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GENETICS AND NEUROSCIENCE (B MAHER, SECTION EDITOR)

Using Induced Pluripotent Stem Cells to Investigate Complex Genetic Psychiatric Disorders

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Abstract
Purpose of Review Induced pluripotent stem cells (iPSCs) can be generated from human patient tissue samples, differentiated into any somatic cell type, and studied under controlled culture conditions. We review how iPSCs are used to investigate genetic factors and biological mechanisms underlying psychiatric disorders, and considerations for synthesizing data across studies.
Recent Findings Results from patient specific-iPSC studies often reveal cellular phenotypes consistent with postmortem and brain imaging studies. Unpredicted findings illustrate the power of iPSCs as a discovery tool, but may also be attributable to limitations in modeling dynamic neural networks or difficulty in identifying the most affected neural subtype or developmental stage.
Summary Technological advances in differentiation protocols and organoid generation will enhance our ability to model the salient pathology underlying psychiatric disorders using iPSCs. The field will also benefit from context-driven interpretations of iPSC studies that recognize all potential sources of variability, including differences in patient symptomatology, genetic risk factors, and affected cellular subtype.

Keywords iPSCs · Schizophrenia · Bipolar disorder · Autism spectrum disorders · Psychiatric · Cellular reprogramming

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Introduction

Psychiatric disorders are associated with a broad range of cognitive, affective, and behavioral symptoms. Like many human diseases, these disorders are believed to arise from a combination of genetic and environmental risk factors. Although a few psychiatric disorders are monogenic, such as Rett Syndrome [1], the vast majority are associated with complex genetic factors, including multiple single nucleotide polymorphisms (SNPs), chromosomal alterations, gene-gene interactions, as well as epigenetic changes that can accumulate throughout life. Most psychiatric disorders are mediated by the cumulative impact of several genetic insults related to common cellular processes or biological pathways, rather than a single gene. Disruption of these processes or pathways beyond a critical threshold could then result in physiological changes that produce the clinical symptomatology. Deciphering the genetic influences that make some individuals more vulnerable to acquiring a psychiatric disorder is a critical step in understanding the biological mechanisms and developing targeted treatments and prevention strategies.

Genome-wide association studies (GWAS) and candidate-gene analysis studies have revealed hundreds of common genetic variants, including SNPs, copy number variations (CNVs), and structural variants, which contribute to the risk for various psychiatric disorders [2]. For the majority of these susceptibility-associated genes and loci, common mutations

are frequently observed but each accounts for only a fraction of the inherited risk of a given disorder [3–6]. Conversely, these studies have also identified a few rare genetic variants that have a high penetrance but account for only a small number of individuals with the disease. Despite the identification of many risk variants, systematic examination of the biological consequences of a given mutation, and the progression of pathology related to psychiatric disease, has been limited by factors that include the inaccessibility of living human neural tissue and the inability to discriminate cause from consequence in post-mortem examination of neural tissue. Although animal models of psychiatric disorders allow for the investigation of biological mechanisms in the context of a developing and intact physiological system, there are significant limitations. At the genetic level, risk genes identified in patients may not be homologous with genes in rodents and many patient-specific genetic mutations and common variations, such as CNVs, are difficult to model in animals. In addition, the neurobiology cannot fully represent the complexity of the human brain, and the behavioral phenotypes cannot fully recapitulate the symptomatology [7]. As a complement to these approaches, cellular reprogramming technology that allows for the generation of human neurons and neural progenitor cells (NPCs) through induced pluripotent stem cells (iPSCs) can reveal new insight into the genetic and physiological factors that mediate psychiatric disorders in humans. As the number of iPSC studies focused on psychiatric disorders is growing rapidly, we are beginning to see points of convergence and divergence related to genotype, cellular phenotype, and clinical diagnoses. In this review, we will focus on three major classifications of psychiatric disorders, schizophrenia (SZ), autism spectrum disorders (ASD), and bipolar disorder (BD) to illustrate how iPSCs can help elucidate the biological and genetic components underlying each disorder and how understanding the mechanisms of one disorder may inform the investigations of others.

Induced Pluripotent Stem Cells

iPSCs are produced by reprogramming somatic cells into an undifferentiated stem cell-like state that retains the genetic information of the human donor, typically through transient expression of specific transcription factors [8]. Similar to embryonic stem cells (ESCs) during fetal development, iPSCs are capable of self-renewal and can differentiate into all somatic cell types, including neurons [9]. Unlike embryonic stem cells, which are only present early in human development, iPSCs can be generated from tissue obtained at any stage of human life. iPSC-based research thus bypasses the ethical issues associated with the study of ES cells, with the additional benefits of producing iPS cell lines that carry a donor's specific genetic information, and the possibility of accessing a detailed clinical history for the donor. Importantly, neurons differentiated from iPSCs show similar developmental properties as neurons differentiated from ESCs, including progressive changes in gene expression and epigenetic profiles [8]. More research is required to understand how developing neurons in culture are related to specific neuronal developmental stages in fetal brains, but transcriptomic comparisons have shown a high level of correlation between iPSC-derived neuronal cultures and fetal tissue up to 12 weeks in utero [10, 11]. For modeling central nervous system disorders, iPSCs can be differentiated into numerous region- and neurotransmitter-specific neural cell types including glutamatergic cortical neurons [12], GABAergic neurons [13], midbrain dopaminergic neurons [14], astrocytes [15], and hippocampal cells [16]. This ability to control fate specification gives us the opportunity to investigate the genetic and physiological underpinnings of early developmental risk for psychiatric disorders at the cellular level in the most disease-relevant human cell types.

Critical Factors in the Design of iPSC-Based Studies of Psychiatric Disorders

Given the heterogeneity of symptoms and genetic variations associated with each psychiatric disorder, the selection of individuals for a study cohort is critical. Most studies to date have relied on one of two non-exclusive strategies for donor selection, each of which can yield important insights into the mechanistic basis of disease. One approach is to select a cohort based on the clinical diagnosis and classification of patient symptoms, while the other focuses on identified genetic risk factors associated with one or more disorders. It is important to consider that many patients who do not have an identified genetic mutation or variant, referred to as idiopathic or sporadic cases, may have a single or combination of variants that has yet to be identified. And it is likely that some of the underlying biological mechanisms related to the causal pathology for a particular disorder, or subset of symptoms, will be shared among patients with identified risk gene mutations and those without any known mutation. In addition, there may be functional overlap as evidenced by independently identified risk genes that have been associated with the same intracellular pathway or cellular phenotype, as well as genetic variants and post-mortem phenotypes that have been associated with multiple neuropsychiatric disorders [17–19]. Several recent studies are summarized in Table 1 showing how genetic risk loci map onto specific psychiatric disorders and cellular phenotypes observed in iPSC studies. Both selection processes are important, and together will provide us with a better understanding of neuropsychiatric disorders.

Following the selection of a group of patients and appropriate control subjects and the generation of iPSC lines, the

Table 1 Overview of genetic variances and iPSC-based cellular phenotypes associated with psychiatric disorders

Genomic locus	Associated psychiatric disorder	Biological role and/or cellular phenotype	iPSC-based studies
DISC1	SZ, BD, MDD	Neuronal migration *Cell proliferation* *Transcriptional regulation* *Synaptogenesis*	[20, 21]
MECP2	ASD	Neuronal maturation Transcriptional regulation *Size of nucleus* *Synaptic maintenance*	[22–25]
22q11.2	SZ, ASD, ADHD, AD, Parkinsons	Axonal development Neuronal migration *Synaptic transmission* *Neuronal differentiation*[a]	[26–28]
FMR1	Fragile X syndrome, ASD	*mRNA regulation* *Synaptic transmission* *Neuronal arborization* *Neuronal differentiation*[a]	[29–31]
15q11.2	SZ, ASD, epilepsy	*Maintenance of synapses and dendrites maintenance* Ionic regulation Radial glial migration *mRNA regulation* *Cytoskeletal development*[a]	[32–34]
15q13.3	SZ, ASD, ADHD, epilepsy	Immunoresponse Neuronal proliferation	[33]
TRPC6	ASD	Ionic regulation *Synapse formation* *Neuronal arborization*	[35]
CACNA1C	ASD, SZ, BD	Synaptic modulation *Neuronal arborization* *Transcriptional regulation* *Ionic regulation* *Neurotransmitter synthesis*[a]	[36–39]
NRXN1	ASD, SZ, BD	Synaptic transmission *Synaptogenesis* *Cell differentiation*[a]	[12, 40]
22q13.3	ASD, ADHD, SZ	*Synaptic modulation* *Synaptogenesis*	[40]
CHD8	ASD	Transcriptional regulation Cell survival *Neuronal differentiation*[a] *Cytoskeletal development*[a]	[41]
CNTNAP2	SZ, ASD, ADHD, epilepsy	Axonal guidance Cell adhesion Dendritic arborization Synaptic development *Neuronal migration*	[42]

Several copy number variations and single gene mutations have been associated with increased risk for psychiatric disorders, often multiple disorders for each variant. We are just beginning to understand the role of these genes in brain development and function. Known biological roles of genetic risk variants found to be present in iPSC-derived neurons or neural progenitor cells are italicized

Abbreviations: *SCZ* schizophrenia, *BD* bipolar disorder, *MDD* major depressive disorder, *ASD* autism spectrum disorder, *ADHD* attention deficit hyperactive disorder, *AD* Alzheimer's disorder

[a] A phenotype observed in iPSC-derived neurons or neural progenitor cells not previously associated with the genetic variant

next step is to determine the cell type of interest for targeted differentiation. Several neural subtypes have been linked to the pathology observed in postmortem analyses of neural tissue from patients. Many of these suggest dysfunction in circuitry mediated by particular neurotransmitters such as GABA, dopamine, serotonin, and glutamate, as well as particular neural regions such as the hippocampus or prefrontal cortex. Differentiating iPSCs to highly enriched populations of a particular neuronal subtype is challenging [43, 44], and the relative purity of the cell cultures should be considered when interpreting results of population-level analyses. Although some common phenotypes and mechanisms are

expected among clinically distinct disorders, there could also be divergence of phenotypes across different kinds of cells derived from the same iPSC lines. Emerging technologies for single cell analysis of transcriptomes and epigenomes will be important to confirm phenotypes in clearly identified cellular subtypes [45].

Finally, decisions about cellular and functional assays to perform can be guided by findings in the literature to test specific hypotheses. Postmortem and imaging studies suggest that many psychiatric disorders are associated with abnormal connectivity and white matter density in the brain, potentially related to changes in neural development and/or synaptic pruning. For example, studies of SZ have often reported decreases in synaptic density and connectivity whereas ASD correlates with an increase in synaptic number and connectivity [46–50]. In addition, many of the GWAS-identified genes for psychiatric disorders are related to synaptic function [51, 52]. Although recent iPSC studies have largely supported these postmortem findings in the case of SZ, some discrepancies exist between the cellular and patient-based studies of ASD. There could be many reasons for this, including the limitations of modeling network connectivity in cellular cultures. Going forward, it will be important to evaluate iPSC studies in the context of all available data related to the donor, and with an appreciation of the constraints imposed by the current state of technology for the cell culture assays (Fig. 1). For example, phenotypes related to neural systems or structural interactions of developing neurons may not be readily observable in monolayer cell cultures. Integrating data from all available sources and advancing technology for cell cultures will be important in determining the genetic and biological underpinnings of psychiatric disorders.

Recent Studies

Schizophrenia

SZ is a disorder marked by heterogeneous symptoms and associated with numerous genetic risk factors. Recent iPSC studies that focused on identified risk factors have revealed how investigations of either rare or common variants can reveal cellular phenotypes and biological pathways that may play a role in conferring susceptibility to SZ. One of the most

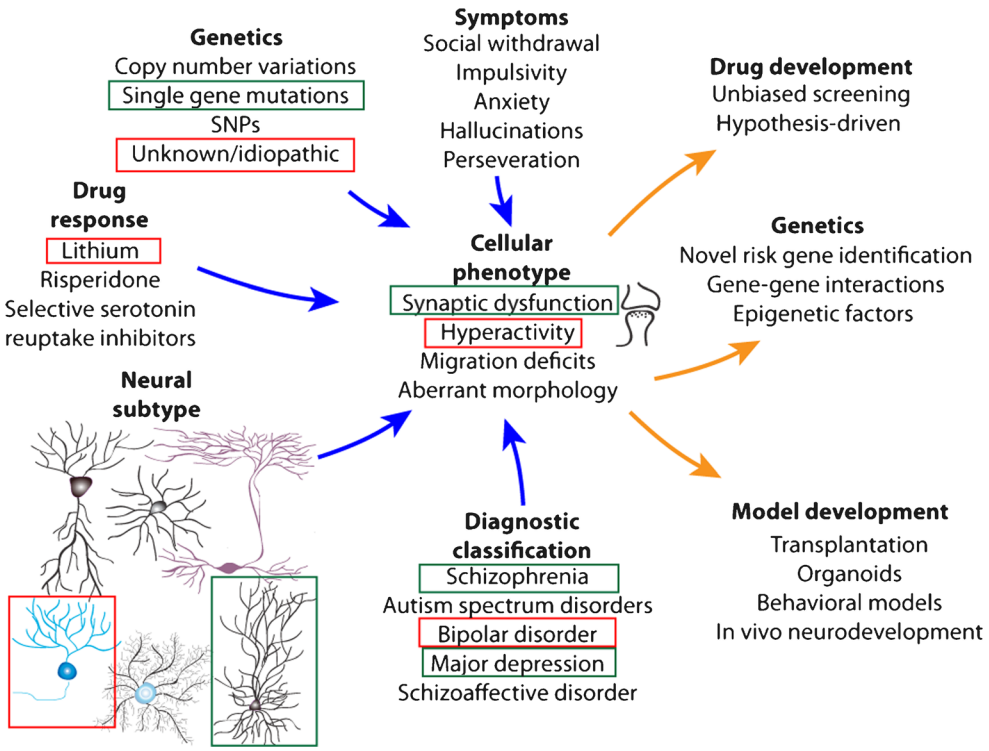

Fig. 1 Relevant factors for interpreting iPSC-based studies of psychiatric disorders. Using psychiatric patient-specific iPSC lines to investigate dysregulation of neural function at the cellular level should be informed by all available information regarding the clinical diagnosis of the donor, including classification of the disorder, constellation of symptoms, known genetic risk factors, and drug treatment sensitivity. In addition, cellular phenotypes may be specific to particular subtypes of neural cells. Several relevant factors and examples are shown. *Red boxes* highlight the findings from a study using bipolar patient-specific iPSCs to generate hippocampal-like neurons [16]. A hyperactivity phenotype was observed in this cell type that could be ameliorated by application of lithium in cells differentiated from iPSC lines generated from lithium-responsive patients. *Green boxes* highlight results from a study focused on iPSC lines with a 4 basepair mutation in *DISC1*, a risk gene for major psychiatric disorders including schizophrenia, bipolar disorder, and major depression [21]. In this study, glutamatergic cortical neurons exhibited synaptic dysfunction due to aberrant expression of presynaptic proteins

well-studied rare variants is *Disrupted-in-schizophrenia 1* (*DISC1*), a risk gene that was first identified due to a balanced translocation that segregated with the occurrence of major psychiatric disorders in a large Scottish pedigree [53]. More recently, an American family was identified in which a 4 basepair deletion in *DISC1* was associated with increased risk for SZ, BD, and major depression [54]. Studies of DISC1 function in animal models of brain development and adult neurogenesis had shown a pivotal role for this gene in cell proliferation, differentiation, migration, neuronal development, and synaptic transmission [55–57], but its role in disease-relevant human neurons was not known. In a recent study using iPSCs generated from the American pedigree family members with and without the *DISC1* mutation, glutamatergic neurons differentiated from the deletion lines exhibited a marked reduction in synaptic development due to a decrease in the presynaptic protein, SV2, and aberrant synaptic function as shown by a decrease in the frequency of spontaneous excitatory events compared to neurons derived from control lines without the mutation [21, 58]. Interestingly, this study also found robust alterations in the transcriptome of these patients, with increases in mRNA for the presynaptic proteins synapsin and synaptoporin, as well as for the transcription factor MEF2C, implicated in synaptic function [59]. These data suggest that *DISC1* influences other genes related to synaptic development through regulation of transcription. Consistent with the postulated role of *DISC1* as a "hub" gene with many interacting partners, enriched categories of differentially expressed genes from gene ontology analysis included "mental disorders," "schizophrenia," and "bipolar disorder." Using the transcription activator-like effector nuclease (TALEN) gene editing approach, isogenic iPSC lines were generated in which the 4 basepair deletion was either repaired in patient lines or introduced in control lines. Several of the synaptic and gene expression phenotypes emerged in neurons derived from the engineered mutant lines and, conversely, these phenotypes were rescued in neurons differentiated from the repaired patient lines. These results demonstrated a causal role of this 4 basepair deletion in dysregulated synaptic development and function and the transcriptomic changes mediated by *DISC1*. These data further illustrate the critical concept that a particular genotype may be causal for cellular phenotypes, but not the disorder itself. Not every member of the American family who harbored the *DISC1* deletion was diagnosed with a psychiatric disorder, again emphasizing that other factors play a role in determining whether behavioral symptoms become manifest in an individual who harbors genetic risk factors.

Human iPSC lines have also been generated from patients with 15q11.2 copy number variations. This CNV plays a potentially diametric role in modulating susceptibility to psychiatric disorders with microdeletions being associated with SZ and microduplications associated with ASD [34, 32]. NPCs derived from iPSC lines with 15q11.2 microdeletion exhibited deficits in apical polarity and adherens junctions which regulate the structural integrity of early neuronal development [34]. This phenotype was found to be due to a loss of function of *CYFIP1*, one of the four genes within this CNV. Restoring CYFIP1 levels could rescue this phenotype, suggesting that CYFIP1 could play a role in neurodevelopmental processes that are compromised in individuals at risk for SZ or ASD due to 15q11.2 CNV. To evaluate the role of this gene in an intact nervous system, short hairpin RNA (shRNA) was used to knockdown Cyfip1 through in utero electroporation into the developing mouse brain. Consistent with observations from the iPSC-based culture assays, radial glial cells exhibited a similar reduction in polarity and aberrant formation of adherens junctions, resulting in migration deficits and disorganized cortical layers. In another study focused on iPSC lines with 15q11.2 deletions, decreases in dendritic spine formation and expression of the postsynaptic protein, PSD95, were observed, suggesting alterations in synaptic communication [32].

In addition to these studies focused on known genetic risk factors, one study compared iPSC-derived neuron lines generated from idiopathic schizophrenia patients to iPSCs from unaffected controls [60]. Interestingly, several cellular phenotypes observed in differentiated neurons from SZ patient-specific iPSCs were similar to those observed in the neurons with known genetic risk factors, including a significant decrease in neuronal connectivity, deficits in synaptic transmission, and transient defects in dendritic structures. Neurons from SZ patient-specific iPSCs exhibited a significant reduction in neurite growth and a decreased ratio of the postsynaptic protein PSD95 to the dendritic scaffold protein, MAP2A. Although both studies revealed an impairment in synaptic transmission, it could be due to different mechanisms acting on either pre- or postsynaptic targets. Follow-up studies on the same cohort of SZ patients and controls revealed aberrant migration with decreased NPC outgrowth, increased signs of oxidative stress in forebrain neurons [10], and altered WNT signaling [61] in gene expression and functional assays. In a recent study, these same lines showed activity-dependent differences in the release of catecholamines, with elevated release of dopamine, norepinephrine, and epinephrine in SZ patient-specific neurons, and a correlated increase in the percentage of tyrosine hydroxylase neurons in heterogeneous neuronal cell cultures [62]. It will be interesting to see whether the catecholamine release phenotype could be affected by the application of antipsychotic medications that act on these neurotransmitter systems.

Together, several studies have shown that iPSC neurons, whether derived from idiopathic or familial patients, exhibit alterations in synaptic formation, neuronal communication, and migration, consistent with hypotheses developed from postmortem, imaging, and genetic studies. Importantly, clear cellular phenotypes were observed in neurons derived from

SZ patient-specific iPSCs, whether the patients had an identified mutation in a single gene such as *DISC1*, a CNV implicated in risk for SZ such as 15q11.2, or had no known genetic risk factor. Although there were broad similarities among the cellular phenotypes in terms of gene expression and synaptic function, there were also clear differences. However, the points of divergence could be attributable in part to the variability across studies in the composition of neural subtypes in the cell populations, the targeted stage of neuronal development, and the cellular assays performed to test specific functions. All of these factors must be considered when drawing comparisons across studies of SZ using patient-specific iPSCs.

Autism Spectrum Disorders

Autism spectrum disorders encompass high and low functioning individuals and syndromic and non-syndromic forms of autism. More so than other forms of ASD, monogenic syndromic autism, including Rett Syndrome, Phelan-McDermid Syndrome, and Fragile X, have been studied using iPSCs. In these disorders, autism is secondary to the genetic condition, which is typically attributed to a mutation in a single gene. Because the genes that underlie the development of these different disorders are largely known, generating iPSCs from these patients can be an important entry point to elucidate the biological pathways associated with ASD. Investigation of iPSC neurons from patients with various forms of monogenic syndromic autism has resulted in a relatively consistent phenotype of decreased synaptic formation and excitatory transmission, similar to what has been found in the study of schizophrenia. In iPSC-derived neurons produced from patients with Rett syndrome, decreased cell body size, decreased frequency and amplitude of spontaneous excitatory neuronal activity, and decreases in the expression of the synaptic protein VGLUT1 were noted [25]. Decreases in frequency and amplitude of spontaneous excitatory neuronal activity, as well as decreased expression of the synaptic proteins, HOMER1 and SYN1 were noted in iPSC-derived neurons from patients with Phelan-McDermid syndrome [40]. And in iPSC-derived neurons produced from Fragile X patients, there was a decrease in dendritic spine number and size as well as decreases in neurite outgrowth [29, 31].

Several studies have focused on the genetic and physiological phenotypes of syndromic autism, but less is known about non-syndromic, idiopathic autism, in which autism is the primary condition. In a recent study, the presence of a de novo disruption in *TRPC6*, a gene not previously associated with ASD, was found in a patient with non-familial non-syndromic autism [35]. The *TRPC6* gene encodes a protein associated with dendritic spine and synaptic formation [63, 64]. Neurons differentiated from this patient's iPSCs with a de novo disruption of *TRPC6* exhibited decreased expression of the synaptic protein VGLUT1 and decreased dendritic growth, which was rescued by overexpressing TRPC6 [35]. These results were replicated in rodents in which TRPC6 was knocked down using shRNA. Interestingly, this paper found evidence that TRPC6 may be regulated by *MECP2*, a gene that is the locus of causal mutations for Rett Syndrome [1]. iPSCs from Rett patients showed decreased TRPC6 expression, and there was significant pulldown of TRPC6 using immunoprecipitation for MECP2 in control lines, suggesting an interaction between the two and highlighting *TRPC6*, as a new gene of interest in the development of ASD. In another study that revealed a potential interaction among autism risk genes, researchers used CRISPR/Cas9 technology to knockout *CHD8* in control iPSC lines to investigate this gene in autism [41]. Mutations in *CHD8* are prominent risk factors for autism and this gene is involved in chromatin remodeling [65]. A partial deletion of *CHD8* affected the expression of genes associated with neuronal development and the WNT signaling pathways, as well as cytoskeletal structure and extracellular matrices, reminiscent of some phenotypes observed in SZ patient-specific iPSC lines [41]. They also identified links between downstream targets of *CHD8* and *TCF4*, which has also been associated with risk for SZ [66], *ANK2* which has been associated with autism and bipolar disorder [67, 68], and several other genes implicated in ASD.

While these studies of iPSC-derived neurons from autistic individuals have consistently revealed decreased synaptic formation and neuronal communication associated with genetic risk factors, the results are in contrast to what has been observed in postmortem studies. However, the molecular and physiological phenotypes observed in iPSC neuronal cultures may not fully capture the long-term consequences of dysregulated neuronal development. Recently, investigators have utilized novel techniques for the development of cerebral organoids, 3D neuronal cultures formed from iPSCs that mimic cortical organization and maturation processes observed in developing human brains [69], and to study the genetics and biological pathways associated with idiopathic autism [70]. In these studies, organoids produced from individuals with idiopathic autism exhibited normal neural organization and excitation at early time points in culture, but an increase in cell proliferation with an increase in synaptic connections over time. These studies also found a significant increase in inhibitory neurons in cultures from autistic patient-specific iPSCs, which was traced back to early expression of the transcription factor, FOXG1, which was found to play a role in cell proliferation and the development of inhibitory neurons [71]. Interestingly, FOXG1 syndrome has also been associated with the syndromic autism disorder, Rett Syndrome [72]. Deficits in cell cycle length in proliferating neural progenitors from patient iPSCs, as compared to control iPSC cultures, were also noted and appeared to recover over time, accompanied by increases in the expression of genes associated with neuronal

differentiation and an increase in synapses in inhibitory cells, seen as an increase in SYN1 in VGAT-positive neurons. Though it is unclear whether these results are more representative of aberrant neuronal development resulting from mutations in genetic risk factors for autism, it is apparent that cellular phenotypes may vary over development and exhibit differences between 2D and 3D cultures. The importance of analyzing different stages of cellular development is also supported by recent work in which iPSC-derived neurons cultured from patients with Rett syndrome exhibited a time-dependent modification in KCC2 expression, a chloride transporter involved in GABAergic function [73].

Bipolar Disorder

Patients with BD typically experience intermittent episodes of major depression and elevated moods or extreme irritability. Expression of psychosis marked by hallucinations and disordered thoughts can occur during bouts of mania, which may resemble some of the positive symptoms of schizophrenia. Emerging data from GWAS and large-scale genetic studies have identified many factors that contribute to risk for BD, including genes that have also been implicated in SZ, such as *CACNA1C* [74, 75]. Observations from imaging and postmortem studies suggest a developmental origin for the disorder and potential mechanistic overlap with some features of SZ including aberrant structural development and synaptic pruning [76].

Comparing iPSC lines derived from three BD patients and matched controls, patient-specific neurons exhibited dysregulated calcium signaling, which was sensitive to lithium pretreatment [77]. Interestingly, this group also found a difference in the response to patterning cues during differentiation suggesting a potential difference in fate specification of the patient-derived iPSCs. Another study observed deficits in neurogenesis that were specific to the BD patient-specific iPSC lines with lower proliferation rates for NPCs [78]. Changes in gene expression were also observed over the course of differentiation and maturation of neural cells from BD iPSCs, with altered levels of DISC1 reported in NPCs and differential expression of several calcium channel subunits in postmitotic neurons. A recent study using BD patient-specific iPSC lines generated from either lithium-responsive or lithium non-responsive patients showed specific deficits in mitochondrial function as well as hyperexcitability in hippocampus-like granule neurons differentiated from all BD lines [16]. Strikingly, in neurons derived from lithium-responsive patient iPSCs, treatment of the cell cultures with lithium was able to rescue the hyperexcitability phenotype. This result illustrates that differences among patients diagnosed with BD with respect to effective treatments could extend to cellular phenotypes as well.

The Future of iPSCs in the Study of Psychiatric Disorders

While many of the phenotypes observed in iPSC studies are consistent with post-mortem and imaging studies of patient brains [12–15], cells derived from patients with some forms of ASD often show a different phenotype than would be predicted. Though the reasons for these discrepancies are being explored, they illustrate some of the potential limitations of the study of iPSCs and resulting neuronal cultures as a tool for studying neuropsychiatric disease. For example, neurons produced from iPSCs are grown in an in vitro environment without the complex developmental cues present in vivo; therefore, they may not accurately represent the types of networks formed endogenously. Furthermore, the limited ability to map neuronal development beyond that seen early in fetal development limits our ability to study the changes associated with crucial later time points in each condition.

Current research in the field is working to overcome these limitations and to advance the use of iPSCs to understand how genetic risk results in physiological differences that turn into behavioral manifestations of neuropsychiatric disorders. As mentioned earlier, the ability to produce 3D organoid structures is a significant advance in the field [69, 11]. While the relationship between these structures and the developing human brain is still being elucidated, organoids provide an opportunity to monitor neuronal migration and development at later time points. Moreover, the identification of additional genetic and environmental factors that are needed to meet the threshold of disease manifestation is still unknown. Finding the answers to these types of questions with iPSCs may require an in vivo setting with complex signaling factors. While human NPCs have been transplanted to rodent brains and are being studied as a potential treatment for neurodegeneration, challenges remain to elucidate relevant developmental properties of transplanted NPCs and to ensure integration of the appropriate cell types into the existent neuronal networks in a functional and predictable manner [79].

Conclusions

The ability to generate human iPSCs has provided researchers with a glimpse into the neurodevelopmental processes that may be affected in patients with psychiatric disorders. Given the challenges associated with examination of neural tissue in patients, the differentiation of iPSCs into neurons with the genetic information of patients allows us to examine the genetic and physiological components that may underlie these disorders. Although we are just beginning to harness the power of iPSC technology for discovery-based studies of psychiatric disorders, several key principles have emerged. It is becoming increasingly clear that there are potential disconnects when mapping

genotype to cellular phenotype to patient symptomatology. As in the case of *DISC1*, genotype may code for some functional dysregulation at the cellular level, without rising to the level of being causal for behavior. On the other hand, for monogenic forms of ASD, causal mutations in the relevant gene for manifestation of the disorder (e.g. MECP2 mutations that lead to Rett syndrome) may not elicit consistent cellular phenotypes across different neural subtypes or patient lines. In the case of BD, we have seen that heterogeneity at the behavioral level with respect to treatment efficacy can be reflected at the cellular level, suggesting mechanistic differences among patients diagnosed with BD. In addition, cellular phenotypes may evolve over time and there is still much work to be done to map neuronal development in culture to specific stages of brain development. All of this argues for a more granular approach to analyzing iPSC-based studies in which the data are interpreted within the context of all potential variables, which should facilitate the identification of meaningful points of divergence and convergence in our investigation of the genetic and mechanistic basis of psychiatric disorders.

Significant advances in our understanding of psychiatric diseases have been made using cellular reprogramming and further optimization of differentiation protocols, single cell analysis, and organoid technology will greatly enhance our capability for discovery. Drawing the relevant connections among genetics, physiology, and the presentation of psychiatric disorders and symptoms will not be easy; however, new studies are finding ways to transplant human iPSC efficiently and effectively into animal models, which may assist in this transition. Despite current limitations, human iPSC-based research has generated critical insight and new hypotheses about the mechanisms underlying psychiatric disorders and genetic risk within a very short period of time. As the methodology becomes more efficient, more researchers will be able to use this approach to contribute to the numerous datasets we need to identify key biological processes that we can target for new therapeutics. One thing is certain, iPSC technology has provided us a new frontier in the study of psychiatric disease, one that may finally provide us with a glimpse into the inner workings of a patient's brain.

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Compliance with Ethics Standards

Conflict of Interest Stephanie J. Temme, Brady J. Maher, and Dr. Kimberly M. Christian declare that they have no conflict of interest.

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