Advancements in Human Brain Organoid Models: Integrating Multi-Omics and Genetic Engineering to Unravel Neurodevelopmental Disorders

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Abstract

Human brain organoids have emerged as transformative tools for modeling early neural development and investigating the molecular basis of neurodevelopmental disorders. Recent advances in stem cell engineering, spatial patterning, and three-dimensional culture have enabled the generation of increasingly complex models that recapitulate features of the embryonic human brain, including region-specific organization, neuronal diversity, and early circuit formation. Simultaneously, the integration of single-cell transcriptomics and multi-omics technologies has enhanced the resolution with which these models can be analyzed, allowing for precise mapping of developmental trajectories and disease-associated perturbations. In parallel, CRISPR-based genome editing has facilitated targeted manipulation of neurodevelopmental genes, enabling mechanistic insights into rare and common disorders such as autism spectrum disorder and microcephaly. This review synthesizes recent progress in the engineering, validation, and application of brain organoid systems, highlighting key studies that combine morphogenetic fidelity with high-throughput genomic and functional analysis. We also discuss current limitations—including variability, incomplete maturation, and ethical considerations—and propose strategies for improving the reproducibility and translational relevance of brain organoids in disease modeling.

Introduction

Understanding the human brain's development—and the origins of its disorders—has long been hindered by ethical, technical, and biological barriers to studying the living human brain in early stages. In recent years, human brain organoids have emerged as a powerful platform to overcome these challenges. These self-organizing, three-dimensional structures, derived from human pluripotent stem cells, can mimic key aspects of in vivo brain development, including region-specific architecture, progenitor zone formation, and early neuronal activity. Their ability to recapitulate spatiotemporal features of neurodevelopment has made them invaluable for modeling neurodevelopmental disorders (NDDs) such as autism spectrum disorder (ASD), microcephaly, and epilepsy.[1]

While their architecture is foundational, the true power of organoids lies in their integration with emerging technologies—namely, single-cell omics and CRISPR-based genome editing—which collectively unlock new dimensions of disease modeling. Advances in single-cell transcriptomics and multi-omics have enabled researchers to map cell-type-specific developmental trajectories and uncover molecular disruptions in patient-derived and engineered models. Simultaneously, the advent of CRISPR-based functional genomics allows for high-throughput perturbation of NDD-associated genes, revealing causal mechanisms underlying both rare mutations and common disease pathways.[2] Organoids not only serve as developmental models but are increasingly used for patient-specific drug testing, opening paths toward personalized interventions for NDDs. Together, these tools allow researchers to precisely track how normal brain development diverges in disease.

In this review, we explore how recent innovations in brain organoid engineering, multi-omic profiling, and genome editing are converging to unravel the complexity of

neurodevelopmental disorders. We highlight foundational studies that demonstrate the importance of morphogenetic fidelity, cellular diversity, and circuit integration, and we examine how transcriptomic and epigenetic signatures across cell types inform our understanding of disorder-specific pathology. Finally, we consider the future of this rapidly evolving field, from overcoming limitations in organoid maturation and reproducibility to designing personalized models of brain disease and repair.

2. Engineering and Refinement of Brain Organoids

2.1. Morphogenetic Fidelity and Tissue Architecture

The spatial architecture of brain organoids, particularly the formation and organization of neuroepithelial rosettes, is central to modeling accurate human cortical development. These rosette structures—radial arrangements of progenitor cells surrounding a central lumen—mimic the neural tube's ventricular zone and provide essential cues for regulated neuronal differentiation.

Lancaster et al. (2023) demonstrated that organoids enriched with well-defined rosette structures exhibit developmental timelines and gene expression trajectories closely aligned with in vivo neurogenesis. In contrast, organoids with disorganized or flattened morphologies showed asynchronous progenitor differentiation and premature neurogenesis, despite being derived from identical pluripotent lines.[1] Complementing these findings, research on "single-rosette" cortical organoids—structures initiated from patterned 2D neuroepithelial monolayers—has shown reproducible morphogenesis with improved structural consistency and predictable lineage progression. Tidball et al. (2023) reported that these SOSR-COs develop with a single, centralized lumen and consistent progenitor zones, enhancing reproducibility and suitability for disease modeling.[3]

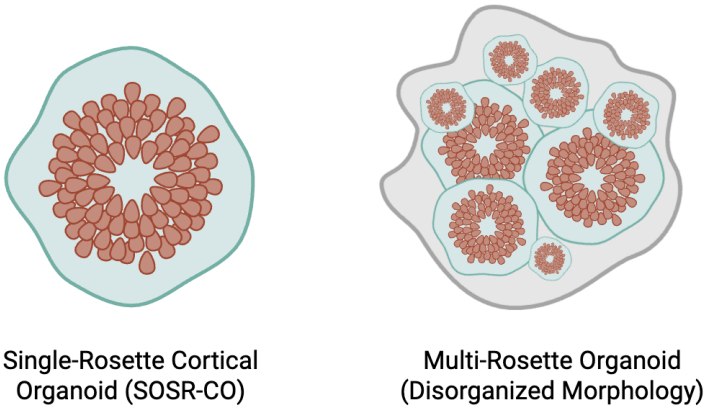

Figure 1. Morphological comparison of cortical organoid structures.

Moreover, bioengineering strategies using micro-patterned substrates or microfluidic devices have further controlled rosette formation. Techniques explored by Cho et al. (2021) have successfully guided the emergence of singular rosettes and promoted tissue folding, thereby improving developmental fidelity.[4] Finally, mechanistic studies using vertex-based

biophysical modeling have shown that rosette formation depends on apicobasal polarity and tissue rigidity, highlighting mechanical constraints as key regulators of neuroepithelial morphogenesis.[5] These findings suggest that multicellular rosette emergence is governed by collective cell mechanics.

2.2. Region-Specific and Assembloid Models

Region-specific organoids and assembloids are emerging as powerful tools to recreate spatial complexity and inter-region interactions in human brain development. Emerging methods such as MERFISH and Slide-seq complement scRNA-seq by localizing expression patterns within intact organoid architecture, adding a new dimension to disease modeling. These methods address a key limitation of early organoid models, which often lacked the regional specification and intercellular dynamics necessary to study integrated circuit development.

One of the most influential advances in this area is the development of dorsal–ventral forebrain assembloids, which combine cortical (dorsal) organoids with subpallial (ventral) organoids to mimic interneuron migration into the cortex. In pioneering work by the Pașca lab, fused human forebrain assembloids were shown to support the generation and tangential migration of GABAergic interneurons—key steps in establishing excitatory-inhibitory balance in cortical circuits. Using a doxycycline-inducible CRISPR-Cas9 system, large-scale perturbation screens identified dozens of genes implicated in interneuron generation and migration. These included cytoskeletal regulators like LNPK, whose disruption impaired endoplasmic reticulum positioning and interneuron motility.[2]

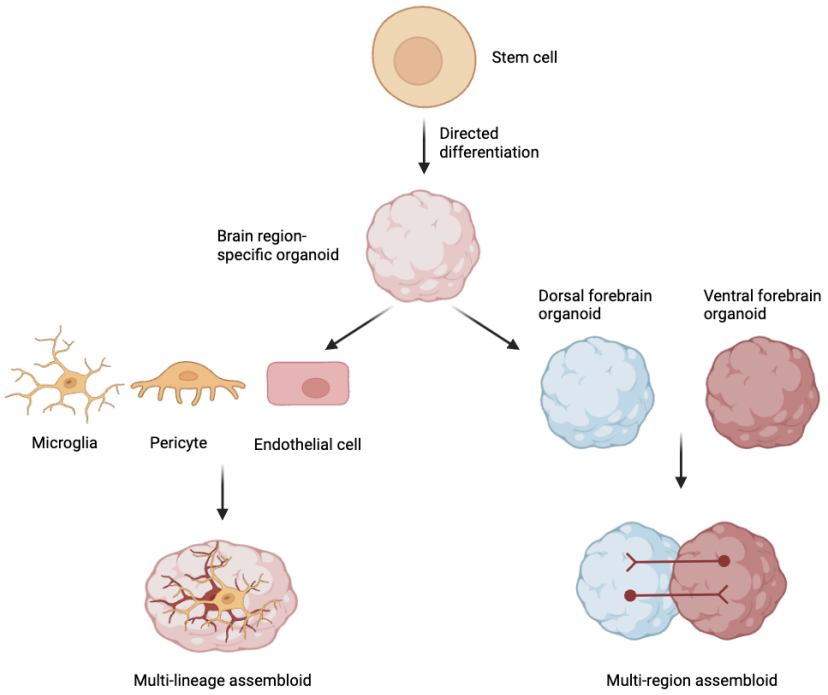

Figure 2. Generation of multi-region and multi-lineage brain assembloids.

Similarly, the Park lab engineered assembloids by fusing regionally patterned dorsal and ventral organoids, demonstrating successful interneuron migration and integration across domains. This model enabled real-time tracking of migration dynamics and synaptic integration, offering a physiologically relevant platform to study disorders characterized by altered excitatory-inhibitory balance, such as epilepsy and autism.[6]

Beyond the forebrain, assembloid approaches have also been extended to include thalamic, hippocampal, and spinal cord regions. These region-specific models enable researchers to study long-range axon projections, circuit connectivity, and synchronized neural activity—phenomena essential for understanding higher-order cognitive functions and their disruption in neurodevelopmental disorders. Together, these advances underscore the importance of cross-regional modeling in capturing the architecture and timing of human brain development. Assembloids offer a powerful platform for investigating neuronal migration, connectivity, and the molecular pathogenesis of NDDs within a more complete and dynamic developmental context.

2.3. Functional Maturation

Achieving functional maturation—particularly the emergence of patterned neural activity—is critical for organoids to model higher-order brain functions and disease-relevant circuitry. Early organoid models lacked synchronized electrical activity, limiting their applicability in modeling disorders affected by electrophysiological disruptions. This gap has spurred the development of protocols aimed explicitly at inducing functional maturation.

To address the gap in circuit-level modeling, recent protocols have focused on promoting electrophysiological activity in organoids. A significant advancement comes from the Muotri lab, which introduced a “semi-guided” cortical organoid approach combining guided differentiation with minimal exogenous patterning, plus neurotrophic support (e.g., BDNF, NT-3). These organoids exhibit complex neural oscillations—rhythmic activity patterns similar to those seen in fetal human brains. Recordings using microelectrode arrays and calcium imaging revealed reproducible oscillatory features, including nested rhythms, suggesting the emergence of immature neural network dynamics.[7] The “semi-guided” strategy balances cellular diversity with structural and functional consistency, making it well-suited for disease modeling, including conditions marked by dysrhythmias such as epilepsy and schizophrenia . Follow-up studies corroborated these findings, showing that spontaneous bursts and rhythmic network events in organoids mimic preterm cortical EEG patterns.[8]

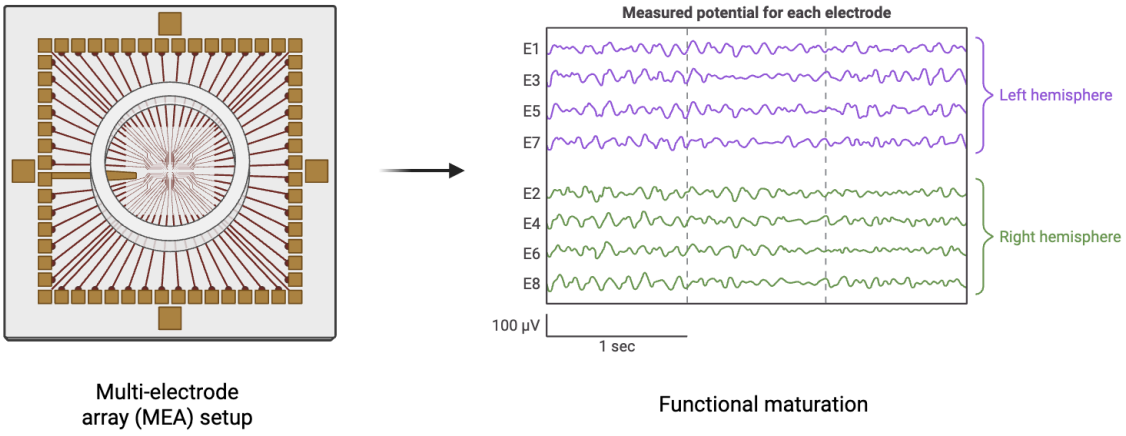

Figure 3. Electrophysiological activity recorded from a brain organoid using a multi-electrode array (MEA).

Beyond oscillations, the maturation push extends to synaptic refinement, astrocyte emergence, and regulated axon pathfinding—all essential for capturing circuit-level behaviors. Efforts to include vascular-like networks, organoid–endothelium co-cultures, and bioengineered scaffolding are further enhancing physiological relevance, supporting long-term viability, nutrient delivery, and deeper tissue complexity.[9]

In summary, advancements in functional maturation—especially through electrophysiologically active semi-guided organoids—are bringing brain organoids closer to in vivo-like neural network paradigms. The integration of functional assays and electrophysiology enhances model fidelity and broadens their utility in investigating circuit-level disruptions in neurodevelopmental and psychiatric disorders. However, while engineering advances have improved organoid fidelity and complexity, deeper understanding of cellular behavior requires molecular dissection. Multi-omics approaches now allow researchers to trace the developmental and pathological trajectories of these refined models at single-cell resolution.

3. Multi-Omics and Single-Cell Profiling

3.1. Single-Cell Transcriptomics

Single-cell RNA sequencing (scRNA-seq) has revolutionized the analysis of human brain organoids, enabling precise tracking of dynamic cellular states and developmental progressions. By profiling gene expression at the single-cell level, scientists can distinguish distinct neural and glial populations, infer lineage relationships, and map the timing and sequence of neurodevelopmental events within organoid models.

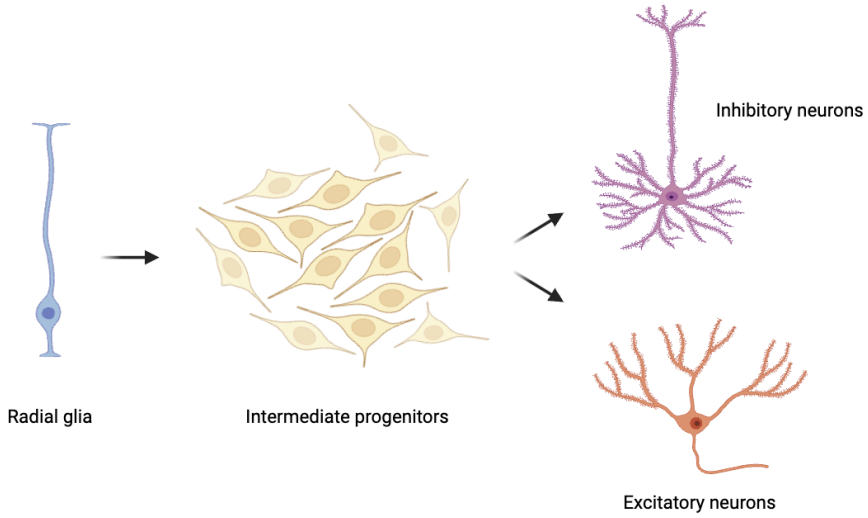

Figure 4. Developmental trajectory of neural lineages in brain organoids.

A landmark contribution constructed a single-cell atlas of the human neocortex during mid-gestation by sequencing over 40,000 cells. This resource identified diverse populations of radial glia, intermediate progenitors, excitatory and inhibitory neurons, and glial subtypes. By integrating chromatin accessibility data (e.g., ATAC-seq), the team reconstructed gene regulatory networks that guide cell fate transitions and highlighted key transcription factors driving neuronal differentiation. Crucially, the atlas linked numerous neuropsychiatric risk genes—including those associated with autism and epilepsy—to specific progenitor or neuronal populations, revealing windows of vulnerability during development.[10]

Building on this foundational atlas, Pașca's team examined ASD organoids to probe whether developmental timing of dysregulation converged across patients. In a complementary effort, the Lab applied scRNA-seq to study autism spectrum disorder (ASD) using human induced pluripotent stem cell (hiPSC)-derived cortical organoids from a large cohort of patients. This time-resolved transcriptomic analysis across four developmental time points (days 25 to 100) revealed both divergent early gene expression patterns across different ASD genotypes and convergent dysregulation of chromatin remodeling and neuronal differentiation pathways as development progressed. Notably, a shared gene co-expression network enriched in high-confidence ASD risk genes—referred to as Module 5 (M5)—was consistently downregulated across ASD genotypes. The functional consequences of this network are explored further in Section 5.1.[11]

Together, these studies demonstrate how single-cell transcriptomics empowers researchers to decode the complexity of human brain development, identify critical regulatory networks, and uncover convergent pathways that underlie diverse neurodevelopmental disorders. As scRNA-seq technologies become more scalable and spatially resolved, they are poised to further refine organoid-based models of disease and development.

3.2. Epigenomic and Chromatin Accessibility Data

Epigenomic analysis complements gene expression profiling by revealing how regulatory mechanisms shape neurodevelopment at the molecular level. Multi-omic tools, particularly those that combine single-nucleus RNA sequencing (snRNA-seq) with chromatin accessibility (snATAC-seq) and DNA methylation profiling, offer a powerful lens into the regulatory programs that shape cell identity and function in both healthy and diseased brains.

A recent study by Adams et al. (2024) performed paired snRNA-seq and snATAC-seq on over 69,000 nuclei from the human substantia nigra, spanning young, aged, and Parkinson's disease (PD) donors. Their analysis revealed that while chromatin accessibility patterns remained globally stable within major cell types, the regulatory connections between accessible regions and gene expression—known as peak–gene associations—underwent significant remodeling with age and disease. These changes were particularly prominent in oligodendrocytes and microglia, two glial cell types increasingly recognized for their role in neurodegenerative disease. The team identified a PD-associated oligodendrocyte subtype, characterized by the loss of CARNS1 and other genes protective against neurodegeneration, highlighting a possible epigenetic transition state that emerges with aging and disease.[12]

Complementary insights into neurodevelopmental gene regulation come from single-cell atlas of the mid-gestation human neocortex. In this study, Bhaduri et al. (2021) combined scRNA-seq and chromatin accessibility data to reconstruct gene regulatory networks governing progenitor differentiation and neuronal lineage specification. They identified key lineage-specific enhancers and transcription factors active in radial glia, intermediate progenitors, and post-mitotic neurons. Notably, many of the enhancer elements overlapped with loci associated with neurodevelopmental disorders such as autism and epilepsy, providing evidence that disease risk is embedded in the regulatory architecture of early brain development.[13]

Together, these studies illustrate how paired multi-omic approaches can map the epigenetic logic behind both normal and pathological brain development. As such tools are increasingly applied to brain organoid systems, they will be critical for understanding how temporal changes in chromatin accessibility and regulatory element usage guide fate decisions—and how these mechanisms go awry in disease.

3.3. Mapping NDD Risk Genes

Identifying how genetic risk variants contribute to neurodevelopmental disorders (NDDs) like autism spectrum disorder (ASD), schizophrenia, and epilepsy requires more than cataloging genes—it demands understanding when, where, and in which cell types these genes act. Multi-omic analysis has become a central approach in bridging genetic findings with functional neurobiology by integrating gene expression, chromatin accessibility, and epigenomic profiling at single-cell resolution.

The single-cell atlas of the human neocortex during mid-gestation represents a landmark in mapping NDD risk genes to specific developmental contexts. By combining scRNA-seq with chromatin accessibility data, they characterized over 40,000 cells, including radial glia,

intermediate progenitors, and various excitatory and inhibitory neurons. Crucially, the team overlaid genome-wide association study (GWAS) and rare variant data for disorders like autism and epilepsy onto their atlas and found that many risk genes were transiently expressed during critical windows—especially during progenitor-to-neuron transitions—highlighting periods of heightened vulnerability during cortical development.[13]

The Arlotta Lab further advanced this approach with their development of brain chimeroids, a system that integrates neural progenitor cells from multiple human donors into a single organoid. This model enabled the study of genotype-specific cellular responses to environmental insults, such as neurotoxic exposures. In their 2024 Nature study, Arlotta's team exposed chimeroids to compounds like ethanol and valproic acid and observed donor-specific variation in transcriptional and epigenetic responses across different cell types. This revealed that genetic background influences susceptibility to environmental triggers and that certain NDD risk genes were differentially regulated in a cell-type- and donor-dependent manner.[14] Such models allow researchers to functionally annotate risk genes within developmentally and genetically diverse contexts—something not possible in homogeneous cell lines or animal models.

Collectively, these studies underscore the value of single-cell, multi-omic platforms in pinpointing the specific cell types and developmental windows during which NDD risk genes exert their effects. This level of resolution is essential for accurately interpreting genetic data and informing the development of targeted therapies for disorders that originate during early brain development. These omics-driven insights identify key molecular regulators, but functional validation is essential. CRISPR-based genome engineering enables precise interrogation of these targets within the organoid context.

4. CRISPR-Based Functional Genomics in Organoids

4.1. Genome Editing for Disease Modeling

The advent of CRISPR-Cas9 genome editing has transformed the utility of human brain organoids by enabling the precise modeling of neurodevelopmental disorder (NDD)–associated genetic variants. In particular, the use of induced pluripotent stem cells (iPSCs) carrying engineered mutations in high-confidence risk genes has allowed researchers to investigate causal relationships between genotype and cellular phenotype in a human-specific context.

Genes such as CHD8, SHANK3, and PTEN—among the most frequently implicated in autism spectrum disorder (ASD)—have been widely studied using this approach. Collectively, these models illustrate how ASD risk genes impact distinct but complementary domains: CHD8 alters early progenitor dynamics, SHANK3 disrupts synaptic connectivity, and PTEN affects growth signaling pathways. Such diversity reflects the syndrome's broad phenotypic spectrum. Studies using CHD8-mutant iPSCs differentiated into brain organoids revealed altered progenitor proliferation, disrupted WNT signaling, and delayed neuronal differentiation.[15] Similarly, mutations in SHANK3, a synaptic scaffolding protein associated with ASD and Phelan–McDermid syndrome, led to impaired synaptogenesis, reduced spine density, and deficits in spontaneous network activity in cortical organoids.[16]

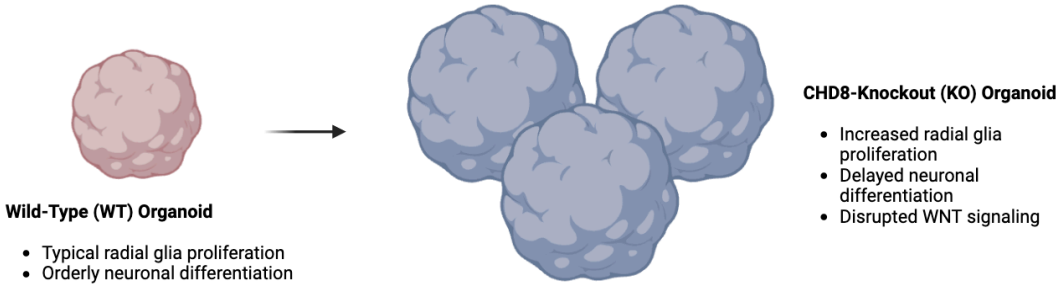

Figure 5. Comparison of wild-type and CHD8-knockout brain organoids.

Loss-of-function mutations in PTEN, a tumor suppressor and negative regulator of the PI3K-AKT-mTOR pathway, have been used to model brain overgrowth syndromes and seizure phenotypes. PTEN-mutant organoids exhibit excessive radial glial proliferation, dysregulated mTOR signaling, and a marked increase in organoid size, mimicking aspects of macrocephaly and cortical dysplasia seen in patients.[17] Beyond single-gene models, researchers are now using genome editing to introduce patient-specific variants or multiple mutations simultaneously, enhancing the relevance of these models to complex and polygenic disorders. Advances in base editing, CRISPR interference (CRISPRi), and inducible systems have further refined this strategy by allowing more controlled temporal and spatial gene perturbation.

These genetically engineered brain organoid models provide critical insight into the cellular mechanisms underlying NDDs, enabling direct comparison of wild-type and mutant phenotypes in an otherwise isogenic background. When combined with single-cell multi-omics, they serve as powerful tools for functionally validating risk genes and uncovering pathogenic pathways.

4.2. High-Throughput CRISPR Screens in Assembloids

High-throughput CRISPR screening has enabled systematic interrogation of gene function at scale, especially in the context of complex human brain development. The Pașca Lab has been at the forefront of integrating CRISPR-based approaches with advanced brain organoid systems, particularly using human forebrain assembloids—fused cortical (dorsal) and subpallial (ventral) organoids—to model GABAergic interneuron development and migration. In their 2023 Nature study, the team utilized a doxycycline-inducible CRISPR-Cas9 system in human induced pluripotent stem cells (hiPSCs) to conduct two large-scale functional screens aimed at uncovering the roles of 425 neurodevelopmental disorder (NDD)-associated genes.[2]

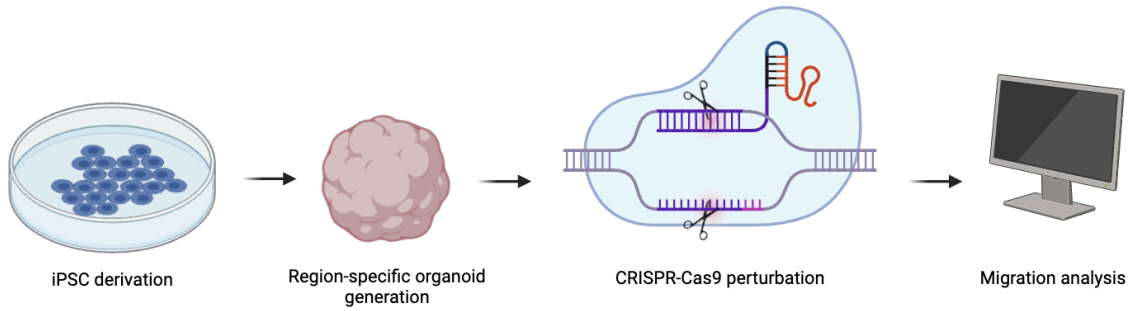

Figure 6. Workflow of CRISPR-Cas9 screening in brain organoids.

The first screen targeted interneuron generation by identifying genes essential for the specification and production of GABAergic interneurons within ventral organoids. This screen revealed 13 key regulators, including CSDE1 and SMAD4, which were found to be critical for maintaining progenitor identity and promoting proper interneuron output. In parallel, the second screen focused on interneuron migration across more than 1,000 assembloids. This screen uncovered 33 genes involved in cytoskeletal regulation, organelle positioning, and cellular motility. Among these, LNPK, a gene involved in endoplasmic reticulum (ER) morphology, emerged as essential for proper ER displacement during neuronal migration. Loss of LNPK disrupted ER positioning and impaired interneuron polarity and motility, revealing a novel role for ER dynamics in migration.[2] By linking NDD risk genes to specific developmental processes in a spatially patterned, human-derived system, this study demonstrated the power of high-throughput CRISPR screening in organoids. The dual-screen framework—simultaneously targeting intrinsic differentiation and dynamic cell behaviors—sets a new standard for functional genomics in brain development and provides a scalable platform for dissecting the cellular basis of neurodevelopmental disorders.

4.3. Validating Mechanisms of Pathogenesis

Moving from association to causation in neurodevelopmental disorder (NDD) research requires functionally testing the impact of specific genes within developmental trajectories. CRISPR-based tools such as CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) have emerged as essential strategies to validate gene function, particularly in human brain organoids where fine-tuned, temporal control over gene expression is critical.[18] Unlike traditional knockout systems, CRISPRi/a platforms modulate gene activity without inducing DNA breaks, making them especially useful for studying dosage-sensitive or early essential genes implicated in NDDs.[19]

One of the most notable applications of CRISPRi in this context comes from the Pașca Lab's 2024 ASD organoid study, which functionally validated the Module 5 (M5) network previously identified by scRNA-seq.[11] The downstream validation of this network is discussed further in Section 5.1, where its functional role in cortical development and neurodevelopmental vulnerability is explored.

Beyond ASD, CRISPRi/a systems have been used to dissect gene networks in schizophrenia, epilepsy, and intellectual disability. While ASD has been a primary focus, these approaches have also shed light on other NDDs, such as schizophrenia. For example, Rajarajan et al. (2021) employed CRISPRa to activate multiple schizophrenia-associated transcription factors in dorsal forebrain organoids, identifying distinct transcriptional cascades that impaired radial glia maturation and promoted premature neuronal differentiation.[18] This functional dissection allowed researchers to prioritize regulatory hubs likely to drive disease risk at the circuit formation level.

Collectively, these studies underscore the transformative role of CRISPRi/a in advancing functional validation within neurodevelopmental research. By enabling precise control over gene expression, these tools establish direct connections between regulatory mechanisms and disease phenotypes—particularly when applied to brain organoids that recapitulate the cellular complexity and structural organization of the developing human brain.

5. Applications to Specific Neurodevelopmental Disorders

5.1. Autism Spectrum Disorder

The integration of improved morphogenetic fidelity, high-resolution omics, and genome engineering has opened new avenues for modeling human neurodevelopmental disorders (NDDs). These systems enable researchers not only to capture disease-relevant phenotypes in a dish, but also to interrogate disorder-specific and convergent mechanisms across multiple genetic backgrounds. The following sections highlight how organoid-based approaches are advancing our understanding of three major NDDs: autism spectrum disorder (ASD), schizophrenia (SCZ), and Parkinson's disease (PD).

Autism spectrum disorder (ASD) is marked by extensive genetic heterogeneity, complicating efforts to uncover shared mechanisms.[20] Emerging research using brain organoids and assembloids, however, has revealed that despite diverse genetic origins, many ASD-linked mutations converge on common developmental processes, particularly affecting progenitor dynamics, neuronal subtype balance, and circuit assembly.[21]

Building on prior transcriptomic analysis (Section 3.1), the Pașca Lab used over 70 iPSC-derived cortical organoids to show that, despite early mutation-specific disruptions, a shared network—Module 5 (M5)—was consistently downregulated. Their analysis linked M5 to chromatin regulators such as ARID1B, CHD8, and SMARCA2, positioning it as a convergence point of early cortical vulnerability in ASD.[11] High-throughput CRISPR perturbation studies have also reinforced these themes. Researchers have used single-cell CRISPR-Cas9 editing to disrupt 36 ASD-associated transcriptional regulators—including ARID1B—within cerebral organoids. They found these perturbations specifically affected dorsal intermediate progenitors and upper-layer excitatory neurons, disrupting gene regulatory networks that align with ASD-driven developmental vulnerability.[22]

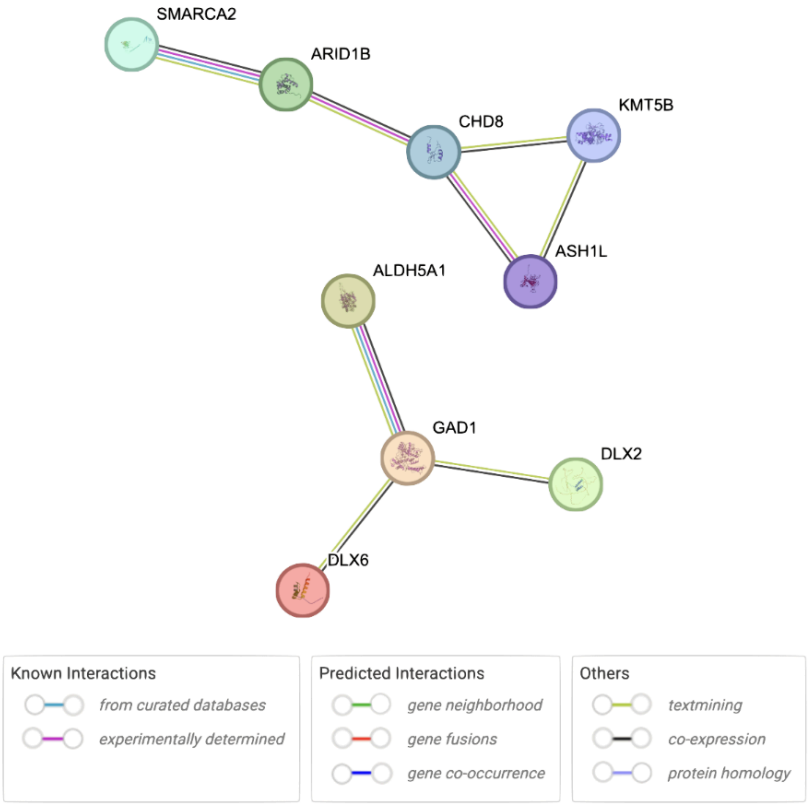

Figure 7. Module 5 (M5) gene co-expression network downregulated in autism organoids.

Finally, specific case-control organoid models—such as the 16p11.2 CNV model—have demonstrated that ~deletion organoids oversized to mimic patient macrocephaly, while duplication organoids remained undersized. These phenotypes correlated with dysregulated pathways in cell motility, synaptic proteins, and RhoA signaling, underscoring synapse-related disruptions tied to ASD risk variants.[23]

Altogether, these studies paint a coherent picture: genetic diversity in ASD causes early divergence in progenitor programs, but later results in convergent derailments of neural subtype balance, progenitor maturation, and circuit integration. Brain organoids and assembloids offer a powerful platform for uncovering these shared vulnerabilities, providing essential insights for stratified therapeutic targeting.

5.2. Microcephaly and Forebrain Expansion

Microcephaly, a neurodevelopmental disorder characterized by reduced brain and head size, has provided critical insight into mechanisms of human-specific forebrain expansion.[24] Brain organoids have become indispensable tools for dissecting the cellular and mechanical factors underlying this condition, enabling comparative studies of human and non-human primate development, as well as modeling pathogenic mutations in key genes.[21]

One of the most foundational studies in this area demonstrated how Zika virus infection causes microcephaly by disrupting radial glial proliferation and inducing premature neuronal differentiation in human cerebral organoids. The study by Qian et al. (2016) revealed that viral infection impairs the integrity of the apical ventricular zone and induces cell death, providing a robust in vitro model that recapitulates clinical phenotypes observed in congenital Zika syndrome.[25]

Beyond infection models, genetic studies using brain organoids have highlighted the role of mitotic spindle orientation and apical-basal polarity in neurogenesis. For example, Lancaster et al. (2013) used patient-derived iPSCs with mutations in CDK5RAP2, a gene involved in centrosome regulation, to model primary microcephaly. These organoids showed premature neurogenesis due to altered cleavage plane orientation in neural progenitors, leading to early depletion of the progenitor pool and a smaller cortical plate.[26]

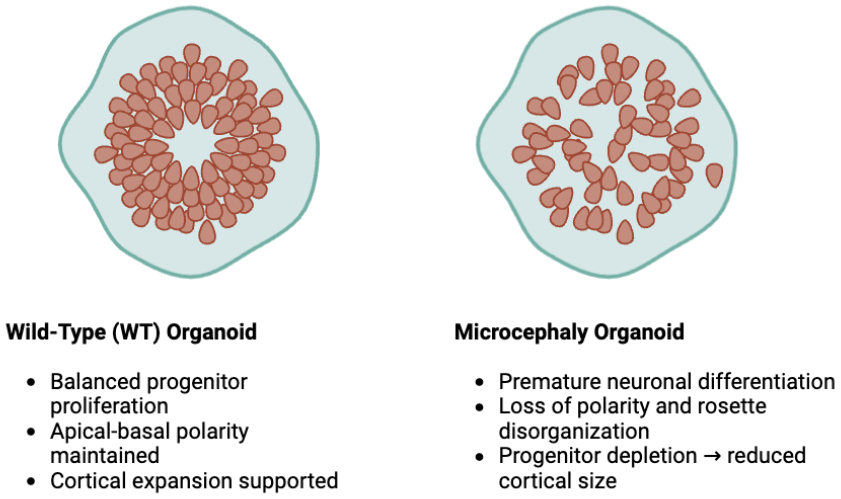

Figure 8. Structural differences between wild-type and microcephaly brain organoids.

Recent studies have further explored how cell shape transitions and mechanical tension contribute to cortical expansion. Rozman et al. (2019) showed that rosette formation and collective cell behavior influence rigidity transitions in organoids. These biomechanical properties, including apical constriction and tension within neuroepithelial rosettes, are essential for proper progenitor layering and cortical morphogenesis. Disruption of these structures—either genetically or through impaired cytoskeletal dynamics—can lead to disorganized architecture and reduced cortical size.[27] Comparative models have also provided clues into species-specific expansion of the human cortex. Otani et al. (2016) developed both human and chimpanzee cerebral organoids and found that prolonged proliferation of basal radial glia—a progenitor type enriched in humans—contributes to the larger cortical size seen in human brains. These differences were linked to species-specific regulation of cell cycle dynamics and mitotic behavior.[28] These findings underscore the advantage of human-derived organoid systems over animal models, which may lack human-specific progenitor dynamics and regulatory networks essential for capturing disease-relevant phenotypes.

Taken together, organoid models have illuminated how a complex interplay between cell polarity, division mode, and tissue mechanics governs cortical size. Disruptions in these processes—whether from viral insult, gene mutation, or biomechanical imbalance—can lead to microcephaly. Conversely, their fine-tuned regulation underlies the evolutionary expansion of the human forebrain.

5.3. Parkinson's and Glial Dysfunction

Recent studies suggest that Parkinson's disease (PD) may have early neurodevelopmental roots, with glial dysregulation and synaptic alterations contributing to the disease's later onset.[29] Thus, organoid systems offer a valuable platform for exploring these developmental underpinnings. Brain organoid models—particularly midbrain-specific organoids—have emerged as powerful systems to investigate both neuronal and non-neuronal contributions to disease mechanisms.

One of the most comprehensive efforts to explore this dimension comes from the Lieber Institute for Brain Development, which used a multi-omic single-nucleus RNA sequencing (snRNA-seq) and ATAC-seq framework to characterize the midbrain transcriptome across 88 individuals, including PD patients and matched controls. This analysis revealed striking alterations in glial cell states, particularly in astrocytes and microglia, during disease progression.[30] In particular, PD-associated regulatory elements were enriched in glial cell types, suggesting that non-neuronal cells may harbor genetic risk loci that modulate inflammation and neuronal vulnerability.

These insights are being incorporated into human brain organoid studies to generate models that include mature glial populations. For example, recent protocols have improved the incorporation of astrocytes and microglia into midbrain-like organoids, which better recapitulate PD-relevant circuitry and immune interactions.[31] When combined with patient-derived iPSCs carrying mutations in genes like LRRK2 or SNCA, these organoids reveal both intrinsic neuronal deficits and glial-mediated neurotoxicity, including elevated cytokine production and impaired synaptic maintenance. Midbrain organoids incorporating astrocytes and patient-derived LRRK2 mutations have revealed synergistic glial–neuronal toxicity, validating prior snRNA-seq findings in an experimentally tractable system.

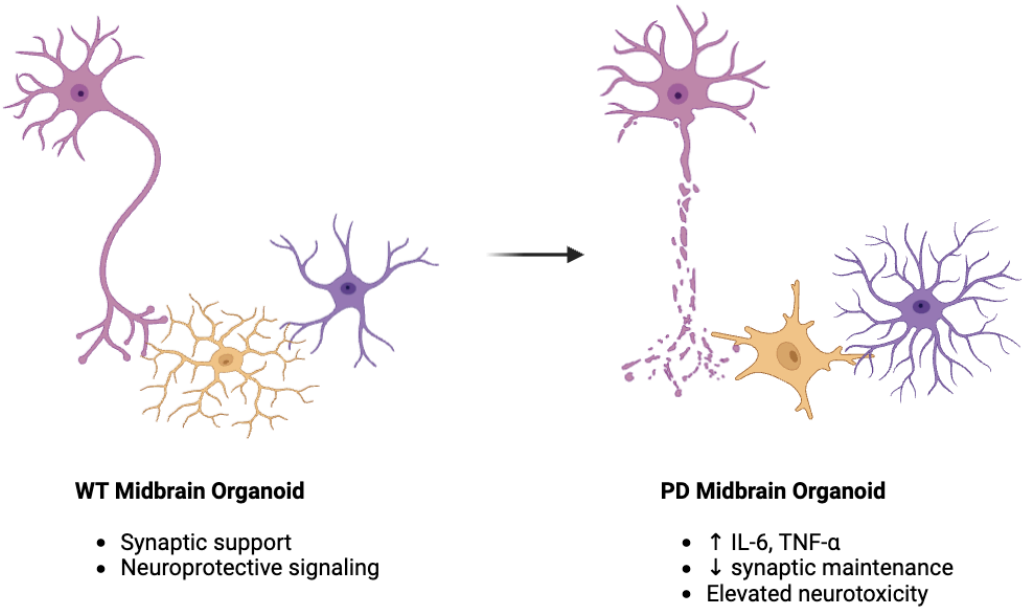

Figure 9. Neuron–glia interactions in wild-type and Parkinson's disease (PD) brain organoids.

Such models are not only redefining our understanding of PD as a multicellular disorder but also enabling the identification of glial-specific therapeutic targets. Given their role in synaptic pruning, immune regulation, and trophic support, glial cells are increasingly implicated in early circuit formation. Dysfunction during early glial maturation may prime the brain for later neurodegenerative vulnerability. As transcriptomic atlases continue to expand, integrating these data with organoid-based perturbation studies promises a deeper understanding of how glial cells contribute to neurodegeneration and how they can be modulated for disease intervention.

6. Limitations and Future Directions

Despite significant advancements, brain organoid models face a number of limitations that restrict their full utility in modeling human neurodevelopment and neurodevelopmental disorders (NDDs). One of the primary challenges remains variability—across cell lines, batches, and protocols—which hinders reproducibility and limits comparative studies. Even under standardized culture conditions, stochastic differentiation events can lead to divergent regional identities or inconsistent proportions of progenitor and neuronal subtypes.[13] This variability complicates the interpretation of gene perturbation studies, especially when attempting to dissect subtle phenotypes associated with polygenic NDDs.

Another critical limitation is the incomplete maturation of organoids. Although significant progress has been made in inducing early developmental structures and primitive circuits, most organoids stall at a fetal-like state. The lack of vasculature and the absence of systemic signals such as blood flow and hormonal cues contribute to metabolic stress, limited growth, and cell death in inner regions. Additionally, glial populations, including astrocytes, oligodendrocytes, and microglia, remain underrepresented or immature in many models, limiting investigations into neuron-glia interactions and late-stage functional development.[31]

As brain organoids begin to exhibit features such as neural oscillations and sensory responses, concerns about sentience and moral status have gained urgency. The emergence of synchronized oscillations, rudimentary sensory responses, or hypothesized pain perception in extended culture raises concerns about consciousness and the moral status of advanced models.[32] While current evidence does not support sentient properties in organoids, these concerns necessitate proactive ethical frameworks as models grow more sophisticated.

To address these limitations, future directions include scaling and standardization of organoid protocols using automated bioreactors and guided differentiation strategies to reduce batch variability. Integration of vascular networks, either through co-culture with endothelial cells or in vivo transplantation, may support more robust maturation and nutrient exchange. Furthermore, incorporation of immune and glial lineages through assembloid strategies is expected to enhance modeling of diseases involving neuroinflammation and synaptic pruning.

From a systems biology perspective, combining brain organoids with multi-omic profiling, spatial transcriptomics, and machine learning approaches—particularly unsupervised clustering and deep learning applied to high-dimensional transcriptomic data—promise to accelerate classification of cellular subtypes and subtle disease phenotypes. As genome editing becomes more precise and high-throughput, organoid-based CRISPR screens may uncover genotype–phenotype relationships at scale.

In parallel, efforts to develop clinical-grade organoids will pave the way for translational applications in drug screening, personalized medicine, and even cell-based therapies. Ultimately, while current brain organoid models are not perfect replicas of the developing brain, they are evolving into indispensable tools that bridge the gap between animal models and human studies. Strategic integration of engineering, computational, and ethical innovations will be key to realizing their full potential in neuroscience research.

7. Conclusion

The development of human brain organoids has ushered in a new era in the study of neurodevelopmental disorders (NDDs), enabling researchers to overcome long-standing ethical and technical barriers to studying the human brain in its earliest stages. Organoids model the critical spatiotemporal aspects of early brain development, offering a scalable platform for studying disorders like autism, microcephaly, and Parkinson’s disease. Recent innovations in organoid engineering—particularly in morphogenetic fidelity, regional specification, and functional maturation—have expanded the scope and depth of these models, allowing unprecedented insight into the architecture and circuitry of the developing brain.

Equally transformative is the integration of single-cell transcriptomics and multi-omic technologies, which provide fine-grained resolution of cell-type-specific developmental trajectories and molecular perturbations. These approaches have enabled the mapping of risk gene expression to precise windows and cell populations in human neurodevelopment, clarifying the etiological underpinnings of complex disorders. In parallel, genome engineering tools like CRISPRi/a and high-throughput screening have revealed causal pathways and mechanistic modules, linking genetic risk to functional outcomes in an experimentally tractable system.

While challenges such as variability, incomplete maturation, and ethical uncertainties remain, ongoing efforts in protocol standardization, vascularization, and multi-lineage integration are steadily improving the reproducibility and physiological relevance of these models. As organoid systems continue to mature, their role will likely expand beyond discovery science into personalized diagnostics, therapeutic screening, and regenerative medicine. By unraveling the developmental roots of neurodevelopmental disorders at both molecular and functional levels, these platforms are not only reshaping our understanding of brain development, but also charting a path toward precision diagnostics, targeted therapies, and ethically guided human-specific neuroscience.

Brain organoids have evolved from experimental models to indispensable tools for understanding the molecular mechanisms driving human brain development and neurodevelopmental disorders. With continued refinement, organoid-based platforms are poised to drive both mechanistic discovery and patient-specific intervention strategies for neurodevelopmental disorders. As these models become increasingly personalized and integrative, they hold the promise of reshaping diagnosis and treatment—not just as tools of discovery, but as platforms for targeted intervention in the earliest stages of human brain development.

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