

# Modeling complex neuropsychiatric disease with induced pluripotent stem cells

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## Abstract

The study of neuropsychiatric diseases and the development of effective treatments have been limited by a lack of appropriate models. Induced pluripotent stem cells (iPSCs) represent a potentially limitless supply of patient-specific cells for the study of neuropsychiatric disorders. In this review, we will discuss the potential and limitations of iPSCs for the development of cell-based models of neuropsychiatric diseases and the identification of novel therapeutics.

## Introduction and context

Neuropsychiatric diseases are amongst the most poorly understood of human ailments. Severe neuropsychiatric diseases such as bipolar disorder, schizophrenia, and autism spectrum disorders (ASDs) exhibit 40-60% concordance in monozygous twins and 60-90% heritability, suggesting an important role for genotype in disease etiology [1]. Indeed, numerous rare monogenic deletions and genomic copy number variations (CNVs) that predispose patients to the development of specific neuropsychiatric diseases have been identified [2]. However, the mechanisms by which these genomic variations cause psychiatric disease are understood in only a few cases. Furthermore, these relatively common and complex diseases are caused by individually rare genomic variations with variable penetrance [2]. As such, the workhorse of modern disease modeling – the laboratory mouse – may be of limited use for the development of appropriate models of these diseases.

Induced pluripotent stem cell (iPSC) technology, which arose from groundbreaking work performed by Yamanaka and colleagues [3], represents an exciting new approach to the modeling of human disease [4,5]. iPSCs are generated from patient-derived somatic cells (such as skin fibroblasts or blood [6]) by exogenous expression of specific

'reprogramming factors' (Yamanaka's original reprogramming of human cells employed *SOX2*, *OCT4*, *KLF4*, and *MYC*, although other reprogramming factors have been described). Patient-derived iPSCs exhibit all of the hallmarks of embryonic stem cells (ESCs), including self-renewal and pluripotency, and therefore represent a potentially limitless number of cells for the modeling of disease. As such, there is great excitement for the potential of iPSCs for modeling neuropsychiatric disorders and for the development of high-throughput screens to identify (a) specific genes that mediate the diseased phenotype and (b) chemicals that may be used as platforms for the development of efficacious pharmacological interventions.

## Major recent advances

### *iPSCs from individuals with neuropsychiatric diseases*

In recent years, iPSCs have been generated from numerous individuals diagnosed with a wide array of diseases. Indeed, a study described the generation of iPSCs from 10 individuals each with a different human disease, including several with psychiatric symptoms, such as Down's syndrome, Duchenne muscular dystrophy, and Parkinson's disease [7]. iPSCs have also been generated from patients with fragile X mental retardation syndrome (FMR) [8].

Our laboratory recently generated iPSCs from individuals with Rett syndrome [9], a severe ASD that has been suggested as an ideal starting point for investigating the potential of iPSCs for modeling neuropsychiatric disease [5,10]. Rett syndrome is monogenic, with the vast majority of cases caused by loss-of-function mutations of the X-linked *MECP2* (methyl CpG binding protein 2) locus; and is caused by inheritance of a highly penetrant dominant allele. It has a relatively severe and consistent phenotype (compared to non-syndromic ASDs) and is an early-onset disease, which may be more easily modeled *in vitro* than a late-onset neuropsychiatric disorder. Therefore, of the iPSC lines derived from individuals with neuropsychiatric disorders that have been published to date, Rett syndrome cell lines represent an excellent model for future investigations.

#### **X-chromosome inactivation status of iPSCs**

Like other X-linked recessive disorders, Rett syndrome offers an exciting advantage for modeling disease because, unlike mouse ESCs, which maintain two active X chromosomes until initiation of differentiation, human ESCs and iPSCs exhibit clone-specific X chromosome inactivation patterns [11,12]. Therefore, isogenic iPSC lines that differ only in their X chromosome inactivation status can be derived from a single tissue donation. Half of the iPSC lines produced from these cells should exclusively express the wild-type locus of the gene of interest, whereas the other half will express the disease-associated locus. Effectively, this approach will control for genetic background, thereby facilitating detection of disease-related phenotypes. For other disorders, iPSCs derived from unaffected, gender-matched parents or siblings must suffice as controls. iPSCs derived from Rett syndrome patients may greatly facilitate identification of disease-relevant phenotypes related to neuropsychiatric disease.

#### **Generation of specific neuronal subtypes from iPSCs**

In order to model disease, reliable and efficacious protocols are required to drive iPSC differentiation to the desired cell type. For example, Parkinson's disease and amyotrophic lateral sclerosis (ALS) result from the selective loss of midbrain dopamine-producing neurons and motor neurons, respectively. Therefore, specific protocols have been developed to drive differentiation of human pluripotent cells to these specific neuronal subtypes [13,14]. In the case of neuropsychiatric diseases, a commonly implicated cell type is glutamatergic forebrain neurons, which are responsible for encoding and transmitting sensory and cognitive information in the mammalian brain.

In recent years, several approaches have been developed to drive neuronal commitment of ESCs and iPSCs, and

to subsequently drive these cells to differentiate into specific neuronal subtypes. For example, two primary approaches have been used to drive ESCs and iPSCs to a *PAX6*<sup>+</sup> neuroepithelial state. To achieve this, Zhang and colleagues [14] used culture conditions designed to mimic embryonic development, with regards to both the kinetics of differentiation and the timing of physical and chemical cues to drive selection of the desired lineages. Colonies of ESCs and iPSCs were allowed to form embryoid bodies to promote specification to one of the three somatic lineages; embryoid bodies were later dissociated and cells were sequentially treated with specific morphogens and culture conditions over a period of 5-12 weeks to promote neuronal induction and differentiation to desired subtypes, such as motor neurons, dopamine-producing neurons [15], and telencephalic glutamatergic neurons [16]. Studer and colleagues [13], on the other hand, directed neuronal cell fate specification under adherent conditions by inhibition of SMAD (small mothers against decapentaplegic) signaling using recombinant Noggin and the drug SB431542. This approach likely increased homogeneity of differentiated cultures, as it obviated the embryoid body procedure used by Zhang and colleagues, which inherently introduced heterogeneity in the population of precursors directed toward specific neuronal cell fates. Similar to neuronal precursors derived from embryoid bodies, those generated by SMAD inhibition were efficiently differentiated into motor neurons and dopamine-producing neurons [13]. Therefore, at least two distinct approaches exist to drive iPSCs towards the neuronal lineage, and (regardless of the approach) these precursors can be directed towards specific neuronal cell types.

The two approaches described above were both used to drive neuronal specification and differentiation of both ESC and iPSC lines. Studer and colleagues used a predominantly qualitative approach in the examination of neuronal cell type specification and did not report significant differences between ESC and iPSC lines in the efficiency of neuronal differentiation [13]. Zhang and colleagues found that both ESCs and iPSCs differentiated to specific lineages with similar kinetics, although iPSCs generally exhibited reduced efficiency of differentiation and notable interline variability [14]. Interestingly, there was no correlation between the factors or approaches used to reprogram somatic cells and the differentiation capacity of iPSCs.

#### **Selecting appropriate neuronal subtypes for disease modeling**

To effectively model disease *in vitro*, iPSC differentiation must be directed to the appropriate cell type. In the case of complex neuropsychiatric disorders, the appropriate

cell type is not always known *a priori*. Furthermore, in several cases, cellular dissection of neuronal disease has revealed cell-nonautonomous etiologies.

The potential for cell-nonautonomous effects in modeling disease have been exemplified by recent studies from groups led by Kevin Eggan, Gail Mandel, and Mario Capecchi. While modeling ALS, which results from the selective loss of motor neurons, Eggan and colleagues found that motor neurons derived from normal ESCs degenerated when cultured with glial cells that encoded an ALS-associated mutation [17]. Similarly, wild-type neurons cultured with *Mecp2*-mutant glia exhibited extensive deficits in dendritic morphology, which is reflective of neuronal phenotypes exhibited by patients with Rett syndrome [18]. Definitive support for the possibility that behavioral phenotypes can arise due to the effects of non-neuronal cells was recently obtained from analyses of *Hoxb8* mutant mice, which exhibit pathological grooming behavior and could therefore be used as a possible model of obsessive compulsive disorder [19]. Interestingly, the only cells within the mouse brain that express *Hoxb8* are bone marrow-derived microglia, and transplantation of wild-type hematopoietic progenitors into lethally irradiated mice resulted in normal grooming behavior in *Hoxb8*-null animals. These studies suggest that, at least in some cases, homogeneous cultures of differentiated neurons may not recapitulate the diseased phenotype *in vitro* when modeling neuropsychiatric disorders.

#### **Genetic manipulation of human iPSCs**

Modeling of disease using iPSCs will be greatly improved by new technological developments that facilitate genetic manipulation of these cells. Compared to their mouse counterparts, human ESCs are notoriously refractory to the application of transgenic technologies such as homologous recombination. However, two recent reports described approaches to temporarily reprogram human cells to a mouse-like pluripotent 'ground state', resulting in human-derived cells with morphological and functional properties similar to those of mouse ESCs [11,20]. Interestingly, among the mouse-like properties exhibited by transiently-reverted human cells was efficient genetic manipulation by homologous recombination [20]. Thus, temporary reprogramming of human cells to a mouse-like state will assist in the future application of gene targeting technology in order to better understand neuropsychiatric disease.

#### **Technical considerations for the use of iPSCs to model disease**

The relative novelty of iPSC technology has resulted in a flood of new data that support the utility of iPSCs to model disease, while also reminding us that this

promising approach faces many hurdles on the course to widespread use.

Many neuropsychiatric disorders have been causatively linked to specific CNVs. For example, inherited and *de novo* CNVs of the 16p11.2 locus have been shown to predispose patients to develop ASDs, schizophrenia, and bipolar disorder [21]. Therefore, clinically diagnosed patients with specific disease-associated CNVs, such as 16p11.2, represent ideal candidates for iPSC-based models of neuropsychiatric disease. However, a recent report suggests that CNV loci are unstable throughout the passaging of ESCs [22]. Therefore, it is essential that iPSCs derived from patients with CNVs associated with psychiatric disease are characterized before phenotypic analyses in order to ensure that patient-specific CNVs are reflected within iPSC lines.

Disease modeling has also been limited by the failure to successfully recapitulate expression of disease-associated genes in reprogrammed cells. A recent report described the generation of iPSCs from patients with fragile X mental retardation syndrome, an ASD that results from loss of function of the X-encoded *FMR1* gene [8]. When ESCs were derived from preimplantation-diagnosed FMR embryos, *FMR1* expression was observed in undifferentiated cells but was silenced upon terminal differentiation. In contrast, iPSCs generated from three FMR patients failed to reactivate *FMR1* expression during reprogramming. These data suggest that (a) iPSCs and ESCs exhibit notable, but poorly understood, biological differences; (b) some diseases are not amenable to modeling using current iPSC technology; and (c) donor cell-specific patterns of gene expression may be retained during the reprogramming process (a conclusion that was supported by two recent reports [6,23]).

An important consideration for the design and interpretation of experiments using iPSCs is heterogeneity (reviewed in [4]). Humans are an outbred population with tremendous genetic variation. In the absence of disease-specific iPSC lines with isogenic controls, appropriate control iPSC lines from unaffected, gender-matched parents or siblings are essential for identifying relevant phenotypes. Also, it is imperative that numerous high-quality iPSC lines (i.e., technical replicates) are derived from both patients and healthy controls. With development of improved neuronal differentiation protocols and/or methods for the isolation and growth of committed neuronal progenitors, researchers may be able to overcome observed deficits in the efficiency of neuronal differentiation of iPSCs, which will hopefully decrease line-to-line variability. Finally, because integrated transgenes may influence gene expression

patterns, transgene-free methods may reduce heterogeneity between iPSC lines used for disease modeling.

Genetic rescue experiments that correct a causative mutation or CNV will be essential for confirming the biological relevance of differences observed between iPSCs from patients and controls. For example, any differences observed between iPSC-derived neurons from patients with Rett Syndrome and their relatives should be ameliorated by restoration of wild-type *MECP2* expression [24]. A key consideration in the design of effective genetic rescue experiments is the choice of transcriptional regulatory elements used to drive expression of the transgene. Neuropsychiatric diseases such as autism can result from both the loss or gain of dosage-sensitive genomic loci, including *MECP2* [25]. Therefore, expression of disease-associated genes using strong, constitutively active promoters may exceed normal expression levels, thereby causing further neuronal dysfunction. As such, an attractive alternative for the design of genetic rescue experiments is to use endogenous regulatory elements to drive appropriate levels of transgene expression in appropriate cell types [24].

#### **Identifying therapeutics using iPSCs**

iPSCs not only represent a useful model for understanding disease but also have tremendous potential for identifying therapeutics for complex neuropsychiatric disorders. Modern libraries of complementary DNA, short hairpin RNA, and small molecules represent heretofore unimaginable possibilities for the development of unbiased high-throughput screens to identify the genes and chemicals that modify the diseased phenotype [26]. However, the development of high-throughput screens requires (a) identification of a relevant cell type affected by the disease-associated mutation, (b) differentiation of iPSCs to the given cell type, (c) development of culture conditions that recapitulate disease-associated phenotype(s) *in vitro*, and most importantly, (d) identification of phenotypes that are experimentally tractable for high-throughput screens.

Two recent studies illustrate the potential for iPSCs in both the modeling of disease and for testing possible therapeutics. Each study characterized iPSCs directed towards disease-relevant cell types, identified disease-associated phenotypes *in vitro*, and corrected these phenotypes using small molecules. However, both studies employed previously identified compounds for alleviation of the diseased phenotype. For example, one report showed that treatment of iPSC-derived motor neurons from patients with spinal muscular atrophy with valproic acid or tobramycin increased expression of the hemizygous-mutated gene that causes the disease [27]. Similarly,

administration of kinetin, a plant-derived compound, partially rescued neurogenesis defects that resulted from mutations associated with familial dysautonomia [28]. Therefore, proof-of-principle has been provided regarding the application of patient-derived iPSCs for the modeling of neuronal disease, and for their potential in the identification of therapeutics.

#### **Future directions**

Modeling of neuropsychiatric disease using iPSCs is still in the earliest stages and widespread implementation of this approach will require theoretical and technical developments in several key areas. As a first step, extensive characterization of iPSC-derived neurons (from patients and controls) will be necessary in order to identify disease-relevant phenotypes. Development of screens for factors that modify neuronal function will require identification of phenotypes that can be analyzed using techniques that are amenable to high-throughput screens, such as high-content imaging. Traditional approaches used to analyze neuronal function, such as electrophysiological recordings, will be of limited use to the large-scale screens envisioned for future gene and drug discovery ventures. Optical analyses of neuronal morphology, synapse formation, and calcium signaling may be better suited to such screens (reviewed in [29]). Optogenetic approaches have been used as a platform for the development of high-throughput screens of synaptic function in the worm *Caenorhabditis elegans* [30] and a recent report described optogenetic stimulation of human ESC-derived neurons, both *in vitro* and *in vivo* [31].

Many challenges must be overcome before iPSCs can achieve their full potential for modeling disease. However, in light of the limited understanding of neuropsychiatric disorders that has arisen from studies of the available models, these efforts are more than justified and should be pursued.

#### **Abbreviations**

ALS, amyotrophic lateral sclerosis; ASD, autism spectrum disorder; CNV, copy number variation; ESC, embryonic stem cell; FMR, fragile X mental retardation syndrome; iPSC, induced pluripotent stem cell; MECP2, methyl CpG binding protein 2; SMAD, small mothers against decapentaplegic.

#### **Competing interests**

The authors declare that they have no competing interests.

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