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Focus: Induced Pluripotency & Cellular Reprogramming

Probing disorders of the nervous system using reprogramming approaches

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Abstract

The groundbreaking technologies of induced pluripotency and lineage conversion have generated a genuine opportunity to address fundamental aspects of the diseases that affect the nervous system. These approaches have granted us unrestricted access to the brain and spinal cord of patients and have allowed for the study of disease in the context of human cells, expressing physiological levels of proteins and under each patient's unique genetic constellation. Along with this unprecedented opportunity have come significant challenges, particularly in relation to patient variability, experimental design and data interpretation. Nevertheless, significant progress has been achieved over the past few years both in our ability to create the various neural subtypes that comprise the nervous system and in our efforts to develop cellular models of disease that recapitulate clinical findings identified in patients. In this Review, we present tables listing the various human neural cell types that can be generated and the neurological disease modeling studies that have been reported, describe the current state of the field, highlight important breakthroughs and discuss the next steps and future challenges.

Introduction

Diseases of the nervous system represent an enormous burden for society in terms of human suffering and financial cost. While significant advancements have been achieved over the last few decades particularly in terms of genetic linkage, clinical classification and patient care, effective treatments are lacking. The inaccessibility of the relevant tissues and cell types in the central nervous system (CNS) and the complex multifactorial nature of most neurological disorders have hampered research progress. While animal models have been crucial in the investigation of disease mechanisms, fundamental developmental, biochemical and physiological differences exist between animals and humans. The importance of utilizing human cells for these purposes is evident by the large number of drugs that show efficacy and safety in rodent models of diseases but subsequently fail in human clinical trials, which are partly attributed to these species differences (Rubin, 2008). Furthermore, the overwhelming majority of neurological disease is of a sporadic nature, rendering animal modeling ineffective, while it is unclear whether the relatively rare monogenic forms of disease truly represent the vast majority of sporadic cases.

The simultaneous development of methods for reprogramming adult cells into induced pluripotent stem cells (iPSCs; Takahashi et al, 2007; Yu et al, 2007; Park et al, 2008) and the directed differentiation of pluripotent stem cells into distinct neuronal subtypes (Williams et al, 2012) suggested an attractive route to a novel model system for the study of neurological disorders. Patient-specific iPSCs can be generated by epigenetic reprogramming of various adult cell types such as skin fibroblasts and blood mononuclear cells and just like embryonic stem cells (ESCs), self-renew indefinitely and retain the potential to give rise to all cell types in the human body (Takahashi et al, 2007). More recently, sophisticated lineage conversion approaches have allowed for the direct generation of neurons and neural cell types from adult cells by means of overexpressing key transcription factors (for a detailed description see Tsunemoto et al, 2014). These methods have overcome some of the limitations of directed differentiation and have enabled for the generation of cell types that in many cases were previously unattainable.

The overwhelming advantages of using iPSCs and lineage conversion to develop models of diseases of the nervous system are that they allow one to study disease mechanisms in the context of human neurons and in the context of each patient's own unique genetic constellation. In many cases, established differentiation protocols allow for the generation of the particular neuronal subtype that is most vulnerable to the particular disease, such as spinal motor neurons (Davis-Dusenbery et al, 2014) and dopaminergic neurons (Kriks et al, 2011). These neurons can be produced in

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abundance from variable genetic backgrounds and could provide useful platforms for drug discovery.

The concept of using iPSCs and lineage conversion to study neurological disease appears straightforward: Both of these approaches allow for the generation of patient-specific neurons, which are relevant to the disease of interest, and when these are compared to neurons generated from healthy controls, any differences identified could be related to the disease. In practice, this approach has been proven to be more challenging than initially believed. What is the right cell type to make and study? How should quality control of neurons be performed? What are the right controls to use when assessing a disease-related phenotype? How do phenotypes identified *in vitro* relate to the clinical presentation of patients? These are just some of the questions that the community has struggled with, since the initial description of iPSCs and the onset of the development of *in vitro* patient-specific disease models. Perhaps the seemingly biggest advantage of this approach —the ability to study disease in the genetic background of the patient—has created the biggest challenge, as genetic background contributes to high variability in the properties of the patient-derived cells. This variability is a reality that neurologists have been facing for years, as often, two patients diagnosed with the same condition might present with very different clinical profiles. The technology of cellular reprogramming has brought this reality of clinical heterogeneity seen in patients from the bedside to the lab bench.

Since the initial description of reprogramming technologies, neuroscientists, neurologists and stem cell researchers have generated and characterized hundreds of patient-specific stem cell lines as well as neuronal cells derived from them (Table 1). The first "wave" of disease modeling studies focused on generating patient-specific human neurons and confirming previously described pathologies (Dimos *et al*, 2008; Ebert *et al*, 2009; Marchetto *et al*, 2010; Brennand *et al*, 2011; Seibler *et al*, 2011; Bilican *et al*, 2012; Israel *et al*, 2012). More recent studies have revealed novel insights into disease mechanisms and employed gene editing approaches to clearly demonstrate the association of identified phenotypes with known genetic variants that contribute to disease (An *et al*, 2012; Corti *et al*, 2012; Fong *et al*, 2013; Reinhardt *et al*, 2013; Kiskinis *et al*, 2014; Wainger *et al*, 2014; Wen *et al*, 2014b). At the same time, there has been tremendous progress in our ability to generate neuronal subtypes both via directed differentiation and through the exogenous expression of transcription factors. Here, we review the current state of disease modeling and neuronal differentiation approaches, highlight breakthrough studies and discuss the shift in focus that is expected over the next few years.

You can study only what you can make

With an eye on modeling neurological disease, stem cell scientists have steadily developed protocols for generating relevant human neural subtypes *in vitro* (Fig 1 and Table 2). Many directed differentiation and lineage conversion studies have focused on cell types that are selectively vulnerable in neurodegenerative or neurological diseases such as spinal motor neurons (amyotrophic lateral sclerosis, ALS), midbrain dopaminergic neurons (Parkinson's disease, PD) and striatal medium spiny neurons (Huntington's disease, HD).

Their selective vulnerability in patients provides confidence that the phenotypes identified in iPSC-derived or lineage-converted cells *in vitro* represent relevant disease processes. In addition, it provides the opportunity to sift out phenotypes that may be disease non-relevant by using neuronal subtypes that are not affected *in vivo* as negative controls.

One important area requiring further development of *in vitro* protocols is region-specific cortical differentiation. Many diseases affect specific regions of the cortex, such as frontotemporal dementia (FTD), which affects the anterior cingulate, orbitofrontal cortex, and temporal lobes, or ALS, which affects layer V neurons in the motor cortex. Thus, region-specific attributes play a large role in the disease vulnerability of neuronal subtypes. While protocols exist to generate neurons from both deep and upper layers of the cortex (Shi *et al*, 2012b; Kadoshima *et al*, 2013), they have not shown to be specific for a given region of the cortex. The identification of marker genes and neuronal projection patterns specific to neurons in different cortical regions will greatly facilitate the development and validation of region-specific cortical neuron protocols.

Adult neural stem cells of the dentate gyrus play a key role in memory formation and pattern separation tasks and could be an important therapeutic target for Alzheimer's disease (AD). Although it is possible to generate human neural stem or progenitor cells *in vitro*, these are likely more embryonic and it is not clear how closely these mimic adult stem cells of the dentate gyrus (Chambers *et al*, 2009, 2011; Shi *et al*, 2012a,b). One reason is that until recently, rigorous molecular characterization of these cells was missing. We are now starting to get a clearer picture. There seem to be several adult neural stem cell populations or states that can be distinguished by markers such as Ascl1 or Gli1, and single-cell RNA sequencing data have been generated (Bonaguidi *et al*, 2011). This new information will serve as a template for generating adult neural stem cells *in vitro*.

A third cell type that has not been produced on a patient-specific level *in vitro* is microglia. Microglia perform inflammatory and non-inflammatory tasks that enable normal neuronal function. Through these roles, they are known to regulate the progression of ALS and AD (Zhong *et al*, 2009; de Boer *et al*, 2014; Johansson *et al*, 2015), and potentially other neurodegenerative diseases. Mouse studies showed that microglia from *SOD1*G93A ALS mice express higher levels of the prostaglandin E2 receptor (Di Giorgio *et al*, 2008; de Boer *et al*, 2014). Similarly, microglia from an AD model sharply upregulate the prostaglandin E2 receptor in response to amyloid-β (Ab) exposure in an age-dependent manner (Johansson *et al*, 2015). Higher prostaglandin E2 signaling in microglia caused reduced microglial cytokine generation, chemotaxis, clearance of Aboligomers, resolution of inflammatory responses to Ab_{42} and trophic factor release (Johansson *et al*, 2015). In both the ALS and AD models, deletion of the prostaglandin E2 receptor significantly slowed disease progression (de Boer *et al*, 2014; Johansson *et al*, 2015). Under normal conditions, microglia are derived from the embryonic yolk sac and go through a maturation process after they enter the nervous system (Nayak *et al*, 2014). The signaling and gene expression changes that occur during this process are not well understood and will need to be characterized further to enable the production of patient-specific microglia.

Table 1.   List of published studies modeling human neurological diseases with iPSCs.

Disease	References	Patient genotype	Cell type analyzed	Identified phenotype	Notable
Alzheimer's Disease	Yagi et al (2011)	PSEN1, PSEN2 mutations	Neurons	Increased amyloid β42 secretion	
Alzheimer's Disease	Israel et al (2012)	APP mutations, sporadic cases	Neurons	Increased amyloid β40, Tau and GSK3β phosphorylation, accumulation of endosomes	One of two sporadic patients exhibited phenotypes
Alzheimer's Disease	Kondo et al (2013)	APP mutations, sporadic cases	Cortical neurons, astrocytes	Accumulated Aβ oligomers, ER & oxidative stress	One of two sporadic patients exhibited phenotypes
Alzheimer's Disease	Muratore et al (2014)	APP mutation	Forebrain neuron	Increase in Aβ42, Aβ38, pTAU	Aβ-antibodies reduce pTAU
Alzheimer's Disease	Sproul et al (2014)	PSEN1 mutation	Neural progenitors	Higher Aβ42/Aβ40 ratio, gene expression differences	Verification of gene expression differences in human AD brains
Alzheimer's Disease	Duan et al (2014)	Sporadic ApoE3/E4	Basal forebrain cholinergic neurons	Higher Aβ42/Aβ40 ratio, increased vulnerability to glutamate-stress	
Alzheimer's Disease	Hossini et al (2015)	Sporadic	Neurons	Gene expression analysis	
Amyotrophic Lateral Sclerosis (ALS)	Dimos et al (2008)	SOD1 mutations	Motor neurons	N.D.	First report of patient-specific neurons
Amyotrophic Lateral Sclerosis (ALS)	Mitne-Neto et al (2011)	VAPB mutations	Fibroblasts, iPSCs, motor neurons	Reduced VAPB protein levels	Although VAPB levels were highest in neurons, the reduction was not specific to neurons
Amyotrophic Lateral Sclerosis (ALS)	Bilican et al (2012)	TDP43 mutations	Motor neurons	Cell death	Real-time survival analysis of HB9^{+} neurons
Amyotrophic Lateral Sclerosis (ALS)	Egawa et al (2012)	TDP43 mutations	Motor neurons	Expression differences, TDP43 pathology, shorter neurites	Rescue by anacardic acid, multiple clones per patient used
Amyotrophic Lateral Sclerosis (ALS)	Sareen et al (2013)	C9orf72 expansion	Motor neurons	RNA foci, hypoexcitability, gene expression differences	Repeat-containing RNA foci colocalized with hnRNPA1 and Pur-α, rescue of gene expression by ASO treatment
Amyotrophic Lateral Sclerosis (ALS)	Donnelly et al (2013)	C9orf72 expansion	Neurons	RNA foci, irregular interaction with ADARB2, susceptibility to glutamate excitotoxicity	Colocalization of repeat with ADARB2 validated in patient motor cortex. Rescue of gene expression by ASO treatment
Amyotrophic Lateral Sclerosis (ALS)	Yang et al (2013b)	SOD1, TDP43 mutations	Motor neurons	Sensitivity to growth factor withdrawal	Rescue by kenpaullone
Amyotrophic Lateral Sclerosis (ALS)	Serio et al (2013)	TDP43 mutations	Astrocytes	Cell death, TDP43 mislocalization	
Amyotrophic Lateral Sclerosis (ALS)	Wainger et al (2014)	SOD1, C9orf72, FUS mutations	Motor neurons	Hyperexcitability	Phenotype rescued by gene correction in SOD1, and by treatment with a Kv7 agonist
Amyotrophic Lateral Sclerosis (ALS)	Kiskinis et al (2014)	SOD1, C9orf72 mutations	Motor neurons	Cell death, reduced soma size, ER stress, mitochondrial abnormalities, gene expression changes	Phenotypes rescued by gene correction in SOD1

Table 1 (continued)

Disease	References	Patient genotype	Cell type analyzed	Identified phenotype	Notable
Amyotrophic Lateral Sclerosis (ALS)	Chen *et al* (2014)	*SOD1* mutations	Motor neurons	Neurofilament aggregation, cell death	Phenotype rescued by gene correction
Amyotrophic Lateral Sclerosis (ALS)	Barmada *et al* (2014)	*TDP43* mutations	Neurons, astrocytes	Sensitivity to TDP43 accumulation	Autophagy stimulation increases survival
Amyotrophic Lateral Sclerosis (ALS)	Devlin *et al* (2015)	*TDP43* and *C9orf72* mutants	Neurons	Electrophysiological dysfunction	Hyperexcitability followed by loss of action potential output
Angelman & Prader–Willi Syndrome	Chamberlain *et al* (2010)	*15q11-q13* deletions	Neurons	*UBE3A* expression	Genomic imprint is maintained in iPSC neurons
Ataxia Telangiectasia	Lee *et al* (2013)	*ATM* mutations	NPCs & neurons	Defective DNA damage response	SMRT compounds rescue phenotype
Best Disease	Singh *et al* (2013)	*BEST1* mutations	RPE cells	Delayed RHODOPSIN degradation, defective Ca^{2+} responses, oxidative stress	
Dravet Syndrome	Higurashi *et al* (2013)	*SCN1A* mutation	Neurons (mostly $GABA^+$)	Reduced AP firing	
Dravet Syndrome	Liu *et al* (2013b)	*SCN1A* mutation	Neurons (GABA & $Glutamate^+$)	Increase Na^+ current density, altered excitability	
Dravet Syndrome	Jiao *et al* (2013)	*SCN1A* mutation	Neurons	Abnormal Na^+ currents, increased firing	
Familial Dysautonomia	Lee *et al* (2009)	*IKBKAP* mutation	Peripheral neurons, neural crest precursors	Mis-splicing & *IKBKAP* expression, neurogenesis & migration defects	Phenotypes are tissue specific
Familial Dysautonomia	Lee *et al* (2012)	*IKBKAP* mutation	Neural crest precursors	*IKBKAP* expression levels	First large-scale drug screening approach, first follow-up study
Fragile X Syndrome	Sheridan *et al* (2011)	*FMR1* expansion	NPCs & neurons	*FMR1* promoter methylation & reduced expression, reduced length of processes	
Fragile X Syndrome	Liu *et al* (2012b)	*FMR1* expansion	Neurons	Decreased PSD95 expression & density, neurite length, electrophysiological defects	
Fragile X Syndrome	Doers *et al* (2014)	*FMR1* expansion	Neurons	Neurite extension & initiation defects	
Friedreich's Ataxia	Liu *et al* (2011)	*FXN* expansion	Peripheral neurons, cardiomyocytes	*FXN* expression, repeat instability	
Friedreich's Ataxia	Hick *et al* (2013)	*FXN* expansion	Neurons, cardiomyocytes	*FXN* expression, mitochondrial dysfunction	
Friedreich's Ataxia	Eigentler *et al* (2013)	*FXN* expansion	Peripheral neurons	*FXN* expression	
Frontotemporal Dementia	Almeida *et al* (2013)	*C9orf72* expansion	Neurons	RNA foci, RAN products, sensitivity to autophagy inhibitors	
Frontotemporal Dementia (Bv)	Gascon *et al* (2014)	Sporadic patients	Neurons	Alterations in miR-124 & AMPAR levels	Confirmation of mouse model findings in iPSC neurons & patients
Frontotemporal Dementia	Raitano *et al* (2015)	PGRN mutation	Cortical & motor neurons	Cortical differentiation defects	Rescue by PGRN expression

Table 1 (continued)

Disease	References	Patient genotype	Cell type analyzed	Identified phenotype	Notable
Gaucher's Disease	Mazzulli et al (2011)	GBA1 mutations	Dopaminergic neurons	Declined proteolysis, increased α-synuclein	Provides links between GD & PD
Gaucher's Disease	Tiscornia et al (2013)	GBA1 mutations	Neurons & macrophages	Reduction in acid-β-glucosidase activity	Identification of two small molecules
Gyrate Atrophy	Meyer et al (2011)	OAT mutation	RPE cells	Decreased OAT activity	Rescued by BAC-mediated introduction of OAT
Hereditary Spastic Paraplegia	Denton et al (2014)	SPAST mutation	Glutamatergic neurons	Axonal swelling, increased levels of acetylated tubulin	
Hereditary Spastic Paraplegia	Zhu et al (2014)	ATL1 mutation	Forebrain neurons	Impaired axonal growth, defects in mitochondrial motility	
Huntington's Disease	Camnasio et al (2012)	HTT expansion	Neurons	Altered lysosomal activity	
Huntington's Disease	Juopperi et al (2012)	HTT expansion	Astrocytes	Cytoplasmic vacuolization	
Huntington's Disease	HD Consortium (2012)	HTT expansion	NPCs & GABA^{+} neurons	Altered gene expression, morphological alterations, survival deficit, sensitivity to stressors	Correlation between repeat length & vulnerability to cell stress
Huntington's Disease	An et al (2012)	HTT expansion	NPCs, neurons	Cell death, gene expression, mitochondrial dysfunction	Genetic correction rescued phenotypes
Huntington's Disease	Guo et al (2013)	HTT expansion	Neurons (GABA[+])	Mitochondrial damage	
Huntington's Disease	Yao et al (2015)	HTT expansion	Striatal neurons	Cell death, caspase-3 activation	Identified Gpr52 as a stabilizer of HTT
Lesch–Nyhan Syndrome	Mekhoubad et al (2012)	HPRT1 mutation	Neurons	Neuronal differentiation efficiency and neurite number defects	Demonstrate that X-inactivation erodes in culture & could affects modeling of X-linked disease
Microcephaly	Lancaster et al (2013)	CDK5RAP2 mutation	Cerebral organoids	Smaller neuroepithelial regions & RGs, premature neurogenesis, RG spindle disarray	Generated 3-dimensional brain structures
Neuronal ceroid lipofuscinosis	Lojewski et al (2014)	CNL2, CNL3 mutations	NPCs, neurons	Morphological abnormalities in ER, Golgi, mitochondria & lysosomes	Rescue by expression of NCL proteins
Niemann–Pick type C1 disease	Trilck et al (2013)	NPC1 mutation	NPCs & neurons	Accumulation of cholesterol	
Parkinson's Disease	Byers et al (2011)	SCNA triplication	Dopaminergic neurons	Oxidative stress, α-synuclein accumulation	
Parkinson's Disease	Nguyen et al (2011)	LRRK2 mutations	Dopaminergic neurons	Oxidative stress, α-synuclein accumulation, sensitivity to stress reagents	
Parkinson's Disease	Seibler et al (2011)	PINK1 mutations	Dopaminergic neurons	Increased mitochondrial copy number, PGC1a upregulation	Rescue by PINK1 overexpression
Parkinson's Disease	Devine et al (2011)	SNCA triplication	Dopaminergic neurons	Upregulation of α-synuclein	

Table 1 (continued)

Disease	References	Patient genotype	Cell type analyzed	Identified phenotype	Notable
Parkinson's Disease	Sanchez-Danes *et al* (2012)	Sporadic & *LRRK2* mutations	Dopaminergic neurons	Reduction in neurite number & density, vacuolization, sensitivity to lysosomal inhibition	A total of 15 patients examined, long-term culture ~75 DIV
Parkinson's Disease	Cooper *et al* (2012)	*PINK1* & *LRRK2* mutations	Dopaminergic neurons	Mitochondrial dysfunction in response to stressors	Pharmacological rescue of phenotypes
Parkinson's Disease	Imaizumi *et al* (2012)	*PARK2* mutations	Dopaminergic neurons	Oxidative stress, mitochondrial dysfunction, Nrf2 induction, α-synuclein accumulation	
Parkinson's Disease	Liu *et al* (2012a)	*LRRK2* mutation	Neural stem cells	Susceptibility to proteosomal stress, differentiation & clonal expansion deficiencies	Genetic correction rescued phenotypes
Parkinson's Disease	Reinhardt *et al* (2013)	*LRRK2* mutation	Dopaminergic neurons	Gene expression differences, ERK phosphorylation & activity	Genetic correction rescued phenotypes
Parkinson's Disease	Su and Qi (2013)	*LRRK2* mutation	Dopaminergic neurons	Mitochondrial damage, shorter neuritis, lysosomal hyperactivity	Pharmacological rescue
Parkinson's Disease	Chung *et al* (2013)	*SNCA* mutation	Cortical neurons	Nitrosative & ER stress	Pharmacological rescue, combination between a yeast and an iPSC platform
Parkinson's Disease	Miller *et al* (2013)	*PINK1* & *PARKIN* mutations	Dopaminergic neurons	TH reduction, dendritic degeneration	Phenotypes induced only after overexpressing progerin
Parkinson's Disease	Ryan *et al* (2013)	*SNCA* mutation	Dopaminergic neurons	Nitrosative stress, gene expression alterations, mitochondrial stress	Genetic & pharmacological rescue of phenotypes
Parkinson's Disease	Flierl *et al* (2014)	*SNCA* triplication	NPCs	Viability, metabolism & stress resistance defects	Rescue by *SNCA* knockdown
Parkinson's Disease	Sanders *et al* (2014)	*LRRK2* mutations	NPCs & neurons	Mitochondrial DNA damage	Genetic correction rescued phenotypes
Phelan–McDermid Syndrome	Shcheglovitov *et al* (2013)	22q13.3 deletion	Forebrain neurons	Defective excitatory synaptic transmission	Rescue by *SHANK3* expression or IGF1 treatment
Retinitis Pigmentosa	Jin *et al* (2011)	*RP1, RP9, PRPH2, RHO* mutations	Rod photoreceptors	Cell death, oxidative & ER stress	Differential response to treatment with α-Tocopherol
Retinitis Pigmentosa	Tucker *et al* (2011)	MAK mutations	Retinal precursors	Defective *MAK* mRNA splicing	
Retinitis Pigmentosa	Jin *et al* (2012)	*RHO* mutations	RPE cells	Cell death & ER stress	
Retinitis Pigmentosa	Tucker *et al* (2013)	*USH2A* mutations	Retinal precursors	*USH2A* transcript defects, ER stress	
Rett Syndrome	Marchetto *et al* (2010)	*MeCP2* mutations	Neurons	*MeCP2* expression, reduced synapses, spine density, soma size, altered calcium signaling	
Rett Syndrome	Ananiev *et al* (2011)	*MeCP2* mutations	Neurons	Reduced nuclear size	
Rett Syndrome	Cheung *et al* (2011)	*MeCP2* deletion	Neurons	*MeCP2* expression, reduced soma size	

Table 1 (continued)

Disease	References	Patient genotype	Cell type analyzed	Identified phenotype	Notable
Rett Syndrome	Kim *et al* (2011c)	*MeCP2* mutations	Neurons	Lower TUJ1 & Na$^+$ channel expression	
Rett Syndrome	Amenduni *et al* (2011)	*CDKL5* mutations	Neurons	No phenotype described	
Rett Syndrome	Ricciardi *et al* (2012)	*CDKL5* mutations	Neurons	Aberrant dendritic spines	
Rett Syndrome	Larimore *et al* (2013)	*MeCP2* mutations	Neurons	Reduced expression of *PLDN*	
Rett Syndrome	Griesi-Oliveira *et al* (2014)	*TRPC6* mutation	NPCs & cortical neurons	Gene expression differences, Ca^{2+} influx defects, decreased axonal length & arborization	Overlap in molecular pathways between *TRPC6* & *MeCPT2*
Rett Syndrome	Williams *et al* (2014)	*MeCP2* mutations	Astrocytes	Mutant astrocytes cause morphological and firing defects in healthy neurons	Demonstrates non-cell autonomous contribution of astrocytes in Rett Syndrome
Rett Syndrome	Djuric *et al* (2015)	*MeCP2e1* mutation	Cortical neurons	Reduced soma size, dendritic density, capacitance & firing defects	Rescue of phenotypes by overexpression of *MeCP2e1*
Rett Syndrome	Livide *et al* (2015)	*MeCP2* & *CDKL5* mutations	NPCs & neurons	Gene expression differences	Identified *GRID1* as a common target in two distinct genetic classes of RTT
Schizophrenia	Brennand *et al* (2011)	Familial & sporadic SCZD patients	NPCs & neurons	Decreased connectivity, neurite number, PSD95 protein, gene expression changes	Recovery after treatment with loxapine
Schizophrenia	Pedrosa *et al* (2011)	22q11.2 deletion & sporadic SCZD	Glutamatergic neurons	No phenotype described	
Schizophrenia	Paulsen Bda *et al* (2012)	SCZD patient	NPCs	Elevated ROS, extramitochondrial consumption	Treatment with valproic acid reduced ROS
Schizophrenia	Robicsek *et al* (2013)	SCZD patients	NPCs, dopaminergic, glutamatergic neurons	Differentiation & maturation deficiencies, mitochondrial defects	
Schizophrenia	Yoon *et al* (2014)	*15q11.2* microdeletion	NPCs	Deficits in adherent junctions & apical polarity	Identified haploinsufficiency of *CYFIP1* as a potential contributor to neuropsychiatric disorders
Schizophrenia	Hook *et al* (2014)	SCZD patients	Neurons	Increased secretion of catecholamines, higher numbers of TH$^+$ neurons	
Schizophrenia	Wen *et al* (2014b)	*DISC1* mutations	Forebrain neurons	Synaptic vesicle release deficits, gene expression changes	Isogenic controls included in this study
Schizophrenia	Brennand *et al* (2015)	Familial & sporadic SCZD patients	NPCs & neurons	RNA & protein-level differences related to cytoskeleton & oxidative stress, aberrant migration	
Spinal Muscular Atrophy	Ebert *et al* (2009)	Type 1 *SMA*	Motor neurons	Cell death, soma size, reduced SMN levels	First study of iPSC-based approach to report a disease-associated phenotype

Table 1 (continued)

Disease	References	Patient genotype	Cell type analyzed	Identified phenotype	Notable
Spinal Muscular Atrophy	Sareen *et al* (2012)	Type 1 *SMA*	Motor neurons	Cell death, increased caspase-8 & 3 activation	Rescue by apoptotic inhibitors
Spinal Muscular Atrophy	Corti *et al* (2012)	Type 1 *SMA*	Motor neurons	Cell death, smaller soma size, reduced axonal length, gene expression and RNA splicing defects	Gene correction, transplantation of iPSC motor neurons extends lifespan of SMA mouse model
Tauopathy	Fong *et al* (2013)	*TAU* mutation	Neurons	TAU fragmentation & phosphorylation, axonal degeneration	Gene editing to correct the mutation & generate a homozygous mutant used as controls
Timothy Syndrome	Pasca *et al* (2011)	*CACNA1C* mutations	NPCs & cortical neurons	Ca^{2+} signaling, activity-dependent gene expression	Rescue by roscovitine treatment
Timothy Syndrome	Krey *et al* (2013)	*CACNA1C* mutations	Cortical neurons	Activity-dependent dendrite retraction	Rescue by GTPase Gem

NPCs, neural progenitor cells; RPE, retinal pigment epithelium; ND, not determined; ASO, allele-specific oligonucleotide; GD, Gaucher's disease; PD, Parkinson's disease; AP, action potential.
The table includes neurodevelopmental and neurodegenerative diseases for which patient-specific iPSCs have been generated and neuronal cells differentiated to develop a cell-based model of disease.

Specificity of phenotypes: the importance of controls

Significant technical advancements achieved over that last few years currently allow for the generation of patient-specific iPSCs that are free from genomic integration of the reprogramming factors (Malik & Rao, 2013). The essential quality of any newly derived iPSC can be easily assessed by (i) immunocytochemistry for pluripotency markers (e.g. NANOG/SSEA3), (ii) a quantitative pluripotency assay such as the *Scorecard* or the *Pluritest* and (iii) analysis of genomic integrity (karyotype, array CGH).

Disease modeling studies based on iPSC technology have relied on the use of diseased cells derived from patients as a model for disease, and cells derived from healthy individuals as controls. However, genetic and potentially epigenetic heterogeneity of iPSC lines contributes to functional variability of differentiated somatic cells, confounding evaluation of disease modeling experiments (Sandoe & Eggan, 2013). Such variability can be introduced at multiple different levels including generation of stem cell lines, continuous *in vitro* culture, variation in cell culture reagents, differential efficiencies of neural generation and genetic background. There are different approaches to overcoming this variation. One approach is through the use of targeted gene editing that results in the generation of a control stem cell line that is isogenic to the patient one, except for the disease-causing mutation. Such an approach effectively minimizes line-to-line differences and is a very important tool for iPSC-based disease modeling.

CRISPR/Cas9, a recent technology that has emerged, allows for the efficient generation of such isogenic stem cell lines (Hsu *et al*, 2014). The system contains two essential components, an enzyme that can cleave DNA such that a double-strand break or a single nick is generated and a guide RNA that targets the enzyme to a specific genomic location. By simultaneously introducing either a single-stranded oligodeoxynucleotide (ssODN) containing the desired edit or a targeting plasmid with larger desired sequence
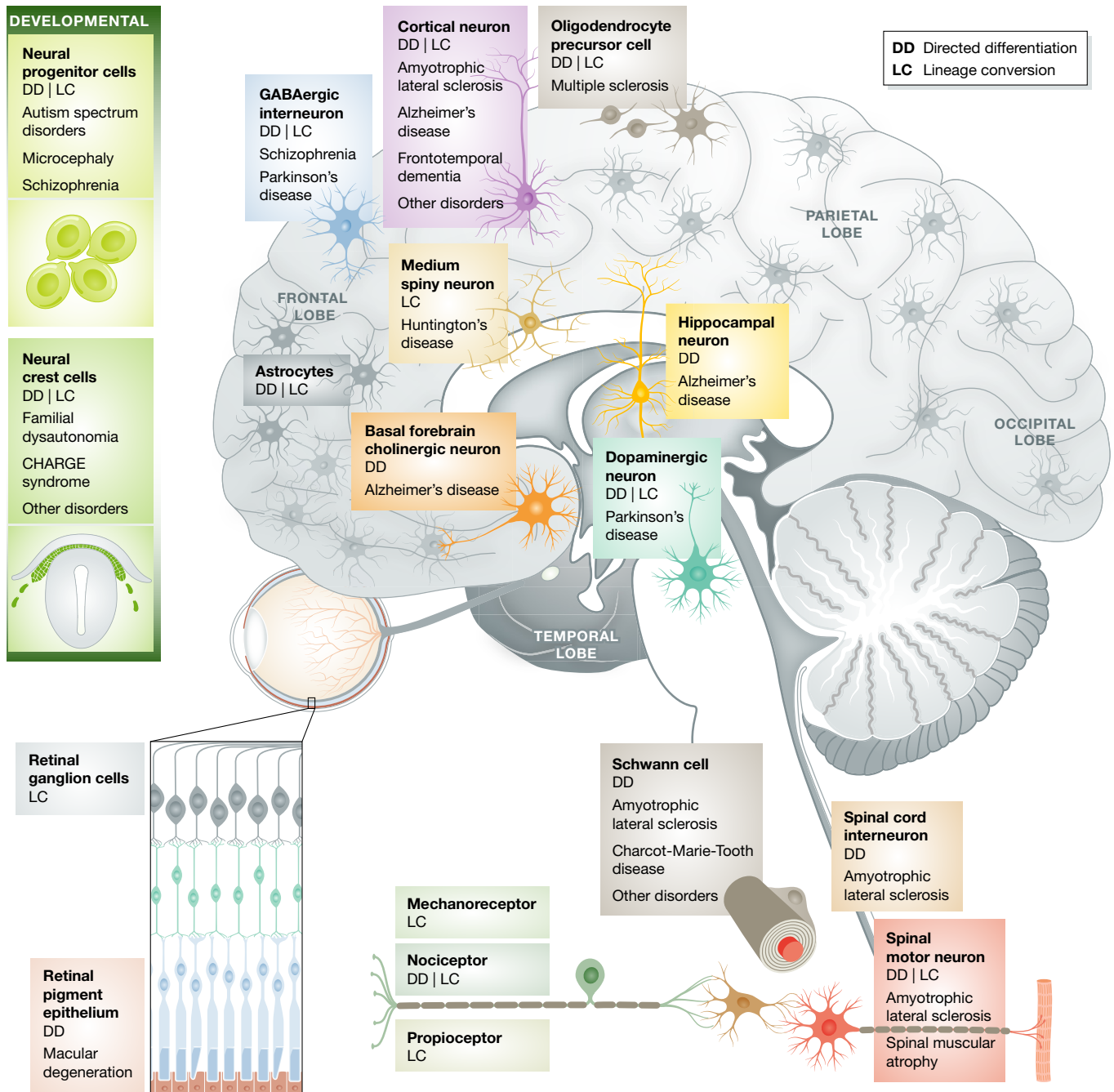
alterations, the genomic sequence can be precisely edited via the cells' own endogenous repair mechanism, homologous recombination. Given the incredible versatility of the CRISPR/Cas9 system and the continuous evolvement of the technical aspects of this approach, it should be expected that every iPSC study that focuses on genetic forms of disease should include an isogenic control cell line. The rescue of a phenotype by genetic correction can lead to the conclusion that the genetic lesion is *necessary* for the onset of the phenotype. The same technique can be used to introduce a disease-associated mutation in a healthy iPSC line in order to assess whether the mutation in itself is *sufficient* for the onset of particular phenotypes.

An alternative approach to the concern of variation would be to utilize multiple stem cell clones from each individual patient and compare the desired measurement against multiple healthy individuals. The use of multiple patient clones would ensure that the phenotype is not an artifact of a defective clonal cell line, while the use of multiple healthy controls should encapsulate sufficient technical and genetic variation, so that the measured cellular properties neuronal firing, dendritic density, etc. will represent a true average. This approach will be important in studies of sporadic disease.

Additionally, approaches that are complementary to the iPSC method should also be considered for the verification of identified phenotypes. These could include the generation of neurons via direct conversion as well as the investigation of human patient material such as postmortem CNS tissue and cerebrospinal fluid (CSF). Other non-invasive techniques such as transcranial magnetic stimulation (TMS) (Fox *et al*, 2014), which allows *in vivo* neurostimulation and neuromodulation, and an electroencephalogram (EGG), can also be used to examine changes associated with electrical excitability of neurons.

An important point to consider when assessing the specificity of an identified phenotype is whether it is only apparent in the cell type known to be most vulnerable to the disease being modeled. In

Figure 1. You can model only what you can make.
A number of different human neural cells can be efficiently generated by directed differentiation (DD) from pluripotent stem cells, or by lineage conversion (LC) from somatic cell types.

ALS patients for example, it is the upper and lower motor neurons that are initially targeted by disease mechanisms and gradually lost, while sensory neurons remain relatively unaffected. It would therefore be predicted that a phenotype that is truly relevant to disease would not be evident in a sensory neuron generated from the same individual. Although this could be a valuable approach, it should be taken with caution for two reasons: firstly because a sensory neuron might simply be resistant to a phenotype, and therefore, it is the

effect of the phenotype on the sensory cell that should be considered and not simply the presence of the phenotype in itself, and secondly because it might be the *in vivo* microenvironment of a sensory neuron that confers resistance and not a cell autonomous trait. Nevertheless, studies have demonstrated neuronal-type specificity of a phenotype including the sensitivity of mutant PD tyrosine hydroxylase (TH)-positive neurons but not TH-negative neurons to H_2O_2-induced toxicity (Nguyen *et al*, 2011), and morphometric

Table 2.   List of neural cells generated by directed differentiation of stem cells and lineage conversion of somatic cells.

Initial cell population	Target cell type	Morphogens/Small molecules	Reprogramming factors	References
Lineage conversion				
Fibroblasts	Neural crest cells		SOX10	Kim *et al* (2011a)
Fibroblasts	Neural stem cells		SOX2	Ring *et al* (2012)
Fibroblasts	Neurons	CHIR99021, SB431542	ASCL1, NGN2	Ladewig *et al* (2012)
Fibroblasts	Neurons		ASCL1	Chanda *et al* (2014)
Pericyte-derived cells	Neurons		SOX2, ASCL1	Karow *et al* (2012)
Fibroblasts	Dopaminergic neurons		ASCL1, BRN2, MYT1L, LMX1A, FOXA2	Pfisterer *et al* (2011)
Fibroblasts	Dopaminergic neurons		ASCL1, LMX1A, NURRL	Caiazzo *et al* (2011)
Fibroblasts	Dopaminergic neurons		Lmx1a, Foxa2, Ascl1, Brn2	Sheng *et al* (2012a)
Fibroblasts	Dopaminergic neurons		Ascl1, Pitx3, Lmx1a, Nurr1, Foxa2, EN1	Kim *et al* (2011b)
Fibroblasts	Dopaminergic neurons		ASCL1, NGN2, SOX2, NURR1, PITX3	Liu *et al* (2012c)
Fibroblasts	Glutamatergic Neurons		ASCL1, BRN2, MYT1L, NEUROD1	Pang *et al* (2011)
Fibroblasts	Glutamatergic neurons		BRN2, MYT1L, miR-124	Ambasudhan *et al* (2011)
Fibroblasts	Glutamatergic neurons	Forskolin, Dorsomorphin	NGN2	Liu *et al* (2013a)
Fibroblasts	Glutamatergic and GABAergic neurons		ASCL1, MYT1L, NEUROD2, miR-9/9*, miR-124	Yoo *et al* (2011)
Fibroblasts	Medium spiny neurons		DLX1, DLX2, MYT1L, CTIP2, miR-9/9*, miR-124	Victor *et al* (2014)
Fibroblasts	Nociceptor, mechanoreceptor, proprioceptor neurons	Brn3a, Ngn1/2		Blanchard *et al* (2015)
Fibroblasts	Nociceptor Neurons	ASCL1, MYT1L, ISL2, KLF7, NGN1		Wainger *et al* (2015)
Fibroblasts	Oligodendrocyte progenitor cells		Sox10, Olig2, Zfp536	Yang *et al* (2013a)
Fibroblasts	Oligodendrocyte progenitor cells		Olig1, Olig2, Nkx2.2, Nkx6.2, Sox10, ST18, Gm98, Myt1	Najm *et al* (2013)
Fibroblasts	Spinal motor neurons		ASCL1, BRN2, MYT1L, NGN2, ISL1, LHX3, NEUROD1	Son *et al* (2011)
Fibroblasts	Astrocytes		Nfia, Nfib, Sox9	Caiazzo *et al* (2015)
Fibroblasts	Neural precursor cells		Brn2, Sox2, FoxG1	Lujan *et al* (2012)
Fibroblasts	Neural progenitor cells	VPA, CHIR99021, RepSox		Cheng *et al* (2014)
Fibroblasts	Neural stem cells		Brn4, Sox2, Klf4, c-Myc, E47	Han *et al* (2012)
Fibroblasts	Neural stem cells		Sox2, Klf4,c-Myc, Oct4	Thier *et al* (2012)
Sertoli cells	Neural stem cells		Ascl1, Ngn2, Hes1, Id1, Pax6, Brn2, Sox2, c-Myc, Klf4	Sheng *et al* (2012b)
Astrocytes	Neuroblasts		Sox2	Niu *et al* (2013)
Hepatocytes	Neurons		Ascl1, Brn2, Myt1l	Marro *et al* (2011)
Fibroblasts	Neurons		PTB repression	Xue *et al* (2013)
Astrocytes	GABAergic neurons		Ascl1, Dlx2	Heinrich *et al* (2010)
Directed differentiation				
Pluripotent stem cells	Forebrain neuronal precursors	SB431542, LDN189193, N2, B27		Chambers *et al* (2009)

Table 2 (continued)

Initial cell population	Target cell type	Morphogens/Small molecules	Reprogramming factors	References
Pluripotent stem cells	Forebrain neurons	SB431542, LDN189193, N2, B27		Chambers *et al* (2009)
Pluripotent stem cells	Telencephalic neurons	N2, B27, IGF1, Heparin, SHH, DKK1, WNT3A, BDNF, GDNF		Li *et al* (2009)
Pluripotent stem cells	Forebrain neural cells	Heparin, N2, B27, BDNF, GDNF, IGF1		Zeng *et al* (2010)
Pluripotent stem cells	Cortical neurons	B27, N2, BSA, sodium pyruvate, 2-mercaptoethanol, Noggin, Y27632		Espuny-Camacho *et al* (2013)
Pluripotent stem cells	Granule cerebellar neurons	FGF2, heparin, N2, Glutamax, FGF8, retinoic acid, ITS, FGF4, WNT1, WNT3A, B27, BMP7, BMP6, GDF7, SHH, NT3, JAG1		Erceg *et al* (2012)
Pluripotent stem cells	Hypothalamic neurons	Neurobasal-A, Glutamax, N2, B27, sodium bicarbonate, dibutyryl cyclic AMP, GDNF, BDNF, CNTF		Merkle *et al* (2015)
Pluripotent stem cells	Dopaminergic neurons	Heparin, N2, serum replacer, cAMP, ascorbic acid, BDNF, GDNF, SHH, FGF8		Yan *et al* (2005)
Pluripotent stem cells	Dopaminergic neurons	LDN193189, SB431542, SHH C25II, purmorphamine, FGF8, CHIR99021, N2, B27, L-Glut, BDNF, ascorbic acid, GDNF, TGFβ3, dibutyryl cAMP, DAPT		Kriks *et al* (2011)
Pluripotent stem cells	Spinal motor neurons	SB431542, LDN189193, N2, B27, retinoic acid, smoothened agonist		Amoroso *et al* (2013)
Pluripotent stem cells	Astrocytes	B27, BMP2, BMP4, LIF		Gupta *et al* (2012)
Pluripotent stem cells	Astrocytes	EGF, FGF, Glutamax, N2, CNTF		Krencik and Zhang (2011)
Pluripotent stem cells	Oligodendrocytes	N2, N1, cAMP, biotin, heparin, retinoic acid, SHH, purmorphamine, FGF2, B27, PDGF, IGF, NT3		Hu *et al* (2009)
Pluripotent stem cells	Hippocampal neurons	DKK1, SB431542, Noggin, cyclopamine, N2, B27, Wnt3a, BDNF, FGF2, ascorbic acid, cyclic AMP, fetal bovine serum		Yu *et al* (2014)
Pluripotent stem cells	Astrocytes (ventralized)	SB431542, LDN189193, RA, SHH, N2, B27, FGF1, FGF2		Roybon *et al* (2013)
Pluripotent stem cells	Basal forebrain cholinergic neurons	RA/SSH/FGF8/BMP9	*Lhx8 & Gbx1*	Bissonnette *et al* (2011)
Pluripotent stem cells	Cortical interneurons	SB431542, LDN189193, XAV939, SHH, purmorphamine, N2, B27		Maroof *et al* (2013)

deficiencies of mutant ALS, ISL-positive motor neurons but not ISL-negative neurons grown in the same culture dishes (Kiskinis *et al*, 2014).

A major advantage of using reprogramming approaches to study a neurological disease is the ability to assess the biological variation associated with a specific neuronal defect. Consider that a

phenotype, for example, defective lysosomal function, has been identified in neurons derived from a patient cell line and that this phenotype is mutation dependent (i.e. it is corrected in an isogenic control line). The first level of biological variation can be addressed by examining neurons derived from a different individual that harbors the exact same mutation in the same gene. If the phenotype is not present, then additional genetic or epigenetic factors might be necessary for the onset of the defect. The next level of biological variability can be addressed by examining neurons from a patient with a different mutation in the same gene. Lastly, the broader relevance of the identified phenotype for the disease can be assessed by examining the lysosomal function of neurons from patients with mutations in different disease-causing genes as well as in a large number of sporadic cases.

A more direct route to the CNS?

Lineage conversion provides a progenitor-free approach for generating various neural types. Lineage conversion relies on the overexpression of transcription factors to internally drive differentiation programs. The forced expression of these factors replaces external developmental morphogens utilized in iPSC differentiation by directly activating downstream genes. Additionally, either purified external cues or other cell types normally present *in vivo* are sometimes added to further guide the developmental trajectory and maturation of various cell types (Son *et al*, 2011; Meyer *et al*, 2014).

An advantage of this approach is that it simplifies the identification of protocols for generating new neural subtypes because it only requires knowledge of transcription factor expression during the terminal stages of development, as opposed to requiring a deep understanding of morphogen signaling dynamics starting from the pluripotent state through the terminally differentiated state. In the same way that identifying the signals that produce the target cell type is simpler for lineage conversion, optimizing the efficiency of their production is more complicated for iPSC-directed differentiation because one must optimize the efficiency of each progenitor step as opposed to one step as in lineage conversion. Due to these advantages, reprogramming biologists have rapidly developed lineage conversion protocols for almost all neural subtypes attainable by directed differentiation just a few years after the initial demonstration of iPSC reprogramming (Takahashi *et al*, 2007), which showed that dramatic changes in cell fate are possible (see Fig 1).

Several groups have taken advantage of the modular nature of transcriptional networks to generate distinct neuronal subtypes. Genetic neuralization through introduction of *BRN2, ASCL1* and *MYT1L* (BAM) to fibroblasts generates induced neurons (iN; Pang *et al*, 2011). The transcription factor *ASCL1* is sufficient in generating iNeurons alone, indicating that it is the key driver in this reprogramming approach (Chanda *et al*, 2014). The addition of *NEUROD1* further enhances this conversion (Pang *et al*, 2011). From iNs, a secondary layer of transcription factors guide cells to particular neurons. Spinal motor neurons have been generated by adding *ISL1, LHX3, NGN2* and *HB9* to the BAM factors (Son *et al*, 2011). Addition of *LMX1A* and *FOXA2* to the BAM cocktail results in dopaminergic neurons (Pfisterer *et al*, 2011). Striatal medium spiny neurons can be generated using a microRNA-based

neuralization platform (Yoo *et al*, 2011) supplemented with *CTIP2, DLX1, DLX3* and *MYT1L* (Victor *et al*, 2014). Neuronal induction has also been achieved through the repression of polypyrimidine tract binding (PTB), a single RNA binding protein (Xue *et al*, 2013). Oligodendrocyte precursor cells follow a separate glial lineage that is independent of BAM-mediated neuralization. Induced oligodendrocyte precursor cells can be made by overexpressing either *SOX10, OLIG2* and *ZFP536* (Yang *et al*, 2013a) or *OLIG1, OLIG2, NKX2.2, NKX6.2, SOX10, ST18, GM98* and *MYT1* (Najm *et al*, 2013). Just like in iPSC differentiation, inductive signals are added during lineage conversion protocols to further guide cells to mature fates.

During development, early neural progenitors produce neurons whereas late progenitors differentiate into astrocytes (Stiles & Jernigan, 2010). iPSC-directed differentiation recapitulates this developmental process. As a result, while the production of neurons from human iPSCs occurs within 30 days, astrocytes only emerge after 3 months (Krencik & Zhang, 2011). Recently, Broccoli and colleagues reported that three transcription factors, *NFIA, NFIB* and *SOX9,* convert fibroblasts into astrocytes (Caiazzo *et al*, 2015). A major advantage of this approach is that it requires < 3 weeks to generate functional astrocytes (Caiazzo *et al*, 2015).

The key consideration in evaluating the utility of lineage-converted cells is how similar they are to their primary counterparts and whether they reliably recapitulate disease phenotypes. We and others have shown that lineage-converted cells such as motor neurons (Son *et al*, 2011), dopaminergic neurons (Kim *et al*, 2011b) and pancreatic beta cells (Zhou *et al*, 2008; Li *et al*, 2014) express transcriptional profiles and DNA methylation patterns (Li *et al*, 2014) very similar to their primary targets. Although bulk analysis of lineage-converted cultures suggested that these cells retained more residual gene expression from the starting somatic cells than iPSC-derived cells (Cahan *et al*, 2014), single-cell studies suggest that this reflects heterogeneous cultures of converted and non-converted cells rather than "confused" or mixed-property cells (Li *et al*, 2014). Detailed epigenetic and single-cell analysis for more lineage-converted cell types will be required to rigorously assess the quality of these cells.

Recent studies have shown that lineage-converted cells are able to recapitulate disease phenotypes and provide insight into pathogenic mechanisms. Induced motor neurons derived from patients with *C9orf72* ALS degenerated rapidly in cell culture relative to control neurons (Wen *et al*, 2014a). In addition, they expressed dipeptide repeat proteins specific to the *C9orf72* form of the disease, indicating that they reproduce the phenotypes observed *in vivo* (Wen *et al*, 2014a). The authors used this model to determine that dipeptide repeat proteins induce toxicity in *C9orf72* ALS (Wen *et al*, 2014a). Meyer and colleagues used lineage conversion to generate astrocytes from sporadic ALS patients (Meyer *et al*, 2014). Astrocytes from the familial *SOD1* form of the disease induce the degeneration of motor neurons (Di Giorgio *et al*, 2008; Marchetto *et al*, 2008; Meyer *et al*, 2014), and the authors used this approach to assess the neurotoxicity of sporadic ALS patient astrocytes. They found that lineage-converted astrocytes from sporadic ALS patients consistently induced neurodegeneration, suggesting that an inherent disease mechanism is maintained in most sporadic patients. These studies demonstrate that lineage-converted cells are effective tools for studying CNS diseases.

Which approach would be better for disease modeling experiments—lineage conversion or iPSC-directed differentiation? It depends on several considerations. Has the disease affected cell type been generated *in vitro* previously? If not, how much is known about their developmental signaling or transcriptional profile? This would dictate which approach would be more effective to pursue. If both developmental signaling and transcriptional profiling are known, then lineage conversion might be a faster route to disease studies.

How many cells are required for the designed study? If a large number of cells are needed, for example for biochemical or epigenetic studies, iPSC-directed differentiation would be more suitable because the number of differentiated cells gets amplified at each progenitor step, whereas lineage conversion does not amplify the number of differentiated cells.

Are lineage-related cell types desirable or undesirable for the specific model? For example, non-cell autonomous neurotoxic stimuli from astrocytes are a key aspect of ALS (Di Giorgio *et al*, 2008; Marchetto *et al*, 2008; Meyer *et al*, 2014) and perhaps AD disease processes. It therefore would be informative to have patient-derived astrocytes included in these disease models. Most iPSC-directed differentiation protocols result in the production of multiple cell types within the same developmental lineage. In addition, several groups have started to develop three-dimensional protocols that produce several cell types from the same tissue that self-organize into structures that mimic the primary tissue (Eiraku *et al*, 2011; Nakano *et al*, 2012; Koehler *et al*, 2013; Koehler & Hashino, 2014). For certain tissues, such as the inner ear, this may enable more relevant disease models.

In contrast, lineage conversion strategies would not be expected to produce developmentally related cell types at a substantial rate nor 3D structures (unless a progenitor was formed that gives rise to self-organizing structures). But this would be desirable for screening applications where pure cultures of one neuronal subtype simplify high-throughput scaling and assay interpretation.

Overall, there are advantages and disadvantages to both approaches for the production of *in vitro* patient-derived cells depending on the disease and application. However, the emergence of the same phenotype in cells derived by both methods would certainly enhance confidence in the results.

A shift in focus: from developing neurons to maturing and aging them

A critical area that deserves further investigation is the maturity and aging of *in vitro* derived cells (Fig 2). We like to think that there are three stages we need to consider when setting up *in vitro* models of disease: the development, the maturation and the natural aging process of a neural cell type. While significant advancements have been achieved in generating and maturing neural cell types—either by directed differentiation or lineage conversion—little has been done in terms of affecting the aging of cells. For late onset diseases such as ALS, FTD, HD, PD and AD, it is possible that changes elicited by aging are required to induce the disease process. Age is the strongest risk factor for neurodegenerative diseases, and although there are rare cases with early onset presentation, the overwhelming majority of patients develop clinical symptoms in the later stages of their lives. The nature of the age-related risk remains largely unknown, and whether it arises from cell autonomous mechanisms or as a result of a systemic dysfunction remains to be determined. A number of studies support the notion that cellular epigenetic
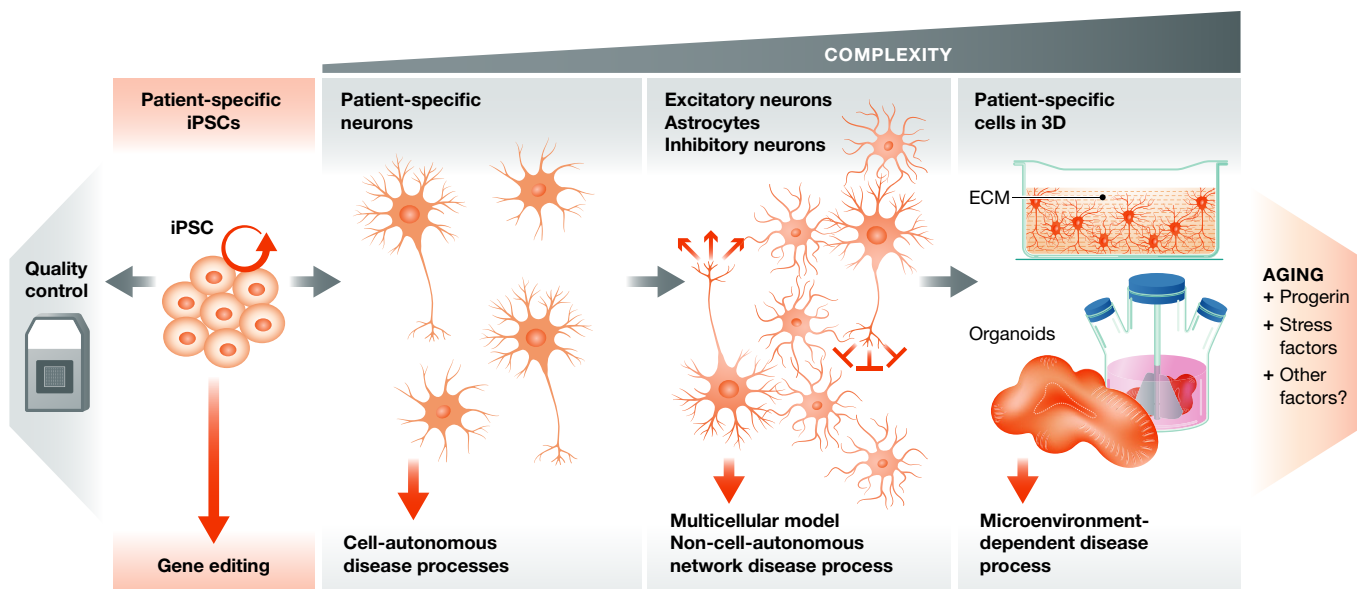
Figure 2. Developing stem cell-based models of neurological disorders.
Patient-specific iPSCs should be properly quality controlled for genomic integrity and pluripotent potential, while gene editing techniques allow for the generation of isogenic controls in cases where the disease-causing allele is known. Simple cell autonomous or more sophisticated multi-cellular and 3D disease models can be developed depending on the hypothesis being addressed. Neuronal maturity increases with the complexity of the cellular system, while methods for effectively aging neurons are lacking.

changes in the CNS correlate with aging. For example, recent work has demonstrated that profound changes in DNA methylation levels occur in the brains of mice with age (Lister *et al*, 2013), while aging oligodendrocytes lose their ability to effectively remyelinate damaged nerves (Ruckh *et al*, 2012). Importantly, under conditions of heterochronic parabiosis in mice, the effects on oligodendrocytes were reversible, implicating some aspect of epigenetic regulation.

Current studies suggest that the transcriptional and electrophysiological properties of both iPSC-derived and lineage-converted neurons are more similar to fetal neurons than adult (Son *et al*, 2011; Takazawa *et al*, 2012). It is likely that extrinsic factors present during normal development or aging are required to activate the maturation process. We and others have shown, for example, that the addition of primary astrocytes to lineage conversion cultures significantly improves the maturation of induced neurons (Son *et al*, 2011; Chanda *et al*, 2013; Wainger *et al*, 2015). Additional progress in generating more mature and aged cells will require a better understanding of the gene expression and functional changes associated with maturation and aging. This has been difficult to obtain for specific neuronal subtypes because of the scarcity of available human tissue. Efforts such as those of the Allen Brain Institute have shed some light on these markers, but future studies will need to analyze specific neuronal subtypes in order to be sure that differences between aged neurons and young neurons are truly due to aging and not different neuronal subtypes.

In addition to glial-derived factors, Rubin and colleagues recently showed that circulatory factors also contribute to the aging process in the CNS (Katsimpardi *et al*, 2014). They were able to identify a single factor, GDF11, which normally declines in expression with age. Interestingly, restoring GDF11 levels in old mice rejuvenated the proliferative and neurogenic properties of neural stem cells in the mouse (Katsimpardi *et al*, 2014). This raises the notion that there may be other factors that control the aging of neurons and could be exploited to regulate this process *in vitro*.

Studer and colleagues took a more intrinsic approach to inducing aging in iPSC-derived neurons by expressing *Progerin*, which is a mutant form of the Lamin A protein that causes accelerated aging phenotypes in humans (Miller *et al*, 2013). Expression of Progerin induced higher levels of DNA damage and mitochondrial reactive oxygen species in dopaminergic neurons derived from PD patients, which enabled the detection of PD-associated disease phenotypes such as dendrite degeneration, mitochondrial enlargement, Lewy body precursor inclusions and suppression of tyrosine hydroxylase expression (Miller *et al*, 2013). It remains unclear whether this approach induces the recapitulation of *bona fide* disease processes, but it represents a new line of targeted aging procedures.

From cell autonomy to more sophisticated systems

Neurons do not exist in isolation in the human nervous system. They form elaborate and functional networks with other neurons and also rely on a sophisticated microenvironment that is created by the interactions with other neural and non-neural cell types, which provide structural, metabolic and functional support as well as effective communication (Abbott *et al*, 2006). Glial cells, astrocytes, oligodendrocytes, microglia and endothelial cells exist in abundance in the nervous system and play vital functional roles. Glial cells buffer harmful ions, astrocytes provide nutrients and circulate neurotransmitters around synapses, oligodendrocytes form myelin sheaths around axons, microglia scavenge and degrade dead cells, and endothelial cells are important in maintaining the blood–brain barrier. Cell–cell interactions and the microenvironment as a whole might mediate important neuroprotective or neurotoxic activities in response to disease or injury. In fact, a number of studies over the last few years have clearly demonstrated that non-cell autonomous processes involving astrocytes, oligodendrocytes and microglia play a critical role in mediating disease progression and potentially onset in neurodegeneration including in ALS, HD, PD, prion disease, the spinal cerebellar ataxias (SCAs) and AD *in vivo* (Ilieva *et al*, 2009). The strength of utilizing iPSCs to study neurological disease is in their ability to generate a range of different cell types from the same genetic background (Fig 2). This allows for the assessment of how a specific genetic lesion, for example, might differentially impact neuronal subtypes. It also allows for a rational step-by step approach to assess how cellular interactions might contribute toward the evolvement of a disease-associated phenotype or a cellular response to stress.

The co-culture of spinal motor neurons with cortical astrocytes has previously been utilized in one of the first stem cell-based models of ALS to demonstrate how mutant or healthy astrocytes significantly compromised or maintained, respectively, the health of a pure population of motor neurons (Di Giorgio *et al*, 2008; Marchetto *et al*, 2008). The co-culture of cortical excitatory with cortical inhibitory neurons and the establishment of functional circuitry might be beneficial when studying epileptic syndromes. The clinical presentation of epileptic patients is the result of the functional control—or lack thereof—of a network of neurons, and recapitulating such a network could be an essential step toward the development of a cellular disease model. The importance of the local microenvironment in neuronal function and potentially dysfunction during disease is also relevant in the context of the three dimensionality that it creates. Neither the brain nor the spinal cord hosts isolated neurons surrounded by an entirely liquid trophic support (akin to culture media) in which nutrients, molecules and proteins can freely diffuse and float around. Recently, Kim, Tanzi and colleagues were able to successfully recapitulate amyloid-β plaques, and tau neurofibrillary tangles—the two pathological hallmarks of AD—in a single 3D human neural cell culture system (Choi *et al*, 2014). Although this system is not based on iPSCs and their cell lines expressed slightly elevated protein levels of *PSEN1* and *APP,* they designed a simple but innovative cell culture system with neurons grown embedded within a 0.3-mm layer of an extracellular matrix composed of BD Matrigel. This viscous layer reduced the diffusion of secreted amyloid-β and led to the accumulation of aggregated plaques. This is the first time this has been achieved in a cell-based *in vitro* system and demonstrates the importance of a 3D environment for disease modeling assays.

The recent description of cerebral organoids generated from human pluripotent stem cells and resembling the three-dimensional regional organization of a developing brain has created an exciting opportunity for iPSC-based disease modeling approaches (Lancaster *et al*, 2013). These brain-like structures, formed by the combination of external growth factor patterning and intrinsic and environmental cues, exhibit distinct regional identities that functionally interact and importantly recapitulate human cortical organization. The
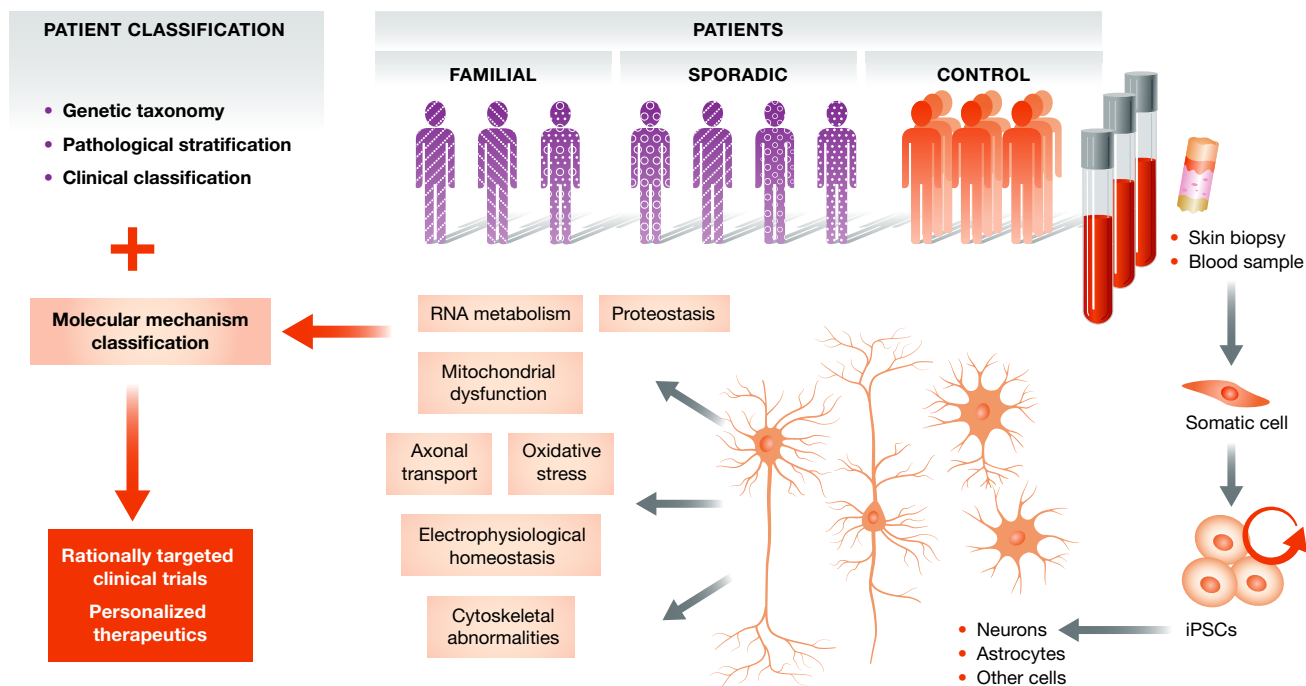
Figure 3. Patient stratification based on the molecular pathways that are affected.
Reprogramming and stem cell-based disease modeling can be utilized to address the level of heterogeneity by defining the molecular mechanisms that lead to disease in different patients. This novel classification of patients could lead to rationally targeted clinical trials and personalized therapeutic approaches.

authors utilized this method to study microcephaly and demonstrate that patient-specific organoids show premature neuronal differentiation and are only capable of developing to a smaller size. Importantly, mouse models have failed to effectively recapitulate these disease phenotypes for microcephaly, probably due to the dramatic differences in the development and regional organization of the brain as mice do not have an outer subventricular zone (SVZ). This system may be suitable for the study of other neurodevelopmental and neuropsychiatric syndromes in which there are moderate but crucial defects in cortical organization and function. This approach may also be useful in recapitulating human neurodegenerative models that primarily affect brain function as it may allow for the establishment of neuronal circuitry as well as biochemical networks.

Patient stratification based on molecular pathways affected

Neurological disorders including schizophrenia, ALS, PD, FTD and epilepsy are often characterized by a profound clinical and genetic heterogeneity, suggesting that they might represent a syndrome rather than a single nosological entity (Fanous & Kendler, 2005; Tremblay *et al*, 2013; Jeste & Geschwind, 2014). The variable combination of positive and negative symptoms in schizophrenia, the variable degree of upper and lower motor neuron dysfunction in ALS, the heterogeneity of cognitive symptoms in PD, the variable rate of progression in FTD and the differential response to anti-epileptic treatments in epileptic syndromes are some examples of the clinical diversity in neurological disorders. In addition,

genetic studies in ALS, for example, have demonstrated that the disease can be caused by mutations in genes that encode proteins involved in diverse cellular functions ranging from RNA metabolism, vesicle transport, cytoskeletal homeostasis and the processing of unfolded proteins (Cleveland & Rothstein, 2001; Pasinelli & Brown, 2006; Sreedharan & Brown, 2013). While progress has been achieved in terms of genetic taxonomy, pathological stratification and the classification of patients based on their clinical presentation, little is known about how similar or different patients are, in terms of the molecular pathways that mediate their disease processes. Reprogramming technologies can be used to develop *in vitro* models of genetic and sporadic disease cases and effectively stratify patients, based on (i) the neuronal subtype that exhibits a disease-associated phenotype and (ii) the pathway that leads to this phenotype in each case (Fig 3). This approach may lead to the identification of overlapping disease mechanisms that will be broadly relevant and represent the best therapeutic opportunities, or toward a personalized approach to clinical trials and therapeutic treatments.

Concluding remarks

Tremendous progress has been achieved in our efforts to develop cellular models of neurological disease since 2007 and the initial description of induced pluripotency and the concept of cellular reprogramming (Takahashi *et al*, 2007). We are now able to generate a wealth of different neural subtypes, have created and characterized hundreds of patient-specific iPSCs and their neural derivatives, have developed efficient gene editing approaches and

are continuously establishing elaborate methods for the functional analysis of neurons. During the next phase in the field, it is imperative that the research community offers unrestricted access to cell lines, human samples and differentiation protocols, maintains close communication and attempts to further establish standards for the quality control of pluripotent stem cells and the neuronal subtypes that are utilized for disease modeling and drug screening experiments. It is also worth pointing out that broad collaborative efforts with substantial financial support need to take center stage in order to address important outstanding questions. What is the variation in the properties of neurons generated from a significant number of healthy individuals? Can we assess the broader relevance of phenotypes identified in genetic types of disease by monitoring hundreds of sporadic cases? Can we predict how patients will respond to a potential therapeutic treatment by studying their stem cell-derived neurons? Can we match an *in vivo* clinical trial with an *in vitro* iPSC-based clinical trial to monitor the correlation of outcome measures? The answers to these questions will help us conclude what are the capabilities and limitations of this promising technological tool. Despite the challenges that have arisen over the last few years, the community has responded with sustained effort and is steadily moving forward toward the development of systems that will have an impact in our efforts to understand and treat diseases that affect the nervous system.

Acknowledgements

Conflict of interest

The authors declare that they have no conflict of interest.

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