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Review

Modeling synaptogenesis in schizophrenia and autism using human iPSC derived neurons

Christa W. Habela [a,b,*], Hongjun Song [a,b,c], Guo-li Ming [a,b,c,d]

[a] Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[b] Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[c] The Solomon Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[d] Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

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ABSTRACT

Schizophrenia (SCZ) and autism spectrum disorder (ASD) are genetically and phenotypically complex disorders of neural development. Human genetic studies, as well as studies examining structural changes at the cellular level, have converged on glutamatergic synapse formation, function, and maintenance as common pathophysiologic substrates involved in both disorders. Synapses as basic functional units of the brain are continuously modified by experience throughout life, therefore they are particularly attractive candidates for targeted therapy. Until recently we lacked a system to evaluate dynamic changes that lead to synaptic abnormalities. With the development of techniques to generate induced pluripotent stem cells (iPSCs) from patients, we are now able to study neuronal and synaptic development in cells from individual patients in the context of genetic changes conferring disease susceptibility. In this review, we discuss recent studies focusing on neural cells differentiated from SCZ and ASD patient iPSCs. These studies support a central role for glutamatergic synapse formation and function in both disorders and demonstrate that iPSC derived neurons offer a potential system for further evaluation of processes leading to synaptic dysregulation and for the design and screening of future therapies.

Contents

1. Introduction

The development of the human nervous system involves the coordinated birth, migration and differentiation of both neurons and glia followed by synaptic integration and appropriate neural circuit formation. Circuitry reorganization continues at individual synapses throughout life in the form of synaptic plasticity of early born neurons, and at the cellular level in the form of the birth and integration of adult-born neurons in the hippocampus, a brain region known to be essential for learning and memory (Bond et al., 2015; Christian et al., 2014). Complex developmental disorders of the nervous system, such as schizophrenia (SCZ) and autism spectrum disorders (ASD), can arise due to

* Corresponding author at: Department of Neurology, Johns Hopkins University School of Medicine, 733 N. Broadway, MRB 706, Baltimore, MD 21205, USA.
E-mail address: chabela1@jhmi.edu (C.W. Habela).

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dysregulation of one, a few or all of these processes. Defects in synapses, including their formation, function, and maintenance, however, are of particular interest not only because they are basic functional units of the brain and continue to be modified by experience throughout life, but also because molecular and structural changes occurring in synapses may be the most immediately targetable for therapeutic interventions after birth.

Dysregulation of synaptic function and structure has been strongly implicated in both ASD and SCZ (Faludi and Mirnics, 2011; Pocklington et al., 2014; Bernardinelli et al., 2014; Spooren et al., 2012). Much of what we have learned about the pathophysiology of these disorders at molecular and cellular levels has come from human postmortem and genetic studies and animal models. While these studies have contributed significantly to our understanding of disease pathogenesis, they are limited in their ability to reveal dynamic neuronal changes in the context of complicated genetic susceptibilities involved in both disorders. The development of techniques to generate induced pluripotent stem cells (iPSCs) from somatic patient tissue has opened the field to new discoveries and therapeutic screening techniques.

2. The synapse in schizophrenia

SCZ is a common and disabling disorder with a frequency of 1% in the worldwide population (Saha et al., 2005). It is clinically characterized by delusions, hallucinations, disorganized behavior and speech as well as diminished emotional expression that results in significant social or occupational dysfunction (Tandon et al., 2013). Mortality for patients with SCZ is 1.5 fold greater than age adjusted control populations, with suicide accounting for 30% of that increase (Brown, 1997). The mainstay of treatment for the disorder has focused on management of delusions, hallucinations and disorganization (positive symptoms) with the use of antipsychotics, which block D2 dopamine receptors. However, these medications are associated with significant side effects and one fifth to one third of patients are treatment resistant (Miyamoto et al., 2012). These medications do not improve symptoms such as anhedonia, apathy, decreased speech content and affective flattening that make up the negative symptoms of schizophrenia, nor do they improve the cognitive deficits frequently seen in patients with the disorder (Leucht et al., 2009; Carbon and Correll, 2014). As such, understanding the underlying pathophysiology with the purpose of developing disease-modifying therapies is of significant importance.

A major hypothesis for the underlying etiology of SCZ is that of synaptic dysfunction (Mirnics et al., 2001). More specifically, SCZ is proposed to result from dysfunctional synaptic transmission, which leads to abnormal connectivity particularly between the prefrontal cortex and the limbic system, striatum and thalamus, and a subsequent excitatory–inhibitory imbalance (Frankle et al., 2003). Understanding how and why these changes occur is one path towards developing more effective treatments. Multiple neurotransmitter systems, including dopamine, glutamate, serotonin and GABA have been implicated in the disease and are reviewed in detail elsewhere (Lisman et al., 2008; Javitt, 2008). The importance of glutamate in the clinical manifestation of the disease is suggested by the recapitulation of symptoms with the addition of drugs that antagonize glutamate signaling (Tsai and Coyle, 2002). Glutamatergic synapses are responsible for the majority of fast excitatory transmission in the brain and are essential for normal cognition. Their function is highly dependent on a unique structure in which a presynaptic axon makes contact with an actin rich protrusion of the post-synaptic dendrite known as a dendritic spine. These spines contain a dense network of scaffolding molecules that anchor ion channels and signaling molecules (the postsynaptic density) opposite the presynaptic terminals (Huganir and Nicoll, 2013). The number of spines and the morphology of individual spines are dynamically altered with sensory experience (Caroni et al., 2012). Synaptic function is dependent on appropriate structural alterations of these synaptic spines and defects in these processes are implicated in multiple developmental disorders (Volk et al., 2015).

The timing of SCZ symptom manifestation in adolescence and young adulthood coincides with a critical period in brain development during which there is an activity-dependent elimination of synaptic spines. During childhood, the number of dendritic spines in the prefrontal cortex is almost double that found in the adult brain (Huttenlocher, 1979). Pruning of these supernumerary spines to adult levels marks adolescence, although this process may extend well into the 3rd decade of life (Petanjek et al., 2011). Structural brain imaging studies have consistently reported volume loss in the prefrontal and temporal cortices of SCZ patient brains (Greenstein et al., 2006a; Cannon et al., 2002). Functional MRI and diffusion tensor imaging studies further indicate altered short distance functional connectivity in the prefrontal, temporal and parietal lobes (Alexander-Bloch et al., 2013; Liu et al., 2008; Zalesky et al., 2011). Together with postmortem studies demonstrating decreased dendritic spine density in the prefrontal cortex (Glantz and Lewis, 2000; Broadbelt et al., 2002; Hu et al., 2015; Sweet et al., 2009; Kolluri et al., 2005; Garey et al., 1998; Konopaske et al., 2014), these data suggest that excessive synaptic elimination during the critical period might play a role in the underlying pathophysiology of the disease (Fig. 1a).

Impaired glutamatergic transmission in SCZ is further demonstrated by postmortem studies showing decreased expression of presynaptic proteins of glutamatergic synapses (Browning et al., 1993; Karson et al., 1999; Glantz and Lewis, 1997; Chambers et al., 2005; Mirnics et al., 2000; Faludi and Mirnics, 2011). At the same time, human postmortem, animal models and genetic studies have also provided evidence for abnormalities in multiple proteins that function in the postsynaptic density including PSD95, Homer and DISC1, and data indicating that antipsychotic drugs modulate these proteins underscores the potential of synaptic modulation as a targetable substrate for therapy (de Bartolomeis et al., 2014). Despite the prevalence and severity of the disorder, a unifying underlying etiology has yet to be established and a combination of genetic risk factors and both prenatal and postnatal environmental influences are likely important (Rapoport et al., 2012). Here we focus predominately on genetic risk factors.

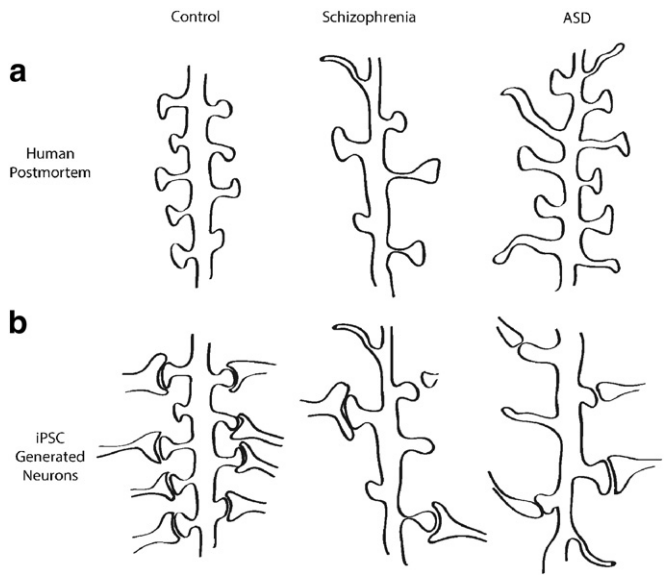

Fig. 1. Structural changes in human postmortem studies (a) and iPSC generated neurons (b). Human postmortem studies demonstrate decreased synaptic spines in schizophrenia. In neurons derived from schizophrenia patients, there are fewer spines and presynaptic terminals. In autism spectrum disorder patients (ASD), postmortem brain studies demonstrate an increase in synaptic spine density as well as more immature synapses while in patient-iPSC derived neurons, there is decreased synaptic density and a more immature phenotype of dendritic spines.

Heritability estimates for SCZ are 73–90% (Sullivan et al., 2003), but despite many studies, including large consortium studies, over the past decades, the underlying genetics of the disorder remain complex. In genetic studies of human SCZ patients, single genes with large effects and high penetrance appear to be rare. This type of genetic susceptibility is exemplified by mutations in the DISC1 gene found to co-segregate with familial schizophrenia, major depression and bipolar disorder in distinct families (Thomson et al., 2013). Over the past decades, the technology has become available to sequence all protein encoding genes in an individual's genome in search of single gene mutations that may be associated with a phenotype in a population, known as whole exome sequencing (WES). Many studies that have attempted to identify genetic causes for SCZ have used genome wide association studies (GWAS). This technique examines single nucleotide polymorphisms (SNP) throughout the genomes of SCZ patients and compares them to unaffected populations in order to identify regions where a predominance of certain alleles is associated with the disease. These SNPs, however, do not necessarily indicate specific gene mutations or functional changes in the genes in those regions, but rather suggests genetic loci where there is the potential for increased risk. More extensive genome variabilities, such as deletions or duplications of larger regions of the genome that are detected by chromosomal microarrays and known as copy number variations (CNVs), have also been increasingly shown to impart susceptibility to SCZ. CNVs may contain a segment of a single gene or many genes whose function may or may not be understood. In SCZ studies, 15–20% of patients carry novel CNVs (Walsh et al., 2008). These are frequently de novo mutations that are not inherited from a parent or parents. When they do occur in families, the penetrance is usually incomplete and phenotypically variable. This makes the determination of pathogenic mechanisms difficult, as there is not always a direct correlation between genotype and phenotype. Despite the complexity of genetic risk, there is mounting evidence that converges on the glutamatergic synapse as an underlying pathological locus in SCZ.

The largest genome wide association study for SCZ to date recently identified 108 genomic loci and suggests that the genetic underpinning of SCZ is likely polygenic in nature (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Interestingly, and consistent with the earlier synaptic hypothesis, many of the genes that were identified at these loci are known to have functions in glutamatergic synaptic transmission and synaptic plasticity. Gene enrichment studies demonstrate that these SCZ risk genes are particularly important in the frontal and temporal lobes — areas shown to have pathological and structural changes in patient brains (Greenstein et al., 2006a; Cannon et al., 2002). Similarly, in a large WES study of SCZ patients, loss of function genetic variation was significantly enriched for genes involved in NMDAR signaling, such as the ARC complex and fragile X mental retardation protein (FMRP) regulated genes (Fromer et al., 2014a). Each of these pathways is critical to the normal development and function of glutamatergic synapses (Lau and Zukin, 2007; Shepherd and Bear, 2011). Finally, gene expression profiling of human postmortem brains shows alterations in the expression of genes associated with synaptic transmission in SCZ patients (Mistry et al., 2013), further supporting a role for the glutamatergic synapse in the disorder.

Integrating human postmortem, imaging and genetic studies spanning the last three to four decades, there is strong evidence that SCZ is, at least in part, a disease of abnormal glutamatergic synapse formation, maintenance and function. Structural studies indicate that there is likely excessive synaptic elimination during a critical period of development that leads to a relative deficit of synaptic spines while genetic and postmortem studies suggest that abnormalities in both pre- and post-synaptic proteins are likely involved if not causative in this process. Despite increased awareness of the various genes or genetic loci linked with these disorders, we have a poor understanding of the dynamic structural and functional changes that these genes bring about in human neurons during disease development.

3. Autism and the synapse

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in communication and socialization along with repetitive or restrictive behaviors (DSMV, 2013). The developmental onset of pathology is still not clear. Symptoms can become evident at 6 to 12 months of life, although diagnosis is often not made until the 4th year of life (Ozonoff et al., 2010). It has a frequency of ~0.5–1% of the population (Lai et al., 2014) and is often associated with other neurodevelopmental disorders, including intellectual disability and epilepsy (Huguet et al., 2013).

Similar to SCZ, many imaging studies have attempted to elucidate structural abnormalities to explain the characteristic clinical symptoms manifest in ASD. Structural MRI analysis demonstrates that in younger children with ASD (2–5 years of age) there is an increase in cerebral gray and white matter volume that is not seen in older adolescents and adults (Courchesne et al., 2001; Carper and Courchesne, 2005). This has led to the hypothesis that ASD is an result of early brain overgrowth, followed by decreased growth or even accelerated volume loss at later time in life (Courchesne et al., 2011; Lange et al., 2015; Zielinski et al., 2014; Wallace et al., 2010). The structural changes are most consistently prominent in the frontal and temporal lobes (Schumann et al., 2010; Carper and Courchesne, 2005; Foster et al., 2015) although variable gray and/or white matter changes have also been reported in other regions including the amygdala, hippocampus, fronto-parietal regions, basal ganglia and cingulate (Ameis and Catani, 2015; Ecker et al., 2015).

Functional imaging techniques, including functional MRI and positron emission topography (PET), have also been used in order to better understand changes in the brains of ASD patients. As with the structural studies, there has been significant variability among results reported from different groups (Ecker et al., 2015). However, an underconnectivity hypothesis is emerging from these studies that suggests a decrease of long-range functional connectivity between the frontal and posterior neural networks (Just et al., 2012). The collective structural and functional studies to date suggest that ASD is a disorder of dysregulated brain growth and connectivity.

In its non-syndromic forms (i.e. not associated with other neurodevelopmental or systemic abnormalities) there are various proposed etiologies for ASD. These include prenatal or post-natal environmental insults, immune system dysfunction, or inadequate psychosocial interactions (Levy et al., 2009). As is the case for SCZ, there is also strong evidence for a genetic basis to the disorder and the heritability for ASD is estimated to be at 50% (Sandin et al., 2014). Approximately 10% of ASD patients carry a novel CNV (Sebat et al., 2009) with 5% of cases carrying loss of function sequence variations in individual genes identified through WES (De Rubeis et al., 2014).

ASD or autistic features are also present in multiple single gene genetic syndromes. Notable single gene syndromic forms of ASD include Fragile X syndrome (FXS), Tuberous sclerosis complex (TSC), and Rett Syndrome. FXS is an X-linked disorder resulting from an expansion of a CGG repeat in the *FMR1* gene that leads to loss of function of the gene and intellectual disability, autistic features, specific facial features, macro-orchidism, and a high prevalence of epilepsy in males, with more subtle symptoms in females. TSC results from a loss of function mutation in either the *TSC1* or the *TSC2* gene and can cause ASD, cognitive impairments and multiple tumor types in the brain and other organ systems. Rett Syndrome is caused by mutations in the *MECP2* gene on the X chromosome. Mutations of *MECP2* are generally lethal in males and result in developmental regression, ASD, intellectual disability, seizures, microcephaly and breathing abnormalities in affected females. Although each of these disorders only accounts for 1–2% of cases of ASD (Kumar and Christian, 2009), they have been particularly important in our understanding of the underlying pathophysiologic processes in ASD due the availability of genetic manipulation in animal models.

Much of what we have learned about the underlying pathophysiology for ASD has come from postmortem and genetic studies in humans and from genetically manipulated animal models. The onset of ASD is much earlier than SCZ, mostly within the first few years of life coinciding with a period of rapid synapse formation and maturation, and prior to the period of spine elimination (Huttenlocher, 1979). Interestingly, in contrast to the decreased synaptic density found in the brains of patients with SCZ, a postmortem study of non-syndromic autistic brains shows increased glutamatergic synaptic spine density (Hutsler and Zhang, 2010). Similarly, in a postmortem study of patients with FXS, in which 20–60% of patients display autism or autistic features, there is an increase in long spines with an immature morphology (Irwin et al., 2001). Similar findings are being reported in animal models of FXS (Comery et al., 1997) as well as TSC (Tang et al., 2014). An increase in spine number and immature spines suggests impaired maturation and pruning. In contrast, in Rett syndrome, a significant monogenic cause of ASD in females, synaptic spine density is lower in cortical and hippocampal regions in both Rett syndrome patients (Belichenko et al., 1994) and animal models (Fukuda et al., 2005; Belichenko et al., 2009; Tropea et al., 2009), suggesting that there might exist two different synaptic phenotypes in ASD or that there might be synaptic degeneration that is unique to Rett syndrome.

Further support for abnormalities in synaptogenesis comes from enrichment analysis of genetic studies in humans. Many of the genes identified by the recent large whole exome sequencing study of ASD patients encode proteins involved in synaptic formation (De Rubeis et al., 2014). Interestingly, 17 of the 107 genes identified in this study demonstrated overlap with studies conducted on patients with SCZ (Table 2). These include *SCN2A*, *RELN*, *NRXN1* and *SHANK3*, all of which have known functions in glutamatergic synaptic transmission (Fromer et al., 2014a; De Rubeis et al., 2014). The latter two genes have also been previously implicated in ASD and are involved in synapse formation and maturation (Sudhof and Malenka, 2008). This convergence of genes involved in the synapse is also found in studies of CNVs in ASD, with overrepresentation of CNVs that disrupt genes associated with the FMRP network and the post synaptic density (PSD) (Pinto et al., 2014).

Taking the genetic and structural data together, both ASD and SCZ seem to be disorders that are characterized, at least in part, by impaired glutamatergic synaptic development and maturation, despite the fact that these are clinically distinct disorders. The abnormalities seem to arise at different time windows of synaptic development and are associated with divergent synaptic phenotypes in postmortem studies (Fig. 1 and Table 1). ASD arises during a period of rapid synapse generation and maturation with postmortem studies demonstrating supernumerary synapse formation, while SCZ onset occurs at the end of the period of synaptic pruning and is characterized by decreased synaptic density (Fig. 1a and Table 1). However, there is significant overlap in both candidate genes and signaling networks involved (Table 2). To address how these changes occur requires a technique that allows for the dynamic evaluation of synaptic development and maturation in the setting of a relevant human genetic background.

4. iPSC studies in schizophrenia and autism

In the past, study of human neurodevelopmental disorders at cellular and molecular levels has been limited by the fact that the direct investigation of human brain cells cannot be undertaken without risk of significant harm to patients affected by these disorders. As a result, we have been relying heavily on postmortem and animal studies in order to better understand these disorders. While these two approaches have led to substantial knowledge and provide important insight into human brain biology and pathology, both of these techniques have inherent limitations. Postmortem tissue is, by definition, not living and is not capable of being manipulated to test experimental hypotheses. There is an inherent risk of variability in postmortem fixation methods, storage duration and storage conditions that can introduce experimental

Table 1
Comparison between schizophrenia and autism spectrum disorder phenotypes in human and induced pluripotent stem cell derived neural cells.

	Schizophrenia	Autism spectrum disorders
Human		
Onset	Adolescence–young adulthood	Late infancy–early toddlerhood
Structural imaging	Volume loss in prefrontal and temporal cortices Gray and white matter implicated	Early increases in volume of frontal and temporal lobes Possible accelerated decreases later Gray and white matter implicated
Functional imaging	Altered short distance connectivity in frontal temporal and parietal lobes	Altered long distance connectivity between frontal and posterior brain regions Decreased probability for activation of social emotional structures
Postmortem synapse phenotype	Decreased synaptic spine density — "excessive dendritic spine pruning" Decreased expression of synaptic proteins	Increased synaptic spine density* — "insufficient pruning" Immature appearing spines *Decreased synaptic spines in Rett syndrome
iPSC		
Pluripotency of iPSCs	Unaffected	Unaffected
Progenitor phenotypes	Unaffected in some studies Genotype specific morphology changes, late stage differentiation and organizational abnormalities	Early increases in proliferation in organoid models
Neuronal differentiation	Technique and/or genotype specific variation in production of neuronal subtypes	Early terminal differentiation in organoid cultures
Synaptic phenotypes	Decreased glutamatergic synaptic density Presynaptic glutamatergic dysfunction at synapses	Decreased glutamatergic synaptic density Presynaptic glutamatergic dysfunction Immature appearing synapses Increased GABAergic synapses in organoid models
Functional connectivity	Decreased	Decreased

Table 2
Examples of overlapping genes between schizophrenia (SCZ) and autism spectrum disorders (ASD) identified by whole exome sequencing. Synaptic genes are in bold. Adapted from de Rubeis et al., 2014.

Overlapping ASD and SCZ genes by WES
CUL3
NRXN1
SCN2A
ARID1B
SHANK3
SYNGAP1
MLL3
RELN
ASH1L
MYT1L
POGZ
BIRC6
PTPRM
C11ORF30
CD163L1
MYH10
AXL

confounds (Mistry and Pavlidis, 2010). Additionally, if pathology is discovered, it is difficult to determine if these are indicative of the causal pathology or changes due to associated comorbidities or attempts to treat the disease that accumulate over time.

Animal models can be both genetically and pharmacologically manipulated, provide the ability to examine many aspects of neurogenesis and synaptogenesis in controlled conditions, and have contributed greatly to our understanding of both ASD and SCZ. However, when behavioral, affective and cognitive changes are a primary phenotype of the disorder in question, animal models may not always be able to recapitulate the human phenotype (Jones et al., 2011). In addition, although single genes with large effect can be manipulated in animal models, many of the genetic abnormalities found in patients with these disorders are large CNV's or multiple single gene mutations that may be technically impossible or too labor intensive to experimentally manipulate. Single gene changes cannot recapitulate the polygenic inheritance patterns that characterize these disorders (O'Tuathaigh and Waddington, 2015) and information may be lost due to single gene changes being made outside of the background genetic context of an affected patient.

Until recently, we have lacked a model in which the structural and functional properties of clinically affected human patients' neurons could be interrogated. The first successful demonstration of reprogramming somatic cells into cells with pluripotent potential (termed induced pluripotent stem cells or iPSCs) was done using mouse fibroblasts (Yamanaka and Takahashi, 2006). This study was rapidly followed by multiple successful attempts to generate iPSCs using human fibroblasts, keratinocytes and lymphocytes (Aasen et al., 2008; Brown et al., 2010; Yu et al., 2007; Takahashi et al., 2007; Staerk et al., 2010; Seki et al., 2010; Lowry et al., 2008; Loh et al., 2010; Chiang et al., 2011). Although there are now several methods with relative benefits and pitfalls that can be used to generate iPSCs (reviewed in (Juopperi et al., 2011), they all have the potential to differentiate into any cell type of the three germ layers and share fundamental properties with human embryonic stem cells. The development of techniques to generate specific subtypes of neurons from patient derived iPSCs has provided a model for the study of dynamic human synaptogenesis in neurodevelopmental diseases.

To date, several studies have used iPSCs to model SCZ and ASD. These studies have helped to confirm a role for synaptic abnormalities in the pathogenesis of these disorders that has been suggested by the structural changes found in postmortem and animal studies. More importantly, they offer possible mechanistic explanations for those changes and the potential for the development of more specific treatments for SCZ and ASD based on the underlying biology.

4.1. Schizophrenia

4.1.1. Phenotypic analysis of neural development using iPSCs

The first few studies demonstrating the generation of iPSCs from patients with SCZ were published in 2011 (Chiang et al., 2011; Brennand et al., 2011; Pedrosa et al., 2011). Some of the SCZ cases described demonstrated familial inheritance and others were described as idiopathic, although it is likely that many of these idiopathic patients may also carry risk-associated alleles. Moreover, different underlying genetic alterations in patients with SCZ may affect neural structure and function through similar mechanisms or pathways.

Reprogramming techniques varied between three initial studies, but each was able to demonstrate pluripotency of resultant iPSCs based on teratoma formation and the ability to be induced into cell types of the three different germ layers. None of the studies reported to date have shown intrinsic deficits in iPSC pluripotency or self-renewal when derived from SCZ patients compared to controls. This is consistent with the fact that SCZ does not have large effects on embryogenesis or affect multiple organ systems. Additionally, there was no difference found between patient and control iPSCs in terms of induction, quantity or morphology.

Brennand and colleagues (Brennand et al., 2011) investigated the phenotype of neural progenitor cells (NPCs) generated from iPSCs derived from control and idiopathic SCZ patients. Similar to later studies (Wen et al., 2014; Topol et al., 2015; Yoon et al., 2014), they did not observe any SCZ-specific changes in the quantity of NPCs generated from iPSCs. However, one study has demonstrated morphologic differences in NPCs between SCZ and control patients and a delay in differentiation of NPCs from early to late progenitors (Robicsek et al., 2013) while a second has demonstrated abnormal organization of NPCs that is dependent on a CNV associated with risk for SCZ (Yoon et al., 2014).

Differentiation into neurons was similar between control and SCZ patient-derived cells in terms of timing and quantity of derived neurons and there was no difference in the ratio of glutamatergic, GABAergic or dopaminergic cells between controls and patient samples in at least three studies (Brennand et al., 2011; Topol et al., 2015; Hartley et al., 2015). The Robicsek study reported defects in dopaminergic differentiation of SCZ patient derived iPSCs (Robicsek et al., 2013), while a second study demonstrated increased dopaminergic survival with elevated catecholamine secretion (Hook et al., 2014). It is unclear whether these differences are methodological or a result of a patient-specific phenotype, but it should be noted that in the Robicsek study, cell lines were derived from keratinocytes rather than fibroblasts and that dopaminergic neurons were directly differentiated from iPSCs without first forming neural progenitor cells. It is possible that this technique amplifies subtle differences that may not be appreciated using other differentiation methods. Notably, in the study by Hartley et al., the argument is made that differences in patient populations and treatment histories may be important. In addition, they suggest that specific patterning towards a midbrain dopaminergic (DA) fate is needed to recapitulate changes in the DA neuronal subtypes most likely to be affected in SCZ (Hartley et al., 2015).

Pedrosa and colleagues found a delay in the loss of the pluripotency gene, *OCT4*, when inducing neuronal differentiation (Pedrosa et al., 2011). However, this study was different from others in that they directly induced neurons from iPSC-derived embryoid bodies following attachment to laminin plates, in the absence of FGF2 and with WNT3A supplementation. This technique bypasses the generation of neural rosettes containing NPCs. Additionally, the patient-derived iPSCs were haploinsufficient for a gene that has previously been shown to be required for silencing *Oct4* in animal models (Melton et al., 2010). A significant alteration in the loss of pluripotency might be explained by a gene specific effect of their model.

Using RNA sequencing in NPCs, a recent study (Topol et al., 2015) found increased β-catenin, the ultimate effector of WNT signaling, as well as increased WNT activity in NPCs from SCZ patients. Despite WNT signaling involvement in neural proliferation and migration, there were no changes in proliferation or migration of NPCs noted in this study. Neuronal differentiation or synaptic functions were not examined in this study, although WNT signaling plays a role in these processes (Rosso and Inestrosa, 2013) and further study is warranted.

4.1.2. Phenotypic analysis of synaptic development of iPSC derived neurons

The development and maturation of synaptic properties of human neurons generated from patient iPSCs have been the focus of most of the studies and have provided important insight into the potential pathological development in SCZ. Although they did not see changes in electrophysiological properties, the first observation by Brennand et al. (2011) was that there was a difference in the functional connectivity between control and schizophrenia patient neurons based on an assay using rabies virus spread between neurons. The rabies virus is spread via synaptic contacts, thus allowing assessment of the connectivity among neurons (Ugolini, 2008). As such, decreased spread of the virus between neurons suggests decreased synaptic strength. Interestingly, this phenotype could be ameliorated by the antipsychotic medication, loxapine, but not by other antipsychotics such as clozapine, risperidone or olanzapine. The effect of loxapine on connectivity suggests that

dopamine or serotonin receptor blockade alters synaptic strength in cultures or that there might be another, more direct effect of loxapine on glutamatergic synapses. As their cultures contained ~10% dopaminergic neurons, this mechanism would need to be further clarified using more pure glutamatergic cultures. In addition to decreased connectivity, they found a decrease in the number of neurites and PSD 95 protein in cells derived from patients with SCZ. This is in agreement with the reduced dendritic arborization that is seen in some postmortem SCZ patient brains and suggests postsynaptic dysfunction at least on the structural level.

A SCZ-specific defect in glutamatergic synaptic maturation has since been reported (Robicsek et al., 2013; Wen et al., 2014). Wen and colleagues approached the question of synaptic development in SCZ using patient-derived iPSCs from an American family (Pedigree H) in which SCZ, bipolar disorder and major depression segregate with a 4 base-pair frameshift deletion in the *DISC1* gene (Chiang et al., 2011; Sachs et al., 2005; Wen et al., 2014). DISC1 localizes to both presynaptic and postsynaptic sites, as well as other subcellular localizations (Soares et al., 2011) and is involved in multiple signaling pathways mediated by NMDA, GABA, GSK3β and WNT (Faigle and Song, 2013; Duan et al., 2007). Using patient-derived iPSCs, Wen and colleagues provided evidence that forebrain-specific glutamatergic neurons from patients carrying the *DISC1* mutation exhibited decreased $SV2^+$ synaptic boutons, suggesting fewer functional glutamatergic synapses. Electrophysiological characterization further showed decreased frequency, but not amplitude, of excitatory spontaneous synaptic currents indicating a presynaptic deficit. This was supported by direct live cell imaging analysis of synaptic vesicle release. The authors further showed that the presynaptic deficits could be rescued in isogenic cell lines in which the mutation was corrected, and the deficits could be induced with an introduction of the mutation into control lines, thus supporting a causal role for the mutant DISC1 protein in synaptic dysregulation. Together, these results provide direct evidence of deficits in presynaptic function at glutamatergic synapses of human neurons from SCZ patients.

Similar to the results from the DISC1 mutant neurons, Yu et al. (Yu et al., 2014) found evidence for presynaptic dysfunction in iPSC derived neurons from four patients with idiopathic and familial SCZ. In this study, they used a hippocampal granule neuron differentiation protocol and successfully demonstrated that these cells expressed granule cell markers after 4 weeks of differentiation. However, for the SCZ patient cells, there was a reduction in the number of cells expressing the postmitotic neuron marker, TBR1, as well as late stage markers of neuronal maturation, NEUROD1 and PROX1, suggesting defects in the development of granule neurons from the NPC stage. PROX1 expressing neurons from both control and SCZ derived iPSCs were able to fire action potentials and had normal current voltage relationships for both potassium and sodium currents suggesting functional neurons were generated in both conditions. However, when these cells were functionally assayed, there was a decrease in the percentage of active cells based on calcium imaging. There was also a decrease in both the amplitude and frequency of excitatory postsynaptic currents in the SCZ patient cells compared to the control cells and thus a decreased probability and magnitude of presynaptic vesicle release. Remarkably, despite different genetic backgrounds, the neurons derived from patients with SCZ in both studies demonstrate similar phenotypes of presynaptic release deficits. This indicates that different genetic or environmental etiologies can result in similar synaptic phenotypes and lends support to the synaptic hypothesis of SCZ. At the same time these findings suggest a common target for therapeutic modification in patients with diverse etiologies.

4.1.3. Transcriptional regulation of synapse-related genes

In postmortem studies, analyzing gene expression in the human brain may be a more sensitive method to detect molecular changes involved in the process of neurodevelopment than conducting somatic genetic analysis in complex genetic disorders. In patients, where direct study of the brain is not possible, gene expression profiling of cells along the neural developmental trajectory from iPSCs, especially neurons, will provide specific insight into the transcriptional and translational states that may be leading to functional neuronal abnormalities. Most of the SCZ iPSC studies to date have examined SCZ-dependent gene expression variation using iPSC models.

RNA sequencing analysis from *DISC1* mutant iPSC-derived glutamatergic neurons in the study by Wen et al. demonstrated differential changes in genes enriched for synaptic spines, nervous system development and synaptic transmission when compared to controls. Genes related to SCZ, bipolar disorder and depression were also altered. The mutant DISC1-dependent expression of multiple presynaptic proteins suggests a novel DISC1 mechanism bringing about synaptic dysfunction through transcriptional dysregulation (Wen et al., 2014). Microarray analysis of neurons generated from SCZ patients in the study by Brennand et al. demonstrated altered expression of multiple genes involved in cAMP, WNT and glutamate receptor signaling — all three pathways important for the development and proper functioning of neuronal synapses, which is also recapitulated in neurons with the DISC1 mutation. Additionally they found consistent changes in some previously reported SCZ associated genes in all lines as well as some genes with differential expression in some but not all of the iPSC lines when compared to controls. Included in those genes that were misexpressed in some lines was *DISC1*. RNA transcripts from the *NRG1* gene, a SCZ susceptibility gene (Harrison and Law, 2006), was increased in all SCZ neuronal lines, but not in progenitors, emphasizing different expression profiles are cell type and developmentally specific.

Expression analysis in iPSC-derived neurons provides several possibilities to identify common pathways that may provide strong evidence for a therapeutic target. A recent study of neurons derived from patients with syndromic SCZ due to a 22q11.2 deletion, as well as neurons from patients with childhood onset SCZ, found that there was differential expression of several miRNAs between control and SCZ-derived cells (Zhao et al., 2015). MiRNAs are small RNA molecules that can interact with specific mRNAs to induce degradation and in so doing, regulate protein expression (Bartel, 2009). In this study, the targets of the differentially expressed miRNA were significantly enriched for SCZ genes and specifically for genes involved in synapse formation. Of the specific targets noted, *DISC1*, *GSK3β* and *NRXN1* as well as several genes involved in glutamatergic signaling are particularly interesting and support a hypothesis that different mechanisms (ie. altered transcription vs. altered mRNA degradation and translation) may affect common pathways in the pathogenesis of SCZ.

As illustrated in Fig. 2 and consistent with what is seen in human genetic and postmortem studies, many lines of evidence in iPSC studies seem to converge on the glutamatergic synapse. Deficits of early development of neural progenitors appears to be genotype specific, and it is possible that there are genotype or technique-dependent changes in the differentiation and survival of select neuronal subtypes. On the other hand, neurons derived from patient iPSCs demonstrate consistent changes in neuronal structure and synapses in all studies in which this was assayed (Fig. 1b). Although most of the studies to date have used unique patients with distinct genetic backgrounds, gene expression analysis appears to converge either on the proteins or the pathways regulating both presynaptic and postsynaptic structure and function.

4.2. Autism

Several studies to date have examined the synaptic connectivity in neurons derived from ASD patient iPSCs. These have, however, predominately been conducted on cells from patients with syndromic forms of the disorder. In contrast to human and animal studies showing increased synaptic density in some, but not all forms of ASD with known genetic etiologies, the iPSC studies of syndromic ASD to date are more consistent. There seems to be a consensus of decreased synaptic connectivity with fewer synapses, and synapses that are

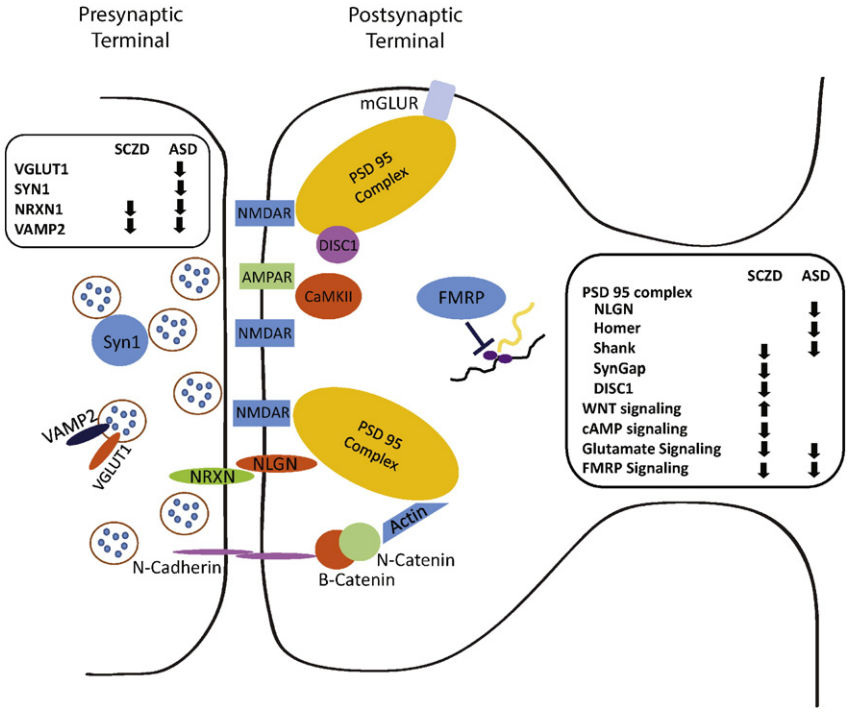

Fig. 2. Synaptic protein dysregulation in iPSC models of schizophrenia and autism. Studies to date demonstrate both presynaptic and postsynaptic protein expression changes in both schizophrenia and autism. Many of these proteins have been implicated in the disorders by genetic and postmortem studies.

more immature in their morphology on differentiated neurons (Fig. 1b). Surprisingly, this is a similar finding to what has been observed in neurons from SCZ patients.

Initial studies in which neurons were generated from Rett patient iPSCs demonstrated decreased cell body size (Marchetto et al., 2010) (Cheung et al., 2011; Ananiev et al., 2011) Much like postmortem and animal studies of Rett syndrome, neurons generated from 3 different patient iPSC lines consistently showed decreased numbers of synapses, decreased synaptic density and decreased connectivity. These changes were limited to excitatory glutamatergic synapses based on decreased amplitude and frequency of spontaneous excitatory post synaptic currents (EPSCs) and decreased VGLUT1 puncta (Marchetto et al., 2010). Treatment with insulin-like growth factor 1 (IGF1) markedly increased the number of glutamatergic synapses (Marchetto et al., 2010). This is particularly interesting in light of animal studies demonstrating that treatment with IGF1 at least partially rescues the Rett phenotype and extends survival (Tropea et al., 2009).

Phelan–McDermid Syndrome (PMDS) is another syndromic cause of ASD resulting from deletion of the 22q13 locus. IPSC-derived neurons from patients with PMDS demonstrated increased input resistance and decreases in the frequency and amplitude of AMPA and NMDAR mediated EPSCs (Shcheglovitov et al., 2013). This corresponded to a decrease in the protein levels for these two receptors as well as decreased HOMER1$^+$ (post synaptic) and SYN1$^+$ synaptic (presynaptic) puncta. This phenotype could partially be rescued by increased expression of SHANK3, a postsynaptic density protein implicated in both autism and schizophrenia and a genetic locus within the 22q13 region. Strikingly, treatment of these neurons with IGF1 also rescued both the decrease in HOMER$^+$ SYN1$^+$ puncta and the glutamatergic synaptic transmission deficits.

IPSCs from a non-syndromic patient with ASD who carries a translocation disrupting the TRPC6 cation channel, demonstrated similar changes. Namely, neurons differentiated from these cells had decreased VGLUT1$^+$ synaptic puncta and decreased neuronal arborization (Griesi-Oliveira et al., 2014). Treatment with IGF1 rescued TRPC6 protein levels as well as synapse number, further supporting a common pathway in genetically distinct patients. Similar effects of IGF1 treatment in different ASD models suggests that targeting the glutamatergic synapse, independent of the underlying genetic defects, may be a potential therapeutic pathway in humans.

In contrast to mouse models and postmortem studies suggesting increased spine density in FXS (Irwin et al., 2001; Comery et al., 1997), iPSC models have shown fewer and shorter spines (Sheridan et al., 2011) and impaired synaptic outgrowth (Doers et al., 2014). The decrease in synaptic spine density and relative immaturity of synapses is similar to findings in other syndromic forms of ASD (Fig. 1b). This contradiction is difficult to explain, but may emphasize the influences of developmental interactions and network integration on synaptic development and possible in vivo compensatory mechanisms to correct synaptic deficits (i.e. increased synapse number to compensate for immature, less functional, synapses).

Differences in spine density between human postmortem studies and in vitro data from iPSC-derived neurons could also be intrinsic to a 2 dimensional (2D) cell culture format itself. A recent study (Mariani et al., 2015) attempted to overcome this using 3 dimensional (3D) cultures. In this study, iPSCs from non-syndromic, idiopathic patients with ASD and large head circumference were used to generate telencephalic organoids. This technique relies on a free-floating culture method to grow neural progenitor cells, which results in a 3D aggregate of cells that autonomously organizes into a layered structure of radial glial cells, intermediate progenitors and, eventually, neurons (Mariani et al., 2012). The 3D organization of these cultures likely better recapitulates normal human development by allowing for cell–cell interactions. Mariani et al. found that in the organoid model of ASD, iPSC-derived NPCs exhibit decreased cell cycle length as well as increased expression of genes related to proliferation and neural stem cell fates early in culture, but not at later stages of development. This return to standard cell cycle lengths was accompanied by upregulation of genes required for neuronal differentiation. Among the genes with greatest differential expression in the ASD organoids were those related to cytoskeletal regulation, synaptic assembly, ion transport and postsynaptic signaling, as well as genes such as NRXN1 and SCN2A that have previously been implicated as autism risk genes (De Rubeis et al., 2014). Interestingly, they found a significant increase in SYN1$^+$ presynaptic puncta, which is

seemingly consistent with the overgrowth hypothesis and the postmortem studies demonstrating increased synaptic spine density (Hutsler and Zhang, 2010). Surprisingly, they showed that the increase of SYN1 is due to an increase of inhibitory pre-synaptic puncta marked by VGAT, but not by VGLUT1 (expressed on excitatory glutamatergic synapses), a result based on the elevated numbers of inhibitory neurons and their precursors in the ASD organoids. In this study, the postsynaptic spine densities were not determined, although at least one NMDA receptor subunit, GRIN1, was found to be upregulated by RNA sequencing. As synaptic spines are generally associated with excitatory synapses and abnormalities in their structure and density are a major phenotype in brains of human ASD patients, direct assessment of dendritic spines would help to clarify this.

In light of the overwhelming evidence from somatic exome sequencing and chromosomal microarray studies indicating alterations in genes involved in the synapse, and significant synaptic phenotypes in iPSCs-derived neural cells, only a few studies to date have analyzed alterations in gene expression in syndromic iPSC models of ASD. IPSC generated neurons from patients with Timothy syndrome, a syndromic form of ASD associated with a calcium channel mutation, demonstrate gene expression changes enriched for ASD susceptibility genes and upregulated in ASD postmortem brains (Tian et al., 2014). Similarly, in a study of iPSC-derived neurons from patients with syndromic ASD secondary to 15q11.2 duplication, transcriptome analysis indicated down regulation of ASD risk genes including *SHANK1* and *NLGN1* (Germain et al., 2014). RNA sequencing analysis of the non-syndromic ASD organoids suggests that there is dysregulation of many of the same genes found in human studies (Mariani et al., 2015). Future analysis of RNA expression of iPSC-generated neurons from other genetic backgrounds may help to further delineate the underlying etiologies of the disorders.

Despite the fact that in non-syndromic ASD and some syndromic forms, the most striking human postmortem phenotype is an increase in glutamatergic synaptic spines, the iPSC studies to date suggest, in contrary, a decrease in glutamatergic synaptic function and possibly decreased synapses, although synaptic spine density has not been directly studied. The available transcriptional analysis has, however, been more consistent with human genetic and postmortem analysis and demonstrates dysregulation of genes involve in glutamatergic synaptic maturation and function (Fig. 2), including members of the SHANK, NRXN and NLGN families, which have also been implicated in SCZ (De Rubeis et al., 2014).

5. Modeling complex neurodevelopmental diseases

Human genetic, structural and functional imaging and postmortem studies have provided evidence supporting a role for abnormal glutamatergic synapse development, maturation and function in both SCZ and ASD. Clinically, the two disorders manifest at different ages with ASD becoming clinically detectable in early childhood and SCZ becoming apparent in adolescence and early adulthood. At the macrostructural and network level, the frontal and temporal lobes and altered connectivity appear to be important in both disorders (Table 1) (Cannon et al., 2002; Alexander-Bloch et al., 2013; Ameis and Catani, 2015). At the microstructural level there are divergent but significant changes in glutamatergic synapses with increased dendritic spines in human ASD brains and decreased spines in SCZ brains (Fig. 1a) (Konopaske et al., 2014; Hutsler and Zhang, 2010). These changes are notably coincident with the clinical changes occurring at the onset or during and after the critical period when synaptic spines are eliminated in ASD and SCZ respectively. One possible explanation for pathologic changes in these disorders is divergent dysregulation of synaptic pruning. However the underlying changes at the molecular and cellular levels are poorly understood.

Over the past few years, various genetic studies in humans and in human postmortem tissue have attempted to address the underlying pathology in these disorders based on gene expression regulation. Consistent with human postmortem studies showing dendritic spine abnormalities, these have consistently implicated genes involved in synaptic development, structure and function in both disorders. Remarkably, despite the clear clinical, structural and pathologic differences between the two disorders, many of the genetic studies to date have found dysregulation of genes involving glutamatergic synapses in common between these two disorders (Table 2) (De Rubeis et al., 2014; Pinto et al., 2014; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Therefore, these genetic studies support the neural development origin of both ASD and SCZ. On the other hand, mutations in similar genes, or even in the same gene, can result in different phenotypes, which calls for future studies on gene regulatory sequences that may regulate gene expression in a context dependent manner and in response to environmental factors.

Instead of static, or snapshot views by postmortem or imaging studies, iPSC-derived neural cells provide a model system to study dynamic structural and functional changes in human cells from affected patients. Although these cells cannot fully recapitulate a complex in vivo system as is possible in animal models, they provide the advantage of patient-specific genetic backgrounds that are likely to predispose to disease. Multiple studies to date have evaluated iPSC-derived neural cells in the context of ASD or SCZ clinical phenotypes. Despite various genetic backgrounds of the patients from which the cells were derived, they have collectively demonstrated a common phenotype of decreased connectivity and functionality of glutamatergic synapses in both disorders (Table 1) (Wen et al., 2014; Brennand et al., 2011; Yu et al., 2007; Marchetto et al., 2010; Shcheglovitov et al., 2013; Griesi-Oliveira et al., 2014; Doers et al., 2014). Further, expression analyses of iPSC-derived neurons from both disorders have found altered gene regulation of genes involved in the development, structure, and function of synaptic spines (Fig. 2) and many shared genes and pathways that have been identified in human genetic studies.

Interestingly, despite different synaptic phenotypes observed in postmortem tissue from SCZ and ASD patients, with a decrease and increase in dendritic spines respectively, iPSC-derived neurons from both disorders suggest decreased glutamatergic synapses and synaptic function (Fig. 1 and Table 1). The apparent discrepancy between human postmortem brains and iPSC-derived neurons in ASD models may be related to in vivo neuronal integration differences or developmental timing of the abnormalities. One could hypothesize that the development of nonfunctional or inappropriate maturation of synaptic spines leads to a compensatory increase in patients, while there is a lack of compensation in a dish. Further studies using 3D organoid cultures may help to resolve this. Of similar importance, establishing and validating protocols that generate neurons with region and subtype-specific characteristics will be critical in revealing cell-type specific and convergent phenotypes.

Although we have focused on genetic susceptibilities in this review, it should be emphasized that both SCZ and ASD are considered to be multifactorial disorders and, with exceptions for syndromic forms of the disorders, such as Rett syndrome where the genetic abnormalities are known to induce widespread disruption of neuronal development, the genetic changes in individuals with SCZ and ASD likely act as risk factors in combination with other factors. In both disorders, environmental influences such as maternal stress, inflammation, or even toxic exposure and postnatal factors are proposed to contribute to the underlying mechanisms (Rapoport et al., 2012; Levy et al., 2009; Ishii and Hashimoto-Torii, 2015). In humans, how individual environmental stressors interact with particular genetic backgrounds to disrupt appropriate developmental processes may be difficult to determine. IPSCs offer an experimental model in which the effects of various environmental stressors can be tested on neuronal development in the context of patient-specific genetic backgrounds. At least one study has looked at differential sensitivity to environmental stressors in NPCs derived from SCZ or control iPSCs (Hashimoto-Torii et al., 2014), how synaptic development is affected, however, has not been examined to date. As

synaptic spines are modulated by experience in human development (Caroni et al., 2012), further study on the effects of environment in the context of genetic susceptibility on synapse formation and functionality may help to elucidate pathologic processes in these disorders and possibly reconcile differences observed between human postmortem tissue and cultured neurons.

Glutamatergic synaptic abnormalities have been implicated in the pathogenic processes of iPSC-derived neurons in both ASD and SCZ. Some of the most striking findings from these studies have been those that indicate potential mechanisms to correct the abnormal synaptic phenotypes. In the study by Wen et al. (2014), isogenic correction of the DISC1 mutation resulted in correction of synaptic abnormalities. Brennand et al. (2011) demonstrated improvement in the SCZ-specific connectivity deficits when neurons were treated with loxapine, a known antipsychotic drug. In two different genetic models of ASD, treatment with IGF1 resulted in correction of the decreased glutamatergic synapses (Marchetto et al., 2010; Shcheglovitov et al., 2013) and in one animal model this translated into an increased survival rates (Tropea et al., 2009). These studies exemplify ways in which iPSCs can be used to screen for potential therapeutic targets, either by identifying critical gene targets or manipulating crucial molecular pathways (Fig. 3). On the other hand, molecules, drugs or genetic manipulations can be screened in multiple iPSC lines to determine where individual pathways intersect to bring about phenotypes.

The proposed environmental and genetic etiologies for both ASD and SCZ are diverse and numerous. Hundreds of genes and genomic regions have been implicated in these disorders, yet the clinical phenotypes and pathologic findings in humans remain relatively consistent. This suggests that these risk factors converge on common pathways at cellular and circuitry levels. As it may be logistically impossible to treat each individual's unique underlying abnormality at the present time, understanding what common networks are involved and the molecular and structural phenotypes that these bring about is essential for the rational design of future therapeutics.

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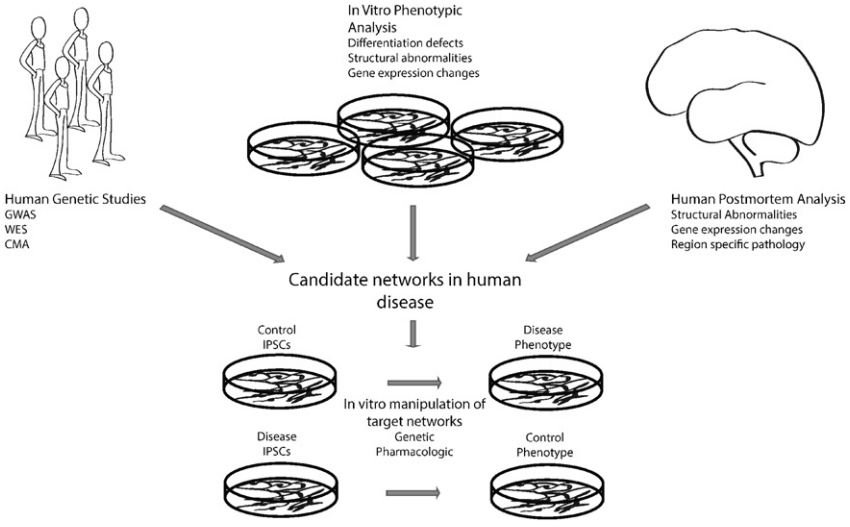

Fig. 3. iPSCs as a tool to identify therapeutic targets in autism spectrum disorder and schizophrenia. Analysis of multiple genetically distinct schizophrenia and autism patient-derived cells may demonstrate common cellular phenotypes. These are more likely to arise from disruption of common networks rather than a single genetic endpoint. Using a combination of human genetic analysis, postmortem studies and iPSC-derived neurons, a more focused network of related proteins or group of networks may be identified based on the intersection of data. In turn, iPSC-derived neurons can be used to independently manipulate those networks in an attempt to recapitulate the pathophysiology or rescue the phenotype before further study in animals and humans.

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