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Stem cells on the brain: modeling neurodevelopmental and neurodegenerative diseases using human iPSCs

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Abstract

Seven years have passed since the initial report of the generation of induced pluripotent stem cells from adult humans, and in the intervening time the field of neuroscience has developed numerous disease models using this technology. Here, we review the progress in the field, and describe both the advantages and potential pitfalls of modeling neurodegenerative and neurodevelopmental diseases using this technology.

Keywords

neurodevelopmental; neurodegenerative; induced pluripotent stem cells; modeling

Introduction

The alleviation of human suffering through the prevention and treatment of disease is the ultimate goal of biomedical research. In order to target the causes of a particular disease, it is beneficial to first understand the molecular mechanisms that underlie the pathology. Studying diseases of the nervous system comes with certain unique challenges due to the complexity of the systems involved and the lack of accessibility of the human brain to direct observation. Recent advances in stem cell technology have allowed researchers to establish human induced pluripotent stem cell (hiPSC) lines from patients (I.-H. Park et al., 2008; K. Takahashi et al., 2007; Yu et al., 2007) and direct the differentiation of these cells to various neuronal and glial fates of the nervous system. The hope is that these advancements will allow researchers to model neurological diseases at a cellular level in a dish, providing an opportunity to both study disease mechanisms and test therapeutic strategies in the cell types most affected in these diseases. As these cells can be maintained and expanded in culture, human iPSCs provide a theoretically unlimited source of disease-relevant human cells for experimentation. Since the initial description of the technology, researchers in the field of neuroscience have established dozens of lines from patients with neurodegenerative and neurodevelopmental diseases, and have modified and expanded neuronal differentiation protocols used with embryonic stem cells (ESCs) to be used with iPSCs. Here, we review the progress in the field in modeling neurologic and psychiatric diseases, providing an overview of the lines created for different diseases and disorders, the cell fates examined for

each, and the cell and molecular phenotypes observed. We highlight the challenges involved in studying phenotypes associated with different categories of genetic alterations, and the potential pitfalls in the interpretations of results obtained with hiPSC models. Finally, we discuss the possibilities for expanding the utility of hiPSCs through manipulations of the *in vitro* environment to generate more physiological models of brain development and aging. As this review focuses on the analysis of stem cells, we will use the term “phenotype” to refer to specific assay outcomes such as gene expression, protein cleavage or phosphorylation, or electrophysiological measures, rather than organismal-level observations. As such, each cell line may display an array of phenotypes that are either a direct result of the genetic lesion (i.e. a truncated protein resulting directly from a nonsense mutation) or are progressively more distantly related to the genetic lesion. We will refer to the former as “proximal” phenotypes, and the latter as “distal” phenotypes. Finally, we include three tables describing published patient-derived iPSC lines and protocols for differentiation to neural fates (Tables 1-3). While these were meant to be an all-inclusive resource for the community, the rapidly growing literature of the iPSC field makes this challenging. We apologize for any unintentional omissions in these tables. For additional information regarding iPSC usage, we direct the reader to reviews pertaining to the careful modeling of disease-associated genetic variants with stem cells (Merkle & Eggan, 2013), direct induction as an alternative to iPSC generation (Tran, Ladrán, & Brennand, 2013), drug screening using stem cells (Marchetto, Winner, & Gage, 2010b), genomic variation between stem cell lines (Vaccarino et al., 2011), methods of iPSC derivation (Tran et al., 2013; Vaccarino et al., 2011), and the study of aging-related disorders using iPSCs (G.-H. Liu, Ding, & Izpisua Belmonte, 2012a).

Examining effects of genetic mutations and modifiers using hiPSCs

The identification of genetic variants that predispose to disease is of tremendous importance when attempting to identify the molecular and cellular underpinnings of a pathological process. Genetic modifiers of various strengths and prevalence have been found for a variety of diseases (Fig. 1a). Different strategies can be (and perhaps should be) used to model disease based upon each of these kinds of variants. The influence of genomic variants on cellular phenotypes in question depends on a number of factors, including: (1) the penetrance of the mutation/variant, (2) the proximity of the phenotype to be studied to the mutation of interest, and (3) the technical and biological reproducibility of the phenotype. For these reasons, the widespread genetic variation that exists between iPSC lines derived from unrelated individuals is likely to affect analyses of weaker disease-predisposing mutations and phenotypes more distant from the mutation. Thus, when studying genetic variants that only mildly increase disease risk or phenotypes far removed from the genetic alteration, it is especially important to control for other genetic variation. Using genetically related, unaffected family-derived control lines would lessen genomic variability, but this is ideally done using gene correction methods (outlined below). On the other hand, rare but highly penetrant variants may be capable of recapitulating disease phenotypes even in the presence of other genomic variation, especially when examining phenotypes proximal to the disease-causing mutation.

For example, fully penetrant mutations have been identified that cause early-onset familial Alzheimer's disease (fAD). Hundreds of such mutations have been identified in Amyloid Precursor Protein (*APP*) and Presenilin 1 or 2 (*PSEN1/2*) (reviewed in (Bertram, Lill, & Tanzi, 2010)). *APP* encodes the precursor protein for β -amyloid ($A\beta$), and presenilins encode the active site of the enzyme that cleaves *APP* to generate $A\beta$ of differing lengths. An example of a so-called “proximal” phenotype to these mutations would be the generation of different lengths of $A\beta$. Based upon pathological findings in fAD patients and animal models, progressively more “distal” phenotypes may include tau phosphorylation, gliosis, neuritic dystrophy, synaptic failure, and ultimately, cell death. Alzheimer's disease genetics also provide an example of a relatively common allelic variant of strong effect. The *APOE* $\epsilon 4$ allele increases risk for AD 3-12 fold, depending on allele dosage, and is present in ~15% of subjects of European ancestry (Mahley & Rall, 2000; Verghese, Castellano, & Holtzman, 2011). A proximal phenotype of *APOE* allelic variation may be expression, secretion, or cholesterol-binding abilities of *APOE* variants, while more distal phenotypes may overlap with those of *APP* and *PSEN* mutations. In order to achieve sufficient statistical power using iPSC modeling, the number of lines required for analysis would vary based upon these variables of penetrance/strength of genetic variant and the proximity of the phenotype to the genetic alteration (schematized in Fig. 1b).

Investigating the proximal effects of strong genetic variants in neurological disease are the “low hanging fruit” that most iPSC studies published to date have presented. Many of these have confirmed the findings from animal models, heterologous cell lines (such as CHO, HEK, HELA, and others) and postmortem studies (see Tables 1 and 2). While it is valuable to re-examine these phenotypes in living human neuronal and glial cells, it will be important to examine additional phenotypes that may or may not be specific to cell type, and identify sites of convergence of multiple predisposing genetic variants (Fig. 2). However, other genetic loci are more likely to impact these phenotypes the further removed they are from the mutation of interest, which underlies the predicted requirement for increased numbers of iPSC lines to study such phenotypes (Fig. 1b). Known genetic variants can be modeled with “isogenic” cell lines, where a patient-derived iPSC line has been gene-corrected (e.g. using zinc-finger nucleases [ZFNs], transcription-activator-like effector nucleases [TALENs], clustered regularly interspaced short palindromic repeat [CRISPR]-Cas, or other methods), reverting a mutant line to the wild-type genotype or vice-versa. It may be more desirable to correct a mutant line than to induce mutation in a wild-type line, as beginning with an iPSC line from a patient manifesting disease ensures that the genetic background is permissive to disease progression in the presence of the mutation in question.

The recent expansion of gene-editing nuclease technologies has greatly enhanced the possibilities for genomic editing in iPSCs. TALENs and ZFNs are similar in action, in that they both involve a custom DNA binding domain conjugated to FokI nuclease, which induces a DNA break upon dimerization with another FokI nuclease within a certain spacer distance. The identification of a simple DNA recognition “code” for TALENs has made TALEN design far simpler and more predictable than ZFN design, which was largely based on testing many nucleases for activity at the target site (Carlson, Fahrenkrug, & Hackett, 2012). For all cleavage-based methods, DNA-binding and cleavage specificity is a concern,

as off-target mutations may induce aberrant phenotypes or even obscure true phenotypes resulting from mutation at the desired site. One proposed method to improve ZFN and TALEN specificity is to use a mutated FokI nuclease, making it an obligate heterodimer (Doyon et al., 2011; Hockemeyer et al., 2011; P. Huang et al., 2011; J. C. Miller et al., 2007; Tesson et al., 2011; Wood et al., 2011). The obligate heterodimer approach ensures that FokI dimerization and nuclease activity can only occur if both the forward *and* reverse TALENs/ZFNs bind to neighboring sites. Other FokI mutations have been engineered to reduce toxicity, enhance nuclease activity, or restrict activity to single-stranded cutting (“nickases”) (Guo, Gaj, & Barbas, 2010; E. Kim et al., 2012; Ramirez et al., 2012; Szczepek et al., 2007; J. Wang et al., 2012). Fortunately, TALENs and ZFNs have not been found to induce off-target genomic mutations with appreciable frequency thus far (Q. Ding, Lee, et al., 2013a; Hockemeyer et al., 2011; Soldner et al., 2011). The recently-discovered CRISPR-Cas system (an RNA-guided nuclease) demonstrates higher genome editing efficiency than TALENs (Q. Ding, Regan, et al., 2013b), but also has increased potential for initiating off-target cleavage events (Fu et al., 2013; Hsu et al., 2013; Mali, Aach, et al., 2013a; Pattanayak et al., 2013). Similar strategies to those used with ZFNs and TALENs have recently been employed to improve CRISPR-Cas specificity, by employing a mutant Cas9 “nickase,” which requires two guide RNA sites in close proximity to create a double-stranded DNA break (Cong et al., 2013; Hsu et al., 2013; Mali, Aach, et al., 2013a; Mali, Yang, et al., 2013b; Ran et al., 2013). The various nuclease activities, specificities, and target site requirements of each system should be examined before attempting genomic editing in hiPSCs. Additional information thoroughly outlining the advantages and disadvantages of ZFNs, TALENs, and CRISPR-Cas methods is provided in other reviews (Carlson et al., 2012; Gaj, Gersbach, & Barbas, 2013; Urnov, Rebar, Holmes, Zhang, & Gregory, 2010).

In addition to the widespread genetic variability between lines from different individuals, it is also important to consider the impact of epigenetic variation on iPSC studies. The parental cell from which iPSCs are derived impacts the resulting epigenome, altering gene expression and potentially cellular functions (Bock et al., 2011; K. Kim et al., 2010; Marchetto et al., 2009). This epigenetic “memory” can impact the study of certain disease loci, which may remain silenced after reprogramming (Amenduni et al., 2011; Ananiev, Williams, Li, & Chang, 2011; Chamberlain et al., 2010; Cheung et al., 2011; Farra et al., 2012; K.-Y. Kim, Hysolli, & Park, 2011; Larimore et al., 2013; J. Liu et al., 2012c; Mekhoubad et al., 2012; Urbach, Bar-Nur, Daley, & Benvenisty, 2010). The reprogramming and differentiation processes can themselves affect genomic and epigenetic stability in ways that affect phenotypes of interest. A 2010 report showed that reprogramming iPSCs derived from patients with CGG repeat expansion in the *FMRI* gene (causing fragile X syndrome, or FXS) fails to reactivate *FMRI* expression (Urbach et al., 2010). This contrasted with results from FXS mutation ESCs, which did express the mutant *FMRI* in the ESC state but silenced it during differentiation. Notably, iPSCs reprogrammed from FXS-ESC-derived differentiated cells also failed to rescue *FMRI* expression, indicating that this disease-associated locus is resistant to reprogramming. Furthermore, different groups have come to variable conclusions regarding repeat instability during reprogramming and differentiation of iPSCs from patients with repeat expansion disorders. In one study of Huntington's

disease, a repeat-expanded *HTT* line showed stable repeat numbers in fibroblasts, iPSCs, and iPSC-derived neurons, with occasional repeat contraction seen with increasing iPSC passage number (loss of 2 out of 44 repeats) (Camnasio et al., 2012). A model of frontotemporal dementia (FTD) using *C9ORF72* repeat expansion lines found differential repeat stability in iPSC reprogramming from fibroblasts of a single patient (200 repeat loss or no loss), as well as repeat contraction with neuronal differentiation (Almeida et al., 2013). Generation of iPSCs from Friedreich's ataxia *FXN* GAA-repeat fibroblasts showed repeat expansion as well as contraction (J. Liu et al., 2011b). Finally, iPSCs derived from FXS patients showed *FMRI* repeat instability during reprogramming in one study (Sheridan et al., 2011) but relative repeat stability in two others (J. Liu et al., 2012c; Urbach et al., 2010). The divergence between studies may arise due to various reasons that are both biological (e.g. differences between patient cells) or technical (e.g. differences in reprogramming or culture methods). Repeat instability may introduce artifactual genomic variation during iPSC culture, or could alternatively recapitulate mosaicism seen in certain disease states (Almeida et al., 2013; Marchetto et al., 2010a; Sheridan et al., 2011).

The X inactivation status of reprogrammed female iPSCs and iPSC-derived differentiated cells introduces another layer of epigenetic variability. Lines derived from the same individual may not be functionally “isogenic” if they each maintain a different inactivated X chromosome. X-inactivation is of particular importance when the disease-causing mutation occurs on a single X chromosome (e.g. *MeCP2* mutation in Rett syndrome, *FMRI* mutation in fragile X syndrome, *HPRT* mutation in Lesch-Nyhan syndrome). When modeling X-linked disorders with female iPSCs, the specific X chromosome that is inactivated is crucial for the presentation of a mutation-dependent phenotype, as only the mutant gene is expressed when one X chromosome is inactivated, and only the wild-type gene is expressed when the other is inactivated, drastically impacting resulting neuronal characteristics (Amenduni et al., 2011; Ananiev et al., 2011; Cheung et al., 2011; Farra et al., 2012; K.-Y. Kim et al., 2011; Larimore et al., 2013; J. Liu et al., 2012c; Marchetto et al., 2010a; Mekhoubad et al., 2012). It is important to note that in *any* female iPSC lines, X-inactivation status can impact neuronal phenotypes, as several neuronal genes are present on the X chromosome, where significant allele-biased expression occurs during neuronal differentiation (Lin et al., 2012). X-inactivation may thus have broad, though subtle, confounding effects on a variety of neuronal phenotypes in any model. This could potentially minimize a genuine disease-associated phenotype or lead to variable results from studies of the same mutation in different lines or labs, such as studies of the same autosomal mutation in a male- vs. a female-derived iPSC line.

Choosing a cell fate: exploring cell type-specific phenotypes

In addition to deriving cells from patients with mutations or diseases of interest, pluripotent stem cells are an exciting tool because of their ability to generate a multitude of cell types from an identical genetic background. This property of iPSCs provides an opportunity for investigating the nature and causes of selective cellular vulnerability seen in neurological diseases using human cells. Differentiating to distinct neuronal or glial subtypes may reveal phenotypes only in a subtype of cell fates, and/or cell autonomous vs. non-autonomous phenotypes. A number of reports have investigated cell type-specificity of phenotypes in

fibroblasts vs. iPSCs vs. differentiated cells (for example: (Bilican et al., 2012; G. Lee et al., 2009; Nguyen et al., 2011)), but fewer have examined phenotypes across distinct neuronal subtypes (Burkhardt et al., 2013; Di Giorgio, Boulting, Bobrowicz, & Eggan, 2008; Nguyen et al., 2011). This underutilized power of iPSCs represents a missed opportunity (but potential future strength) to investigate the molecular mechanisms underlying selective vulnerability in neurological diseases.

The ability to make a specific cell fate is of particular interest in the study of diseases with well-characterized and limited affected cell populations. A 2011 paper utilized dopaminergic neuron-directed differentiation to study effects of the Parkinson's disease-predisposing *LRRK2* G2019S mutation (Nguyen et al., 2011). *LRRK2* G2019S-homozygous dopaminergic (DA) neurons were more susceptible to H₂O₂-induced cell death than wild-type DA neurons. However, both wild-type and *LRRK2* G2019S tyrosine hydroxylase (TH)-negative neurons were less susceptible to peroxide-induced cell death than TH-positive neurons. *LRRK2* G2019S DA neuron-enriched cultures also showed increased α -synuclein protein levels and increased expression of oxidative stress pathway genes (the latter phenotype seen in day 35, but not day 50 or 55, neurons). A 2010 study examined cellular phenotypes of amyotrophic lateral sclerosis (ALS), a motor neuron disorder, in motor neurons from sporadic and familial ALS patient-derived iPSCs. Burkhardt, et al. reported TDP-43 aggregates in select sporadic ALS iPSC-derived motor neurons. These aggregates were less commonly found in other types of neurons in the same cultures (neurons lacking ISLET1/HB9 expression) and were absent in wild-type and *SOD1*-mutant iPSC-derived motor neurons (Burkhardt et al., 2013). Neural crest-directed differentiation was used by the Studer lab to investigate the molecular basis of familial dysautonomia, a fatal peripheral neuropathy, in *IKBKAP*-mutant iPSCs (G. Lee et al., 2009). *IKBKAP* mutant iPSC-derived neural crest precursors (the progenitor cells of the peripheral nervous system) displayed abnormal *IKBKAP* splicing, decreased *ASCL1* expression, and reduced neuronal differentiation and migratory capacity. These studies and others (Tables 1 and 2) demonstrate the capacity of iPSC disease models to recapitulate cell-type specific phenotypes.

Cell-type-specific directed differentiation is of limited utility when studying diseases without a clear fate-restricted cellular origin, as is the case with many developmental and neuropsychiatric diseases. In these instances, it may be possible to identify alterations present in broad groups of neurons (e.g. altered spine density), which may only noticeably affect specific circuits *in vivo*. In some cases, it is valuable to study specific neuronal subtypes in the context of a milieu of cell types. The differentiation protocols currently available produce cultures that are heterogeneous to variable extents, whether they contain a wide variety of neural cell types or cells of more fate-restricted neural lineages (Table 3). As alluded to above, the cellular context of the cells to be studied may significantly affect the phenotype of interest. For example, the presence of a pool of neural progenitor cells in a neuronal culture could mask disease-associated phenotypes by continuing to produce newborn neurons (discussed in (Sandoe & Eggan, 2013)). This will particularly confound analyses of phenotypes that dramatically change over the course of neuronal maturation, such as neurite architecture, soma size, synaptic density, electrophysiological activity, and

gene expression (Espuny-Camacho et al., 2013; Shi, Kirwan, Smith, Robinson, & Livesey, 2012; Takazawa et al., 2012), and could in theory mask a cell death phenotype by replenishing viable neurons. Alternatively, some phenotypes may *only* be discernible in a heterogeneous culture. Non-cell autonomous effects have been carefully studied in models of ALS, which have revealed cytotoxicity of *SOD1*-mutant astrocytes on motor neurons (Di Giorgio et al., 2008; Di Giorgio, Carrasco, Siao, Maniatis, & Eggan, 2007; Marchetto et al., 2008). The Eggan lab found that wild-type hESC- derived motor neurons displayed increased cell death when cultured with *SOD1* G93A-overexpressing human astrocytes relative to wild-type astrocytes. Interestingly, *SOD1* wt-overexpressing astrocytes did not cause motor neuron death, interneurons were not susceptible to *SOD1* G93A astrocyte-induced cytotoxicity, and *SOD1* G93A-overexpressing fibroblasts did not induce motor neuron death (Di Giorgio et al., 2008). In parallel, the Gage lab showed similar cytotoxic effects of *SOD1* G37R- (but not wild-type *SOD1*-) overexpressing human astrocytes on hESC-derived motor neurons, whereas GABAergic neurons were unaffected (Marchetto et al., 2008). These studies highlight the importance of choice of cell type when studying a human disease process. The predicted pathogenic and/or affected cell subtypes should be carefully evaluated when deciding what cell types will be used to interrogate disease-relevant processes.

Studying selective neuronal vulnerability or cell fate-specific phenotypes is limited to those cell fates that can be generated using available protocols (Table 3). The breadth of neuronal and glial cell fate protocols is constantly expanding, and these are continually being improved to yield higher efficiencies at lower cost. However, this perpetual refinement of protocols also presents a challenge to researchers - how do we compare similar cell fates generated by distinct protocols? Differences in differentiation methods (e.g. use of small molecules or genes, monolayer vs. aggregate, small molecule/growth factor concentrations and sources, differentiation time, cell purification by MACS/FACS, lab environment) could considerably alter the population of resulting neurons. In addition, different iPSC lines have incredibly variable neuronal differentiation capacities (Amenduni et al., 2011; Bock et al., 2011; Boulting et al., 2011; Di Giorgio et al., 2008; Hu et al., 2010; Koyanagi-Aoi et al., 2013; Meyer et al., 2009; Osafune et al., 2008), which can complicate analyses of presumed neuronal differentiation-impairment resulting from mutation (K.-Y. Kim et al., 2011; Pedrosa et al., 2011; Robicsek et al., 2013). As a result, it is essential that as a research community we thoroughly characterize the cell populations we study (by gene expression studies, immunostaining, electrophysiological characterization, etc.) and maintain a high level of transparency in data reporting (recording number of lines used, number of differentiations, and number of cells/wells studied for each experiment).

Specificity of phenotype

There are many factors that may contribute to the presence or absence of any particular phenotype in iPSC-derived cells. Technical variables can be a prominent source of phenotypic variation between iPSC lines or within one iPSC line used in multiple laboratories. Each step from somatic cell harvesting to phenotypic assay introduces multiple variables that may differ between lines, differentiation rounds, and labs. Included among the many technical variables are: somatic cell source and age at harvest, reprogramming

method, iPSC culture conditions, differentiation method (including use of small molecules and exogenous transcripts and/or proteins), and phenotypic assay protocol. These factors can influence the epigenome and genome, potentially altering specific cellular phenotypes, including the capacity to differentiate to a particular cell lineage. Indeed, recent studies have shown that different iPSC lines each have their own propensities to differentiate to particular cell lineages (addressed above), although this can be partially overcome by modified protocols, such as extended iPSC passaging (Takako Kondo, Johnson, Yoder, Romand, & Hashino, 2005), DMSO treatment prior to differentiation (Chetty et al., 2013), and FGF2 and/or dual SMAD inhibition during differentiation (Boulting et al., 2011; Hu et al., 2010). This is a vital concern when studying a potential phenotype of altered differentiation potential, and requires that multiple distinct lines and/or isogenic lines be used.

Of equal importance to technical variables are the biological causes of phenotypic variation between iPSC lines (Fig. 2). These biologically-significant variable phenotypes can be grouped as follows: (1) a phenotype that is unique to cell lines from that particular person, (2) a phenotype specific for a mutation of interest, and (3) a phenotype of a pathological process. A person-specific phenotype would likely result from genomic or epigenomic variation other than the mutation of interest. In contrast, a phenotype could be a biological result of a certain mutation, but may not result from other mutations linked to the same disease. For example, the *SOD1* G93A mutation causing ALS could result in a specific biochemical phenotype that does not occur with other ALS-causing mutations in *SOD1* or ALS-causing mutations in other genes, such as *TDP-43* or *C9ORF72*. Findings such as these reveal interesting aspects of the basic molecular and cell biology of the proteins implicated in disease, but may reduce the possibility that the unique phenotype is absolutely required for disease pathogenesis. Finally, a phenotype may be characteristic of a specific disease, such as ALS, or a disease spectrum, such as neurodegeneration.

Although it would be time- and resource-intensive to tease out each of these possibilities in any one study, compilation of data from multiple studies can reveal patterns that indicate into which group a specific phenotype falls. Targeted strategies can be revealing, including using multiple iPSC lines with the same mutation, iPSC lines with different disease-causing mutations, and iPSCs derived from patients with sporadic disease. When possible, rescue experiments in which the suspected disease cause is corrected can demonstrate the dependence of an observed phenotype on a mutation or other characteristic. Complementary methods are also critical for determining the relevance of any iPSC-derived finding. This can include non-iPSC cell culture, postmortem human tissue studies, and animal models, each of which provide a distinct set of advantages and disadvantages when compared to iPSCs. For example, disease processes characterized by loss of a developmentally- and regionally-restricted cell type can be studied using xenografting of hiPSC-derived cells into rodent disease models, followed by examination for symptomatic alleviation. Examples of this sort have been carried out in the literature in the study of spinal muscular atrophy (SMA) (Corti et al., 2012), Huntington's disease (An et al., 2012; Jeon et al., 2012), Parkinson's disease (Kriks et al., 2011), and congenital hypomyelination (S. Wang et al., 2013). One such report generated iPSCs from *SMN1* mutation-homozygous SMA patients (SMA-iPSCs) and gene-corrected one copy of the *SMN2* gene to an *SMN1*-like sequence by

single-stranded oligonucleotide treatment (TR-iPSCs), enabling expression of a functional homolog of SMN1 from one *SMN2* allele (Corti et al., 2012). SMA-iPSC-derived, but not isogenic TR-iPSC-derived, spinal motor neurons displayed degenerative phenotypes *in vitro*. Corti, et al. subsequently transplanted SMA-iPSC- or TR-iPSC-derived motor neurons into SMA transgenic mice, and found that TR-neurons engrafted with higher efficiency than SMA-neurons, while both rescued deficits in SMA transgenic mice in proportion to motor neuron engraftment (Corti et al., 2012). These types of iPSC-based studies that incorporate non-iPSC methods will expand our knowledge of the characteristics and potential applications of iPSC-derived cells.

Limitations of the dish: bringing *in vitro* closer to *in vivo*

Although iPSCs are an exciting and appealing tool for studying the molecular and cellular phenotypes underlying human disease states, we must recognize the many caveats and limitations that accompany this method. As iPSCs are an *in vitro* system, they lack many of the characteristics of a developing and mature brain. The microenvironment of the developing brain cannot yet be fully recapitulated, but certainly affects the extracellular cues presented to differentiating cells. The lack of physically disparate regional identities also obfuscates area-specific phenotypes. For example, it is difficult to study neuronal circuitry using iPSCs, particularly when investigating phenotypes unique to specific neuronal circuits of the adult brain. However, it may be possible to reproduce inter-regional cellular connections using co-culture of cells resulting from distinct directed differentiation methods. Adding to the challenge of recapitulating the endogenous nervous system, the cell types that can be made using iPSCs have intrinsic limitations. No matter the extent of characterization of gene expression, protein expression, morphology, and electrophysiology, it is nearly impossible to map an iPSC-derived cell to a corresponding *in vivo* cell fate. Although a cell may express a set of proteins and display firing characteristics of a layer V excitatory pyramidal neuron, it is impossible to know if this neuron is a faithful representation of a neuron that exists *in vivo* at any time over the life of a human. This theoretically insurmountable obstacle is minimized with detailed phenotypic examination of the cell types to be studied, but must always be acknowledged when interpreting data.

One strategy to bring more *in vivo* relevance to iPSC use is xenografting of iPSC-derived neurons into the developing or mature rodent CNS environment. Transplantation has been utilized by several groups to characterize the differentiation and functional capacity of neural cell types resulting from differentiation (Carri et al., 2013; Erceg et al., 2010; Espuny-Camacho et al., 2013; Goulburn et al., 2011; Goulburn, Stanley, Elefanty, & Anderson, 2012; Juopperi et al., 2012; Kirkeby et al., 2012; Krencik, Weick, Liu, Zhang, & Zhang, 2011; Kriks et al., 2011; Kumamaru et al., 2012; W. Li et al., 2011; Maroof et al., 2013; Morgan et al., 2012; Nicholas et al., 2013; Swistowski et al., 2010; Takazawa et al., 2012; Tønnesen et al., 2011; S. Wang et al., 2013). In addition, a handful of studies have used xenografting of wild-type or gene-corrected iPSC-derived cells into disease-model rodent brains to observe resulting engraftment and behavioral rescue (An et al., 2012; Corti et al., 2012; Jeon et al., 2012; Kriks et al., 2011; S. Wang et al., 2013). It would be of interest to extend these studies to examine cellular defects relating to processes such as

neuronal migration and maturation, axon guidance, and synapse formation in mutant vs. wild-type iPSC-derived cells.

The developmental timeline of human stem cells *in vitro* is restrictive, as iPSC/ESC neuronal differentiation recapitulates early *in vivo* neurodevelopment, producing embryonic-like neurons that proceed through the neurodevelopmental stages of neural progenitor cell proliferation, neuronal migration, and neurite outgrowth and arborization (Dhara & Stice, 2008; Eiraku et al., 2008; Gaspard et al., 2008; Krencik & Zhang, 2006; Lancaster et al., 2013; H. Liu & Zhang, 2011; Mariani et al., 2012; Maroof et al., 2013; Nicholas et al., 2013; Watanabe et al., 2005). However, using currently available methods, these cells lack many characteristics of adult neurons (Erraji-Benchekroun et al., 2005), complicating the study of adult-onset diseases using stem cell-derived neurons. Human neurodevelopment continues for decades postnatally, with continuing changes in synapse number, myelination of axons, and neuronal maturation (Jaaro-Peled, 2009; Pescosolido, Yang, Sabbagh, & Morrow, 2012; Tau & Peterson, 2010). In contrast, hiPSC-derived neurons used to study mechanisms of disease are often differentiated for anywhere from 14 to greater than 100 days (Chambers et al., 2009; Shi et al., 2012; Zeng et al., 2010). These differentiation times fall far short of the delayed onset of symptoms that accompanies many neurological diseases studied with iPSCs (fragile X syndrome: neonatal (Jacquemont, Hagerman, Hagerman, & Leehey, 2007), Rett syndrome: 6-18 months (Amir et al., 1999; Chahrour & Zoghbi, 2007), schizophrenia: 15-30 years (Stilo & Murray, 2010), Huntington's disease: average ~40 years (Myers, 2004; Walker, 2007), ALS: average ~60 years (Chiò et al., 2013; Huisman et al., 2011), Parkinson's disease: 60 years (de Lau & Breteler, 2006), Alzheimer's disease: 65 years (Thies, Bleiler, Alzheimer's Association, 2013)), calling into question whether cellular phenotypes of these disorders could be observed *in vitro* in a matter of weeks or months. The *in vitro* differentiation process is more relevant for neurodevelopmental and neuropsychiatric disorders, which are often hypothesized to stem (at least in part) from defects in early brain development (Table 2) (Brandon & Sawa, 2011; Lewis & Levitt, 2002; Mitchell, 2011; Pescosolido et al., 2012). However, even late-onset neurological diseases can have early cellular endophenotypes that manifest before clinically-observed symptoms. For example, neurons derived from patients with late-onset disorders can reveal early mechanisms underlying disease pathophysiology prior to the development of overt pathology, such as altered gene expression and protein processing (Table 1).

While certain molecular phenotypes may be observable in neurons at early developmental stages, other downstream phenotypes may only be observed in fully-mature, “aged” neurons. To this end, a number of methods are currently being developed to accelerate the aging and maturation process of hiPSC-derived neurons. Xenografting stem cell-derived neurons into rodent brain stimulates neuronal maturation (Espuny-Camacho et al., 2013; Tabar et al., 2005), and could be used to ‘age’ neurons prior to assaying for a phenotype of interest. In addition, neurons may be artificially aged in culture by presenting cells with exogenous stressors (see Table 1). Aging could also be accelerated by introducing mutations in genes implicated in cellular aging regulation, such as *LMNA*, which is mutated in the premature aging disorder Hutchinson-Gilford progeria syndrome [HGPS]. HGPS iPSC-derived cells display premature senescence, dysmorphic nuclei, DNA damage, increased

mitochondrial reactive oxygen species, and reduced telomere length, thus recapitulating aspects of accelerated cellular aging *in vitro* (G.-H. Liu et al., 2011a; J. D. Miller et al., 2013; J. Zhang et al., 2011). Such ‘aging’ mutations could theoretically be manipulated to hasten neuronal aging in culture. Indeed, a recent study was able to recapitulate aspects of neuronal aging *in vitro* by overexpressing progerin (the truncated transcript resulting from HGPS-associated *LMNA* mutations) in control and Parkinson's disease (PD) iPSC-derived cells. In this report, progerin-induced aging was able to reveal PD-specific phenotypes that were previously unobservable in wild-type and PD-derived neurons (J. D. Miller et al., 2013). These strategies facilitate the study of late-onset disorders *in vitro* on a more practical timescale.

The recent published protocol to produce cerebral “organoids” presents the possibility of studying a disease phenotype in a specific cell type or group of cell fates in the context of a three-dimensional (3D) model of human neurodevelopment (Lancaster et al., 2013). These organoids also facilitate the study of phenotypes which may only manifest in a 3D system, or which are easier to study in a 3D context, such as alterations in neural progenitor proliferation, neuronal migration, cortical layering, and axon guidance. While these processes can occur to varying extents in two-dimensional culture systems, they are likely more ordered and closer to their *in vivo* counterparts when occurring in 3D. This is of interest especially when studying common neurodevelopmental and neuropsychiatric disorders, which often do not display striking neuroanatomical phenotypes, but are characterized by subtle perturbations in cortical organization (Insel, 2010; S. Kim & Webster, 2010; Oblak, Rosene, Kemper, Bauman, & Blatt, 2011). Cerebral organoids do recapitulate some area-restricted neuronal identities (e.g. prefrontal lobe, hippocampal, and ventral cortical neuron marker expression), indicating the possibility of using organoids to study inter-regional phenotypes. No current method can exactly replicate the *in vivo* ratios of relevant cell fates, extra-cerebral influences on disease processes (e.g. immune cells, blood-brain barrier), or the 3D structure of neurodevelopment, preventing the study of human disease-associated states in a precise human *in vivo* environment. However, further characterization of this recent technique compared to more traditional differentiation protocols and *in vivo* neurodevelopment will help identify those phenotypes that are better studied in this 3D environment.

Another approach to make *in vitro* systems more physiological involves tissue engineering to mimic *in vivo* structures “on a chip”. Microfluidic devices can be used to improve nutrient delivery and waste removal in tissues or cultures *in vitro* (Y. Huang, Williams, & Johnson, 2012), simulate the blood-brain barrier (Griep et al., 2013), model a neurovascular unit (Achyuta et al., 2013), and manipulate neuronal connectivity (Berdichevsky, Staley, & Yarmush, 2010) or organization (Kunze, Giugliano, Valero, & Renaud, 2011). These devices could be used to characterize the behavior of iPSC-derived vs. rodent brain-derived neurons, as well as provide opportunities to investigate disease-associated phenotypic alterations that may not be accessible in traditional culture systems. Although these have yet to be widely used in the field, tissue engineering presents many exciting possible avenues for future applications with iPSC-derived neurons (Khademhosseini, Langer, Borenstein, & Vacanti, 2006).

Concluding remarks

Since the first descriptions of human iPSC methodologies, hundreds of laboratories in academia and industry have adopted the technology for the study of neurological and psychiatric disorders. Lines from over one hundred patients with brain diseases have been published (Tables 1 and 2), but thousands more are in the process of being characterized. As the number of iPSC lines developed goes through exponential expansion, it is crucial that the iPSC community develop a standard format for sharing information about these lines. The format used should allow the information to be described using a consistent vocabulary and to be easily discovered online. There are already several repositories that share information on their individual websites (coriell.org, atcc.org, wicell.org, hsci.harvard.edu, and nyscf.org), and informational sites that share data about existing lines (umassmed.edu/iscr, nimhstemcells.org, and eagle-i.net). While the number of lines is still manageable, these groups should work together to drive the standardization of cell line description and of the format for sharing iPS cell information.

While many have embraced the potential of this technology, others remain skeptical that hiPSCs will reveal significant insights into the mechanisms of and treatments for neurological diseases. As with any new technology, the early years of adoption have resulted in a wave of studies of varying caliber. Several important initial studies with hiPSCs confirmed known effects of certain genetic variants, but for the first time in *human* neurons, while others were able to reveal novel insights into the effects of such variants on neuronal function. In some studies reporting novel phenotypes in patient-derived lines, concerns have been raised about the specificity of the phenotype for the disease state or even for the genetic alteration being studied. For example, it has been described that first-generation neuronal differentiation protocols show variable efficiencies, even between multiple lines derived from the same subject (see Tables 1 and 2 and (Hu et al., 2010)). Therefore, phenotypes relating to neuronal differentiation (such as neurite outgrowth, neuronal marker expression, electrophysiology) must be carefully interpreted in the context of appropriate controls.

Despite the concerns mentioned above, human iPSCs allow unprecedented investigation into the causative events in disease progression. In addition, for the first time, this technology allows neuroscientists to test therapeutics in the cell types of interest derived from the patients to be treated. As differentiation protocols become more efficient and reliable, and novel strategies are optimized to make the *in vitro* environment more physiological, the insights garnered from studies using hiPSCs will be broadened.

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Figure 1.

A) Distributions of disease-predisposing genetic variants and allele frequency. Nearly all identified rare variants that confer an increased disease risk are high in penetrance, such as autosomal dominant mutations causing early-onset familial Parkinson's disease (e.g. in *SCNA*), early-onset familial Alzheimer's disease (e.g. in *APP* or *PSEN1/2*), or familial ALS (e.g. in *SOD1*). There also are low frequency variants, with lower penetrance than the aforementioned rare alleles, which greatly increase disease risk, such as *LRRK2* mutation in Parkinson's disease. Relatively common alleles have been identified that carry a substantially increased risk, such as the *APOE* ε4 allele, with an allelic odds ratio of ~4 for Alzheimer's disease (Bertram et al., 2010). Many genome-wide association study (GWAS)-identified loci mark common variants of weak effect, as is the case for most SNPs associated with neuropsychiatric disease. Finally, there almost certainly exist rarer variants than those currently known, which confer a small increase in disease risk. However, current methods are unable to discern such genetic variants due to lack of statistical power. B) Estimate of the number of disease and control-derived iPSC lines needed to attribute a phenotype to the genotype under examination. For strong genetic variants with high increased disease risk and penetrance, fewer lines will generally be needed. Similarly, when analyzing phenotypes that are closer functionally to the genetic alteration of interest, fewer lines will be required. The graph above relays an estimate of how the variables of variant strength and phenotypic 'distance' might combine to achieve statistically significant results, based upon published studies. Example phenotypes listed pertain to the study of a familial Alzheimer's disease mutation, i.e. *APP* mutation.

Figure 2. Schematic of biological causes of observed phenotypes

Given the study of any particular mutation, several genuine phenotypes attributable to biological (rather than technical) causes may exist. This diagram depicts a subset of meaningful biological causes of phenotypes, which each should be considered when interpreting an observed phenotype. On the bottom from left to right: patients with a particular disease-causing mutation (mutation 1, purple), those with a distinct disease-causing mutation (mutation 2, orange), others with sporadic disease (green), patients experiencing a disease process that encompasses the disease in question (blue), and finally unaffected individuals. Above, hypothetical phenotypes altered in iPSC-derived cells from the individual directly below are shown in boxes. In the given set of individuals, some phenotypes will be common among certain sets of iPSCs, whereas others will be unique. Combinations of lines with similar and distinct origins help to parse out how the phenotype relates to the mutation of interest (e.g. phenotypes b, d, h), the disease of interest (e.g. phenotype c), an overarching disease process (e.g. phenotype e), or that particular individual's genetic background (e.g. all other phenotypes listed above).

Table 1

Human iPS cell lines created to study neurodegenerative diseases

	Novel iPSC lines generated (# of subjects per genotype/ phenotype)	Differentiated cell type(s) identified and analyzed	Phenotypes described	Reference
Alzheimer's disease				
2011	1x PSEN1 A246E, 1x PSEN2 N141I	Neurons	A β generation	(Yagi et al., 2011)
2012	2x sporadic AD, 2x APP duplication, 1x wt	Neurons, co-culture with astrocytes	A β generation, Tau phosphorylation, GSK3 β phosphorylation, endosome size	(Israel et al., 2012)
2013	1x APP homo E693, 1x APP V717L, 2x sporadic AD, 3x wt	Cortical neurons	A β generation, localization, and oligomerization state, levels of cellular stress	(Takayuki Kondo et al., 2013)
Amyotrophic lateral sclerosis				
2008	2x SOD1 L144F	Motor neurons and astrocytes	ND	(Dimos et al., 2008)
2010	16x idiopathic ALS, 10x wt	Cortical neurons, motor neurons, co- cultured with astrocytes	TDP-43 aggregation	(Burkhardt et al., 2013)
2011	4x VAPB P56S, 3x wt	Motor neurons	VAPB protein levels, VAPB aggregates	(Mitne-Neto et al., 2011)
2012	1x TARDBP M337V, 2x wt	Motor neurons	TDP-43 protein levels, cell death	(Bilican et al., 2012)
2013	4x C9ORF72 GGGGCC expansion (3x ALS, 1x ALS/FTLD, 1x >70, 3x >800 repeats), 4x wt	Motor neurons	C9ORF72 GGGGCC repeat stability, C9ORF72 expression, RNA foci, RNA binding protein sequestration, gene expression, motor neuron survival	(Sareen et al., 2013)
2013	4x C9ORF72 GGGGCC expansion (1x >620, 2x >850, 1x >1150 repeats), 2x SOD1 A4V, 2x SOD1 D90A, 5x wt	Motor neurons	C9ORF72 expression, C9ORF72 GGGGCC repeat stability, RNA foci, gene expression, RNA binding protein sequestration, C9ORF72 RAN translation, excitotoxicity	(Donnelly et al., 2013)
Ataxia telangiectasia				
2012	1x ATM het, 1x ATM homo (ATM c.7004delCA and/or ATM c.7886delTATTA)	Neurons	Radiation-induced signaling, radiosensitivity, DNA damage signaling pathways, mitochondrial and pentose phosphate pathways	(Nayler et al., 2012)
2013	1x ATM homo c.103C>T, 1x ATM compound het c. 967A>G;c.3802delG, 1x wt	NPCs and neurons	Activation of ATM activity, DNA repair, mitochondrial function	(P. Lee et al., 2013)
Best vitelliform macular dystrophy				
2013	1x BEST1 A146K, 1x BEST1 N296H, 2x wt	RPE, co-cultured with photoreceptor outer segments	Fluid flux, rhodopsin degradation, stimulated calcium responses, oxidative stress measures	(Singh et al., 2013)
Familial dysautonomia				
2009	3x IKBKAP homo c. 2507+6T>C, 1x wt	Neural crest, NPCs	IKBKAP splicing, cell motility	(G. Lee et al., 2009)
Friedreich's ataxia				
2010	2x FXN homo GAA (330;380 and 541;420)	No neurons created	Global gene expression, FXN expression, FXN GAA repeat instability, MMR enzyme expression and localization to FXN gene	(Ku et al., 2010)

	Novel iPSC lines generated (# of subjects per genotype/phenotype)	Differentiated cell type(s) identified and analyzed	Phenotypes described	Reference
2011	2x FXN homo GAA expansion (527;1058 and 751;1027)	Neurons, neural crest, peripheral sensory neurons, cardiomyocytes	FXN expression, FXN GAA repeat instability	(J. Liu et al., 2011b)
2013	2x FXN homo GAA expansion (800;600 and 900;400), 2x wt (<20 FXN GAA)	Neurons, cardiomyocytes	FXN expression, FXN GAA repeat stability, morphology, viability, electrophysiological properties, mitochondrial structure and membrane potential, MMR enzyme levels	(Hick et al., 2013)
2013	2x FXN homo GAA expansion (500;750 and 580;620)	Neural crest, peripheral sensory neurons	FXN expression	(Eigentler, Boesch, Schneider, Dechant, & Nat, 2013)
Frontotemporal dementia				
2012	1x FTD sporadic, 1x GRN S116X, 1x wt	Neurons (majority glutamatergic, some GABAergic)	GRN/PGRN expression, sensitivity to cellular stress, S6K2 expression	(Almeida et al., 2012)
2013	2x C9ORF72 >1,000 GGGGCC	Neurons (majority glutamatergic, some GABAergic), some astrocytes	C9ORF72 expression, C9ORF72 GGGGCC repeat stability, RNA foci, RNA binding protein sequestration, C9ORF72 RAN translation, viability in response to inhibitors of autophagy	(Almeida et al., 2013)
Gaucher's disease				
2013	1x GBA1 compound het L444P;G202R (acute neuronopathic [type 2] Gaucher's disease)	Dopaminergic neurons, macrophages	Acid- β -glucosidase activity	(Tiscornia et al., 2013)
Gyrate atrophy				
2011	1x OAT homo A226V	RPE	OAT activity	(Meyer et al., 2011)
Hereditary spastic paraplegia				
2013	1x SPAST c.683-1G>T, 1x wt	Telencephalic glutamatergic neurons	Axonal swellings, mitochondrial transport, spastin protein levels, tubulin acetylation	(Denton et al., 2014)
Huntington's disease				
2012	3x HTT CAG expansion (180;18, 109;19, 60;18), 3x wt (HTT 33;18, 28;21, 21;17 CAG).	NPCs, forebrain neurons, striatal neurons	Adhesion, cytoskeletal properties, electrophysiological activity, gene expression profiles, ATP levels, susceptibility to glutamate toxicity and other stressors	(HD iPSC Consortium, 2012)
2012	1x HTT 73;19 CAG expansion, corrected to HTT 21;20 CAG	NPCs, striatal neurons	Gene expression, cell death following growth factor withdrawal, mitochondrial bioenergetics, xenografting into striatum	(An et al., 2012)
2012	2x HTT CAG expansion (50;ND, 109;ND CAG), 1x wt (HTT 28;ND CAG)	Neurons, astrocytes	Clear cytoplasmic vacuole formation	(Juopperi et al., 2012)
2012	3x HTT CAG expansion (42;44, 39;42, 17;45), 2x wt (HTT 15;17, 15;18 CAG)	Neurons	Lysosomal activity	(Camnasio et al., 2012)
Neuronal ceroid lipofuscinosis				
2013	2x CLN2 compound het c. 509-1G>C;R208X (late-infantile NCL), 3x CLN3 homo 1.02 kb del (juvenile NCL), 1x CLN3 compound	NPCs, neurons	Organelle morphology, lysosomal storage material	(Lojewski et al., 2013)

	Novel iPSC lines generated (# of subjects per genotype/phenotype)	Differentiated cell type(s) identified and analyzed	Phenotypes described	Reference
	het c.1056+3 A>C;D416G (juvenile NCL), 1x CLN3 het 1.02 kb del, 1x wt			
Niemann-Pick type C1 disease				
2013	1x NPC1 compound het c.1628delC;E612D, 1x wt	NPCs, neurons	Electrophysiological properties, cholesterol accumulation	(Trilck et al., 2013)
Parkinson's disease				
2009	5x idiopathic PD, 3x non-PD	Dopaminergic neurons	ND	(Soldner et al., 2009)
2011	1x LRRK2 homo G2019S, 1x wt	Dopaminergic neurons and other midbrain neuronal types	α -synuclein accumulation, measures of oxidative stress, cell death in response to stressors	(Nguyen et al., 2011)
2011	2x PINK1 homo Q456X, 1x PINK1 homo V170G, 1x wt	Dopaminergic neurons	Stress-induced Parkin mitochondrial translocation, mitochondrial copy number, and PGC-1 α expression	(Seibler et al., 2011)
2011	1x SCNA A53T with gene correction	Dopaminergic neurons	ND	(Soldner et al., 2011)
2011	1x SCNA triplication, 1x wt	Dopaminergic neurons	Expression of α -synuclein and other triplicated genes	(Devine et al., 2011)
2011	1x SCNA triplication, 1x wt	Dopaminergic neurons	α -synuclein expression, oxidative stress, peroxide-induced cell death	(Byers et al., 2011)
2012	7x idiopathic PD, 4x LRRK2 G2019S, 4x wt	Ventral midbrain dopaminergic neurons	α -synuclein accumulation, cell death, autophagy	(Sánchez-Danés et al., 2012)
2012	2x PINK1 homo Q456X, 1x LRRK2 homo G2019S, 2x LRRK2 R1441C, 2x wt	Neurons, dopaminergic neurons	Response to stressors as measured by, mitochondrial respiration, proton leakage, mitochondrial mobility	(Cooper et al., 2012)
2012	1x PARK2 homo ex2-4 del, 1x PARK2 homo ex6-7 del	Dopaminergic neurons	Oxidative stress, mitochondrial morphology and homeostasis, changes in Nrf2 pathway, α -synuclein accumulation	(Imaizumi et al., 2012)
2012	1x LRRK2 G2019S with gene correction	NPCs, neurons	LRRK2 activity, nuclear morphology, susceptibility to proteosomal stress, neuronal differentiation	(G.-H. Liu et al., 2012b)
2013	2x LRRK2 G2019S with gene correction, 4x wt	Dopaminergic neurons	Neurite outgrowth, cell death in response to certain toxins, tau and α -synuclein expression	(Reinhardt et al., 2013)
2013	1x LRRK2 homo G2019S, 1x wt	Dopaminergic neurons	Mitochondrial morphology, ATP levels, mitochondrial ROS generation, lysosomal activity	(Su & Qi, 2013)
2013	3x LRRK2 G2019S with gene correction, 1x wt	NPCs, dopaminergic neurons	Mitochondrial DNA damage	(Sanders et al., 2013)
2013	1x PINK1 homo Q456X, 1x PARK2 homo c.1072delT, 1x PARK2 R275W, 2x wt	Dopaminergic neurons, fibroblasts	Age-associated markers, dendritic length, gene expression, xenografting into striatum, cell death, neuromelanin accumulation, mitochondrial size & ROS, Lewy body-precursor inclusions	(J. D. Miller et al., 2013)
Retinitis pigmentosa				
2011	1x RP1 c.2161insC, 2x RP9 H137L, 1x PRPH2 W316G, 1x RHO G188R	Rod photoreceptor cells	Cell degeneration, oxidative stress, ER stress, response to antioxidants	(Jin et al., 2011)

	Novel iPSC lines generated (# of subjects per genotype/phenotype)	Differentiated cell type(s) identified and analyzed	Phenotypes described	Reference
2011	1x MAK homo ex 9 353bp Alu repeat ins, 1x non-MAK RP	Postmitotic retinal cells	MAK expression	(Tucker et al., 2011)
2012	1x RHO G188R	Rod photoreceptor cells	RHO protein distribution, ER stress, cell degeneration	(Jin, Okamoto, Xiang, & Takahashi, 2012)
Spinal muscular atrophy				
2012	2x type I SMA (1x homo SMN1 del, 1x unknown genotype) with targeted conversion of SMN2 to SMN1, 1x SMN1 het del, 1x wt	Spinal motor neurons, co-cultured with myotubes	Motor neuron number and size, axon length, endplate size and number (NMJ) when co-cultured with myotubes, number of nuclear gems, xenografting into spinal cord	(Corti et al., 2012)
Tauopathy				
2013	1x MAPT A152T with gene correction to wt or MAPT homo A152T	Neurons	Tau fragmentation, phosphorylation, axonal degeneration, cell death	(Fong et al., 2013)

This table outlines published studies that generated novel hiPSC lines to study a subset of neurodegenerative diseases. The number of lines listed indicates the number of distinct *subjects* from whom iPSC lines were derived (i.e. "1x" may represent a single line or multiple clonal lines derived from a single subject). All mutations are heterozygous unless otherwise indicated (het: heterozygous, homo: homozygous). The differentiated cell types are listed as identified in the original paper. A-T: ataxia telangiectasia, AD: Alzheimer's disease, ALS: amyotrophic lateral sclerosis, ER: endoplasmic reticulum, FA: Friedreich's ataxia, FD: familial dysautonomia, FTD: frontotemporal dementia, HD: Huntington's disease, MMR: mismatch repair, NCL: neuronal ceroid lipofuscinosis, ND: no data, NMJ: neuromuscular junction, NPC: neural progenitor cell, PD: Parkinson's disease, ROS: reactive oxygen species, RP: retinitis pigmentosa, RPE: retinal pigment epithelium, SMA: spinal muscular atrophy, TH: tyrosine hydroxylase, wt: wild-type.

Table 2

Human iPSC cell lines created to study neurodevelopmental and neuropsychiatric diseases

	Novel iPSC lines generated (# of subjects per genotype/phenotype)	Differentiated cell type(s) identified and analyzed	Phenotypes described	Reference
Angelman/Prader-Willi syndromes				
2010	2x AS maternal 15q11-13 del, 1x PWS paternal 15q11-13 del, 1x wt	Neurons, astrocytes	Methylation imprint, UBE3A and UBE3A-ATS expression	(Chamberlain et al., 2010)
Dravet syndrome				
2013	1x SCN1A c.IVS14+3A>T, 1x SCN1A Y325X, 3x wt	Forebrain neurons (primarily GABAergic)	SCN1A expression, sodium current density, various electrophysiological measures of excitability	(Y. Liu, Lopez-Santiago, et al., 2013b)
2013	1x SCN1A F1415I, 1x SCN1A Q1923R, 1x wt	Primarily glutamatergic neurons, some GABAergic; co-cultured with astrocytes	Various electrophysiological measures of excitability	(Jiao et al., 2013)
2013	1x SCN1A R1645X	GABAergic neurons	SCN1A expression, various electrophysiological measures of excitability	(Higurashi et al., 2013)
Fragile X syndrome				
2010	3x FXS (FMR1 >200 CGG), 2x wt	No neurons created	CpG methylation in the FMR1 promoter region, FMR1 expression	(Urbach et al., 2010)
2011	3x FXS (FMR1 > 200 CGG, 1x FMR1 142 CGG derived from mosaic FXS patient), 1x wt	NPCs, neurons	CpG methylation in the FMR1 promoter region, FMR1 expression, neuronal differentiation	(Sheridan et al., 2011)
2012	1x pre-FXS (F) with derivation of isoautosomal lines containing either FMR1 94 CGG- or FMR1 30 CGG-active chrX	Neurons, astrocytes	FMR1 mRNA expression, PSD95 expression, synaptic density, neurite length, spontaneous Ca ⁺⁺ transient frequency and amplitude, altered response to glutamate uptake	(J. Liu et al., 2012c)
Lesch-Nyhan syndrome				
2013	1x HPRT1 -/Y (M), 1x HPRT1 +/- (F) with derivation of isoautosomal lines containing either wt- or mutation-active chrX (both M & F lines: complex HPRT1 rearrangement involving ex6-9 inv & del), 1x wt (M), 1x wt (F)	Neurons	HGPRT activity, X-inactivation status, neuronal differentiation, neurite length	(Mekhoubad et al., 2012)
Phelan-McDermid syndrome				
2013	2x 22q13 del (1x 825 kb del, 1x 871 kb del)	Mature forebrain neurons	SHANK3 expression, action potential characteristics, sE/IPSCs, presence of structural and functional synapses	(Shcheglovitov et al., 2013)
Microcephaly				
2013	1x CDK5RAP2 compound het E1516X;R1558X	Cerebral organoids	Neuroepithelial and progenitor region sizes, neuronal outgrowth, RG and neuronal number, neurogenic divisions, RG spindle orientation	(Lancaster et al., 2013)
Rett syndrome				
2009	1x MeCP2 R306C (F)	No neurons created	ND	(Hotta et al., 2009)

	Novel iPSC lines generated (# of subjects per genotype/phenotype)	Differentiated cell type(s) identified and analyzed	Phenotypes described	Reference
2010	1x MeCP2 T158M (F), 1x MeCP2 Q244X (F), 1x MeCP2 R306C (F), 1x MeCP2 c.1155del32 (F), 3x wt (M), 2x wt (F)	Neurons	MeCP2 expression, X-inactivation, glutamatergic synapse number, spine density, soma size, Ca ²⁺ oscillations, sE/IPSP frequency	(Marchetto et al., 2010a)
2011	1x MeCP2 ex3-4 del (F), isoautosomal lines with either wt- or mutation-active chrX	Neurons	MeCP2 RNA and protein expression, soma size, X-inactivation	(Cheung et al., 2011)
2011	1x CDKL5 T288I (M), 1x CDKL5 Q347X (F) with isoautosomal lines with either wt- or mutation-active chrX	Neurons	X-inactivation	(Amenduni et al., 2011)
2011	1x MeCP2 T158M (F), 1x MeCP2 Q244X (F), 1x MeCP2 R306C (F), 1x MeCP2 X487W (F), 1x MeCP2 c.705delG, isoautosomal lines with either wt- or mutation-active chrX	Neurons	X-inactivation, neuronal maturation	(K.-Y. Kim et al., 2011)
2011	1x MeCP2 T158M (F), 1x MeCP2 V247X (F), 1x MeCP2 R306C (F), 1x MeCP2 R294X (F), isoautosomal lines with either wt- or mutation-active chrX	Neurons	Nuclear size, X-inactivation	(Ananiev et al., 2011)
Schizophrenia				
2011	2x SCZ (DISC1 c.2420_2423del), 1x wt	No neurons created	ND	(Chiang et al., 2011)
2011	1x childhood onset-SCZ, 2x SCZ/SCZoid, 1x SCZ-affective, 5x wt	NPCs, neurons	Connectivity, neurite number, PSD95 and glutamate receptor levels	(Brennan et al., 2011)
2011	1x SCZ 22q11.2 del, 1x childhood-onset SCZ, 1x SCZ, 2x wt	Glutamatergic neurons	ND	(Pedrosa et al., 2011)
2012	4x SCZ, 4x wt	No neurons created	ND	(Vitale et al., 2012)
2012	1x SCZ, 1x wt	NPCs	Extramitochondrial oxygen consumption, ROS generation	(Paulsen et al., 2012)
2013	3x SCZ, 2x wt	NPCs, glutamatergic neurons, dopaminergic neurons	Differentiation capacity, cell area, neurite length & number, monoamine levels, mitochondrial distribution	(Robicsek et al., 2013)
Timothy syndrome				
2011	2x CACNA1C c.1216G>A, 3x wt	Cortical neurons	Ca ²⁺ signaling, activity-dependent gene expression, cell fate and differentiation to lower cortical layer and callosal projection neurons	(Pacini et al., 2011)

This table outlines published studies that generated novel hiPSC lines to study a subset of neurodevelopmental and neuropsychiatric disorders. The number of lines listed indicates the number of distinct *subjects* from whom iPSC lines were derived (i.e. “1x” may represent a single line or multiple clonal lines derived from a single subject). All mutations are heterozygous unless otherwise indicated (het: heterozygous, homo: homozygous). The differentiated cell types are listed as identified in the original paper. AS: Angelman's syndrome, F: female, FXS: fragile X syndrome, ND: no data, NPC: neural progenitor cell, pre-FXS: FMR1 premutation carrier, M: male, PWS: Prader-Willi syndrome, RG: radial glia, ROS: reactive oxygen species, SCZ: schizophrenia, SCZoid: schizoid, sE/IPSCs: spontaneous excitatory/inhibitory post-synaptic currents, wt: wild-type.

Table 3

Protocols for human ES or iPS cell differentiation

Cell type	References
Forebrain neuronal precursors or neurons	(Chambers et al., 2009; Eiraku et al., 2008; Elkabetz et al., 2008; Emdad, D'Souza, Kothari, Qadeer, & Germano, 2012; Erceg et al., 2008; Espuny-Camacho et al., 2013; Gupta et al., 2012; Hu et al., 2010; Juopperi et al., 2012; Kirkeby et al., 2012; Lancaster et al., 2013; X.-J. Li et al., 2009; Marchetto et al., 2010a; Mariani et al., 2012; Pankratz et al., 2007; Shi et al., 2012; Sonntag et al., 2007; Wada et al., 2009; Wernig et al., 2008; Yuan et al., 2011; Zeng et al., 2010; S. C. Zhang, Wernig, Duncan, Brüstle, & Thomson, 2001; X.-Q. Zhang & Zhang, 2009)
Motor neuron precursors or neurons	(Chambers et al., 2009; Elkabetz et al., 2008; Erceg et al., 2008; Hester et al., 2011; Hu et al., 2010; Hu & Zhang, 2009; H. Lee et al., 2007b; X.-J. Li et al., 2005; 2008; Nizzardo et al., 2010; Singh Roy et al., 2005; Wada et al., 2009; Zeng et al., 2010)
Dopaminergic neurons	(Chambers et al., 2009; Cho, Hwang, & Kim, 2008a; Cho et al., 2008b; Cooper et al., 2010; Elkabetz et al., 2008; Erceg et al., 2008; Iacovitti, Donaldson, Marshall, Suon, & Yang, 2007; Karumbayaram et al., 2009; Kirkeby et al., 2012; Ko et al., 2007; Kriks et al., 2011; Morizane, Doi, & Takahashi, 2013; C.-H. Park et al., 2005; Perrier et al., 2004; Roy et al., 2006; Sonntag et al., 2007; Swistowski et al., 2010; Wernig et al., 2008; Yan et al., 2005; Zeng et al., 2010; X.-Q. Zhang & Zhang, 2009)
GABAergic neuronal precursors or neurons (enriched)	(Goulburn et al., 2011; 2012; X.-J. Li et al., 2009; Y. Liu, Liu, et al., 2013a; Maroof et al., 2013; Nicholas et al., 2013)
Medium spiny neurons	(Aubry et al., 2008; Carri et al., 2013; Delli Carri et al., 2013; Ma et al., 2012)
Forebrain cholinergic neurons	(Bissonnette et al., 2011; Nilbratt, Porras, Marutle, Hovatta, & Nordberg, 2010; Wicklund et al., 2010)
Serotonergic neurons	(Erceg et al., 2008)
Caudal neurons	(Kirkeby et al., 2012; Pankratz et al., 2007)
Cerebellar neurons	(Erceg et al., 2010; Erceg, Lukovic, Moreno-Manzano, Stojkovic, & Bhattacharya, 2007)
Astrocytes (observed or enriched)	(Czepiel et al., 2011; Emdad et al., 2012; Erceg et al., 2008; Gupta et al., 2012; Juopperi et al., 2012; D. S. Lee et al., 2006; Shi et al., 2012; Swistowski et al., 2010; S. Wang et al., 2013; Wernig et al., 2008; Zeng et al., 2010; S. C. Zhang et al., 2001)
Oligodendrocytes (observed or enriched)	(Czepiel et al., 2011; Erceg et al., 2008; Kang et al., 2007; Krencik et al., 2011; Nistor, Totoiu, Haque, Carpenter, & Keirstead, 2005; Ogawa, Tokumoto, Miyake, & Nagamune, 2011; Swistowski et al., 2010; S. Wang et al., 2013; Wernig et al., 2008; S. C. Zhang et al., 2001)
Neural crest precursors or derivatives (including peripheral neurons, nociceptors, melanocytes, Schwann cells)	(Chambers, Mica, Lee, Studer, & Tomishima, 2013; Chambers et al., 2012; Eigentler et al., 2013; G. Lee, Chambers, Tomishima, & Studer, 2010; G. Lee et al., 2007a; K. S. Lee et al., 2012; J. Liu et al., 2011b; Q. Liu et al., 2012d; Menendez et al., 2013; Menendez, Yatskevych, Antin, & Dalton, 2011; Mica, Lee, Chambers, Tomishima, & Studer, 2013; Pomp, Brokhman, Ben-Dor, Reubinoff, & Goldstein, 2005; Ziegler, Grigoryan, Yang, Thakor, & Goldstein, 2011)
Cranial placode derivatives	(Dincer et al., 2013)
Retinal cells	(Buchholz et al., 2009; Hiram et al., 2009; Jin et al., 2011; Kokkinaki, Sahibzada, & Golestaneh, 2011; Lamba, Karl, Ware, & Reh, 2006; Meyer et al., 2009; 2011; Nakano et al., 2012; Osakada et al., 2008; Osakada, Ikeda, Sasai, & Takahashi, 2009a; Osakada et al., 2009b; Phillips et al., 2012; Tucker et al., 2011)

Dozens of protocols have been published for the production of various types of neurons from human ESCs and iPSCs. As many hESC differentiation protocols have been successfully used in hiPSCs, protocols using both cell types are included here. Readers should refer to the papers listed for further information on the efficiency of directed differentiation to each cell fate listed. For GABAergic neuron generation, only protocols that specifically direct differentiation to an inhibitory fate (rather than observation of inhibitory neuron generation) are listed. In contrast, studies that reported generation of astrocytes or oligodendrocytes are included whether they intentionally biased cells toward these cell fates, or if these cells were observed as a by-product of another differentiation method.