Modeling Heterogeneous Patients With a Clinical Diagnosis of Schizophrenia With Induced Pluripotent Stem Cells

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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.

Key Words: Clinical heterogeneity, genetics, human induced pluripotent stem cells, mouse model, neuronal differentiation, schizophrenia

Schizophrenia (SZ) is a devastating mental condition characterized by psychotic manifestations, including delusions and hallucinations. Although onset typically occurs in late adolescence and early adulthood (1), abnormal neurodevelopmental processes are thought to 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, although most cases show initial deterioration followed by remission, relapse, or recovery (6,7). Patients show a heterogeneous response to treatment, which does not fully ameliorate the symptoms (8). Although antipsychotics can reduce positive symptoms in some patients (but do not improve negative symptoms or cognitive deficits), a 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 5 years of their first psychotic episode (10). Only a small fraction of subjects diagnosed with SZ fully recover (11).

Schizophrenia 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 γ-aminobutyric acid (GABA) (16,17). Although frequently confounded by variables such as patient treatment history, addiction, and poverty, postmortem 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; human induced pluripotent stem cell (hiPSC)-based studies will not replace these traditional models but might 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, might 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 interpatient variation, we discuss how selecting subjects with common clinical manifestations or rare genetic variants will increase the likelihood of finding biologically meaningful insights

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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
Postmortem	Captures genetic and epigenetic variation; ultimate source of information	Confounded by patient treatment history, addiction, and poverty; reveals endpoint 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 nonresponsiveness 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

hiPSC, human induced pluripotent stem cell; SZ, schizophrenia.

from the necessarily small hiPSC-based studies that can be undertaken at this time. Third, we will describe how selecting genetically homogenous patient cohorts has already been successfully employed for autism spectrum disorders (ASDs). 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 state (32–34). This ability to reprogram patient somatic cells into hiPSCs provides a limitless source of live human cells for modeling complex genetic conditions, where many genes might 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. Although 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: messenger RNA (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); nonintegrating Sendai virus reprogramming has been shown to be a reliable alternative (37,38). Messenger RNA and Sendai viral reprogramming represent the best and most robust methods available to date. Integration-free reprogramming is now straightforward, although 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 with episomal reprogramming methods (44,45). Although episomal reprogramming remains less efficient than other methods, it can be improved with 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 seem to have no detectable EBV elements (45). It might 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. Although CNVs have been associated with the reprogramming of hiPSCs (47), more CNVs are present in early-passage hiPSCs than in higher-passage hiPSCs, because most novel CNVs generated during the reprogramming process are subsequently lost (48). We recommend using at least three hiPSC lines/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. We believe that, with carefully designed and controlled experiments, 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 into 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 into 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 into several neuronal populations as well as astrocytes, it might be possible to