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Single Cell RNA Sequencing and Emerging Transcriptomic Technologies in Alzheimer's Disease Cerebral Organoids:

A Mini Review

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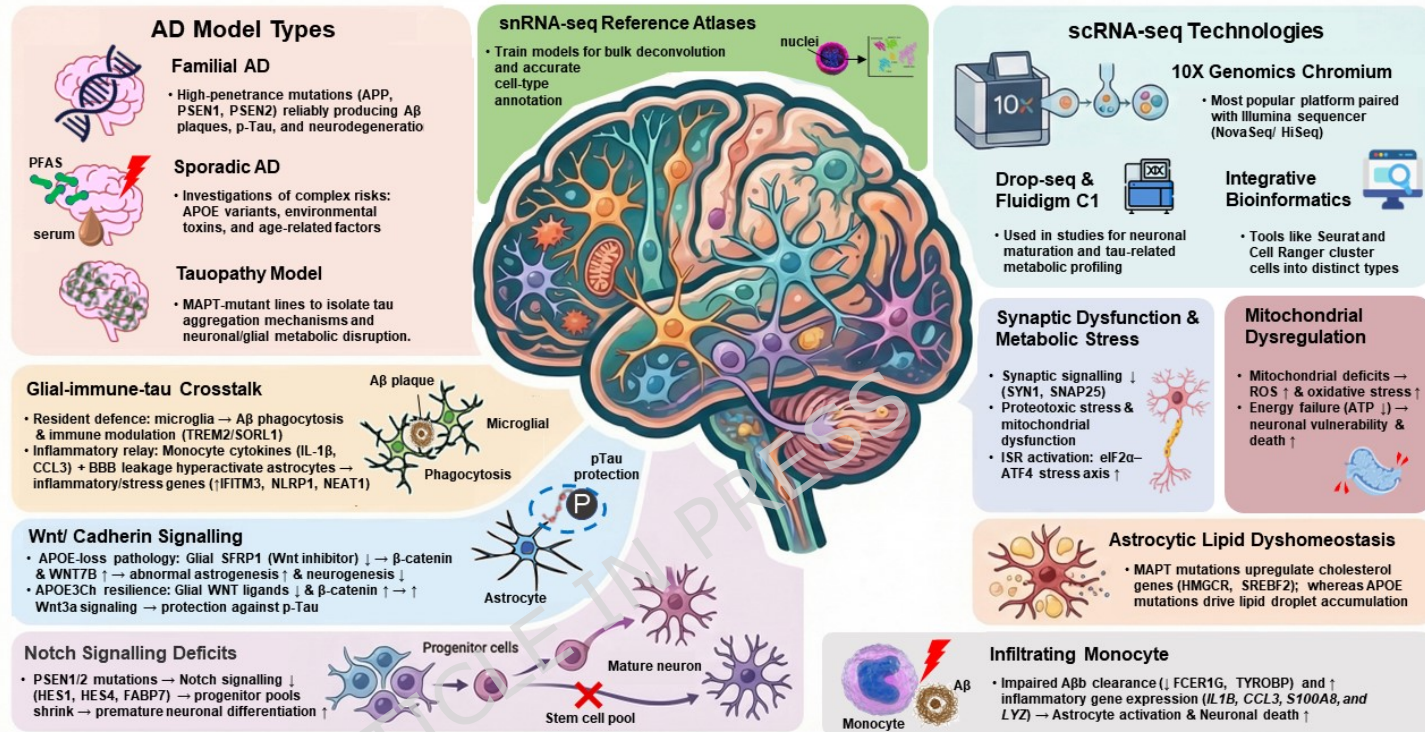
Abstract (237 words)

Alzheimer's disease (AD), the most common cause of dementia worldwide, presents a formidable challenge due to its complex, multifactorial etiology, and limited translational success of existing models. Recent advances in stem cell biology have enabled the generation of three-dimensional human cerebral organoids which exhibit multicellular complexity and self-organisation, recapitulating key aspects of fetal brain development *in vitro*. This mini review highlights the landscape of AD modelling in human cerebral organoids. While studies using single cell RNA sequencing (scRNA-seq) form the core of this review, we also discuss single-nucleus RNA sequencing, computational deconvolution, and spatial approaches as translationally relevant extensions. We categorise current organoid research into distinct model types: (1) familial AD models driven by *APP*, *PSEN1*, and *PSEN2* mutations; (2) sporadic AD models incorporating genetic *APOE* variants and environmental risk factors (serum exposure, pollutants, and viral reactivation); (3) tauopathy models highlighting astrocyte-driven metabolic disruption; (4) models featuring glial-immune interactions; and (5) emerging models of mitochondrial dysfunction as an early disease initiator. Across these categories, single cell technologies reveal critical alterations in lipid metabolism, synaptic programs, and glial-immune crosstalk. Finally, we review methodological innovations—drawing on non-AD developmental organoid studies—such as region-specific patterning, morphodynamic control, and enhanced cellular diversity, which offer a roadmap for improving the physiological fidelity of next-generation AD organoid models. Ultimately, integrating these advanced cerebral organoid platforms with single cell technologies offer a powerful toolkit to dissect early disease mechanisms and accelerate the development of personalised therapeutics.

Keywords: Alzheimer's disease, cerebral organoids, *in vitro*, single cell, next-generation sequencing

1 Graphical Abstract: Single-Cell RNA Sequencing Landscape in Alzheimer's Disease Organoid Models

Single-Cell Transcriptional Landscape of Alzheimer's Disease Organoid Model



Early developmental disturbances caused by familial AD mutations and APOE deficiency impair neural progenitor maintenance and alter neuronal and astrocytic differentiation. During disease progression, systemic environmental triggers converge with dysregulated immune interactions to drive severe pathology. While resident microglia exert neuroprotective effects via active A β phagocytosis, infiltrating peripheral monocytes and reactive astrocytes promote a destructive neuroinflammatory cascade, impaired amyloid- β clearance, and lipid metabolic dysfunction. These converging inflammatory, mitochondrial, and metabolic stressors drive tau hyperphosphorylation and activate the neuronal integrated stress response, leading to synaptic dysfunction, energy failure, and neuronal loss.

2 Introduction

Alzheimer's disease (AD) is the most prevalent form of dementia that accounts for 60% to 70% of all cases. It currently affects over 55 million people worldwide, reflecting a 271% increase from 20.3 million in 1990 (GBD 2016 Dementia Collaborators 2019). This number is expected to surge to 152.8 million by 2050, with the steepest rises projected in low- and middle-income regions, particularly North Africa and Eastern Sub-Saharan Africa (GBD 2019 Dementia Forecasting

Collaborators 2022). In parallel, the global economic impact of Alzheimer's and other dementias is estimated to reach \$14.5 trillion between 2020 and 2050, equivalent to 0.42% of annual global gross domestic product (GDP) (Chen et al. 2024a). Despite decades of research, effective treatments remain elusive due to the disease's complex and heterogeneous aetiology. Hallmark features include amyloid-beta ($A\beta$) plaques, neurofibrillary tangles of hyperphosphorylated tau, neuroinflammation, synaptic dysfunction, and progressive neuronal loss, many of which manifest years before clinical symptoms appear (Pradeepkiran et al. 2024; Gyimesi et al. 2024). This underscores an urgent need for experimental models that could capture early-stage AD pathogenesis at the single cell level.

Traditional experimental systems, such as monolayer cell cultures and animal models, have provided accessible platforms for studying AD pathology in the absence of post-mortem or longitudinal human studies (Liu et al. 2024b). Human neural cell lines are widely used for molecular assays, but they suffer from limited physiological relevance (Fabbri et al. 2023). These immortalised cells, often derived from malignant origins, lack the spatial organisation, cellular diversity, and stable gene expression patterns characteristic of native human brain tissue and may exhibit genetic instability that compromises data interpretation (Gordon and Amini 2021). On the other hand, animal models particularly transgenic mouse models, knock-in/knockout models, and specialised strains like *SAMP8* or seeding models have been instrumental in elucidating key AD hallmarks (Serneels et al. 2020; Xia et al. 2022; Zhong et al. 2024). Nevertheless, while these models successfully recapitulate many molecular and pathological features of AD, they do not fully reproduce the complexity of sporadic late-onset AD or the human brain's unique developmental trajectory, prolonged aging process, cellular diversity, and regional cytoarchitecture. Furthermore, species-specific differences in gene regulation, neuronal and glial biology, immune responses, and brain connectivity can influence disease manifestation and therapeutic responses, limiting the direct translation of preclinical findings to humans (Polis and Samson 2024; Granzotto et al. 2024).

3 Single Cell Transcriptomic Landscape in 3D Cerebral Organoids

Cerebral organoids are three-dimensional (3D), self-organising tissues derived from human pluripotent stem cells (iPSCs) that have emerged as a valuable human-specific model for studying AD pathology (Lancaster et al. 2013). Unlike other region-specific organoid models, such as midbrain, hypothalamic, or spinal organoids, which represent brain areas less implicated in AD, these miniaturised brain-like structures recapitulate key features of early cortical development, including neuroepithelial organisation, layered neuronal differentiation, and region-specific transcriptional patterning. They also maintain greater cellular diversity and human-relevant gene expression than traditional animal models or immortalised cell lines (Luo et al. 2016). In the context of AD, cerebral organoids are particularly relevant due to many early and well-established AD-related pathological processes involving the cerebral cortex and its interaction with cortico-hippocampal networks (Liu et al. 2024a; Ji et al. 2025; Ellingford et al. 2026). Although cerebral organoids might not represent the entire spectrum of AD which notably features early degeneration of the basal forebrain cholinergic systems and eventually impacts widespread brain regions as the disease progresses—they provide an invaluable, human-relevant platform for interrogating the cortical and early network-level mechanisms that are highly relevant to AD (Chen et al. 2021; Vanova et al. 2023). Recent innovations in organoid culture systems, including stem cell

optimisation, refined morphogen delivery strategies, modulation of the extracellular matrix (ECM) niche, and manipulation of tissue architecture to better mimic *in vivo* brain morphogenesis, have significantly advanced the physiological relevance of cerebral organoids (Pagliaro et al. 2025). Complementary approaches such as co-culture with microglia-like cells, incorporation of vascular elements, exposure to systemic factors, and the integration of spatio-temporal dynamics have further enhanced cellular heterogeneity, neuronal functionality, and maturation (Saglam-Metiner et al. 2023).

Single cell RNA sequencing (scRNA-seq) has become indispensable in cerebral organoid research, enabling precise mapping of cell-type diversity, developmental trajectories, molecular states, and protocol-dependent effects, as well as benchmarking against fetal brain tissue and high-throughput screening of disease-associated genes (Li et al. 2023; Sato et al. 2023). Unlike bulk RNA sequencing, which averages gene expression across mixed cell populations, scRNA-seq captures transcriptomic signatures at the single cell resolution, making it especially powerful for dissecting subtle but disease-specific cellular heterogeneity (Tzec-Interián et al. 2025). While scRNA-seq has been widely applied to study neurodevelopment, neuroinflammation, and tumourigenesis in cerebral organoids, its application in AD-specific models remains relatively limited but is rapidly expanding (Davis et al. 2025; Narasipura et al. 2025; Peng et al. 2025). Focusing on this emerging intersection, this mini-review explores how scRNA-seq applied to AD-specific cerebral organoids enables the interrogation of genetic, environmental, immunological, and metabolic processes at the single cell level.

3.1 Familial Alzheimer's Disease: *APP*, *PSEN1* and/or *PSEN2* Mutation Models

Among the earliest and most well-defined applications of scRNA-seq in AD cerebral organoids has been the modelling of fAD, where autosomal dominant mutations in *APP*, *PSEN1*, or *PSEN2* provide a genetically tractable entry point for dissecting pathogenic cascades. For example, Vanova *et al.* (2023) explored the early impact of *PSEN1* (A246E) and *PSEN2* (N141I) mutations in iPSCs-derived cerebral organoids from fAD patients and isogenic controls. By integrating multi-omics tools, including scRNA-seq, mass spectrometry, immunohistochemistry, and pseudotime trajectory analysis, the study identified not only classical AD-like features such as elevated A β _{42/40} ratios and tau phosphorylation (pSer202/pThr205), but also unexpected developmental abnormalities, such as defective tissue patterning, premature neuronal differentiation, and reduced retinal-like zones. scRNA-seq analyses revealed disrupted progenitor-to-neuron transitions and premature neuron development, linked to downregulation of Notch pathway genes (*HES1*, *HES4*, *FABP7*), implicating an early failure to maintain progenitor pools. These findings show that fAD mutations may impair brain development long before symptomatic onset, suggesting early developmental perturbations may contribute to AD vulnerability (Vanova et al. 2023).

Following this, Choe and the co-investigators advanced the field by developing a more mature, and neuropathologically robust fAD model. Pathogenic mutations in *APP* and *PSEN1* were introduced into human pluripotent stem cells, which were then differentiated into cerebral organoids. These isogenic lines exhibited hallmark AD pathologies, including extracellular A β plaques, phosphorylated tau (pTau), neurofibrillary tangle-like structures, and neurodegeneration. ScRNA-seq revealed diverse neural and non-neural populations, with transcriptional signatures

reflecting disease progression. Treatment with β - and γ -secretase inhibitors ameliorated A β and tau pathology, underscoring the model's translational potential for preclinical drug screening (Choe et al. 2024).

Unlike earlier efforts that primarily modelled fAD using defined mutations and static time points, the integration of longitudinal single cell data enhances the ability of cerebral organoids to capture dynamic transcriptional changes, cell fate disruptions, and glial dysfunction which are features increasingly recognised in the pathophysiology of sAD.

3.2 Sporadic Alzheimer's Disease: *APOE* Genotypes and Environmental Factors

3.2.1 *APOE*-Linked Genetic Risk and Resilience

While fAD models leverage deterministic mutations in *APP* and *PSEN* genes, sAD, which accounts for over 95% of cases arises from complex interactions among common genetic risk variants and environmental influences (Mayeux and Stern 2013). Among these, *APOE* polymorphisms stand as the most significant genetic modifiers of AD susceptibility (Corder et al. 1993). In 2023, Zhao and co-researchers demonstrated that *APOE* knockout in cerebral organoids impairs excitatory neuron development and disrupts lipid metabolism through the activation of the eukaryotic initiation factor 2 (*EIF2*)-mediated integrated stress response (ISR). Using scRNA-seq on isogenic iPSC-derived organoids, they found that *APOE* deficiency led to altered cell-type composition, reduced neuronal maturity, and upregulation of *activating transcription factor 4* (*ATF4*) target genes in neurons. Dysregulated cholesterol efflux and intracellular lipid accumulation further implicated *APOE*'s metabolic role in early-stage AD pathology. Notably, treatment with the ISR inhibitor partially restored neuronal differentiation and mitigated stress-related transcriptomic signatures, identifying the ISR as a modifiable therapeutic axis (Zhao et al. 2023).

Building on the role of *APOE* in disease modulation, Pérez-Corredor *et al.* investigated a rare case of genetic resilience using iPSC-derived cerebral organoids from an individual carrying both a *PSEN1* fAD mutation and the *APOE3* Christchurch (*APOE3Ch*) variant, who remained cognitively intact into his seventies. Their scRNA-seq and transcriptional network analyses revealed that *APOE3Ch* organoids exhibited enhanced Wnt/ β -catenin signalling in both neurons and astrocytes, which are the pathways critical for neuronal differentiation, synaptic stability, and glial homeostasis. Interestingly, this correlated with significantly lower levels of pTau, suggesting that Wnt pathway enhancement may underlie *APOE3Ch*-mediated protection from tauopathy. These protective effects were further validated using isogenic *APOE3Ch*-edited lines, reinforcing the utility of cerebral organoids and single cell technologies in unraveling mechanistic insights into genetic resilience in AD (Perez-Corredor et al. 2024).

3.2.2 Serum Exposure, Pollutants, and Latent Viral Infection Lead to sAD

Beyond genetic risk, scRNA-seq-based cerebral organoid models have also illuminated how systemic environmental stressors contribute to sAD pathology. For instance, Chen *et al.* established a non-genetic sAD model by exposing human iPSC-derived cortical organoids to

human serum, mimicking blood-brain barrier (BBB) breakdown. This triggered hallmark AD features, including A β accumulation, pTau elevation, synaptic loss, and neuronal apoptosis. scRNA-seq revealed cell-type-specific transcriptional responses, with both neurons and astrocytes showing downregulation of synaptic genes (*SYN1*, *SNAP25*) and astrocytes exhibiting an upregulation of inflammatory transcripts (*IFITM3*, *NLRP1*, *MT2A*). Serum exposure increased *BACE1* and *GSK3 α/β* levels, which in turn promoted A β and pTau pathology. However, inhibition of these pathways failed to reverse synaptic degeneration, suggesting the existence of multifactorial disease mechanisms (Chen et al. 2021). Additionally, the observed transcriptomic alterations closely mirrored those found in postmortem Alzheimer's disease cortical tissue, thereby reinforcing the physiological relevance and translational validity of the model (Grubman et al. 2019).

Expanding on environmental modelling, researchers from Sichuan University, China, investigated per- and polyfluoroalkyl substances (PFAS) exposure on human cerebral organoids. Chronic exposure to PFOA, PFOS, and PFHxS led to neuronal loss, increased A β and pTau, and disruptions in lipid metabolism. These effects were supported by integrated scRNA-seq and lipidomic analyses, highlighting lipid dysregulation as a key mediator of environmentally induced AD-like neurodegeneration (Lu et al. 2024).

On the other hand, Feng et al. employed an integrated two-part single cell resolution strategy to investigate how human herpes simplex virus (HSV) infection drives transposable element (TE) dysregulation in AD. Single-nucleus RNA sequencing of post-mortem AD brains from the ROS/MAP and MSBB cohorts revealed widespread TE dysregulation, notably astrocyte-specific upregulation of *LINE1* elements in HHV-positive samples. To validate these findings, the authors analysed existing bulk and scRNA-seq datasets from HSV type1-infected human iPSC-derived forebrain organoids, which showed similar astrocyte-specific *LINE1* activation that was partially reversed by antiviral treatment with valacyclovir or acyclovir. Valacyclovir also rescued tau-associated neuropathology, linking HHV infection, TE activation, and tau pathology in sAD (Feng et al. 2025). Notably, while the study integrates post-mortem snRNA-seq with organoid-based scRNA-seq models, these modalities differ substantially in transcriptomic composition, and scRNA-seq datasets from live organoid systems are not directly comparable to snRNA-seq profiles from post-mortem tissue due to weak cross-modality correlations and fundamental biological differences (Chen et al. 2024b; Ivich and Greene 2026). This limitation should be considered when interpreting cross-system concordance in AD-associated HSV-TE mechanisms.

Despite such inherent methodological limitations, these environmental models illustrate how diverse insults, namely serum factors, pollutants, and latent viruses, can initiate AD-like pathology through converging molecular pathways, including synaptic dysfunction, lipid imbalance, and tau accumulation. These sporadic triggers recapitulate molecular pathways similarly implicated in familial tauopathies, suggesting a potential pathogenic framework underlying both sporadic and familial forms of the disease.

3.3 Tauopathy and Developmental Models: Insight into Cell-Type Pathways

Building on insights from fAD and sAD models, recent efforts have shifted toward modelling tau pathology, a hallmark more closely linked to neurodegeneration and cognitive decline (Langerscheidt et al. 2024). Tauopathies, whether familial and sporadic, converge on pathological tau aggregation as a shared endpoint (Yancopoulou and Spillantini 2003). When combined with scRNA-seq, cerebral organoid models enable high-resolution mapping of tau-associated cellular vulnerabilities and developmental trajectories in the human brain. To demonstrate this, Fiock et al. (2020) profiled tau expression during cortical development and observed its dynamic upregulation in maturing excitatory neurons. By comparing cerebral organoids with human fetal brain tissue, they validated organoids as a relevant model for studying early tau regulation in neurodevelopment (Fiock et al. 2020).

In contrast, Glasauer et al. developed a familial tauopathy model (specifically modelling frontotemporal dementia) using human cerebral organoids harboring *MAPT* mutations (*V337M* and *R406W*). By employing isogenic iPSC lines with both heterozygous and homozygous variants, they captured gene dosage effects across a developmental time-course (4–8 months). Combination of scRNA-seq and lipidomics revealed early, astrocyte-specific upregulation of cholesterol biosynthesis genes (*HMGCR*, *ACAT2*, *STARD4*, *LDLR*, and *SREBF2*). While astrocytes exhibited metabolic dysfunction, pyramidal neurons showed a coordinated downregulation of glycolysis-related genes (*PGK1*, *ENO2*) and GABA receptor components, suggesting both cell-autonomous and non-cell-autonomous effects of tau mutations (Glasauer et al. 2022).

By juxtaposing findings from Glasauer et al. and Lu et al. (from section 3.2.2), it becomes evident that astrocytic lipid dysregulation emerges downstream of tau pathology in both sporadic (environmental toxicant-induced) and familial tauopathies (genetic *MAPT* mutation-driven, such as frontotemporal dementia). Despite distinct upstream triggers, both models converge on common astrocyte-mediated metabolic disruptions, defining a shared mechanistic axis in tau-driven neurodegeneration across distinct disease classifications (Glasauer et al. 2022; Lu et al. 2024).

3.3.1 Glial-Immune-Tau Crosstalk: A Frontier for Cerebral Organoid Models

Glial pathology is increasingly recognised as a key driver of Alzheimer's disease. Astrocyte-mediated lipid dysregulation and tau toxicity, unfolding within an immune-competent microenvironment, underscore critical pathological mechanisms in AD. Nevertheless, no cerebral organoid models to date have recapitulated tauopathy within a fully integrated immune context. To address this gap, Cakir et al. leveraged cortical organoid by introducing microglia-like cells via the transcription factor *PUL1* overexpression, thereby enabling the study of innate immune interactions. These cells exhibited key morphological and transcriptomic features of native microglia, including the capacity to phagocytose A β aggregates. Using CRISPR interference and scRNA-seq, the authors performed functional screening of AD-associated genes such as *TREM2* and *SORL1*, elucidating their roles in modulating A β toxicity. This microglia-containing organoid platform provided a tractable system for examining glia–neuron crosstalk and revealed both protective and dysregulated immune responses in early AD-like pathology (Cakir et al. 2022).

Similarly, Tian *et al.* developed a novel and scalable human cortical organoid microphysiological system (hCO-MPS) to assess peripheral immune contributions to AD. This platform integrates 3D-printed scaffolds into a 96-well format to support high-throughput culture

of viable, doughnut-shaped cortical organoids. Co-culture with AD patient-derived monocytes revealed increased infiltration, reduced A β clearance, and elevated *IL1B* and *CCL3* secretions, inducing astrocyte activation and neuronal apoptosis. scRNA-seq further identified transcriptional states associated with oxidative stress, glial reactivity, and neuronal death, implicating peripheral immune cells as active drivers of neuroinflammation and neural injury in AD (Tian et al. 2025).

3.4 Mitochondrial Dysregulation as AD Initiators: A Paradigm Shift

While A β and tau aggregation dominate the landscape of AD research, accumulating evidence implicates that mitochondrial dysregulation may act as an early contributing factor of AD rather than a downstream consequence. In this emerging view, mitochondrial impairment is not merely a downstream consequence of amyloid or tau pathology, but a central orchestrator of early AD-like pathogenic events (Casley et al. 2002; Aleardi et al. 2005; Manczak et al. 2011). This finding aligns with the mitochondrial cascade hypothesis, which states that inherited or acquired mitochondrial deficits initiate oxidative stress and drive sAD pathology (Swerdlow and Khan 2004; Swerdlow et al. 2014).

Supporting this model, Pérez et al. use pitrilysin metalloproteinase 1 (*PITRM1*) knockout iPSC-derived cerebral organoids to demonstrate that loss of mitochondrial protease induces widespread proteostatic stress, along with hallmark AD pathologies including A β and tau accumulation, and neuronal death. Notably, scRNA-seq further revealed cell-type-specific responses, including astrocytic immune activation, highlighting mitochondrial dysfunction as a driver of early, cell-intrinsic AD features (Pérez et al. 2021). This indicates that organoids offer a uniquely human, multicellular system to dissect these processes at single cell resolution. As such, they represent a critical platform for future investigations into the initiating role of mitochondrial dysfunction in AD.

4 Methodological Innovations Shaping Future Cerebral Organoids for AD Research

Building on evidence that mitochondrial dysfunction, tau pathology, and glial-immune crosstalk are central to early AD processes, cerebral organoids provide a promising platform to model these mechanisms in a physiologically relevant, three-dimensional context that captures aspects of early brain development and cell-type diversity (Pérez et al. 2021; Cakir et al. 2022; Tian et al. 2025). However, the next leap in AD modelling cannot rely solely on disease-specific studies; it requires adopting breakthroughs from fundamental developmental biology. Current cerebral organoid models are limited by incomplete brain regionalisation, a lack of immune components, and difficulties achieving robust, cell-type-resolved analysis (Quadrato et al. 2016; Velasco et al. 2019; Louey et al. 2021). Recent biological and computational innovations are expanding what cerebral organoids can model, particularly in terms of regional identity, neuronal subtype diversity, and morphogenetic precision. These advances hold particular promise for improving the accuracy and scalability of AD-specific organoid models. For instance, Bertacchi et al. demonstrated that *FGF8* signalling can induce co-development of telencephalic and mesencephalic domains in cerebral organoids derived from human iPSCs. Within these spatially distinct regions, *FGF8* modulates the balance of GABAergic and glutamatergic neurons, alters spontaneous network activity, and regulates genes linked to neurodevelopmental disorders. These findings not only

enhance region-specific patterning but also support the generation of multi-regional cerebral organoids suitable for studying circuit-level AD dysfunctions, such as excitatory–inhibitory imbalance and anterior-posterior vulnerability gradients, which are phenomena implicated in selective neuronal susceptibility in AD (Bertacchi et al. 2024).

Although AD research has traditionally prioritises cortical regions, increasing clinical evidence suggests that AD is a multi-systemic disorder, where non-cognitive symptoms such as weight dysregulation, severe sleep/circadian disturbances, and hypothalamic-pituitary axis disruption often emerge before memory impairment (Lv et al. 2022; Yang et al. 2025). These early symptoms are driven by early-stage vulnerability in the hypothalamus and its dopaminergic circuits (Khairy and Salama 2023; P and Vellapandian 2024). To provide a platform for capturing these often-overlooked early disease stages, Sarrafha et al. developed a blueprint for creating hypothalamus organoids enriched in diverse dopaminergic neuron subtypes. Through dual-SMAD and *WNT* inhibition alongside *SHH* activation, their protocol achieves robust neurochemical subtype diversity. Importantly, their scRNA-seq analysis revealed significant metabolic reprogramming within these dopaminergic populations, including upregulated shifts in glycolysis and oxidative phosphorylation alongside a targeted downregulation of cholesterol biosynthesis (Sarrafha et al. 2023). Because these exact metabolic and lipid pathways are increasingly implicated as core drivers of AD pathology across cortical models (Wang et al. 2024, 2025), adopting this targeted hypothalamic protocol provides the precise methodological framework needed to model the earliest systemic symptoms and region-specific metabolic vulnerabilities of AD.

These region- and subtype-specific innovations are further strengthened by the work of Jain *et al.* in 2025, who introduced a live imaging platform to capture the morphodynamics of cerebral organoid development, adding a biophysical dimension to these patterning strategies. Using light-sheet microscopy and fluorescently labeled hiPSCs, they showed that extracellular matrix (ECM) cues and mechanosensitive signalling (e.g., *WNT*, *YAP1*) govern lumen formation, telencephalic expansion, and caudal identity suppression. Their results underscore the importance of biophysical context in shaping organoid morphology and regional fate that provides a scalable, ECM-guided strategy to improve telencephalic fidelity in cerebral organoids used for AD modelling (Jain et al. 2025).

Finally, on the computational front, tools such as scRNA-seq-informed bulk RNA deconvolution now enable cell-type-specific transcriptomic analysis even when full single cell profiling is impractical (Dai et al. 2024). These approaches are essential for connecting disease-relevant phenotypes, such as microglial activation, astrocyte reactivity, and/or neuronal stress response, to specific cellular compartments within heterogeneous organoid models. Complementary methods, including single-nucleus RNA sequencing and spatial transcriptomics, will further enhance the resolution and interpretability of these complex systems.

Together, these advances represent a next-generation toolkit for cerebral organoid-based AD research: combining molecular patterning, neurochemical enrichment, morphodynamic control, and computational resolution to create human brain models that are not only more regionally

accurate and functionally relevant but also increasingly scalable for mechanistic and translational studies in AD.

To capture the rapid evolution of this niche field, **Table 1** presents a comprehensive summary of the currently available literature utilising scRNA-seq in human AD-specific cerebral organoids. Rather than selectively sampling the literature, this table outlines the entire current landscape of familial, sporadic, and tauopathy AD organoid models profiled at single cell resolution. Furthermore, a highly selective subset of non-AD organoid studies are included to address current limitations in disease modelling. The inclusion of these non-AD studies is specifically justified by their introduction of critical methodological innovations—such as enhanced regional patterning, morphodynamic control via extracellular matrix cues, and computational bulk deconvolution techniques—which provide a direct roadmap for the next generation of physiologically accurate AD models. Complementing this, **Figure 1** provides a chronological overview of these landmark studies, illustrating the rapid evolution and expanding complexity of scRNA-seq applications in AD organoid research over time.

5 Conclusion

Cerebral organoid models, combined with scRNA-seq, represent a transformative platform for investigating the multifaceted pathogenesis of AD. By enabling single cell resolution of cell-type-specific trajectories, developmental disruptions, and context-specific responses to genetic and environmental insults, these models address key limitations of traditional systems. Recent findings including the upstream role of mitochondrial dysfunction, the convergence of environmental and genetic risk pathways, and the impact of glial-immune interactions underscore the potential of cerebral organoids to capture early, modifiable stages of disease. Moreover, innovations in organoid engineering, brain regionalisation techniques, and computational analysis significantly enhances the scope and fidelity of these models.

Looking ahead, integrating immune competence, metabolic stressors, and tauopathy will be essential for capturing the full spectrum of AD pathology. By combining insights from developmental biology, engineering, and data science, next-generation cerebral organoids hold great promise not only for understanding disease mechanisms but also for accelerating therapeutic discovery.

6 Table

Table 1: Summary of Single Cell Transcriptomic Studies in Cerebral Organoids:
Applications, Cell Type, Methodologies, and Key Findings in Models of Alzheimer's Disease, Tauopathies, and Neurodevelopment

Type	Role of scRNA-seq	Type/ Number of cells analysed	Technology & sequencer ^a	Take home
fAD	Analysis of the specific cell types within the CO, revealing a heterogeneous composition of normal brain cells	70-day-old <i>CAG-APP^{SweF/Lon-IRE5}-PSEN1M146L/L286V</i> (CAP) cerebral organoids; cell number not stated	Chromium (10x Genomics); sequencer not stated	- Overexpression of Aβ and apoptosis - Pathological γ-secretase preclinical

Type	Role of scRNA-seq	Type/ Number of cells analysed	Technology & sequencer ^a	Take home
fAD	- Identification of a heterogenous mixture of cells - Identification of developmental trajectories, showing that clusters enriched in APOE3Ch cells represented later stages, suggesting altered developmental timing or premature differentiation. - Identification of regulation of Cadherin and Wnt signalling pathways by APOE3Ch	6,400 cells from 6 pooled organoids per iPSC-derived cell line (total 8 lines from 2 patients, α and ω): α-APOE3/PSEN1 E280A, α-APOE3Ch/PSEN1 E280A, α-APOE3Ch/PSEN1 WT, α-APOE3Ch/PSEN1 E280A (duplicate clone), ω-APOE3/PSEN1 E280A, ω-APOE3/PSEN1 E280A (duplicate clone), ω-APOE3/PSEN1 WT, ω-APOE3/PSEN1 E280A	Chromium (10x Genomics); sequencer not stated	- APOE3 timing - Enhanced protection
fAD	- Identification of heterogeneity of NDC#1- and AD#1-iPSC-derived organoids - Confirmation of the altered developmental phenotype and signs of premature onset of differentiation in a subset of neurons via integrated data analysis (NDC#1-CO and AD#1-CO)	2,298 NDC cells and 2,795 AD cells retained post-QC; derived from 18 pooled day-60 organoids per sample	Chromium (10x Genomics); NextSeq 500 (Illumina)	- PSEN1 accumulation - Reduced pool differentiation
A β -induced AD	- Integration of organoid-derived microglia-like cells (oMG) and primary microglia signatures to assess differences between innate oMG and PU.1-induced microglia - Identification of developmental trajectories, segregating cells into microglia, neurons, astrocytes, and other lineages to trace lineage commitment (linking GFP/PU.1 expression to microglial fate) - Evaluation of the transcriptional response and protective role of microglia following Aβ₁₋₄₂ exposure - Analysis of high-throughput genetic perturbations (CROP-seq) to identify the specific roles of AD-associated risk genes (e.g., <i>SORL1</i>, <i>TREM2</i>) in cholesterol metabolism and Aβ response	13,416 hCO cells and 13,546 mhCO cells for baseline characterisation; 49,867 cells analysed for the non-treated vs. A β -treated comparison	Chromium (10x Genomics); NovaSeq S4 (Illumina)	- PU.1-induced neuroprotection - This model of AD risk
sAD/ immune-driven	- Investigation of mechanisms of increased AD monocyte infiltration into the human cortical organoid microphysiological systems (hCO-MPSs) - Analysis of neuronal and monocyte interactions in the hCO-MPS model to reveal immune-driven neurodegeneration	Cells from hCO-MPSs that were cocultured with human peripheral monocytes; number not stated	Chromium (10x Genomics); sequencer not stated	- Infiltration show in - Monocyte apoptosis pathway
sAD/ viral induced	- Profiling of transcriptomes from 60-day-old organoids at 3 days post infection and clustering with distinct cell types - Mechanistic validation of snRNA-seq, pinpointing cell types in the HSV-1/HHV-infected human organoid model that drive TE dysregulation and to assess how antiviral drugs modulate these effects - Identification of cell type-specific transcriptional changes in transposable elements (TEs) and genes following HHV infection - Investigation of the underlying mechanisms of acyclovir and valacyclovir on HSV-1-infected human brain organoids to assess how antiviral drugs modulate TE dysregulation	Three public datasets: - scRNA-seq (GSE129308): 24,660 soma with NFTs, 38,465 neighbouring NFT-free neurons from AD donors and 63,110 control NFT-free somas - snRNA-seq (GSE138852): 13,214 nuclei of entorhinal cortex from control and AD brains of twelve individuals - snRNA-seq (GSE148822): 482,472 nuclei from non-demented control brains and AD brains containing only amyloid-β plaques or both amyloid-β plaques and tau pathology Validation = droplet-based scRNA-seq public dataset (GSE163952): human iPSC-derived organoid cells; number not stated	- GSE129308 (Otero-Garcia et al. 2022): Chromium (10x Genomics); NovaSeq 6000 (Illumina) - GSE138852 (Grubman et al. 2019) & GSE148822 (Gerrits et al. 2021): Chromium (10x Genomics); NextSeq 500 (Illumina) Validation scRNA-seq public datasets: - GSE163952 (Rybak-Wolf et al. 2023): Drop-seq (ChemGenes Corp.); NextSeq 500 (Illumina)	- Latent type-specific transposon - Antiviral partially downst
sAD/ environmental induced	- Prediction and validation of cell-type-specific neurotoxicity of PFASs - A powerful methodology to evaluate the health risks associated with environmental contaminants, particularly those with neurotoxic potential	4 organoids per group (control and 900 $\times$ PFAS); 14,556 cells from control group and 14,030 cells from 900 $\times$ PFAS group	Chromium (10x Genomics); NovaSeq X Plus (Illumina)	- Chronic neurodegeneration - Pathological dysregulation of sphingolipids

Type	Role of scRNA-seq	Type/ Number of cells analysed	Technology & sequencer ^a	Take home
sAD	- Elucidation of the cell-type-specific effects of APOE deficiency in human cerebral organoids - Identification of changes in cellular composition, demonstrating that excitatory neurons were reduced while astrocytes, radial glia, and inhibitory neurons increased	27,199 single cells in total (14,920 cells from control Day-90 cerebral organoids and 12,279 cells from isogenic APOE ^{-/-} Day-90 cerebral organoids)	Chromium (10x Genomics); HiSeq 4000 (Illumina)	- APOE^{-/-} and triglyceride signalling - Activation and differentiation phenotypes
sAD	- Identification of a heterogeneous mixture of cells - Evaluation of synaptic and immune transcriptomic alterations in serum-exposed sAD organoids to reveal reduced synaptic function in neurons and induced immune responses in astrocytes	26,631 cells (9,338 BO2_control; 5,412 BO2_serum; 6,535 BO3_control; 5,346 BO3_serum)	Chromium (10x Genomics); NovaSeq 6000 (Illumina)	- Serum-induced breakdown of pTau, and - Synaptic function GSK3β mechanism
Tauopathy/familial	- Profiling of human cerebral organoids from MAPT-mutant lines vs isogenic controls across development - Identification of a heterogeneous cell population - Identification of cell-type-specific pathway changes, specifically uncovering cholesterol biosynthesis dysregulation in astrocytes and glycolysis/synaptic drops in neurons - Nomination of disease-relevant mechanisms downstream of tau mutations	76,111 total cells post-filtering, with a mean of 1,228 cells $\pm$ 239.6 SD per sample	Drop-seq (Macosko et al. 2015); NextSeq 500 (Illumina)	- Familial APOE^{-/-} biosynthesis - Pyramidal downregulation of components
Tauopathy/developmental	- Evaluation of tau (MAPT) mRNA expression patterns as a function of lamina and neuronal maturation during fetal brain development and in iPSC-derived cortical organoid - Confirmation of low amounts of tau mRNA in very early neuronal precursors (radial glia and intermediate progenitors)	2 public datasets: 48 human fetal cortex samples aged 5.8–37 postconceptional weeks; number not stated (Nowakowski et al. 2017) - hiPSC-derived cortical organoids derived from three different donors at 105 day of <i>in vitro</i> differentiation; number not stated (Yoon et al. 2019)	- Fluidigm C1 (Fluidigm) and HiSeq2500 (Illumina) (Nowakowski et al. 2017) - BD Rhapsody (BD Biosciences) (Yoon et al. 2019)	- Organoid upregulation of brain disease - Human conservation of neuronal disease
Familial risk mitochondrial dysfunction	Characterisation of cell-type-specific pathogenetic mechanisms, mitochondrial dysfunction, and AD-like pathology in PITRM1-deficient organoids.	Total 15,183 cells from 2-month-old cerebral organoids (WT+KO pooled; per-genotype counts not reported; ~18,000 cells loaded per sample pre-QC)	Chromium (10x Genomics); sequencer not stated	- PITRM1 mitochondrial proteome - Knockdown of tau pathology NAD⁺
Developmental/regional modelling (neurodevelopment disorder)	Investigation of the transcriptomic signature of cerebral organoids upon FGF8 treatment to quantify regional and neuronal subtype diversity shifts, illuminating mechanisms of regional vulnerability	18,590 cells analysed from Day 69 organoids (from two independent batches per condition [WNTi; WNTi+FGF8], each batch pooling 2–3 organoids)	Chromium (10x Genomics); sequencer not stated	- FGF8 signalling distinct - A multi-selective
Non-AD (developmental modelling/ Parkinson's disease)	- Characterisation of neuroendocrine and dopaminergic diversity in hypothalamic organoids - Identification of molecularly distinct hypothalamic DN subtypes and their respective developmental maturities	21,001 cells from D85 hypothalamus organoids (TH ^{hi} group: 10,464 cells, $\approx$ 2,647–4,015 per replicate; TH ^{lo} group: 10,537 cells, $\approx$ 3,262–3,663 per replicate)	Chromium (10x Genomics); sequencer not stated.	- Guided neuronal development - DNs expression including downregulation
Non-AD (developmental modelling)	- Mapping of regionalisation and morphogenetic transitions in unguided brain organoids. - Evaluation of cell-state changes associated with tissue transitions and the molecular processes affected by different extracellular matrix (ECM) and signalling perturbations.	- Time-course (D5, D7, D11, D16, D21, D30): Per-day cell counts not reported; pooled organoids = 24 (D5/D7/D11), 12 (D16), 10 (D21), 6 (D30). - Day 13 – Matrix: Matrigel 8,095; No-matrix 8,343; Agarose 3,725. - Day 10 – YAP (Py-60): DMSO 2,085; Py-60 day-5 763; Py-60 day-7 1,955. - Day 55 – Hashed (WT+KO totals): Matrigel 5,221 (3,560+1,661); No-matrix 5,377 (1,283+4,094); CHIR 5,084 (3,310+1,774); Py-60	Chromium (10x Genomics); sequencer not stated.	- Extraction of format fidelity - Mechanism of the balance pattern

Type	Role of scRNA-seq	Type/ Number of cells analysed	Technology & sequencer ^a	Take home
		2,782 (1,399+1,383); DMSO 3,540 (2,087+1,453). • Extended Data (hashed subset): Matrigel 1,183; No-matrix 1,285; Py-60 1,189.		
Non-AD (protocol that support AD studies)	• Evaluation of nine existing sample-wise cell deconvolution methods (DSA, OLS, CIBERSORT, dtangle, MuSiC, Bisque, bMIND, swCAM, TCA) on human brain transcriptomic data using matched bulk RNA-seq, sc/snRNA-seq, and IHC data to establish best practices and explore biological applications	Matched datasets used for benchmarking/analysis: ROSMAP bulk RNA-seq n=1112; ROSMAP snRNA-seq 48 donors / 69,611 nuclei; IHC n=49. CMC bulk n=572; CMC snRNA-seq 101 donors / 569,289 nuclei. Brain organoids bulk n=130; organoid scRNA-seq 72 samples / 490,844 cells. Across all, ≈1,130,767 nuclei/cells from 149 adult brains and 72 organoid samples	snRNA-seq and scRNA-seq (public datasets downloaded from Synapse); specific sequencer and single cell platforms are not stated.	• Benchr the opt RNA d • Accura proport profilin

^a denotes by scRNA-seq platform (brand name) and sequencing platform (brand name)

Abbreviations: A β = amyloid beta, AD = Alzheimer's disease, APOE = apolipoprotein E, BACE1 = beta-secretase 1, CCL3 = C-C motif chemokine ligand 3, CO = cerebral organoids, CROP-seq = CRISPR droplet sequencing, DMSO = dimethyl sulfoxide, DN = dopaminergic neuron, ECM = extracellular matrix, EIF2 = eukaryotic initiation factor 2, fAD = familial Alzheimer's disease, FGF8 = fibroblast growth factor 8, GFP = green fluorescent protein, GSK3 = glycogen synthase kinase 3, hCO = human cortical organoid, hCO-MPSs = human cortical organoid microphysiological systems, HHV = human herpesvirus, hiPSC/iPSC = human induced pluripotent stem cell, HSV-1 = herpes simplex virus type 1, IHC = immunohistochemistry, IL-1 β = interleukin-1 beta, KO = knockout, LINE-1 = long interspersed nuclear element-1, MAPT = microtubule-associated protein tau, mhCO = microglia-containing human cortical organoids, NDC = non-demented control, NFTs = neurofibrillary tangles, NMN = nicotinamide mononucleotide, oMG = organoid-derived microglia-like cells, PFAS/PFASs = per- and polyfluoroalkyl substances, PITRM1 = pitrilysin metalloproteinase 1, PSEN1 = presenilin 1, PSEN2 = presenilin 2, pTau = phosphorylated tau, PU.1 = PU box-binding transcription factor, QC = quality control, RNA-seq = RNA sequencing, ROSMAP = Religious Orders Study and Memory and Aging Project, sAD = sporadic Alzheimer's disease, scRNA-seq = single cell RNA sequencing, SD = standard deviation, snRNA-seq = single-nucleus RNA sequencing, SORL1 = sortilin related receptor 1, TEs = transposable elements, TH = tyrosine hydroxylase, TREM2 = triggering receptor expressed on myeloid cells 2, WT = wild type

7 Figure

The Evolution of Single-Cell Sequencing in Alzheimer's Organoid Research

A chronological overview of how scRNA-seq technologies have advanced our understanding of Alzheimer's disease using human organoid models.

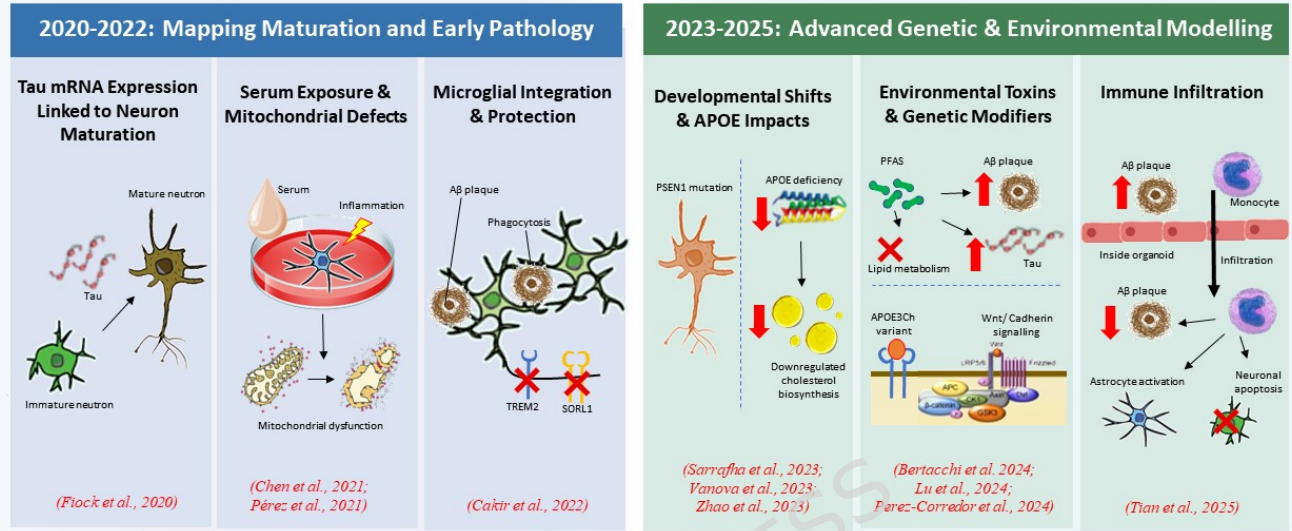


Figure 1: Chronological Overview of scRNA-seq in AD Organoid Research. The timeline illustrates the rapid evolution of scRNA-seq in AD organoid models. 2020–2022: studies established baseline cellular maturation and early disease mechanisms, including tau-associated neuronal maturation, sporadic AD induction via serum exposure and mitochondrial dysfunction, and neuroprotective effects of microglial integration. 2023–2025: advances enabled sophisticated genetic, environmental, and multicellular modelling, revealing the effects of familial AD mutations, APOE deficiency, PFAS exposure, APOE3 Christchurch, Wnt/Cadherin signalling, and monocyte-mediated neuroinflammation on neuronal, glial, and lipid pathways.

8 Supplementary material

Supplementary Table 1: Cell Clusters, Marker Genes, and Detailed Research Findings in Single Cell Transcriptomic Studies of Alzheimer's Disease Cerebral Organoids

Ref.	Cell clusters	Genes involved	Detailed research findings
(Choe et al. 2024)	17 clusters: - Stem cells - Radial glial cells - Neuroepithelial cells - Pericytes - Neuronal progenitor cells - Intermediate progenitor cells - Astrocytes - Macroglia - Choroid plexus - Mesenchymal - Endothelial cells	<i>APP, PSEN1</i> , lineage markers	Overexpression → AD-like pathology: fAD gene overexpression in hPSC-COs boosts Aβ_{40/42} and PHF-tau vs controls (CAP > CA); abundant amyloid deposits (Amylo-Glo, thioflavin-S, 6E10/4G8) Neurodegeneration signals up: More NFT-like structures and higher CASP3 (apoptosis) Drug-responsive model: BACE1 inhibitor IV or γ-secretase inhibitor (Compound E) lowers Aβ and eases PHF-tau/NFT-like changes → suitable for drug testing Maturation/overexpression needed: 70-day patient-derived hiPSC COs without overexpression

Ref.	Cell clusters	Genes involved	Detailed research findings
	• Microglia • Five neuron subtypes		show no A β /PHF-tau increase vs wild-type → possibly need overexpression or longer culture
(Perez-Corredor et al. 2024)	15 clusters with 6 major cell types: • Neuronal (0, 1, 8, 10, 12, 13, 14) • Neuroblast (2) • Radial glia (3, 5, 7) • Neuro endothelial (4, 11) • Oligodendrocyte (6) • Progenitor (9)	<i>PSEN1, APOE3Ch, cadherins, WNT2B, WNT4, WNT7B, β-catenin, APP, MAPT, RELN</i>	• Shifted development: APOE3^{Ch} organoids skew toward later cell states (clusters 2–3), indicating altered timing/identity • Core pathways hit: Wnt and Cadherin signalling are the most affected across neurons and glia • Ligands down, β-catenin up: Glial WNT2B/WNT4/WNT7B transcripts drop; β-catenin mRNA is unchanged, but protein increases and spreads in rosettes, seen in patient brain tissue • APOE-driven tau effects: Together with pTau data, supports a PSEN1-independent, APOE-dependent influence on tau-related signalling
(Vanov a et al. 2023)	11 clusters with 9 major types from integrated NDC#1-CO and AD#1-CO sequencing data: • NSCs; radial glia (0, 7) • Immature/mature neurons (1, 5) • Intermediate progenitors; neuroblasts (2) • NSCs; radial glia; choroid plexus (3) • Retinal progenitor cells (4) • Retinal progenitor cells; neural retina (neurons, photoreceptor cells, bipolar cells) (6) • Meningeal cells; glial cells (OPCs, microglia); mesoderm (8) • Forebrain neurons; retinal neurons (9) • Meningeal cells; Mesoderm (10)	<i>PSEN1, PAX6, SLX3, RAX, CRX, HES1, HES4, HES5, FABP7</i>	• Amyloid pathology: Early Aβ accumulation (elevated A$\beta_{42/40}$ ratio) and increased APP-CTFs; drug-responsive to β/γ-secretase inhibitors • Neurodegeneration markers: Elevated phospho-tau and neurofilament proteins; reduced CHAT; increased stress, apoptosis, and senescence • Developmental trajectory: Premature neuronal maturation with reduced retinal-like cell populations • Signalling defects: Reduced Notch activity in progenitors (reduced HES1/HES4 and FABP7 expression)
(Cakir et al. 2022)	14 annotated cell-type clusters: • Cortical neurons • Interneurons • Neuron-non-committed • Neural progenitors • Astrocytes • Glial progenitors • BMP-responsive cells • Cilia-bearing cells • Unfolded-protein-response-related cells • Unassigned • Proteoglycan-expressing cells • <i>C1QA</i>⁺, <i>C1QC</i>⁺, <i>CCL3/4</i>⁺ microglia • <i>TREMI</i>⁺, <i>NFKB1</i>⁺ microglia precursor A1 • <i>CXCL1</i>⁺, <i>MYB</i>⁺, <i>IRF8</i>⁺ microglia precursor A2	<i>TREM2, TYROBP, IL1B, TNF, CD68, CX3CR1, P2RY12</i>	• Microglia-like cells in organoid: PU.1 induction creates mhCOs with bona fide microglia and precursors matching yolk-sac-like and human fetal microglia profiles • Protective Aβ response: On Aβ_{1-42} exposure, these microglia activate and phagocytose more Aβ, reducing surface apoptosis and easing neuronal injury • Closer to primary cells: PU.1-induced microglia resemble primary microglia more than oMG, and Aβ pushes them even further toward this state • Key genes control protection: Endocytosis genes (<i>PICALM</i>, <i>SORL1</i>, <i>BIN1</i>) link to cholesterol pathways during Aβ stress. Knocking down <i>TREM2</i> or <i>SORL1</i> removes microglial protection and increases Aβ-toxicity; <i>SORL1</i> loss lowers CYP46A1 → impaired cholesterol turnover
(Tian et al. 2025)	Seven cell types in hcO-MPSs: • Neurons • Astrocytes • Other glial cells • Monocytes • Intermediate progenitors • Progenitors • Others	<i>CCR1, SEMA6B, FGR, APLP2, NFI, NUMB, PECAM1, CYP1B1, FOXO3, FCER1G, FCGR2A, TYROBP, GAS6, RAB5A, RAB11A, CLU, LRP2, S100A8, LYZ, ATF4, JUN, GADD45G, ITGB1, RPS3, ANP32A, IL1B, CCL3.</i>	• Stronger entry, weaker cleanup: AD monocytes infiltrate organoids more than controls ($\uparrow$ with Aβ), but once inside they phagocytose less Aβ, downregulate phagocytosis/Aβ-catabolism genes, upregulate inflammatory genes, as well as triggering astrocyte activation and neuronal apoptosis • Key mediators: IL-1β and CCL3 are elevated (RNA + protein), supporting a peripheral monocyte-driven neuroinflammation pathway in AD

Ref.	Cell clusters	Genes involved	Detailed research findings
(Feng et al. 2025)	Six clusters at occipital cortex region • Endothelial • Mural cells • Astrocytes • Microglia • Oligodendrocytes • Neurons Five clusters at occipitotemporal cortex region • Endothelial • Mural cells • Astrocytes • Microglia • Oligodendrocytes Four cell types in HSV-1-infected and uninfected organoids: • Early neuron • Progenitors • Choroid plexus • Mesodermal	<i>IRF8, STAT6, TLR2, PPARA, APP, CLU, TGFB2, ITPKB, NEAT1, LPAR1, IL4R, EPHB2, NFAT5, YWHAQ, NRXN1, LIPA10, LIMCa, LIMA5, LIMB3, LIMEd, LIMA9, LIMA2, LIM4c, L2b</i>	• Cell-type TE boost in HHV⁺ AD: LINE-1 elements rise in astrocytes, microglia, and oligodendrocytes (e.g., LIPA10/LIMCa/LIMA5); microglia also show immune TFs (IRF8) and inflammatory pathways • Genomic links & staging: L2b ↔ ITPKB and LIM4c ↔ NEAT1; LIM4c tracks Braak stage. NEAT1 is specifically up in astrocytes across regions (OC/OTC) • Model + rescue: HSV-1–infected forebrain organoids replicate LINE-1 upregulation in microglia/astrocytes, and antivirals (valacyclovir/acyclovir) reduce LINE-1 activity and tau-related pathology
(Lu et al. 2024)	18 clusters with 5 major cell types: • Neural progenitor cells (15, 17) • Intermediate progenitors (8) • Neurons (0, 2, 3, 4, 6, 7, 9, 12, 13, 14) • Astrocytes (1, 16) • Radial glia (5, 10, 11).	<i>APP, MAPT, SMPD1, SPTLC1</i>, oxidative stress genes	• Cell mix shifts to glia: PFAS reduces neurons/radial glia and increases astrocytes. • AD-like gene programs: Neurons show mitochondrial stress ↑ and synaptic genes ↓; astrocytes show immune genes ↑ and synapse terms ↓—matching AD cortex • Pathology + energy hits: Aβ_{40/42} and pTau rise; lipid droplets build up; mitochondria malfunction • Lipid pathway link to Aβ/tau: Lipidomics (517 lipids) shows glycerophospholipid/sphingolipid disruption; 74 lipids ↑ at high dose (900×); gene shifts (SPHK1, SMPD2, BACE1, APH1A) connect sphingolipid changes to Aβ/tau
(Zhao et al. 2023)	19 clusters with 5 major cell types: • Excitatory neurons (0, 1, 2, 3, 5) • Radial glia (4, 7, 9, 11, 17) • Astrocytes (10, 12, 13) • Intermediate progenitor cells (6) • Inhibitory neurons (8, 14, 15) • Undecided cluster (16, 18)	<i>APOE, SFRP1, ABCA1, ABCG1, DCX, SLC17A7, TBRI, BCL11B, SATB2, SOX2, S100B, GFAP, HMGCS, MSMO, HMGCR, IDII, FDPS, SQLE, FDFT1</i>	• Cell mix shifts: Fewer excitatory neurons; more astrocytes, radial glia, and inhibitory neurons • Stress pathway on: EIF2–ISR is activated in APOE^{−/−} organoids; ISRIB reverses it. • More astrogenesis via Wnt: APOE loss ↓SFRP1 → ↑Wnt/β-catenin in astrocytes → more astrocytes • Lipid/cholesterol dysregulation: Excitatory neurons ↑cholesterol-biosynthesis genes; astrocytes accumulate lipids and ↑ABCA1/ABCG1 • APOE4 genotype: APOE4 organoids mirror the APOE^{−/−} phenotype (impaired neurogenesis and cholesterol defects) when compared to isogenic APOE3 organoids → supports a loss-of-function mechanism for APOE4
(Chen et al. 2021)	14 clusters (no clear definition): • Six neuronal subclusters • Four astrocytes subclusters • Four neuroepithelial subclusters	<i>BACE1, GSK3α, GSK3β, HRK, CNR1, GABRB1, GABRG2, GRIA1, GRIA2, LRRC7, LRRTM2, LRRTM4, MT2A, NEAT1, PRDX6, NLRP1, IFITM3, GABRA2, GLUL, GPM6A, NLGN1, NRXN1, PTN</i>	• Serum drives parallel AD-like changes: Aβ and pTau both increased. BACE1 inhibitor IV lowers Aβ (not pTau); GSK3 inhibitor (CHIR99021) lowers pTau (not Aβ) → partly independent pathways • Synapse programs drop; astrocytes inflame: Neuronal synaptic genes fall (e.g., CNR1, GABA/AMPA components, LRRC7/LRRTM2/4). Astrocytes show stronger immune signatures (e.g.,

Ref.	Cell clusters	Genes involved	Detailed research findings
			MT2A, NEAT1, PRDX6, NLRP1, IFITM3) and fewer synapse-related terms, echoing AD cortex • Functional decline not rescued: After serum, synapses shrink ($\downarrow$SYN1 puncta) and networks weaken ($\downarrow$spikes, bursts, firing, synchrony). Neither BACE1 nor GSK3 inhibition restores synapses $\rightarrow$ multiple parallel pathologies
(Glasauer et al. 2022)	14 neuronal populations: • Excitatory pyramidal neurons (BCL11B, SOX5, TBR1, SATB2, FEZF2, SLCC17A7) • GABAergic neurons (GAD1, GAD2, DLX1,2,5,6, ETV1, PBX3, ARX, SP9) • Inhibitory neurons (GAD1, GAD2 but lacking IN-GE markers) • Largely unidentified excitatory neurons • Unidentified neurons (SYT1, CPLX2, SYNJ2) • Neural progenitor cell 1 • Neural progenitor cell 2 • Radial glia (VIM, SOX2, PAX6, EMX2) • Actively proliferating cells (RGC/NPC cycling; VIM, EMX2, DLX1, TOP2A, CENPF) • Largely unidentified glia (TTR, HTR2C, TM4SF1, EFNB3, WNT2B, BMP7) • Oligodendrocyte progenitors (OLIG1, OLIG2, PDGFRA) • Astrocytes (SLC1A2, GFAP, S100B, AQP4) • Glial progenitor cells (SLC1A3, GFAP, S100B) • Mesenchymal cells (LUM, DCN)	<i>MAPT, HMGCR, ACAT2, STARD4, LDLR, SREBF2, APOE, ALDOA, ALDOC, ENO1, ENO2, HK1, PFKF, PGK1, LDHA, GABRA2, GABRG2, NPAS4</i>	• Astrocyte cholesterol program up: MAPT-mutant organoids show higher expression of cholesterol genes (e.g., HMGCR, SREBF2), more HMGCS1⁺ astrocytes, and this intensifies from 4$\rightarrow$8 months without changing overall cell proportions • Neuronal metabolism/synapses down: Pyramidal neurons reduce glycolysis genes and GABA receptor genes $\rightarrow$ coordinated neuron–glia metabolic and synaptic disruption • Progressive lipid imbalance: By ~7 months, lipidomics shows higher cholesterol and late-pathway intermediates (desmosterol, 7-/8-DHC, cholestanol) $\rightarrow$ ongoing cholesterol dyshomeostasis • APOE-independent effect: Astrocyte cholesterol-gene upregulation with APOE downregulation occurs regardless of APOE genotype tested
(Fiock et al. 2020)	Four cell type based on classic neuronal maturation markers: • GFAP⁺/NES⁺ radial glia • TBR2⁺ intermediate progenitors • TBR1⁺ immature neurons • SYN⁺ mature neurons	<i>MAPT, TBR1, SYN, GFAP, NES, EOMES (TBR2), SYP.</i>	• Tau rises with neuronal maturation: MAPT is low in radial glia and TBR2⁺ progenitors, higher in immature TBR1⁺ neurons, and highest in mature SYN1⁺ neurons • Layer pattern: Tau mRNA is higher in SVZ and especially cortical plate vs ganglionic eminence • Confirmation in tissue and organoids: RNAscope shows weak MAPT in VZ/SVZ-like zones and strong in cortical plate-like regions; organoids increase over time (D35 < D50 < D134) • Protein lags mRNA: Little tau protein in germinal zones (IHC/Western), more in white matter/cortical plate $\rightarrow$ mRNA up before robust protein • Conserved tau maturation trajectory: Human fetal brain and organoids share a conserved tau-upregulation trajectory during neuronal maturation—useful as a baseline for disease models
Pérez et al. (2021).	26 transcriptionally distinct clusters with five major cell types: • Three neurons sub-annotations: ◦ Layer I neurons (cluster 18, 21) ◦ Upper-layer neurons (cluster 0, 1, 2, 17) ◦ Dopaminergic neurons (cluster 15) • Progenitors & radial glia (cluster 4, 6, 10, 16, 24) • Astrocytes (cluster 9, 13)	<i>PITRM1, ATF4, DDIT3, HSP60, HSPA9, ERO1, LONP1, CLPP, APP</i>	• Broad energy-pathway disruption: scRNA-seq (26 clusters) shows a similar cell mix across genotypes, but mitochondria/oxidative phosphorylation/sirtuin programs are consistently dysregulated in neurons, glia, astrocytes, and microglia • Synapse & signalling changes: Neurons have altered synaptogenesis/LTP; astrocytes show dysregulated iNOS, PPAR, TNFR1, RAR, chemokine, and IL-17A pathways

Ref.	Cell clusters	Genes involved	Detailed research findings
	- Microglia (cluster 22, 25) - Other glia (cluster 3, 11, 23)		Pathology up, partial rescue: KO organoids $\uparrow$APP, $\uparrow$A$\beta_{40/42}$ and A$\beta_{42/40}$, and tau hyperphosphorylation. NMN lowers A$\beta_{42/40}$ and pTau/total-tau and reduces cleaved caspase-3⁺ cells
(Bertachi <i>et al.</i> 2024).	15 transcriptionally distinct clusters with two major classes: - Neural progenitors (cluster 2, 5, 8, 9, 12, 13, 14, 15) - Neurons (cluster 1, 3, 4, 6, 7, 10, 11) Axis/region notes (from sub-analysis): - Ventral GABAergic identity (cluster 6, 7) - Mixed dorsal/ventral and posterior features (cluster 8, 9, 10, 11)	<i>FGF8, FOXG1, OTX2, ASCL1, DLX2, PBX3, EMX1, NEUROG2, SOX5, LHX2, ETV1, NR2F1, FGFR3, ZIC1, ZIC3</i>	Enhanced regional diversity: FGF8 boosts brain regional heterogeneity vs WNTi alone Coexistence of distinct regional identities: scRNA-seq finds FOXG1⁺ telencephalic plus OTX2/IRX3/EN1/EN2 non-telencephalic states, IF shows adjacent FOXG1⁺ and FOXG1⁻/OTX2⁺ zones Diversification of neuronal lineage specification: Glutamatergic and GABAergic lineages co-exist, with FGF8 promoting antero-ventral patterning
(Sarrafha <i>et al.</i> 2023).	Broad cell types (12 total; cluster number not specified): - Neurons - Astrocytes - Radial glial cells - Intermediate progenitor cells - OPCs - Oligodendrocytes - Ependymal cells - Tanycytes - Endothelial cells - VLMCs - Microglia - Unassigned Neuronal subtypes (17 total; cluster number not specified): - Neuroendocrine-like cells - Unknown neuron subtype - Unclassified - Dopaminergic neurons - SF1 neurons - SST neurons - GABAergic neurons - Preoptic-area neurons - TH⁺ GABAergic neurons - Glutamatergic neurons - TRH neurons - MCH neurons - OXT neurons - HCRT neurons - NTS neurons - POMC neurons. - NPW neurons (plus TAC1 and midbrain neurons listed as minor categories)	<i>TH, NKX2-1, OTP, POMC, TRH, GFAP, SOX9, SLC17A6 (VGLUT2), GAD2, DLX5, SST, TAC1, HCRT, NR5A1, FOXG1, RAX, DCX, ASCL1, TOP2A, OLIG2, MOBP, CCDC153, COL1A1, DCN, AIF1, DDC, KCNJ6, PRDM12, NR2F2, KCNJ3, NR5A2, TENT5A, CREB3</i>	Validation of hypothalamic specification and dopaminergic heterogeneity: D85 organoids contain diverse cell types plus 17 neuronal subtypes; dopaminergic neurons split into 7 subtypes based on OTP/RAX states (four OTP⁺RAX⁺ groups, OTP⁺RAX⁻, OTP⁻RAX⁺, and KISS1-like), defined by marker combos (SHH, PRDM12, ASCL1, NR2F2, KCNJ3/KCNJ6) Trajectory inference of dopaminergic maturation: RNA-velocity/pseudotime tracks OTP⁺RAX⁺ $\rightarrow$ OTP⁺RAX⁻ / OTP⁻RAX⁺, with KISS1-like neurons the most mature Metabolic reprogramming in dopaminergic neurons: Dopaminergic neurons upregulate glycolysis, gluconeogenesis, and oxidative phosphorylation, while EIF2 signalling, cholesterol biosynthesis, tRNA charging, and mevalonate–geranylgeranyl pathways are down—consistent with higher energy demand
(Jain <i>et al.</i> 2025).	Major cell types identified (cluster number not specified): - Early neuroectoderm - Late neuroectoderm - Telencephalic progenitors - Telencephalic neurons	<i>YAP1, WLS, POU5F1, ITGA5, PROM1, THY1, SOX2, TCF7L2, OTX2, ZIC2, CYP26A1, ITGA6, SOX21, COL1A1, COL11A1, LAMA5,</i>	Extracellular matrix–dependent regulation of neuroepithelial morphogenesis and regional specification: Exogenous ECM incorporation (e.g., Matrigel or laminin-PEG) accelerates lumen expansion, promotes neuroepithelial maturation, and biases patterning toward telencephalic identity,

Ref.	Cell clusters	Genes involved	Detailed research findings
	• Diencephalic progenitors • Diencephalic neurons • Mesencephalic/Rhomencephalic progenitors & neurons • Neural crest & peripheral nervous system neurons • Retinal progenitors • Choroid plexus	<i>HAPLN3, MMP16, IGFBP2, SIX3, LHX2, NRG1, FOXH1, HESX1, RSPO3, GPC3, SFRP2, NPTX1, PRTG, PODXL, RAX, PAX6, SOX10</i>	whereas absence of ECM or use of agarose results in reduced lumen growth, delayed fusion, and increased neural crest and caudal identities. • Mechanotransductive control of patterning via YAP–WNT signalling axis: In the absence of an extrinsic extracellular matrix, YAP1 activity is elevated and directly induces WLS expression, promoting caudalisation of the developing neuroepithelium. Genetic ablation of WLS prevents this shift, restoring telencephalic identity even under matrix-deprived conditions.
(Dai <i>et al.</i> 2024)	Eight major brain cell classes evaluated (cluster number not specified): • Excitatory neurons • Inhibitory neurons • Astrocytes • Oligodendrocytes • OPCs • Microglia • Endothelial cells • Pericytes Note: Organoid benchmarking also referenced radial-glia/neuronal subclasses but without fixed cluster numbers.	<i>NRGN, GAD1, CALM2</i>	• Benchmarking of deconvolution methods: dtangle best for cell-type fractions; bMIND best for per-sample cell-type expression ($p \approx 0.62$ ROSMAP, 0.75 CMC, 0.85 organoids). • Enhanced genetic signal detection through deconvolution: Mapped 25,273 decon-eQTLs (8 cell types); they explain more SCZ GWAS signal than single cell eQTLs; decon + bulk $\approx 63\%$ heritability vs $\sim 49\%$ with bulk alone. • Robustness across datasets with reduced sensitivity for rare populations: AD/SCZ/development DE results replicate in bulk; accuracy drops for rare cell types ($<5\%$). • Recommended analytical framework for brain tissue deconvolution: Use dtangle (fractions) + bMIND (expression) for brain deconvolution.

Abbreviations: A β =amyloid beta, AD=Alzheimer's disease, APOE = apolipoprotein E, APP = amyloid precursor protein, BACE1 = beta-secretase 1, bMIND=Bayesian Model-based INtegration from Deconvolution, CMC=CommonMind Consortium, CO=cerebral organoid, DE=differential expression, DHC=dehydrocholesterol, dtangle=deconvolution method for cell-type heterogeneity, ECM=extracellular matrix, eQTLs=expression quantitative trait loci, fAD=familial Alzheimer's disease, FGF8 = fibroblast growth factor 8, GWAS=genome-wide association study, hcO-MPSs=human cortical organoid–microphysiological systems, HHV=human herpesvirus, hiPSC=human induced pluripotent stem cell, hPSC=human pluripotent stem cell, HSV-1=herpes simplex virus type 1, IF=immunofluorescence, IHC=immunohistochemistry, KO=knockout, LTP=long-term potentiation, mhCOs=microglia-containing human cortical organoids, mRNA=messenger RNA, NDC=non-demented control, NFTs=neurofibrillary tangles, NMN=nicotinamide mononucleotide, NSCs=neural stem cells, OC=occipital cortex, oMG= organoid-derived microglia-like cells , OPCs=oligodendrocyte precursor cells, OTC=occipitotemporal cortex, PFASs=per- and polyfluoroalkyl substances, PHF-tau=paired helical filament tau, pTau=phosphorylated tau, PSEN1 = presenilin 1, PSEN2 = presenilin 2, ROSMAP=Religious Orders Study and Memory and Aging Project, scRNA-seq=single cell RNA sequencing, SCZ=schizophrenia, SVZ=subventricular zone, TEs=transposable elements, TFs=transcription factors, VLMCs=vascular and leptomeningeal cells, VZ=ventricular zone, WNTi=WNT inhibition

9 Ethical and consent to participate

An ethical waiver was granted because this study did not involve human and animal subjects.

10 Data and software availability

Not applicable.

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13 Author contributions

Maizatul Fazilah binti Abdul Razak: Conceptualisation, Writing - Original draft, Visualisation, Writing - Review & editing; **Francis Yew Fu Tieng:** Conceptualisation, Writing - Original draft, Visualisation, Writing - Review & editing, Project administration, Supervision, and Funding Acquisition. All authors have agreed to the final submitted version.

14 Conflict of interest disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

15 Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (<https://chatgpt.com/>) to proofread the final manuscript. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication. Additionally, all figures were created using Microsoft PowerPoint. No BioRender software was used and no BioRender licence applies. No AI tools were used in the generation of these figures. NotebookLM (<https://notebooklm.google.com/>) was used only as a supportive tool for ideation during preparation, while the final design and composition were performed entirely by the authors.

16 Consent to publish declaration

Not applicable.

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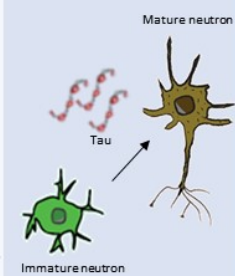
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The Evolution of Single-Cell Sequencing in Alzheimer's Organoid Research

A chronological overview of how scRNA-seq technologies have advanced our understanding of Alzheimer's disease using human organoid models.

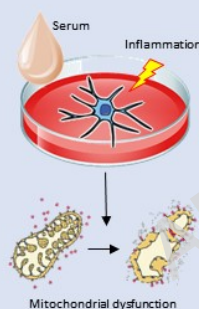
2020-2022: Mapping Maturation and Early Pathology

Tau mRNA Expression Linked to Neuron Maturation



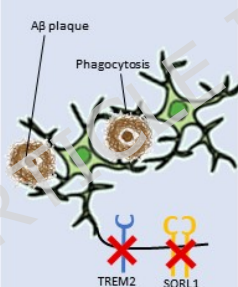
(Fiocik et al., 2020)

Serum Exposure & Mitochondrial Defects



(Chen et al., 2021;
Pérez et al., 2021)

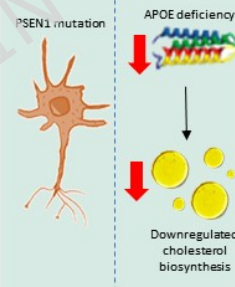
Microglial Integration & Protection



(Cakir et al., 2022)

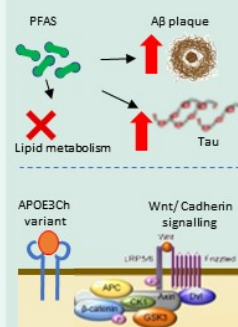
2023-2025: Advanced Genetic & Environmental Modelling

Developmental Shifts & APOE Impacts



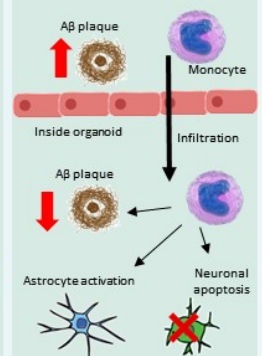
(Sarrafina et al., 2023;
Vanova et al., 2023;
Zhao et al., 2023)

Environmental Toxins & Genetic Modifiers



(Bertacchi et al. 2024;
Lu et al., 2024;
Perez-Corredor et al., 2024)

Immune Infiltration



(Tian et al., 2025)

Single-Cell Transcriptional Landscape of Alzheimer's Disease Organoid Model

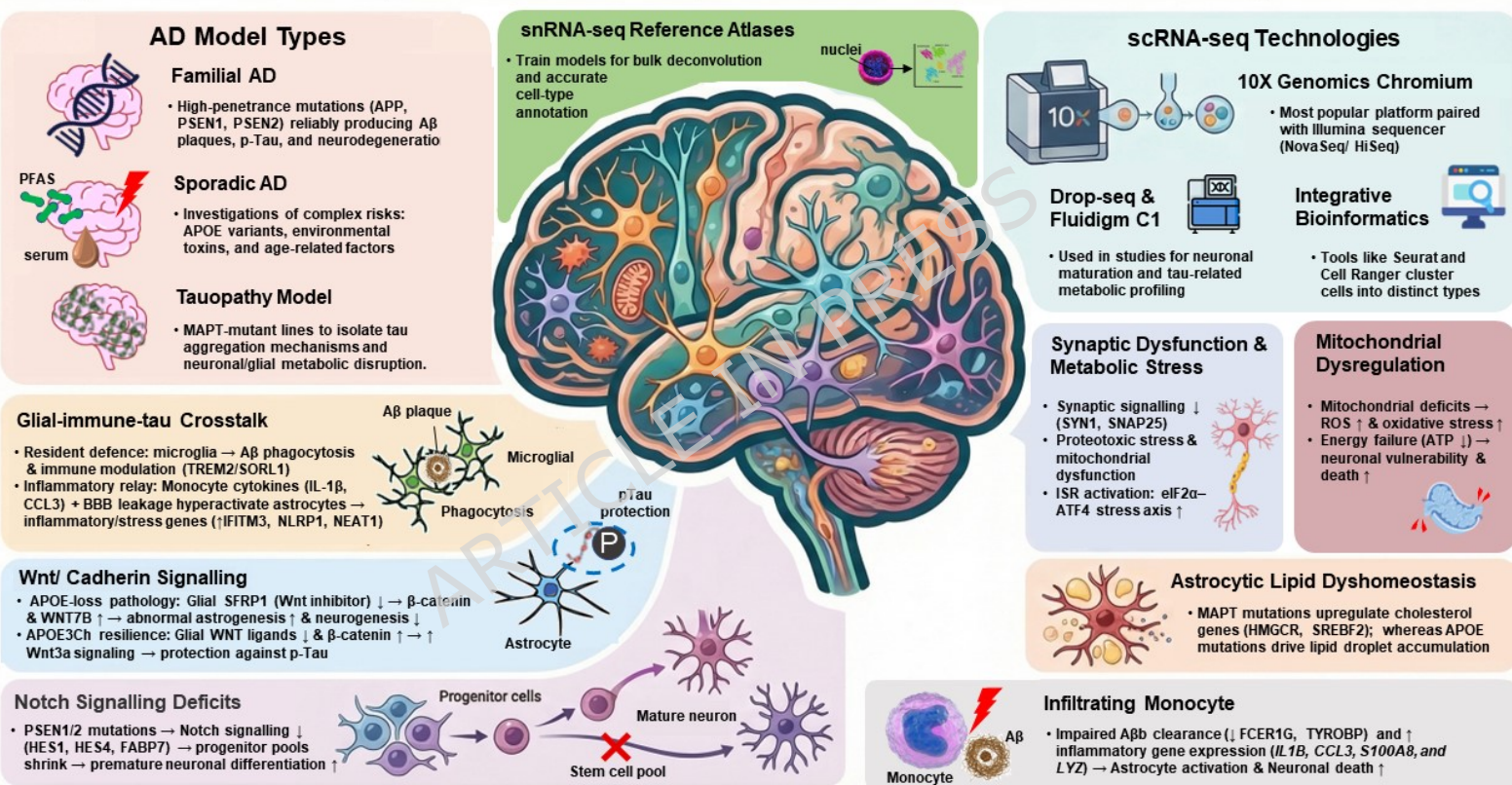


Table 1: Summary of Single Cell Transcriptomic Studies in Cerebral Organoids: Applications, Cell Type, Methodologies, and Key Findings in Models of Alzheimer's Disease, Tauopathies, and Neurodevelopment

Type	Role of scRNA-seq	Type/ Number of cells analysed	Technology & sequencer ^a	Take home
fAD	Analysis of the specific cell types within the CO, revealing a heterogeneous composition of normal brain cells	70-day-old <i>CAG-APP^{SweFLon-IRES-PSEN1M146L/L286V}</i> (CAP) cerebral organoids; cell number not stated	Chromium (10x Genomics); sequencer not stated	- Overexpression accelerates increases - Pathological and γ-secretase for prote
fAD	- Identification of a heterogenous mixture of cells - Identification of developmental trajectories, showing that clusters enriched in APOE3Ch cells represented later stages, suggesting altered developmental timing or premature differentiation. - Identification of regulation of Cadherin and Wnt signalling pathways by APOE3Ch	6,400 cells from 6 pooled organoids per iPSC-derived cell line (total 8 lines from 2 patients, α and ω): α-APOE3/PSEN1 E280A, α-APOE3Ch/PSEN1 E280A, α-APOE3Ch/PSEN1 WT, α-APOE3Ch/PSEN1 E280A (duplicate clone), ω-APOE3/PSEN1 E280A, ω-APOE3/PSEN1 E280A (duplicate clone), ω-APOE3/PSEN1 WT, ω-APOE3/PSEN1 E280A	Chromium (10x Genomics); sequencer not stated	- APOE3 timing - Enhanced protection
fAD	- Identification of heterogeneity of NDC#1- and AD#1-iPSC-derived organoids - Confirmation of the altered developmental phenotype and signs of premature onset of differentiation in a subset of neurons via integrated data analysis (NDC#1-CO and AD#1-CO)	2,298 NDC cells and 2,795 AD cells retained post-QC; derived from 18 pooled day-60 organoids per sample	Chromium (10x Genomics); NextSeq 500 (Illumina)	- PSEN1 accumulation - Reduced pool differentiation
A β -induced AD	- Integration of organoid-derived microglia-like cells (oMG) and primary microglia signatures to assess differences between innate oMG and PU.1-induced microglia - Identification of developmental trajectories, segregating cells into microglia, neurons, astrocytes, and other lineages to trace lineage commitment (linking GFP/PU.1 expression to microglial fate) - Evaluation of the transcriptional response and protective role of microglia following Aβ₁₋₄₂ exposure - Analysis of high-throughput genetic perturbations (CROP-seq) to identify the specific roles of AD-associated risk genes (e.g., <i>SORL1</i>, <i>TREM2</i>) in cholesterol metabolism and Aβ response	13,416 hCO cells and 13,546 mhCO cells for baseline characterisation; 49,867 cells analysed for the non-treated vs. A β -treated comparison	Chromium (10x Genomics); NovaSeq S4 (Illumina)	- PU.1-induced neurotoxicity - This is on AD
sAD/ immune-driven	- Investigation of mechanisms of increased AD monocyte infiltration into the human cortical organoid microphysiological systems (hCO-MPSs) - Analysis of neuronal and monocyte interactions in the hCO-MPS model to reveal immune-driven neurodegeneration	Cells from hCO-MPSs that were cocultured with human peripheral monocytes; number not stated	Chromium (10x Genomics); sequencer not stated	- Infiltration show in - Monocyte neuron inflammation
sAD/ viral induced	- Profiling of transcriptomes from 60-day-old organoids at 3 days post infection and clustering with distinct cell types - Mechanistic validation of snRNA-seq, pinpointing cell types in the HSV-1/HHV-infected human organoid model that drive TE dysregulation and to assess how antiviral drugs modulate these effects - Identification of cell type-specific transcriptional changes in transposable elements (TEs) and genes following HHV infection - Investigation of the underlying mechanisms of acyclovir and valacyclovir on HSV-1-infected human brain	Three public datasets: - scRNA-seq (GSE129308): 24,660 soma with NFTs, 38,465 neighbouring NFT-free neurons from AD donors and 63,110 control NFT-free somas - snRNA-seq (GSE138852): 13,214 nuclei of entorhinal cortex from control and AD brains of twelve individuals - snRNA-seq (GSE148822): 482,472 nuclei from non-demented control brains and AD brains containing only amyloid-β plaques or both amyloid-β plaques and tau pathology	- GSE129308 (Otero-Garcia et al. 2022): Chromium (10x Genomics); NovaSeq 6000 (Illumina) - GSE138852 (Grubman et al. 2019) & GSE148822 (Gerrits et al. 2021): Chromium (10x Genomics); NextSeq 500 (Illumina) Validation scRNA-seq public datasets:	- Latent type-specific transp - Antiviral partial down

Type	Role of scRNA-seq	Type/ Number of cells analysed	Technology & sequencer ^a	Take home
	organoids to assess how antiviral drugs modulate TE dysregulation	Validation = droplet-based scRNA-seq public dataset (GSE163952): human iPSC-derived organoid cells; number not stated	GSE163952 (Rybak-Wolf et al. 2023): Drop-seq (ChemGenes Corp.); NextSeq 500 (Illumina)	
sAD/ environmental induced	- Prediction and validation of cell-type-specific neurotoxicity of PFASs - A powerful methodology to evaluate the health risks associated with environmental contaminants, particularly those with neurotoxic potential	4 organoids per group (control and 900× PFAS); 14,556 cells from control group and 14,030 cells from 900× PFAS group	Chromium (10x Genomics); NovaSeq X Plus (Illumina)	- Chronic neurodegeneration and synaptic dysfunction - Pathological dysregulation of sphingolipids
sAD	- Elucidation of the cell-type-specific effects of APOE deficiency in human cerebral organoids - Identification of changes in cellular composition, demonstrating that excitatory neurons were reduced while astrocytes, radial glia, and inhibitory neurons increased	27,199 single cells in total (14,920 cells from control Day-90 cerebral organoids and 12,279 cells from isogenic APOE ^{-/-} Day-90 cerebral organoids)	Chromium (10x Genomics); HiSeq 4000 (Illumina)	- APOE4 neurodegeneration and proliferation - Activation of response to homeostatic APOE
sAD	- Identification of a heterogeneous mixture of cells - Evaluation of synaptic and immune transcriptomic alterations in serum-exposed sAD organoids to reveal reduced synaptic function in neurons and induced immune responses in astrocytes	26,631 cells (9,338 BO2_control; 5,412 BO2_serum; 6,535 BO3_control; 5,346 BO3_serum)	Chromium (10x Genomics); NovaSeq 6000 (Illumina)	- Serum barrier of Aβ - Synaptic GSK3 disease
Tauopathy/ familial	- Profiling of human cerebral organoids from MAPT-mutant lines vs isogenic controls across development - Identification of a heterogeneous cell population - Identification of cell-type-specific pathway changes, specifically uncovering cholesterol biosynthesis dysregulation in astrocytes and glycolysis/synaptic drops in neurons - Nomination of disease-relevant mechanisms downstream of tau mutations	76,111 total cells post-filtering, with a mean of 1,228 cells ± 239.6 SD per sample	Drop-seq (Macosko et al. 2015); NextSeq 500 (Illumina)	- Familial APOE4 cholesterol - Pyramidal downregulation of components
Tauopathy/ developmental	- Evaluation of tau (MAPT) mRNA expression patterns as a function of lamina and neuronal maturation during fetal brain development and in iPSC-derived cortical organoid - Confirmation of low amounts of tau mRNA in very early neuronal precursors (radial glia and intermediate progenitors)	2 public datasets: 48 human fetal cortex samples aged 5.8–37 postconceptional weeks; number not stated (Nowakowski et al. 2017) - hiPSC-derived cortical organoids derived from three different donors at 105 day of <i>in vitro</i> differentiation; number not stated (Yoon et al. 2019)	- Fluidigm C1 (Fluidigm) and HiSeq2500 (Illumina) (Nowakowski et al. 2017) - BD Rhapsody (BD Biosciences) (Yoon et al. 2019)	- Organic upregulation of foetal tau - Human conservation of neuronal disease
Familial risk mitochondrial dysfunction	Characterisation of cell-type-specific pathogenetic mechanisms, mitochondrial dysfunction, and AD-like pathology in PITRM1-deficient organoids.	Total 15,183 cells from 2-month-old cerebral organoids (WT+KO pooled; per-genotype counts not reported; ~18,000 cells loaded per sample pre-QC)	Chromium (10x Genomics); sequencer not stated	- PITRM1 mitochondrial proteome - Knockdown and tau by NA
Developmental/ regional modelling (neurodevelopment disorder)	Investigation of the transcriptomic signature of cerebral organoids upon FGF8 treatment to quantify regional and neuronal subtype diversity shifts, illuminating mechanisms of regional vulnerability	18,590 cells analysed from Day 69 organoids (from two independent batches per condition [WNTi; WNTi+FGF8], each batch pooling 2–3 organoids)	Chromium (10x Genomics); sequencer not stated	- FGF8 distinct domain - A multi-selective dynamic
Non-AD (developmental modelling/ Parkinson's disease)	- Characterisation of neuroendocrine and dopaminergic diversity in hypothalamic organoids - Identification of molecularly distinct hypothalamic DN subtypes and their respective developmental maturities	21,001 cells from D85 hypothalamus organoids (TH^{hi} group: 10,464 cells, ≈2,647–4,015 per replicate; TH^{lo} group: 10,537 cells, ≈3,262–3,663 per replicate)	Chromium (10x Genomics); sequencer not stated.	- Guided dopaminergic varying - DNs expression reprogram

Type	Role of scRNA-seq	Type/ Number of cells analysed	Technology & sequencer ^a	Take home
Non-AD (developmental modelling)	- Mapping of regionalisation and morphogenetic transitions in unguided brain organoids. - Evaluation of cell-state changes associated with tissue transitions and the molecular processes affected by different extracellular matrix (ECM) and signalling perturbations.	- Time-course (D5, D7, D11, D16, D21, D30): Per-day cell counts not reported; pooled organoids = 24 (D5/D7/D11), 12 (D16), 10 (D21), 6 (D30). - Day 13 – Matrix: Matrigel 8,095; No-matrix 8,343; Agarose 3,725. - Day 10 – YAP (Py-60): DMSO 2,085; Py-60 day-5 763; Py-60 day-7 1,955. - Day 55 – Hashed (WT+KO totals): Matrigel 5,221 (3,560+1,661); No-matrix 5,377 (1,283+4,094); CHIR 5,084 (3,310+1,774); Py-60 2,782 (1,399+1,383); DMSO 3,540 (2,087+1,453). - Extended Data (hashed subset): Matrigel 1,183; No-matrix 1,285; Py-60 1,189.	Chromium (10x Genomics); sequencer not stated.	- glycolysis - Extraction - luminescence - region - Mechanism - control - caudal
Non-AD (protocol that support AD studies)	Evaluation of nine existing sample-wise cell deconvolution methods (DSA, OLS, CIBERSORT, dtangle, MuSiC, Bisque, bMIND, swCAM, TCA) on human brain transcriptomic data using matched bulk RNA-seq, sc/snRNA-seq, and IHC data to establish best practices and explore biological applications	Matched datasets used for benchmarking/analysis: ROSMAP bulk RNA-seq n=1112; ROSMAP snRNA-seq 48 donors / 69,611 nuclei; IHC n=49. CMC bulk n=572; CMC snRNA-seq 101 donors / 569,289 nuclei. Brain organoids bulk n=130; organoid scRNA-seq 72 samples / 490,844 cells. Across all, ~1,130,767 nuclei/cells from 149 adult brains and 72 organoid samples	snRNA-seq and scRNA-seq (public datasets downloaded from Synapse); specific sequencer and single cell platforms are not stated.	- Benchmarking - as the - bulk R - Accuracy - proportion - profilin

^a denotes by scRNA-seq platform (brand name) and sequencing platform (brand name)

Abbreviations: A β = amyloid beta, AD = Alzheimer's disease, APOE = apolipoprotein E, BACE1 = beta-secretase 1, CCL3 = C-C motif chemokine ligand 3, CO = cerebral organoids, CROP-seq = CRISPR droplet sequencing, DMSO = dimethyl sulfoxide, DN = dopaminergic neuron, ECM = extracellular matrix, EIF2 = eukaryotic initiation factor 2, fAD = familial Alzheimer's disease, FGF8 = fibroblast growth factor 8, GFP = green fluorescent protein, GSK3 = glycogen synthase kinase 3, hCO = human cortical organoid, hCO-MPSs = human cortical organoid microphysiological systems, HHV = human herpesvirus, hiPSC/iPSC = human induced pluripotent stem cell, HSV-1 = herpes simplex virus type 1, IHC = immunohistochemistry, IL-1 β = interleukin-1 beta, KO = knockout, LINE-1 = long interspersed nuclear element-1, MAPT = microtubule-associated protein tau, mhCO = microglia-containing human cortical organoids, NDC = non-demented control, NFTs = neurofibrillary tangles, NMN = nicotinamide mononucleotide, oMG = organoid-derived microglia-like cells, PFAS/PFASs = per- and polyfluoroalkyl substances, PITRM1 = pitrilysin metalloproteinase 1, PSEN1 = presenilin 1, PSEN2 = presenilin 2, pTau = phosphorylated tau, PU.1 = PU box-binding transcription factor, QC = quality control, RNA-seq = RNA sequencing, ROSMAP = Religious Orders Study and Memory and Aging Project, sAD = sporadic Alzheimer's disease, scRNA-seq = single cell RNA sequencing, SD = standard deviation, snRNA-seq = single-nucleus RNA sequencing, SORL1 = sortilin related receptor 1, TEs = transposable elements, TH = tyrosine hydroxylase, TREM2 = triggering receptor expressed on myeloid cells 2, WT = wild type