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How induced pluripotent stem cells are informing drug discovery in psychiatry

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Summary

Compared with other medical fields, psychiatry is particularly challenging for rational drug discovery. The therapeutic endpoints are abstract measures of cognitive and behavioral performance, for which we have a very limited understanding of the underlying biological mechanisms. Existing preclinical disease models are also limited in their translational fidelity. Recently, there have been active discussions on the use of human induced pluripotent stem cells (iPSCs) as a catalyzing research tool in psychiatry, but very few review articles in the field have given specific considerations to their use at the interface between psychiatric research and drug discovery. Here, we discuss recent perspectives emerging from this interface. For physicians and researchers on the clinical side, we explain how iPSC-based experimental approaches are placed at the crossroads with psychiatric genetics and how representative studies in the field are addressing biological mechanisms underlying psychiatric disorders. For researchers who directly work with iPSCs and aspire to develop new research techniques, we direct their attention to the utility of this approach for unmet needs in drug discovery workflows.

Key words: *induced pluripotent stem cells; psychiatric genetics; disease modeling; neurobiology; drug discovery*

Introduction

Mental and behavioral disorders are among the top medical conditions resulting in disabilities [1, 2], homelessness [3, 4], and risk for suicide [5]. Six decades have elapsed since the last revolution of pharmaceuticals in psychiatry, which was catalyzed by the serendipitous finding of substances that affect cognitive function and behavior, such as chlorpromazine, imipramine and iproniazid. These substances act by modulating dopamine, serotonin and other monoamine systems in the brain. Current psychiatric medications share similar mechanisms of action as well as limitations in efficacy. For example, available antipsychotic drugs are ineffective in improving negative as well as cognitive symptoms associated with schizophrenia. Existing antidepressants require weeks, or longer courses of administration, to manifest effects in patients with major depression. For some conditions, like the core social disability of autism, there are no pharmaceutical treatments at all.

Rational drug discovery requires a deep understanding of the biology of the disease in question. Disease biology is integrated into every stage of a drug discovery workflow, starting with the validation of a therapeutic target and extending all the way to the determination of biological factors driving safety and efficacy in clinical trials. In recent years, as research in psychiatric therapeutics advances, there has been an increasing emphasis on neurobiology and genetics. Conceptually, the conventional symptom-based disease categories may be transformed into new categories that incorporate cognitive neuroscience metrics, genetic underpinning, and neurobiological data [6]. In practice, new laboratory techniques and collaborative efforts in psychiatric genetics are catalyzing a rapid growth in knowledge around the neurobiological basis of mental and behavioral disorders. Among these new frontiers, a unique approach is the use of patient-derived or genetically engineered human induced pluripotent stem cells (iPSCs) for disease modeling and rational drug discovery. This review article will discuss the utility of human iPSCs in psychiatry research, summarize state of the art studies, and point to particular areas to watch from a drug discovery perspective.

The enabling power of human iPSCs in psychiatric research

In psychiatry, research involving patients is normally conducted in a clinical setting. Occasionally, researchers may have the ability to work with postmortem or surgically removed brain tissues from diagnosed patients. However, neither method is sufficient to empower researchers with the desirable number of research samples and versatile experimental techniques. Although animal models in psychiatry and drug discovery provide better versatility for experimental designs, they have the caveat of biological divergence from human clinical subjects. Specifically, genetic or extrinsic factors that contribute to a psychiatric condition in humans may not be faithfully correlated with experimental observations in a lab animal.

These limitations are being circumvented by the advent of human iPSC cells and techniques to differentiate the iPSCs

into various brain cell types. In particular, patient-derived neuronal tissue may be readily accessible to the research community for reproducible investigations. The ease of expansion of human iPSCs makes it possible to scale up research studies and to run primary screening and secondary assays in drug discovery workflows. An unlimited number of *in-vitro* experimental techniques can be applied to iPSC-derived neural tissue, to chase down details of a molecular or cellular event or to assess pathway-level abnormalities associated with a disease. Importantly, the neural differentiation of human iPSCs recapitulates many aspects of human brain development. It is now possible to design studies to follow disease-associated developmental events such as neurogenesis, and synapse formation and maturation.

Human iPSCs at a crossroads with human genetic variation and psychiatric genetics

Human genetic variation, including single nucleotide polymorphisms (SNPs) and structural changes such as copy number variants (CNVs), are building blocks in the genetic basis of health and disease. In the past decade, there have been significant advances in the delineation of common versus rare genetic variations between individuals, as well as populations [7–10]. Rare variants often play a causal role in severe neurodevelopmental disorders, for example, the *MeCP2* mutations that underlie Rett syndrome and the *FMR1* mutations that are responsible for fragile X mental retardation. Now, a growing list of rare variants distributed amongst hundreds of genes is unfolding from exome and whole-genome sequencing studies on neurodevelopmental disorders, including both syndromic and non-syndromic cases [11–15]. In contrast, common variants in a specific gene do not necessarily reconstitute a causal relationship with disease, but instead confer elevated or reduced risk amongst the whole of all other genetic variants in an individual. However, genome-wide common variants identified between large cohorts of patients and healthy subjects may converge on biological pathways that have significant implications on both etiology and drug discovery. For example, a recent mega-analysis of common variants between 36 989 cases and 113 075 controls led to the identification of 108 genomic loci in association with schizophrenia risk [16]. The genomic loci are enriched with genes expressed in the brain, as well as in tissues involved in immunity, highlighting dopamine receptor DRD2 signaling, glutamatergic synaptic transmission, and immune regulation as biological mechanisms for rational discovery of therapeutic targets for schizophrenia.

The advances in psychiatric genetics have an unprecedented potential to aid the investigation of disease biology, the identification of new drug targets, and the design of intelligent clinical trials. The key to unlock this potential are experimental studies that connect genetic variants to neurobiological function. Human iPSCs have an unfolding potential to empower such studies. For rare variants that play a causal role in disease, patient-iPSC derived neural tissues enable disease modeling in a dish. For the investigation of common variants, especially SNPs, human iPSCs also have an important utility. SNPs are often present in a block of linkage disequilibrium and they collectively share the association with disease risk. Such genomic organization can be faithfully represented in human iPSCs, but are very challenging to recapitulate with genetic manipulation in model organisms or cell lines. Similarly, human iPSCs are great research materials for the functional analysis of CNVs. Between human individuals, overlapping CNVs may have variable DNA breakpoints, thus including or excluding certain genes. A collection of iPSCs bearing overlapping CNVs may be used to associate cellular phenotypes with specific CNVs and the genes therein.

The genetic background of an individual may either enhance or mitigate penetrance of specific genetic variants (i.e., phenotypic manifestation). In order to associate cellular phenotypes with genetic variants in human iPSC-based studies, the effect of genetic background should be controlled for. This may be achieved by inclusion of sufficient numbers of study subjects and choice of the proper controls (such as unaffected family members in a study of *de-novo* genetic variants). On the contrary, it could be meaningful to explore the relationship between genetic background and phenotypic divergence between patient individuals using iPSC-derived neural tissue. From a drug discovery perspective, this is tied to the need for understanding the heterogeneity of a patient population and may inform the clinical investigation of mechanism-driven therapeutics in relevant patient groups. Going further, iPSCs may serve as experimental materials for the identification of specific modifier genes in the genetic background. Modifier genes may shed light on additional mechanisms to target or to avoid during drug discovery and even provide guidance on patient stratification in clinical studies.

Neurobiological mechanisms identified in existing iPSC-based models of psychiatric disorders

In this section, we discuss several selected studies (table 1) that have demonstrated specific cellular phenotypes in patient iPSC derived neural tissue and translational potentials for drug discovery. Comprehensive reviews of iPSC-based research in psychiatry (and neurology) can be found in multiple earlier articles [17–20].

Neurodevelopmental syndromes attributed to high-penetrance single nucleotide variations and CNVs

Rett syndrome is a neurodevelopmental disorder characterized by autistic behaviors, stereotypical hand movements and multiple developmental delays. Rett syndrome almost exclusively affects females. The genetic cause lies in mutations in the X-chromosome encoded protein Methyl CpG Binding Protein 2 (MECP2), a key transcriptional regulator acting during brain development. Marchetto et al. [21] pioneered the use of patient-derived iPSCs to model Rett syndrome. The researchers demonstrated that during neural differentiation of patient-derived iPSCs, one of the two X-chromosomes was randomly inactivated, recapitulating X-inactivation in females. This resulted in random silencing of the wild-type or mutated MECP2 allele and a mosaic neuronal culture resembling the cellular composition of the brain of a Rett syndrome patient. Importantly, excit-

atory (but not inhibitory) synaptic activity was significantly reduced in patient iPSC-derived neuronal networks in comparison with controls. In parallel, patient iPSC-derived neurons had much fewer dendritic spines and glutamatergic synaptic terminals as well as smaller soma size (also see work by Cheung et al. [22], Ananiev et al. [23] and Kim et al. [24]).

Reminiscent of these findings, Shcheglovitov et al. [25] demonstrated for Phelan McDermid syndrome, another neurodevelopmental disorder with high-prevalence of aut-

Table 1: An overview of induced pluripotent stem cells (iPSCs)-based disease models discussed in this article.

Authors/year	Disease / genetic underpinning	iPSC-derived cell type(s)	Methods for phenotyping	Cellular phenotypes
Marchetto and Carromeu et al. (2010) [21]	Rett syndrome / MECP2	Mixture of excitatory and inhibitory neurons, without characterization of brain regional specification	Patch clamp electrophysiology; Light microscopy (including calcium imaging)	Reduction in the frequency of spontaneous excitatory synaptic currents, fewer dendritic spines and glutamatergic synaptic terminals, and smaller cell soma size
Cheunget al. (2011) [22]	Rett syndrome / MECP2	Neuronal subtypes not characterized	Light microscopy	Smaller cell soma
Ananiev et al. (2011) [23]	Rett syndrome / MECP2	Neuronal subtypes not characterized	Light microscopy	Smaller cell nuclei
Kim et al. (2011) [24]	Rett syndrome / MECP2	Neuronal subtypes not characterized	Light microscopy Real-time qPCR	Fewer cells expressing neuronal marker Tuj1 and neuronal sodium channels SCN1A and SCN1B
Shcheglovitov et al. [25]	Phelan McDermid syndrome / SHANK3	CaMKII-GFP labeled forebrain neurons expressing cortical neuronal markers	Patch clamp electrophysiology; Light microscopy	Reduced formation and activity of excitatory synapses. Rescue of the synaptic defect *in vitro* with IGF1.
Pasca et al. (2011) [29]	Timothy syndrome / CACNA1C	Forebrain neurons expressing cortical layer markers; mainly excitatory neurons	Patch clamp electrophysiology; Light microscopy (including calcium imaging); Transcriptome profiling by Illumina microarrays and Fluidigm chips.	Increased ratio of neurons with upper cortical layer identity over those with lower layer identity. Increased ratio of neurons characteristic of subcortical projection cells over those characteristic of callosal projection cells. Increase in voltage-dependent calcium influx. Transcriptome-level changes in gene expression with or without KCl stimulation.
Sheridan and Theriault et al. (2011) [30]	Fragile-X syndrome / FMR1	Neuronal subtypes not characterized. Neural progenitors expressed NESTIN and SOX1.	Light microscopy; promoter methylation, mRNA expression, and protein expression analysis for FMR1	FMR1 promoter hyper-methylation correlated with loss of mRNA and protein expression. Reduced neurite length.
Wen et al. (2014) [31]	Schizophrenia and major depression (existing in a pedigree) / DISC1	Forebrain neurons expressing cortical layer markers; mainly excitatory neurons	Light microscopy (including FM1-43 live imaging for synaptic vesicle release); patch clamp electrophysiology Transcriptome profiling by RNA-seq and qPCR confirmation; protein expression analysis for DISC1	Impaired synaptic vesicle release. Reduction of SV2-positive synaptic puncta. Dysregulation of genes required for presynaptic function. Findings were further consolidated by isogenic cell lines edited for the DISC1 gene.
Krencik et al. (2015) [42]	Costello syndrome / HRAS	Forebrain astrocytes that express S100B, CD44, and GFAP	Light microscopy; Transcriptome profiling by Affymetrix microarray; grafting of human iPSC-derived astrocytes to mouse hippocampal organotypic slices	Astroglial hyperplasia (proliferation, cell morphology, and astroglial marker expression). Increased expression of extracellular matrix remodeling factors and proteoglycans.
Pak et al. (2015) [32]	ASD and schizophrenia / NRXN1	Neurons generated by NgN2-mediated reprograming of hESCs; neurons generated by conventional differentiation of hESCs via a neural progenitor stage	Light microscopy; Western blotting; patch clamp electrophysiology	Reduced frequency of miniature excitatory synaptic currents owing to a smaller probability of neurotransmitter release
Madison et al. (2015) [37]	Bipolar disorder (type I) / undefined	CNS neural progenitors	Light microscopy; RNAseq	Reduced proliferation of neural progenitors and reduced neuronal differentiation; downregulation of genes involved in WNT signaling; phenotypic rescue by a WNT pathway activator CHIR-99021
Mertens and Wang et al. (2015) [38]	Bipolar disorder (type I) / undefined	Neurons resembling hippocampal dentate gyrus granule cells	RNAseq; light microscopy; mitochondrial membrane potential assay; patch clamp electrophysiology	Hyperexcitability; increased expression of genes involved in mitochondrial function, PKA/PKC signaling and action potential generation; phenotypic rescue by lithium

ASD = autism spectrum disorder; CNS = central nervous system; hESC = human embryonic stem cell; IGF1 = insulin-like growth factor 1; PKa/PKC = proteinkinase A / protein kinase C; qPCR =real-time quantitative polymerase chain reaction; SV2 = synaptic vesicle protein 2

ism, that excitatory synaptic density and activity were reduced in patient iPSC-derived neurons. Phelan McDermid syndrome is associated with heterozygous deletions within the cytogenetic band 22q13.3. The deletions vary in size but all encompass the SHANK3 gene, which encodes a scaffolding protein involved in the formation and function of excitatory synapses. Shcheglovitov et al. showed that restoring SHANK3 expression, via a lentiviral vector, rescued the synaptic defect in patient iPSC-derived neurons, thus confirming the critical role of SHANK3 in pathogenesis. Interestingly, in both the Rett and Phelan McDermid studies, insulin-like growth factor 1 (IGF1) application to patient iPSC-derived neurons lead to an elevation of synapse number and/or function, corroborating the therapeutic rationale around IGF1 signaling that is already suggested by mouse models [26, 27].

Timothy syndrome is a rare disease characterized by co-occurrence of autism and non-neurological symptoms affecting the development of heart, digits, face, and teeth. Gain-of-function mutations in the voltage-gated calcium channel CACNA1C (Cav1.2) are responsible for the multisystemic symptoms. Yazawa et al. [28] generated cardiomyocytes from Timothy syndrome iPSCs and demonstrated calcium-dependent defects in cardiac physiology. Pasca et al. [29] used the same set of iPSC lines to uncover cellular phenotypes associated with the development of cerebral cortex. Specifically, the iPSCs were differentiated into cortical neurons, which were then analyzed for cortical layer designation and neuronal subtype identity. Compared with the control samples, cultures of Timothy syndrome neurons contained a smaller fraction representing lower cortical layers (layers IV, V, and VI) and a larger fraction representing upper layers (layers II and III). Furthermore, for lower layer neurons, the Timothy syndrome samples showed a decrease in the fraction positive for special AT-rich sequence-binding protein 2 (SATB2) (corresponding to callosal projection neurons) and an increase in the COUP-TF interacting protein 2 (CTIP2)-positive fraction (corresponding to subcortical projection neurons). These findings coordinated with changes in voltage-dependent calcium influx and gene expression profiles. In particular, the expression of tyrosine hydroxylase was ectopically upregulated in cortical neurons derived from Timothy syndrome iPSCs, resulting in an increase in catecholamine production. This phenotype was reversed by roscovitine, an L-type calcium channel modulator that accelerates channel inactivation and a cyclin-dependent kinase inhibitor.

Fragile-X Syndrome is a common form of inherited intellectual disability with comorbidities overlapping with autism spectrum disorders. The term fragile-X reflects a cytogenetic defect due to overexpansion of CGG repeats (>200 copies) in 5'UTR region of the *Fragile X Mental Retardation Gene 1* (*FMR1*) gene. Sheridan and Theriault et al. [30] showed that the CGG repeat number consistently correlated with the level of FMR1 promoter methylation and gene expression in donor fibroblasts, fibroblast-derived iPSCs, and iPSC-derived neural progenitors. In particular, a male patient included in this study carried mosaic CGG repeat numbers in his fibroblasts: while some fibroblasts had normal lengths of CGG repeats, the rest shared a pathogenic, high repeat-number. The researchers generated a pair of iPSC clones that represented the two subpopulations of fibroblasts. Compared with the unaffected clone, the high-CCG-repeat clone gave rise to neurons bearing significantly shortened neurites, establishing an FMR1-dependent cellular phenotype easily identifiable in a culture dish.

An emerging trend is to use a family-based study design, where iPSCs are made from both affected and unaffected family members. The study by Wen et al. [31] set an informative example. The researchers generated iPSCs from four family members: two members were affected by a frameshift mutation of the gene *Disrupted in Schizophrenia 1* (*DISC1*) and diagnosed with schizophrenia and major depression, respectively; the other two members were asymptomatic and without the mutation. The mutant *DISC1*-impaired synaptic vesicle release in iPSC-derived forebrain neurons, which were paralleled with a dysregulation of genes required for presynaptic function and a reduction of synaptic vesicle protein 2 (SV2)-positive synaptic puncta. These findings were further confirmed in isogenic iPSCs created by gene editing – editing the *DISC1* gene in iPSCs from unaffected family members to carry the frameshift mutation resulted in synaptic phenotypes resembling those in iPSCs from the patients. Correcting the *DISC1* mutation in patient iPSCs led to rescue of the synaptic phenotypes. This work exemplifies a nice cosegregation of a genetic variant (the DSIC1 frameshift mutation) with cellular phenotypes in iPSC-derived neurons. It is interesting to note that there was no phenotypic difference identified between the family member diagnosed with schizophrenia and the member diagnosed with major depression, leaving a clue for additional disease-underlying mechanisms not explored within this study.

Another study by Pak and colleagues [32] further showcased the use of gene editing techniques to recapitulate disease-associated mutations in human pluripotent stem cells. The researchers introduced heterozygous conditional mutations of the *NEUREXIN1* (*NRXN1*) gene to a human embryonic stem cell line (H1), using gene-targeting constructs carried by recombinant adeno-associated viruses. Heterozygous loss-of-function mutations in *NRXN1* have been associated with autism spectrum disorders and schizophrenia [33–35]. *NRXN1* encodes a cell-adhesion molecule located in the presynaptic terminal that physically interacts with postsynaptic proteins such as neuroligins and LRRTMs (leucine rich repeat transmembrane neuronal proteins). No phenotype of synaptic transmission was found in cultured mouse cortical neurons missing one or both copies of the mouse homolog Nrxn1α (examined in the same study). However, in human neurons derived from the *NRXN1* mutant stem cells, there was a significant decrease in the frequency of miniature excitatory synaptic currents attributable to a smaller probability of neurotransmitter release. It was worth noting that this study also provided an example for the use of neurons acquired from a rapid neural induction of pluripotent stem cells by the transcription factor neurogenin 2 (NgN2) [36]. The aforementioned synaptic phenotypes were consistently observed in the NgN2-induced neurons and in neurons generated from conventional neural differentiation, but the NgN2 induction method shortened the cell culture time by half (about 20

days), a technical advantage that may be desirable for many future studies.

The examples above demonstrated clear cellular phenotypes attributable to single disease genes. The findings and methodologies therein also coincided with emerging themes in psychiatric genetics. There are a prevalent number of genes, identified as schizophrenia risk genes in the recent genome wide association studies and the autism risk genes from a growing list of exome and genome sequencing studies, that encode proteins for synapse formation and function, voltage-gated ion channels, and transcriptional and epigenetic regulators [13, 16]. Furthermore, there is a growing effort to investigate mosaic genetic variants in psychiatric disorders. The existing iPSC-based studies prove the feasibility of investigating the biology in all of these domains. Many of the experimental approaches are also amenable to adaptation to cell-based phenotypic assays in a drug discovery workflow.

Understanding pathogenic mechanisms for complex and polygenic psychiatric disorders.

For most individuals affected by a psychiatric disorder, possible genetic underpinnings are not known. With the assistance of genomic technologies, it is possible to assess the presence or absence of many genetic risk factors, but their causal roles in pathogenesis remain to be elucidated. Furthermore, the genetic risk factors likely confer aggregative effects in the context of environment, life experiences, and the homeostatic or plastic properties of the nervous system. Are there meaningful ways to design iPSC-based studies to tackle the complexities and identify converging pathogenic mechanisms? Here we discuss two recent examples that each demonstrated promising approaches.

Madison and colleagues [37] established iPSCs for two siblings affected by bipolar disorder (type I) and their two unaffected parents, who belong to a larger pedigree with additional affected individuals. SNP array analysis of fibroblasts from the four family members revealed the presence of several bipolar disorder risk alleles and multiple CNVs with LOD score (log base 10 of odds) greater than 10 and size greater than 10 kb. However, none of these genetic variances were unique to the patients. In other words, no specific genetic underpinning was associated with the disease in this study. However, a set of cellular phenotypes were identified in iPSC-derived central nervous system (CNS) neural progenitors from both patients in comparison with their unaffected parents. These included a reduced proliferation of neural progenitors and reduced neurogenesis upon postmitotic differentiation, which were paralleled by changes in gene expression profiles. Specifically, patient neural progenitors differentially expressed genes encoding WNT pathway components and ion channel subunits (including several voltage-gated calcium channels and sodium channels). CHIR-99021, a compound activating WNT signaling, was found to rescue the proliferation deficit in patient-derived neural progenitors. This work sets another example for family-based study design, for the benefits of enriching the load of known or unknown genetic risk factors and exploring disease or case specific mechanisms within a more uniform genetic background. In the future, it will be desirable to expand the investigation to additional families to address whether the neural progenitor phenotypes are common in the patient population.

In another recent study, Mertens and Wang et al. [38] also investigated cellular phenotypes associated with type I bipolar disorder, using iPSC-derived excitatory neurons representing six patients and four unaffected individuals. The excitatory neurons in this study are characteristic of hippocampal dentate gyrus granule cells and express the Prox1::eGFP fluorescent reporter [39]. Gene expression profiling by RNAseq revealed that the top-ranking changes in patient-derived neurons included upregulation of multiple mitochondrial genes, which was paralleled by observations of elevated mitochondrial membrane potential and smaller mitochondrial size, likely in favor of energizing neuronal activity. In addition, a set of genes involved in proteinkinase A/ proteinkinase C (PKA/PKC) signaling and action potential generation were also upregulated in patient-derived neurons. The gene expression data collectively led to a hypothesis that intrinsic excitability would be altered in patient-derived neurons. Indeed, the researchers observed an overall increase in neuronal excitability from multiple parameters in patch clamp electrophysiology as well as calcium imaging. The most intriguing experiment was testing the effects of lithium on these cellular phenotypes. Lithium is a treatment option for patients with bipolar disorder, but the underlying mechanisms of action are not fully understood, and a fraction of patients are refractory to lithium treatment. Mertens and colleagues found that lithium could suppress neuronal hyperexcitability in neurons derived from patients who responded to lithium treatment, but no effect was observed in neurons from lithium-refractory patients. Furthermore, only in neurons derived from lithium-responsive patients, lithium normalized expression of genes involved in mitochondrial function, PKA/PKC signaling and action potential generation. Therefore the neuronal phenotypes identified in this study may guide further mechanistic investigation of lithium and, potentially, serve as an endophenotype for the evaluation of new therapeutic candidates.

Reconstituting the interplay between brain cell types

So far, our discussion has been confined to the utilities of iPSC-derived neurons, without considering disease-associated roles of other cell types in the brain. In previous efforts based on human embryonic stem cells (hESCs) to model amyotrophic lateral sclerosis, there were important examples showing that human or mouse primary astrocytes carrying mutant *SOD1* alleles trigger cell death in wild-type hESC-derived motor neurons [40, 41]. Such non-cell-autonomous mechanisms, especially those tied to neuron-glia interaction, could also be relevant in iPSC-based investigations of psychiatric disorders. Krencik et al. [42] took an informative step in this direction in their work to model Costello syndrome using patient-derived iPSCs. Costello syndrome, caused by activating mutations in the *Harvey Rat Sarcoma viral oncogene homolog (HRAS)*, is characterized by multisystemic symptoms. These include intellectual disability, short stature, large mouth, loose and soft skin, and unusually flexible joints, as well as heart hypertrophy, arrhythmia and structural defects [62]. *HRAS* controls cell division and growth, but it was not understood

how HRAS hyperactivity leads to intellectual disability in Costello syndrome. Krencik and colleagues found that iPSCs from patients differentiated more rapidly into astrocytes than control iPSCs. The patient astrocytes exhibited hyperplasia and produced more extracellular matrix remodeling factors and proteoglycans. The finding was corroborated by observations in mice, where mouse cortical astrocytes expressing mutant *HRAS* made extra perineuronal net proteoglycans in an experience-independent manner, and parvalbumin expression was constitutively elevated in cortical interneurons. This work provided mechanistic insights into the neurocognitive aspect of Costello syndrome, because previous work had established that the perineuronal net regulates the functional maturation of parvalbumin-positive inhibitory interneurons and neural circuit plasticity [43, 44].

As a future outlook, iPSC-based disease models have the potential to incorporate many brain cell types generated from directed differentiation [45–50], follow regional specification of the human cerebral cortex [51], and build in neuroanatomical components that make up disease relevant circuits (e.g. the corticostriatal circuit, the corticothalamic circuit, the cerebellar circuit etc.). Furthermore, iPSC-derived neural tissue may be spatially patterned or compartmentalized within an engineered cell culture environment to mimic the endogenous histology and neurite orientation [17]. Alternatively, iPSC-derived three-dimensional brain organoids may naturally recapitulate many organizational principles of the human brain in development. Lancaster et al. [52] developed a neural differentiation and culture system to convert human iPSCs into “cerebral organoids”, which are comprised of multiple self-assembled embryonic-stage brain regions, including a readily identifiable cortex. The cortical structure was populated with neural progenitors, including outer radial glial stem cells, as well as spatially organized cortical neurons expressing subtype markers. As an example for the utility of this model system, the researchers found that neurogenesis occurred prematurely in organoids derived from individuals affected by microcephaly. Pasca et al. [53] developed a simpler procedure that specifically generates three-dimensional cultures of the cerebral cortex from iPSCs, named human cortical spheroids. Importantly, the spheroids are laminated with cortical neurons of deep layer and superficial layer identities. Additionally, the spheroids can be sectioned into slices for electrophysiological experiments, resembling an acute slice preparation from rodent brains. Cortical neurons on the slice generated complex synaptic events and spontaneous network activity, indicating emergence of circuit properties.

The use of iPSCs in evaluating therapeutic strategies

Primary hit finding

An iPSC-based phenotypic assay may serve as primary screening assay, identifying hit molecules that act in the human neurobiological context and bypass the gap frequently seen between a primary screen in heterologous cells and validation in native tissues. This latter application requires assay automation to support screening of large libraries of compound or biologics.

In the field of neurodegeneration, Lee and colleagues [54] did pioneering work to demonstrate the feasibility of running iPSC-based drug screens at the scale of thousands of compounds. It is worth noting that the screening assay quantified gene expression levels, for which there are well established instrumentation and methodology for further laboratory automation if larger scale screens are desirable. However, for assays that quantify neuronal activities, such as synaptic transmission, action potentials and voltage-dependent calcium transients, laboratory automation is more challenging for several reasons intrinsic to neurobiology. First, neuronal activities must be tracked over a time period, adding demands on temporal resolution to assay development and choice of instrumentation. Second, neuronal activities have spontaneous and stochastic properties, which increases assay noise and compromises reproducibility and quality. Third, the heterogeneous cellular composition of neural tissues makes it difficult to monitor specifically cell type-specific and disease-relevant activities, such as the excitatory versus inhibitory transmissions that were specifically measured in the studies of Rett syndrome [21] and Phelan-McDermid syndrome [25]. Efforts addressing these challenges are unfolding in the field, such as commercially available multielectrode arrays coupled to multiwell culture plates, custom-made [55] or commercial multiwell devices [56] to evoke and image neuronal calcium transients, the Optopatch system [57], which integrates patterned optogenetic stimulation and optical recording of neuronal action potentials via fluorescent voltage sensors, and the multiwell automated neurotransmission assay system (MANTRA) [58].

The lengthy laboratory procedure to differentiate human iPSCs into neural tissues also adds special considerations to assay development for iPSC-based large-scale primary screens. In conventional cell-based assays, a cell line is easily expandable to any necessary scale and is almost invariant from passage to passage. However, like primary neurons, iPSC-derived neurons are postmitotic, and their availability directly limits the scale of a screen. If a screen is split over multiple stages or runs in an iterative manner, there is a further need to ensure consistency of iPSC neural differentiation from batch to batch. One strategy to establish a large batch of uniform of iPSC-derived neurons is to expand neural progenitors to sufficient numbers out of a single, large-scale neural differentiation and cryopreserve this neural progenitor pool in many aliquots for repeated assay runs. In a true high-throughput screen, up to the million-compound scale, it would be impractical to prepare manually the required number of neural progenitors, differentiate them into neurons, and establish the neuronal culture in a large number of multiwell assay plates. Existing laboratory robots can prepare simple cell lines for high-throughput assays scheduled in advance. It remains to be seen how robotics and associated data tracking tools may adapt to the more elaborate iPSC neural differentiation procedure that spans over weeks of time and with possible staggering of multiple batches of assays. Alternatively, it could be meaningful to explore the advantage of cellular reprogramming methods that convert iPSCs into functional

neurons or other cell types in fewer steps and less amount of time [36].

For novel hits from a phenotypic screen, the pharmacological target and mechanism(s) of action remain to be elucidated. There are target-specific screening panels that can help profile the pharmacological activities of a molecule against many G-protein coupled receptors, kinases, enzymes and ion channels. Alternatively, drug-bound iPSC-derived neural tissue may be used for direct target identification through drug-target cross linking, co-purification, mass spectrometry and proteomic analysis.

Lead identification and optimization

iPSC-based phenotypic assays may also be used to validate and optimize candidate molecules established in upstream assays. Typically, the potency of a candidate molecule will be determined in a concentration response curve. It would be meaningful to compare the potency data from human iPSC derived neural tissue with those from animal tissues as well as cell lines heterologously expressing the target of interest. Here, human-specific aspects in disease mechanisms or target biology may come into play. The developmental stage represented by iPSC-derived neural tissue may also influence the potency data, especially when the target is a hetero-multimeric neurotransmitter receptor or ion channel for which the subunit composition changes during brain development.

Aiding proof-of-concept trials.

Proof of concept (PoC) trials aim at examining target engagement and evidence for therapeutic benefit in a small cohort of patients with genetic or biological characteristics that match the intended mechanism(s) of action of an investigational drug. A well designed and executed PoC trial should provide a timely answer regarding the clinical validity of the therapeutic hypothesis and increase the pharmacological knowledge of the investigational drug. Adverse events from a PoC trial would advise against pursuing larger scale efficacy trials, protect other patients and healthy volunteers from unnecessary risks, and save on further research and development and clinical resources. Theoretically it is possible to prescreen a candidate therapeutic against iPSC lines derived from many patients and healthy subjects, targeting one or multiple validated cellular phenotypes as the experimental readout. The responder rate in an iPSC-based prescreen may help reveal patient heterogeneity that cannot be sufficiently predicted by other patient stratification methods. With additional profiling experiments in the collection of iPSC lines, it may even be possible to identify translational biomarkers that cosegregate with the prescreen findings. All this information may inform the design of a PoC trial in the clinic. In order to power such an iPSC-based study with enough numbers of cell donors, it would be necessary to establish cell banks hosting large collections of iPSC cell lines [59]. Learning from the research fields utilizing model organisms, it would also be desirable to have libraries of isogenic iPSC lines that each carries a disease-relevant genetic variant.

Aiding the interpretation of clinical data with iPSC-based findings

If iPSC lines are available from patients who benefitted from a therapeutic, or who responded with a pharmacodynamic marker, versus those who did not, retrospective studies can be carried out in iPSC-derived tissues to explore cellular and molecular mechanisms of the efficacy or pharmacodynamic data. This point has been covered in an earlier review on iPSC-aided drug development in general [60]. Such applications could be particularly valuable in psychiatric drug discovery, where the complex etiology and clinical spectrum require focused research and development efforts for correctly delineated patient populations. The study of lithium in bipolar-disorder iPSCs [38] that we discussed earlier has provided a proof-of-principal example.

Cautions

Although iPSCs-based experimental systems provide human-specific and individual-specific biological contexts for research and drug discovery, they should not be simply viewed as “disease in a culture dish”, at least in the realm of psychiatry, where the ultimate goal is to understand and discover treatments for abnormalities in behavior and cognition. It will be more rigorous if we take experimental observations from iPSCs as molecular and cellular correlates of disease, which through proper validation and integration with other study approaches may lead to the discovery of disease mechanisms or tools and methodology for drug discovery.

We propose a set of key questions to be considered for the use of iPSCs across different stages psychiatric drug discovery (fig. 1). Reflecting on how other experimental systems, including the variety of animal models, have improved over time, we would anticipate a similar evolution of iPSC-based systems in the years to come and many lessons to be learned.

Conclusion

In this review, we have made an effort to reflect on the fast-evolving field of iPSC-related methodology to model and

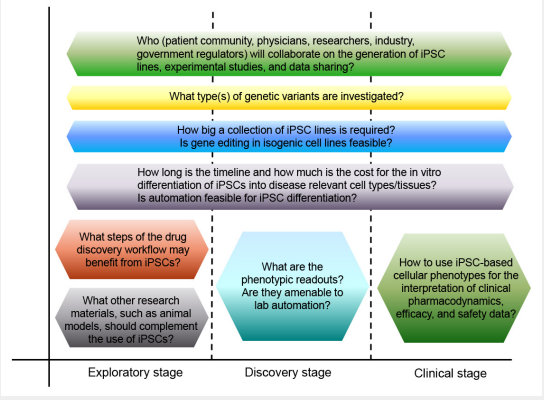

Figure 1

Key questions to consider for the use of human induced pluripotent stem cells (iPSCs) in psychiatric drug discovery. Some questions are relevant to a specific stage of a drug discovery project, while others are overarching for the entire workflow.

discover treatments for psychiatric disorders. Most of the research studies we reviewed were the first of their kind for a particular disease class. It will be important to watch for follow-up studies, new research techniques, and emerging cellular principles that distinguish between or unify disease classes. We emphasized that the use of iPSCs should go hand-in-hand with advances in human genetics, no matter if it is for a single-gene or genetically complex disorder. We shared our views on how iPSCs can be used in several steps of psychiatric drug discovery, with a focus on the "standard" workflow that starts with a defined therapeutic mechanism or hypothesis. There are many other important (prospective) applications of iPSC that we did not discuss within this scope, such as evaluating therapeutics derived from natural products, generating specific neuronal subtypes for therapeutic transplantation [61], and aspects related to drug safety and adverse effects [60]. But, we hope our review may serve as a primer, helping bridge future research efforts using iPSCs in basic science, drug discovery, and clinical development for psychiatric disorders.

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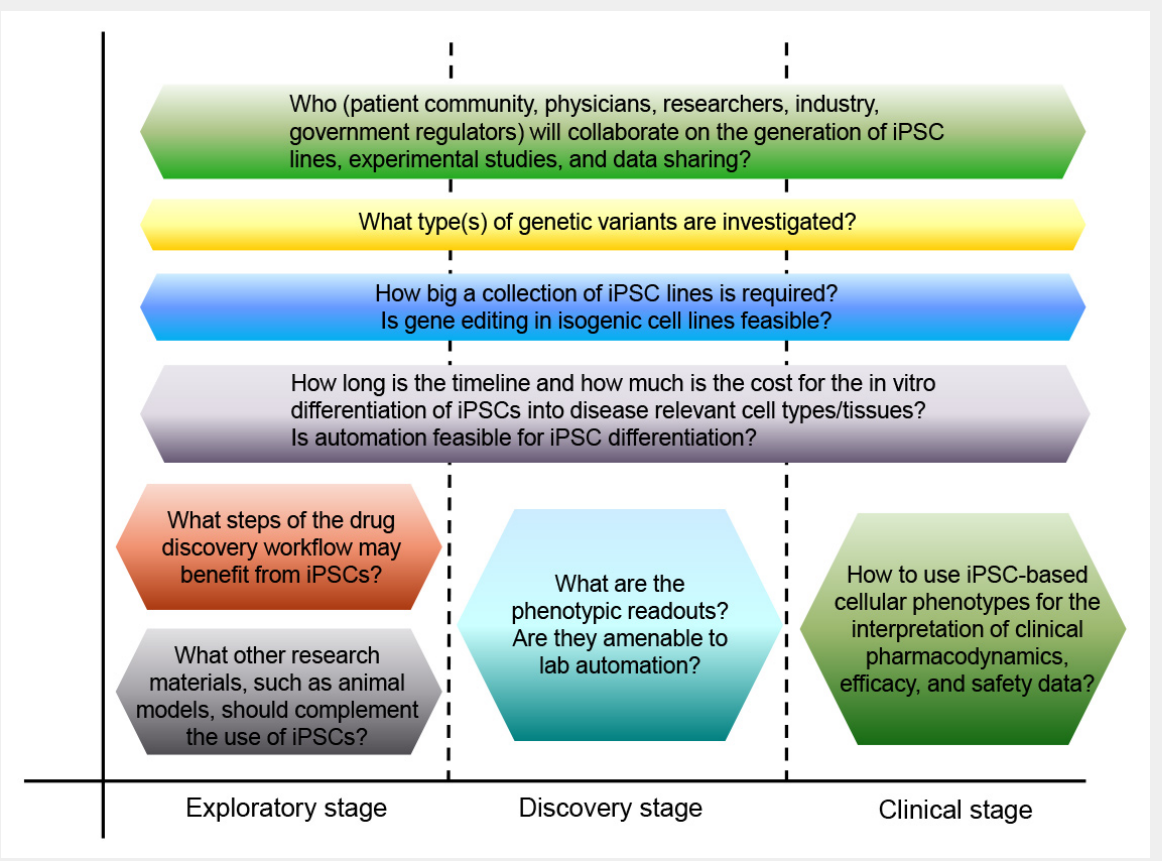
Figures (large format)



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