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EXPERT REVIEW

Modeling psychiatric disorders: from genomic findings to cellular phenotypes

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Major programs in psychiatric genetics have identified >150 risk loci for psychiatric disorders. These loci converge on a small number of functional pathways, which span conventional diagnostic criteria, suggesting a partly common biology underlying schizophrenia, autism and other psychiatric disorders. Nevertheless, the cellular phenotypes that capture the fundamental features of psychiatric disorders have not yet been determined. Recent advances in genetics and stem cell biology offer new prospects for cell-based modeling of psychiatric disorders. The advent of cell reprogramming and induced pluripotent stem cells (iPSC) provides an opportunity to translate genetic findings into patient-specific *in vitro* models. iPSC technology is less than a decade old but holds great promise for bridging the gaps between patients, genetics and biology. Despite many obvious advantages, iPSC studies still present multiple challenges. In this expert review, we critically review the challenges for modeling of psychiatric disorders, potential solutions and how iPSC technology can be used to develop an analytical framework for the evaluation and therapeutic manipulation of fundamental disease processes.

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A NEED FOR DISEASE MODELS

Psychiatric disorders are associated with major economic, societal and personal burdens. As a group, they constitute 13% of the global burden of disease, and are the leading cause of disability worldwide.[1,2] Multiple lines of investigation from brain imaging, studies of post-mortem brain tissue and genetic studies implicate aberrant cellular function in the most serious psychiatric disorders (for example, schizophrenia (SCZ), bipolar disorder, autism spectrum disorder (ASD), anorexia nervosa and major depressive disorder). However, these implications have not been tested *in vitro,* and this relative lack of understanding of disease mechanisms hampers the development of treatment. Induced pluripotent stem cells (iPSC) technology is an exciting and very promising tool to generate new disease models, with the ultimate goal of creating a new generation of pathophysiology-relevant assays for *in vitro* drug screening.[3] iPSC-based investigation has added advantages of permitting temporal analyses of neurodevelopmental deficits that are not as readily available in animal studies and human studies, allowing longitudinal cell studies that follow the progress of disease processes from initiation to their end point.[4–8] However, to develop iPSC-based assays that truly reflect the pathophysiology of psychiatric disorders, we need a precise understanding of which molecular pathways and cellular structures are involved.

Here we review the search for cellular models and phenotypes in the context of the current state of the art for SCZ genetics and understanding gained from SCZ-related animal models (Figure 1). We will discuss current capabilities and further developments needed, potential pitfalls for stem cells reprogramming, culturing and *in vitro* differentiation; and the establishment of relevant cellular phenotypes that can be translated into disease models (Figure 1), and ultimately into pharmaceutical targets for psychiatric disorders.

ADVANCES IN PSYCHIATRIC GENOMICS: THE CASE OF SCZ

Decades of twin/family studies have compellingly established that psychiatric disorders are heritable.[9] However, the identification of causal genetic variants has, until recently, been notably difficult. Unprecedented advances in the past decade have shown that psychiatric disorders are complex and influenced by the combination of hundreds of common genetic variants each of relatively small impact on disease risk and occassionally by rare variants with larger effects.[10] The field has made major advances in identification of these risk variants, although it is clear that there are more to be found, and how they combine together to create a polygenic risk is currently unknown.

Genetic epidemiology provides strong support for a genetic component for SCZ (with a heritability of ~0.64 in Nordic population samples and 0.81 in a twin study meta-analysis).[9–13] Common variation assessed by genome-wide association studies

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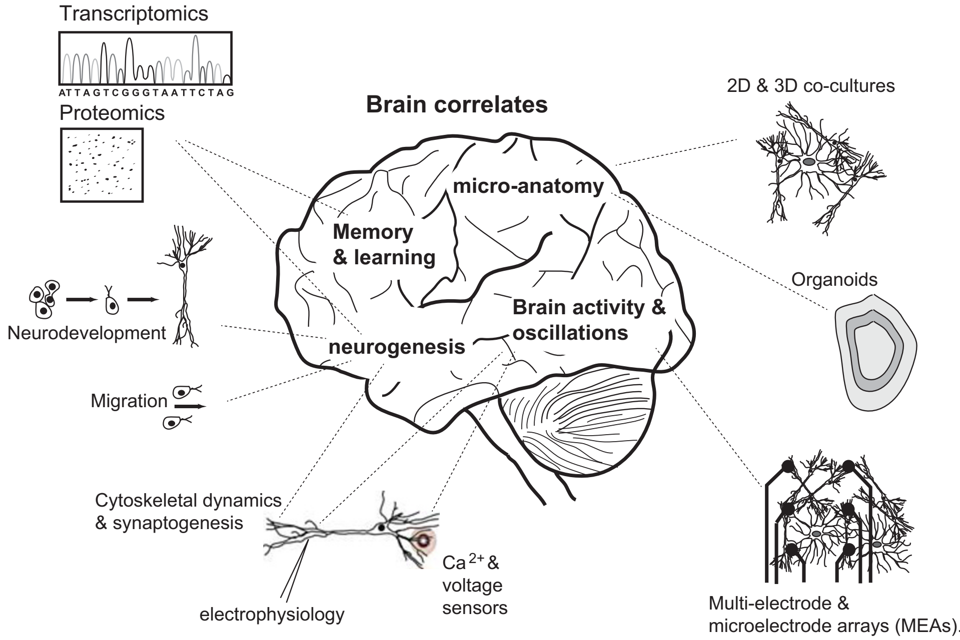

Figure 1. Brain correlates of *in vitro* iPSC cell phenotypes. Current analysis of patient iPSC offers a range of potential methods of cell phenotyping that correlates to potential changes in brain pathology associated with psychiatric disorders. Gene expression (transcriptomic) and protein expression (proteomic) profiling of *in vitro* neurodevelopment or iPSC-derived mature neuronal and glial cultures correlates with brain development and processes associated with adult neurogenesis, such as some aspects of memory and learning. Cell analysis of neurodevelopment, cell migration, cytoskeletal dynamic and synaptogenesis informs on the basic processes by which neurogenesis builds and remodels the brain. Functional activity is measured by electrophysiological recording (for example, patch-clamp) and calcium or voltage sensors (dyes and genetically encoded markers). Multicellular interactions (connectomics) can be investigated as structural interactions in two-dimensional (2D), 3D and organoid cell co-culture, and at the functional level using multi-electrode and microelectrode array (MEA) recordings. iPSC, induced pluripotent stem cell.

(GWAS) yields single nucleotide polymorphism-based heritability estimates for SCZ over 0.30, and have yielded 108 independent genomic risk loci.[14,15] Critically, most loci identified in GWAS are broad (median 129 kb) with small impact on SCZ risk (median relative risk 1.08).[14] Whole-exome sequencing studies for SCZ identified no specific genes but implied a role for functional gene sets, for example, voltage-gated calcium channels, ARC-associated scaffold and FMRP interactors.

Copy number variation (CNV) studies for SCZ have yielded a dozen CNVs that are robustly associated with SCZ, but also with other psychiatric disorders.[10,16,17] Findings from recent studies suggest that a high polygenic burden adds to the SCZ risk in carriers of CNVs, suggesting cumulative effects between common and rare risk variants.[14,18,19,20]

SCZ, but also ASD, converge on common pathways; such as within synaptogenesis and synapse function[21,22] and epigenetic processes,[22,23] with many genes being highly expressed during fetal cortical development.[24–26]

Despite these unprecedented advances in the genetics of SCZ, very few of the current findings unequivocally implicate specific individual genes that are easily 'actionable' for biological, clinical or therapeutic studies. To be of value, such studies need to show strong linkage between the genetic variation and a discriminative phenotype that is relevant for the disorder. This connection is both crucial for understanding the molecular pathways that lead to SCZ and essential to develop iPSC-based assays that reflect SCZ pathophysiology. A single variant of small effect is unlikely to yield a measurable cellular phenotype, we therefore aim to model either the cumulative effect of hundreds of risk variants of small effect or a single variant of high penetrance or large effect.

iPSC technology[27,28] has provided a highly promising tool to investigate human disorders, and is especially well-suited to deal with disorders that are not caused by a single mutation, such as mental disorders.[4,29] As iPSC studies rely on cells from patients, one can select patients with a high genetic propensity for the disease, either due to the accumulation of many common variants of small effect or due to carrier status of a rare variant of large effect. In addition, by utilizing cells from patients with a targeted set of risk alleles, one also captures the complete genetic background of an individual, which includes possible genetic modifiers that are currently unidentified.[4] Several initial iPSC studies for SCZ have already been carried out and have yielded proof-of-principle by successfully identifying differences in synaptic functions in iPSC-derived cells from patients. However, these initial studies also clearly illustrate some of the pitfalls of iPSC studies for identifying cellular traits associated with SCZ. We will discuss these pitfalls in more detail below, after first examining the alternative approach of using animal models for SCZ and ASD.

RODENT MODELS IN THE STUDY OF SCZ AND AUTISM

Several approaches have been employed to produce rodent models for SCZ and ASD. Rodents provide a number of general advantages with respect to animal husbandry and handling, well-established behavioral and physiological tests, and the availability of transgenic manipulation.[30,31] For example, maternal stress and malnutrition, infection and hypoxic insult at birth have all been implicated as developmental triggers of SCZ, and these can be replicated in rodent models through manipulations such as prenatal drug administration, disruption of neurogenesis during gestational periods, neonatal ventral hippocampal lesions, post-weaning social isolation and perinatal or maternal immune activation (reviewed in ref. 30). Genetic manipulation has also been employed to target several genes implicated in SCZ or ASD in transgenic mice.[31–42] An important caveat with respect to transgenic models is that these are relatively easy to generate, and

Table 1. Comparing advantages of transgenic mouse and human cell-based models

Experimental feature	*Advantage*	
	Transgenic mouse	*Human iPSCs in vitro*
Assessment of monogenic effects	High	High
Assessment of polygenic effects congruent with human disease	Low	High
Recapitulates genetic complexity of human disease	Nil	High
Recapitulates physiological complexity of human disease	Moderate	Low
Replication of age-dependence of human disease	Low	Low
Behavioral assessment	High	Nil
Cognitive assessment	Low	Nil
Neuroanatomical assessment	High	Nil
Neurophysiological assessment at cellular/subcellular level	High	High
Neurophysiological assessment at network level	Moderate	High
Neurophysiological assessment of neural networks with high throughput	Low	High
Neurophysiological assessment at systems level	High	Nil
Biochemical/epigenetic assessment at cellular/subcellular level	Moderate	High
Pharmacological assessment/drug testing relevant to human disease	Moderate	High

Abbreviation: iPSC, induced pluripotent stem cell.

thus may fuel research efforts that are actually red herrings because the genes in question are not convincingly implicated in the human diseases.[43]

Although rodents provide tractable and accessible platforms, these are not without significant shortcomings. First, although each of the rodent models that are already established replicates certain neurophysiological, neuroanatomical and/or behavioral features of genetic mutations implicated in SCZ or ASD, none of them fully recapitulates the complexity of these disorders. Thus, the knowledge contributed by animal models to the etiology of SCZ and ASD is by its nature fragmentary, with each model providing a specific facet that needs to be integrated into a greater whole that reflects the heterogeneity implicit in the disorder itself. Second, animal models cannot be interrogated with the necessary depth. Indeed, how can one gauge effects on thought processes, perception and abstract learning in animals, and when these can only be conveyed fully through language? As a consequence, many core features of psychiatric disorders can only be assessed indirectly or obliquely in animal models, with an artificial focus on simpler behavioral and physiological features that can be easily identified. The extent to which these can be translated to the more complex symptomatology of the human conditions is not always clear. Third, induction of disease states in rodents may involve acute pharmacological or other insults that do not accurately replicate the causes of psychiatric disorders in humans. Even transgenic approaches targeting the same genes may be inaccurate, since a manipulation as coarse as a single gene knockout is unlikely to capture the complexity of the genetic causes of psychiatric disorders. Moreover, the genomic landscapes of the genes in question may differ in rodents and humans, and genetic differences will also be compounded where genes and environment interact extensively in disease development. Fourth, rodents and humans have vastly different lifespans, which may not be appropriately congruent with respect to the timeline of disease development. Last, the pharmacology of potential drug treatments may differ in the two species, creating false positives and negatives in preclinical studies.

In conclusion, only a small percentage of psychiatric disorders is caused by single gene variations and can be modeled with transgenic mice. Indeed, many clinical studies based on promising drug targets found in animals failed human translation. As a consequence, the difficulties in modeling polygenic risk gene variants and the human genetic background have made animal models less attractive in modeling complex neurological disorders. Nevertheless, transgenic mouse models advanced our understanding of potential mechanisms regulated by genes involved in psychiatric disorders. Table 1 compares some of the principal advantages of transgenic mouse models and human iPSC-based models.

CELL PHENOTYPING OF PATIENT IPSC

The key challenge for iPSC-based disease modeling is to identify one or more relevant cellular phenotypes that accurately represent the disease pathophysiology. Increasing numbers of reports have demonstrated that for many diseases specific pathophysiology can be captured in human iPSC-based disease models. These range from cardiovascular disease,[44,45] cancer,[46,47] ocular disease,[48,49] diabetes mellitus[50,51] and neurological disorders of the brain.[52,53] Can the same approach be applied to complex psychiatric disorders?

The problem is that almost all psychiatric disorders are characterized by clinical signs and symptoms, but lack independent verification from objective biomarkers. Thus, how might these clinical phenotypes manifest themselves in terms of cell behavior? The identity of robust cellular 'readouts', which typify any psychiatric disorder, is a crucial unsolved problem and an area of intense study[54] (Table 2). When satisfactorily answered, this will herald a new degree of biological objectivity and quantification for the study of psychiatric disorders.

The aim is to find a single or small number of cell phenotypes or parameters that strongly associate with psychiatric disorders, and establish a cellular profile characteristic of cells derived from the general patient population. Although a consensus set of cellular phenotypes for psychiatric disorder is yet to be established, we can define some of their desired characteristics. First, cellular phenotypes have to relate to the biological pathways identified by genetics. Second, although there are many risk genes in disparate biological pathways, at some level, phenotypes should converge onto a much smaller grouping. Third, phenotypes need to be quantifiable. Finally, to be useful for drug development cellular phenotypes should be reversed by pharmacological treatment, although not necessarily by drugs in current use.

Although human iPSC-based approaches underrepresent the complexity of the human central nervous system, cellular phenotypes are likely to lie more proximal to molecular disease mechanisms than phenotypes seen at the level of a tissue or organism,[55] and thus may bypass compensatory homeostatic

Table 2. Current *in vitro* and iPS models

Cell type (iPSCs, ESCs)	*Number of lines*	*Phenotype*	*Reference*
Schizophrenia			
Schizophrenia iPSC (genotype unknown)	Healthy controls (6) Patients (4)	(1) SCZ human iPSC neurons showed diminished neuronal connectivity in conjunction with decreased neurite number, PSD95-protein levels and glutamate receptor expression. (2) Gene expression profiles of SCZ human iPSC neurons identified altered expression of many components of the cyclic AMP and WNT signaling pathways. (3) Key cellular and molecular elements of the SCZ phenotype were ameliorated following treatment of SCZ iPSC neurons with the antipsychotic loxapine. (4) Discovery-based approaches-microarray gene expression and stable isotope labeling by amino acids in cell culture (SILAC) quantitative proteomic mass spectrometry analyses: abnormal gene expression and protein levels related to cytoskeletal remodeling and oxidative stress, and subsequently aberrant migration and increased oxidative stress in SCZ iPSC NPCs observed. (5) SCZ cases showed elevated levels of secreted DA, NE and Epi. Consistent with increased catecholamines, the SZ neuronal cultures showed a higher percentage of tyrosine hydroxylase (TH)-positive neurons, the first enzymatic step for catecholamine biosynthesis. (6) Impaired differentiation into hippocampal granule cells. (7) Decreased amplitude and frequency of sEPCs in hippocampal granule cells. (8) Increased cell-to-cell variation in the HSF1 activation level among neural progenitor cells (NPCs) differentiated from iPSCs derived from schizophrenia patients.	56,66,124–126
Schizophrenia iPSC: 15q11.2 microdeletion haploinsufficiency of *CYFIP1* that encodes a subunit of the WAVE complex that regulates cytoskeletal dynamics.	Healthy controls (3) Patients (3)	(1) Deficits in adherens junctions and apical polarity. (2) Targeted human genetic association analyses revealed an epistatic interaction between CYFIP1 and WAVE signaling mediator ACTR2 and risk for schizophrenia.	127
Schizophrenia iPSC 22q11.2 microdeletion (del)	Healthy controls (2) Patients (3) Healthy controls (6) Patients (3)	(1) A significant delay in the reduction of endogenous OCT4 and NANOG expression during differentiation. (2) A number of genes involved in synaptogenesis that have been implicated in SCZ and ASD are also increased in these early-differentiating neurons, including NRXN1, NLGN1, RELN, CNTNAP2 and CTNNA2. (1) 45 differentially expressed miRNAs were detected (13 lower in SZ and 32 higher). (2) A significant increase in the expression of several miRNAs was found in the 22q11.2 del neurons that were previously found to be differentially expressed in autopsy samples and peripheral blood in SZ and autism spectrum disorders (for example, miR-34, miR-4449, miR-146b-3p and miR-23a-5p).	128,129
Schizophrenia iPSC (genotype unknown)	Control (1): aged match male Patient (1): female SCZ patient	(1) Extra-mitochondrial oxygen consumption is increased in SCZ NPCs compared with control NPCs (2) NPCs from a SCZ patient had higher ROS levels, which were reverted by valproic acid. (3) NPCs from SCZ patient have higher levels of potassium and zinc. (4) Valproate normalized the elevated zinc and potassium levels.	130,131
Schizophrenia iPSC DISC1 mutations	Controls (3): 2 from same pedigree and 1 unrelated control Patients (2): with the frameshift DISC1 mutation in same pedigree. Isogenic iPS cell lines (3): 1 TALENs-corrected *DISC1* iPS cell line; 2 TALENs-introduced *DISC1* mutation (4-bp deletion) Control (1): healthy human iPSC line YZ1 Isogenic *DISC1* mutations (2): 1 TALENs-introduced exon 8 frameshift; 1 CRISPR/Cas-introduced exon 2 frameshit	(1) Density of SV2+ synaptic boutons is decreased in the SCZ neurons. (2) Frequency, but not amplitude, of spontaneous synaptic currents is decreased, suggesting presynaptic release defects. (3) TALEN genome-editing shows that the DISC1 mutation is necessary and sufficient for these changes. (4) Schizophrenia neurons show widespread transcriptional disturbances. (1) An increased level of canonical Wnt signaling in neural progenitor cells. (2) Decreased expression of fate markers such as Foxg1 and Tbr2 in both mutants. (3) Both gene expression changes are rescued by antagonizing Wnt signaling in a critical developmental window. (4) Subtly alters neuronal fate but not neuronal maturity.	58,132
Schizophrenia iPSC (genotype unknown)	Healthy control (2) Patient (3): clozapine-treated schizophrenia patients	(1) 12-day-old SCZ NPCs show decreased expression of nestin and increased expression of PAX6 compared with control NPCs, suggesting a delay in differentiation. (2) SCZ NPCs differentiate into dopaminergic neurons (DaNs) with a lower yield than control NPCs.	133

Table 2. (Continued)

Cell type (iPSCs, ESCs)	Number of lines	Phenotype	Reference
		(3) SCZ glutamatergic neurons (GluNs) express lower levels of TBR, PSD95 and synapsin1 than control GluNs. (4) The mitochondrial membrane potential has a lower magnitude in SCZ NPCs, GluNs and DaNs compared with control equivalent neural cells. (5) The distribution of mitochondria inside neurons is more variable in SCZ NPCs, GluNs and DaNs compared with control.	
Schizophrenia iPSC (genotype unknown)	Controls (6) Patients (4)	(1) Expression of genes in the Wnt signaling pathway is increased in SCZ NPCs. (2) Activity of the Wnt-β–catenin signaling cascade, as measured by the TOPFLASH assay, is increased in SCZ NPCs compared with control.	61
ASD			
Timothy syndrome (TS) iPSC: mutations in the L-type calcium channel, Cav1.2.	Healthy controls (2) Patients (2)	(1) Showed the TS-associated transcriptional changes. (2) Activity-dependent dendrite retraction (3) Defects in calcium-channel function (4) Altered activity-dependent gene-expression/dendritic retraction (5) Abnormality of lower cortical layer and callosal projection differentiation (6) Abnormal catecholaminergic differentiation	134,135,57
Rett syndrome: MECP2 null	Healthy controls (1) Patients (1)	(1) A reduction in soma size. (2) Fewer synapses, reduced spine density, smaller soma size, altered calcium signaling and electrophysiological defects (3) Reduced synaptic density was restored by treatment of IGF1 or gentamycine. (4) Defect in neuronal maturation. (5) Smaller nucleus size (6) Impaired AKT/mTOR activity (7) Mitochondria deficit (8) Decreased transcription in neurons.	136–140
Atypical Rett syndrome iPSCs: mutations of the cyclin-dependent kinase-like 5 (CDKL5) and netrin-G1 (NTNG1) genes	from two female patients: Healthy CDKL5 (2) Mutant CDKL5 (2)	(1) Exhibit aberrant dendritic spines (2) Impairs synaptic activity (3) A significantly reduced number of synaptic contact	141
Phelan–McDermid syndrome iPSCs: deletion of *SHANK3*	Control: normal iPSC (1) and ESC (1) Patients (2)	(1) Impaired excitatory (both AMPA and NMDA-mediated) but not inhibitory synaptic transmission mainly due to loss of function of SHANK3. (2) Reintroduction of SHANK3 and IGF1 application restore excitatory synaptic transmission	142
Fragile X syndrome iPSC	Control: wild-type-FMR1(2) Patients (3)	(1) DNA methylation and transcriptional silencing even in the pluripotent stage. (2) Neurons showed reduced neurite numbers and neurite lengths (3) Fewer and shorter processes	143–145
Fragile X-associated tremor/ataxia syndrome (FXTAS) iPSC:(FMR1)	From 1 patient: Control: wild-type-FMR1 (1) Premutation FMR1 (1)	(1) Shorter neurite length (2) Fewer PSD95-positive synaptic puncta (3) Sustained calcium response after glutamate application	146
15q11-q13.1 duplication (Dup15q) syndrome, (CNV), iPSC	Control (1) Patients (4)	(1) Gene copy number does not consistently predict expression levels in cells with interstitial duplications of 15q11-q13.1. (2) mRNA-Seq experiments show that there is substantial overlap in the genes differentially expressed between 15q11-q13.1 deletion and duplication neurons.	147
ASD (NRXN1 mutation)	Control: normal iPSC (1) and human ESC (1) Mutants: NRXN1 knockdown in neural stem cells (2)	(1) Reduced glial differentiation (2) Altered gene expression related to cell adhesion and neuron differentiation	148
ASD (NRXN1 mutation)	Control: human ESC (1) Mutants: human ESC (2) Heterozygous Conditional NRXN1 mutations	(1) Decrease the frequency of spontaneous mEPSCs in neurons without affecting synapse density. (2) Impaired evoked neurotransmitter release but not the readily releasable pool of vesicles. (3) Increased CASK protein levels in neurons.	149
Idiopathic ASD (deletions in Chromosomes 10 or 14)	Control: unaffected, first-degree family members (1–3) Patients (4)	(1) Significantly perturbed in transcriptional regulation of cell proliferation/cell fate, neuronal differentiation/process outgrowth and synaptic transmission. (2) A significant decrease in cell-cycle length in ASD-derived iPSCs and derived neuronal progenitors. (3) Accelerated or increased neuronal differentiation and synaptic connections. (4) An increase in the number of inhibitory synapses in ASD-derived neurons.	100

Table 2. (Continued)

Cell type (iPSCs, ESCs)	Number of lines	Phenotype	Reference
		(5) The number of cells immunoreactive for ASCL1/MASH1 and NKX2.1 (two TFs expressed by GABAergic progenitor cells) and the neurotransmitter GABA was also increased in ASD-derived organoids. (6) FOXG1 overexpression causes deregulated cell differentiation in ASD organoids	
Williams–Beuren syndrome	Control (1) Patients (3)	(1) Profound alteration in action potentials, with prolonged repolarization times and a deficit in voltage-activated K+ currents. (2)136 negatively enriched gene sets, including gene sets involved in neurotransmitter receptor activity, synaptic assembly and potassium channel complexes.	150

Abbreviations: ASD, autism spectrum disorder; iPSC, induced pluripotent stem cell; NPC, neural progenitor cells; SCZ, schizophrenia.

processes that buffer the effects of deleterious genetic variants in whole tissues and organs (Table 2). Identification of cellular phenotypes may therefore offer a more direct readout of the pathophysiological process (Figure 1). This, of course, would need to be validated against clinical data. We discuss below various ways to phenotype iPSC-derived patients cells and to interrogate the phenotypes to extract information about the disorder.

‘OMIC’ APPROACHES TO CELL PHENOTYPING

Post-genomic technologies offer a battery of approaches for profiling cell difference at both population and single-cell level. Advances in RNA sequencing technologies and transcriptomics provide one of the easiest and highest throughput approaches to cell phenotyping, and potentially could be compared with transcription profiles from brain biopsies or post-mortem tissue. Transcriptome studies of both SCZ and ASD patient-derived cells have identified hundreds of gene expression differences.[56–59] To date, however, there is no definitive or consensus RNA-based transcription profile associated with SCZ or ASD-derived iPSC, a situation that is mirrored by post-mortem brain tissue profiling.[60] Trends point to expression changes of genes involved in synaptic structure, adhesion and transmission, and specific cell signaling pathways particularly those associated with glutamate, Wnt and cAMP signaling.[61] These studies also often reveal overlap with risk genes identified by both GWAS and CNV studies, and may point to convergence on biological pathways rather than on individual genes.

Mapping and measuring DNA methylation may extend this analysis to provide unique epigenetic signatures. For example, the methyl-cytosine-binding protein MeCP2 is causative of Rett’s syndrome and is associated with ASD.[62] Histone protein modifications can be profiled using ChIP-seq and a number of histone methyl transferase enzymes are associated with neuropsychiatric disorders. Either alone, or more likely when combined with expression data, epigenetic profiling may identify developmental and activity-dependent cellular phenotypes.[63–65]

Proteomic technologies have also been used to investigate patient iPSC phenotypes, backing up the results of transcriptional profiling. Use of SILAC (stable isotope labeling by amino acids in cell culture) in mass spectrometry to quantitate changes in protein levels of neural progenitor cells (NPC) has shown decreases in the SCZ-associated NLGN3 protein, and increases in the actin cytoskeletal regulators Cofilin and Profilin, and proteins associated with oxidative stress.[66] Protein profiling can be extended further to protein function by investigating protein interactomes and phosphoproteomes. An analysis of the protein complexes in neuron-like SH-SY5Y cells showed a convergence of proteins encoded by ASD associated genes onto a small number of protein complexes.[67] Phosphoproteomic analysis of iPSC-derived from Phelan–McDermid syndrome (PMDS) patients, in which the post-synaptic density protein *SHANK3* is lost, and of neurons from Shank3 knockdown mice, has revealed elevated activity of protein kinase CLK2 and demonstrated its potential as a therapeutic target.[68]

NEURODEVELOPMENTAL DEFICITS

A major advantage of patient-derived iPSC studies is the possibility to follow neurodevelopment *in vitro*. Transcriptional analyses are beginning to show that differences in neurodevelopment may arise prior to overt neuronal differentiation, leading to altered timing or cell differentiation fates of NPC.[66] This fits with the potential neurodevelopmental component of psychiatric conditions and the profile of genetic risk. Transcriptional differences can readily be confirmed using the extensive range of antibodies to neurodevelopmental marker proteins. Interestingly, a number of CNV cases, such as PMDS and Timothy Syndrome, in which there is a deficit in the voltage-gated calcium channel, *CACNA1C* [57] show gene expression changes at the NPC stage. This may reflect a feedback onto neurodevelopmental signaling due to electrophysiological activity or cell contact-dependent gene expression[69] in determining developmental timing and differentiation. Such studies raise the possibility that transcriptional profiling of developmentally regulated gene expression in patient-derived iPSC may reveal quantitatively robust and disease-relevant phenotypes.

In addition to neuronal deficits, abnormalities of all three glial cell types have been observed in SCZ patients.[70] Post-mortem studies indicate that oligodendrocyte numbers are reduced, and that oligodendrocyte maturation and morphology is impaired in SCZ patients,[71,72] although neuroleptic treatment and aging might have confounded these results. Results from large-scale expression analyses and GWAS for SCZ,[73–75] implicate changes in genes that regulate cell-cycle control and oligodendrocyte maturation, suggesting impaired cell-cycle exit and re-entry.[71] Altered astrocyte numbers are also found in the brains of SCZ patients after autopsy with early studies reporting astrogliosis,[76] while more recent studies indicate astrocyte cell loss in selected (sub)cortical and callosal regions.[77] GWAS demonstrates genetic variants in genes involved in astrocyte function, including signal transduction, tyrosine kinase signaling, G protein–coupled receptor signaling, small GTPase-mediated signaling, cell adhesion and gene transcription.[73] These findings are supported by results from expression studies that showed altered expression levels of astrocyte-associated genes, including *GFAP*,[78] glutamine synthetase[79] and *S100B*.[80] The involvement of biological pathways associated with inflammation and immunity in the development of SCZ is receiving increased attention, and is supported by patient genetic studies, with variants found in several cytokine genes,[81,82] as well as the major histocompatibility complex region[83] where structural variants of the complement component 4 (C4) gene lead to increased activity.[84] Previous studies have indicated microglial activation and altered microglia-related gene expression in postmortem brain tissue (reviewed by ref. 70). As severe infections and aberrant immune responses are risk factors for SCZ, this may point to gene–environment interactions for SCZ and the use of anti-inflammatory drugs in treatment strategies.[85]

ALTERED NEURONAL CELL BIOLOGY AND FUNCTION

Beyond transcriptomics and neurodevelopment, other cell parameters may also be effective measures of cellular phenotype. Brennand *et al*.[66] noted reduced migration in neural precursor cells from four SCZ patients, using a variety of assay formats.[66] The molecular mechanism for this is not entirely clear but correlates with increased expression of adhesion molecules. Its relevance may be significant, since interneurons migrate from specific progenitor domains to populate cortical and other regions during brain development. Changes in morphology due to alterations of the cytoskeleton have also been observed, suggesting more than just an adhesion effect.[86] Cytoskeletal effects could manifest later in development as changes in neuroarchitecture and particularly in dendritic spine morphology and dynamics. If of sufficient magnitude, such structural sequelae could lead to macroscopic changes in brain anatomy that may correlate with larger scale changes detected by human brain imaging.[87] Computed tomography and magnetic resonance imaging analyses have revealed structural changes in the brain of SCZ patients, such as enlarged lateral and third ventricles, smaller cortical volumes, smaller gray matter volumes and larger basal ganglia (reviewed by ref. 88). Morphometric changes generally do not progress overtime and therefore may match histological findings in postmortem tissue, which include altered cortical and hippocampal pyramidal neuron size, decreased interneuron numbers and reduced dendritic spine densities.[88,89]

At the subcellular level, altered synaptogenesis, synaptic vesicle release and mitochondrial function have all been observed in patient-derived iPSCs.[58,62] Recently, mitochondrial abnormalities have been detected using RNA sequencing and mitochondrial assays, and hyperexcitability has been demonstrated by using both patch-clamp recording and Ca^{2+} imaging in immature neurons from patients with bipolar disorder.[90]

The gold standard for electrophysiological assessment is patch clamping recording, and mature differentiated neurons are assessable with this technique. However, this approach requires obtaining high-resistance seals between the electrode tip and the neuron surface for full effect, limiting throughput, even in automated systems. An alternative is to use optical recording of electrical events to monitor cell activity. This is most commonly done indirectly by imaging calcium fluctuations, using calcium sensitive fluorescent dyes or genetically encoded calcium indicators.[91] The latter approach has the added advantage that genetically encoded calcium indicators can be selectively expressed in specific cell types. Calcium recording, however, can only capture events that involve changes in intracellular calcium concentration, meaning that hyperpolarization and inhibitory synaptic events go largely unrecognized, and it has low-temporal resolution. Voltage-sensitive indicators on the other hand provide direct information about changes in membrane potential irrespective of the cause and the sign, permitting the assessment of both excitatory and inhibitory synaptic interactions and depolarizing and hyperpolarizing neurotransmitter and drug effects.[92,93]

Currently, the range of phenotypes seen in cellular studies of neuropsychiatric disorders is diverse and variable, and there is a need for a more systematic investigation across a range of phenotypes. For example, effects on single-cell electrophysiological parameters appear variable with no clear pattern. What is clear, however, is that there do not seem to be major deficits in the basic electrophysiological behavior of neurons. Where differences are emerging is in synaptic function and connectivity, findings that fit the types of pathways implicated by genetic analysis. This may point to the major problem with single-cell measurements, as the biology of neuropsychiatric disorders is ultimately an emergent property of cell connectivity and network activity.

CELL INTERACTIONS AND NEURAL NETWORKS

Two general approaches can be taken to assay neural networks and cell connectivity; one focuses on structural interaction, the other on functional connectivity, although ideally these could be combined to provide both structural and functional assemblies. Conventional two-dimensional (2D) monocultures are limited in their ability to form dynamic anatomical connections and may not follow the same neurodevelopmental pathway, as cells within the brain are both constrained by, and receiving signals from the extracellular matrix and neighboring cells. 3D culture methods are being explored to better mimic tissue architecture, and to study cellular properties and network interactions in health and disease. Ideally, these iPSC-based 3D culture platforms would involve co-cultures of appropriate neuronal and glial cell types in a mechanically appropriate matrix with soluble and extracellular matrix-derived signals to those extant in the developing brain. They would also be compatible with optical imaging for morphological and electrophysiological analysis. Current approaches are based on the use of biomaterials to support 3D network organization and/or the use of neural stem cell aggregates to reconstruct complex *in vivo*-like structures (organoids). The range of biocompatible materials being tested for 3D culture systems includes hydrogel-based materials, 3D electrospun polymers, synthetic scaffolds, silica beads and microfluidic bioreactors. At present, we lack a full understanding of how biomaterials affect cell properties, and 3D cultures systems have so far not been widely explored for iPSC-based disease modeling.[94–96] Organoids and aggregate cultures give prospects to reconstruct *in vivo*-like neural circuits and to achieve insights into the signal integrated on multiple levels.[97] Through self-organization of complex tissue patterns, attempts have been made to replicate various brain regions to generate models of ASD.[98–100] Of particular note, 3D human 'cortical spheroids' generate a laminated cerebral cortex-like structure containing electrophysiologically mature neurons that form functional synapses.[98] However, it is difficult to control the size and internal laminar structure of the spheroids, and robust and reproducible methods need to be developed for quantification of both structure and physiology.

Functional network studies have substantial potential as drug-screening platforms. Ideally, they should measure such network behavior as the degree of connectivity (spread of impulses through the network) and the synchrony and oscillation frequency of neuronal firing.[101] In principle, these could mirror at the cellular level the types of brain activity measured by electroencephalogram, but at much higher spatial resolution. Human iPSCs can be developed into functional neuronal network on *in vitro* microelectrode arrays,[102–104] where network behavior has been shown to be sensitive to reduced expression of post-synaptic genes associated with SCZ and bipolar disorder.[105] Network level properties may be utilized to model the beta and gamma oscillation perturbations observed in patients.[106] Calcium- and voltage-sensitive imaging either instead of or in conjunction with microelectrode array recordings provide powerful options to observe activity oscillations in neuronal networks. Many of molecular phenotyping methods, such as transcriptomic and cell morphology analyses can be combined with these electrophysiological assays in high throughput to facilitate a multimodal assessment of many patient-derived iPSC lines.

CHALLENGES FOR IPSC-BASED DISEASE MODELS

Modeling psychiatric disorders at the cellular level is not without difficulties and there are many potential sources of error. First, to faithfully model diseases it is important that variation detected between the iPSC lines reflects the underlying genetic differences associated with the disorder, and is not introduced by cell reprogramming or downstream effects of cell culturing and differentiation protocols. This is particularly crucial when cellular differences between cases and controls are expected to be subtle. Substantial experimental variation between iPSC lines can arise from inconsistency in iPSC reprogramming protocols, parental somatic cell type[107] and persistent epigenetic modifications;[108,109] interline variability due to genetic instability, mosaicism or accumulation of mutations during cell line expansion;[110] and intra-line variability arising during prolonged cell culturing and differences in growth conditions.[107,111–113] However, methods for reprogramming have steadily improved since Takahashi and Yamanaka[27] first described iPSC, and recent studies show that by following standardized protocols reprogramming and cell culturing consistency can be achieved and intra-line variation reduced.[114–116] Evidence for the equivalence of human iPSC and embryonic stem cells indicate that reprogramming can instate a pluripotent state similar to that of the inner cell mass of an early human embryo.[117] Interestingly, the epigenetic erasure that occurs during the reprogramming process appears to make gene expression in iPSC more dependent on the genotype compared with gene expression in the cells used for reprogramming, in which interline variability is much larger.[118] The maturity of iPSC-derived neurons is a concern in studies of neuronal networks since they initially appear to most closely resemble fetal brain cells.[66,98,119,120] Different methods have been developed to promote the maturation of iPSC-derived neurons, that is, transplantation into rodent brains may mature them into GABAergic interneuron for up to 7 months.[120] Aging of iPSC-derived dopaminergic neurons by induction of progerin expression revealed disease phenotypes such as pronounced dendrite degeneration, progressive loss of tyrosine hydroxylase expression, enlarged mitochondria and Lewy body-precursor inclusions.[121]

Second is the question of choosing the best patient and control cell combinations. Early iPSC studies did not always fully consider possible confounders, such as differences in genetic background, unmatched age, sex, and ancestry between patients and control individuals, as well as differences in passage number of the iPSC lines.[74] It has become clear that these factors need to be carefully considered when selecting appropriate healthy control iPSC.[75] For many studies, samples derived from healthy family members of the patient with similar genetic background but not diagnosed with disease currently represent the most feasible control.[77]

An ideal control would be an isogenic iPSC line generated by correcting the genetic lesion(s) of the patient-derived iPSC line. In recent years, novel techniques for genome-editing have greatly increased level of efficiency of gene targeting *in vitro*. Using engineered endonucleases such as zinc finger nucleases, TALENs or CRISPR/Cas9, it is now possible to genome edit iPSC with high specificity.[76] For monogenic diseases, isogenic gene-corrected iPSC lines represent an ideal control population. However, for complex disorders with multiple genetic loci contributing to the disease, editing approaches face limitations. Although editing of multiple loci is feasible,[122] gene correction of a larger number of disease-associated variants in a single iPSC line remains problematic. In principle, a disease versus control scenario could also be generated by active introduction of candidate mutations into 'healthy' iPSC via gene editing. However, at present this route too appears only feasible for diseases with relatively small numbers of highly penetrant mutations. Furthermore, it comes with the significant disadvantage that phenotypic alterations in such *in vitro*-mutated iPSC cannot be correlated with the clinical history of an individual patient.[78]

Currently, parallel studies on isogenic gene-corrected iPSC for selected variants and cells derived from unaffected family members will remain the most feasible controls for comparative phenotypic analysis of patient-derived iPSC. These studies could be supplemented by the reverse experiment of using genome editing to introduce additional gene mutations into patient or non-patient cell lines with a high polygenic risk score derived by classic reprogramming to create artificial 'hyperphenotypes', where the effects of different patient backgrounds can be studied on highly penetrant disease-associated variants (Figure 2a). However, such approaches still face several limitations such as difficulties in engineering large chromosomal deletions. Further challenges include the large number of single nucleotide polymorphisms in linkage disequilibrium and limited information to guide the choice of relevant variants (Table 3).

Finally, inter-individual variability of patients with similar diagnosis and subtle differences in the clinical disease progression will result in quantitative, and perhaps qualitative, differences in cell phenotypes between iPSC lines derived from different patients.[110] It is therefore necessary to have the capacity to handle large-sample sizes for modeling these complex disorders. Comprehensive exploration of the steadily increasing number of risk loci in iPSC-based models will only be possible using large cohorts of patients and controls. To assess the combined impact of genetic variants on a single background or to decipher the single contribution of each variant, it will be necessary to explore novel technical solutions that enable much higher throughput. To that end, automated modules covering key reprogramming steps such as transfection, media changes, splitting and colony picking are already being implemented.[123] It is foreseeable that automation will move towards large-system integrations enabling fully automated production of iPSC on industrial production-line platforms such as the StemCellFactory (www.stemcellfactory.de; Figure 2b). While automated cell culture provides key advantages with respect to standardization and parallelization, large-system

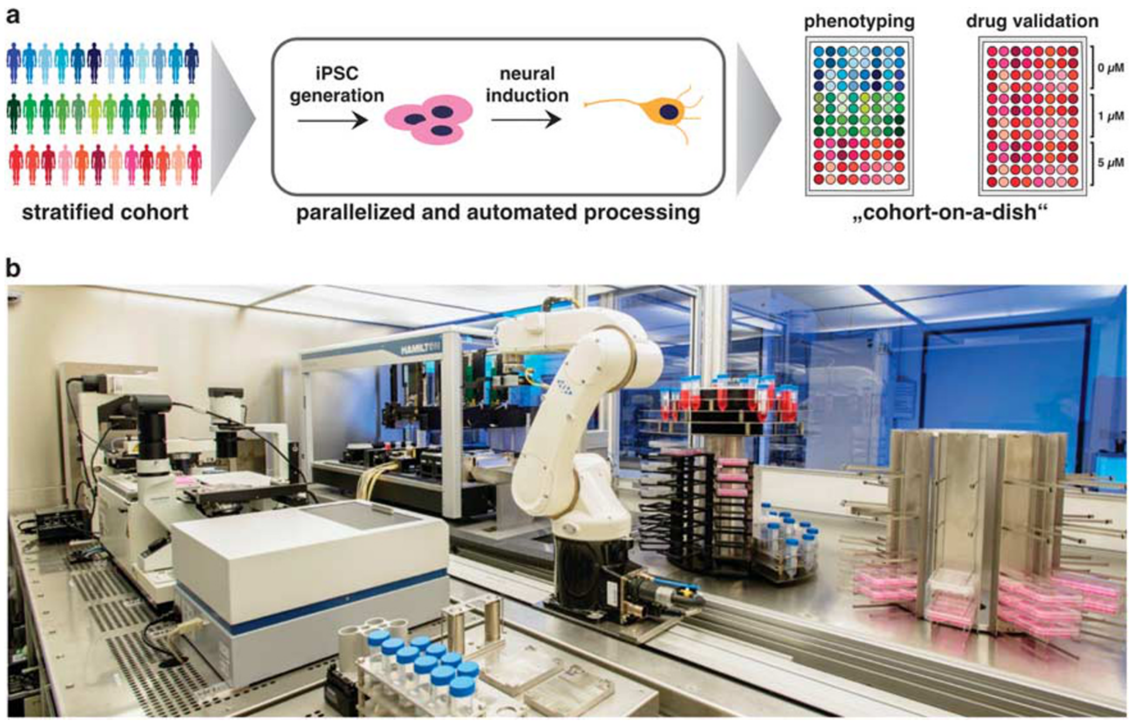

Figure 2. Automated production and differentiation of iPSCs. (**a**) Conventional disease modeling or drug evaluation approaches mostly rely on a small number of disease-specific, as well as control iPSC lines and largely ignore the impact of genetic variability on pathological pathways or drug targets. Parallelization of reprogramming and subsequent differentiation would allow assessing phenotypic variation or to validate candidate drugs on multiple genomic backgrounds, for example, stratified patient or control cohorts. (**b**) Fully integrated robotic systems such as the StemCellFactory (www.stemcellfactory.de) are expected to allow high-throughput reprogramming and differentiation under controlled and standardized conditions, and thus to minimize line-to-line heterogeneity induced by non-standardized manual handling steps. Kindly provided by Andreas Elanzew, Simone Haupt (Life & Brain, Bonn, Germany) and the Fraunhofer Institute for Production Technology (IPT). iPSC, induced pluripotent stem cell.

Table 3. Potential and limitations of gene editing strategies at mono- and multigenic level

Modification	*Potential*	*Limitations*	*Alternatives*
Monogenic	Genetic correction of patient backgrounds provides ideal isogenic controls for *in vitro* disease modeling (reduced experimental 'noise')	Strategy cannot be faithfully applied to diseases based on large CNVs (for example, chromosomal deletion syndromes).	(i) Inducible expression of candidate transgene targeted to genomic 'safe harbor' locus[151] (ii) Engineering allelic series into isogenic standard background[149]
Multigenic	Introduction of additional risk variants or protective alleles into patient backgrounds could provide mechanistic insight into disease modulation and serve as a tool to aggravate or mitigate *in vitro* phenotypes	Variant modeling studies are complicated by (i) the large number of SNPs in linkage disequilibrium and (ii) limited information to guide the choice of relevant variants.	Automated high-throughput *in vitro* analysis of patient cohorts stratified according to risk and/or protective factors (Figure 2).

Abbreviation: CNV, copy number varient; SNP, single nucleotide polymorphism.

Table 4. Advantages and challenges of automated cell culture systems for cell reprogramming and differentiation

Advantages	*Challenges*
High degree of standardization	Requires robust cell culture protocols amenable to robotic handling
High level of parallelization enabling handling of large cohorts	Requires complex, self-scheduling software
Little hands-on time; 24/7 operation; remote, web-based control	High cost; requires trained engineering staff and manual emergency plans for cases of catastrophic machine failure
Can accommodate genetic modification	Special requirements for viral transduction systems (for example, Sendai virus)
Facilitates seamless bar code-based documentation of all handling steps	Requires innovative fast imaging strategies and handling/storage of large data volumes

integration units for robotic reprogramming come with their own challenges (Table 4).

CONCLUSIONS AND FUTURE PERSPECTIVES

The proof-of-concept emerging from many recent studies that have attempted to mimic aspects of psychiatric disorders *in vitro* using patient-derived cells is very encouraging (Table 2). Increased standardization, proper controls and new integrative robotic systems will give solutions to many problems. However, there remain a number of considerable challenges ahead.

Strategies moving forward need to take into account the genetic characteristics of the patient population in which genetic risk is largely polygenic, and a mixture of many common variants of small effect, as well as few rare variants of large effect. In contrast, *a priori* we would expect to find the most robust phenotypes in cells derived from patients carrying highly genetically penetrant rare variants and cell models created using genome editing of isogenic iPSC lines. It will be important to connect the knowledge gained from single gene deficits and that gained from the accumulated effects of multiple subtle genetic risk alleles.

Both the selection of patients carrying rare variants of large effect and the selection of patients of extremely high polygenic risk require large patient populations to optimize the selection. When genetic risk in selected patients is not sufficiently causal, any iPSC experiment will require the analysis of large numbers of patient cell lines. An important step is to have robust protocols for reprogramming and differentiation of large numbers of patient samples. This will require standardization and rigorous quality control to reduce technical variation to an acceptable minimum. Given the high current reagent costs for stem cell research, the unit price per patient cell assay needs to drop substantially before this will be feasible. These processes need to integrate well with global efforts in patient recruitment and accompanying clinical phenotyping and genomic analysis.

Beyond the issues of variability and capacity lies the key question of what is the relevant cellular phenotype or phenotypes. We have discussed what is currently possible and under development, and how these might relate to function and physiology in the intact brain. However, these investigations have only just begun, and are likely to require multiple lines of converging evidence, carried out in numerous centers and with validation against clinical and animal model studies, before consensus cellular phenotypes can be established and accepted.

Finally, we need to consider what constitutes success in this enterprise? Although iPSC-based systems provide a powerful route to identifying molecular mechanisms underlying genetic and other disease-related risks, in isolation they do not provide information about brain physiology, higher order neuronal circuitry and function or human psychology. Success might simply be to create a reliable experimental link between genetics and patient studies via cell physiology. Alternatively, we could set more ambitious goals using iPSC to inform connectomic and neuro-computational modeling, predict patient drug responses and promote preclinical drug discovery. True success would be achieved if analysis of iPSC-derived neuronal networks became a standard assay for neurophysiologists, forming an integral component of diagnostic and precision medicine for neuropsychiatric disorders and facilitating the first advent of new drugs screened on patient iPSC reach the clinic.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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