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Review

Contribution of induced pluripotent stem cell technologies to the understanding of cellular phenotypes in schizophrenia

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ABSTRACT

Schizophrenia is one of the leading causes of disability among mental disorders, contributing to a substantial socioeconomic burden. Our understanding of the mechanisms of the pathogenesis of the disease has largely been limited by its inherent complexity imparted by the polygenicity and interactions with environmental factors. Since pathobiological events are initiated in the schizophrenic brain long before the onset of the psychotic manifestations, characterizing these processes is limited, mainly due to a lack of access to neuronal tissues. Induced pluripotent stem cell (iPSC) technologies have provided an unprecedented opportunity to establish pluripotent stem cells from patients with schizophrenia and differentiate them into neuronal lineage, enabling an in vitro recapitulation of the pathogenesis of the disease. Despite the inherent challenges, patient-derived iPSC studies of schizophrenia have been instrumental in unraveling the cellular and molecular phenotypes that might be involved in the biological causality. Here we review the literature and focus on studies that have utilized patient-derived iPSCs to model the pathogenesis of schizophrenia. We also discuss the challenges in modeling cellular phenotypes of schizophrenia.

1. Introduction

Schizophrenia is a highly heritable and complex psychiatric disorder characterized by deficits in behavior and cognitive abilities that afflicts approximately 1% of the global population (Owen et al., 2016). Although the heritability estimate ranges to 80% (Hilker et al., 2018; Sullivan et al., 2003), studies that attempted to delineate the genetic component of schizophrenia liability have not yet defined a unitary mechanism for the pathophysiology due to substantial polygenic components (Giegling et al., 2017; Gratten et al., 2014). However, epidemiological data have shown an increased risk of schizophrenia in the offspring of mothers who experienced prenatal insults such as starvation, malnutrition, and maternal infection, supporting a role for early life neurodevelopmental defects (Brown and Derkits, 2010; St Clair et al., 2005; Susser et al., 1996; Susser and Lin, 1992). Interestingly, recent large-scale genetic studies concur with previous findings that many schizophrenia-associated genes are enriched for neurodevelopmental and neurotransmitter functions (Schizophrenia Working Group of the Psychiatric Genomics, 2014).

Schizophrenia-associated pathobiological processes initiate long before the onset of psychosis, therefore, characterization of these events could enable them to be targeted for intervention modalities. Although both genetic and pharmacological animal models have been critical in discerning the neurobiological perturbations underlying schizophrenia phenotypes, an ideal preclinical disease model that recapitulates the pathogenesis, course of disease and clinical phenotypes has not been possible until now. Additional constraints include methodological limitations to model combinations of multiple genetic variants with varying effect sizes identified from human genetic studies in animals, as well as the inadequacy of behavioral tests in animals aligning with Diagnostic and Statistical Manual of Mental Disorders criteria (Jones et al., 2011). Further limitations include the human-specific nature of the schizophrenia and the phylogenetic differences in neurobiological substrates for the disease across lower species (Dolmetsch and Geschwind, 2011).

Unlike neurodegenerative diseases, schizophrenia does not have a characteristic neuropathology as a cause or consequence that can be traced back to its origin. Postmortem tissue samples have provided substantial evidence for the current understanding of alterations at the cellular and molecular levels in schizophrenia (Bakhshi and Chance, 2015). However, measuring pathobiological end points, which are usually confounded by the use of antipsychotic drugs, substance abuse, and nutritional changes, makes postmortem samples disadvantageous for deciphering the pathogenesis. Also, obtaining brain tissue biopsies is not feasible in patients due to ethical concerns and limitations in the expansion of post-mitotic neurons in vitro. Currently, noninvasive,

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structural, and functional neuroimaging is instrumental in localizing abnormalities in the brain regions, but they lack the resolution required to delineate the neuronal and synaptic pathology (Falkenberg et al., 2014). Thus, an alternative model is essential to model molecular players with respect to the developmental course of the disease.

2. Induced pluripotency technologies

The ground-breaking discovery of induced pluripotency by coaxing terminally differentiated adult somatic cells back to a pluripotent state by introducing four specific transcription factors to form pluripotent stem cells has opened a new avenue of modeling schizophrenia and other complex neuropsychiatric disorders (Takahashi et al., 2007; Takahashi and Yamanaka, 2006). Similar to embryonic stem cells, induced pluripotent stem cells (iPSCs) are capable of differentiating into any cell type of the three germ layers, with controlled manipulation of growth factor treatments, modulators of specific signaling pathways, and culture conditions. Thus, human iPSC (hiPSC) technology permits the establishment of a renewable source of pluripotent cells, that can be further differentiated to neural progenitor cells (NPCs), glutamatergic neurons, dopaminergic neurons, and other neural subtypes (Mertens et al., 2016). In due course, different methodologies have been adopted to the establish iPSCs (Malik and Rao, 2013) from a wide variety of somatic cell sources (Raab et al., 2014). Subsequently, transcription factor-mediated direct reprogramming (transdifferentiation) of fibroblasts to induced-neuronal cells was also achieved, surpassing the pluripotent state (Pang et al., 2011; Vierbuchen et al., 2010).

Thus hiPSC technologies provide the prospect to study the recapitulation of molecular and cellular aspects of schizophrenia pathogenesis in relevant neural cells (Fig. 1). This in vitro modeling also facilitates the screening of novel drug candidates to rescue the molecular, cellular, and functional abnormalities manifested in schizophrenia-derived hiPSCs, and provides the possibility for personalized medicine (Zhang et al., 2017). Recently, clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9) mediated genetic manipulation techniques have been widely employed to correct the risk allele(s) in schizophrenia hiPSCs or introduce schizophrenia-associated variant alleles in normal hiPSCs to create isogenic hiPSC lines, allowing the elucidation of molecular and cellular phenotypes imparted by risk alleles (Hockemeyer and Jaenisch, 2016; Wen et al., 2014). Genome-wide association studies and exome sequencing studies with large sample sizes continue to identify novel/additional risk loci. Such results demand functional evaluation pertaining to their corresponding neurobiological phenotypes (Giegling et al., 2017; Li et al., 2017; Schizophrenia Working Group of the Psychiatric Genomics, 2014). Recently developed in vitro three-dimensional (3D) brain organoid cultures further allow modeling of spatiotemporal cellular organization of multiple subtypes of neural cells, recapitulating many features of in vivo neurodevelopment (Lancaster et al., 2013; Paşca et al., 2015; Quadrato et al., 2017; Renner et al., 2017). Thus, the combination of hiPSC technologies along with genome editing technologies and 3D brain organoid cultures offers an unprecedented opportunity to model the disease pathogenesis more precisely (Shi et al., 2017). Here, we review the studies that utilized patient-derived hiPSCs to study schizophrenia-relevant cellular phenotypes.

3. Cellular phenotypes in hiPSC models of schizophrenia

The discovery of iPSC technology rapidly led to a variety of Mendelian and complex genetic disorders being modeled using hiPSCs (Park et al., 2008). Schizophrenia was the first neuropsychiatric disorder to be modeled using hiPSCs (Brennand et al., 2011; Chiang et al., 2011). Chiang et al. were the first to demonstrate the generation of hiPSCs from adult schizophrenia patients harboring a Disrupted-In-Schizophrenia-1 (*DISC1*) frameshift mutation using the integration-free episomal vector approach (Chiang et al., 2011). Subsequently, Brennand et al. successfully established hiPSCs derived from fibroblasts of one childhood-onset and three familial schizophrenia patients (with no genome information) (Brennand et al., 2011). Later, many hiPSCs from schizophrenia patients harboring penetrant genetic variants and with non-specified genetic composition were established with the aim of understanding cellular and molecular phenotypes in relation to neuronal development.

3.1. Reduced neural connectivity

The first comprehensive evaluation of cellular and molecular phenotypes of neural cells from schizophrenia patient-derived hiPSCs was carried out by Brennand et al. (Brennand et al., 2011). Although there were no defects in differentiation propensities of hiPSCs into NPCs or neurons, a significant reduction in neuronal connectivity was observed. Interestingly, treatment with loxapine, but not other antipsychotics, was shown to rescue attenuated neuronal connectivity in hiPSC-derived neurons from all patients. In addition, loxapine treatment also increased the expression of several glutamate receptor genes (Brennand et al., 2011). Consistent with observations from animal models and postmortem studies (Jaaro-Peled et al., 2009), reduced neurite outgrowth and a decreased number of neurites was evident in iPSC-derived neurons that were predominantly glutamatergic. The molecular perturbations in patient-derived neurons were also in line with the evidence from postmortem brain studies, where glutamate, cyclic 3′, 5′-adenosine monophosphate (cAMP), and WNT signaling were shown to be affected (Freyberg et al., 2009). Remarkably, synaptic phenotypes were unaffected, with no disparities in the electrophysiological properties and intact expression of synaptic proteins in patient hiPSC-derived neurons. Concurring with these results, there was no reduction in postsynaptic density protein 95 (PSD-95)-synaptic density, despite a reduction in PSD95 protein levels in hiPSC-derived neuronal dendrites from schizophrenia patients. Taken together, these initial results support findings from established schizophrenia mouse models and showed that neurons from patient-derived hiPSCs had similar deficits in cellular and molecular phenotypes.

Reduced neural connectivity was consistently replicated in other neural subtypes, such as hippocampal dentate gyrus (DG) granule neurons derived from schizophrenia hiPSCs (Yu et al., 2014). In the schizophrenia hiPSCs as reported previously (Brennand et al., 2011), defective DG granule neurons generation from NPCs were observed along with a marked reduction in the expression of key genes involved in the regulation of DG granule neuronal differentiation (*NEUROD1*; neuronal differentiation 1), proliferation, and survival (*FOXG1*; forkhead box G1), and downregulation of DG granule neuron markers, prospero homeobox 1 (PROX1) and T-box, brain 1 (TBR1) (Hodge et al., 2012; Shen et al., 2006; Yu et al., 2014). Although patient-derived hiPSCs were capable of forming hippocampal DG granule cells, their proportion was less, corresponding to the abnormal hippocampal neurogenesis reported in schizophrenia (Allen et al., 2016b; Tamminga et al., 2010; Yu et al., 2014). While there were no significant electrophysiological abnormalities, spontaneous neurotransmitter release was significantly lower in schizophrenia hiPSC-derived DG granule neurons, substantiating the dampened neuronal connectivity as reported previously (Brennand et al., 2011; Yu et al., 2014). However, activity-dependent neurotransmitter release from hiPSC-derived neurons, whose origin were the same schizophrenia samples (Brennand et al., 2011; Yu et al., 2014), showed elevated levels of secreted catecholamines, namely, dopamine, norepinephrine, and epinephrine, when compared with the controls (Hook et al., 2014). In agreement with this, a larger proportion of differentiated neurons were tyrosine hydroxylase-positive. This indicates a pathological regulation of catecholamine neurotransmitters in schizophrenia and also reflects a hyperdopaminergic state, the target of antipsychotics (Howes et al., 2012).

All these initial reports consistently support the reduced neuronal connectivity in schizophrenia hiPSC-derived neurons from neuronal

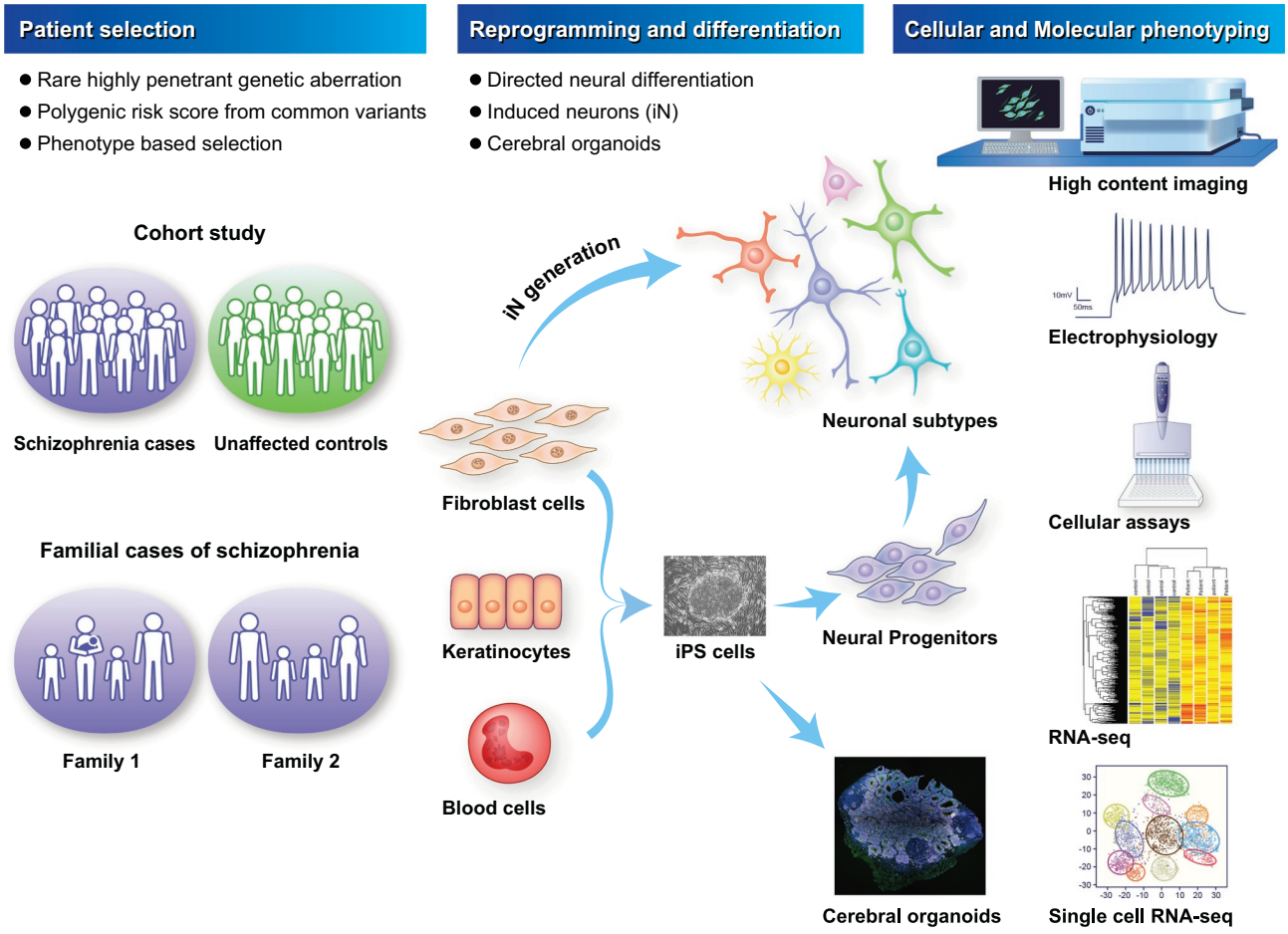

Fig. 1. Schematic representation of strategies for cell-based modeling of schizophrenia using patient-specific hiPSCs.
Appropriate patient selection strategies form the crux of modeling in vitro cellular phenotypes of schizophrenia, and should incorporate: (1) genetically characterized schizophrenia patients, that is, subjects with highly penetrant risk variants (CNVs or mutations) or general schizophrenia cases with high polygenic risk scores based on common genetic variants, and (2) patients selected based on the phenotypes manifested. The study design could be either a cohort based case-control study to increase the statistical power, or a familial sample-based study to minimize confounding genetic heterogeneity. Biopsies are taken from the patient for the desired somatic cells, which are further reprogrammed to obtain hiPSCs by ectopic co-expression of OCT4, SOX2, KLF4, and cMYC. Alternatively, somatic cells can also be transdifferentiated to different neuronal fates, thus generating induced neurons. Isogenic hiPSC lines could also be generated by genome editing techniques, either by correcting the mutation in the patient hiPSCs or introducing the mutation into control hiPSCs. These patient-specific hiPSCs could be further differentiated into the desired neural cell types relevant for the disease pathobiology for phenotypic evaluation. For the hiPSC-derived neurons to acquire functional maturity, appropriate cell culture strategies such as coculture with other neural cell types or 3D brain (cerebral) organoid culture can be performed to achieve the relevant phenotypes. Cellular phenotypes can be evaluated by high content imaging for (1) gauging morphological characteristics of hiPSCs, NPCs, and neurons, (2) assaying the cellular dynamics of hiPSCs across neural fate differentiation, and (3) expression of relevant neural/fate-specific markers. Further electrophysiological measurements could be employed to evaluate the physiological properties of hiPSC-derived neurons and assess the connectivity. High-throughput biochemical assays can be employed to evaluate phenotypes pertaining to synaptic events, metabolic activity, and other cellular processes relevant to the disease pathology. Similarly, molecular phenotypes associated with the pathogenesis of schizophrenia can be determined by population-based and single cell transcriptome sequencing technologies.

tracing experiments and neurotransmitter release. The inability to replicate the reduced connectivity electrophysiologically could be attributed to the relatively immature nature of differentiated neurons or the limitation to form functional neural circuits in two-dimensional (2D) culture. Moreover, the differentiated neurons are constituted of a mixture of excitatory, inhibitory, and dopaminergic neurons, whose proportion in the culture also could hinder the functional connectivity. Although these initial studies could provide crucial evidences pertaining to the cellular and molecular phenotypes in schizophrenia pathogenesis, recapitulating the functional neuronal development in vitro is challenging.

3.2. Synaptic dysfunctions

DISC1 was identified as the major risk gene for psychiatric disorders, found disrupted at the chromosomal breakpoint of a balanced chromosomal translocation, which co-segregated in a large family manifesting schizophrenia, bipolar disorder, and major depression (Thomson et al., 2013). Wen et al. generated hiPSCs from four members of a family with a *DISC1* frameshift mutation who developed psychiatric disorders, including schizophrenia, and further differentiated them into forebrain cortical neurons (Wen et al., 2014). Similar to previous reports, no difference was observed in differentiation propensity. Strikingly, the density of synaptic vesicle protein $(SV2)^{+ve}$ synaptic boutons, activity-induced neurotransmitter release, and frequency of excitatory spontaneous synaptic currents were significantly reduced in *DISC1* mutant neurons compared with the controls. However, these defects were rescued by the isogenic correction of the *DISC1* mutation in hiPSCs (Wen et al., 2014). Additionally, *DISC1* mutants showed dysregulation of genes implicated in psychiatric disorder pathogenesis that are enriched for synaptic transmission, nervous system development, and dendritic spine function pathways. Similarly, another study that modeled *DISC1* mutation in normal hiPSCs also showed altered NPC identity and neuronal fate that could result in abnormal forebrain

specification (Srikanth et al., 2015). These were accompanied by elevated levels of WNT signaling, resulting in increased neural proliferation that was rescued by early inhibition of WNT signaling. However, there were inconsistencies in terms of molecular perturbations between hiPSC derived-neurons from genetically defined schizophrenia and from non-specified cases (Brennand et al., 2011; Wen et al., 2014). These disparities could be attributed to the underlying genetic heterogeneity of the subjects studied. Even the discordance was evident for cellular and molecular phenotypes of neural cells derived from normal hiPSCs edited for *DISC1* mutations and hiPSCs derived from familial samples of *DISC1* mutant schizophrenia patients (Srikanth et al., 2015). This phenotypic discrepancy could be, at least in part, due to the polygenic nature of schizophrenia, where genetic aberrations in addition to *DISC1* are required for recapitulating the disease phenotypes in vitro.

Similarly, higher levels of postsynaptic density protein, the striatal-enriched protein tyrosine phosphatase ($STEP_{61}$) expressed mainly in forebrain neurons, are consistently observed in schizophrenia (Boulanger et al., 1995; Carty et al., 2012). $STEP_{61}$ regulates synaptic function by promoting the internalization of *N*-methyl-D-aspartate (NMDA) receptors through dephosphorylation of glutamate ionotropic receptor NMDA type subunit 2B (GRIN2B), extracellular signal-regulated kinase (ERK) 1/2, and FYN proto-oncogene, Src family tyrosine (Fyn) kinases. Increased levels of $STEP_{61}$ were replicated in hiPSC-derived neurons from two different cohorts of schizophrenia, concomitant with reduced ubiquitination of $STEP_{61}$ (Xu et al., 2018). Furthermore, inhibition of $STEP_{61}$ by genetic or pharmacological manipulation increased the phosphorylation of $STEP_{61}$ targets and the spontaneous neuronal activity in hiPSC-derived neurons. Deficits in synaptic function in schizophrenia were further underscored by dysregulation of genes pertaining to synaptic structure and function, consistently across many other patient derived hiPSC studies.

3.3. Aberrant neurodevelopment

3.3.1. Cytoskeletal abnormalities in 15q11.2 deletion

Considering the genetic heterogeneity of schizophrenia, modeling genetic variants with high penetrance offers the opportunity to characterize the genetic effects on disease-specific cellular abnormalities. Among them, the 15q11.2 deletion copy number variation (CNV) imparts high risk for neuropsychiatric disorders including schizophrenia, with a prevalence ranging from 0.57%–1.27% in patients (Cox and Butler, 2015; Kirov, 2015; Stefansson et al., 2008). This CNV spans four genes tubulin gamma complex associated protein 5 (*TUBGCP5*), cytoplasmic FMR1 interacting protein 1 (*CYFIP1*), NIPA magnesium transporter 1 (*NIPA1*), and NIPA magnesium transporter 2 (*NIPA2*). Patient hiPSC-derived neural progenitors exhibited deficits in adherens junctions and apical polarity, resulting from haploinsufficiency of *CYFIP1*, suggestive of aberrant neuronal maturation (Yoon et al., 2014). CYFIP1 is a subunit of the WAVE regulatory complex (WRC), which plays a crucial role in cortical development by regulating cytoskeletal dynamics during the migration of radial glial cells. The role of CYFIP1 in maintaining dendritic complexity and spine characteristics has also been well described in rodent models (De Rubeis et al., 2013; Pathania et al., 2014). The abnormal phenotype of disrupted adherens junction in the neural rosettes was rescued by forced expression of *CYFIP1*. A subsequent study further showed the reduced CYFIP1 and PSD-95 protein levels and altered dendritic morphology in hiPSC-derived neurons from 15q11.2 deletion subjects (Das et al., 2015). Deficits in CYFIP1 expression thus affect the regulation of neural stem cells function and thereby elicit abnormal corticogenesis. Moreover, in schizophrenia patients, reduced neural stem cell proliferation was reported in postmortem brain samples (Allen et al., 2016a; Reif et al., 2006). Consistent with this, proteomic analysis in schizophrenia postmortem brain samples has also shown reduced levels of CYFIP1, suggesting its role in pathogenesis of schizophrenia by affecting cortical development (Hirayama-Kurogi et al., 2017).

3.3.2. Reduced neural migration in CNTNAP2 deletion

Large heterozygous deletions in the genomic interval spanning the gene for contactin-associated protein-like 2 (*CNTNAP2*), that is involved in axon guidance, dendritic arborization, spine development, and organization of myelinated axons, represent another predisposing factor for neuropsychiatric disorders (Rodenas-Cuadrado et al., 2014). NPCs and neurons derived from hiPSCs of heterozygous deletion carriers with discordant clinical presentation for schizoaffective disorder showed decreased expression of exons mapped in the hemizygous region (Lee et al., 2015). Interestingly, in those affected, there was a compensatory increase in expression of the specific exons of *CNTNAP2* in forebrain neurons. Besides allele-specific expression in *CNTNAP2* deletion carriers, with predominant inclusion of mutant allele, was consistent with the clinical presentation of schizoaffective disorder. NPCs from the affected carrier also showed reduced neural migration, and this phenotype correlated well with *CNTNAP2* expression patterns (Lee et al., 2015). However, in neurogenin 2 (Ngn2)-induced glutamatergic neurons, overall *CNTNAP2* expression was increased in deletion carriers irrespective of clinical presentation. Notably, the deletion was shown to affect expression of genes involved in synaptic transmission, neuronal development, and neuronal activity in Ngn2-induced glutamatergic neurons (Flaherty et al., 2017). This was further corroborated by an increased spontaneous network level activity in both hiPSC-derived forebrain and glutamatergic neurons from the deletion carriers, consistent with the disrupted neuronal synchrony in *Cntnap2*-knockout mice (Peñagarikano et al., 2011).

3.3.3. 22q11.2 deletion and neurodevelopment defects

One of the highest risk factors for developing schizophrenia is 22q11.2 deletion, which is present in 0.3% of patients with schizophrenia, although with varying penetrance (Anne S. Bassett et al., 2017; Balan et al., 2014; Kirov, 2015). This deletion has been widely modeled using hiPSCs. Since DiGeorge Syndrome Critical Region Gene 8 (DGCR8), which is involved in micro-RNA (miRNA) biogenesis, is located in the 22q11.2 deletion region, disturbances in miRNA-mediated regulation of neurodevelopmental process were more evident. Pedrosa et al. were the first to establish hiPSCs from 22q11.2 deletion subjects and showed that there was a significant delay in the fading out of endogenous pluripotency markers such as OCT4 and NANOG during glutamatergic neuronal differentiation (Pedrosa et al., 2011). Notably, the firing properties of specific neuronal lineage and the maturation stage were not different between cases and controls. However, the dynamics of gene expression relevant for GABAergic, glutamatergic, and dopaminergic specification were dysregulated in iPSC-derived neurons when they acquired electrical properties (Belinsky et al., 2014).

The contribution of neurodevelopmental defects to schizophrenia manifestation was further emphasized in 22q11.2 deletion hiPSCs, where defects in neurosphere size, neural differentiation efficiency, neurite outgrowth, cellular migration, and the neurogenic-to-gliogenic competence ratio was observed compared with the controls (Toyoshima et al., 2016). Reduced expression of miR-17/92 cluster and miR-106a/b that negatively regulate p38α (MAPK14; Mitogen-Activated Protein Kinase 14) was observed in 22q11.2-derived neurospheres, resulting in elevated gliogenic differentiation propensity. This was further substantiated by an increased expression of the gliogenic marker Glial Fibrillary Acidic Protein (GFAP) and reduced Microtubule Associated Protein 2 (MAP2) expression in postmortem brains from schizophrenia. The dampened neurogenic-to-gliogenic cellular competence was rescued by p38 inhibitors (Toyoshima et al., 2016).

Another study showed increased expression of miRNAs such as miR-34, miR-4449, miR-146b-3p, and miR-23a-5p in hiPSC-derived glutamatergic neurons from 22q11.2 deletion, which was in parallel with the expression in peripheral blood samples and postmortem brain samples

of patients with schizophrenia and autism (Zhao et al., 2015). From the predicted targets of the dysregulated miRNA, genes involved in neurotransmitter function, synaptogenesis, and neuronal differentiation were likely to be affected. However, experimentally, neurons from early differentiation stages showed differential expression of genes enriched for apoptosis, MAPK signaling, and cell cycle and survival pathways (Lin et al., 2016). Overexpression of genes for MAPK signaling-related events and p38 activation was in agreement with an earlier report of hiPSC-derived neurons from 22q11.2 deletion (Toyoshima et al., 2016). Co-expression analysis of differentially expressed genes along with the developing brain transcriptome converged onto gene networks involving Cell Division Cycle 45 (CDC45)-mediated cell cycle pathway involved in embryonic brain development, and the Proline Dehydrogenase (PRODH)-mediated pathway, contributing to adolescent brain functions (Lin et al., 2016). Interestingly, ERK/Fibroblast Growth Factor (FGF) signaling, which is known to be dysregulated in other hiPSC models of schizophrenia (Narla et al., 2017; Xu et al., 2018), was one among the top co-expressed differentially expressed genes.

3.3.4. Neurodevelopmental defects mediated by miRNA

Aberrant neural migration is consistently observed in schizophrenia animal models (Muraki and Tanigaki, 2015) and is recapitulated in schizophrenia hiPSCs (Brennand et al., 2015; Lee et al., 2015; Murai et al., 2016; Stachowiak et al., 2017; Topol et al., 2015a; Toyoshima et al., 2016; Yoon et al., 2014). Han et al. demonstrated the role of miR-19, which is involved in the neural migration (Han et al., 2016). Similar to the findings in postmortem brain samples of schizophrenia, miR-19 was found to be overexpressed in schizophrenia patient-derived NPCs, and was involved in the migration of newborn neurons in the adult brain (Beveridge et al., 2009; Han et al., 2016). miR-19 is highly expressed in NPCs and is downregulated during neural differentiation in the adult hippocampus. It targets Rap guanine nucleotide exchange factor 2 (RAPGEF2) and regulates cellular migration and modulates the positioning of newborn neurons in the granule cell layer of the DG (Han et al., 2016). Increased expression of miR-19 resulted in a reduction of *RAPGEF2*, thereby regulating Rap1 signaling that is involved in neuronal migration phenotypes (Ye et al., 2014). Thus, miR-19 forms a major hub for abnormal cellular phenotypes manifested in schizophrenia.

Another brain-specific miRNA dysregulated in schizophrenia is miR-219, which inhibits neural stem cell proliferation and promotes neural differentiation (Beveridge et al., 2009; Murai et al., 2016; Santarelli et al., 2011). Neural stem cell proliferation and self-renewal is maintained by expression of T Cell Leukemia Homeobox (TLX) and its downstream effector Platelet Derived Growth Factor Receptor Alpha (PDGFRα), which is further targeted by miR-219. Thus, PDGFRα expression is regulated by TLX through miR-219, forming a regulatory cascade. Similar to the results observed in postmortem brain tissues from schizophrenia, increased miR-219 expression was observed in neural stem cells derived from schizophrenia hiPSCs with a *DISC1* frameshift mutation. This results in abnormal neural stem cell proliferation in schizophrenia, limiting the neural stem cell pool. However, the proliferative defect could be rescued by the treatment of miR-219 inhibitor. Therefore, the TLX–miR-219 cascade forms a critical pathway in regulating cell proliferation in schizophrenia subjects with *DISC1*-mutations (Murai et al., 2016).

Upstream of the TLX–miR-219 cascade, another miRNA, miR-9, that negatively regulates neural stem cell proliferation by suppressing TLX expression, was found to be downregulated in NPCs, but not in neurons derived from schizophrenia hiPSCs (Topol et al., 2016; Zhao et al., 2009). Transcriptome analyses have shown that the targets of miR-9 are enriched in differentially expressed genes in schizophrenia NPCs. Notably, overexpression of miR-9 was sufficient to rescue aberrant radial migration exclusively in schizophrenia NPCs (Topol et al., 2016). Thus, the observed cellular phenotype is largely mediated by the network of genes regulated by miR-9.

3.3.5. Environmental insults in neurodevelopment defects

Prenatal environmental insults represent an important risk factor for schizophrenia by affecting neurodevelopmental pathways (Owen et al., 2016; Weinberger, 2017). Among them maternal immune activation (MIA) resulting from bacterial, viral and other parasitic infections during gestation is known to be a prominent risk factor for schizophrenia and other neurodevelopmental disorders in offspring (Estes and McAllister, 2016). However, comprehensive studies that effectively modeled MIA to evaluate cellular and molecular deficits relevant for schizophrenia using hiPSCs-derived neurons are limited (Lin et al., 2014; Zuiki et al., 2017). Nevertheless, the effect of environmental insults has been retrospectively evaluated in hiPSCs derived from schizophrenia patients. It has been suggested that environmental factors could influence long interspersed nucleotide element-1 (LINE-1) retrotransposition, which is thought to be involved in somatic mosaicism in neurons (Singer et al., 2010). This, in turn, has implications in generating neuronal diversity and also poses risks for neurological disorders (Lee, 2016). Bundo et al. showed an elevated copy number of LINE-1 retrotransposons in hiPSC-derived neurons from 22q11.2 deletion patients with schizophrenia and in an animal model of maternal immune activation (Bundo et al., 2014). They also showed that increased LINE-1 retrotransposition events in postmortem brain samples from patients with schizophrenia that were preferentially observed in the regions harboring genes involved in synaptic functions (Bundo et al., 2014).

Induction of subthreshold environmental stressors (alcohol, methylmercury) in hiPSCs derived from schizophrenia patients showed differential sensitivity among NPCs, evidenced by the cell-to-cell variation of heat shock factor 1 (HSF1) activation level (Hashimoto-Torii et al., 2014). Expression of HSF1 plays a crucial role as a response to environmental challenges in brain cells. However, this study lacked functional extrapolation with respect to synaptic or cellular phenotypes. Likewise, herpes simplex virus type 1 (HSV-1), that causes latent infection in neurons with periodic reactivation, can lead to encephalitis accompanied by cognitive impairments. Schizophrenia patients who experienced HSV-1 infection showed reduced prefrontal cortical gray matter volume, and impaired working memory and executive functions (Prasad et al., 2011; Prasad et al., 2006). Characteristic features of latent HSV-1 infection were modeled in hiPSC-derived neurons from a schizophrenia patient, where altered neural transcriptomes that were enriched for glutamate receptor signaling, mitochondrial dysfunction, and axonal guidance signaling, were observed (D'Aiuto et al., 2015). Furthermore, NPCs were seen more susceptible to infection than the differentiated neurons. This might contribute to deficits in proliferation and differentiation, which are plausible causal factors for cognitive impairments resulting from HSV-1 infection as evidenced from functional magnetic resonance imaging (fMRI) studies (D'Aiuto et al., 2015). Although, modeling environmental insults in hiPSCs yielded reasonable hints for neurodevelopmental defects pertaining to schizophrenia, recapitulating in vivo conditions remains challenging. Future studies employing brain organoid models might aid in circumventing this shortcomings to an extent.

3.4. Mitochondrial dysfunction

Since neural activity is highly energy demanding, impairments in mitochondrial oxidative phosphorylation result in abnormal neural functions associated with an increased risk of developing schizophrenia (Flippo and Strack, 2017). Paulsen et al. observed a two-fold increase in extramitochondrial oxygen consumption and elevated levels of reactive oxygen species (ROS) in NPCs differentiated from hiPSCs established from a clozapine resistant schizophrenia patient (Paulsen Bda et al., 2012). This was more pronounced in NPCs than in iPSCs or fibroblasts, and was rescued by treatment with valproic acid, suggestive of mitochondrial deficits associated with aberrant neurogenesis (Paulsen Bda et al., 2014).

Another group used hair follicle keratinocytes to establish iPSCs from patients with schizophrenia, and further differentiated the cells into dopaminergic and glutamatergic neuronal cells (Robicsek et al., 2013). Although there were no differences in the reprogramming ability between cases and controls, an impaired ability to differentiate into dopaminergic neurons as well as maturational defects in glutamatergic neurons were observed, which were resolved into pervasive mitochondrial dysfunction in schizophrenia-derived cells. A diminished turnover of dopamine and glutamate in patient-derived cells was also observed, possibly stemming from mitochondrial dysfunction (Robicsek et al., 2013). Moreover, dopaminergic precursor cells showed an increased cell area in schizophrenia cases as well as smaller and fewer neurites in dopaminergic neurons, indicating defects in the neurodevelopmental process. Most importantly, dissipation of the mitochondrial membrane potential, together with uneven mitochondrial cellular distribution, was observed through the differentiation into dopaminergic neurons and in glutamatergic cells derived from schizophrenia patients. However, the impaired mitochondrial network connectivity was specific to dopaminergic neurons. This hiPSC model also showed synaptic defects, where the patient-derived glutamatergic cells displayed significantly lower expression of synapsin1 and PSD-95, unlike in previous reports (Brennand et al., 2011). Mitochondrial damage and increased oxidative stress were consistently shown in schizophrenia hiPSC models by other groups (Brennand et al., 2015; D'Aiuto et al., 2015; Lin et al., 2016). Taken together, hiPSC models could be used to represent the intricate relationships between neural differentiation and mitochondrial function with respect to aberrant neurodevelopment, as well as the vulnerability of specific neural cells to oxidative stress in schizophrenia.

3.5. Molecular signatures relevant to cellular phenotypes

Advanced technologies in DNA/RNA sequencing at both population and single-cell levels offer the opportunity to characterize the molecular phenotypes of schizophrenia hiPSC-derived cells relevant to the pathogenesis of the disease. High-throughput transcriptomic phenotyping of hiPSC-derived cells enables the characterization of even subtle molecular perturbations, and allows their comparison with those of postmortem brain samples, which could be complemented by proteomics technologies. Interestingly, many of the identified dysregulated genes overlap with the risk genes identified in large-scale genetic studies.

Neurons differentiated in vitro from hiPSCs are immature compared with those in the human brain. For example, gene expression comparisons of hiPSC-derived neurons to the Allen BrainSpan Atlas indicate that they most resemble first trimester fetal brain tissue, i.e. 8–16 weeks post conception for hiPSC-derived NPCs and 8–24 weeks post conception for hiPSC-derived 6-week-old neurons (Brennand et al., 2015). Thus hiPSC-based models may be better suited for the study of disease predisposition rather than modeling the late features of schizophrenia. In addition, a significant fraction of the gene signature of schizophrenia hiPSC-derived neurons is conserved in schizophrenia hiPSC-derived NPCs (Brennand et al., 2015). Interestingly, altered WNT signaling is a consistent molecular phenotype evident from transcriptomic data of NPCs and neurons derived from schizophrenia hiPSCs (Brennand et al., 2011; Freyberg et al., 2009; Topol et al., 2015b). This pathway has roles in neural patterning, proliferation and migration, and synapse formation. Functional assays delineated increased canonical WNT signaling along with increased β-catenin protein levels compared with control NPCs, although there were sample-to-sample variations (Topol et al., 2015b). Furthermore, combined transcriptomic and proteomic analyses using schizophrenia hiPSC-derived NPCs revealed dysregulated cytoskeletal remodeling and oxidative stress-related functions (Brennand et al., 2015). It is known that oxidative stress negatively regulates WNT signaling (Zhang et al., 2012); therefore, the variations in the activation of canonical WNT signaling among the samples could be attributed to the differential oxidative stress in NPCs. Increased levels of canonical WNT signaling in NPCs were also observed in cells where *DISC1* mutations were introduced via genome-editing (Srikanth et al., 2015).

Increased expression of protein translational machinery (ribosomal proteins and translation initiation factors) and a resultant increase in global protein synthesis and protein levels were observed exclusively in schizophrenia hiPSC-derived NPCs, but not in hiPSCs or in differentiated neurons (Topol et al., 2015a). Co-expression analysis of the perturbed proteins showed modules enriched for ribosome/RNA binding, nucleosome, chromatin, and nucleocytoplasmic transport, along with schizophrenia-associated proteins. Although treatment with rapamycin reduced global protein synthesis, it could not rescue the reduced radial migration observed in NPCs (Brennand et al., 2015). However, loxapine treatment, which was effective in improving neural connectivity, aggravated the reduced migration in NPCs.

Transcriptome analysis of schizophrenia NPC-derived neuron committed cells (NCCs) revealed a preneuronal genomic etiology of schizophrenia, where unrelated schizophrenia cases shared a common signature of dysregulated genes and coordinated gene networks (Narla et al., 2017). The nuclear (n) form of Fibroblast Growth Factor Receptor 1 (FGFR1), a pan-ontogenic gene regulator, that integrates pathways involved in schizophrenia pathogenesis, was overexpressed in schizophrenia NCCs and it over-targeted genes dysregulated in schizophrenia. Thus nFGFR1 was suggested to be the most common dysregulated upstream signaling pathway responsible for the perturbation of gene expression observed in schizophrenia NCCs. Notably, the upregulated genes were enriched for differentiation, maturation of neurons, and axonal guidance, whereas downregulated genes were involved in glial differentiation and myelination. However, this may contradict observations from 22q11.2 deletion hiPSCs that showed an obvious increased gliogenic competency (Toyoshima et al., 2016), possibly due to sample heterogeneity. In the same sample set, changes in activity-induced gene expression were attenuated in neurons from schizophrenia hiPSCs compared with control samples (Roussos et al., 2016).

Furthermore, differential methylation of genes involved in the chromatin remodeling complex was observed during differentiation of hiPSCs to NPCs from a schizophrenia patient previously reported to have increased mitochondrial oxygen consumption and elevated ROS levels (Maschietto et al., 2015; Paulsen Bda et al., 2012). Also, treatment by histone deacetylase inhibitor (valproic acid) rescued oxidative stress in schizophrenia NPCs to control levels (Maschietto et al., 2015). Thus, the role of an epigenetic component in oxidative stress pertaining to schizophrenia pathogenesis may be important in the early neuronal stage. The resultant transcriptomic changes during neuronal differentiation that are specifically disrupted in schizophrenia showed enrichment for metabolic processes, signal transduction, nervous system development, regulation of neurogenesis, and neuronal differentiation. However, these results should be interpreted cautiously as the results stemmed from a single sample that could be confounded by patient-specific features and epigenetic reshuffling during reprogramming.

Even though many typical and atypical antipsychotics are used for routine pharmacological management of schizophrenia, approximately 30% of patients continue to be unresponsive to two or more adequate antipsychotic trials (Meltzer, 1997). While clozapine is efficacious for treating drug-resistant schizophrenia, some patients remain resistant, and severe side effects such as agranulocytosis hinder its routine use. Several hiPSC lines have been established from schizophrenia patients with differential clozapine responses (Marcatili et al., 2016; Marsoner et al., 2016; Nakazawa et al., 2017). Nakazawa et al. established hiPSCs from immortalized B cells from monozygotic twin cases with treatment-resistant schizophrenia and discordant for clozapine response. Transcriptome analysis in hiPSC-derived Ngn-2-induced glutamatergic neurons showed differential expression of several genes encoding homophilic cell adhesion molecules including protocadherins that are thought to be responsible for the integrity of synapses and dendrites. These transcripts also showed specificity with respect to drug response

profiles (Nakazawa et al., 2017).

3.6. Evidence from brain organoid models in schizophrenia

Spontaneous self-organization of neural cells in a 3D microenvironment to generate brain organoids has been demonstrated to model the neural circuits and regional connectivity, which are known to be affected in schizophrenia patients (Bagley et al., 2017; Birey et al., 2017; Lancaster et al., 2017; Lancaster et al., 2013; Paşca et al., 2015). Brain organoids are capable of generating diverse neural cell types and resemble gene expression program in early brain development (Camp et al., 2015; Luo et al., 2016; Quadrato et al., 2017). Furthermore organoid culture for prolonged time period resulted in mature cellular phenotypes and establishment of interconnected functional neural networks across the organoid (Paşca et al., 2015; Quadrato et al., 2017; Sloan et al., 2017). Since the brain organoids can recapitulate in vivo neurodevelopment more accurately than conventional directed neuronal differentiation paradigms, they have an immense potential to model the inherent early neurodevelopmental deficits in schizophrenia hiPSCs.

Recently, Stachowiak et al. modeled schizophrenia hiPSCs using brain (cerebral) organoids and found deficits in cellular compostion, although there were no gross morphological differences (Stachowiak et al., 2017). Abnormal scattering of NPCs from the ventricular zone was evident into the intermediate and cortical zones of schizophrenia cerebral organoids. This was also accompanied by restricted neuronal growth, resulting in truncated cortical development and decreased intracortical connectivity. Consistent with earlier reports in schizophrenia hiPSCs, nFGFR1 signaling was dysregulated in schizophrenia hiPSC-derived cerebral organoids, contributing to abnormalities in the fine cortical architecture (Narla et al., 2017; Stachowiak et al., 2017). However this initial study was limited by functional connectivity assays. Future studies should benefit from the recent advancements in 3D brain organoid cultures, which could test progenitor zone organization, proliferation, neural migration, establishment of synaptic interconnectivity, and cortical structural organization.

4. Challenges in modeling cellular phenotypes in vitro: limitations and prospects

Advances in hiPSC technology have provided unprecedented opportunities to model cellular phenotypes relevant to the pathogenesis of schizophrenia with the same genetic architecture of a patient. This makes hiPSCs a unique model that enables the mechanistic understanding of the disease directly in the context of a human system in a high-throughput manner (Dolmetsch and Geschwind, 2011). Moreover, it also complements and bridges studies utilizing patient and animal models. In spite of these advantages, modeling of schizophrenia using hiPSCs is not without limitations, mainly due to the complexity of the disease itself. Schizophrenia is a behavioral and cognitive disorder and is characterized by a constellation of symptoms with multiple etiologies and prognosis, making it highly heterogeneous (Owen et al., 2016). This heterogeneity and a limited understanding of the complexities in schizophrenia etiopathogenesis hinder the in vitro modeling of the entire phenotypic spectrum.

Ideally, recapitulating disease-associated cellular phenotypes in vitro should reflect the biological causality and not be confounded by the effects of reprograming, differentiation, or cell culture-induced changes. However, the reprogramming of patient somatic cells resets the epigenetic status to achieve pluripotency and also establishes new epigenetic marks depending on the lineage (Coskun et al., 2012; Hewitt and Garlick, 2013) and could dilute the epigenetic contribution to the risk of psychosis when modeled in vitro. Considering the risk imparted by multiple common variants of small effects in schizophrenia, random viral integrations across the genome upon reprogramming might confound the cellular phenotype. Furthermore, acquisition of genetic aberrations is also quite common in hiPSCs and may result in pseudophenotypes or amplify the cellular phenotypes associated with the disease (Liang and Zhang, 2013). Therefore, strict quality control measures using whole-genome sequencing are essential to confirm that risk loci are spared from viral integration and mutations during reprogramming. Recent studies in iPSCs have revealed a preferential occurrence of mutations in structurally condensed lamina-associated heterochromatic domains, suggesting a bias in mutation hotspots favored by epigenetic marks (Yoshihara et al., 2017). Alternatively, reprogramming methodologies employing integration-free approaches (Schlaeger et al., 2014) can circumvent these shortcomings and could be effectively scaled up for high throughput derivation of iPSCs (Paull et al., 2015). While lineage-specific transdifferentiation of somatic cells to neurons could evade the epigenetic resetting, the propensity of acquiring mutations during the process is not known (Vierbuchen et al., 2010). However, the differences between the disease predisposing epigenetic profiles of neural cells and starting somatic cells make it less favorable to accommodate epigenetic components in the transdifferentiation model. Direct neuronal reprograming from fibroblasts has been demonstrated in childhood-onset schizophrenia patients harboring CNVs, which was further modeled to evaluate in vitro Toxoplasma infection (Passeri et al., 2016; Passeri et al., 2015). Furthermore, the effect of *MIR137* on synaptic phenotypes has also been tested on induced-neurons harboring gain of function genetic variants (Siegert et al., 2015). But, the inability to model protracted neurodevelopment in this approach limits its utility in modeling diseases like schizophrenia.

To date, the majority of hiPSC studies in schizophrenia have favored fibroblasts as a source of starting somatic cells for reprogramming, but keratinocytes and blood cells have also been used by some groups (Table 1). Notably, altered dopaminergic differentiation efficiency was observed in hiPSCs derived from fibroblasts and keratinocytes (Hook et al., 2014; Robicsek et al., 2013). It is known that the epigenetic memory of the original lineage of somatic cells could also contribute to the variability in cellular phenotypes in terms of incomplete reprogramming or the tendency to differentiate toward their parental lineage (Kim et al., 2011; Polo et al., 2010). However, it is uncertain whether these disparities were due to the source of the cells, donor genotype, or the patient-specific disease phenotype. Taken together, all these factors can contribute significantly to clonal variations derived from the same hiPSCs (Brennand et al., 2012). Interestingly, recent reports also suggest donor-specific genetic variations mainly contribute to the iPSC variability, rather than the source of cells, as evidenced from transcriptomic and epigenetic profiles of late-passage iPSCs (Kyttälä et al., 2016).

Intraclonal variations could also arise during differentiation, where the inherent genetic/epigenetic constitution could alter the differentiation propensities, leading to disparities in neural cell type compositions (Hoffman et al., 2017). Unfortunately, none of the available neuronal differentiation strategies are perfect enough to yield uniform neuronal cell types. Accordingly, lineage-specific differentiation of schizophrenia hiPSCs has shown a heterogeneous population of neurons, where neuronal subtype composition also significantly differed among the studies. The majority of hiPSC studies in schizophrenia queried neuronal phenotypes, which are predominantly glutamatergic. Other neuronal subtypes relevant for schizophrenia such as GABAergic interneuron subtypes are less investigated, owing to their lower yield. This is highly important because pathological cellular phenotypes can be captured in relevant neural cells that could otherwise result in pseudophenotypes. For example, differences in dopaminergic neuronal differentiation efficiencies between control and schizophrenia-derived hiPSCs (Hook et al., 2014) were not replicated in the same samples, suggesting an incompetency of the neuronal differentiation protocol used in previous reports (Hartley et al., 2015). Hartley et al. emphasized that understanding schizophrenia-related pathological changes in dopaminergic neurons requires precise fate specification toward a

Table 1
Summary of human induced pluripotent stem cell (hiPSC)-based studies of schizophrenia.

No	Author	Patients	Controls	Genetic aberration	Source of somatic cells	Cell types investigated	Phenotypes relevant for schizophrenia
1	Chiang et al., 2011	Two affected siblings; 1 male chronic undifferentiated schizophrenia and 1 female chronic paranoid schizophrenia	One control	*DISC1* mutation	Fibroblasts	–	First report on hiPSC lines derived from schizophrenia patients
2	Brennand et al., 2011	Four subjects; 1 (male childhood onset schizophrenia), 2 affected siblings (1 male schizophrenia and 1 female schizoaffective disorder) and 1 male schizophrenia patient	Seven controls	Uncharacterized	Fibroblasts	Mixed neuronal cells; predominantly glutamatergic neurons	Significant reduction in neuronal connectivity Reduced neurite outgrowth and decreased number of neurites Decreased PSD95 expression Glutamate, cAMP and WNT signaling pathways were affected Loxapine treatment attenuated neuronal connectivity and restored dysregulated gene expression
3	Pedrosa et al., 2011	Three subjects; one 22q11.2 deletion patient with schizophrenia and two patients from Brennand et al. (2011) (one childhood onset schizophrenia, and one adult schizophrenia)	Two controls	One subject with 22q11.2 deletion and the rest uncharacterized	Fibroblasts	Glutamatergic neurons	First report on hiPSC lines derived from 22q11.2 deletion subjects with schizophrenia Delayed fading out of endogenous pluripotency markers such as OCT4 and NANOG during glutamergic neuronal differentiation
4	Paulsen Bda et al., 2012	One clozapine resistant schizophrenia patient	One control	Uncharacterized	Fibroblasts	hiPSC-derived NPCs	Two-fold increase in extra-mitochondrial oxygen consumption and elevated level of reactive oxygen species (ROS) in hiPSC-derived NPCs, which was rescued by the treatment with valproic acid
5	Robicsek et al., 2013	Three subjects with paranoid schizophrenia	Two controls	Uncharacterized	Hair follicle keratinocytes	Dopaminergic neurons and glutamatergic neurons	Impairment in differentiation ability, to dopaminergic neurons and maturational defects in glutamatergic neurons Smaller and lesser number of neurites in dopaminergic neurons Dissipation of mitochondrial membrane potential and impaired mitochondrial network connectivity, specifically in dopaminergic neurons
6	Belinsky et al., 2014	One 22q11.2 deletion patient (same patient from Pedrosa et al., 2011)	One control	22q11.2 deletion	Fibroblasts	Young post-mitotic neurons	Gene expression relevant for GABAergic, glutamatergic and dopaminergic specification were dysregulated in the schizophrenia hiPSC-derived neurons when they acquired electrical properties

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Table 1 (*continued*)

No	Author	Patients	Controls	Genetic aberration	Source of somatic cells	Cell types investigated	Phenotypes relevant for schizophrenia
7	Bundo et al., 2014	Two 22q11.2 deletion patients with schizophrenia	Two controls	22q11.2 deletion	Fibroblasts	Mixed neural cell types (neuronal and glial cells)	Elevated copy number of LINE-1 retrotransposons in the hiPSC-derived neurons from 22q11.2 deletion patients with schizophrenia
8	Hashimoto-Torii et al., 2014	Patients from Brennand et al. (2011)	Five controls from Brennand et al., (2011)	Uncharacterized	Fibroblasts	hiPSC-derived NPCs	Differential sensitivity to the induction of subthreshold environmental stressors in hiPSC-derived NPCs from schizophrenia, when compared to the controls, as evidenced by the variation of HSF1 activation level
9	Hook et al., 2014	Three subjects; one childhood onset schizophrenia and two schizophrenia patients from Brennand et al. (2011)	Three controls	Uncharacterized	Fibroblasts	Mixed neuronal cells; mainly dopaminergic neurons	Activity dependent neurotransmitter release from the hiPSC-derived neurons showed elevated levels of catecholamines Larger proportion of differentiated neurons from schizophrenia-derived hiPSCs were tyrosine hydroxylase positive dopaminergic neurons
10	Paulsen Bda et al., 2014	Patients from Paulsen Bda et al. (2012)	Controls from Paulsen Bda et al. (2012)	Uncharacterized	Fibroblasts	hiPSC-derived NPCs	Elevated levels of trace elements; potassium and zinc, resultant of ROS generation in hiPSC-derived NPCs, which was bought down by valproic acid treatment
11	Wen et al., 2014	Quad family; Father (Major depression) and daughter (Schizophrenia) with *DISC1*mutation	Mother and sibling from the same quad family with no DISC1 mutations, and one control out of the family	*DISC1* 4 bp deletion	Fibroblasts	Forebrain neurons constituting mixed neuronal cell types; predominantly glutamatergic	Significant reduction in the density of SV2 + ve synaptic boutons, activity-induced neurotransmitter release, and frequency of excitatory spontaneous synaptic currents in *DISC1* mutant neurons Correction of *DISC*1 mutation in hiPSCs rescued these defects Dysregulation of genes involved in synaptic transmission, nervous system development, and dendritic spine function pathways
12	Yoon et al., 2014	Three schizophrenia patients with 15q11.2 deletion	Five controls from family members	15q11.2 deletion	Fibroblasts	hiPSC-derived NPCs	Patient hiPSC-derived NPCs exhibited deficits in adherens junctions and apical polarity, resulting from haploinsufficiency of *CYFIP1* Forced expression of *CYFIP1* rescued the deficits

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Table 1 (*continued*)

No	Author	Patients	Controls	Genetic aberration	Source of somatic cells	Cell types investigated	Phenotypes relevant for schizophrenia
13	Yu et al., 2014	Patients from Brennand et al. (2011)	Four controls from Brennand et al. (2011)	Uncharacterized	Fibroblasts	Hippocampal dentate gyrus granule neurons	Defective dentate gyrus granule neurons generation from schizophrenia hiPSC-derived NPCs Dysregulated expression of genes involved in dentate gyrus granule neuron differentiation, proliferation, and survival Reduced spontaneous neurotransmitter release in schizophrenia hiPSC-derived dentate gyrus granule neurons
14	Brennand et al., 2015	Patients from Brennand et al. (2011)	Six controls from Brennand et al. (2011)	Uncharacterized	Fibroblasts	hiPSC-derived forebrain NPCs	Transcriptomic and proteomic analyses of schizophrenia hiPSC-derived NPCs revealed dysregulated cytoskeletal remodeling and oxidative stress-related functions Gene expression analysis of hiPSC-derived NPCs and neurons correlated with fetal brain tissue
15	D'Aiuto et al., 2015	One schizophrenia patient	One control	Uncharacterized	Fibroblasts	hiPSC-derived NPCs and glutamatergic neurons	Altered neural transcriptomes enriched for glutamate receptor signaling, mitochondrial dysfunction and axonal guidance signaling were observed for HSV-1 infection in schizophrenia hiPSC-derived neurons NPCs were more susceptible to infection than the differentiated neurons
16	Das et al., 2015	A subject with schizoaffective disorder and the mother, both harboring 15q11.2 deletion	One control from D'Aiuto et al. (2015)	15q11.2 deletion	Fibroblasts	hiPSC-derived neurons	Altered dendritic morphology and reduced levels of CYFIP1 and PSD-95 protein in hiPSC-derived neurons
17	Hartley et al., 2015	Patients from Brennand et al. (2011)	Three controls from Brennand et al. (2011)	Uncharacterized	Fibroblasts	Dopaminergic neurons	Reported no differences in dopaminergic neuronal differentiation efficiencies between control and schizophrenia-derived hiPSCs
18	Lee et al., 2015	A female subject with schizoaffective disorder and her unaffected father, both harboring heterozygous deletion of *CNTNAP2*, and unaffected mother without deletion	Five unrelated controls	*CNTNAP2* deletion	Fibroblasts	hiPSC-derived forebrain NPCs, Ngn2-induced excitatory neurons, oligodendrocyte precursor cells (OPC),and mixed neural cells differentiated from OPCs comprising of mature oligodendrocytes, astrocytes, and neurons	Reduced neural migration observed in hiPSC-derived forebrain NPCs from schizoaffective disorder subject harboring *CNTNAP2* deletion and this phenotype correlated with the exon and allele specific expression patterns of *CNTNAP2* in hiPSC-derived NPCs, neurons and OPCs

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No	Author	Patients	Controls	Genetic aberration	Source of somatic cells	Cell types investigated	Phenotypes relevant for schizophrenia
19	Maschietto et al., 2015	One treatment resistant schizophrenia patient under clozapine treatment	One unrelated control	Uncharacterized	Fibroblasts	hiPSC-derived NPCs	Differential methylation of the genes involved in the chromatin remodeling complex during differentiation of schizophrenia derived-hiPSCs to NPCs Valproic acid treatment rescued the oxidative stress in schizophrenia NPCs to control levels Dysregulated genes in schizophrenia-derived NPCs enriched for metabolic processes, signal transduction, nervous system development, regulation of neurogenesis and neuronal differentiation
20	Topol et al., 2015 a	Patients from Brennand et al. (2011)	Six controls from Brennand et al. (2011)	Uncharacterized	Fibroblasts	hiPSC-derived forebrain NPCs and neurons	Increased expression of protein translational machinery and resultant increase in global protein synthesis and protein levels were observed exclusively in schizophrenia hiPSC-derived NPCs Rapamycin treatment reduced global protein synthesis, but could not rescue the reduced radial migration observed in the NPCs
21	Topol et al., 2015 b	Patients from Brennand et al. (2011)	Six controls from Brennand et al. (2011)	Uncharacterized	Fibroblasts	hiPSC-derived forebrain NPCs and neurons	Increased canonical WNT signaling along with increased β-catenin protein levels in hiPSC-derived forebrain NPCs No difference in neuronal differentiation propensity between schizophrenia-derived hiPSCs and controls
22	Zhao et al., 2015	Six subjects; four 22q11.2 deletion patients (3 with schizoaffective disorder and one schizophrenia) and two childhood onset schizophrenia patients	Six controls	22q11.2 deletion, childhood onset schizophrenia patients were uncharacterized	Fibroblasts	Mixed population of glutamatergic and GABAergic neurons	Overexpression of miRNAs; miR-34, miR-4449, miR-146b-3p, and miR-23a-5p in hiPSC-derived glutamatergic neurons from 22q11.2 deletion patients with schizophrenia
23	Han et al., 2016	Patients from Brennand et al. (2011)	Four controls from Brennand et al. (2011)	Uncharacterized	Fibroblasts	hiPSC-derived hippocampal NPCs	Elevated expression of miR-19 in schizophrenia patient-derived NPCs, which targets Rap guanine nucleotide exchange factor 2 (RAPGEF2) and regulates cellular migration
24	Lin et al., 2016	Patients from Zhao et al. (2015) and additional two childhood onset schizophrenia patients	Seven controls (6 from Zhao et al., 2015)	22q11.2 deletion, Childhood onset schizophrenia patients were genetically uncharacterized, except for one case of 22q11.2 deletion	Fibroblasts	Mixed population of glutamatergic and GABAergic neurons	Differential expressed genes in the neurons from early differentiation stages were enriched for apoptosis, MAPK signaling, and cell cycle and survival pathways

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No	Author	Patients	Controls	Genetic aberration	Source of somatic cells	Cell types investigated	Phenotypes relevant for schizophrenia
25	Marcatili et al., 2016[a]	One treatment resistant schizophrenia patient responsive for clozapine treatment	One control	Uncharacterized	Peripheral Blood Mononuclear Cells	–	No phenotype reported
26	Marsoner et al., 2016[a]	One clozapine resistant schizophrenia patient	–	Uncharacterized	Peripheral Blood Mononuclear Cells	–	No phenotype reported
27	Murai et al., 2016	Patients from Wen et al. (2014)	Controls from Wen et al. (2014)	*DISC1* mutation	Fibroblasts	hiPSC-derived NPCs	Abnormal neural stem cell proliferation resulting from increased expression of miR-219 in schizophrenia hiPSC-derived NPCs with *DISC1* frameshift mutation, which regulates *TLX* expression Treatment of miR-219 inhibitor rescues proliferative defect in schizophrenia hiPSC-derived NPCs
28	Nakazawa et al., 2017	Monozygotic twins with treatment resistant schizophrenia; Clozapine resistant	Clozapine responsive	Uncharacterized	Lymphoblastoid B-cell line	Ngn2-induced excitatory neurons	Differential expression of several genes encoding homophilic cell adhesion molecules including protocadherins that are responsible for the integrity of synapses and dendrites Expression of transcripts correlating with drug response profiles
29	Roussos et al., 2016	Patients from Brennand et al. (2011)	Four controls from Brennand et al. (2011)	Uncharacterized	Fibroblasts	Forebrain NPC-derived mixed population of glutamatergic and GABAergic neurons	Activity induced gene expression changes were attenuated in neurons from schizophrenia-derived hiPSCs, compared to control samples
30	Topol et al., 2016	Patients from Brennand et al. (2011) and 10 childhood onset schizophrenia patients	Six controls from Brennand et al. (2011) and 10 additional controls	Six subjects with childhood onset schizophrenia harbored CNVs in 22q11.2, 1p33, 16p11.2, 3p25.3, and 2p16.3. The remaining subjects were genetically uncharacterized	Fibroblasts	Forebrain NPCs, and mixed population of NPC derived glutamatergic and GABAergic neurons	Downregulation of miR-9 in schizophrenia hiPSC-derived NPCs, that negatively regulates neural stem cell proliferation by suppressing TLX expression Overexpression of miR-9 rescued the aberrant radial migration in schizophrenia NPCs

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No	Author	Patients	Controls	Genetic aberration	Source of somatic cells	Cell types investigated	Phenotypes relevant for schizophrenia
31	Toyoshima et al., 2016	Patients from Bundo et al. (2014)	Three controls	22q11.2 deletion	Fibroblasts	Mixed neural cell types (neuronal and glial cells)	Defects in neurosphere size, neural differentiation efficiency, neurite outgrowth, cellular migration and the neurogenic-to-gliogenic competence ratio in hiPSC-derived from 22q11.2 deletion patients with schizophrenia Reduced expression of miR-17/92 cluster and miR-106a/b that negatively regulates p38α (MAPK14) in 22q11.2 hiPSC-derived neurospheres, resulting in elevated gliogenic differentiation The dampened neurogenic-to-gliogenic cellular competence could be rescued by p38 inhibitors
32	Xu et al., 2018	Patients from Brennand et al. (2011) and 9 childhood onset schizophrenia patients from Topol et al. (2016)	Fourteen controls	Five subjects with childhood onset schizophrenia harbored CNVs in 1p33, 16p11.2, 3p25.3, and 2p16.3. The remaining subjects were genetically uncharacterized	Fibroblasts	hiPSC-derived forebrain neurons and Ngn2-induced excitatory neurons	Increased levels of post synaptic density protein, $STEP_{61}$ in schizophrenia hiPSC-derived neurons, concomitant with reduced ubiquitination of $STEP_{61}$ Inhibition of $STEP_{61}$ increased phosphorylation of $STEP_{61}$ targets and the spontaneous neuronal activity in hiPSC-derived neurons.
33	Flaherty et al., 2017	Patients from Lee et al. (2015)	One unrelated control	*CNTNAP2* deletion	Fibroblasts	hiPSC-derived forebrain neurons and Ngn2-induced excitatory neurons	*CNTNAP2* deletion affected expression of genes involved in synaptic transmission, neuronal development and neuronal activity in Ngn2-induced glutamatergic neurons Increased spontaneous network level activity in both hiPSC-derived forebrain and glutamatergic neurons in the deletion carriers
34	Narla et al., 2017	Patients from Brennand et al. (2011)	Four controls from Brennand et al. (2011)	Uncharacterized	Fibroblasts	Neuron committed cells	The nFGFR1, a pan-ontogenic regulator, which integrates pathways involved in schizophrenia pathogenesis, was overexpressed in schizophrenia NCCs and it over-targeted genes dysregulated in schizophrenia Upregulated genes were enriched for differentiation, maturation of neurons and axonal guidance, whereas downregulated genes were involved in glial differentiation and myelination

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Table 1 (*continued*)

No	Author	Patients	Controls	Genetic aberration	Source of somatic cells	Cell types investigated	Phenotypes relevant for schizophrenia
35	Hoffman et al., 2017	Childhood onset schizophrenia patients; 10 from Topol et al. (2016) and 4 additional samples	Twelve controls	SZ-relevant CNVs, including 22q11.2 deletion, 16p11.2 duplication, 15q11.2 deletion, and NRXN1 deletion (2p16.3)	Fibroblasts	Forebrain NPCs, and mixed population of NPC derived glutamatergic and GABAergic neurons	Transcriptional signatures of schizophrenia in hiPSC-derived NPCs and neurons showed concordance with post-mortem adult brain samples, after adjusting cell type composition, which reduced the stochastic effects of the differentiation process
36	Stachowiak et al., 2017	Patients from Brennand et al. (2011)	Four controls from Brennand et al. (2011)	Uncharacterized	Fibroblasts	Cerebral organoids	First report to model schizophrenia hiPSCs using cerebral organoids Abnormal scattering of NPCs from ventricular zone was evident into intermediate and cortical zones of schizophrenia cerebral organoid Restricted neuronal growth, resulting in truncated cortical development and decreased intracortical connectivity nFGFR1 signaling was observed to be dysregulated in schizophrenia hiPSC-derived cerebral organoids

NPC, Neural Progenitor Cells.

[a] These studies only established, hiPSCs from schizophrenia patients but did not differentiated them to neural lineage.

midbrain dopaminergic lineage (Hartley et al., 2015). Thus, improvement of current protocols exploiting the gene regulatory networks for fate-specific differentiation with reduced cellular heterogeneity is warranted (Luginbühl et al., 2017).

Although hiPSC-derived neurons require longer duration in culture for maturity, they still resemble fetal stage neurons. This limits modeling of cellular phenotypes associated with schizophrenia that generally emerge during late adolescence. Strategies such as progerin overexpression, telomere shortening, prolonged in vitro culture, and direct neuronal reprogramming have potential for studying neurons at more mature stages (Mertens et al., 2015; Miller et al., 2013; Petrini et al., 2017; Vera et al., 2016). Nevertheless, the simplicity of hiPSC-derived neuronal differentiation models compared with in vivo neural development demands a coculture system with multiple neural cell types for functional maturity of neurons and to recapitulate circuit level deficits. In line with this, several schizophrenia hiPSC studies have employed coculture with primary astrocytes for neuronal maturity. However, hiPSC-derived neurons generally lack the myelination essential for neuronal maturation, particularly in a targeted neuronal subtype differentiation approach. Therefore, coculturing schizophrenia hiPSC-derived oligodendrocyte precursor cells and neurons offers the prospect of neuronal myelination (Thiruvalluvan et al., 2016). Additionally, modeling schizophrenia-related deficits in myelination and synaptic pruning also benefits from coculturing with appropriate cell types (Prytkova and Brennand, 2017). Similarly, a more realistic manifestation of downstream events including environment insult such as viral infection requires the concerted action of neurons along with immune cells and glial cells warranting appropriate coculture systems (D'Aiuto et al., 2015).

Promisingly, recent studies have shown that brain organoids are capable of generating mature neural cell types including astrocytes and oligodendrocytes in a spatiotemporal manner and can make neural connectivity within organoid tissues (Birey et al., 2017; Lancaster et al., 2017; Quadrato et al., 2017; Renner et al., 2017). Although 2D culture offers cost-effective simple model, they are incapable to recapitulate cellular architecture and the microenvironment during the neurodevelopment events. Due to a relatively easier setup and scalability for high throughput analysis, 2D culture of hiPSCs has been a convenient option to study neurodevelopment in vitro. However, recapitulation of in vitro cortical development from iPSCs along with self-organized developmental patterning provided brain organoids an additional advantage, which otherwise is not possible in 2D culture (Lancaster et al., 2017; Renner et al., 2017). Moreover, protracted in vitro maturation in 3D culture also favors functional maturity (Paşca et al., 2015). Recently, astrocyte lineage cells originated from long term culture of hiPSC-derived human cerebral cortical spheroids for 20 months, resembled primary human astrocytes from transcriptional profile and physiological characteristics (Sloan et al., 2017). Yet, there are several limitations to be addressed in 3D brain organoid culture methodology (Di Lullo and Kriegstein, 2017). One main issue is that the events triggering and regulating self-organization of stem cells in 3D is largely unknown (Renner et al., 2017). Moreover, comprehensive information on cellular composition and identity is limited in the case of brain organoids, demanding an extensive single cell transcriptomic profiling (Quadrato et al., 2017). Furthermore, brain organoids generally lack cortical layer organization, although the neuronal population from all cortical layers is present. Transcriptomic studies in brain organoids have shown collinearity with early to mid-stages of fetal brain development which limits modeling the post-natal stages, where extensive synaptic pruning and neural circuit refinement occurs. Nevertheless, overcoming these shortcomings by methodological advancements would make brain organoids a promising strategy for modeling schizophrenia pathogenesis in the near future. Only one study has modeled schizophrenia in brain organoids, and reports of unaddressed phenotypes are awaited in future independent studies (Stachowiak et al., 2017).

Above all, variability in cellular phenotypes owing to the inherent complexity of schizophrenia can be minimized to an extent by appropriate patient selection strategies based on genetic risk or uniform diagnostic phenotype (Hoekstra et al., 2017). Highly penetrant genetic aberrations will reflect its large biological effects as detectable phenotypes in vitro, and thus advantageous for hiPSC modeling. Several schizophrenia hiPSC studies have modeled highly penetrant risk variations including 22q11.2 deletion, 15q11.2 deletion, *CNTNAP2* deletion, and *DISC1* mutations. However, substantial pleiotropy exists for such genetic variations and not all individuals were diagnosed with schizophrenia in these studies (Jonas et al., 2014; Thomson et al., 2013; Wen et al., 2014). This is reflected in the molecular profile of 22q11.2 deletion hiPSC-derived neurons, where no overlap among the differentially expressed miRNA was observed. The existence of additional risk variants in these subjects might also contribute to the pleiotropic effects (Balan et al., 2014; Rodríguez-López et al., 2017). Since subjects harboring highly penetrant risk variants form only a subset of schizophrenia patients, the cellular and molecular phenotypes observed in these hiPSC models might have limited implication for general schizophrenia. Alternatively, “general” schizophrenia cases can be selected on the basis of a uniform phenotype or high polygenic risk score based on common genetic variants. However, in all these strategies, hiPSCs from unaffected controls were compared, rendering adjustment of their genetic background challenging. Isogenic lines with genome-edited mutations are appropriate for modeling single gene mutations, such as in *DISC1* (Srikanth et al., 2015; Wen et al., 2014). However, the cellular phenotypes observed are limited only to the gene function and may only unmask certain aspects of the disease pathogenesis. Furthermore, generating isogenic lines modeled for larger CNVs is tedious. For general schizophrenia, utilizing familial samples as hiPSCs would minimize the confounding genetic heterogeneity to some extent.

Interpreting the findings from small cohorts in the context of a general patient population should be carried out cautiously. A large volume of hiPSC studies in schizophrenia have revealed changes in cellular phenotypes between patient-derived neural cells and controls. However, to date, all of these studies have been underpowered, considering the small sample size of the studies and polygenic genetic components (Hoffman et al., 2017). This highlights the need to establish large schizophrenia hiPSC cohorts with more biological replicates to increase the statistical power, albeit a time-consuming process. It should be noted that the majority of schizophrenia hiPSCs studies have used the same patient samples as reported in the initial study by Brennand et al. (Brennand et al., 2011) (Table 1). This limits our observations on cellular phenotypes of schizophrenia and relies on only a subset of patients. Furthermore, disparities were also reported in cell lines derived from the same samples (Hartley et al., 2015). Therefore, future extensive accumulation of hiPSC studies accommodating different phenotypic spectrum of schizophrenia is needed to unmask genotype-related phenotypes. Moreover, advances in comprehensive analytical technologies are essential to unravel precise cellular phenotypes aligning with behavioral phenotypes.

5. Summary

Advances in patient-specific hiPSC technology, together with genome editing, 3D brain organoid culture, and deep sequencing of transcriptomes at population or single cell levels have provided a unique opportunity to characterize cellular and molecular phenotypes relevant to the pathogenesis of schizophrenia. As well as substantiating observations from the postmortem brain samples and animal models of schizophrenia, hiPSC studies have provided novel insights pertaining to the neuronal, synaptic, developmental, and metabolic pathologies. However, hiPSC modeling of schizophrenia has been hindered by the inherent complexity of the disease, along with methodological limitations resulting in variability in cellular phenotypes. Technological advances in reducing this variability and appropriate patient selection strategy will, in turn, form a strong basis for evaluating robust cellular

phenotypes. Nevertheless, modeling a behavioral disorder in vitro has its own drawbacks as the cellular equivalent for behavioral phenotypes is unknown. Alternatively, assessing higher order neuronal circuitry and function is limited in culture conditions. These limitations warrant technological advancements in 3D culture to recapitulate in vivo neural development. Furthermore, large independent studies accommodating a wide phenotypic spectrum of schizophrenia with sufficient statistical power is needed to consolidate multiple lines of evidences from clinical and animal model studies. Thus, multidisciplinary innovative approaches in hiPSC modeling of schizophrenia will enable pathobiological events pertaining to initiation and progression to be deciphered which might be amenable for therapeutic targeting.

Abbreviations

iPSCs	induced pluripotent stem cells
hiPSC	human iPSC
NPCs	neural progenitor cells
CRISPR	clustered regularly interspaced short palindromic repeats
Cas9	CRISPR-associated protein 9
2D	two-dimensional
3D	three-dimensional
DISC1	Disrupted-In-Schizophrenia-1
cAMP	cyclic 3′, 5′-adenosine monophosphate
DG	dentate gyrus
NEUROD1	neuronal differentiation 1
FOXG1	forkhead box G1
PROX1	prospero homeobox 1
TBR1	T-box, brain 1
SV2	synaptic vesicle protein
STEP61	striatal-enriched protein tyrosine phosphatase
NMDA	*N*-methyl-D-aspartate
GRIN2B	glutamate ionotropic receptor NMDA type subunit 2B
Fyn	FYN proto-oncogene, Src family tyrosine kinases
CNV	copy number variation
TUBGCP5	tubulin gamma complex associated protein 5
CYFIP1	cytoplasmic FMR1 interacting protein 1
NIPA1	NIPA magnesium transporter 1
NIPA2	NIPA magnesium transporter 2
WRC	WAVE regulatory complex
CNTNAP2	contactin-associated protein-like 2
Ngn2	neurogenin 2
DGCR8	DiGeorge Syndrome Critical Region Gene 8
miRNA	micro-RNA
GFAP	Glial Fibrillary Acidic Protein
MAP2	reduced Microtubule Associated Protein 2
MAP K14	Mitogen-Activated Protein Kinase 14
CDC45	Cell Division Cycle 45
PRODH	Proline Dehydrogenase
FGF	Fibroblast Growth Factor
RAPGEF2	Rap guanine nucleotide exchange factor 2
TLX	T Cell Leukemia Homeobox
PDGFRα	Platelet Derived Growth Factor Receptor Alpha
MIA	maternal immune activation
LINE-1	long interspersed nucleotide element-1
HSV-1	herpes simplex virus type 1
fMRI	functional magnetic resonance imaging
ROS	reactive oxygen species
NCCs	neuron committed cells
FGFR1	Fibroblast Growth Factor Receptor 1

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Conflict of interests

The authors report no conflict of interest.

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