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Modeling heterogeneous patients with a clinical diagnosis of schizophrenia using induced pluripotent stem cells

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Abstract

Schizophrenia (SZ) is a devastating complex genetic mental condition that is heterogeneous in terms of clinical etiologies, symptoms and outcomes. Despite decades of postmortem, neuroimaging, pharmacological and genetic studies of patients, in addition to animal models, much of the biological mechanisms that underlie the pathology of SZ remain unknown. The ability to reprogram adult somatic cells into human induced pluripotent stem cells (hiPSCs) provides a new tool that supplies live human neurons for modeling complex genetic conditions such as SZ. The purpose of this review is to discuss the technical and clinical constraints currently limiting hiPSC based studies. We posit that reducing the clinical heterogeneity of hiPSC-based studies, by selecting subjects with common clinical manifestations or rare genetic variants, will help our ability to draw meaningful insights from the necessarily small patient cohorts that can be studied at this time.

Keywords

schizophrenia; human induced pluripotent stem cells; neuronal differentiation; clinical heterogeneity; mouse model; genetics

Introduction

Schizophrenia (SZ) is a devastating mental condition characterized by psychotic manifestations, including delusions and hallucinations. While onset typically occurs in late adolescence and early adulthood (1), abnormal neurodevelopmental processes are thought to

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initiate in childhood in some cases of SZ (2, 3). There is substantial variation in the type and severity of symptoms and cognitive deficits among individuals classified with SZ (4, 5). The disease course varies between individuals, though the majority of cases show initial deterioration followed by either remission, relapse, or recovery (6, 7). Patients show a heterogeneous response to treatment, which does not fully ameliorate the symptoms (8). While antipsychotics can reduce positive symptoms in some patients (but do not improve negative symptoms or cognitive deficits), one-third of patients do not experience any symptom amelioration through medication (5, 9). Less than 20% of SZ patients experience symptom remission and adequate social functioning within five years of their first psychotic episode (10). Only a small fraction of subjects diagnosed with SZ fully recover (11).

SZ is a polygenic condition with an estimated heritability as high as 80% (12). Although many candidate susceptibility genes have been identified, the individual effect sizes are modest (13). Subjects with SZ are three times more likely to have genomic mutations that disrupt genes involved in neurodevelopmental pathways (14): single nucleotide polymorphisms and copy number variations (CNVs) have both been implicated in conferring risk of SZ. Damaging de novo mutations in persons with SZ converge in a network of genes coexpressed in the prefrontal cortex during fetal development (15); one prevailing hypothesis is that disruptions in fetal prefrontal cortical development underlie SZ.

Despite the heterogeneity in clinical features, several lines of evidence, including postmortem, brain imaging, pharmacological, genetic and animal studies, have identified some levels of common phenotypes of disease: synaptic deficits, interneuron abnormalities, enlarged ventricles and changes in neurotransmission involving dopamine, glutamate and GABA (16, 17). Though frequently confounded by variables such as patient treatment history, addiction and poverty, post-mortem studies have yielded many valuable insights into the neuronal pathology of disease, but have revealed less about disease initiation or progression. Animal models of SZ have recapitulated some of the behavioral traits, neuronal phenotypes and molecular signatures relevant to SZ, and proven valuable for studying the aberrant connectivity and function of specific neural networks in disease; however, they are typically used to study highly penetrant (and rare) SZ genes, failing to capture the polygenicity of SZ (16, 18, 19). Methodological approaches used to study SZ to date have significant limitations; hiPSC-based studies will not replace these traditional models but may supplement them (Table 1).

Genetic and epigenetic variations underlie differences in clinical outcome and treatment responsiveness (20-25) and the explanation for the 41-65% discordance rate of SZ between monozygotic twins sharing identical genetic predisposition to disease remains unclear (26). Environmental stressors, such as cannabis use, maternal immune activation and birth complications, may also contribute to SZ (27-30), and recent efforts have attempted to combine animal models with environmental stressors (31). Although, these studies can provide important insights into biological mechanisms that underlie at least in part the pathology of SZ, it is still difficult to fully recapitulate the heterogeneity of the disease and address mechanisms of this “human” condition. Cell-based studies have the potential to combine both environmental and genetic influences by using cells from patients with known genetic backgrounds.

This review will first broadly introduce the methods and practical limitations of hiPSC-based studies. Second, with specific reference to SZ, a complex genetic disorder characterized by large inter-patient variation, we discuss how selecting subjects with common clinical manifestations or rare genetic variants will increase the likelihood of finding biologically meaningful insights from the necessarily small hiPSC-based studies that can be undertaken at this time. Third, will describe how selecting genetically homogenous patient cohorts has already been successfully employed for autism spectrum disorders. Finally, findings from recent hiPSC-based studies of SZ will be summarized and future directions of this work will be discussed.

Generation and differentiation of hiPSCs

Reprogramming of somatic cells

In 2012, the Nobel Prize in Medicine was awarded for the discovery that differentiated cells could be reprogrammed back to a pluripotent state. The transient expression of just four factors (Oct3/4, Klf4, Sox2 and c-Myc) is sufficient to directly reprogram adult somatic cells into an induced pluripotent stem cell (iPSC) state (32-34). This ability to reprogram patient somatic cells into human induced pluripotent stem cells (hiPSCs) provides a limitless source of live human cells for modeling complex genetic conditions, where many genes may be interacting to produce the disease state, such as SZ.

Early methods of reprogramming relied upon constitutive retroviral or lentiviral expression systems, with two potential limitations: insertional mutagenesis upon viral integration and persistent viral expression of reprogramming factors. Though these first-generation methods were ultimately sufficient to generate cell-based models of several psychiatric disorders (35, 36), integration-free methods have now been developed. Of numerous protocols reported (37-42), two are commonly used: mRNA and Sendai virus reprogramming. Synthetic modified mRNA reprogramming is efficient, but requires daily transfections in addition to a proprietary media formulation during the reprogramming process (42); non-integrating Sendai virus reprogramming has been shown to be a reliable alternative (37, 38). mRNA and Sendai viral reprogramming represent the best and most robust methods available to date. Integration-free reprogramming is now straightforward, though at present, only Sendai reprogramming is effective for both human fibroblast and blood cells (43).

Epstein-Barr virus (EBV) immortalized lymphoblastoid B-cell lines have been widely banked for studying a variety of diseases. Reprogramming methods have recently been reported for EBV immortalized cell lines, but so far have only been demonstrated using episomal reprogramming methods (44, 45). Though episomal reprogramming remains less efficient than other methods, it can be improved using a cocktail of small molecules (46). It remains to be shown whether functionally mature neurons can be generated from EBV-derived hiPSCs; however, it should be noted that these hiPSCs appear to have no detectable EBV elements (45). It may now be possible to generate hiPSCs from countless EBV cell lines generated by clinicians and geneticists around the globe for the study of SZ.

It is important to note that genetic and epigenetic mutations can and do occur during the reprogramming process. Though, CNVs have been associated with the reprogramming of

hiPSCs (47), more CNVs are present in early-passage hiPSCs than in higher passage hiPSCs as most novel CNVs generated during the reprogramming process are subsequently lost (48). We recommend utilizing at least three hiPSC lines per individual, to reduce the likelihood that a rare *de novo* mutation might affect disease-specific hiPSC lines in a meaningfully different way than control hiPSC lines. With carefully designed and controlled experiments, we believe that rare random mutations should not interfere with the ability to draw meaningful conclusions from hiPSC-based studies of psychiatric disorders.

Neuronal differentiation of hiPSCs

Neural populations generated through differentiation protocols are invariably extremely mixed. Although the relative frequency of a specific neuronal cell type might be favored, the populations generally remain composed of other types of neurons, as well as astrocytes, oligodendrocytes, neural precursors and even non-neural cells. In fact, even state-of-the-art hiPSC neural differentiation protocols produce heterogeneous neural populations of mixed spatial and temporal identities.

Strong evidence now links SZ to aberrant activity of three neural populations: cortical glutamatergic and GABAergic neurons as well as midbrain dopaminergic neurons. Both cortical glutamatergic and GABAergic neuronal populations and midbrain dopaminergic neuronal populations can now be efficiently differentiated *in vitro* from hiPSCs. Pluripotent stem cells can be differentiated to pyramidal cortical neurons in the presence of dual SMAD inhibition, FGF2 and vitamin A (Figure 1) (49, 50), and GABAergic interneurons with dual SMAD inhibition and combined stimulation of WNT and SHH signaling (51, 52). Midbrain dopaminergic (mDA) neurons are particularly relevant to the study of SZ, and efficient protocols have been developed to differentiate pluripotent stem cells to mDA neurons through neural induction in the presence of dual SMAD inhibition followed by mDA specification via activation of SHH and WNT signaling (53, 54).

Because hiPSCs can be efficiently differentiated to several neuronal populations as well as astrocytes, it may be possible to identify the specific neuronal subtype(s) whose aberrant activity contributes to SZ initiation and progression.

Scalability of hiPSC generation, NPC generation, and neural differentiation

Kits for both mRNA and Sendai viral-based reprogramming methods are now commercially available. Though the efficiency of reprogramming still varies between experiments, the process is now reasonably robust and scalable. Yields and purity of independent neuronal differentiations remain more variable. Commercial products, developed based on published methods (55), such as the AggreWell™800 system claim to standardize aggregate size, leading to yields of up 90% pure neural rosette cultures that can be enzymatically separated from non-neural progenitor cells (NPCs). A FACS-based method purifies NPCs using antibodies for the cell surface signature CD184+/CD271-/CD44-/CD24+, generating a replicative population that is 99.1% pure for the NPC marker Nestin (56); upon neuron- or glia-specific differentiation, this population can be sorted for neurons (CD184-/CD44-/CD15LOW/CD24+) or glia (CD184+/CD44+), though the regional patterning of all three populations remains unclear (56). Both methods eliminate the need for trained selection of

ideal neural rosettes by morphology alone, allowing this technology to more easily be shared between research groups. Our hope is that an enhanced understanding of the timing and concentration of specific growth factors involved in patterning specific neuronal identities may obviate the need for such purification techniques.

Direct induction of iNPCs or iN cells from fibroblasts

An alternative to hiPSC reprogramming and differentiation is the direct induction of induced neuronal (iN) cells from fibroblasts. Early reports demonstrated that iNeuron induction was fast, occurring in as little as six days, but inefficient and yielding functionally immature neurons (57). Methods were quickly refined: the addition of key microRNAs yielded iN cells with functional synapses (58, 59); pools of dopamine neuron-specific transcription factors could be used to induce predominantly dopaminergic iN cells (60, 61); and the addition of puromycin selection ultimately generated yields nearing 100% (62). One must consider that the generation of iN cells bypasses neuronal development, eliminating the ability to test cellular phenotypes such as neuronal maturation that may contribute to SZ progression. Additionally, the generation of terminally differentiated neurons limits the cellular material available that can be derived from patient fibroblasts. Now, several combinations of neural transcription factors can be used to induce neural progenitor cells (iNPCs) from fibroblasts (63-65), though patterning of specific regional identities has not yet been shown. As the efficiency, purity and patterning of iNPC methods advance, we predict that this method may supplant hiPSC-based studies.

Constraints of hiPSC-based studies

Technical limitations

Cell surface markers to determine the exact temporal and regional identity of any individual neuron in a heterogeneous hiPSC derived neural population are critically lacking; in live cultures, one cannot distinguish whether a neuron is glutamatergic, GABAergic or dopaminergic. Compounding this, no existing *in vitro* neural differentiation is yet fully efficient. Experimental variability is currently exacerbated by neural heterogeneity in *in vitro* derived populations, which results because current neuronal differentiation protocols are not 100% efficient and, in contrast to the hematopoietic system, cell surface markers by which specific subtypes of neurons might be purified have not been developed.

To some extent, all *in vitro* cultures of hiPSC-derived neurons are heterogeneous, though the relative ratios of glutamatergic, GABAergic, dopaminergic and other neurotransmitter producing neurons can be biased via directed differentiation. While *in vitro* derived neurons can generally be patterned as forebrain, midbrain or hindbrain neural cells, specific regional patterns such as layer IV cortical neurons or basal ganglionic eminence cannot yet be generated. hiPSC-derived neurons require months to fully mature *in vitro* (49-52, 66) and lack myelination (67, 68). Though electrophysiologically mature and capable of spontaneous action potentials and synaptic activity, these neurons still most resemble fetal neural tissue (51, 52, 66). Within a single culture, neurons can still be of variable functional maturity, as neuronal activity is required for neural maturation, thus, *in vitro* neuronal maturation occurs

first in clusters of early maturing neurons. hiPSC neuronal populations are characterized by mixed spatial patterning and temporal maturity.

Many strategies for the purification of heterogeneous NPC and neuron populations have been considered. There is a dearth of validated immunohistochemical markers, particularly cell surface markers, by which to indicate the range of regional patterns or neurotransmitter fates that can be differentiated from each NPC line. The most reliable method is indirect, relying on analysis of the composition of differentiated neuronal cultures.

Intra-patient and inter-patient variability, described below, are two additional types of variables that constrain hiPSC-based studies. Intra-patient exists because individual hiPSC lines vary genetically, epigenetically and in propensity towards neural differentiation. To some extent, this variability may be unavoidable, though it is currently exacerbated by the heterogeneity of cellular subtypes in hiPSC neural populations. While unpublished data by others and us suggests that intra-patient variability is less than inter-patient variability, the optimal number of hiPSC lines to generate and study per individual remains unresolved.

Limitations due to clinical heterogeneity

The sample size of hiPSC-based studies remains relatively very small compared with the standards of genome-wide association studies of complex genetic disorders. Though ultimately methods will have to be developed to permit comparisons among thousands of patients, to date, technical constraints greatly limit hiPSC generation, NPC differentiation and cellular phenotyping (listed in order of increasing difficulty). Consequently, given the small sample size (typically 1-4 patients) of recent hiPSC-based studies, a major concern is whether the findings are representative of the larger patient population. Inter-patient variability results from the heterogeneity between patients with SZ (Table 2). There are two strategies for hiPSC disease modeling of heterogeneous patient populations: 1) use a genetically homogenous patient cohort sharing a single genetic lesion and characterize the effect relative to isogenic lines generated through gene targeting technologies; and 2) use a patient cohort selected on the basis of a shared clinical phenotype and compare to individuals without the phenotype. The first strategy parallels traditional mouse based studies of SZ that investigate the effects of rare loci, while the second takes full advantage of the ability of hiPSC-based studies to investigate complex genetic disorders without full knowledge of all the genes involved.

By modeling specific aspects of SZ, rather than capturing the entire diversity of this disorder, researchers might be able to reduce the inter-patient variability in hiPSC-based studies. In the short-term, selecting homogenous patient cohorts characterized by common genetic mutations, or by shared neurophysiological endophenotypes and/or pharmacological responses, can address this. Neurophysiological characterization of patients with SZ have identified abnormal responses to paired auditory stimuli (69, 70) and defects in oculomotor control (71) - growing evidence suggests these endophenotypes may be heritable (72). Though strong evidence supports the pharmacogenetics of lithium-responsive Bipolar Disorder (73), new data supports the heritability of antipsychotic resistance in SZ as well (23). Of course, additional factors, ranging from epigenetic effects to circuit-based plasticity (derived from experience), may contribute to the heterogeneity of SZ. Nonetheless, though

such risk factors are likely to be lost as part of the reprogramming process, we predict that some, even if not all, key mechanisms contributing to SZ can be studied using hiPSCs.

Multiple levels of etiological cause might contribute to SZ, increasing from biochemical to cellular to neuronal network and brain circuits. As the complexity of this “causal action” grows, it will become more difficult to resolve biological meaning through cell-based models (74). Critics may also question the relevance of months old *in vitro* derived neurons in the study of SZ, a condition whose hallmark symptom of psychosis typically appears in late adolescence. For this reason, we posit that current hiPSC-based approaches may be most appropriately used in the study of SZ predisposition.

Future applications of cell-based models

Whether they are differentiated from hiPSCs or induced as iNPCs or iN cells, future applications of cell-based models will require greater maturity and more specific regional patterning of neurons. Better markers will facilitate either the purification or labeling of live human neurons with particular patterning and/or neurotransmitter profiles. This will facilitate combining specific subtypes of neurons, via improved microfluidic or micropatterned devices, into increasingly complex *in vitro* networks. Finally, improved genetic tools, ranging from optogenetic stimulators of specific populations of neurons (75, 76), to genetically encoded indicators of neuronal activity in defined neuronal subtypes (77), will permit more sophisticated analysis of neuronal activity in these artificial networks.

Utility of using a Homogeneous Patient Population

An alternate way to address the question of heterogeneity is to utilize patients with higher genetic homogeneity. Patients carrying rare variants, such as CNVs, that are associated with SZ may represent unique sub-groups in the broad diagnostic classification currently used in clinical psychiatry. Nonetheless, we can expect that they are more homogeneous populations suitable for biological study. CNVs associated with SZ include deletions at 1q21.1, 2p16.3, 15q13.3, 22q11.2 and a 16p11.2 duplication (78). Although some CNVs are not specific to SZ and are also present in individuals with neurodevelopmental disorders, like autism, they may represent a unique population for the generation of hiPSCs to uncover novel pathways relevant to the pathology of SZ.

Although this perspective is widely shared among SZ researchers, only two preliminary studies have so far been reported. One study utilized hiPSC from SZ patients carrying a rare variant in the *DISC1* gene (79). As this variant is seen more frequently in the general population than in the patient group (80), it is unclear whether the variant may directly link to the disease pathology. Another small study included hiPSCs from one subject with SZ carrying the 22q11.2 deletion together with those from two other patients not carrying the deletion, showing that the 22q11.2 deletion subject had a delay in down regulating OCT4 and NANOG expression (81).

Cell-based models of autism spectrum disorders (ASD), looking at Timothy syndrome and Rett syndrome, have successfully utilized this approach. The success through the study of Timothy syndrome, a rare autosomal dominant disorder characterized by physical

malformations (e.g., heart failure) and ASD-like neuropsychiatric manifestations (82), may provide us with many lessons. A recent paper reported that the findings from rat and mouse neurons could be recapitulated with hiPSCs from individuals with Timothy syndrome (83): in this study, the authors differentiated hiPSCs into cortical neurons and showed that neurons from individuals with Timothy syndrome retract their dendrites in response to depolarization, whereas control neurons responded with dendrite growth. This defect was independent of calcium but likely caused by an inability of the $Ca_v1.2$ channel to interact with a RGK protein, Gem. An earlier study found that the Timothy syndrome derived neurons have defects in calcium signaling, abnormalities in differentiation, abnormal expression of tyrosine hydroxylase and increased secretion of dopamine and norepinephrine (84); the increased proportion of dopaminergic neurons could be reversed by roscovitine. Since these studies were able to use cells from two individuals with a monogenic neurodevelopmental disorder, their findings contribute significantly to the pathophysiology of Timothy syndrome and perhaps other autism spectrum disorders. Mutations in the methyl CpG binding protein (MeCP2) gene underlie Rett Syndrome, a form of ASD. Consistent with animal models and postmortem human brain tissue (85), four groups have now detected a decrease in cell soma size of neurons and glutamatergic connections in patient-derived cells compared to non-affected controls, which, in one study, was ameliorated through treatment with Insulin Growth Factor 1 (36, 86-88). We believe that these studies are good examples of how using hiPSCs from individuals with the same genetic mutation can contribute to a better understanding of the pathogenesis of SZ.

Common Phenotypes and Molecular Pathways in Heterogeneous SZ Patients

As discussed earlier in this review, brain imaging studies have revealed decreased whole brain volume in SZ (89-91). This may reasonably correspond to smaller neuronal somas, reduced dendritic arborization, reduced dendritic spine density, and increased neuronal density, which have been observed in autopsied brains from SZ patients (92-97). Consistent with these observations, mouse models that capture key features of SZ, including *Disc1*, *Neuregulin-1* and *ErbB4*, reveal reduced dendritic complexity and synaptic deficits in adult mice, but also aberrant neurite outgrowth and axon targeting in fetal mice (16, 98, 99). What postmortem analysis of SZ has failed to resolve is whether disease progression reflects developmental aberrations during neuronal differentiation or activity-dependent atrophy of neuronal dendrites or synapses in mature neurons, a question that may be resolved using human cell-based models of SZ.

A number of groups have now published studies of hiPSC-derived neurons from sporadic SZ. We generated hiPSCs from four patients with sporadic forms of SZ and showed that SZ-hiPSC neurons had reduced neuronal connectivity and altered gene expression profiles (Figure 2 A and B) (35). Specifically, we observed reduced neurite outgrowths, reduced PSD95 dendritic protein levels and reduced rabies virus trans-neuronal tracing. The defects in neuronal connectivity and gene expression were ameliorated following treatment with the antipsychotic drug loxapine. Another group reported a two-fold increase in extra-mitochondrial oxygen consumption as well as elevated levels of reactive oxygen species in

NPCs derived from hiPSCs from one SZ patient (Figure 2 C and D) (100). A third group has independently verified both phenotypes, reporting impaired synaptic maturation and mitochondrial dysfunction in SZ hiPSC neurons from three patients (101). Findings from these studies now await replication across larger patient cohorts.

Future Directions in Cell-Based Models of SZ

It is now time to bring together the fields of neurobiology and human genetics. Beyond correlating *in vitro* phenotypes to patient clinical information, researchers can test the relationship of neuronal gene expression, neural phenotypes, and pharmacological response. By examining the effects of genetic variants in hiPSC-derived neurons, we can assay gene pathway perturbations in both the specific patient in whom a novel lesion was identified and across cohorts of SZ patients. As the adoption of more scalable methods permits the design of larger studies, it will become possible to directly correlate genotype to neural gene expression. Hypotheses generated through whole genome-based genetic studies will be directly tested in the cell types most relevant to SZ, allowing causal networks to be validated and manipulated *in vitro* in order to identify intervention strategies. Though hiPSC-derived neurons have not yet been shown to recapitulate the pathology of SZ at the circuit level, which underlies the pathophysiology of the condition after onset, nonetheless, we believe that hiPSCs can contribute to our understanding of the cellular pathology associated with SZ.

Beyond mechanistic insights into SZ, the promise of hiPSC neurons is that they may serve as a platform for high throughput screening to identify new therapeutics with which to treat this disorder. While it is true that hiPSC neurons are a limitless source of live human neurons for drug screening, the technology for long-term differentiation and synaptic phenotypic assays have not yet proven scalable; however, we do not expect this to be the ultimate constraint. Rather, what remains to be demonstrated is whether patient pharmacological response (or treatment-resistance) correlates to cellular phenotypic drug response *in vitro*. Nonetheless, we remain cautiously optimistic that hiPSCs may prove to be a direct tool for drug discovery.

There is vast clinical heterogeneity between subjects with the same diagnosis of SZ, to the extent that the clinical criteria defined in the Diagnostic and Statistical Manual of mental disorders (DSM) does not stem from the disease etiology. To overcome this problem, in the present review article we have proposed two major strategies, selecting subjects with common clinical manifestations or with rare genetic variants, in order to increase the homogeneity of the study subjects. Human cell-based approaches can also be applied in conjunction with Research Domain Criteria (RDoC), where basic dimensions of functioning are utilized as the basis for classification (102). We suggest that cell-based models are productively used when study groups are correlated to specific phenotypic or genetic dimensions.

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Figure 1. Cortical differentiation of hiPSCs. **(A)** Fold differences in neuronal gene expression compared with undifferentiated hiPSCs for dorsally and ventrally enriched telencephalic genes (49). **(B)** Temporal and spatial similarity of hiPSC derived cortical tissue to developing human brain samples (49). **(C)** Top, Glutamatergic neurons differentiated from hiPSCs display vGLUT-positive puncti. Middle, By day 50, corticothalamic projection neurons (TBR1-positive) differentiated from hiPSCs. Bottom, By day 70, astrocytes (S100, red) had appeared (50). Adapted from Mariani et al 2012 PNAS and Shi et al 2012 Nature Neuroscience.

Figure 2.

SZ hiPSC neural cells show reduced neuronal connectivity and increased oxidative stress.

(A) Representative images of patient-specific hiPSCs (left), NPCs (middle) and neurons (right) (35). (B) Neuronal connectivity of control and SZ hiPSC neurons were assessed by transneuronal tracing of transgenic Rabies-RFP. Treatment with the antipsychotic loxapine resulted in significant improvement in SZ hiPSC neuronal connectivity (35). (C) Increased extra-mitochondrial respiration in SZ hiPSC NPCs relative to controls exacerbated by treatment with the antipsychotic VPA. (100). (D) Increased reactive oxygen species generated by SZ hiPSC NPCs relative to controls is partially ameliorated by treatment with VPA (100). Adapted from Brennand et al 2011 Nature and Paulsen et al Cell Trans.

Table 1
Comparison of common methodological approaches by which to study SZ

Methodological Approach	Advantages	Disadvantages
Genetic	Captures full diversity of SZ in unbiased manner	Need for large patient cohorts to achieve genome-wide significance; lacks ability to functionally validate findings
Post-mortem	Captures genetic and epigenetic variation; ultimate source of information	Confounded by patient treatment history, addiction and poverty; reveals end-point of disease
Neuroimaging	Ability to study live human patients; Ability to observe neuronal connectivity and test activity of key metabolites	Small effect sizes
Pharmacological	Ability to study live human patients; ability to test effect of new drugs on specific endophenotypes of disease	Can be difficult to resolve non-responsiveness from intolerable side effects; does not necessarily reveal cell of origin
Animal studies	Ability to test neurocircuitry-based effects of highly penetrant SZ genes on specific types of neurons and defined circuits	Does not capture genetic heterogeneity of disease; phenocopies only part of the human disease
hiPSC	Source of live human neurons with which to test hypotheses; captures full genetic contribution to disease	Neurons have a heterogeneous identity, networks are artificial, immature and lack myelination; methods lack scalability

Table 2
Inter-patient heterogeneity: sources and solutions

Sources of inter-patient heterogeneity	Possible ways to overcome inter-patient heterogeneity in research
Type and severity of symptoms	Select subgroups with common clinical manifestations Utilize RDoC and related approaches
Disease course	Select subgroups with a common clinical course
Treatment response	Select subgroups with similar medications and pharmacological responses
Polygenicity	Select subgroups carrying the same genetic mutation (i.e. CNVs) Utilize RDoC and related approaches
Environmental stressors	Select subgroups exposed to the same environmental stressors Test the effects of environmental stressors (such as cannabis) in hiPSCs to clarify the cellular impacts of the environmental factors