EXPERT REVIEW

Opportunities and limitations for studying neuropsychiatric disorders using patient-derived induced pluripotent stem cells

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Neuropsychiatric disorders affect a large proportion of the global population and there is an urgent need to understand the pathogenesis and to develop novel and improved treatments of these devastating disorders. However, the diverse symptomatology combined with complex polygenic etiology, and the limited access to disorder-relevant cell types in human brains represent a major obstacle for mechanistic disease research. Conventional animal models, such as rodents, are limited by inherent species differences in brain development, architecture, and function. Advances in human induced pluripotent stem cells (hiPSCs) technologies have provided platforms for new discoveries in neuropsychiatric disorders. First, hiPSC-based disease models enable unprecedented investigation of psychiatric disorders at the molecular, cellular, and structural levels. Second, hiPSCs derived from patients with known genetics, symptoms, and drug response profiles offer an opportunity to recapitulate pathogenesis in relevant cell types and provide novel approaches for understanding disease mechanisms and for developing effective treatments. Third, genome-editing technologies have extended the potential of hiPSCs for generating models to elucidate the genetic basis of rare monogenetic and complex polygenic psychiatric disorders and to establish the causality between genotype and phenotype. Here we review opportunities and limitations for studying psychiatric disorders using various hiPSC-derived model systems.

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INTRODUCTION

Psychiatric disorders with neurodevelopmental origins, such as autism spectrum disorder (ASD), schizophrenia (SCZ), depression, and bipolar disorder (BD), have a devastating impact on patients' lives and represent a heavy social and economic burden for societies worldwide. Investigating mechanisms of these disorders remains a challenge due to a lack of access to disease-relevant human brain tissues, heterogeneous phenotypes among individuals, and the inability to accurately recapitulate disease phenotypes in classic experimental model systems [1, 2]. Conventional animal models, such as the most commonly used mouse models, provide valuable opportunities to elucidate the role of a single gene in brain development and dysfunction via loss- or gain-of-function studies. However, accurately modeling human neuropsychiatric disorders in animals is challenging due to species-specific differences, complexity of genetic and social contributions, and a lack of biomarkers and reliable tests. In the past, the best opportunity to access patient-specific brain tissues has been to obtain postmortem tissues, which serve as valuable sources to examine pathological changes in patients. These tissues, however, only represent the disease endpoint and offer limited direct insight into the disease pathogenesis process that often occurs early in life. Over the past decade, the rapid progress in human induced pluripotent stem cell (iPSC) technology has opened a new field in neuropsychiatric disorder research. Human iPSCs (hiPSCs) can be generated from somatic cells by the introduction of reprogramming factors [3]. Patient iPSC-derived two-dimensional (2D) and three-dimensional (3D) neural cultures provide promising opportunities to study disease-relevant cell types, to investigate disease etiology, and to establish a scalable platform for drug screening in vitro [4–6]. In this review, we focus on opportunities using patient iPSC-derived models to better understand the etiology of psychiatric disorders and discuss many challenges that remain to be solved before translating iPSC-based discoveries into clinics.

VARIOUS HIPSC-BASED MODELS FOR PSYCHIATRIC DISORDER RESEARCH

Limited access to disease-relevant tissues and cell types in humans is a major impediment to understand the pathogenesis of developmental psychiatric disorders and to develop effective treatments. Human brain biopsies and postmortem tissues have been studied, providing important clues about disease pathology in a single snapshot, but with limitations. For example, obtaining human brain biopsies is highly invasive with little yield, and postmortem tissues typically represent the endpoint of diseases with influences from other comorbid conditions and treatments [7]. Both samples are hard to manipulate for mechanistic studies or drug screening. Human iPSC-based models can bypass these

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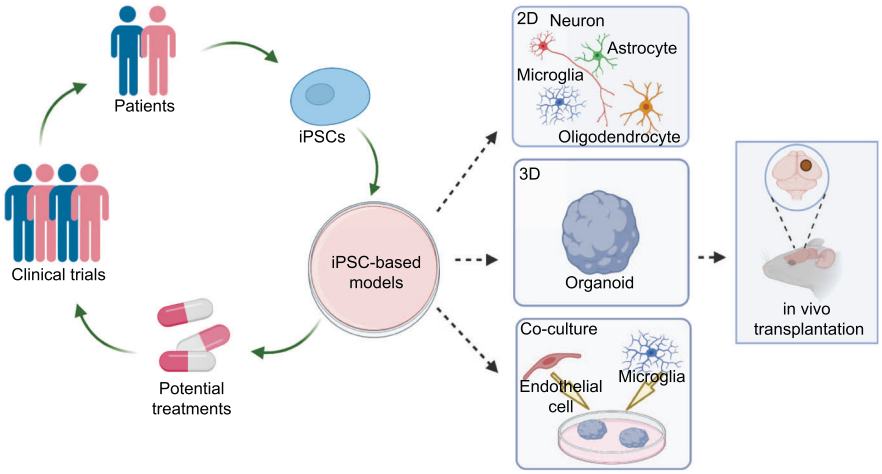

Fig. 1 Diagram of hiPSC-based models. iPSCs could be derived from patients with neuropsychiatric disorders by the introduction of reprogramming factors. These iPSCs could be used in multiple platforms for disease modeling in vitro, including 2D models (neurons, astrocytes, oligodendrocytes, and microglia), 3D models, and co-culture models (organoid co-cultured with microglia or endothelial cells). Organoids could be transplanted into the mouse brain to obtain vascularization and microglia infiltration and test the impact on animal behavior. Identification of phenotypes in patient iPSC-based models would accelerate the development of potential treatments and clinical trials, leading to personalized treatments for patients with neuropsychiatric disorders.

limitations and provide an exciting opportunity to generate in vitro culture systems to understand neuropsychiatric disorders at molecular and cellular levels through a relatively long, albeit limited, developmental course.

Human iPSC-derived 2D models

Over the past decade, efficient protocols have been developed and optimized to differentiate hiPSCs into multiple subtypes of neurons, including cortical glutamatergic neurons and GABAergic neurons, dopaminergic neurons, hippocampal neurons, as well as glial cells, including astrocytes and oligodendrocytes [8] (Fig. 1). It should be noted that generating all brain-relevant cell types with regional and subtype specificity remains a challenge.

Dysfunction of cortical glutamatergic neurons and cortical GABAergic neurons have been found in many neuropsychiatric disorders [1, 5, 9–11]. For example, the glutamate and GABA hypotheses for SCZ were proposed many years ago [12, 13]. Cortical glutamatergic neurons are generated from the dorsal pallium in vivo [5]. Specification of cortical glutamatergic neurons from hiPSCs is achieved by first inhibiting the bone morphogenetic protein (BMP) and tumor growth factor-β (TGF-β) pathways, commonly referred to as "dual-SMAD inhibition", followed by specification into specific cortical layer identities, including deep layer neurons (e.g., $TBR1^+$, $CTIP2^+$) and upper layer neurons (e.g., $BRN2^+$, $CUX1^+$, $SATB2^+$) [14–16]. GABAergic interneurons within the telencephalon populate from one of two embryonic subcortical progenitor zones in the ventral pallium, the medial and caudal ganglionic eminences (MGE and CGE) [17]. Within the cortex, parvalbumin fast-spiking interneurons and somatostatin interneurons arise from the MGE [5]. Several groups were able to generate MGE-like neural progenitors from hiPSCs using small molecules inhibitors for WNT and SMAD signaling coupled with timely sonic hedgehog (SHH) exposure to induce the ventral telencephalic fate [18–20]. Interestingly, GABAergic interneurons take longer to mature than glutamatergic neurons in vitro, mimicking human neural development in vivo [20].

Changes in dopaminergic (DA) synaptic transmission have also been linked to neuropsychiatric disorders [21, 22]. Multiple groups have developed and optimized efficient protocols to differentiate hiPSCs into midbrain DA neurons [23–25]. For example, hiPSCs are first directed to differentiate into floor-plate precursors with activation of SHH and WNT/β-CATENIN signaling, followed by differentiation into functional midbrain neurons expressing the DA neuronal markers tyrosine hydroxylase (TH) and pituitary homeobox 3 (PITX3) [23].

Hippocampal neurons are another disease-relevant neuronal subtype [26, 27]. Embryonic lineage tracing studies have shown that hippocampal neurons are generated from the dorsal-medial region of the developing telencephalon known as the medial pallium, which is composed of the hippocampal neuroepithelium, the cortical hem, and the choroid plexus [28]. The dentate gyrus of the hippocampus is one of two regions in the mammalian brain where neurogenesis continues to occur throughout life [7, 29]. Aberrations in hippocampal neurogenesis have been reported in SCZ [30, 31]. Two differentiation approaches based on free-floating embryoid bodies (EBs) and monolayer neural progenitor cells (NPCs) were developed to generate $PROX1^+$ dentate granule neurons. These neurons formed functional networks in vitro and were able to functionally integrate into the mouse dentate gyrus after transplantation [26]. One study reported a differentiation protocol for generating CA3 pyramidal neurons from hiPSCs and recapitulating hippocampal connectivity in vitro by applying co-culture of dentate granule neurons and CA3 pyramidal neurons, providing opportunities for modeling diseases with hippocampal vulnerability [32]. Another study also reported a protocol for generating both hippocampal granule- and pyramidal-like neurons from human embryonic stem cells (hESCs) [28].

Glial cells, including astrocytes, oligodendrocytes, and microglia, play essential roles in multiple processes, such as synapse formation, maturation and pruning, and blood-brain barrier formation [33]. Understanding glial dysfunction could illuminate neuropsychiatric disorder phenotypes associated with neuronal aberrations and open new avenues for therapies. Many efficient protocols for glia differentiation have been developed and optimized. Astrocytes are a major component of the human brain and provide metabolic and trophic support to neurons [34]. During embryonic development, astrocytes are generated from radial glial cells at the subventricular zone (SVZ). Radial glial cells give birth to neurons in early embryonic development and to glial cells in the late embryonic and early postnatal stages. An efficient protocol to differentiate functional astrocytes from hiPSCs has been described [35]. Using this method, glial progenitor cells can be propagated, expanded, and frozen as intermediates. Oligodendrocytes support signal transduction by forming insulating myelin

sheaths and providing metabolic support to axons. Oligodendrocytes are generated from glial-restricted progenitor cells, known as oligodendrocyte precursor cells [36]. The process of oligodendrocyte differentiation from hiPSCs starts with neural induction, followed by SHH activation. Oligodendrocytes generated from hiPSCs can produce myelin sheaths when co-cultured with neurons or transplanted into the mouse brain [37–39]. Microglia are the brain's resident macrophages that perform essential functions in maintaining homeostasis and modulating neuronal circuits [40]. Microglia originate almost exclusively from erythro-myeloid progenitors generated during primitive hematopoiesis from yolk-sac cells [41, 42]. Recent protocols have differentiated hiPSCs into microglia-like cells by first generating primitive hematopoietic cells and then differentiating those cells into microglia, recapitulating environmental cues present in the developing embryo [43–46].

Rapid and efficient protocols have been developed by several groups to generate induced neurons (iNs) from hiPSCs without the requirement of going through neural stem/progenitor stages. For example, a relatively homogeneous population of cortical excitatory iNs (cortical layers 2/3-like) can be derived from hESCs or hiPSCs by forced expression of a single transcription factor, neurogenin-2 (NGN-2) [47]. Forced expression of two transcription factors, ASCL and DLX2, in hiPSCs, allows for the generation of GABAergic iNs [48]. These neurons express genes representing inhibitory neuronal subtypes of the telencephalon and diencephalon, but not of the hindbrain or spinal cord. Expression of a combination of ASCL1 and mesencephalic factors LMX1A and NURR1 leads to the generation of peripheral dopaminergic neurons, whereas co-expression of ASCL1, LMX1A, NURR1, EN1, FOXA2, and PITX3 results in the generation of dopaminergic neurons with midbrain characteristics [49]. Another group reported a rapid (5 weeks) and efficient (~90%) induction of induced dopaminergic neurons (iDANs) from hiPSCs by transient overexpression of ASCL1, LMX1B, and NURR1 via a single doxycycline-inducible lentiviral vector [50]. Transcriptome analysis of these iDANs revealed a fetal midbrain dopaminergic neuron identity. Induced glial cell protocols have also been published recently to generate homogeneous populations of induced astrocytes (iAstrocytes) from hiPSCs by overexpression of transcription factors SOX9 and NFIB [51]. These cells exhibit molecular and functional properties resembling those of adult human astrocytes. Induction of three transcription factors, SOX10, OLIG2, NKX6.2, in iPSC-derived neural progenitor cells was shown to be sufficient to generate $O4^+$ oligodendrocytes (OLs). This approach yields up to 70% $O4^+$ OL within 28 days of differentiation and produces myelin-like structures around iPSC-derived neurons [52]. A rapid induced microglia (iMG) approach was reported to differentiate hiPSCs into microglia within 10 days by forced expression of both SPI1 and CEBPA [53]. Another study established a more efficient 8-day protocol to differentiate hiPSCs into microglia-like cells by overexpressing six transcription factors, including Hematopoietic Transcription Factor PU.1, MAF BZIP Transcription Factor B, CCAAT Enhancer Binding Protein Alpha, CCAAT Enhancer Binding Protein Beta, Interferon Regulatory Factor 5 and Interferon Regulatory Factor 8 [54]. These induced microglia-like cells show robust expression of microglia markers and recapitulate cellular functions of human microglia, such as response to inflammatory stimuli, phagocytic capabilities, and can be co-cultured with iPSC-derived neurons. Rapid conversion of hiPSCs into various functional neural cell types creates another resource for cellular modeling of human neurological and psychiatric disorders.

Together, these hiPSC-based monolayer cultures often generate highly enriched, uniform populations of targeted cell types, which make the system more suitable for mechanistic studies and large-scale screening. In addition, more complex 2D systems can be built by co-culturing different cell types together for the examination of cell-cell interactions. Further characterization of these hiPSC-derived cell types for comparison with in vivo human counterparts in term of both identities and maturation stages will help to improve differentiation protocols and models of various disorders.

Human iPSC-derived 3D models

Brain organoids [55], self-organized cell aggregates derived from hESCs or hiPSCs, can be generated by "unguided/minimally guided" [56] or "guided" protocols [57–60] to mimic human fetal brain development. Guided approaches using different patterning factors generate more homogeneous organoids for distinct brain regions, including the cortex, hippocampus, thalamus, hypothalamus, and cerebellum [28, 58, 61–64], which are emerging as powerful tools for studying neurodevelopment in distinct brain regions and modeling related disorders, whereas unguided protocols give rise to organoids with the most diverse cell types representing various brain regions [56] (Fig. 1).

Cortical (or forebrain) organoids are the best studied to date, with relatively robust patterning paradigms [57, 58, 65]. Neocortex is the brain region that is the most disproportionally expanded during evolution and is directly connected to higher cognitive functions. Aberrant cortical development is considered to be a major contributor to neuropsychiatric disorders. Cortical organoid models offer promising opportunities to study the etiology of neurodevelopmental disorders, as they recapitulate spatial and temporal features of cortical development. For example, forebrain organoids generated from hiPSCs have been shown to exhibit multiple human-specific features, including generation of a particularly large and well-defined outer SVZ zone with outer radial glial cells [58, 59], co-expression of upper and deep cortical layer markers before layer segregation, and the presence of human-specific astrocyte subtypes [65]. To address the challenge of studying neuronal interactions between different brain regions, several groups have developed an "assembloid" method by fusing region-specific organoids [66]. For example, dorsal forebrain-MGE assembloids have been created to investigate the migration and integration of interneurons, and cortical-thalamic assembloids have been shown to mimic connections between the thalamus and cortex [62, 67, 68]. Future development of additional region-specific or subregion-specific organoids will undoubtedly expand the toolbox to study multiple brain regional interactions using this assembly approach.

Together, brain organoids recapitulate brain tissue structure and the developmental trajectory with diverse cell types, facilitating not only neurodevelopmental and functional studies of each individual cell type, but also examination of cell-cell interactions and circuitry formation in the presence of both neuronal and glia cell types. Brain organoids generated from patient iPSCs may provide the opportunity to bring novel insights into mechanisms of neurodevelopment disorders. Future optimization of protocols with better reproducibility and more similarities with in vivo conditions in term of cell type characteristics, diversity, and cytoarchitectural organization will significantly advance the field.

Human iPSC-derived co-culture models and transplantation into animals

Brain region-specific organoids are relatively homogeneous for neural precursors due to well-developed neuroectoderm specification protocols, which, however, result in the absence of other non-neural lineage cell types, such as microglia and vasculature cells. Reconstituting these cells remains an important endeavor to more accurately mimic in vivo cellular diversity and niche environments (Fig. 1).

Recently developed and optimized protocols to differentiate human microglia-like cells has created the possibility of adding microglia-like cells to brain organoids [69]. Microglia-like cells

Table 1. Comparison of 2D, and 3D culture models and transplantation into animals.

	2D	3D	Transplantation
Advantages	Relatively homogenous populations	Cellular diversity	In vivo environment
	High reproducibility	More cell-cell interaction	Vascularization
	Relatively inexpensive	Cellular organization and cytoarchitecture	Microglia infiltration
	Easy for scale-up	Cellular migration patterns	Extended cell maturation
	Easy to set up		Axonal projection
Disadvantages	Less cell diversity	Low reproducibility	Technical challenging
	Less cell-cell interaction	Lack of functional vascularization	Small scale
	Lack of cytoarchitecture	Lack of microglia and other cell types	Not complete human features
	In vitro system	In vitro system	More expensive
		Expensive	

co-cultured with forebrain organoids showed improved maturation and the response to pro-inflammatory stimuli [70]. Vascularization is particularly necessary for oxygen penetration, nutrient supply [71, 72], and neuronal development [73–75]. The lack of oxygen and nutrient penetration limits the long-term development of organoids. Co-culture of brain organoids with endothelial cells responsible for lining major blood vessels and capillaries is one way to vascularize brain organoids. A few studies demonstrated that hiPSC-derived endothelial cells co-cultured with brain organoids showed robust engraftment with the formation of capillary-like structures [76, 77], although active perfusion cannot be achieved.

Besides assembloids and co-culture approaches, several groups have transplanted brain organoids into mouse brains, which would establish efficient perfusion and enable the recapitulation of intricate network connectivity under in vivo conditions, facilitating mechanistic studies into the pathogenesis of neurodevelopmental psychiatric disorders [78] (Fig. 1). One study reported an efficient method for transplanting human brain organoids into the superficial cortex of the adult mouse brain [79]. The grafted organoids integrated into the mouse brain, exhibited progressive neuronal differentiation and maturation of human neurons, and host microglia and blood vessels penetrated into the grafts. In vivo extracellular recording combined with optogenetics showed functional synaptic connectivity between grafted organoids and the mouse brain. This study generated the exciting possibility of disease modeling under an in vivo environment. Although widespread axonal extension outside the graft area was observed, region-specific long projections were not reported in this study. Another group optimized a culturing protocol capable of efficiently generating small human cerebral organoids [80]. Organoids were transplanted into the medial prefrontal cortex, a deep region of the cerebral cortex associated with a variety of neurodevelopmental disorders. Under these conditions, human neurons extended long projections into the lateral hypothalamus region, and importantly, the grafted human organoids formed mutual synaptic connections with mouse neurons. Interestingly, mice transplanted with organoids showed an increase in freezing time in response to auditory conditioned stimuli, suggesting that grafted human organoids may functionally integrate into the mouse brain circuits and contribute to physiological functions. In a more recent study, hiPSC-derived cortical organoids were transplanted into the primary somatosensory cortex of early postnatal athymic rats [81]. Neurons from transplanted organoids underwent substantial maturation and received thalamocortical and corticocortical inputs that are capable of producing sensory responses in human cells. Transplanted organoids extended axons throughout the rat brain that could drive reward-seeking behaviors, suggesting that transplanted organoids can engage host circuits that control behavior. Together, these early studies provide the foundation to investigate behavior related to neurological diseases using transplantation approaches in the future. Caveats of the in vivo transplantation are also noted. For example, immune-deficient animals or immune suppression is needed. The difference in brain development between species results in the early depletion of progenitors and a potential loss of human-specific features. Furthermore, the communication between human and mouse neural cells remains to be characterized in detail.

Together, hiPSC-derived 2D, 3D, and in vivo transplantation models provide platforms to investigate different aspects of developmental psychiatric disorders. Although each model may lead to unique insights and recapitulate diverse phenotypes of neurodevelopmental disorders, each has its own strengths and limitations (Table 1). Therefore, different models need to be carefully selected to address specific questions.

INSIGHTS INTO PSYCHIATRIC DISORDERS FROM PATIENT-DERIVED IPSC RESEARCH

While hiPSC-derived 2D or 3D models can be used to decipher molecular and cellular events related to normal brain development, iPSC-based models derived from patients with psychiatric disorders carrying patient-specific mutations and genetic backgrounds provide a renewable cellular resource that has been largely inaccessible and an unprecedented opportunity to model molecular, cellular and cytoarchitectural deficits in these developmental disorders. They can also serve as a platform to model drug responses (Fig. 2).

Modeling molecular and cellular deficits

It is now possible to gain a deeper understanding of the pathogenesis of psychiatric disorders in living human cells by differentiating patient-derived iPSCs into disease-relevant cell types with disease-permissive genetic contexts. NPCs derived from hiPSCs in culture can self-renew or further differentiate into neurons, astrocytes, or oligodendrocytes [82]. Dysfunction along the NPC proliferation and differentiation cascade has been related to many neurodevelopmental disorders [83].

SCZ is a chronic psychiatric disorder that influences early brain development [84] and it was among the first neuropsychiatric disorders for which patient-specific iPSC lines were generated [85] and investigated in vitro [86, 87]. Specifically, patient iPSC-based cell models have been used to identify molecular and cellular deficits of SCZ, including transcriptional dysregulation of synaptic genes, alteration of cell-specific gene networks, reduced synaptic vesicle release, aberrant synaptic formation, reduced neuronal activity, and impaired mitochondrial function [26, 87–89] (Fig. 2). One significant challenge is to correlate the emergent phenotypes to the clinical characteristics of donor patients. A recent study analyzed gene expression and indices of cellular function in iPSC-derived NPCs and cortical neurons generated from 13 individuals

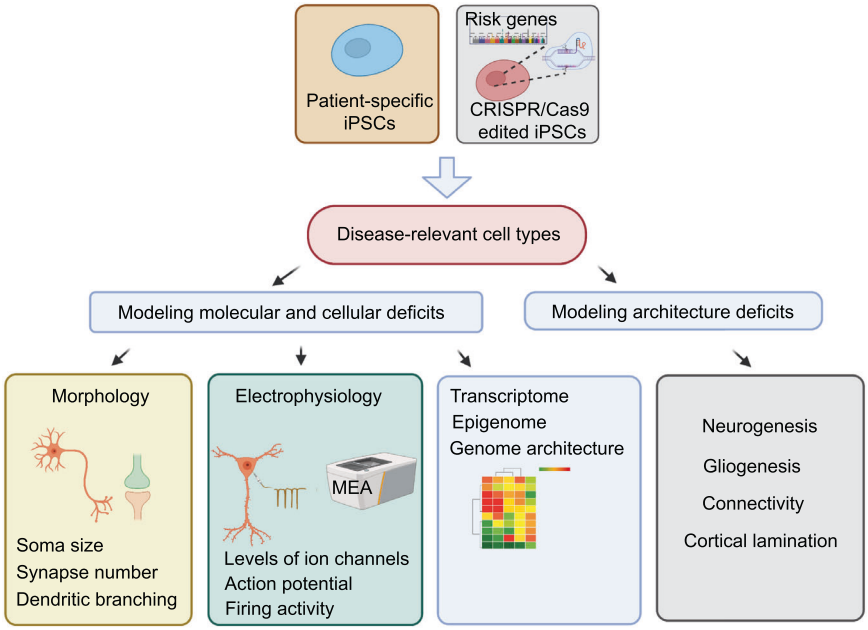

Fig. 2 Molecular, cellular, and architectural read-outs for iPSC-based disease modeling. Patient-derived iPSCs and genome-edited iPSCs based on human genetics data could be differentiated into disease-relevant cell types, which could model molecular, cellular, and architecture deficits of neuropsychiatric disorders.

with high polygenic risk scores (PRSs) for SCZ and a clinical diagnosis of SCZ, along with 15 neurotypical individuals with low PRS [90]. Their analysis revealed that electrophysiological data is associated with diagnosis and/or predictive of the severity of clinical and cognitive features of individual donors. These initial results suggested that neurophysiological measures might be related to the patient's personal clinical characteristics, which may help to develop novel biomarkers and therapeutic targets. The number of patients examined in this study was, however, relatively low, and additional studies are needed to increase the power and relate molecular and cellular phenotypes to patient symptoms in the future.

ASD comprises of a group of complex neurodevelopmental disorders that affect more than 1% of children in the United States [91]. An increase in brain size of autistic individuals in the first three years of life has been suggested to be the first clinical sign [92]. One study generated iPSCs from 8 ASD patients who displayed brain overgrowth early in life. Consistent with clinical presentations, ASD patient iPSC-derived NPCs showed increased cell proliferation, which was due to the dysregulation of a β-CATENIN/BRN2 transcriptional cascade. Furthermore, ASD patient iPSC-derived neurons displayed reduced synaptogenesis leading to functional deficits in neuronal networks [93] (Fig. 2). Although growing evidence supports the notion that NPCs derived from ASD patients with macrocephaly undergo rapid cell cycle progression [93, 94], little is known about the underlying mechanisms and downstream consequences. One recent study revealed a previously unknown mechanism by which accelerated S-phase progression in ASD-derived NPCs may contribute to DNA damage via increased replication stress [95]. Using fluorescence-activated cell sorting (FACS) analysis and a DNA combing assay, it was shown that ASD-derived NPCs exhibited accelerated S-phase progression, increased replication stress, and chronic DNA damage. To understand how altered replication affects genome stability, they used a high throughput genome-wide translocation sequencing assay to map double-strand break (DSB) sites at nucleotide resolution in NPCs. They found that replication stress in ASD-derived NPCs increased DSBs in long ASD genes leading to expression changes and functional defects. Their findings provide a mechanism linking hyperproliferation of NPCs with the pathogenesis of ASD. These studies also suggest that idiopathic ASD can be modeled by patient iPSCs to reveal novel cellular and molecular mechanisms that may contribute to brain abnormalities.

Several large, rare copy number variations (CNVs) have been identified as significant genetic risk factors for developmental and psychiatric disorders, such as SCZ and ASD [96–99]. We know little about how these CNVs affect neural development, since CNVs contain multiple, and sometimes over a hundred, genes and are difficult to model in animal models due to differences in chromosome composition. Patient-derived iPSCs capture identical risk alleles as the donor individual and provide disease-relevant human cell types to facilitate molecular and cellular investigations. One study established iPSC lines from three individuals carrying 15q11.2del that is linked to SCZ and compared them with iPSCs from five individuals without the CNV. Patient iPSC-derived NPCs exhibit deficits in adherens junctions and apical polarity due to WAVE complex destabilization. Haploinsufficiency of cytoplasmic FMR1-interacting protein 1 (CYFIP1), one of the genes within 15q11.2, is an underlying cause of these defects by altering cytoskeletal dynamics [100]. These findings provided insight into how CYFIP1 regulates neural stem cell function and may contribute to the susceptibility of neuropsychiatric disorders.

Psychosis is a shared clinical feature of both SCZ and BD. BD is a common neuropsychiatric disorder characterized by chronic recurrent episodes of depression and mania. Recent development and progress in single-cell RNA sequencing (scRNA-seq) technology enables the identification of cell-type-specific pathophysiology (Fig. 2). Brain organoids derived from patient iPSCs are an optimal tool to identify the cell-type-specific pathophysiology of SCZ and BD. ScRNA-seq analysis of cerebral organoids from iPSCs generated from monozygotic twins discordant for psychosis showed enhanced GABAergic specification and reduced cell proliferation following diminished WNT signaling in patient iPSC-derived cerebral organoids, which was confirmed in iPSC-derived forebrain neuronal cells [101]. Two additional monozygotic twin pairs discordant for SCZ also confirmed excess GABAergic specification of the patients' iPSC-derived NPCs. Their results suggest that unbalanced specification of excitatory and inhibitory

neurons during cortical development may contribute to psychosis. Organoids derived from ASD patient-derived iPSCs were investigated for neurodevelopmental alterations in individuals with macrocephaly. ASD patient iPSC-derived organoids exhibited an accelerated cell cycle and overproduction of GABAergic inhibitory neurons [94]. Overexpression of the transcription factor FOXG1 was correlated with the overproduction of GABAergic neurons.

In addition to NPCs and neurons, glial cells have also been shown to play important roles in neurodevelopmental disorders. Synapse density is reduced in postmortem cortical tissue from SCZ patients, suggesting that dysregulation of synaptic pruning may contribute to the onset of SCZ pathophysiology. Using a reprogrammed in vitro model to study SCZ-specific microglia-mediated synapse engulfment, increased synapse elimination was observed in patient-derived neural cultures [102]. Together, patient-derived iPSC models are promising for studying psychiatric disorders, allowing for deeper probes into the human-specific molecular mechanisms and cellular phenotypes of disease-relevant cells.

Modeling cytoarchitecture deficits

Brain organoids generated from patient-derived iPSCs exhibit cytoarchitectural features resembling the developing human brain, which may help to reveal critical disease phenotypes that are hard to recapitulate in animal models and 2D cultures.

Brain organoids were first applied to model disorders with large structural deficits, as the phenotypes are usually easy to observe (Fig. 2). For example, larger organoids have been generated from iPSC lines derived from patients with macrocephaly, while smaller organoids have been generated from microcephaly patient iPSC lines [103, 104]. One study reported the effects of CNTNAP2 on embryonic cortical development using forebrain organoids derived from ASD patients with a homozygous protein-truncating mutation in CNTNAP2. Forebrain organoids showed an increase in volume and total cell number that is driven by increased NPC proliferation [103]. Reciprocal deletion and duplication of the 16p11.2 region is the most common CNV associated with ASD. Importantly, deletion and duplication were associated with macrocephaly and microcephaly in human carriers, respectively [105, 106]. Consistently, the 16p11.2 deletion organoids were larger in size, whereas 16p11.2 duplication organoids exhibited a smaller size, recapitulating patients' brain size phenotypes [104].

Brain organoids provide a new tool to study developing cortical morphology of disease-associated mutations. Rare mutations in disrupted in schizophrenia 1 (DISC1) have been associated with SCZ, ASD, and other major mental disorders [89]. Forebrain organoids derived from patients with a 4-base pair frameshift deletion in DISC1 exhibited a delay in cell cycle progression related to disrupted protein interactions between DISC1 and NED1/NDEL1 [107]. Laminar disorganization is frequent in ASD patients [108]. The sliced neocortical organoid (SNO) system that leads to formation of well-separated upper and deep cortical layers provides a platform to test whether other psychiatric disorders have similar abnormalities [65]. SNOs derived from patients with the DISC1 mutation exhibited deficits in both cortical neuron subtype specification and laminar distribution related to disrupted WNT/β-CATENIN signaling [65] (Fig. 2).

Together, these examples highlight how brain organoid models can recapitulate the human brain not only at the cellular level, but also in terms of brain architecture and developmental trajectory, providing access to more advanced features of human-specific brain developmental processes implicated in neuropsychiatric disorders. Future optimization of organoid protocols with extended neuronal maturation and similar architecture and neuronal circuit formation as in the human brain will significantly advance the field.

Modeling drug treatment responses

Some BD patients show remarkable improvement with lithium treatment, but others are refractory to the same treatment. Hippocampal dentate granule neurons differentiated from BD patient iPSCs showed hyperexcitability in electrophysiological recordings [27, 109]. Interestingly, this hyperexcitability phenotype could be selectively reversed by lithium treatment only in the neurons derived from patients who also responded to lithium treatment. Cortical interneuron migration depends on L-type calcium channel (LTCC) function, and genetic variants that alter LTCC function have been implicated in various neuropsychiatric disorders [110, 111]. An early study used forebrain assembloids derived from patients with Timothy syndrome (TS), a rare and highly penetrant neurodevelopmental disorder caused by a mutation in the LTCC $Ca_v1.2$, and found inefficient migration with reduced saltation length but increased saltation frequency of TS interneurons [68]. More recently, they found that acute pharmacological modulation of $Ca_v1.2$ could regulate the saltation length, but not the frequency, of interneuron migration, indicating that defects in TS interneuron saltation length and frequency are driven by distinct molecular pathways [112].

Together, these studies indicate that patient iPSC-based models can capture molecular, cellular, and structural phenotypes of neurodevelopmental and neuropsychiatric disorders, and provide an opportunity for investigating the underlying mechanisms for treatment development.

ENHANCING HIPSC MODELS WITH GENOME-EDITING FOR PSYCHIATRIC DISORDER RESEARCH

Combined with recent advances in genome-editing technologies, iPSC-based models became even more powerful as tools to model genetically complex diseases, including neuropsychiatric disorders. Since the development of genome editing tools such as zinc-finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), and more recently, clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) technology, they have been widely applied to better understand the function of risk genes of neuropsychiatric disorders, and to address the causal relationship between genotype and phenotype (Fig. 2). For example, one early study generated iPSC lines from four members of an American family in which a 4 base-pair deletion of DISC1 co-segregated with SCZ and major depression, as well as isogeneic lines via TALEN-based genome editing to correct the mutation in patient lines or to introduce the same mutation in control lines. These iPSC lines were differentiated into forebrain cortical neurons and showed that the mutation is directly responsible for synapse deficits and transcriptional dysregulation. This study was the first to illustrate how a rare genetic mutation for psychiatric disorders is causal for molecular and cellular phenotype in human neurons [89].

While a wide range of genes are identified as risk factor for ASD [91, 113–116], their impact on different cell types in the brain could be diverse and complex. To understand how genetic changes perturb brain development and affect clinical symptoms of ASD, a multiplex "cell village" approach using CRISPR/Cas9 was developed to study a group of genes implicated in ASD. Thirty isogenic disease iPSC lines with 27 ASD mutations were co-cultured together and differentiated into prefrontal cortex (PFC) lineages. They defined a subgroup of ASD mutations that increase PFC neurogenesis, while another subgroup decreases it. Remarkably, there is also a segregation of clinical profiles in language acquisition of patients with subclass-specific mutations. This study not only provided neurodevelopmental clues on genetic heterogeneity associated with ASD, but also suggested converging molecular pathways of diverse ASD-associated mutations [117]. Another study used human cortical organoid models combining

single-cell RNA-seq to probe the impact of genetic background as well as risk mutations on different cell types, and came to similar conclusions [118]. Three ASD risk genes, SUV420H1, ARID1B, and CHD8, were introduced by CRISPR/ Cas9 technology into multiple control iPSC lines with different genetic backgrounds. Although the three mutations are known to act through largely different molecular pathways, their impact on cell-type-specific neurodevelopmental abnormalities is found to be mostly shared across ASD risk genes.

Hundreds of risk loci have been identified in major psychiatric disorders [115, 119, 120]. Most single-nucleotide polymorphisms (SNPs) have been mapped to non-coding regions by genome-wide association studies (GWAS), making their functional study challenging. Human iPSC-based models combined with genome editing technologies provide promising tools to investigate the function of variants in the native genomic context. One study prioritized ~100 putatively functional SCZ-SNPs located in neuronal open chromatin regions, including rs1198588, at a leading risk locus flanking MIR137. HiPSC-derived excitatory neurons with CRISPR/Cas9-edited rs1198588 showed altered MIR137 expression, dendrite arborization, and synapse maturation, suggesting a causal function for this SNP in neuromorphological development [121]. Another study used CRISPR-mediated gene editing to study one SCZ-SNP (FURIN rs4702) and demonstrated the downstream effects of the allelic conversion of rs4702 showing reduced neurite length and neuronal activity in hiPSC-derived NGN2 excitatory neurons [122].

Advances in the genome editing field with invention of new approaches, such as base editing and prime editing, enable predefined nucleotide exchanges in genomic sequences without generating DNA DSBs [123] (Fig. 2). Base editing uses catalytically inactive Cas9 (dCas9) fused to DNA deaminases to induce base substitutions at the target site and has been used in hiPSCs [124–126]. Base editing is currently restricted to changing single bases and only certain nucleotide changes can be achieved. More recently, prime editing has been developed [127], which uses a Cas9-nickase fused to a reverse transcriptase and an extended prime editing guide RNA. Prime editing can introduce all types of base-to-base conversions and the insertion and deletion of small fragments of DNA without DSBs. These methods will allow for the efficient base editing of hiPSCs for disease modeling and cell-based therapies.

CRISPR/Cas9 technologies have gone beyond gene editing. CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) allow the modulation of the expression of one gene or multiple genes from the endogenous genomic loci [128, 129]. CRISPRi or CRISPRa is achieved by repressor or activator transcription domains fused to a catalytically dCas9 and guide RNAs directed to the promoter or regulatory regions of specific genes. The simultaneously modulated expression of different genes through CRISPRi/a provides the possibility to unravel the contribution of common risk variants to complex genetic disorders. Schrode et al. demonstrated functional validation of four top-ranked SCZ expression quantitative trait loci (eQTLs) genes (FURIN, SNAP91, TSNARE1, and CLCN3) via single and combinatorial CRISPRi/a manipulations in iPSC-derived NGN2 excitatory neurons [122]. CRISPRi/a effected small changes in target genes that resulted in convergent downstream transcriptomic differences capturing the effects observed in the postmortem brain. This study also identified the cell-type-specific effects of common variants and demonstrated a synergistic effect between SCZ eQTL genes that converge on synaptic function, supporting the notion that synergy between risk variants may impact SCZ risk.

These early studies highlight the potential of genome editing technology, in particular CRISPR/Cas9, to complement patient iPSC-based models for understanding the genetic basis of complex psychiatric disorders.

LIMITATIONS AND FUTURE DIRECTIONS

With the rapid advancement of applying hiPSC-based models to study human brain development and mechanisms underlying various psychiatric diseases, we are still facing many challenges that need to be addressed for clinical and pharmacological applications. We discuss these limitations and offer some suggestions for minimizing their impact.

Inherent variability among hiPSC-based models remains a major challenge for reproducibility. The variability arises from several aspects, such as genetic heterogeneity from donors, reprogramming methods, and differentiation protocols. To address these limitations, first, multiple lines derived from donors of different genetic backgrounds are needed when reporting a new protocol. Second, when comparing phenotypes between control and patient lines, it is necessary that the lines are derived from the same source (skin or blood cells), same reprogramming method, matched gender, and similar age. If possible, multiple in-family and/or isogenic controls from genome editing technology should be used for comparison [89]. Third, CRISPR-edited lines should be screened for off-target effects in major regulatory and protein-coding regions of the genome. In addition, it is imperative to generate more than one clone for each line.

Multiple non-neural lineage cell types are absent in brain region-specific organoids, most notably microglia that are essential for maintaining homeostasis [130]. Although iPSC-derived microglia can be introduced into organoids [70, 131, 132], or host microglia can be found in transplanted organoids in the mouse brains [79], there are limitations in these approaches. For example, there are limited numbers and a lack of functional validation of those introduced microglia cells in organoids in vitro and the non-human nature of the microglia after transplantation into animals. In addition, there is a lack of subtype diversity of microglia [133]. Future studies are needed to either co-develop microglia and neural cells together or to identify the best timing for introducing microglia cells. A mouse model with human microglia may also be beneficial for transplantation purposes.

A lack of vascularization to facilitate oxygen and nutrient exchange has been known to cause a hypoxic necrotic core that impedes long-term organoid cultures. While co-cultured brain organoids with iPSC-derived endothelial cells showed formation of capillary-like structures [76, 77], no perfusion is achieved. Bioengineering approaches such as microfluidic systems to introduce flow through either endothelial-based capillary or artificial vasculature would be a significant advance toward achieving long-term cultures of brain organoids.

Although patient iPSC-based 2D and 3D models provide unprecedented opportunities for uncovering disease mechanisms in relevant human cell types, behavioral deficits, the hallmarks of psychiatric disorders, cannot be modeled in vitro. Humanized animal models or non-human primate models are likely required for accessing disease-relevant behaviors. A recent study generated a mouse model carrying the same mutation in the DISC1 locus as that found in patients. This humanized mouse model not only showed similar molecular changes and synaptic deficits as patient iPSC-derived neurons with the DISC1 mutation, but also exhibited deficits in behavioral tests [134]. Importantly, pharmacological targets that were capable of reversing phenotypes of patient iPSC-derived neurons in vitro were also effective in rescuing synaptic and behavioral deficits in the mouse model, suggesting that iPSC-based systems and disease mouse models are complementary for translational studies. Human cellular models have been slowly adapted as a drug screening platform based on molecular or cellular phenotypes, and patient iPSCs offer the opportunities to develop patient-specific treatments, however, the validity of this approach remains to be demonstrated in future studies. With further improvement in iPSC technology and the combination of iPSC-based models with genome editing or other advances

techniques, patient iPSCs have the potential to contribute to novel therapies for heterogeneous and genetically complex psychiatric disorders.

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YH wrote the manuscript with contributions from all co-authors.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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