Human Molecular Genetics, 2014, Vol. 23, Review Issue 1 ***R17–R26***
doi:10.1093/hmg/ddu204
Advance Access published on May 13, 2014

Human-induced pluripotent stem cells: potential for neurodegenerative diseases

Christopher A. Ross[1,2,*] and Sergey S. Akimov[1]

[1]Division of Neurobiology, Department of Psychiatry and [2]Departments of Neurology, Neuroscience and Pharmacology, and Program in Cellular and Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MA, USA

Received April 24, 2014; Revised and Accepted April 28, 2014

The cell biology of human neurodegenerative diseases has been difficult to study till recently. The development of human induced pluripotent stem cell (iPSC) models has greatly enhanced our ability to model disease in human cells. Methods have recently been improved, including increasing reprogramming efficiency, introducing non-viral and non-integrating methods of cell reprogramming, and using novel gene editing techniques for generating genetically corrected lines from patient-derived iPSCs, or for generating mutations in control cell lines. In this review, we highlight accomplishments made using iPSC models to study neurodegenerative disorders such as Huntington's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Fronto-Temporal Dementia, Alzheimer's disease, Spinomuscular Atrophy and other polyglutamine diseases. We review disease-related phenotypes shown in patient-derived iPSCs differentiated to relevant neural subtypes, often with stressors or cell "aging", to enhance disease-specific phenotypes. We also discuss prospects for the future of using of iPSC models of neurodegenerative disorders, including screening and testing of therapeutic compounds, and possibly of cell transplantation in regenerative medicine. The new iPSC models have the potential to greatly enhance our understanding of pathogenesis and to facilitate the development of novel therapeutics.

INTRODUCTION

The cell biology of brain diseases has been very difficult to study till recently, owing to the inaccessibility of the human brain *in vivo*, the challenges in working with human postmortem brain tissue and the incomplete success of many cell and animal models to date. Therefore, the advent of human stem cell technologies has the potential to revolutionize the field. Brain diseases are especially attractive for stem cell applications, as pluripotent stem cells appear to have an inherent propensity to differentiate into neuronal lineages. Furthermore, many brain diseases have genetic forms and thus are amenable to the use of modern genome-editing technologies. This article will focus on neurodegenerative diseases, whereas neurodevelopmental and psychiatric disorders will be the subject of the accompanying article (1).

There are many approaches for generating stem cells for use in brain disease research (2–6). In this review, we will discuss induced pluripotent stem cells (iPSCs). The ability to generate pluripotent stem cells from adult fully differentiated cells such as skin fibroblasts was a major advance in medicine in recent years (7–10). True pluripotency of iPSCs has been demonstrated by successful production of viable mice from iPSCs using 'tetraploid complementation' (11,12). Concern about the persistence of the reprogramming vectors in chromosomal DNA has been alleviated by the development of techniques for reprogramming without viral vector integration (13–16). The efficiency of reprogramming is improving (17). The demonstration that iPSCs can be generated from patients (Fig. 1) has opened the era of studying cellular disease phenotypes in patient-derived cell models (10,18). iPSCs can be generated from patients of advanced age, indicating their usefulness for such late onset diseases as neurodegenerative diseases (19).

Critical for the ability to model neurodegenerative diseases is the capacity to generate defined neuronal populations from pluripotent cells. While much progress has been made, current protocols are still often lengthy, expensive and complex and yield incompletely homogeneous populations of neurons. Nevertheless, it has become possible to differentiate human pluripotent stem cells into cells with phenotypes resembling dopaminergic neurons

*To whom correspondence should be addressed. Email: caross@jhu.edu

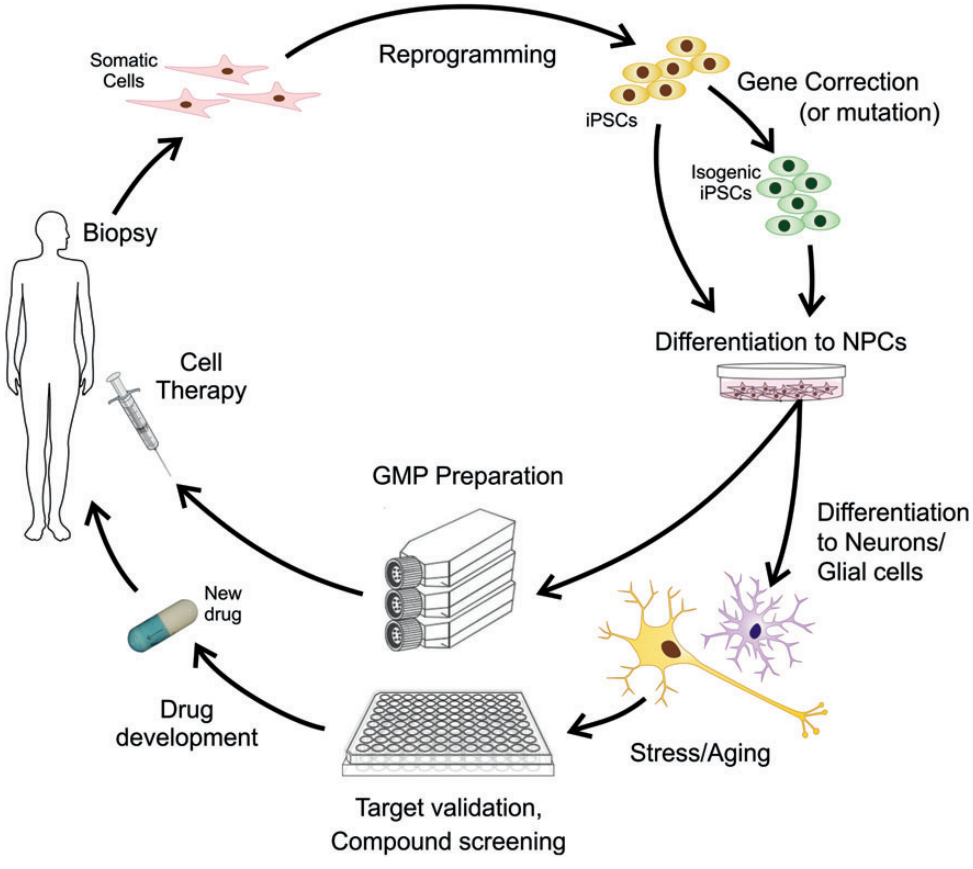

Figure 1. Schematic diagram showing uses of human somatic cells such as adult skin fibroblasts reprogrammed into pluripotent stem cells (iPSCs). The iPSCs derived from a donor carrying a mutation in a disease risk gene can be genetically corrected using homologous recombination, ZFN, TALEN or CRISPR/Cas9. The resulting isogenic cells can be used as normal controls or potentially for cell transplantation therapy. These techniques can also be used to introduce disease-related mutations into the genomes of healthy donor derived iPSCs. The genetically modified cells and parental iPSCs can be differentiated into neural stem (precursor) cells (NSCs). The NSCs can be expanded, sorted for appropriate neural precursors, and further differentiated into mature disease-specific neural subtypes. For research into neural degenerative disorders, cells stressors or artificial cell aging may be applied to enhance disease-specific phenotypes. The cells can be used for compound screening for drug discovery and testing of novel therapeutics.

(20–22), glutamatergic neurons (20), GABAergic neurons (23), motor neurons (22,24) and medium spiny neurons of the striatum (25,26). Human pluripotent stem cells can also be differentiated *in vitro* into astroglial and oligodenrocyte precursors able to further differentiation into mature astrocytes and oligodendrocytes *in vivo* (23,27–29).

The opportunities for studying genetic neurodegenerative diseases have also been greatly enhanced by modern techniques of genome editing. These include homologous recombination (30,31) and the more recent and efficient techniques involving zinc finger nucleases (32,33), transcription activator-like effector nucleases (TALENs) and the highly efficient and low-cost technique termed Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). The CRISPR/Cas9 system consists of the CRISPR-associated (Cas) nuclease Cas9 and a short guide RNA (gRNA) to target the bacterial double-stranded DNA endonuclease Cas9 to specific genomic sequence (34,35). The CRISPR/Cas9 approach has been criticized for off-target effects (36); however, further advances in the technique strikingly reduced the off-target mutagenesis (37). These genome-editing techniques have many potential applications for the studying of neurodegenerative diseases, including correction or generation of genetic mutations, and addition of experimental alterations to cell lines with genetic mutations, with applications for disease modeling, or potentially for cell transplantation approaches.

One issue in modeling of neurodegenerative disorders with iPSCs is the late onset of many of the diseases, often with aging as a potential risk factor. Stressors such as oxidative stress, growth factor deficiency, excitotoxicity and proteasome or autophagy inhibition have been used to reveal disease-associated phenotypes in many iPSC models (Fig. 2). Recently, expression of progerin, a protein causing premature aging, has been used to model aging in Parkinson disease iPSCs (38).

There are many possible applications of stem cell technology for neurodegenerative disease research. These include study of basic disease mechanisms, identification of therapeutic targets and development of assays for screening for therapeutics. Cells may also be developed for cell transplantation (sometimes termed 'cell replacement') therapeutics. There have been several recent reviews of the application of iPSCs to neurodegenerative disease research (3,5,18,39,40). In this article, we will focus on recent accomplishments in the iPSC field toward insights into pathogenesis and leads toward therapeutics.

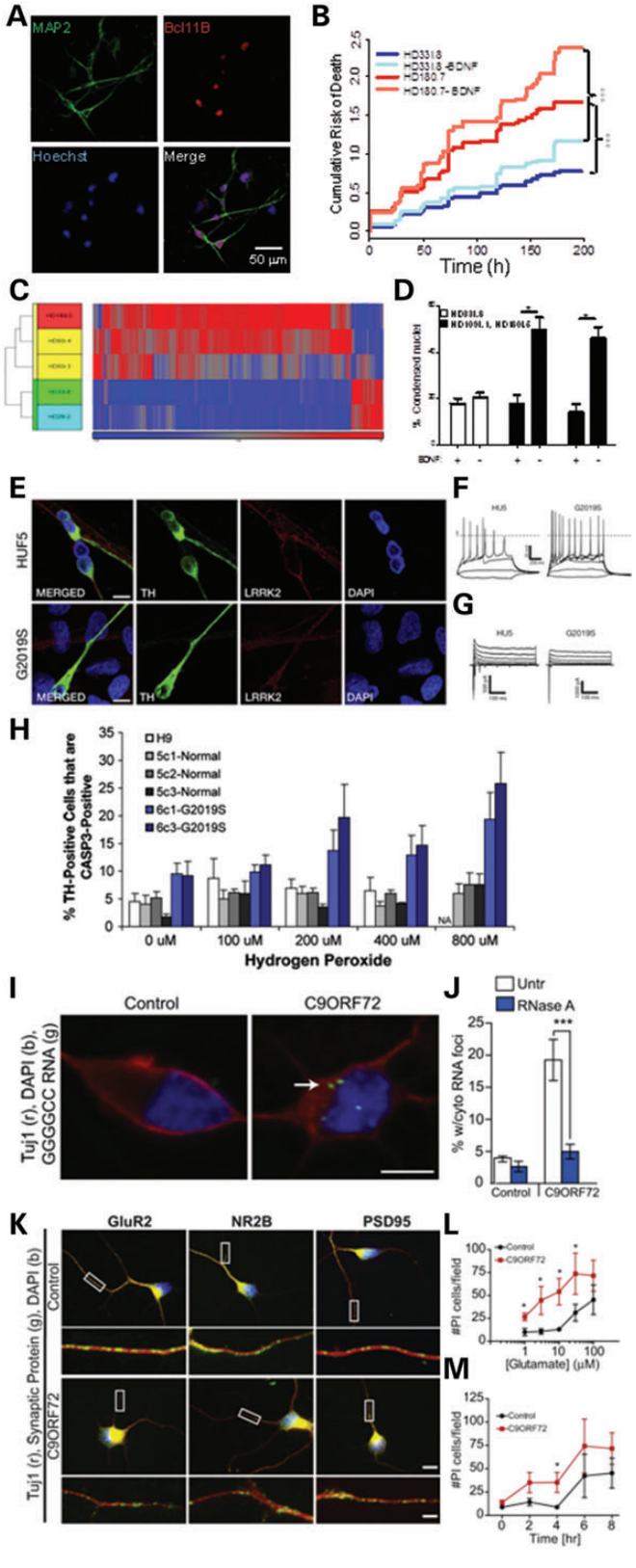

Figure 2. iPSCs derived from HD, PD and ALS patients have been successfully differentiated into mature neurons with relevant properties, and show disease-specific phenotypes. (**A**–**D**). HD iPSC model (26). (A) HD iPSCs with 109 CAG repeats can be differentiated into mature, striatal-like neurons that express medium-spiny-neuron markers MAP2a/b and Bcl11B. (B) Time-lapse experiment. The risk of death was significantly higher for the HD180i.7 line (180 CAG) compared to the HD33i.8 line (33 CAG) over time in culture. After BDNF removal, the risk of death was significantly greater for the HD180i.7 line compared to the HD33i.8 line. (C) Hierarchical clustering of genes from striatal-like cells is represented by the vertical bars (yellow for HD and green for control). HD and control cells are clearly separated. (D) Quantification of condensed nuclei as a measure of cell toxicity showed that both HD109i.1 and HD180i.5 lines had significantly more cell death after BDNF withdrawal, whereas the HD33i.8 control line showed no change. (**E**–**H**). PD iPSC model (57). (E) Differentiation of iPSCs into midbrain dopaminergic (mDA) neurons, and expression of LRRK2 protein in dopaminergic neurons. (F–G) Electrophysiological recordings of control HUF5 and PD G2019S iPSCs differentiated to DA neurons demonstrates that they can fire action potential. (H) Quantification of cells double positive for TH and CASP3 in clones of normal (wild-type) H9 and HUF5-iPSC versus disease G2019S-iPSC-derived DA neurons, with several H_2O_2 concentrations. (**I**–**M**). ALS-FTD model (75). (I) C9ORF72 ALS cells show cytoplasmic RAN translation peptides. C9ORF72 iPSN cells contain cytoplasmic GGGGCC RNA foci, while control iPSNs do not. (J) Quantification of cytoplasmic RNA foci reveals that cytoplasmic foci are RNase A sensitive. (K) C9ORF72 ALS iPSC neurons are highly susceptible to glutamate toxicity. Immunofluorescent staining of control and C9ORF72 iPSC cultures show expression of glutamate receptors GluR2, NR2B, and postsynaptic density protein PSD95 at comparable levels. Box indicates region of high magnification seen below each image. (L) Dose response curve of control and C9ORF72 iPSC neurons revealed that C9ORF72 iPSC neurons are highly susceptible to glutamate excitotoxicity. (M) Glutamate-induced excitotoxicity of C9ORF72 iPSNs shows statistically significant cell death after 4 hr of 30 mM glutamate treatment when compared to control iPSNs.

HUNTINGTON'S DISEASE

Huntington's disease (HD) is an excellent model disease for study, because it is caused by mutations in a single gene and because the length of the triplet repeat expansion determines age of onset and severity of progression (41,42). Furthermore, there are protocols for differentiating human pluripotent stem cells into a phenotype similar to those of medium spiny neurons (24,25,43), the cell type most affected in the disease. A comprehensive review of HD modeling with iPSCs has previously been published (44).

The NINDS HD iPS Cell Consortium has conducted an extensive series of studies of cells generated from patients with a range of CAG repeat expansion lengths (26). The HD iPSCs showed patterns of gene and protein expression distinct from controls, and consistent with pathways of cell function known to be altered in HD, such as proteostasis, neurodevelopment, cell transport, RNA metabolism and cellular metabolism. In addition, the patterns of gene and protein expression varied with CAG-expansion length—with cells with longer expansions having greater perturbations in gene and protein expression patterns. Differentiated HD neural cells showed disease-associated phenotypes, including changes in electrophysiology, metabolism, cell adhesion and cell toxicity. The longest repeat lines were most vulnerable to cell stressors, including withdrawal of nutrients from the cell media, especially BDNF, again mirroring the repeat-expansion-length dependence in human HD patients. Other stressors causing CAG-expansion-dependent toxicity were glutamate toxicity, oxidative stress and autophagy inhibition.

A simultaneous publication using a single line with expanded repeats was notable for demonstrating correction of the repeat expansion mutation, using homologous recombination. Cells with expanded repeats, but not control cells or corrected cells, had changes in gene expression, including cadherin family and TGF-beta pathways. In addition, HD cells, but not control or corrected

cells, had abnormalities of oxygen consumption rate and enhanced cell toxicity with cell stressors and BDNF withdrawal (31).

Other studies have generated HD iPSCs and observed phenotypes such as alteration in lysosomal activity (45), mitochondrial fragmentation (46) and alterations in transcription repressor activity (47). Gene expression changes in HD iPSCs are consistent with reports of changes in FOXO interaction networks (48). HD likely does not affect only neurons. Indeed, when HD iPSCs are differentiated into astrocytes, signs of cellular pathology ensue (49).

HD iPSCs have been used for some initial studies of experimental therapeutics. Using a luciferase assay measuring REST activity, a screen identified two small molecules, which partly reversed phenotypes in the HD iPSCs. This compound also altered REST-regulated genes in the prefrontal cortex of mice with quinolinate-induced striatal lesions, including increasing expression of BDNF (47). Treatment of HD iPSCs with an inhibitor of mitochondrial-fission-related protein Drp1 improved mitochondrial measures and increased cell viability in HD cell culture models, including iPS-cell-derived neurons from HD patients. This compound also had beneficial effects in HD transgenic mice (46). Thus, these two studies show the potential for use of HD iPSCs not just for studies of pathogenesis but also for testing experimental therapeutics.

The studies mentioned earlier involved disease modeling for testing therapeutics. Another approach is cell transplantation with the hope of yielding 'cell replacement.' HD iPSC-derived neuronal precursors were transplanted into a rat model of HD with excitotoxic striatal lesions. There was behavioral recovery; however, the transplanted cells developed signs of HD pathology themselves (50). This indicates the importance of mutation correction prior to cell transplantation.

PARKINSON'S DISEASE

Parkinson's disease (PD) is an interesting model for application of iPSC technology, because protocols for generating dopamine neurons (which are not the only neurons affected, but which are preferentially vulnerable) are relatively robust and reproducible (20–22). In addition, because PD has a mix of several different genetic causes as well as, more commonly, predominantly sporadic causes, there is the opportunity to study phenotypes in both genetic and sporadic cases and to use the different genetic forms as part of an effort to piece together pathogenic pathways involving their gene products. There have been several reviews of PD iPSCs studies (51–53).

The first genetic cause identified for familial Parkinsonism (generally showing more widespread brain pathology than most cases of idiopathic PD) was alpha-synuclein. Parkinsonism can be caused by mutations in alpha-synuclein or by overexpression of normal alpha-synuclein via gene duplication or triplication, consistent with a gain-of-function mechanism. One of the first iPSC models of genetic PD involved a patient with triplication in the alpha-synuclein gene (54). In the study, dopamine neurons showed accumulation of alpha-synuclein, overexpression of markers of oxidative stress and enhanced sensitivity to cell toxicity induced by hydrogen peroxide compared with controls. These findings were all consistent with the idea of a genetic gain of function and the concept that cell toxicity can be, at least in part, a cell autonomous process.

A subsequent study generated cortical neurons from iPSCs reprogrammed from patients with the most common alpha-synuclein mutation, A53T. These cells showed increased nitric oxide and 3-NT levels compared with controls. They also showed evidence of ER stress. A small molecule (NAB2) ameliorated some of these phenotypes (55). In another study, mutant alpha-synuclein was genetically corrected using gene editing techniques. The mutant alpha-synuclein cells also exhibited markers of increased nitrosative stress. Rather remarkably, the authors reported critical role of the MEF2C-PGC1alpha transcriptional pathway in mitochondrial toxin-induced apoptosis in A53T mutant alpha-synuclein neurons and via high-throughput screening identified a small molecule isoxazole as potent to activate MEF2 transcription and protect the neurons from apoptosis (56).

A frequent cause of familial PD arises from mutations in LRRK2—accounting for up to a quarter of cases in some populations. The most common LRRK2 mutation is G2019S. Dopamine neurons derived from G2019S LRRK2 iPSCs showed increased expression of alpha-synuclein, suggesting a connection between these two risk genes in a pathogenic pathway, as had been previously hypothesized (57). The mutant neurons had increased expression of oxidative stress response genes and were more sensitive than control neurons to cell toxicity caused by cell stress agents such as hydrogen peroxide, the proteasome inhibitor MG132 and 6-hydroxy dopamine. The differences were quite striking, indicating that the LRRK2 iPSC PD model is likely to be a robust cell model of PD. Another study showed similar phenotypes from patients with idiopathic PD and patients with mutant LRRK2 (58). Interestingly, both models had elevated levels of alpha-synuclein and alpha-synuclein accumulation, consistent with the idea that alpha-synuclein is downstream from LRRK2.

A subsequent study also reported phenotypic differences between LRRK2 mutant cells and control cells, including changes in respiration, though the differences seemed less robust (59). This paper also reported on patients with PINK1 mutations. The authors found some differences in the metabolic parameters of the PINK1 neurons compared with the LRRK2 neurons, but they found similar cellular vulnerabilities. The cell vulnerabilities could be rescued by co-enzyme Q10 or rapamycin. Interestingly, both the LRRK2 mutant cells and the PINK1 mutant cells could be rescued by the LRRK2 kinase inhibitor GW5074. Thus, the PD iPSC models suggest a pathogenic pathway in which PINK1 is upstream of LRRK2 and LRRK2 is upstream of alpha-synuclein. Another suggestion of a pathogenic pathway as determined in human PD iPSCs is that Parkin recruitment is impaired in neurons derived from mutant PINK1 patients. This would place Parkin in the pathogenic pathway also, downstream of PINK1. PD iPSCs from Parkin patients also showed evidence of increased oxidative stress and enhanced activity of the related NRF2 pathway (60).

Several papers have now shown that the LRRK2 mutations can be corrected by genome-editing techniques (32,33,61). There have been several studies showing transplantation of iPSCs into PD models. An early study showed that neuronal cells derived from human iPSCs could be transplanted into the fetal mouse brain. The cells migrated into several different brain regions, with differentiation into both glia and neurons. When iPSCs were induced to differentiate into dopamine

neurons and transplanted into a rat model of PD, there was some improvement into the behavioral phenotype. The cells were prescreened by fluorescence-activated cell sorting to separate out pluripotent cells, which might have the propensity for tumor formation (62). Another study showed that PD iPSCs differentiated into dopamine neurons could be transplanted into the adult rodent striatum, and a few of these cells developed axons projecting into the striatum. 6-OHDA-lesioned rats transplanted with the neurons showed reduced amphetamine- and apomorphine-induced rotational asymmetry (63). Another study also showed that transplantation of human protein-based (derived without any viral or other DNA-based vectors) iPSCs into rats with striatal lesions could rescue motor deficits (64).

AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA

Amyotrophic lateral sclerosis (ALS) is also an attractive topic for modeling using iPSCs, because there are several genetic mutations that cause ALS and also frontotemporal dementia, as well as sporadic cases with no known mutations. Furthermore, protocols for generating motor neurons are well established (65), making possible relatively consistent studies from different laboratories. Two studies showed disease-related phenotypes in cells programmed from patients with mutations in TDP-43 (66,67). Both studies found elevated levels of detergent insoluble TDP-43 protein, consistent with previous reports of aggregates in cells from ALS patients. Both studies also showed decreased survival and increased vulnerability to cell stressors. These studies are consistent with the hypothesis that TDP-43 mutations act via a genetic gain-of-function and suggest that effects may be cell autonomous. In addition, the Egawa *et al*. study demonstrated amelioration of the cellular phenotypes with a small molecule showing the potential for use of these cells for screening therapeutic drugs (66). Another study found increased sensitivity to staurosporine in iPSCs from patients with TDP-43 mutations as well as alterations in levels of TDP-43 itself and a micro RNA (miR-9), a target of TDP-43 (68).

iPSC models have also been made from ALS8 patients with mutations in the vamp-associated protein B/C (VAPB) (69). VAPB protein levels were reduced in the ALS8-derived motor neurons compared with control cells from normal siblings, which is in agreement with reduction of VAPB in sporadic ALS.

About 20% of ALS cases caused by genetic mutations are associated with the Cu/Zn superoxide dismutase (SOD1) gene. As it has been recently shown, iPSCs derived from ALS donors carrying A4V or D90A mutations and differentiated to motor neurons demonstrate presence of small SOD1 aggregates in cytoplasm and nuclei but not in mitochondria. SOD1 ALS motor neurons exhibited neurofilament aggregation in cytoplasm and neurites followed by neurite degeneration that were rare in non-motor neuron ALS cells or wild-type and isogenic control motor neurons (70). The ALS motor neurons also showed decreased stability of neurofilament-L mRNA and binding of its 3′ UTR by mutant SOD1 destabilizing the mRNA. Notably, the described changes were prevented in genetically corrected ALS iPSCs and mimicked by expression of mutant SOD1 in normal human embryonic stem cells.

iPSCs have also been used to generate functional astrocytes from patients with ALS TDP-43 mutations. These astrocytes showed similar mislocalization of TDP-43 as neurons and also showed decreased survival and responsiveness to stress (71). More recently, iPSCs from patients with TDP-43 mutations were found to have abnormal axonal transport (72).

The discovery that expansion of hexanucleotide repeat in the C9ORF72 causes ALS and frontotemporal dementia has led to an expansion of the research as well. Cells from patients with this mutation showed RNA foci, and evidence of RNA toxicity, indicating a gain-of-function mechanism (73,74). RNA toxicity from the repeat expansion could be ameliorated using antisense oligonucleotides (73,75), indicating that antisense oligos may be an attractive therapeutic strategy. More recently, iPSCs have been used as just one component in a study showing that the repeat expansion causes abnormal structures of both DNA and RNA and causes nucleolar stress (76).

Importantly, models of sporadic ALS have also been generated (77). Motor neurons derived from three sporadic ALS patients showed de novo TDP-43 aggregation. Notably, the aggregates recapitulated pathology in postmortem tissue from one of the donors from which the iPSCs were derived. A high-content chemical screen using the TDP-43 aggregate endpoint both in lower motor neurons and upper motor neuron-like cells identified FDA-approved small molecule modulators including Digoxin, demonstrating the feasibility of patient-derived iPSC-based disease modeling for drug screening.

Transplantation of normal iPSC-derived neural stem cells has been successfully used to improve ALS phenotype in a mouse model (78).

ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a complex disorder with Mendelian causes, genetic risk factors such as ApoE and sporadic age-associated incidence in the majority of cases. Progress in iPS cell models of AD has been previously reviewed (79).

AD iPSC models have been generated from patients with APP mutations, including an E693 deletion mutation and APP gene duplication (80,81). Neurons from the E693 deletion case showed endoplasmic reticulum and oxidative stress. Intracellular A-beta oligomers were identified. Cells from the patients with the APP duplication showed increased A-beta 1–40 peptide but also increased phospho-tau and increased active GSK3 beta. There were also changes in the endosomal compartment. Some of these changes could be reversed by treatment with beta-secretase inhibitors but not gamma-secretase inhibitors.

Models have also been generated using fibroblasts from patients with presenilin mutations, both in PS1 and PS2 (82–84). Cells with presenilin mutations had increased amyloid beta 42 secretion consistent with pathogenic hypotheses about AD. One of these studies used TALEN technology to generate and study an allelic series of PS1 mutations as well as PS null mutant (83). The data were consistent with the idea that mutations do not cause a simple loss of function. They used exome sequencing to show that genome editing with TALEN did not cause a significant excess of additional mutations.

AD iPSCs have also been generated from patients with the V717I mutation. This mutation also resulted in dramatic increase

of APP expression and A-beta levels as well as elevated beta-secretase cleavage of APP resulting in increased levels of both APPs-beta and A-beta. The mutation altered the initial cleavage site of gamma-secretase resulting in an increased generation of A-beta 42 and in this case also A beta 38 as well as total and hyper phosphorylated Tau. Treatment with A-beta-specific antibodies reversed the phenotype of increased total Tau (85).

Of note, studies have also been successful in generating models of AD using sporadic AD patients as well as familial mutations. In two studies in which AD iPSCs from sporadic cases were compared with familial cases with APP mutations, the phenotypes appeared similar including significantly higher levels of the pathological markers, accumulation enlarged RAB5-positive early endosomes (one of two sporadic cases) or endoplasmic reticulum and oxidative stress associated with accumulation of A-beta oligomers (80,81). Another study used patients with sporadic AD, but with focus on patients with ApoE3/E4 genotypes (86). Cells were differentiated into a phenotype comparable with that of basal forebrain cholinergic neurons. These cells showed increased vulnerability to glutamate toxicity and increased intracellular free calcium as well as increased A-beta 42 to A-beta 40 ratios. Thus, studies of AD have successfully modeled several mutations in Mendelian forms as well as the risk factor ApoE and sporadic cases without any known genetic predisposition.

OTHER POLYGLUTAMINE DISEASES

There have been several models of ploygluta mine disorders besides HD. It may be interesting in the future to compare the phenotypes among the different polyglutamine diseases. Unfortunately, most of them affect brainstem and cerebellar neurons, for which there are not good differentiation protocols at present.

An iPSC model for SCA3 (Machado–Joseph Disease) has been generated using fibroblasts from four patients and two controls. The SCA3 cells showed calpain-dependent cleavage of ataxin-3, and SDS insoluble aggregates somewhat similar to the aggregate and inclusions seen in SCA3 postmortem brain that could be abolished by calpain inhibition (87). There has also been a model generated from SCA2 (88). The SCA2 iPSCs showed abnormal rosette formation but successfully differentiated into neural stem cells and subsequent neural cells. Terminally differentiated SCA2 neural cells showed decreased lifespan compared with control cells.

iPSCs have been also generated from a patient with SCA7 (89). No phenotype has yet been reported. This could potentially be an interesting model as this disease also affects the retina and it is possible to differentiate iPSCs to a retinal neuron-like phenotype.

An iPS cell line has been generated from patient with spinal and bulbar muscular atrophy caused by an expanding repeat in the androgen receptor (90). This model also showed aggregation of androgen receptors, which is seen in the human disease. In this study, a cell line has also been derived from a patient with Dentato-Rubral and Pallido-Luysian Atrophy (DRPLA) but did not show any abnormal phenotype. This could potentially be of interest to study in relation to HD as DRPLA also affects the basal ganglia, though less selectively than in HD.

SPINOMUSCULAR ATROPHY

Perhaps the first neurologic disease with a characterized iPSC model is spinomuscular atrophy (SMA) (91). SMA is an interesting model for study as it is an early onset motor neuron disease and can be compared with the late onset disease ALS. Neuronal cultures derived from SMA patients reproduce disease-specific phenotypes (91–95). Cells from these patients exhibited a reduced capacity to form motor neurons and abnormalities of neurite outgrowth and calcium regulations. These features could be corrected by ectopic expression of the survival of motoneuron protein (92) and genome editing (93).

PROSPECTS FOR THE FUTURE

Research using iPSCs for neurodegenerative diseases has gotten off to a very good start. It was initially uncertain whether any phenotypes could be seen for these late onset diseases in human neuron differentiated for weeks or months rather than many years. However, robust phenotypes are being identified, usually under various stresses, which appear to correspond well to known aspects of the relevant diseases. Moreover, in most cases, rescue of the phenotypes has been demonstrated using gene correction or known therapeutics. This initial state of validating the models can now begin to be succeeded by a stage in which the cell models are used to identify novel pathways or therapeutic targets and to screen or test for novel candidate therapeutics. Thus, the immediate benefit of iPSC technology is likely to be for disease modeling and small molecule therapeutic development.

Another goal is to use the cells for cell transplantation, with the hope for ‘cell replacement’. This is a considerably more daunting task, with many obstacles. For genetic diseases such as HD or rare Mendelian variants of PD or AD, the new genomic editing technologies do offer the possibility to correct the mutations in cells from individual patients. Cells would then need to be tested for introduction of other mutations or chromosomal rearrangements, or possibly epigenetic changes, likely using whole-genome sequencing as well as other methods. They would then need to be grown in cell culture using good manufacturing practices. They would need to be differentiated to appropriate neural precursors and sorted away from undifferentiated cells to minimize a risk of teratoma formation and recognition by the immune system (62,96,97).They would then need to be delivered to the right area of the brain, and much preclinical work would need to be done to make sure that cells could be safely introduced into the brain and that the cells would not form tumors or trigger an immune response. Then, it would need to be shown that they could form functional connections. This is a daunting set of challenges, yet at least in theory might be achievable.

How to treat the vast majority of patients with sporadic disease is still uncertain. It is possible that reprogramming may make the cells less vulnerable to the pathogenic process. While significant improvement in motor function was achieved after cell transplantation into PD patients, for many diseases, cell-to-cell communication contributes to pathogenesis; transplantation of healthy cells into brains of patients with diseases results in communication of the disease phenotype to the transplanted cells (98,99).

At least in the immediate future, the most promising application of iPSCs is likely to be for disease modeling. It will be important to establish protocols to generate additional cell types relevant to other diseases—for instance, for AD, hippocampus and entorhinal cortex, and for polyglutamine diseases, brainstem and cerebellum. These protocols will need to be much faster, and more reproducible, and they will need to yield a much more homogeneous population of neurons. Identification of appropriate markers of such populations and generation of corresponding high-quality antibodies is also important. It will be necessary to have reproducible assays, which can be screened or used for testing at least in low- to moderate-throughput mode. Successful approach toward such protocols has been recently demonstrated (100,101).

It is interesting that commonalities are emerging among the models of the different diseases, for instance involving metabolic abnormalities and oxidative stress. This may even extend to models of schizophrenia. A study of iPSCs from schizophrenia patients identified cellular phenotypes in iPSC neural precursor cells (NPCs) from four schizophrenia patients compared with controls (102,103). The schizophrenia NPCs showed abnormal gene expression and protein levels related to cytoskeletal remodeling and oxidative stress and correspondingly showed aberrant migration and increased oxidative stress markers. The reports of mitochondrial abnormalities and oxidative stress are intriguing. They are consistent with previous reports in smaller studies (104,105). It raises the very interesting possibility of pathogenic overlap with neurodegenerative diseases. A cautionary note though is that even though the hypothesis has been active for many years, it has still not been productive of major therapeutic advances.

One important goal would be to establish models that have more than one cell type. This could involve populations of neurons and glia, as might be important for studying ALS. It might also involve populations of two defined classes of neurons with appropriate connections between them, such as cortico-spinal connections or cortico-striatal connections for ALS and HD, or nigro-striatal connections for PD. Nanotechnology is likely to provide novel techniques or engineering cultures into a more brain-like configuration. Another approach for better mimicking cell–cell and cell–extracellular environment in brain could be using 3D cultures *in vitro* (106), and chimeric models with the human cells inoculated into animal brain using immunocompromised mice or human immune system mice (107). The cell transplantation experiments are important because of incomplete maturation of neural cells, especially glial cells *in vitro*, and because of significantly longer survival of the grafted cells compared with *in vitro* cultures. Transplantation of normal iPSC-derived precursors into disease model mice versus transplantation of affected donor derived cells into control mice could help in understanding of interaction between the specific neural subtype and healthy or affected environment. Another interesting approach is using co-culture of iPSCs with control of animal disease model-derived live organotypic brain slices (108). This could greatly aid in studying neurodegenerative diseases.

Whether or not iPSCs prove useful for cell transplantation therapy, it appears increasingly likely that iPSC models of neurodegenerative disease will help accelerate the search for novel targets of genuinely human relevance. Finally, the hope is that iPSCs will accelerate the testing of small molecule therapeutics, perhaps in some cases even bypassing the mouse model stage, so that therapeutics can reach human patients more quickly.

Conflict of Interest statement. None declared.

FUNDING

This work was supported by the NIH/NINDS HD iPS Cell Consortium grant U24NS078370, the NIH/NINDS grant R21NS083365, the NIH/NINDS grant R01NS076631, and the Johns Hopkins University Brain Sciences Initiative.

REFERENCES

1. Winner, B., Marchetto, M.C., Winkler, J. and Gage, F.H. (2014) Human-induced pluripotent stem cells pave the road for a better understanding of motor neuron disease. *Hum. Mol. Genet.*, **23**, R27–R34.
2. Jakel, R.J., Schneider, B.L. and Svendsen, C.N. (2004) Using human neural stem cells to model neurological disease. *Nat. Rev. Genet.*, **5**, 136–144.
3. Hargus, G., Ehrlich, M., Hallmann, A.L. and Kuhlmann, T. (2014) Human stem cell models of neurodegeneration: a novel approach to study mechanisms of disease development. *Acta Neuropathol.*, **127**, 151–173.
4. Gage, F.H. and Temple, S. (2013) Neural stem cells: generating and regenerating the brain. *Neuron*, **80**, 588–601.
5. Qiang, L., Fujita, R. and Abeliovich, A. (2013) Remodeling neurodegeneration: somatic cell reprogramming-based models of adult neurological disorders. *Neuron*, **78**, 957–969.
6. Hermann, A. and Storch, A. (2013) Induced neural stem cells (iNSCs) in neurodegenerative diseases. *J. Neural. Transm.*, **120**(Suppl 1), S19–S25.
7. Takahashi, K. and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, **126**, 663–676.
8. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K. and Yamanaka, S. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, **131**, 861–872.
9. Yu, J., Vodyanik, M.A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J.L., Tian, S., Nie, J., Jonsdottir, G.A., Ruotti, V., Stewart, R. *et al.* (2007) Induced pluripotent stem cell lines derived from human somatic cells. *Science*, **318**, 1917–1920.
10. Park, I.H., Arora, N., Huo, H., Maherali, N., Ahfeldt, T., Shimamura, A., Lensch, M.W., Cowan, C., Hochedlinger, K. and Daley, G.Q. (2008) Disease-specific induced pluripotent stem cells. *Cell*, **134**, 877–886.
11. Zhao, X.Y., Li, W., Lv, Z., Liu, L., Tong, M., Hai, T., Hao, J., Guo, C.L., Ma, Q.W., Wang, L. *et al.* (2009) iPS cells produce viable mice through tetraploid complementation. *Nature*, **461**, 86–90.
12. Boland, M.J., Hazen, J.L., Nazor, K.L., Rodriguez, A.R., Gifford, W., Martin, G., Kupriyanov, S. and Baldwin, K.K. (2009) Adult mice generated from induced pluripotent stem cells. *Nature*, **461**, 91–94.
13. Stadtfeld, M., Nagaya, M., Utikal, J., Weir, G. and Hochedlinger, K. (2008) Induced pluripotent stem cells generated without viral integration. *Science*, **322**, 945–949.
14. Yu, J., Hu, K., Smuga-Otto, K., Tian, S., Stewart, R., Slukvin, II. and Thomson, J.A. (2009) Human induced pluripotent stem cells free of vector and transgene sequences. *Science*, **324**, 797–801.
15. Fusaki, N., Ban, H., Nishiyama, A., Saeki, K. and Hasegawa, M. (2009) Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. *Proc. Jpn Acad. Ser. B Phys Biol. Sci.*, **85**, 348–362.
16. Warren, L., Manos, P.D., Ahfeldt, T., Loh, Y.H., Li, H., Lau, F., Ebina, W., Mandal, P.K., Smith, Z.D., Meissner, A. *et al.* (2010) Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell*, **7**, 618–630.
17. Rais, Y., Zviran, A., Geula, S., Gafni, O., Chomsky, E., Viukov, S., Mansour, A.A., Caspi, I., Krupalnik, V., Zerbib, M. *et al.* (2013) Deterministic direct reprogramming of somatic cells to pluripotency. *Nature*, **502**, 65–70.

18. Svendsen, C.N. (2013) Back to the future: how human induced pluripotent stem cells will transform regenerative medicine. *Hum. Mol. Genet.*, **22**, R32–R38.
19. Yagi, T., Kosakai, A., Ito, D., Okada, Y., Akamatsu, W., Nihei, Y., Nabetani, A., Ishikawa, F., Arai, Y., Hirose, N. *et al.* (2012) Establishment of induced pluripotent stem cells from centenarians for neurodegenerative disease research. *PloS one*, **7**, e41572.
20. Zeng, H., Guo, M., Martins-Taylor, K., Wang, X., Zhang, Z., Park, J.W., Zhan, S., Kronenberg, M.S., Lichtler, A., Liu, H.X. *et al.* (2010) Specification of region-specific neurons including forebrain glutamatergic neurons from human induced pluripotent stem cells. *PloS one*, **5**, e11853.
21. Kriks, S., Shim, J.W., Piao, J., Ganat, Y.M., Wakeman, D.R., Xie, Z., Carrillo-Reid, L., Auyeung, G., Antonacci, C., Buch, A. *et al.* (2011) Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. *Nature*, **480**, 547–551.
22. Chambers, S.M., Fasano, C.A., Papapetrou, E.P., Tomishima, M., Sadelain, M. and Studer, L. (2009) Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat. Biotechnol.*, **27**, 275–280.
23. Liu, H. and Zhang, S.C. (2011) Specification of neuronal and glial subtypes from human pluripotent stem cells. *Cell Mol. Life Sci.*, **68**, 3995–4008.
24. Ebert, A.D., Shelley, B.C., Hurley, A.M., Onorati, M., Castiglioni, V., Patitucci, T.N., Svendsen, S.P., Mattis, V.B., McGivern, J.V., Schwab, A.J. *et al.* (2013) EZ spheres: a stable and expandable culture system for the generation of pre-rosette multipotent stem cells from human ESCs and iPSCs. *Stem Cell Res.*, **10**, 417–427.
25. Aubry, L., Bugi, A., Lefort, N., Rousseau, F., Peschanski, M. and Perrier, A.L. (2008) Striatal progenitors derived from human ES cells mature into DARPP32 neurons in vitro and in quinolinic acid-lesioned rats. *Proc. Natl. Acad. Sci. USA*, **105**, 16707–16712.
26. HD iPSC Consortium. (2012) Induced pluripotent stem cells from patients with Huntington's disease show CAG-repeat-expansion-associated phenotypes. *Cell Stem Cell*, **11**, 264–278.
27. Krencik, R., Weick, J.P., Liu, Y., Zhang, Z.J. and Zhang, S.C. (2011) Specification of transplantable astroglial subtypes from human pluripotent stem cells. *Nat. Biotechnol.*, **29**, 528–534.
28. Hu, B.Y., Du, Z.W. and Zhang, S.C. (2009) Differentiation of human oligodendrocytes from pluripotent stem cells. *Nat. Prot.*, **4**, 1614–1622.
29. Wang, S., Bates, J., Li, X., Schanz, S., Chandler-Militello, D., Levine, C., Maherali, N., Studer, L., Hochedlinger, K., Windrem, M. *et al.* (2013) Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. *Cell Stem Cell*, **12**, 252–264.
30. Zou, J., Maeder, M.L., Mali, P., Pruett-Miller, S.M., Thibodeau-Beganny, S., Chou, B.K., Chen, G., Ye, Z., Park, I.H., Daley, G.Q. *et al.* (2009) Gene targeting of a disease-related gene in human induced pluripotent stem and embryonic stem cells. *Cell Stem Cell*, **5**, 97–110.
31. An, M.C., Zhang, N., Scott, G., Montoro, D., Wittkop, T., Mooney, S., Melov, S. and Ellerby, L.M. (2012) Genetic correction of Huntington's disease phenotypes in induced pluripotent stem cells. *Cell Stem Cell*, **11**, 253–263.
32. Soldner, F., Laganiere, J., Cheng, A.W., Hockemeyer, D., Gao, Q., Alagappan, R., Khurana, V., Golbe, L.I., Myers, R.H., Lindquist, S. *et al.* (2011) Generation of isogenic pluripotent stem cells differing exclusively at two early onset Parkinson point mutations. *Cell*, **146**, 318–331.
33. Reinhardt, P., Schmid, B., Burbulla, L.F., Schondorf, D.C., Wagner, L., Glatza, M., Hoing, S., Hargus, G., Heck, S.A., Dhingra, A. *et al.* (2013) Genetic correction of a LRRK2 mutation in human iPSCs links Parkinsonian neurodegeneration to ERK-dependent changes in gene expression. *Cell Stem Cell*, **12**, 354–367.
34. Mali, P., Yang, L., Esvelt, K.M., Aach, J., Guell, M., DiCarlo, J.E., Norville, J.E. and Church, G.M. (2013) RNA-guided human genome engineering via Cas9. *Science*, **339**, 823–826.
35. Hou, Z., Zhang, Y., Propson, N.E., Howden, S.E., Chu, L.F., Sontheimer, E.J. and Thomson, J.A. (2013) Efficient genome engineering in human pluripotent stem cells using Cas9 from Neisseria meningitidis. *Proc. Natl. Acad. Sci. USA*, **110**, 15644–15649.
36. Fu, Y., Foden, J.A., Khayter, C., Maeder, M.L., Reyon, D., Joung, J.K. and Sander, J.D. (2013) High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat. Biotechnol.*, **31**, 822–826.
37. Ran, F.A., Hsu, P.D., Lin, C.Y., Gootenberg, J.S., Konermann, S., Trevino, A.E., Scott, D.A., Inoue, A., Matoba, S., Zhang, Y. *et al.* (2013) Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. *Cell*, **154**, 1380–1389.
38. Miller, J.D., Ganat, Y.M., Kishinevsky, S., Bowman, R.L., Liu, B., Tu, E.Y., Mandal, P.K., Vera, E., Shim, J.W., Kriks, S. *et al.* (2013) Human iPSC-based modeling of late-onset disease via progerin-induced aging. *Cell Stem Cell*, **13**, 691–705.
39. Jung, Y.W., Hysolli, E., Kim, K.Y., Tanaka, Y. and Park, I.H. (2012) Human induced pluripotent stem cells and neurodegenerative disease: prospects for novel therapies. *Curr. Opin. Neurol.*, **25**, 125–130.
40. Kim, S.U., Lee, H.J. and Kim, Y.B. (2013) Neural stem cell-based treatment for neurodegenerative diseases. *Neuropathology*, **33**, 491–504.
41. Ross, C.A. and Tabrizi, S.J. (2011) Huntington's disease: from molecular pathogenesis to clinical treatment. *Lancet Neurol.*, **10**, 83–98.
42. Ross, C.A., Aylward, E.H., Wild, E.J., Langbehn, D.R., Long, J.D., Warner, J.H., Scahill, R.I., Leavitt, B.R., Stout, J.C., Paulsen, J.S. *et al.* (2014) Huntington disease: natural history, biomarkers and prospects for therapeutics. *Nat. Rev. Neurol.*, **10**, 204–216.
43. Carri, A.D., Onorati, M., Lelos, M.J., Castiglioni, V., Faedo, A., Menon, R., Camnasio, S., Vuono, R., Spaiardi, P., Talpo, F. *et al.* (2013) Developmentally coordinated extrinsic signals drive human pluripotent stem cell differentiation toward authentic DARPP-32+ medium-sized spiny neurons. *Development*, **140**, 301–312.
44. Kaye, J.A. and Finkbeiner, S. (2013) Modeling Huntington's disease with induced pluripotent stem cells. *Mol. Cell Neurosci.*, **56**, 50–64.
45. Camnasio, S., Delli Carri, A., Lombardo, A., Grad, I., Mariotti, C., Castucci, A., Rozell, B., Lo Riso, P., Castiglioni, V., Zuccato, C. *et al.* (2012) The first reported generation of several induced pluripotent stem cell lines from homozygous and heterozygous Huntington's disease patients demonstrates mutation related enhanced lysosomal activity. *Neurobiol. Dis.*, **46**, 41–51.
46. Guo, X., Disatnik, M.H., Monbureau, M., Shamloo, M., Mochly-Rosen, D. and Qi, X. (2013) Inhibition of mitochondrial fragmentation diminishes Huntington's disease-associated neurodegeneration. *J. Clin. Invest.*, **123**, 5371–5388.
47. Charbord, J., Poydenot, P., Bonnefond, C., Feyeux, M., Casagrande, F., Brinon, B., Francelle, L., Auregan, G., Guillermier, M., Cailleret, M. *et al.* (2013) High throughput screening for inhibitors of REST in neural derivatives of human embryonic stem cells reveals a chemical compound that promotes expression of neuronal genes. *Stem Cells*, **31**, 1816–1828.
48. Parmentier, F., Lejeune, F.X. and Neri, C. (2013) Pathways to decoding the clinical potential of stress response FOXO-interaction networks for Huntington's disease: of gene prioritization and context dependence. *Front. Aging Neurosci.*, **5**, 22.
49. Juopperi, T.A., Kim, W.R., Chiang, C.H., Yu, H., Margolis, R.L., Ross, C.A., Ming, G.L. and Song, H. (2012) Astrocytes generated from patient induced pluripotent stem cells recapitulate features of Huntington's disease patient cells. *Mol. Brain*, **5**, 17.
50. Jeon, I., Lee, N., Li, J.Y., Park, I.H., Park, K.S., Moon, J., Shim, S.H., Choi, C., Chang, D.J., Kwon, J. *et al.* (2012) Neuronal properties, in vivo effects, and pathology of a Huntington's disease patient-derived induced pluripotent stem cells. *Stem Cells*, **30**, 2054–2062.
51. Jacobs, B.M. (2014) Stemming the hype: what can we learn from iPSC models of Parkinson's disease and how can we learn it? *J. Parkinson's Dis.*, **4**, 15–27.
52. Badger, J.L., Cordero-Llana, O., Hartfield, E.M. and Wade-Martins, R. (2014) Parkinson's disease in a dish - Using stem cells as a molecular tool. *Neuropharmacology*, **76**(Pt A), 88–96.
53. Cooper, O., Parmar, M. and Isacson, O. (2012) Characterization and criteria of embryonic stem and induced pluripotent stem cells for a dopamine replacement therapy. *Prog. Brain Res.*, **200**, 265–276.
54. Byers, B., Cord, B., Nguyen, H.N., Schule, B., Fenno, L., Lee, P.C., Deisseroth, K., Langston, J.W., Pera, R.R. and Palmer, T.D. (2011) SNCA triplication Parkinson's patient's iPSC-derived DA neurons accumulate alpha-synuclein and are susceptible to oxidative stress. *PloS one*, **6**, e26159.
55. Chung, C.Y., Khurana, V., Auluck, P.K., Tardiff, D.F., Mazzulli, J.R., Soldner, F., Baru, V., Lou, Y., Freyzon, Y., Cho, S. *et al.* (2013) Identification and rescue of alpha-synuclein toxicity in Parkinson patient-derived neurons. *Science*, **342**, 983–987.
56. Ryan, S.D., Dolatabadi, N., Chan, S.F., Zhang, X., Akhtar, M.W., Parker, J., Soldner, F., Sunico, C.R., Nagar, S., Talantova, M. *et al.* (2013) Isogenic human iPSC Parkinson's model shows nitrosative stress-induced dysfunction in MEF2-PGC1alpha transcription. *Cell*, **155**, 1351–1364.

57. Nguyen, H.N., Byers, B., Cord, B., Shcheglovitov, A., Byrne, J., Gujar, P., Kee, K., Schule, B., Dolmetsch, R.E., Langston, W. *et al.* (2011) LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. *Cell Stem Cell*, **8**, 267–280.
58. Sanchez-Danes, A., Richaud-Patin, Y., Carballo-Carbajal, I., Jimenez-Delgado, S., Caig, C., Mora, S., Di Guglielmo, C., Ezquerra, M., Patel, B., Giralt, A. *et al.* (2012) Disease-specific phenotypes in dopamine neurons from human iPS-based models of genetic and sporadic Parkinson's disease. *EMBO Mol. Med.*, **4**, 380–395.
59. Cooper, O., Seo, H., Andrabi, S., Guardia-Laguarta, C., Graziotto, J., Sundberg, M., McLean, J.R., Carrillo-Reid, L., Xie, Z., Osborn, T. *et al.* (2012) Pharmacological rescue of mitochondrial deficits in iPSC-derived neural cells from patients with familial Parkinson's disease. *Sci. Transl. Med.*, **4**, 141ra190.
60. Imaizumi, Y., Okada, Y., Akamatsu, W., Koike, M., Kuzumaki, N., Hayakawa, H., Nihira, T., Kobayashi, T., Ohyama, M., Sato, S. *et al.* (2012) Mitochondrial dysfunction associated with increased oxidative stress and alpha-synuclein accumulation in PARK2 iPSC-derived neurons and postmortem brain tissue. *Mol. Brain*, **5**, 35.
61. Sanders, L.H., Laganiere, J., Cooper, O., Mak, S.K., Vu, B.J., Huang, Y.A., Paschon, D.E., Vangipuram, M., Sundararajan, R., Urnov, F.D. *et al.* (2014) LRRK2 mutations cause mitochondrial DNA damage in iPSC-derived neural cells from Parkinson's disease patients: reversal by gene correction. *Neurobiol. Dis.*, **62**, 381–386.
62. Wernig, M., Zhao, J.P., Pruszak, J., Hedlund, E., Fu, D., Soldner, F., Broccoli, V., Constantine-Paton, M., Isacson, O. and Jaenisch, R. (2008) Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proc. Natl. Acad. Sci. USA*, **105**, 5856–5861.
63. Hargus, G., Cooper, O., Deleidi, M., Levy, A., Lee, K., Marlow, E., Yow, A., Soldner, F., Hockemeyer, D., Hallett, P.J. *et al.* (2010) Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. *Proc. Natl. Acad. Sci. USA*, **107**, 15921–15926.
64. Rhee, Y.H., Ko, J.Y., Chang, M.Y., Yi, S.H., Kim, D., Kim, C.H., Shim, J.W., Jo, A.Y., Kim, B.W., Lee, H. *et al.* (2011) Protein-based human iPS cells efficiently generate functional dopamine neurons and can treat a rat model of Parkinson disease. *J. Clin. Invest.*, **121**, 2326–2335.
65. Dimos, J.T., Rodolfa, K.T., Niakan, K.K., Weisenthal, L.M., Mitsumoto, H., Chung, W., Croft, G.F., Saphier, G., Leibel, R., Goland, R. *et al.* (2008) Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science*, **321**, 1218–1221.
66. Egawa, N., Kitaoka, S., Tsukita, K., Naitoh, M., Takahashi, K., Yamamoto, T., Adachi, F., Kondo, T., Okita, K., Asaka, I. *et al.* (2012) Drug screening for ALS using patient-specific induced pluripotent stem cells. *Sci. Transl. Med.*, **4**, 145ra104.
67. Bilican, B., Serio, A., Barmada, S.J., Nishimura, A.L., Sullivan, G.J., Carrasco, M., Phatnani, H.P., Puddifoot, C.A., Story, D., Fletcher, J. *et al.* (2012) Mutant induced pluripotent stem cell lines recapitulate aspects of TDP-43 proteinopathies and reveal cell-specific vulnerability. *Proc. Natl. Acad. Sci. USA*, **109**, 5803–5808.
68. Zhang, Z., Almeida, S., Lu, Y., Nishimura, A.L., Peng, L., Sun, D., Wu, B., Karydas, A.M., Tartaglia, M.C., Fong, J.C. *et al.* (2013) Downregulation of microRNA-9 in iPSC-derived neurons of FTD/ALS patients with TDP-43 mutations. *PloS one*, **8**, e76055.
69. Mitne-Neto, M., Machado-Costa, M., Marchetto, M.C., Bengtson, M.H., Joazeiro, C.A., Tsuda, H., Bellen, H.J., Silva, H.C., Oliveira, A.S., Lazar, M. *et al.* (2011) Downregulation of VAPB expression in motor neurons derived from induced pluripotent stem cells of ALS8 patients. *Hum. Mol. Genet.*, **20**, 3642–3652.
70. Chen, H., Qian, K., Du, Z., Cao, J., Petersen, A., Liu, H., Blackbourn, L.W.t., Huang, C.L., Errigo, A., Yin, Y. *et al.* (2014) Modeling ALS with iPSCs reveals that mutant SOD1 misregulates neurofilament balance in motor neurons. *Cell Stem Cell*, in press.
71. Serio, A., Bilican, B., Barmada, S.J., Ando, D.M., Zhao, C., Siller, R., Burr, K., Haghi, G., Story, D., Nishimura, A.L. *et al.* (2013) Astrocyte pathology and the absence of non-cell autonomy in an induced pluripotent stem cell model of TDP-43 proteinopathy. *Proc. Natl. Acad. Sci. USA*, **110**, 4697–4702.
72. Alami, N.H., Smith, R.B., Carrasco, M.A., Williams, L.A., Winborn, C.S., Han, S.S., Kiskinis, E., Winborn, B., Freibaum, B.D., Kanagaraj, A. *et al.* (2014) Axonal transport of TDP-43 mRNA granules is impaired by ALS-causing mutations. *Neuron*, **81**, 536–543.
73. Sareen, D., O'Rourke, J.G., Meera, P., Muhammad, A.K., Grant, S., Simpkinson, M., Bell, S., Carmona, S., Ornelas, L., Sahabian, A. *et al.* (2013) Targeting RNA foci in iPSC-derived motor neurons from ALS patients with a C9ORF72 repeat expansion. *Sci Transl Med*, **5**, 208ra149.
74. Almeida, S., Gascon, E., Tran, H., Chou, H.J., Gendron, T.F., Degroot, S., Tapper, A.R., Sellier, C., Charlet-Berguerand, N., Karydas, A. *et al.* (2013) Modeling key pathological features of frontotemporal dementia with C9ORF72 repeat expansion in iPSC-derived human neurons. *Acta Neuropathol.*, **126**, 385–399.
75. Donnelly, C.J., Zhang, P.W., Pham, J.T., Heusler, A.R., Mistry, N.A., Vidensky, S., Daley, E.L., Poth, E.M., Hoover, B., Fines, D.M. *et al.* (2013) RNA toxicity from the ALS/FTD C9ORF72 expansion is mitigated by antisense intervention. *Neuron*, **80**, 415–428.
76. Haeusler, A.R., Donnelly, C.J., Periz, G., Simko, E.A., Shaw, P.G., Kim, M.S., Maragakis, N.J., Troncoso, J.C., Pandey, A., Sattler, R. *et al.* (2014) C9orf72 nucleotide repeat structures initiate molecular cascades of disease. *Nature*, **507**, 195–200.
77. Burkhardt, M.F., Martinez, F.J., Wright, S., Ramos, C., Volfson, D., Mason, M., Garnes, J., Dang, V., Lievers, J., Shoukat-Mumtaz, U. *et al.* (2013) A cellular model for sporadic ALS using patient-derived induced pluripotent stem cells. *Mol. Cell Neurosci.*, **56**, 355–364.
78. Nizzardo, M., Simone, C., Rizzo, F., Ruggieri, M., Salani, S., Riboldi, G., Faravelli, I., Zanetta, C., Bresolin, N., Comi, G.P. *et al.* (2014) Minimally invasive transplantation of iPSC-derived ALDHhiSSCloVLA4+ neural stem cells effectively improves the phenotype of an amyotrophic lateral sclerosis model. *Hum. Mol. Genet.*, **23**, 342–354.
79. Young, J.E. and Goldstein, L.S. (2012) Alzheimer's disease in a dish: promises and challenges of human stem cell models. *Hum. Mol. Genet.*, **21**, R82–R89.
80. Kondo, T., Asai, M., Tsukita, K., Kutoku, Y., Ohsawa, Y., Sunada, Y., Imamura, K., Egawa, N., Yahata, N., Okita, K. *et al.* (2013) Modeling Alzheimer's disease with iPSCs reveals stress phenotypes associated with intracellular Abeta and differential drug responsiveness. *Cell Stem Cell*, **12**, 487–496.
81. Israel, M.A., Yuan, S.H., Bardy, C., Reyna, S.M., Mu, Y., Herrera, C., Hefferan, M.P., Van Gorp, S., Nazor, K.L., Boscolo, F.S. *et al.* (2012) Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. *Nature*, **482**, 216–220.
82. Yagi, T., Ito, D., Okada, Y., Akamatsu, W., Nihei, Y., Yoshizaki, T., Yamanaka, S., Okano, H. and Suzuki, N. (2011) Modeling familial Alzheimer's disease with induced pluripotent stem cells. *Hum. Mol. Genet.*, **20**, 4530–4539.
83. Woodruff, G., Young, J.E., Martinez, F.J., Buen, F., Gore, A., Kinaga, J., Li, Z., Yuan, S.H., Zhang, K. and Goldstein, L.S. (2013) The presenilin-1 DeltaE9 mutation results in reduced gamma-secretase activity, but not total loss of PS1 function, in isogenic human stem cells. *Cell Rep.*, **5**, 974–985.
84. Sproul, A.A., Jacob, S., Pre, D., Kim, S.H., Nestor, M.W., Navarro-Sobrino, M., Santa-Maria, I., Zimmer, M., Aubry, S., Steele, J.W. *et al.* (2014) Characterization and molecular profiling of PSEN1 familial Alzheimer's disease iPSC-derived neural progenitors. *PloS one*, **9**, e84547.
85. Muratore, C.R., Rice, H.C., Srikanth, P., Callahan, D.G., Shin, T., Benjamin, L.N., Walsh, D.M., Selkoe, D.J. and Young-Pearse, T.L. (2014) The familial Alzheimer's disease APPV717I mutation alters APP processing and Tau expression in iPSC-derived neurons. *Hum. Mol. Genet.*, in press.
86. Duan, L., Bhattacharyya, B.J., Belmadani, A., Pan, L., Miller, R.J. and Kessler, J.A. (2014) Stem cell derived basal forebrain cholinergic neurons from Alzheimer's disease patients are more susceptible to cell death. *Mol. Neurodegener.*, **9**, 3.
87. Koch, P., Breuer, P., Peitz, M., Jungverdorben, J., Kesavan, J., Poppe, D., Doerr, J., Ladewig, J., Mertens, J., Tuting, T. *et al.* (2011) Excitation-induced ataxin-3 aggregation in neurons from patients with Machado-Joseph disease. *Nature*, **480**, 543–546.
88. Xia, G., Santostefano, K., Hamazaki, T., Liu, J., Subramony, S.H., Terada, N. and Ashizawa, T. (2013) Generation of human-induced pluripotent stem cells to model spinocerebellar ataxia type 2 in vitro. *J. Mol. Neurosci.*, **51**, 237–248.
89. Luo, Y., Fan, Y., Zhou, B., Xu, Z., Chen, Y. and Sun, X. (2012) Generation of induced pluripotent stem cells from skin fibroblasts of a patient with olivopontocerebellar atrophy. *Tohoku J. Exp. Med.*, **226**, 151–159.
90. Nihei, Y., Ito, D., Okada, Y., Akamatsu, W., Yagi, T., Yoshizaki, T., Okano, H. and Suzuki, N. (2013) Enhanced aggregation of androgen receptor in induced pluripotent stem cell-derived neurons from spinal and bulbar muscular atrophy. *J. Biol. Chem.*, **288**, 8043–8052.

91. Ebert, A.D., Yu, J., Rose, F.F. Jr., Mattis, V.B., Lorson, C.L., Thomson, J.A. and Svendsen, C.N. (2009) Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature*, **457**, 277–280.
92. Chang, T., Zheng, W., Tsark, W., Bates, S., Huang, H., Lin, R.J. and Yee, J.K. (2011) Brief report: phenotypic rescue of induced pluripotent stem cell-derived motoneurons of a spinal muscular atrophy patient. *Stem cells*, **29**, 2090–2093.
93. Corti, S., Nizzardo, M., Simone, C., Falcone, M., Nardini, M., Ronchi, D., Donadoni, C., Salani, S., Riboldi, G., Magri, F. *et al.* (2012) Genetic correction of human induced pluripotent stem cells from patients with spinal muscular atrophy. *Sci Transl Med*, **4**, 165ra162.
94. McGivern, J.V., Patitucci, T.N., Nord, J.A., Barabas, M.E., Stucky, C.L. and Ebert, A.D. (2013) Spinal muscular atrophy astrocytes exhibit abnormal calcium regulation and reduced growth factor production. *Glia*, **61**, 1418–1428.
95. Zanetta, C., Riboldi, G., Nizzardo, M., Simone, C., Faravelli, I., Bresolin, N., Comi, G.P. and Corti, S. (2014) Molecular, genetic and stem cell-mediated therapeutic strategies for spinal muscular atrophy (SMA). *J Cell Mol Med*, **18**, 187–196.
96. Araki, R., Uda, M., Hoki, Y., Sunayama, M., Nakamura, M., Ando, S., Sugiura, M., Ideno, H., Shimada, A., Nifuji, A. *et al.* (2013) Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. *Nature*, **494**, 100–104.
97. Guha, P., Morgan, J.W., Mostoslavsky, G., Rodrigues, N.P. and Boyd, A.S. (2013) Lack of immune response to differentiated cells derived from syngeneic induced pluripotent stem cells. *Cell stem cell*, **12**, 407–412.
98. Kordower, J.H., Chu, Y., Hauser, R.A., Freeman, T.B. and Olanow, C.W. (2008) Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nature medicine*, **14**, 504–506.
99. Li, J.Y., Englund, E., Holton, J.L., Soulet, D., Hagell, P., Lees, A.J., Lashley, T., Quinn, N.P., Rehncrona, S., Bjorklund, A. *et al.* (2008) Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nature medicine*, **14**, 501–503.
100. Lee, G., Ramirez, C.N., Kim, H., Zeltner, N., Liu, B., Radu, C., Bhinder, B., Kim, Y.J., Choi, I.Y., Mukherjee-Clavin, B. *et al.* (2012) Large-scale screening using familial dysautonomia induced pluripotent stem cells identifies compounds that rescue IKBKAP expression. *Nature biotechnology*, **30**, 1244–1248.
101. Desbordes, S.C. and Studer, L. (2013) Adapting human pluripotent stem cells to high-throughput and high-content screening. *Nature protocols*, **8**, 111–130.
102. Brennand, K.J., Simone, A., Jou, J., Gelboin-Burkhart, C., Tran, N., Sangar, S., Li, Y., Mu, Y., Chen, G., Yu, D. *et al.* (2011) Modelling schizophrenia using human induced pluripotent stem cells. *Nature*, **473**, 221–225.
103. Brennand, K., Savas, J.N., Kim, Y., Tran, N., Simone, A., Hashimoto-Torii, K., Beaumont, K.G., Kim, H.J., Topol, A., Ladran, I. *et al.* (2014) Phenotypic differences in hiPSC NPCs derived from patients with schizophrenia. *Molecular psychiatry*, in press.
104. Paulsen Bda, S., de Moraes Maciel, R., Galina, A., Souza da Silveira, M., dos Santos Souza, C., Drummond, H., Nascimento Pozzatto, E., Silva, H. Jr., Chicaybam, L., Massuda, R. *et al.* (2012) Altered oxygen metabolism associated to neurogenesis of induced pluripotent stem cells derived from a schizophrenic patient. *Cell transplantation*, **21**, 1547–1559.
105. Robicsek, O., Karry, R., Petit, I., Salman-Kesner, N., Muller, F.J., Klein, E., Aberdam, D. and Ben-Shachar, D. (2013) Abnormal neuronal differentiation and mitochondrial dysfunction in hair follicle-derived induced pluripotent stem cells of schizophrenia patients. *Molecular psychiatry*, **18**, 1067–1076.
106. Zhang, D., Pekkanen-Mattila, M., Shahsavani, M., Falk, A., Teixeira, A.I. and Herland, A. (2014) A 3D Alzheimer's disease culture model and the induction of P21-activated kinase mediated sensing in iPSC derived neurons. *Biomaterials*, **35**, 1420–1428.
107. Legrand, N., Ploss, A., Balling, R., Becker, P.D., Borsotti, C., Brezillon, N., Debarry, J., de Jong, Y., Deng, H., Di Santo, J.P. *et al.* (2009) Humanized mice for modeling human infectious disease: challenges, progress, and outlook. *Cell host & microbe*, **6**, 5–9.
108. Novozhilova, E., Olivius, P., Siratirakun, P., Lundberg, C. and Englund-Johansson, U. (2013) Neuronal differentiation and extensive migration of human neural precursor cells following co-culture with rat auditory brainstem slices. *PloS one*, **8**, e57301.