

Development and Application of Brain Region-Specific Organoids for Investigating Psychiatric Disorders

Zhijian Zhang, Xin Wang, Sean Park, Hongjun Song, and Guo-Li Ming

ABSTRACT

Human society has been burdened by psychiatric disorders throughout the course of its history. The emergence and rapid advances of human brain organoid technology provide unprecedented opportunities for investigation of potential disease mechanisms and development of targeted or even personalized treatments for various psychiatric disorders. In this review, we summarize recent advances for generating organoids from human pluripotent stem cells to model distinct brain regions and diverse cell types. We also highlight recent progress, discuss limitations, and propose potential improvements in using patient-derived or genetically engineered brain region-specific organoids for investigating various psychiatric disorders.

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Psychiatric disorders, including depression, bipolar disorder, schizophrenia, and other psychoses, cause great suffering to patients and their families and impose a growing emotional and economic burden worldwide (1). Characterized by significant cognitive, emotional, and behavioral disturbances, these disorders have been considered as developmental brain disorders (2,3). Understanding underlying pathological mechanisms is crucial for developing effective treatments. Most psychiatric disorders are polygenic, and even mutations of a single gene can contribute to different symptoms. Mutations in *DISC1*, for instance, have been associated with autism spectrum disorder (ASD), schizophrenia, bipolar disorder, and major depression (4,5). This makes psychiatric disorders difficult to recapitulate in traditional genetically modified animal models. Evolutionary increase in the complexity of human brain architecture and functions also makes it challenging to investigate the pathogenesis of human-specific psychiatric disorders with animal models, and many therapies developed based on animal studies have failed in clinical trials (6–8). Hence, utilization of patient brain tissues is important to overcome interspecies differences for both pathological research and therapeutic development. While the availability and usage of human fetal brain materials are subject to ethical and practical limitations, recent advancements in human stem cell-based models provide an alternative approach. For example, human induced pluripotent stem cells (hiPSCs) derived from patients with psychiatric disorders can be differentiated into neurons or glia in 2-dimensional cultures or 3-dimensional (3D) organoids for analyses (9,10). Monolayer cultures usually possess limited cellular diversity, endogenous cell-cell interactions, and tissue organization, whereas 3D brain organoids recapitulate several key characteristics of the developing human brain. Because psychiatric disorders often affect cellular architecture functions

and interactions of multiple brain regions, brain organoids may provide new avenues for psychiatric research and drug development (11).

Growing numbers of organoid protocols have been developed to recapitulate distinct brain regions and subregions (12) and applied to psychiatric disorder research. In this review, we first briefly summarize current protocols for generating different brain region-specific organoids, then outline achievements and prospects for applying these models to investigate psychiatric disorders. We conclude with limitations and potential future improvements in using brain region-specific organoids for psychiatric disorder research.

GENERATION OF BRAIN REGION-SPECIFIC ORGANIDS

Current brain region-specific organoid protocols are largely developed by following the *in vivo* developmental trajectory with temporally defined patterning by different chemical cues (Figure 1), providing an extensive and growing toolbox for psychiatric disorder research (Table 1).

Cerebral Cortex

The Sasai group developed the first guided cortical organoid protocol by inhibiting transforming growth factor β (TGF- β) and Wnt signaling (13). Treatment with retinoic acid at a later culture stage leads to subregion specificity resembling the human prefrontal cortex (14). Cortical organoids were also generated with SMAD inhibition, followed by a combination of early Wnt inhibition and FGF2/EGF treatment (15,16), or continuous TGF- β inhibition, and later Wnt activation (17,18). These protocols produce self-organized ventricular structures with cortical-specified neural progenitor cells and neuronal zones with different cortical neural types, with some containing

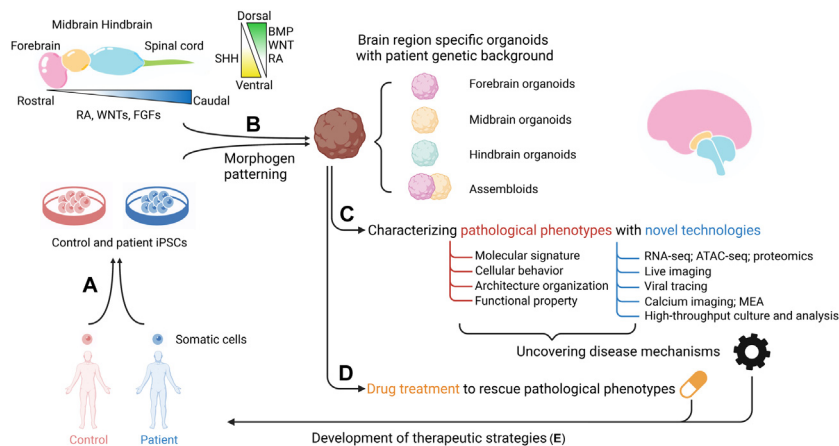


Figure 1. Patient-derived brain region-specific organoids for studies of psychiatric disorders and therapeutic strategies. **(A)** Human iPSCs are established from donor tissue of patients or healthy family control subjects. **(B)** Distinct brain region-specific organoids are then generated by patterning with proper morphogens based on the endogenous human brain development process using these human iPSCs. **(C)** Comparative analysis between patient and control organoids can be performed to reveal pathological phenotypes and mechanisms at molecular, cellular, structural, and functional levels. **(D)** Based on the knowledge acquired from these studies, brain region-specific organoids can be used for testing drug responses and large-scale drug screening. **(E)** Eventually, brain region-specific organoids can be used to develop novel and personalized therapeutic strategies for patients. ATAC-seq,

assay for transposase-accessible chromatin sequencing; iPSCs, induced pluripotent stem cells; MEA, microelectrode array; RA, retinoic acid; RNA-seq, RNA sequencing.

cortical neurons of all 6 layers (17) and some exhibiting distinct superficial and deep cortical layers (19). These cortical organoids have been widely used to study various brain disorders, such as microcephaly (20–22), lissencephaly (23,24), ASD (16,25,26), schizophrenia (19,27), fragile X syndrome (28), Angelman syndrome (29), Alzheimer’s disease (30), and frontotemporal dementia (31) (Table 1).

Hippocampus and Choroid Plexus

Hippocampal development is regulated by BMP signals secreted from the choroid plexus tissue (32). Hippocampal and choroid plexus organoids can be induced by combinational and timed treatment with BMP and Wnt signaling (33–35). Choroid plexus organoids could provide a valuable tool to assess drug permeability into the brain.

Ganglionic Eminence

Human cortical interneurons are mainly derived from the ganglionic eminence of the ventral pallium (36–38), which is patterned by high SHH and low Wnt activity. Medial ganglionic eminence organoids have been generated by activating the SHH pathway (13,39–41) and employed to model interneuron migration deficiency in Timothy syndrome. The lateral ganglionic eminence generates the striatum, which contains inhibitory medium spiny neurons that receive unidirectional inputs from excitatory cortical neurons. These features have been recapitulated in striatal organoids generated with a combination of TGF- β activation and Wnt inhibition and later retinoic acid activation (42), which have been used to model 22q13.3 deletion syndrome (22q13.3DS).

Thalamus and Hypothalamus

Thalamic and hypothalamic organoids can be generated using dual-SMAD inhibitors, followed by insulin treatment and then MEK-ERK inhibition for caudal forebrain fate induction (43), or SHH and Wnt activation for ventral rostral forebrain induction (17). Organoids resembling the arcuate nucleus, a subregion of the hypothalamus, were generated using dual-SMAD inhibition followed by extended Wnt inhibition and triple SHH activation for modeling Prader-Willi syndrome (44).

Midbrain

Organoid protocols aimed at generating midbrain dopaminergic neurons have been developed using SHH activation (17,45–50) or combined treatment with FGF8 (17,45,46,50). Midbrain organoids have been used to study Parkinson’s disease (48–52) and treatments (52).

Hindbrain

Cerebellar organoids modeling the dorsal hindbrain have been generated using TGF- β inhibition, FGF2, and insulin for early cerebellar neuroepithelium induction, followed by FGF19 and SDF1 treatments for dorsal tissue differentiation (53). These organoids produced various cerebellar specific neural precursors and provide useful tools for studies of cerebellar-associated diseases. Ventral hindbrain organoids have been generated using SHH for ventralization and retinoic acid for caudalization (54) and contain various hindbrain-specific cell types, including serotonin-synthesizing neurons, which are promising for studies and drug tests of human 5-HT-related diseases, such as depression.

Assembloids

Various brain region-specific organoid subtypes can be assembled (assembloids) to model cross-regional interactions, such as cell migration, axonal projection, and synaptic formation, as well as functional interactions, for relevant brain disorders. Many cortical interneurons are derived from the ventral forebrain and migrate into the dorsal cortex during development. Several studies using dorsoventral forebrain assembloids showed the recapitulation of the saltatory (39) and tangential (40,41) migration of interneurons and its regulation by neurotransmitter signaling (55). Corticostriatal assembloids form unidirectional axonal projections and functional connections from excitatory cortical neurons to striatal GABAergic (gamma-aminobutyric acidergic) medium spiny neurons (42), resembling the in vivo network connectivity in forebrains. Thalamocortical assembloids recapitulate reciprocal thalamocortical axonal projections (43), and corticomotor assembloids model cortical-spinal-muscle circuit connections that can functionally control muscle contraction (56).

Application of Brain Organoids for Psychiatric Research

Table 1. Brain Disorder Modeling With Brain Region–Specific Organoids

Brain Organoid	Brain Region–Specific Organoid	Psychiatric Disorder	Genetic Background	Findings	Reference
Forebrain Organoids	Cortical organoids	Angelman syndrome	Engineered <i>UBE3A</i> KO hESCs and isogenic control cells	Hyperactivity of neurons and networks, which can be rescued by paxilline	Sun <i>et al.</i> (29)
		Frontotemporal dementia	Patient iPSCs with tau-V337M and isogenic corrected iPSCs	Affected synaptic maturation and excitatory neuron survivability, which can be rescued by the PIKfyve kinase inhibitor apilimod	Bowles <i>et al.</i> (31)
		Autism spectrum disorder	Multiple patient iPSCs	Accelerated cell cycle and overproduction of inhibitory neurons	Mariani <i>et al.</i> (16)
			Engineered heterozygous mutation in <i>SUV420H1</i> , <i>ARID1B</i> , or <i>CHD8</i> with multiple parental iPSCs	Convergent phenotypes with asynchronous development of inhibitory and excitatory neurons	Paulsen <i>et al.</i> (25)
			Patient iPSCs with a <i>CNTNAP2</i> mutation and isogenic corrected iPSCs	Increased neural progenitor proliferation and organoid volume	de Jong <i>et al.</i> (26)
		Schizophrenia	Patient iPSCs with a <i>DISC1</i> mutation	Disruption of cell cycle with delayed mitosis	Ye <i>et al.</i> (27)
				Deficits in cortical neuron fate specification	Qian <i>et al.</i> (19)
		Fragile X syndrome	Patient iPSCs and isogenic corrected iPSCs	Dysregulated neurogenesis, neuronal maturation, and neuronal excitability	Kang <i>et al.</i> (28)
		Alzheimer's disease	Multiple patient iPSCs	Modeled amyloid aggregation and hyperphosphorylated tau protein, which is rescued by treatment with β - and γ -secretase inhibitors	Raja <i>et al.</i> (30)
		Lissencephaly/Miller-Dieker syndrome	Patient iPSCs with heterozygous deletion of chromosome 17p13.3 and rescue cell lines expressing <i>LIS1</i> or <i>YWHAE</i>	Reduced organoid size caused by premature neurogenesis and can be rescued by Wnt activation	Iefremova <i>et al.</i> (24)
				Reduced organoid size, increased apoptosis and horizontal divisions, and defects in neuronal migration and mitotic spindles	Bershteyn <i>et al.</i> (23)
		22q11.2 deletion syndrome	Patient iPSCs	Abnormal neuronal excitability–related gene expression, neuronal excitability, and calcium signaling	Khan <i>et al.</i> (76)
		Microcephaly	Multiple engineered iPSCs with a <i>WDR62</i> mutation	Reduction in organoid size due to decreased proliferation and premature differentiation of neural progenitor cells	Zhang <i>et al.</i> (20)
				Reduction in organoid size; neurogenesis defects; defective neuronal activity	Li <i>et al.</i> (21)
			Patient iPSCs from multiple families with missense or frameshift mutations in <i>NARS1</i>	Disruption of protein synthesis; reduction in organoid size due to reduced RGC proliferation	Wang <i>et al.</i> (22)
Engineered iPSCs with a homozygous mutation in <i>IER3IP1</i>	Reduced organoid size and rosette size, which can be restored using ISRIB; increased unfolded protein response and ER stress; decreased extracellular matrix protein		Esk <i>et al.</i> (101)		
Rett syndrome	Patient iPSCs with mutations in <i>MECP2</i> and isogenic corrected iPSCs	Cell migration deficits in distances, speeds, and radial trajectories	Yildirim <i>et al.</i> (84)		
Cortical organoids and ventral forebrain organoids	Tuberous sclerosis complex	Patient iPSCs with mutations in <i>TSC2</i> and isogenic corrected iPSCs	Brain tumors and cortical malformations caused by overproliferation of caudal late interneuron progenitor	Eichmuller <i>et al.</i> (125)	
Retinal organoids	Retinitis pigmentosa	Patient iPSCs with frameshift mutations in <i>RPGR</i> and isogenic corrected iPSCs	Photoreceptor defects in morphology, localization, transcriptional profiling, and electrophysiological activity	Deng <i>et al.</i> (126)	
ARC organoids	Prader-Willi syndrome	Patient iPSCs with major or minor deletion on the chromosome 15q11-q13	Differentiation and functional deficits	Huang <i>et al.</i> (44)	

Table 1. Continued

Brain Organoid	Brain Region–Specific Organoid	Psychiatric Disorder	Genetic Background	Findings	Reference
Midbrain Organoids	Midbrain organoids	Parkinson's disease	Gene edited iPSCs with LRRK2-G2019S mutation	Accumulation of α -synuclein; mimicked the gene expression profiles in patients	Kim <i>et al.</i> (48)
			Patient iPSCs with LRRK2-G2019S mutation	Decreased number and complexity in the expression of mDANs	Smits <i>et al.</i> (49)
			Patient iPSCs with SNCA triplication and CRISPR/Cas9-corrected isogenic control iPSCs	Elevated accumulation of α -synuclein and reduction in dopaminergic neurons	Mohamed <i>et al.</i> (50)
			Patient iPSCs with a <i>PINK1</i> mutation and CRISPR/Cas9-corrected isogenic control iPSCs	Imbalanced proliferation, apoptosis, and mitophagy; reduced dopaminergic differentiation, which can be rescued by treatment with HP- β -CD	Jarazo <i>et al.</i> (52)
Assembloids	Cortical organoids + microglia assembloids	Alzheimer's disease	APP duplicated patient organoids co-cultured with APOE3- or APOE4-carrying microglia-like cells	Increased A β aggregates and hyperphosphorylation of tau in organoids cocultured with APOE4-microglia	Lin <i>et al.</i> (127)
	Cortical organoids + blood vessel assembloids	Alzheimer's disease	A mixture of control and inducible ETV2-expressing hESCs	A β_{1-42} oligo treatment led to disruption of tight junctions	Cakir <i>et al.</i> (62)
	Corticoventral forebrain assembloids	Timothy syndrome	Patient iPSCs with a <i>CACNA1C</i> mutation	Abnormal interneuron migration saltation; L-type calcium-channel blocker rescued saltatory length	Birey <i>et al.</i> (39)
				Abnormal interneuron migration saltation; GABA _A receptor antagonism rescued saltatory frequency	Birey <i>et al.</i> (83)
	Corticostriatal assembloids	Phelan-McDermid syndrome	Patient iPSCs with chromosome 22q13.3 deletion	Corticostriatal connectivity defects with abnormal calcium activity	Miura <i>et al.</i> (42)
	Cerebral-ganglionic eminence assembloids	Rett syndrome	Patient iPSCs with mutations in <i>MECP2</i>	Transcriptomic differences; epileptiform-like neural network activity that can be rescued by treatment with pifithrin- α	Samarasinghe <i>et al.</i> (90)
Cortical or ventral forebrain organoids and assembloids	Rett syndrome	Patient iPSCs with mutations in <i>MECP2</i>	Premature development of the deep cortical layer; neural function defects; impaired interneuron migration	Gomes <i>et al.</i> (86)	

A β , amyloid- β ; APOE3, *APOE* ϵ 3; APOE4, *APOE* ϵ 4; ARC, arcuate nucleus; CRISPR, clustered regularly interspaced short palindromic repeats; ER, endoplasmic reticulum; GABA, gamma-aminobutyric acid; hESCs, human embryonic stem cells; iPSCs, induced pluripotent stem cells; ISRIB, integrated stress response inhibitor; KO, knockout; mDAN, midbrain dopaminergic neuron; RGC, retinal ganglion cell.

Hypothalamic-pituitary projections and functional units were also modeled with assembloids (57). We expect many more brain region-specific protocols and assembly approaches to be developed, which will help to build organoids with more complex cellular populations and regional interactions for studies of relevant psychiatric disorders.

CO-CULTURED ORGANIDS

Patterning methods produce cell populations with similar regional identities and reduce the variability of organoids; however, this consistency comes at the cost of restricted cellular and brain regional diversity. Neuroectoderm derived brain region-specific organoids rarely produce tissues with nonneuroectodermal origin, such as microglia and vasculature. A co-culture strategy can overcome this caveat and enhance cellular diversity. For example, hiPSC-derived microglia or

macrophage precursors readily integrate into dorsal and ventral forebrain (58,59), or midbrain organoids (60). Co-culturing primitive neural and macrophage progenitors produced cortical organoids with a controllable ratio of microglia (61). Brain organoids with microglia showed improved synaptic pruning, tissue maturation, and functionality. Cortical organoids with vascular-like structures can be generated by co-culturing human stem cells with inducible hETV2-expressing cells (62) or human umbilical vein endothelial cells (63). Blood vessel-associated endothelial cells and pericytes are rarely produced in current brain organoid protocols and have not yet been modeled in co-culture. Transplanting organoids into rodent brains also results in host integrated functional vessels with circulation (64–67).

Taken together, progress in patterning methods and co-culture strategies provides a versatile toolbox of brain organoid protocols that allow heterogeneous cellular diversity and

Application of Brain Organoids for Psychiatric Research

multiple brain region interactions. With these advances, brain organoid technology is moving toward making a significant impact on psychiatric disorder research.

PATIENT iPSC-DERIVED BRAIN REGION-SPECIFIC ORGANOID FOR PSYCHIATRIC DISORDER RESEARCH

Brain region-specific organoids derived from patients with clinical profiles provide a powerful platform to maintain the patient genomic context to recapitulate known disease phenotypes, investigate underlying mechanisms, and develop rational drug treatments (Figure 1). These studies can provide insights at multiple levels for psychiatric disorders.

At the Molecular Level

Brain development is dynamically governed by comprehensive molecular processes, which are ultimately coded in the genome. Rapid advances in high-throughput molecular profiling technologies make it possible to systematically compare molecular signatures of human fetal brain tissue with organoids at the bulk or single cell level. Numerous studies showed that (epi)transcriptomic and epigenomic profiles of brain region-specific organoids reliably reflected the developmental trajectory of the human fetal brain (14,68–75), laying the foundation for applying patient-derived brain organoids to study pathological molecular events of psychiatric disorders within patient-specific genomic contexts.

Brain region-specific organoids derived from patients with monogenic etiology have been extensively used to investigate downstream molecular consequences. For example, cortical organoids derived from patients with a homozygous mutation in *CNTNAP2* revealed differentially expressed genes for cell proliferation and neuronal differentiation, which are enriched for ASD-associated genes (26). Organoids generated from isogenic corrected patient iPSCs or isogenic mutant iPSCs with various genetic backgrounds can be further used to determine causality and understand mechanisms underlying disease phenotypes.

Brain region-specific organoids derived from patients with known large-scale genomic variants allow identification of causal gene(s) and relevant mechanisms for certain pathological phenotypes. For example, cortical organoids derived from patients with heterozygous deletion of chromosome 17p13.3 showed a reduced expansion rate caused by increased asymmetric cell division in ventricular neural stem cells (23,24). Re-expression of *LIS1* or *YWHAE*, two genes within 17p13.3, in patient-derived organoids partially restored the expansion and cell division defects, revealing their causal roles in controlling ventricular neurogenesis and cortical expansion. Similarly, deletion of the *DGCR8* gene was identified as the cause for abnormal neuronal excitability and associated gene expression of 22q11DS with patient-derived cortical organoids (76).

Brain region-specific organoids derived from idiopathic patients can also be used to identify common molecular mechanisms of pathogenesis. Compared with those from unaffected family relatives, cortical organoids derived from multiple idiopathic patients with ASD with macrocephaly showed upregulated expression of genes involved in cell proliferation, neuronal differentiation, and synaptic assembly, as well as

overproduction of GABAergic neurons (16). *FOXP1* is among the top upregulated genes, and downregulation of *FOXP1* in patient-derived organoids rescued the dysregulation of GABAergic neuronal fate. This study highlights the importance of brain region-specific organoids for revealing convergent molecular pathways for psychiatric disorders with unidentified genomic variations.

With advances in multiomics approaches, including at the single cell level, we expect that many omics datasets, including transcriptomic, epigenomic, proteomic, and metabolomic signatures of human brain tissues and organoids, will help to decipher and cross-validate mechanisms of psychiatric disorders at the molecular level.

At the Cellular Level

Deficits in cellular development processes, such as proliferation, differentiation, migration, axonal projection, and maturation, have been broadly linked to various psychiatric disorders (16,77–80). Several studies have demonstrated the utility of brain organoids in recapitulating these developmental processes and elucidating their pathological roles in psychiatric disorders. For example, fragile X syndrome-derived cortical organoids exhibit dysregulated neural progenitor proliferation, neural differentiation, and maturation (28). Arcuate organoids derived from patients with Prader-Willi syndrome also exhibit aberrant neural differentiation and selective loss of POMC-expressing neurons (44). Cortical organoids from patients with Miller-Dieker syndrome with lissencephaly showed a reduced organoid expansion rate due to premature neural differentiation (24), increased apoptosis, and defects in cell division and neuronal migration (23). Excitation/inhibition imbalance is known to be associated with psychiatric disorders (81,82). Overproduction of inhibitory neurons caused by increased inhibitory neuron differentiation and early precursor proliferation was identified as a common feature in cortical organoids derived from patients with ASD (16). Dorsoroventral forebrain assembloids derived from patients with Timothy syndrome exhibit abnormal interneuron migration (39,83).

Together, these studies demonstrated that patient-derived brain region-specific organoids can reveal cellular mechanisms for psychiatric disorders. Leveraging various co-culture or multiregion organoids and innovative imaging technologies, such as label-free live imaging (84), will allow more complex cellular diversity and interactions to be visualized in organoids, which will provide novel insights into cytopathological mechanisms of psychiatric disorders.

At the Architectural Level

With highly ordered spatial organization and interactions of various cell types, organoids can model many architectural features of the developing human brain and disease-associated deficits. Cortical organoids faithfully resemble early cerebral development by displaying polarized laminar structures, including the subventricular zone (SVZ) harboring proliferating apical radial glia cells, the outer SVZ harboring outer radial glia cells, and the cortical plate harboring cortical neurons (85). Altered thickness or areas of these laminar structures have been shown in organoids from patients with Rett syndrome (86), microcephaly (22), and periventricular

heterotopia (87). The cortical plate expands in an inside-out manner and separates into 6 cortical layers. A slicing method developed to bypass diffusion limitations and allow for long-term maintenance generates cortical organoids with laminar expansion and segregation into distinct cortical layers (19). This study further showed that sliced cortical organoids from psychiatric patients with a *DISC1* mutation exhibited aberrant cortical layer distribution due to deficits in cortical fate specification (19). At later stages of development, the human cerebral cortex folds and generates gyrifications. *PTEN* mutations have been demonstrated to expand SVZ/outer SVZ layers and promote surface folding in unpatterned brain organoids (88); however, the developmental process could be different in this system because this folding is absent in organoids without the *PTEN* mutation. A recent on-chip approach to generate cortical folding-like structures by growing organoids in Matrigel-filled thin microchannels showed that a *LIS1* heterozygous mutation resulted in lissencephalic organoids with reduced convolutions and cell elasticity (89).

Accumulating evidence is redefining psychiatric disorders as brain circuit disorders (1). Incorporating circuit abnormalities into phenotypic assessments and further tuning these aberrant circuits will substantially contribute to precision diagnostics and therapeutics for psychiatric disorders. Synaptic deficits have been widely identified in patient-derived brain organoids (28,29,31,86). While abnormal long-distance projection synaptic connections were only recently modeled with patient-derived dorsoventral forebrain assembloids (90), other types of assembloids also demonstrated reciprocal thalamocortical (43) and unidirectional corticostriatal projections (42). Furthermore, xenotransplantation could promote circuit maturation and incorporate human organoids within host brain circuits (65,67). In the future, more advanced organoid cultures, combined with transsynaptic viral tracers, such as rabies and herpes simplex virus, can be used to reveal long-distance projection circuits communicating among various brain regions, or even sensory organs to model pathological defects in psychiatric disorders.

At the Functional Level

Brain organoids not only recapitulate many cellular and architectural features of the developing brain, but also exhibit some functionalities, such as releasing various signaling molecules (33,91), producing neural and synaptic activities (15,17), generating complex network dynamics (29,90,92), and even sensing light stimuli (91) and controlling muscle movement (56). Recent advances have just started to realize the potential of organoids for studying functional deficiencies in psychiatric disorders. Whole-cell patch-clamp recording or calcium imaging has revealed altered neural activities at the single cell level from patient organoids in various diseases, such as fragile X syndrome (28), Timothy syndrome (39), microcephaly (21), and 22q11DS (76). Complex neural network activity involves interactions among distinct brain regions and cell types. Medium spiny neurons in corticostriatal assembloids from patients with 22q13.3DS exhibited elevated spontaneous calcium activity but reduced global network synchronization (42). Cortical organoids derived from patients with Timothy syndrome showed hypersynchronous network calcium dynamics,

which were further exacerbated in cortical-subpallial assembloids (83), consistent with clinical features of frequent recurrent seizures in patients with Timothy syndrome (93). Similarly, dorsoventral forebrain assembloids derived from patients with Rett syndrome displayed hypersynchrony and epileptiform-like network activities, consistent with the common symptom of epilepsy in patients (90).

Collectively, patient-derived brain region-specific organoids are shedding light on functional abnormalities in psychiatric disorders. Nevertheless, sophisticated brain functions are built on highly organized tissue with diverse cell types, fully mature cells, and complex circuit connections, and shaped through interactions with sensory, motor, and other systems. Future efforts, such as long-term cultures and xenotransplantation strategies, will hopefully enhance the potential of brain organoids in functional studies of psychiatric disorders.

Responses to Drug Treatment

Patient-derived brain region-specific organoids are becoming increasingly important for developing therapeutic strategies. So far, progress has been made by testing specific drugs to restore phenotypic abnormalities observed in patient-derived organoids. *MECP2* deficiency has been reported to activate the TP53 pathway and promote neuronal senescence (94). The key physiological phenotype of epileptiform-like network activity in assembloids from patients with Rett syndrome with *MECP2* mutations can be restored by a putative TP53 inhibitor, pifithrin- α , and a commonly used antiepileptic medication, sodium valproate (90). Tissue growth defects caused by impaired N-cadherin/ β -catenin signaling in cortical organoids derived from patients with Miller-Dieker syndrome can be ameliorated by the GSK3 β inhibitor CHIR99021 (24).

Scalability and high-throughput phenotypic analyses are major roadblocks that hinder the effective applications of brain organoids in drug discovery. Renner *et al.* (95,96) developed a robotic technology to control initial cell dispensing, organoid patterning, feeding, staining, and analysis, thus creating a scalable workflow for generation and maintenance of human midbrain organoids that is amenable to automatic high-content optical analysis.

Human brain organoids could also play invaluable roles for testing drug toxicity. Due to the inaccessibility of human embryos, certain approved drugs, such as thalidomide and isotretinoin, used by pregnant women resulted in severe birth defects, although they were preclinically tested to be non-teratogenic in animal models (97). Thus, fully exploiting the potential of brain organoids for screening teratogens or drug toxicity will be crucial to avoid similar outcomes in the future.

GENETICALLY ENGINEERED BRAIN REGION-SPECIFIC ORGANOID FOR PSYCHIATRIC DISORDER RESEARCH

Human iPSCs and derived brain organoids provide remarkable accessibility for genetic engineering. In addition to revealing disease phenotypes in patient-derived organoids, engineered brain organoids from healthy individuals are increasingly used in investigating roles of genetic risk variants linked to human brain disorders (Figure 2).

Application of Brain Organoids for Psychiatric Research

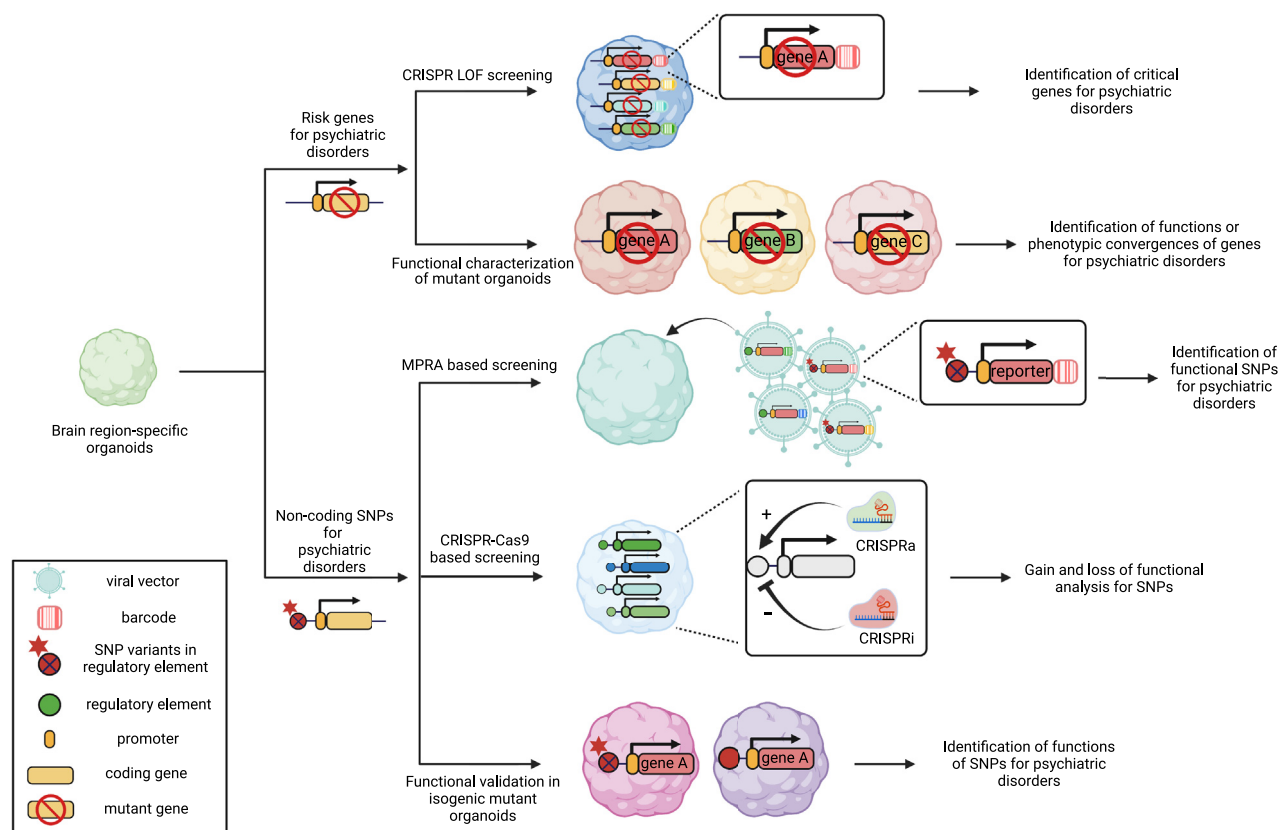


Figure 2. Genetically engineered brain organoids derived from healthy donors for functional studies of risk gene variants. Engineered brain organoids can be used for highly efficient functional screening and in-depth phenotypic convergence studies of protein-coding risk genes and noncoding SNP variants. CRISPR, clustered regularly interspaced short palindromic repeats; CRISPRa, clustered CRISPR activation; CRISPRi, CRISPR interference; LOF, loss of function; MPRA, massively parallel reporter assay; SNP, single nucleotide polymorphism.

Analyses of Functions of Psychiatric Disorder Risk Genes in Human Brain Development

Advancements in human genetics have led to the identification of an ever increasing number of genes linked to psychiatric disorders. Brain organoids provide powerful platforms for analyzing risk gene functions in diverse cell types at single-cell resolution in a human tissue context. CRISPR (clustered regularly interspaced short palindromic repeats) technology not only permits genomic editing of a single gene, but also enables systematic assays of many candidate genes in a multiplex fashion (98,99). A recent study highlighted how brain organoids could provide insight into phenotypic convergences of multiple risk genes (25). Heterozygous mutations of *SUV420H1*, *ARID1B*, or *CHD8*, top ASD risk genes linked to different biological functions (100), were introduced to iPSCs from different healthy donors. Analysis of proteomic and single-cell transcriptomic profiles from generated cortical organoids showed that although the 3 genes function through distinct molecular pathways, they exhibited common phenotypes of asynchronous development of inhibitory and excitatory cortical neurons. Scalability was further demonstrated in another study, in which 173 microcephaly candidate genes were screened for their effects on cell proliferation using a

CRISPR/Cas9-mediated loss-of-function assay in healthy human cortical organoids (101). This study applied a dual barcoding strategy to link individual lineages with a given gene deletion to their lineage sizes, thus overcoming the impact of inherently unequal proliferation dynamics.

Thus, multiplexed CRISPR-based gene editing in brain organoids may provide a framework for high-throughput causative screens, in-depth functional characterization of risk genes, and mechanism discovery for psychiatric disorders.

Linking Functional Single Nucleotide Polymorphisms to Target Genes in Specific Cell Types

Most psychiatric disease-associated variants reside in non-coding regions (102,103). Chromatin structure mapping studies suggest that human psychiatric disorder-associated noncoding single nucleotide polymorphisms (SNPs) are enriched in enhancer-promoter interactions in a cell type-specific manner (104,105), which can regulate target genes over a long distance via 3D chromatin structures (106). Functions of noncoding SNP candidates have been explored in hiPSCs and derived brain cell cultures by leveraging CRISPR-based genetic engineering (107,108). High-throughput

massively parallel reporter assays (MPRAs) have been widely used for large-scale screening of enhancer–promoter interactions in mammalian brain cells (109–113). An MPRA library can be designed by fusing DNA-barcoded reporter genes with candidate regulatory elements with either reference or risk SNP variants and then integrated into brain organoids. By quantifying barcoded transcripts through single-cell sequencing, MPRA can provide biological relevance of SNPs to disease-related gene expression at a large scale and in specific cell types. However, MPRA is limited in recapitulating the size and endogenous chromatin structural context of regulatory elements. CRISPR/Cas9–based *in situ* SNP editing (114) overcomes this limitation and can be used for in-depth functional validation of candidate SNPs identified from MPRAs or other screening models. This method, however, is low throughput and lacks scalability (115). CRISPR-based tiling deletion (116) or CRISPR activation/interference (114,117,118) in regulatory elements can be an alternate for increasing the throughput for functional validations of risk SNPs but with a lower depth. Taken together, noncoding, disorder-associated SNP functions can be potentially studied in depth with high throughput by integrating these genetic engineering approaches in hiPSC-derived organoids, although currently these technologies have only been applied to 2-dimensional cultures.

CURRENT LIMITATIONS AND POTENTIAL IMPROVEMENT

The field of applying brain organoids for psychiatric research is still nascent, and current technologies have considerable limitations. First, human brain organoids can only mimic fetal or, at most, early postnatal brain development, rather than mature brains (119), whereas most psychiatric disorders manifest only in postnatal or adult stages. Sustaining long-term healthy cultures through methods such as slicing (19) and air-liquid interface (120), or xenotransplantation, can promote maturation to a limited degree. Recently, a cocktail of pharmacological compounds targeting several signaling pathways significantly accelerated functional and structural maturation in both human cortical organoids and various hiPSC-derived cell types (121), providing a new strategy for promoting organoid maturation. Second, organoids only recapitulate limited human brain architecture. Although layer segregation has been reported, a fully defined 6-layer structure with distinct radial unit/column distributions and phenotypic normal gyrification has not been demonstrated in human cortical organoids. Further efforts are needed to improve organoid cultures to show these key architectural features, potentially with bioengineering approaches. Third, despite the emergence of various brain region-specific organoid and assembloid methods, brain organoids with multiple well-organized regions have not been reported. Assembloids do not model the intrinsic multiregion developmental process that generates a smooth continuum and structural intermediaries among different regions, and thus are unable to recapitulate neural circuit formation dynamics during brain region development and could potentially yield artificial connections. A recent study using bioengineering approaches to apply spatial gradient morphogens generated neural tube–like organoids with precursor cells representing

multiple brain regions (12). Future studies aiming to maintain these multiregional neural tubes will potentially produce multiregional or whole brain organoids, which could provide better insight into endogenous multiregion brain development and interactions as well as disease processes. Fourth, upregulated cellular stress has been reported in brain organoids, which may hinder cell-type specification (72,119,122). Cellular stress can be reduced by improving culture conditions, such as using sliced cultures (19), or by xenotransplantation (122). On the other hand, a recent study revealed that cellular stress is restricted to a specific subpopulation and can be removed using bioinformatic analysis (123), and some studies have argued that it could reflect a distinct *in vitro* homeostatic metabolic state (119) and does not affect most cellular identity acquisition process in organoids (124). Last, we need to be cautious in the interpretation of data coming just from organoid models. For instance, the high concentration of patterning molecules used in organoid protocols could mask the pathology caused by altered patterning during development in some disease conditions. Organoids lack certain cell types, which are critical for developing psychiatric disorders. A combinational use of organoids as well as *in vivo* animal models could help to address this concern. Together, limitations and challenges exist, and future improvements are needed to make brain organoids a better model for psychiatric research.

SUMMARY

Various methods have been developed to model distinct brain (sub)regions with diverse resident cell types using organoids. Both patient-derived and genetically engineered brain region-specific organoids have been applied in pathological and pharmacological studies of psychiatric disorders, and novel insights have been gained in the understanding of disease pathogenesis. Only by fully acknowledging the limitations can we properly interpret data obtained from organoid studies. We envision that future improvements will further unlock the potential of this technology and leap toward a deeper understanding of psychiatric disorders and will develop better and more rational therapeutics.

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ARTICLE INFORMATION

From the Department of Neuroscience and Mahoney Institute for Neurosciences, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania (ZZ, XW, SP, HS, G-LM); Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania (HS, G-LM); Institute for Regenerative Medicine, Perelman School of Medicine, University of Pennsylvania,

Philadelphia, Pennsylvania (HS, G-LM); Epigenetics Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania (HS); and the Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania (G-LM).

Address correspondence to Guo-Li Ming, M.D., Ph.D., at gming@penncmedicine.upenn.edu.

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Application of Brain Organoids for Psychiatric Research

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