic partitioning to combine single cells, reverse transcription (RT) reagents, gel beads with barcoded oligonucleotides, and oil on a microfluidic chip to create reaction vesicles known as gel beads-in-emulsion (GEMs) or, droplets. Ideally, each droplet contains a single cell, a barcoded gel bead, and reagents including reverse transcriptase, polymerase, and nucleotides. Within each GEM, the cell is lysed, the gel bead is dissolved to release the identically barcoded RT oligonucleotides into solution so the reverse transcription of the polyadenylated mRNA occurs. Next-generation sequencing (NGS) libraries are created from these barcoded cDNAs and profiled by a single cell profiler, such as the 10x Genomics Chromium Platform. The mRNA reads, typically tagged with cellular barcodes or a unique molecular identifier (UMI), are aligned to the reference genome to trace back to their cells of origin. The resulting output is a count matrix representing cells by genes, which serves as input for downstream analysis (Figure 1A).

While single-cell studies offer a more nuanced understanding of cell type–specific responses to AD, no single dataset can capture the full heterogeneity of the disease. Our lab is addressing this challenge by aggregating hundreds to thousands of samples, which involves collecting datasets from various sources, performing quality control (QC), and integrating them for a comprehensive view.

3.1.2 | Demonstration and walk-through of snRNA-seq analysis approaches

For demonstrating the pipeline, we have merged snRNA-seq data from the following publications in the field of AD and dementia associated disorders: Morabito et al. (2020), Swarup et al. (2021), Mathys et al. (2019), Leng et al. (2021), Miyoshi et al. (2023), and Seattle Alzheimer's Disease Brain Cell Atlas (SEA-AD),[23,42,55–58] totaling 349 samples, 1.3 million cells, and 25k genes after the initial QC process (Figure 1B). While analysis approaches are continually evolving, we have adopted the following workflow for single cell/nucleus RNA-seq

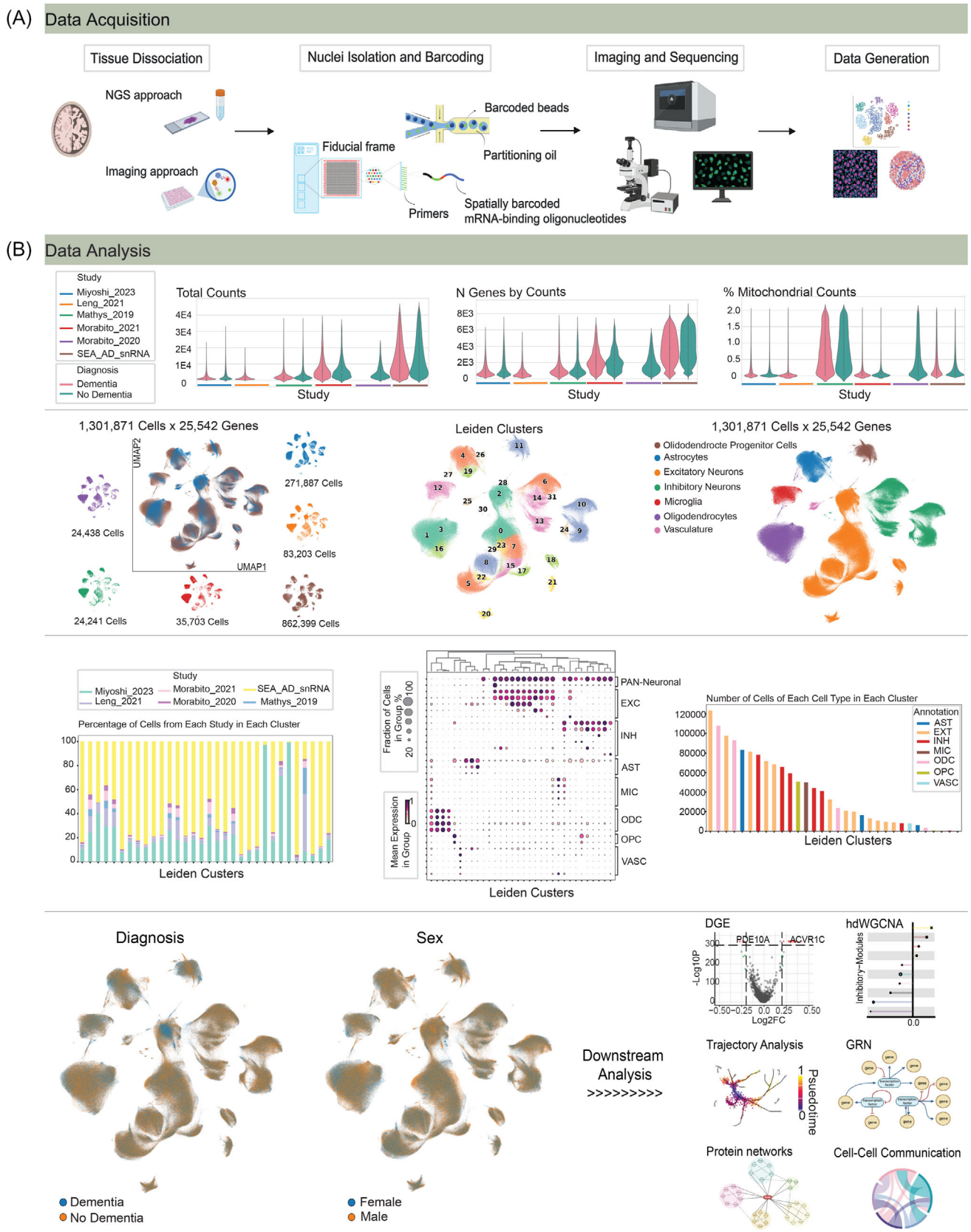

FIGURE 1 Schematic workflow of single-cell/nucleus data acquisition and analysis. (A) Workflow of snRNA-seq and spatial transcriptomics. (B) Demonstrated tentative workflow of snRNA-seq data analysis utilizing Scanpy toolset.[121] Publicly available datasets from six dementia and AD studies initially go through CellBender[59] to remove noise and are then merged. Data are next QC-filtered to control for high mitochondrial counts, UMI counts, genes that are only expressed in very few cells, as well as the total number of transcripts in a cell, and are visualized using violin plots. Resulting dataset contains 1.3 million cells each harboring 25.5k genes. After normalizing the data, data are corrected for batch effects emerging

studies, inspired by the guidelines by Luecken et al.[54] Sequencing can introduce random noise, potentially obscuring biological signals. During QC, ambient RNA and technical artifacts are removed using software including CellBender and SoupX.[59–60] Technical variances in sample and library preparation, biological phenomena, or a mix of the two, can result in some cells having a low number of detected genes, a high fraction of mitochondrial counts, or low count depth. These cells are commonly classified as low-quality cells and are filtered out. Additionally, empty droplets and doublets are identified by transcript counts, or by using algorithms including scDblFinder[61] and Scrublet[62] that infer doublets by simulating doublets through combining counts from random pairs of cells.[61,62] Next, a k-nearest-neighbor (KNN) classifier is built based on the observed cells and simulated doublets, and the algorithm assigns a doublet score to each cell.[61,62] Post-QC, the data undergo normalization and scaling to ensure comparability across cellular profiles, preparing them for subsequent analytical steps. Datasets with multiple samples often contain technical variations that may be mistaken as actual biological signals, which are mitigated using tools including Harmony and Scanorama.[63,64] For more complex computations including integration and batch correction for bigger datasets, deep learning tools include scANVI, scVI, and scGen are employed.[65–68] Dimensionality reduction techniques including principal component analysis (PCA) are applied to the data for visualization, alongside methods including UMAP, t-SNE, and PHATE.[69]

To discern biologically relevant clusters, cells with similar expression patterns are grouped together using Louvain and Leiden community detection-based algorithms.[70] These methods are applied to the KNN graph, and depending on the chosen resolutions, varying numbers of clusters are obtained. The Leiden algorithm is a successor of Louvain and is recommended over Louvain.[70] Clusters are annotated with cell type identities based on specific gene markers, or through label transfer from other references. Statistical tests such as generalized mixed effect models including MAST are conducted to compare clusters, and find differential gene expression patterns in various cell states. An alternative approach in snRNA-seq in getting DGE involves aggregating counts per sample into creating pseudobulks, followed by bulk DGE analysis using DEseq2, edgeR, or Limma.[71–73] Downstream analysis includes summarizing gene expression findings using gene set enrichment analysis platforms like MSigDB, Gene Ontology, KEGG, or Reactome to identify regulated processes in AD.[74–77] Cell-cell communications across cell types and in different brain regions are inferred using ligand-receptor databases such as CellChat, CellPhoneDB, CellTalkDB, and SingleCellSignalR to understand cellular cross talks in AD.[78–81] Sun et al. consolidated data from the four databases to highlight interacting cell pairs via ligand receptor signaling pathways.[82] Changes in cell expressions, as indicated by variations in gene marker expression profiles, can be used to identify co-expressed modules associated with cell types. To infer GRN in AD, high dimensional WGCNA (hdWGCNA), developed by our lab, generates co-expressed networks and identifies cell type–specific co-regulated modules that can be associated with cognitive decline and AD pathology.[13] This suggested pipeline produces results that ultimately must be validated before drawing substantive conclusions.

3.1.3 | SnRNA-seq findings in human samples

Recent studies have used snRNA-seq from postmortem human brain samples at various stages of AD to gain deep insights into cell type–specific changes in the disease. We have summarized some of the recent studies in Table 1. Otero–Garcia et al. reported on high throughput profiling of NFT-bearing neurons compared to NFT-free somas from the same sample tissue.[40] Single soma RNA-seq in these samples revealed subpopulations of excitatory and inhibitory neurons with varying susceptibilities to NFT formation, with subclusters of the excitatory neurons showing higher propensity for NFT formation.[40] Synaptic transmission was the common pathway in NFT-affected neuronal cell types.[40] Despite the neuropathological features known today, the presence of the histopathology does not always directly correlate with cognitive decline or an AD diagnosis.

Sustained inflammatory response fuels neurodegeneration in AD.[17,43] Glial cells, particularly microglia and astrocytes, profoundly contribute to this inflammatory response by dysregulating neuronal Ca^{2+} homeostasis, Aβ clearance, and overall brain function.[17] Depending on the disease stage and regional location, these cell types can exhibit multiple reactive phenotypes, both neurotoxic and neuroprotective. Olah et al. explored microglial heterogeneity using scRNA-seq and reported clusters enriched for disease-related genes and signatures.[14] Additionally, vascular factors contribute to AD through arterial hardening, known as atherosclerosis, leading to reduced blood flow and oxygen. The decomposition of the blood-brain barrier (BBB) impairs the clearance of toxic aggregates and disrupts glucose supply. Consequently, loss of neuronal connections and cell death results in brain atrophy and volume loss.[83]

RNA in situ hybridization results have demonstrated a reduction in excitatory neurons and the downregulation of *NTNG1*, which is involved in the regulation of neurite outgrowth reported by Mathys et al.[56] AD transcriptomics studies have also shown an upregulation of *LINGO1*, which is a negative regulator of myelination.[56] Lipids, critical for membrane structural integrity, are a known risk factor

from integrating samples across different studies. Next, data are clustered using the Leiden algorithm,[70] resulting in 32 clusters, which are then annotated for cell types using known gene markers. Overlaying metadata including diagnosis (AD vs control) and sex on the clusters can provide insight into which cell types are enriched in AD versus control. Downstream analysis includes finding control versus AD cell type–specific differentially expressed genes, gene regulatory network analysis, trajectory analysis, and GO enrichment to further understand the AD associated changes and cell states transitioning from control to AD. AD, Alzheimer's disease; DGE, differential gene expression; GO, gene ontology; GRN, gene regulatory networks; hdWGCNA, high dimensional weighted correlation network analysis; NGS, next-generation sequencing; QC, quality control; snRNA-seq, single-cell RNA sequencing; UMI, unique molecular identifier.

TABLE 1 Recent transcriptomics, multi-omics, and spatial single nuclei RNA-Seq studies.

Study (Author, Year)	Cohort size + Assay	Brain region	Main takeaways
(Mathys et al., 2019)	48 individuals, snRNA-seq	Prefrontal cortex (PFC)	Profiled 80,660 single nucleus transcriptomes. Suggest a myelination-related processes in AD pathogenesis. Sex-specific differential transcriptional response to AD pathology. Males and Females differed in EX and OLIG responses to AD pathology. Significant association between the volume of white matter lesions in females and lower cognition.
(Grubman et al., 2019)	12 individuals, snRNA-seq	Entorhinal cortex	Profiled 13,214 nuclei. In AD individuals, *APOE* is repressed in OPC and AST, upregulated in MIC in AD. High expression of *LINGO1* and *NEAT1* in AD subclusters. *TFEB* gene, upregulated in diseased astrocytes, acts upstream of ten GWAS loci for AD (*BIN1*, *CLDN11*, *POLN*, *STK32B*, *EDIL3*, *AKAP12*, *HECW1*, *WDR5*, *LEMD2*, and *DLC1*).
(Del-Aguila et al., 2019)	Three individuals, snRNA-seq	Parietal lobe	Single-nuclei molecular atlas of AD brains carrying pathological mutations in *PSEN1* and related sporadic AD. Shows a decrease in EX neurons in brain carrier of *PSEN1*
(Zhou et al., 2020)	32 individuals, snRNA-seq	Dorsolateral PFC	Analyzed 66,311 nuclei from human brain AD. NEFL and NEFM were underrepresented in AD compared to normal samples. Higher AST in AD. Upregulation of *SORL1*, *A2M*, *CHI3LI*, *IRF8*, *IBA1*, *CD68*, *HLA-DR*, and homeostatic genes (*TMEM119*, *CX3CR1*, *P2RY12*) in AD. Signature of human glial cells in AD is distinct from DAM in 5XFAD model. Downregulation of genes promoting myelination in AD, for example, *STMN4*, *SEMA3B*, *MIR219A2*
(Morabito et al., 2020)	Five individuals, snRNA-seq	Frontal cortex	Profiled 27,321 nuclei on healthy aged human brains, excitatory, and inhibitory neuronal subpopulations significantly decreased with late stage pathology
(Leng et al., 2021)	10 individuals, snRNA-seq	Caudal entorhinal cortex (EC) and superior frontal gyrus (SFG)	Profiled 42,528 cells from the EC and 63,608 cells from the SFG. A specific subpopulation of excitatory neurons in the entorhinal cortex, characterized by *RORB* expression is highly susceptible to AD. Also found an AST subpopulation in AD with decreased homeostatic gene expression (reactive AST).
(Lau et al., 2021)	21 individuals, snRNA-seq	PFC	Profiled 169,496 nuclei. Reduced proportions of neuroprotective astrocytes and oligodendrocytes as well as the increased proportions of endothelial cells. Dysregulated pathways in endothelial cells are associated with angiogenesis and antigen presentation.
(Davila-Velderrain et al., 2021)	112 individuals, snRNA-seq	Hippocampus and entorhinal cortex	Profiled 489,558 nuclei. Early stage AD is characterized by dysregulation of cellular and cholesterol metabolism and is a diverging stage. Late stage AD is characterized by alterations in neurotransmission, cellular stress, apoptosis, and DNA damage across cell types. CA1 pyramidal neurons most transcriptionally altered, CA3 and dentate gyrus granule neurons the least.
(Yang et al., 2022)	17 individuals, snRNA-seq	Hippocampus and superior frontal cortex	Profiled 143,793 nuclei. Defined two subtypes of human pericytes, marked by solute transport and ECM organization. Selective vulnerability of ECM-maintaining pericytes and gene expression patterns that implicate dysregulated blood flow in AD. Vascular cells having an auxiliary role through shared endocytosis and inflammatory pathways
(Otero-Garcia et al., 2022)	Eight individuals, snRNA-seq	PFC	NFTs represent a cellular response rather than a direct cause of cell death
(Alsema et al., 2020)	27 individuals, snRNA-seq	Superior parietal lobe superior frontal gyrus	Transcriptomic differences between AD and controls were not detected but were present between AD and controls with Aβ plaques and/or hyperphosphorylated tau
(Marinaro et al., 2020)	16 individuals, snRNA-seq	PFC	Studied monogenic AD, found reduction in neurons (NeuN+) in monogenic AD patients with *PSEN1* or *APP* mutations, lower mRNA of neuronal and glial nuclei. *CLU*, *PTK2B*, *ABCA7*, *BIN1*, upregulated, and *SORL1*, *APP*, *PICALM*, *CNTNAP2*, *MEF2C* downregulated in neurons.

(Continues)

TABLE 1 (Continued)

Study (Author, Year)	Cohort size + Assay	Brain region	Main takeaways
(Gerrits et al., 2021)	18 individuals, snRNA-seq	Occipitotemporal cortex and fusiform gyrus	Profiled 482,472 nuclei. 2500 AD-associated differentially expressed genes in microglia. Two distinct AD-associated microglia profiles were identified that are associated with either amyloid-β (AD1) or hyperphospho-tau (AD2). AD1-microglia are similar to phagocytic/activated profiles in amyloid mouse models. AD2-microglia have not been identified before and may be tissue supportive or responsive to neuronal loss.
(Olah et al., 2020)	17 individuals, snRNA-seq	14 Dorsolateral PFC and 3 temporal cortex	Explored microglial heterogeneity from 16,242 cells, resulting in nine microglial subpopulation, with 1 subtype showing altered frequency in AD histologically. Unlike many mouse models with accelerated amyloid or tau proteinopathy, there does not seem to be a strong proliferative component to microglia in AD based on histological studies
(Xu et al., 2021)	Five individuals, snRNA-seq	PBMCs	Profiled 36,849 peripheral blood mononuclear cells from AD patients with amyloid-positive status and normal controls with amyloid negative status. Speculated that the peripheral adaptive immune response, especially mediated by T cells, may have a role in the pathogenesis of AD
(Gate et al., 2020)	18 individuals, snRNA-seq	Peripheral CD8+ TEMRA; CSF cells	Identified an adaptive immune signature of AD that consists of increased peripheral CD8+ TEMRA cell.
(Smith et al., 2019)	12 individuals, snRNA-seq	Entorhinal and somatosensory cortex	Profiled 52,706 astrocytes and 27,592 microglia. Soluble biomarkers of AD in astrocytes (*CLU*) and microglia (*GPNMB*). Astrocytes and microglia involved in pathological protein clearance and inflammation. Also showed glial transcriptional diversity in AD.
(Dileep et al., 2023)	47 individuals, snRNA-seq	PFC	Profiled 5821 cells post filtering. DNA double-strand breaks lead to mosaic genome structural variations and the disruption of 3D genome organization in neurons. Observed increased mosaic gene fusions caused by genome structural variations in excitatory neurons associated with increased cohesin, DNA damage, and senescence-like gene expression in AD.
(Mathys et al., 2023)	427 individuals, snRNA-seq	PFC	Profiled 2.3 million nuclei. Coordinated increase of the cohesin complex and DNA damage response factors in AD. Somatostatin inhibitory neuronal subset depleted in AD, while another inhibitory neuronal subtype linked with preserved cognitive in aged individuals.
(Corces et al., 2020)	39 individuals, Multi-omics	Isocortex, striatum, hippocampus, and substantia nigra	snATAC-seq + H3k27ac HiChIP + Bulk ATAC-seq Analyzed profile accessibility of 70,631 individual cells. Provided epigenetic characterization of the role of inherited noncoding variation in AD and PD. Predicted functional SNPs, nominated gene and cellular targets for each noncoding GWAS locus such as *BIN1* in AD and *STAB1* in PD. Epigenomic analysis of the *MAPT* locus showed a long-distance putative regulatory element located 650 kb upstream of the *MAPT* gene that showed elevated interaction with the *MAPT* promoter specifically in the H1 haplotype. They also identified H2-specific 3D interactions between a putative domain boundary upstream of *MAPT* and the region surrounding the *KANSL1* promoter located 330 kb downstream of *MAPT*
(Morabito et al., 2021)	20 individuals, Multi-omics	PFC	snRNA-seq + snATAC-seq Multi-omics profile of 191,890 in late-stage AD. Identified cCREs and associated target genes in AD in a cell-type specific manner. Observed differential enrichment of *SREBF1* motif with decreased accessible binding sites in AD and decreased gene expression of *SREBF1* in oligodendrocytes.

(Continues)

TABLE 1 (Continued)

Study (Author, Year)	Cohort size + Assay	Brain region	Main takeaways
(Anderson et al., 2023)	15 individuals, Multi-omics	Dorsolateral prefrontal cortex	snRNA-seq + snATAC-seq Multi-omics profile of 105,332 nuclei in AD. Identified cell-type specific peak-gene TF trios to identify key TF's driving AD and identified *ZEB1* and *MAFB* in AD specific trios in neurons and microglia respectively.
(Gabitto et al., 2023)	28 individuals, Multi-omics	Middle temporal gyrus	snRNA-seq + snATAC-seq Report high-quality expression profiles for roughly 1.2 million nuclei (14k per donor), chromatin landscapes for 580,000 nuclei (7k per donor), and combined expression and epigenomic profiles for 140,000 nuclei (5k per donor). Results suggest that SA donors undergo global chromatin repression and shutdown of transcription, consistent with previous reports studying familial AD in which chromatin re-organization triggered neuronal identity repression and dedifferentiation
(Sun et al., 2023)	557 individuals, Multi-omics	PFC, mid-temporal cortex, angular gyrus, entorhinal cortex, thalamus, and hippocampus	snRNA-seq + snATAC-seq Reported that microglia may retain a relatively permissive chromatin landscape that is crucial to allow dynamic state transitions in response to microenvironment changes. Transitions can be mediated via the transcriptional activity of master regulator TFs. Poor capture of microglia transcriptional state diversity by chromatin accessibility
(Xiong et al., 2023)	92 individuals, Multi-omics	PFC	snRNA-seq + snATAC-seq Multi-omics profile of 850,00 nuclei in AD. Showed enrichment of AD-risk loci in microglial enhancers with specific TFs such as *SPI1*, *ELF2*, and *RUNX1*. Demonstrated the loss of cell-type identity driven by epigenomic erosion in late-stage AD
(Miyoshi & Morabito et al., 2023)	Human: 39 Mice: 80, Spatial Transcriptomics	Human: cortex Mice: coronal sections of brain hemisphere	Using 10x Genomics Visium, authors studied spatial and temporal dynamics of gene expression in the disease pathogenesis of both sporadic AD and AD in DS by using spatial transcriptomic. Found systems-level differences in the transcriptome between female and male.
(Chen et al., 2020)	Mice: 12 Human: 3 end stage AD and 3 non-demented, Spatial Transcriptomics	Mouse: sequential 10-μm coronal sections Human: Superior Frontal Gyrus	Using reverse transcription followed by sequencing, this paper characterizes two gene co-expression networks responsive to Ab deposition. (1) The 57 plaque-induced genes (PIGs) are involved in complement, endosomes, oxidation-reduction, and inflammation. (2) Oligodendrocyte genes (OLIGs) are involved in myelination and are depleted in high amyloid accumulation.
(Choi et al., 2023)	Mice $n = 4$, Spatial Transcriptomics	10-μm coronal sections	Using 10x Genomics Visium, the authors reported gene patterns that change according to disease progression in each brain region. Initial molecular changes related to glial cell activation in WM before the changes in GM.
(Chen et al. 2022)	Human: 3 Control, 3 AD, Spatial transcriptomics	Middle temporal gyrus	Using 10x Genomics Visium, authors reported anatomical architecture of cortical laminae and the WM, identified unique gene signatures and biological pathways that may contribute to the vulnerability of various AD pathology.

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E; AST, astrocytes; cCRE, candidate cis-regulatory element; CSF, cerebrospinal fluid; DAM, disease-associated microglia; DSBs, double stranded breaks; EX, excitatory neurons; GM, gray matter; GWAS, genome-wide association studies; MIC, microglia; NEFL, Neurofilament Light chain; NEFM, Neurofilament Medium Chain; NFT, neurofibrillary tangle; OPC, oligodendrocytes; PD, Parkinson's disease; SA, severly affected; SNP, single nucleotide polymorphism; snRNA-seq, single-nucleus RNA-sequencing; TEMRA, effector memory T cells re-expressing CD45RA; TF, transcription factor; WM, White matter.

in neurodegenerative disease as disruptions in lipid metabolism can adversely affect myelin homeostasis.[84] A commonly referred risk factor in AD is the *APOE* gene, mainly expressed by astrocytes and microglia. Apolipoproteins, such as apoB, apoE, and apoJ, as well as apoC3 and apoA1, combine to form soluble lipoproteins such as high-density lipoprotein (HDL), playing a vital role in lipid transport in the blood and cerebrospinal fluid (CSF).[85]

GWAS have identified the ε4 allele of *APOE* as a genetic risk factor for sporadic late-onset AD,[26] while the *APOE* ε2 allele has emerged as a strong genetic protective factor from extensive large-scale

meta-analyses.[26] NPC intracellular cholesterol transporter 1 (*NPC1*), which encodes a cholesterol transporter, has been implicated in AD. Loss of *NPC1* triggers enhanced phagocytic uptake and impaired myelin turnover in microglia that precede neuronal death.[86] AD is also characterized by DNA damage at neuronal enhancers and promoters. Immune-related genes including *TREM2* (involved in lipid metabolism in microglia), *CD33*, and *HLA-DR* as well as microglia with disease-associated microglia (DAM) transcriptional profiles that are localized to plaques also are highlighted in AD. Zhou et al. (2020) found that certain genes, such as *HLA-DR* and *APOE* in microglia, and *QDPR*, *CA2*, and *SLC38A2* in oligodendrocytes—previously noted by Mathys et al. as upregulated—also showed increased levels in their AD samples.[51,56] The consolidated findings from Lau et al., Mathys et al. (2019), and Grubman et al. (2019), suggesting that despite differences in study cohorts, brain regions sampled, preparation methods, and sequencing techniques, there is a consistent observation: Dysregulated pathways in neurons are commonly associated with synaptic signaling, while those in oligodendrocytes frequently pertain to myelination issues.[49,56,87]

Additionally, some microglia in AD display binding affinity for *SPI1*, which encodes the transcriptional repressor PU.1. In brain endothelial cells forming the BBB, genes like *CLDN5* and *SLC2A1*, along with various adhesion molecules, exhibit altered expressions. The contractility of pericytes, which controls vascular dynamics, is also implicated in the reduced cerebral blood flow associated with AD.[20]

3.1.4 | SnRNA-seq findings in mouse models

Mouse models have been fundamental in advancing our understanding of AD and are typically developed using transgenes that overexpress human AD associated genes. Mathys et al. used CK-p25 mouse models, which exhibit a predictable pattern of neurodegeneration within a compressed time frame.[88] Two weeks post p25 induction, in these mice show DNA damage and increased Aβ levels, progressing to neuronal and synaptic loss with cognitive impairment by 6 weeks, with NFT-like pathology 27 weeks post p25 induction.[88] The authors used this mouse model to study the microglial response to neuronal cell death since the p25 transgene is strictly expressed only in excitatory neurons.[88] The authors identified two distinct neurodegeneration-associated microglia cell states that differ from those in a healthy brain: an early-response state and a late-response state.[88] Late-response microglia express elevated gene expression levels in several genes that were also observed to be upregulated in DAM including *Cd9*, *Itgax*, *Clec7a*, *Cd63*, *Spp1*, *Fth1*, *Axl*, *Lpl*, *Cst7*, *Ctsb*, *Apoe*, that is also accompanied by morphological changes.[88] Of the 278 genes significantly upregulated in DAM, 202 were also significantly upregulated in late-response microglia, suggesting a notable overlap between the expression profiles of DAM and late-response microglia. This observation is consistent with the idea that the DAM program may be a primed set of genes that is expressed in response to various homeostatic disturbances.[88]

Loss of TREM2 function in AD mouse models hampers the microglial ability to surround Aβ plaques, proliferate, and transition to the DAM state.[51] The DAM subtype was introduced using the 5xFAD transgenic mouse model, which harbors five mutations in *APP* and *PSEN1* human genes resulting in excessive amyloid pathology, but no tangles.[44,89] Using this mouse model, Zhou et al. investigated gene-expression changes in AD pathology and TREM2 using snRNA sequencing.[51] They discovered a *Trem2*-dependent DAM and a novel reactive oligodendrocyte population characterized by Serpina3n+C4b+ markers in mice, with implications for axonal myelination and metabolic responses to neuronal degeneration.[51] Astrocyte profiles suggested a disruption in metabolic coordination with neurons.[51] Moreover, TREM2-R47H and TREM2-R62H carriers displayed a diminished reactive microglia phenotype, highlighting TREM2's role in AD across both mouse and human species.[51] Overall, scRNA-seq and related techniques have revolutionized our understanding of AD by providing a granular view of cellular behavior, heterogeneity, and spatial context. These findings hold the potential to propel forward diagnostics and therapeutic strategies, ultimately improving patient outcomes.

3.2 | Spatial transcriptomics reveal the role of cellular interactions and spatial heterogeneity in AD

In AD, microglia and astrocytes undergo abnormal proliferation and morphological changes giving rise to gliosis, another prominent AD histopathology.[20] Following this ideation, isolation of single cells and cell types allow for understanding specific cell type responses and reactions to AD pathology. A successful scRNA-seq experiment requires the isolation of cells from whole tissue, a challenging task due to the need to avoid cellular stress or death. Neurons, astrocytes, and oligodendrocytes require specialized tissue dissection protocols for isolation compared to microglia; however, single-nucleus approaches have emerged as a viable alternative.[49] A major limitation of single-nucleus studies is the loss of spatial and environmental context. This is particularly relevant when examining the impact of amyloid plaques on the surrounding cells, which is not discernible once nuclei are isolated from their native environment.[90] The role of plaque-associated glial responses in AD is an ongoing area of study. A study by Serrano-Pozo et al. suggested that the microglial response is proportional to the size of dense-core plaques, indicating a potential chemotactic effect of Aβ on microglia. By contrast, the size of these plaques does not appear to have a similar influence on the astrocyte response.[91] The authors also reported a significantly increased density of astrocytes and microglia in the vicinity of dense-core plaques, compared to regions located further away (more than 50 μm).[91] Considering the importance of molecular proximity and its effects on interactions, performing transcriptomics on intact tissues would yield a more accurate understanding of cell changes in response to environmental cues such as the plaques and toxic aggregates. Methods that enable extraction of spatial information in intact tissue are referred to as spatially resolved transcriptomics, or spatial transcriptomics (ST). Capturing information about cell position and its location relative to neighboring cells and tissues provides

valuable insight for understanding the cell state. In the field of neuroscience, ST removes the need for tissue dissociation of neurons, and preserves the spatial information of cells.

3.2.1 | Methods in ST

Currently, there are two common methods to profile transcriptomes while preserving spatial information. The first is by imaging mRNAs in situ via microscopy. This is the foundation of imaging-based spatial transcriptomics technologies. In situ imaging of mRNAs necessitates a strategy for differentiating among various mRNA species, for which there are two primary methods. One is hybridization of mRNAs to fluorescently labeled, gene-specific probes. Hybridization refers to polymerization of single-stranded mRNAs to single-stranded probes with a complementary sequence. This spatial transcriptomics technique is thus called in situ hybridization (ISH). The other is in situ sequencing (ISS) of amplified mRNAs, in which transcripts are directly sequenced inside a tissue block or section by sequencing by ligation (SBL) technology. Among imaging-based technologies we highlight ISH-based methods and ISS-based methods. Second, the other broad method of spatial transcriptomics is to extract mRNAs from the tissue while preserving spatial information and subsequently profile mRNA species via NGS techniques. This is the foundation of sequencing-based spatial transcriptomics technologies (sequencing referring to NGS rather than ISS). Common methods of preserving spatial information are (1) via direct capture and recording of location, such as via microdissection and microfluidics; and (2) via ligation of mRNAs to spatially barcoded probes in a microarray. An important goal in the field of neurodegenerative research and single-molecule analysis is to achieve true single-cell resolution and to capture the entire genomic expression profile. Table 2 represents currently available and widely used spatial technologies.

Current spatial analysis methods are limited to DGE comparisons between conditions and clusters.[52] To overcome patient-specific heterogeneity observed in clustering analysis, non-negative matrix factorization (NMF) was utilized to identify shared expression modules.[92] Although matrix factorization is commonly used in scRNA-seq analysis, the most suitable approach for inferring biologically meaningful gene expression programs remains unclear. A method called consensus NMF (cNMF) accurately inferred identity and activity programs, including their relative contributions in each cell.[92] Additionally, Morabito et al. introduced the hdWGCNA method to identify co-expression network modules,[13] showcasing its application in a mouse brain spatial dataset. In sequencing-based spatial transcriptomics, expression profiles are localized to spots, each of which may contain more than one cell. The study addressed the issue of data sparsity in single-cell and spatial transcriptomics by using "metacells," which are created by aggregating transcriptionally similar cells. In a parallel approach, "metaspots" are formed by aggregating adjacent spatial transcriptomic spots.[13] Module eigengenes (MEs) were used as metrics to summarize the gene expression of a given co-expression module. Other analyses included using CellChat[78] to gain better insights into the functional communication between cell types and their surrounding locations.

3.2.2 | Findings from ST studies

An important question in AD is the relationship between the neurodegeneration process in AD and amyloid plaques, and whether proximity would induce trigger the pathogenic response by Aβ deposition. Chen et al. used a combination of spatial transcriptomics and in situ sequencing on mouse and human brain to demonstrate multicellular gene co-expression networks in AD.[90] In detail, the authors obtained three adjacent coronal sections by cryo-sectioning mouse brains from $APP^{NL\text{-}G\text{-}F}$ and C57BL/6 mice 3, 6, 12, and 18 months of age. Every coronal section contained more than 500 transcriptomic profiles of individual tissue domains (TDs), adding up to 10,327 transcriptomic profiles over 20 coronal sections. Each TD was annotated with spatial, pathological, and cellular information. With ST, the authors measured in situ in hundreds of small TDs genome wide transcriptomics changes induced by amyloid plaques. An orthogonal in situ sequencing method visualizing hundreds of selected transcripts with cellular resolution was also performed. The analysis showed two gene co-expression networks highly responsive to accumulating amyloid plaques. Utilizing WGCNA analysis, the authors identified 57 plaque-induced genes (PIGs) over multiple cell types including microglia and astroglia and in pathways related to oxidation-reduction and inflammation.[90] PIGs are gradually co-expressed with increasing Aβ load in $APP^{NL\text{-}G\text{-}F}$ mice. The second network, oligodendrocyte genes (OLIGs), included genes involved in myelination and mainly expressed by oligodendrocytes. An OLIG is activated under mild amyloid stress but becomes depleted in microenvironments with high amyloid accumulation. This study corroborates the association between DAM and amyloid plaques. The authors detected $31{,}283 \pm 7441$ unique molecular identifiers and 6578 ± 987 unique genes per TD. They aligned each coronal section with 14 anatomical brain regions defined by the Allen Brain Atlas. Each TD was assigned to one of them. The number of TDs varied between 112 (entorhinal cortex) and 2114 (thalamus).[90] Their spatial transcriptomics analysis therefore shows that that proximity to amyloid plaques induce gene expression in inflammation, lysosomal degradation, and endocytosis. Authors also report oligodendrocytes specific changes, specifically, increase in myelination gene expression in response to proximity with plaques.[90]

In a study conducted by Lu et al., scRNA-seq and spatial transcriptomics on mouse brain tissue were conducted to investigate transcriptional changes in major brain cell types and regions in response to acute peripheral inflammation.[93] Their findings reveal cell type and spatial-specific molecular responses, suggesting dysregulation of the BBB and blood-CSF barrier, as well as molecular alterations in the amyloid plaque microenvironment. Spatial transcriptomics unveiled locally specific transcriptional signatures in the APP/PS1 mouse brains, with notable gene expression responsiveness to staph infection observed in the ventricular surroundings. The data further demonstrated that the choroid plexus (CP) and its ventricular microenvironment responded

TABLE 2 Spatially resolved transcriptomics platforms.

Platform	Capture/ Profile area	Tissue	Type	Advantages and limitations
Vizgen Merscope	1 cm^2	Frozen/FFPE	In situ imaging	- Up to 500 targets - mFISH - Single cell resolution - Not whole genome
NanoString CosMx	1.5 cm^2	Frozen/FFPE	In situ imaging	- 1000 RNA and over 60 proteins - mFISH - Single cell resolution
Akoya Biosciences (Codex)	Resolution of 260 nm	FFPE	In situ imaging	- Ab-based - RNA and protein detection - 50 + cellular markers at the single-cell level
Rebus Biosystems	3 cm^2	Frozen	In situ imaging	- mFISH - Up to 30 genes simultaneously - No protein markers
10x Xenium	12×24 mm^2	Frozen/FFPE	In situ imaging	- RNA and protein detection - mFISH - single cell resolution
10x Visium	6.5 mm^2 (5000 spots)	Frozen/FFPE	Spatial NGS	- Whole Transcriptome Mapping - RNA and protein detection - Not single cell resolution.
Curio	3 mm^2	Fresh frozen	Spatial NGS	- Whole genome - single cell resolution
NanoString GeoMX	5 μm^2 to a maximum of 660×785 μm^2	Frozen/FFPE	Spatial NGS	- Simultaneous ST (thousands to tens of thousands of genes) and SP (1 nuclear and 3 surface markers)

Abbreviations: FFPE, Formalin-Fixed Paraffin-Embedded; mFISH, multicolor fluorescence in situ hybridization; NGS, next-generation sequencing; SP, spatial proteomics; ST, spatial transcriptomics.

to acute staph inflammation, displaying upregulated specialized CP epithelial cell-related genes and ion transport genes.[93]

Understanding regional susceptibility in AD has become possible by the means of spatial RNA-seq. In a recent publication by Miyoshi & Morabito et al., researchers conducted a comprehensive transcriptomic survey of AD using ST and snRNA-seq techniques. They analyzed cortical samples from various AD stages, including early-stage AD, late-stage AD, and AD in individuals with Down syndrome.[58] Their study spotlighted significant changes in *ANGPTL* and *CD99* signaling, revealing how astrocytes influence brain vascular integrity in AD. By pinpointing downstream targets of AD-related astrocyte changes, the researchers shed light on the modulation of astrocytes in AD. Utilizing multiscale co-expression network analysis, they identified 166 gene modules across different cortical layers, which they further condensed into 15 cortex-wide "metamodules." Crucially, these modules not only revealed spatial patterns of gene expression but also unveiled temporal patterns.[58] To enhance cross-species comparisons, the researchers performed ST on 5xFAD and wild-type mouse models. These comparisons illustrated dysregulated transcriptomic programs common to different species in AD. The researchers identified an upregulation of the glial metamodule M11 in the cortical upper layers during the disease progression. This module contained DEGs shared between sporadic AD and AD in individuals with Down syndrome. Cell signaling analysis revealed *CD99* and *ANGPTL4* as hub genes in M11. Furthermore, the study highlighted the presence of M11 in regions with amyloid deposition in both mouse and human samples. This underscores the pivotal role of M11's associated biological processes and genes in AD pathophysiology.[58]

3.3 | Single-nuclei multi-omics approaches uncover complex regulatory mechanisms in AD

In the quest to understand the basis of AD, the analysis of snRNA-seq has been instrumental in elucidating aberrant gene expression profiles particularly in the context of different cell types in the brain.[56] While snRNA-seq has been helpful in this aspect, it does have limitations in providing a more comprehensive understanding of the underlying mechanisms responsible for driving these gene expression changes. Interestingly, a large proportion of genetic variants identified through GWAS for AD are located within the noncoding regions of the genome and might exert phenotypic effects by disrupting gene regulatory elements such as promoters, enhancers, and silencers. For instance, the intronic variant rs405509 within the promoter region of the *APOE* gene, the major risk gene for AD, is associated with an increased risk for AD in different populations.[22,27,94,95] Consequently, there has been an increasing emphasis on using single-nucleus assay for transposase-accessible chromatin sequencing (snATAC-seq), a sequencing technique that captures chromatin accessibility that contain active regulatory elements, to study AD. In a study by Corces et al.,

snATAC-seq was performed on samples spanning different regions of the brain, uncovering AD-associated single nucleotide polymorphisms (SNPs) enriched in microglia-specific chromatin accessible regions.[25] This finding lends further support to the increasingly recognized role of microglia in AD.[20,96] Furthermore, studies on mouse models of AD have also proposed that alterations in chromatin accessibility may be a driver for AD pathogenesis.[97] While ATAC-seq can be instrumental in identifying genomics loci that might be linked to AD, it falls short in identifying downstream gene targets.

The integration of multiple single-cell omics modalities, commonly referred to as single-cell multi-omics (scMulti-omics), is essential towards uncovering intricate relationships and interactions between different molecular layers providing valuable insights into the regulatory networks of diverse cell types and cell states. In the realm of AD research, the most prevalent multi-omics approach involves the concurrent capture of gene expression (via snRNA-seq) and chromatin accessibility (via snATAC-seq) from the nucleus of the same cells or similar cell populations as shown in Figure 2. The complementary information of snRNA-seq and snATAC-seq helps capture distinct aspects of cellular biology and this combination provides a more holistic view of regulatory mechanisms at the cell-type level. Several advantages arise from an integrative analysis of the two modalities such as the ability to correlate gene expression and chromatin accessibility in the same cell, which provides insights into regulatory mechanisms driving gene expression. Studies have also shown improved abilities in cell-type annotation and identifying finer cell subtypes using both snRNA-seq and snATAC-seq.[98] With snRNA-seq, the clusters are annotated based on expression levels of known cell-type marker genes, whereas in snATAC-seq the clusters are annotated based on chromatin accessibility at the promoter regions of the known cell-type marker genes, and combining the two contrasting approaches leads to more robust cell-type annotations.

ScMulti-omics datasets are generally classified into two categories: matched and unmatched. In matched datasets, multiple omics modalities are simultaneously captured from the same individual cells, whereas in unmatched datasets, different omics modalities are obtained separately from different but comparable samples. Unmatched datasets exhibit a higher variability compared to matched datasets, since different omics layers are obtained from distinct cells and experimental protocols. Yet, analysis of unmatched datasets is a popular approach due to the substantial volume of published single modality data that can be leveraged to unravel meaningful biological insights.

The first attempt to use both snATAC-seq and snRNA-seq on the AD brain to characterize diseases-associated cellular dysregulation was done by our group.[23] Through an integrated analysis of both modalities, we identified target genes of candidate cis-regulatory elements (cCREs) by curating a subset of co-accessible peaks in which one of the peaks was situated within a promoter element. By further correlating the expression of the candidate target gene to chromatin accessibility for the subset of co-accessible links, we curated a set of gene-linked cCREs (gl-cCREs). From our analysis, a substantial overlap between cCRE-linked genes and genes upregulated in AD within specific cell types was observed, highlighting a potential role of CREs in disease-related gene expression changes. Additionally, we constructed cell-type specific TF-gene networks to get a more holistic view of the regulatory landscape and identified several genes, especially *SREBF1* whose motifs were significantly reduced in AD and the gene expression to be downregulated in AD oligodendrocytes. Anderson et al. (2023) recently published a matched multi-omics study of snRNA-seq and snATAC-seq on both control and AD samples and introduced an approach called peak-gene-TF "trios" that involves (1) establishing a correlation between accessibility of a linked peak and expression of associated genes, (2) correlating accessibility of linked peak to expression of TF whose motif resides within that peak, and (3) correlating expression of the TF and the linked gene.[99] Using this approach, cell type–specific and AD-specific trios were identified and interestingly *ZEB1* and *MAFB* were found to be enriched in AD-specific trios in neurons and microglia respectively. Additionally, they performed stratified linkage disequilibrium score (sLDSC) regression and identified that AD-specific microglia-linked peaks were enriched for AD-associated SNPs. Xiong et al. similarly observed a significant enrichment of microglial enhancers at AD risk loci containing increased binding sites for two microglial transcriptional regulators, *RUNX1* and *SPI1*.[100] More interestingly, they reported significant loss in cell identify in late-stage AD characterized by epigenetic alterations such as decreased enrichment at transcription start sites.

However, around 30% of the noncoding variants associated with AD are located within known enhancers and function as expression quantitative trait loci (eQTLs) for at least one gene.[101] Interestingly, the majority of these genes are differentially expressed in AD and colocalize with their eQTL variants within the same topological associated domains (TADs). Several tools have been developed to discern long-range chromatin interactions such as Hi-C, ChIA-PET, Hi-ChIP, and so on., that offer a critical lens into the intricate orchestration of genomic functionality, specifically by pinpointing genes that are under the influence of distal regulatory elements.[102–104] To study the role of cell type–specific promoter-enhancer interactions in the context of AD, a study used a combination of epigenetic assays such as ATAC-seq, PLAC-seq, and HiChIP.[105] By deleting a microglia-specific enhancer 30 kb upstream of *BIN1* promoter, they observed a reduced *BIN1* expression in microglia, but not in neurons or astrocytes. In another study, Hi-C was performed on AD brain samples and a notable enrichment of chromatin loops upstream of the *BIN1* promoter overlapping AD risk variants was reported, lending further support to the existence of long-range regulatory mechanisms related to *BIN1*.[106] In a very recent study, analysis of snRNA-seq and Hi-C from AD brain samples revealed increased somatic mosaic gene fusion events due to DNA double stranded breaks (DSBs) in excitatory neurons. Regions with altered 3D genome organization were enriched for DSBs leading to genome instability, and align with gene expression changes, specifically cohesin which is involved in chromatin looping and DSB repair.[107]

However, several challenges persist in the successful application of scMulti-omics to study AD. First, multimodal data integration is one of the major obstacles in accurate multi-omics analysis. The integration of multiple modalities, each with its own technical protocols and

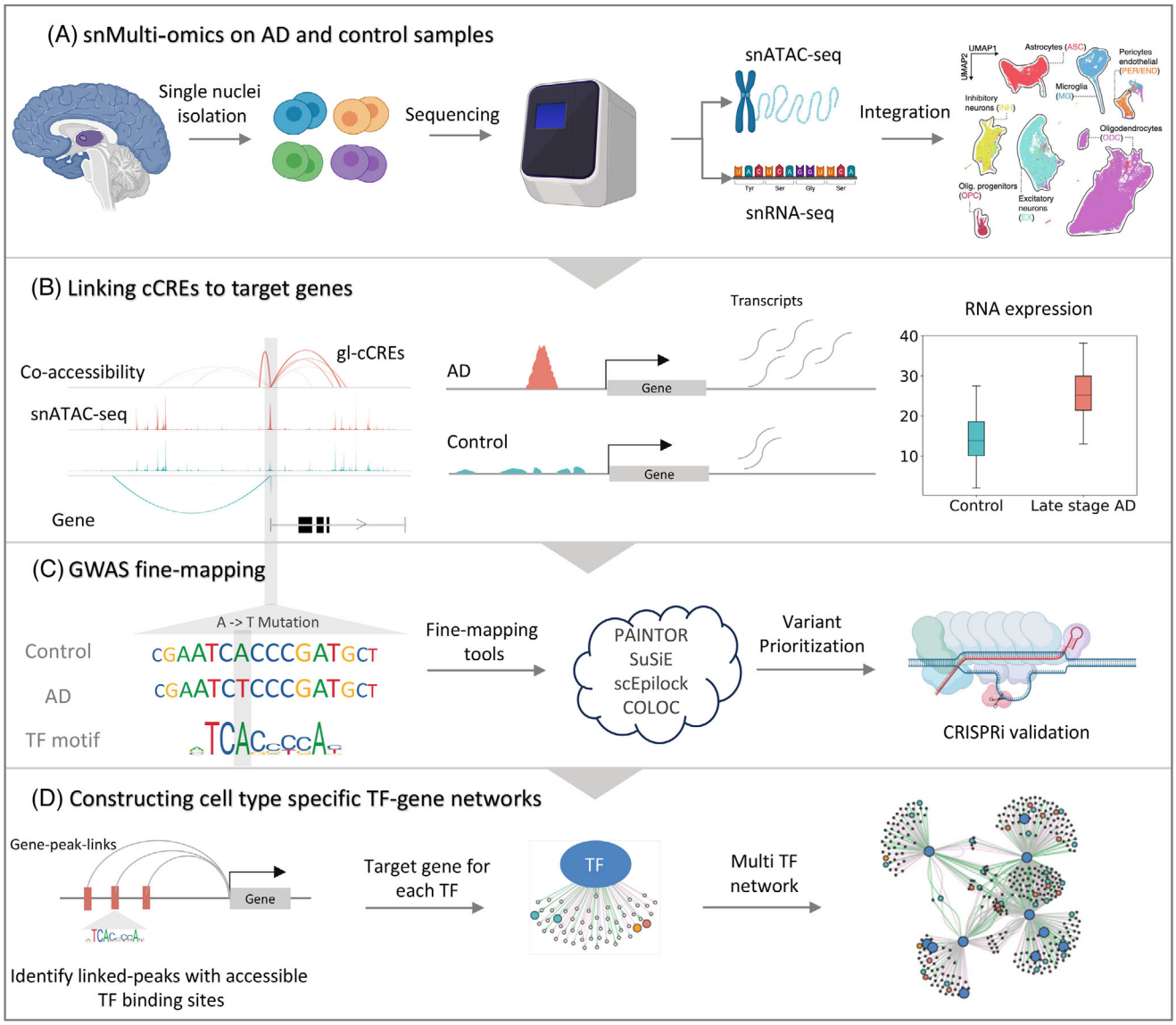

FIGURE 2 Schematic workflow of scMulti-omics (RNA+ATAC) data acquisition and analysis. (A) Single cells are isolated from specific brain regions and single-cell multi-omics sequencing is performed, followed by computational integration of the two modalities. (B) ATAC peaks are linked to target gene via correlation of chromatin accessibility and gene expression to subset a list of gl-cCREs. Further analysis to link aberrant chromatin accessibility to differential gene expression across control and AD samples in individual cell types. (C) Cell type–specific GWAS fine-mapping to identify variants that influence AD using computational and experimental approaches. (D) Gene-peak links are identified to construct transcription factor gene networks in each cell-type. AD, Alzheimer's disease; ATAC, assay for transposase-accessible chromatin; cCRE, candidate cis-regulatory element; gl-cCRE, gene-linked cCRE; GWAS, genome-wide association studies; scMulti-omics, single-cell multi-omics; snATAC-seq, single-nucleus ATAC sequencing; snRNA-seq, single-nucleus RNA sequencing; TF, transcription factor.

throughput levels, is particularly challenging.[108] Effective integration must address and reconcile these technical differences and batch effects, while preserving the biologically relevant information. Additionally, certain modalities might be affected by confounding factors across features which adds additional challenges. Integration strategy can be broadly classified into vertical or diagonal depending on whether the different data modalities are captured from either the same (matched) cells or different (unmatched) cells. Vertical integration strategies leverage the clear correspondence between molecular profiles within matched multimodal experiments. These strategies establish certain cells or cell clusters as anchors to link the different data modalities. Examples of such methods include SCENIC+, scMVAE, MIRA, and so on.[109–111] In unmatched datasets, there are no anchors to link either the cells or features in the high-dimensional space, making diagonal integration more difficult to validate and interpret. Diagonal integration typically aims to construct a low-dimensional latent space that captures the correlation between the data modalities, however even if gene expression and chromatin accessibility are correlated there is no guarantee that the latent representation can capture this information, making it a difficult endeavor. Commonly used diagonal

integration methods include GLUE, LIGER, Cobolt, MultiVI, and Seurat V5, to name a few.[112–116] Second, current computational analysis methods are limited in their ability to learn the intricate relationship and cross talk between different data modalities. For instance, computational methods for variant fine-mapping can potentially benefit from incorporating multi-omics data; however, existing methods do not leverage multi-omics information.[117–119] This acts as a barrier in the application of sc-multi-omics for inferring the underlying biological mechanism, and their response to external stimuli. Third, as data complexity increases, such as when dealing with multiple modalities and multiple samples, the need for computational efficiency becomes paramount. Ensuring scalability to effectively manage vast amounts of data becomes imperative in such scenarios. The rising popularity of large language models (LLMs) has spurred numerous efforts to apply these models in developing computational methods for single-cell multi-omics. An example is scGPT, a pretrained generative model that utilizes a masked language model training strategy.[98] When fine-tuned, scGPT has surpassed state-of-the-art methods in various tasks, including cell-type annotation and data integration, demonstrating the potential of LLMs in genomic applications. Enformer presents another use of language model, specifically tailored for predicting gene expression from DNA sequences.[120] The Enformer architecture uniquely combines convolutional neural networks (CNNs) with transformers to enhance computational efficiency. However, the application of these models in genomics is not without its challenges. The quadratic complexity inherent in transformer models, coupled with their substantial requirements for graphics processing unit resources and extensive training data, presents significant hurdles. Moreover, a critical limitation of these models lies in their current lack of mechanistic interpretability regarding their predictions, which is a vital aspect for broader application and understanding in genomics. Despite these challenges, it is important to recognize that the development of computational methods for the analysis and integration of sc-multi-omics is still in its infancy and represents an exciting and rapidly evolving avenue, with the potential for significant improvements in the near future.

4 | CONCLUSION AND FUTURE DIRECTIONS

As we continue to learn about the complexities of AD and related dementias, several areas of research can be explored to better capture the disease. One area emphasizes the need for an integrative approach that melds mouse and human cell type–specific data to improve the representation of AD in mouse models for translational research. Looking forward, some key open questions to focus on include: (1) How can we more accurately model human-specific AD changes in mouse models to enhance translational validity? (2) What are the shared and unique gene expression patterns between mouse models and human AD, and how do these findings translate into human-specific pathological signatures? and (3) To what extent can module preservation and weighted co-expression network analysis illuminate human-specific changes, and how can these findings be validated using unexplored datasets and wet lab experimentation?

As we aim to achieve true single-cell resolution with spatially resolved transcriptomics and harness long-read sequencing to identify isoforms within tissue structures, the challenge is to refine these technologies. Future directions should also aim to integrate spatial long-read transcriptomics to unravel the role of alternative splicing in AD pathology at a single-cell resolution. The burgeoning field of spatial transcriptomics, despite its rapid expansion and influx of new technologies and datasets, faces limitations in resolution, sensitivity, throughput, and accessibility. Compatibility with paraffin-embedded tissues opens a retrospective window into decades of biobanked samples, and future innovations may allow for the reconstruction of 3D organ-level atlases and real-time visualization of transcriptomic changes.

Finally, a comprehensive understanding of AD will require the integration of transcriptomics with epigenetics and proteomics at single-cell resolution. Single multi-omics approaches, such as snATAC-seq, sn-ChIP-seq, and innovative technologies like Paired-Tag, hold promise for creating cell type–resolved maps of chromatin state and interactions. Optimizing protocols to concurrently preserve RNA quality and chromatin state is a critical hurdle to overcome. Moreover, spatial proteomics at single-cell resolution may elucidate how and to what extent AD risk transcripts are translated into proteins and how these proteins interact with pathological processes. These are the frontiers that future studies should navigate to uncover the molecular intricacies of AD and propel us towards effective interventions.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Samuel Morabito and Zechuan Shi for their valuable feedback and discussions. Funding for this work was provided by National Institutes on Aging 1RF1AG071683, U54 AG054349-06 (MODEL-AD), Adelson Medical Research Foundation funds to V.S, National Institute of Health (NIH) Institutional National Research Service Award (T32) 1T32GM136624-01 to N.R., and by National Institute of Health grant R01NS128523 to J.Z.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. Author disclosures are available in the Supporting Information.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Rahimzadeh N, Srinivasan SS, Zhang J, Swarup V. Gene networks and systems biology in Alzheimer's disease: Insights from multi-omics approaches. *Alzheimer's Dement*. 2024;20:3587–3605.
https://doi.org/10.1002/alz.13790