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REVIEW

Modeling neurodegenerative disorders in adult somatic cells: A critical review

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Abstract Development of new therapeutic targets for neurodegenerative disorders has been hampered by a reliance on *post mortem* tissue that is representative of end-stage disease, or on animal models that fail to provide faithful analogs. However, rapid advances in cellular genetic reprogramming, in particular the induction of somatic cells into stem cells, or directly into neurons, has led to intense interest in modeling of human neurodegeneration *in vitro*. Here, we critically review current methods and recent progress in cellular models of Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Several challenges are identified, including technical variability, lack of degenerative phenotypes, neurodevelopmental age and establishing ground truths for models of sporadic disease. Recommendations for evaluating neurodegenerative cellular models are proposed along with suggestions for future research.

Neurodegenerative disorders (NDDs) place an enormous burden on affected individuals, their families and the community at large. For example, they are the leading neurological cause of lost disability-adjusted life years in developed countries (Collins et al., 2011). Yet unlike researchers in the fields of cancer or cardiovascular disease, the tissue of interest (neuronal circuits) is generally inaccessible, except at *post mortem*. The history of neuropathology is therefore a catalog of end-stage disease with the pivotal upstream biological events, those most likely amenable to therapeutic targeting, obscure. For these reasons, modeling the pathogenesis of NDDs in adult somatic cells has generated much excitement. This brief review aims at a critical assessment of progress toward this goal.

NDDs are characterized by three core features, each with important ramifications for *in vitro* models. The first is not simply age-associated onset (generally in later life), but accelerating prevalence with advanced years. In other words, age is their primary risk factor. In the case of Alzheimer's disease (AD), this is extreme: AD prevalence doubles every five years after the age of 60, and is approximately 10–20 times stronger than any other risk factor (Brayne, 2007). As will be briefly reviewed below, this makes the issue of "developmental age" when modeling neurons intrinsic to their validity.

The second core criteria is frequency and severity of neuropathology compared to age-matched controls. Unfortunately, distinctions are blurred because there are generally no absolute thresholds when distinguishing normal from abnormal. Indeed, it is very common for healthy elders' brains to express AD pathology of some degree; hence, it could be argued that a model cell line from "healthy controls" ought to also express some pathology. The underlying point is that clinical phenotype is not decisive: as many as 30% of individuals who die with moderate or severe AD pathology were asymptomatic in life (Mrc, 1998). Rather, it is a matter of degree, in which case those with more severe pathology are at higher risk of clinical symptoms. Accordingly, an *in vitro* model that is exclusively engineered to express neuropathology does not guarantee a clinical outcome from positive "hits" in drug screening.

An important caveat to NDD pathology is co-morbidity. In this review, for the sake of brevity we are focusing on AD, Parkinson's disease (PD) and Amyotrophic Lateral Sclerosis (ALS). The defining classical pathology of AD is intracellular hyperphosphorylated tauopathic neurofibrillary tangles (NFTs) and extracellular insoluble amyloid plaques (varying from diffuse to compact to neuritic); for PD, it is intracellular α-synuclein Lewy bodies, and in ALS it is ubiquitin positive

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cytoplasmic inclusions in anterior horn cells of spinal cord (and in the brain in some cases). However, it is rare for any particular individual to exhibit just one class of pathology. Those diagnosed with AD may have frequent Lewy bodies and those with PD, amyloid plaques. One could say that it is somewhat of a "dirty secret" that NDDs are so promiscuous. Whether neuronal models of NDDs can or should recapitulate this is debatable. Occam's razor would argue that one or two within-class pathologies is admirable; on the other hand, if, as often argued, such models permit a prized insight into the etiology of human brain disease, then a wild type pathological landscape in all its fecundity is superior.

Finally, NDDs by their nature display progressive neuronal and synaptic loss at an industrial scale. In early AD there is ~10 million lost neurons in the CA1 subfield of the hippocampus alone (West et al., 1994); in symptomatic PD, 80% of substantia nigra neurons have died, and in ALS there is an estimated 50%–80% culling of motor neurons in the motor cortex, brainstem and spinal cord (Dauer and Przedborski, 2003;Coan and Mitchell, 2015). Neuronal and synaptic losses are the closest biologic correlate of clinical symptoms (Crystal et al., 1996; Price et al., 2001), and so this phenotype is arguably one of the most important when evaluating neuronal models of NDDs. Similarly, there are stereotyped patterns in the spatiotemporal evolution of neuropathology and neuronal loss. In AD, it generally starts in the entorhinal and hippocampal areas, then temporal cortex, insula and eventually most cortex (Braak and Braak, 1998). In sporadic PD, it tends to begin in the brain stem, then midbrain and finally meso- and neocortex (Braak et al., 2004). For ALS, a complex pattern has been proposed moving from spinal-, brainstem- motor nuclei to prefrontal cortex, then striatum and even hippocampus (Braak et al., 2013). How this can be modeled in a dish is not clear, especially when the gold standard is often the "pure culture" of a single neuronal subtype. Perhaps multicellular organoids (Lancaster et al., 2013; Lancaster and Knoblich, 2014) will be required to model neurodegenerative progression, but current techniques do not permit this.

Basic methods

Induced pluripotent stem cells (iPSCs)

In 2007, Yamanaka and colleagues successfully generated human iPSCs from adult fibroblasts for the first time (Takahashi et al., 2007). Briefly, retrovirus-mediated transduction of four transcription factors–Oct3/4, Sox2, Klf4 and c-MYC, was shown to be sufficient to reprogram the somatic nucleus to a pluripotent undifferentiated state (Fig. 1). These iPSCs are similar (but not identical) to native human embryonic stem cells (ESCs) in morphology, protein and gene expression, proliferation and telomerase activity.

Current protocols permit generation of stable patient-specific iPSC lines, albeit at low efficiency rates of 0.005% following transduction (Takahashi et al., 2007; Soldner et al., 2009). These reprogrammed cells can be maintained *in vitro* for comparable time length to human ESCs, with their endogenous genome and its transcriptional feedbacks largely intact (Takahashi et al., 2007). Their pluripotency is confirmed by the capacity to differentiate into cells of all three germ layers following *in vivo* teratoma assay. Subsequently, patient-derived iPSCs can be differentiated into different neuronal subtypes by coculture with appropriate growth factors or feeder cells.

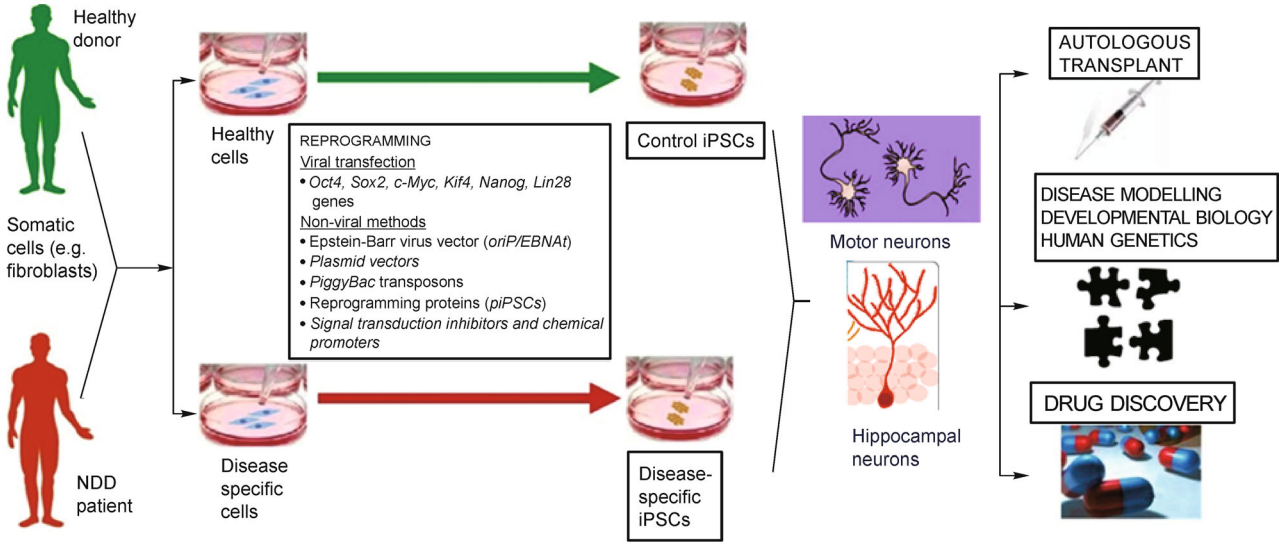

Figure 1 Generation of human iPSCs from dermal fibroblasts and potential downstream applications of different iPSC-derived progenies. Adapted from Liras et al. (2013).

Several general problems continue to hamper iPSCs approaches for modeling pathogenic mechanisms. Because the underlying mechanism of nuclear reprogramming is unknown, randomly incorporated factors can and do get spontaneously reactivated post-differentiation, confounding or masking downstream disease phenotypes (Okita et al., 2007; Sommer et al., 2012). Also, the inclusion of the proto-oncogene c-MYC, and the act of genetic integration itself, may inactivate tumor suppressor genes and increase the likelihood of tumorgenesis (Byrne, 2008) further confounding phenotypes. To circumvent this issue, non-integrating delivery methods such as adenoviruses (Zhou and Freed, 2009) and episomal protein delivery (Kim et al., 2009), or removing c-MYC from the reprogramming mixture itself (Nakagawa et al., 2008; Soldner et al., 2009) have been investigated, however, all report a further decrease in efficiency. Efficiency of reprogramming is important to disease modeling not only from an experimental feasibility point of view, but also in terms of ability to replicate results widely between laboratories.

More recently, small molecule cocktails added in combination with viral mediated transcription factors have been shown to enhance the efficiency of iPSC reprogramming by up to 200-fold (Lin et al., 2009; Li et al., 2009). Hou *et al*. (2013) demonstrated that these small compounds could entirely substitute for the traditional genetic reprogramming factors and was sufficient to generate iPSC from somatic cells through the targeting of specific signaling pathways, an approach that requires replication.

This brings up the key issue of model replicability. Without replicability there can be no validity, and current iPSCs methods suffer from lack of (within-line) clone-to-clone reproducibility (Narsinh et al., 2011; Thatava et al., 2013) as well as patient-to-patient or synthesized line-to-line reliability (Schuster et al., 2015; Kyttälä et al., 2016). Further development of transduction methods and systematic replicability are therefore major technical challenges for disease modeling.

Induced neuronal cells (iNs)

While somatic cells can be induced back to a more primitive pluripotent state, reprogramming technology also permits direct conversion (trans-differentiation) of cells from one somatic lineage (e.g., fibroblasts) into another (e.g., neuronal). It is thereby a way of sidestepping the 'backward' pluripotent step and moving directly to the cell type of interest. Conceptually, this method is attractive because of potential time and resource efficiency and it avoids the risk of tumorigenesis.

Vierbuchen and colleagues (2010) used a lentiviral vector packaged with five transcription factors (Brn2, Ascl1, Myte1l, Olig2 and Zic1) to directly convert mouse fibroblast into neurons. Eventually, three BAM "master factors" (Brn2, Ascl1 and Myt1l) were identified to be sufficient for the generation of these induced neuron (iN) cells, with serotonergic, dopaminergic and motor subtypes, albeit with efficiency varying widely between 2 and 50% (Vierbuchen et al., 2010). These may comprise a hierarchical set of neuronal-specific lineage regulators, in which Ascl1 is upstream and essential for recruitment of Brn2 to its target (Wapinski et al., 2013). A detailed mechanistic understanding of how BAM-mediated neuronal specification proceeds is still elusive.

Results are more limited when starting with mature adult human tissue. Human iNs have only been able to attain immature neuronal protein expression and morphology (Pang et al., 2011). However, the addition of NeuroD1 to BAM-factors (BAMN) resulted in trans-differentiation of fetal and adult human fibroblasts to neurons expressing mature neuronal markers NeuN and MAP2. These cells also form functional synapses and integrate into neuronal networks. Pharmacological application of GABA and AMPA inhibitors halted inhibitory and excitatory post-synaptic potentials, respectively (Pang et al., 2011). The use of BAMN is therefore a promising application for generating functional neurons from adult human fibroblasts.

The use of micro-RNA (miRNA) for reprogramming is yet another alternative approach that is less genomically invasive than using viral vectors. This provides a non-integrating and self-regulating means of converting human somatic cells into iNs without risk to genetic integrity (Yoo et al., 2011; Lau et al., 2014). Several miRNAs have been demonstrated to promote reprogramming by negatively regulating genes involved in the differentiated state and enhance reprogramming efficiency (Anokye-Danso et al., 2012). miRNA-124 has been a popular choice due to its specific expression in cells of neuronal lineage. When combined with BAM factors, mature neurons expressing MAP2 and NeuN can be produced, displaying synaptic functionality with mainly GABAergic or glutamatergic characteristics (Lau et al., 2014).

While such non-viral reprogramming methods preserve genetic integrity and are easier to synthesize, modify and standardize, it should be noted that they do not resolve other issues faced by iPSCs or iNs. Further, miRNAs are involved in many cellular processes and thus the exact molecular mechanisms underlying their pro- reprogramming effects are still unclear (Anokye-Danso et al., 2012). There is also the question of robustness and whether the generated neurons behave (let alone model disease) similarly to neurons of ESC or iPSC origins.

A major disadvantage of directly reprogrammed iNs is that the fate of the neuronal sub-population is difficult to manipulate and once the cells have differentiated into a particular subtype, their fate (and number) is sealed. Like for iPSCs, methods for producing such cells continue to diverge rather than converge (Ladewig et al., 2012; Liu et al., 2013) and this methodological variability is likely to have a material effect on NDD model outcomes.

Clearly, each reprogramming technology has its own set of advantages and disadvantages and it remains an empirical question as to which strategy is best suited for a particular application. In the absence of a gold standard, a utilitarian approach is recommended: does the model elucidate a veridical disease pathway; does it help develop a new effective treatment?

Key challenges in neurological disease modeling

From an experimental point of view, two fundamentally different strategies can be trialled when modeling NDDs. One is to culture somatic cells from *bona fide* patients, reprogram these, grow the cells into mature neurons and compare to disease-free healthy control neurons. This strategy is preferred for sporadic/idiopathic disease which comprise > 90% of all NDD, since the underlying polygenetic-environmental interaction driving pathogenesis is unknown. The downside of this approach is that the two groups of cells are necessarily non-isogenic. Whether this in fact matters in these complex disorders is open to debate.

A second approach involves attempted rescue of a disease phenotype in familial, genetically-defined NDDs with gene editing (Soldner et al., 2011; Ryan et al., 2013; Sproul et al., 2014; Ring et al., 2015). Here, the contrast is between isogenic cell lines and hence allows strong conclusions about causality. Yet as mentioned, these revelations can in the best case only generalize to a small minority of NDD patients, particularly familial AD and PD.

A major challenge inherent to all reprogramming approaches is the reversion to some kind of ground state. For iPSCs, reversion is all the way back to an embryonic-like state (Silva et al., 2008), for iNs, it is a neural progenitor-like state Ring *et al*. (2012). However, as mentioned at the outset, age is the number one factor driving NDD pathogenesis. Even in the earliest familial cases of ALS, this may be in the late 30s or early 40s. For sporadic cases, it is at least in the 60s.

So what is the "developmental age" of modeled neurons? There remains some debate, not least because age is a multidimensional construct and complicated by reprogamming-related cellular rejuvenation (Marion et al., 2009; Suhr et al., 2010; Lapasset et al., 2011; Mahmoudi and Brunet, 2012; Miller et al., 2013; Vera and Studer, 2015; Mertens et al., 2015). In this context, age - and lifestyle - associated epigenetic signatures could potentially be lost or altered during the iPSC reprogramming process, but might be retained in directly converted iNs, assuming that these characteristics are similar between fibroblasts and neurons (Sommer et al., 2012; Kyttälä et al., 2016). What ought to be avoided is circular logic: model X recapitulates disease phenotype Y; therefore it is developmentally mature. Simply designing models for a given phenotype is perilous, particularly for the sporadic diseases, in the same way that transgenic mice models of NDDs have produced little clinical advancements (Wilcock, 2010; Breitner, 2015). A better approach is for neuronal maturity to be evaluated against a panel of indicators (e.g., loss of developmentally immature transcription factors, loss of embryonic neuronal patterning markers, etc.) that are orthogonal to the NDD phenotype.

Linked to this is the fanciful idea that age may be "accelerated," not least because of the second law of thermodynamics. Rather, researchers can trial the use environmental stressors (oxidants, free radicals, hypoxemia, carboxia, alcohol, etc.) to attempt to make the experiment more tractable, revealing, and realistic given at least 30% of all sporadic AD is attributable to environmental factors (Silva et al., 2008).

Neuronal cell models of AD

Genetic mutations linked to early onset (< 60 years of age) familial AD (FAD) include the Amyloid precursor protein (APP), Presenilin 1 (PSEN1) and Presenilin 2 (PSE2) (Tanzi and Bertram, 2005). These represent < 5% of all AD, the rest being sporadic AD (SAD). Several genetic variants have been proposed as SAD susceptibility or risk factors by genome wide association studies (Lambert et al., 2009; Harold et al., 2009), but results have not been consistent (Gandhi and Wood, 2010), with the exception of ApoE-ε4 that is common and exhibits a strong effect size (Tsai et al., 1994).

Overall, human iPSC-derived neurons from FAD patients with PSEN1 and PSEN2 mutations exhibit abnormal levels of Aβ42/40 ratio in the absence of changes to tau (Yagi et al., 2011). iPSCs with APP mutations displayed increased levels of Aβ peptides and abnormal Aβ42/40 ratio, as well as elevated levels of total tau, and phosphorylated-tau (p-tau) (Israel et al., 2012; Muratore et al., 2014). Interestingly, while animal models increasingly suggest that tauopathy is a critical partner in Aβ toxicity (Ittner and Götz, 2011), only a small number of iPSC studies have demonstrated such a connection (Israel et al., 2012; Choi et al., 2014; Kim et al., 2015). It is also notable that to date no AD cell model has demonstrated *bona fide* neurofibrillary tangles as opposed to incipient tauopathy.

Biochemical changes to Aβ42 and Aβ40 in modeled cells have been contradictory. Cells derived from a population of FAD patients with APP mutation found elevated Aβ42/40 ratio only in the one case with the APP-V717L mutation (Kondo et al., 2013). It is thereby possible that variability in disease phenotypes can, in part, be due to the specific nature of APP cleavage site dysfunction (Kondo et al., 2013; Muratore et al., 2014).

In studies of SAD, variability has been the rule rather than the exception. In general, phenotypes have not been replicable between patients (Israel et al., 2012). By definition, these studies mix patients with different AD-related genetic mutations along with patients where there is no known genotype (Kondo et al., 2013). SAD can thereby harbour multiple genetic variants (Israel et al., 2012), many of which

Table 1 Key studies using induced pluripotent stem cell and directly induced neuron techniques to generate patient-specific *in vitro* models of SAD and FAD

Study	Cell type. Generation and differentiation methods	Efficiency	Disease phenotypes	Key findings and significance
Yagi et al. (2011)	FAD iPSC derived neurons (PS1/PS2 mutation)	~80%	• Elevated Aβ42/40 ratio • No difference in NFTs • Responds to pharmacological agents	Secretase inhibitors significantly reduced Aβ levels
Israel et al. (2012)	Patient iPSC derived neurons (APP^{DP}) Patient derived iPSC induced neurons (SAD)	> 90%	• Elevated Aβ-peptides in fibroblast and neurons • Elevated p-tau and total tau • Elevated levels of GSK-3β • γ- and β-secretase inhibitors reduced Aβ-peptides, p-tau/total tau and GSK-3β • Early RAB5 + endosomes found in one patient • Elevated Aβ-peptides neurons only • Elevated levels of p-tau and GSK-3β in patient 2 only • γ- and β-secretase inhibitors reduced Aβ-peptides, p-tau/total tau and GSK-3β in one patient only • Early RAB5 + endosomes found in one patient	Neurons from APP^{DP} differed significantly from patient FBTs in levels Aβ-peptides, suggesting the disease phenotype may be specific to neuronal cells. The study allows comparison between SAD and FAD patients, as well as within SAD population itself. SAD patient derived cells indicate SAD patient could harbor more genetic variants, which can be clarified in future studies.
Kondo et al. (2013)	FAD (APP) iPSC-derived neurons and astrocytes SAD (genotype unknown) iPSC-derived neurons and astrocytes	N/A	• Strong decrease in intracellular Aβ42 and Aβ40 species (cortical neurons) • Elevated extracellular Aβ42 levels and increased Aβ42/40 ratio • Intracellular accumulation of Aβ Oligomers found in neurons and astrocytes derived from one patient. • No change in extracellular Aβ levels • Decreased intracellular Aβ in one SAD derived neurons • Intracellular accumulation of Aβ Oligomers found in neurons and astrocytes derived from one patient	The study suggested different types of APP mutation consequently lead to different APP metabolism. Further genetic analysis of neurons with significantly high levels of intracellular Aβ oligomers showed upregulation of oxidative stress, and downregulation of glycosylation. Pharmacological agents were effective in reducing such oxidative stress, including the clinical trial drug DHA, which improved viability of cells with E693 mutation.
Ooi et al. (2013)	SAD iPSC-derived neurons	~66%	• Successful generation of iPSC-derived cholinergic neurons from SAD patients, which confirmed to be morphologically similar to control iPSCs and ESCs. • No disease phenotype characterization were carried out	Comparison between feeder-derived iPSC and feeder-free (ff) iPSC shows ff-iPSC helps to generate homogenous populations of patient-specific pluripotent cells. This can be advantages when using iPSC derived neurons for regenerative medicine.
Honda et al. (2016)	hESC-derived neurons (PS1)	~80%	• Increased extracellular levels of Aβ42/40 ratio • Responds to γ-secretase inhibitors • Synaptic activity alterations	One of the few studies to address electrophysiological abnormalities in AD cell models
Muratore et al. (2014)	FAD patient iPSC derived neurons	90%	• Elevated extracellular levels of Aβ42 and Aβ38, with a 1.6 fold increase in Aβ42/40 ratio. Intracellular and membrane-associated Aβ levels are substantially lower. • The increase in levels of secreted Aβ occurred with increasing differentiation time • Elevated levels of total-tau and p-tau, which were reversed by administration of Aβ antibodies during early time points only.	The study examined findings on cleavage of APP site associated with APPV717I mutation. Findings suggest mutation increases β-secretase cleavage and alters γ-secretase cleavage site. This effect is also found to be linked when γ-secretase inhibitor also prevented increased of β-secretase processing of APP.

(*Continued*)

Study	Cell type. Generation and differentiation methods	Efficiency	Disease phenotypes	Key findings and significance
Duan et al. (2014)	Patient iPSC derived cholinergic neurons (ApoE3/E4)	> 95% (~66% ChE neurons)	• Increased Aβ42/40 ratio due to elevated levels of Aβ42 secretion (except one SAD and one FAD patient) • SAD derived ChE neurons were more susceptible to calcium or glutamate toxicity associated cell death	The study used age-matched healthy iPSC derived control as well as 2 FAD patient derived cell lines for positive control. It is one of rare studies that have utilized a number of AD patients, and has allowed comparison of disease phenotypes between the SAD risk allele (ApoE4) and FAD mutations.
Choi et al. (2014)	ReN-derived neurons induced with multiple FAD mutations (PS1 & APP)	N/A	• Elevated levels of extracellular Aβ42, Aβ40 and Aβ42/40 ratio • Pharmacological agents reduced Aβ levels • Elevated levels of Aβ and p-tau, p-tau to total tau ratio increased with enhanced expression	3D cell culture model with FAD mutations compared to 2D showed more accumulation of tau isoforms. 3D matrices are also found to promote neuron maturation.
Hossini et al. (2015)	Patient iPSC derived neurons (SAD)	N/A	• Upregulated GSK-3beta and accumulation of tau • Altered expression of Alanine, glutamate and Aspartate metabolism • Upregulated oxidative stress and reaction to free radicals	The first study to generate a protein interaction network on SAD patient-derived neuronal cell lines. Study analysis of differentially expressed genes in iPSCs to control cell lines found upregulation and downregulation of a number of genes not related to typical FAD mutation (APP, PS1 and PS2). Further analysis showed these included downregulation of genes related to Ubiquitin-proteasome system.
Hu et al. (2015a)	Patient fibroblast derived neurons (APP^{DP}) Patient fibroblast derived neurons (PS1 mutation)	5-15%	• Elevated levels of extracellular Aβ42 and increased Aβ42/40 ratio • Elevated levels of p-tau • No change in GSK-3β level • Elevated levels of extracellular Aβ42 and increased Aβ42/40 ratio • No change in p-tau • No change in GSK-3β level	Induced cells do not pass a stage of pluripotency. Comparisons between AD patients with different genetic mutations show difference in p-tau levels.
Kim et al. (2015)	ReN-derived neurons with multiple FAD mutations (PS1 and APP)	N/A	• Increased Aβ42 and Aβ40 levels in undifferentiated FAD cells • Extracellular Aβ and p-tau aggregation observed at 6 weeks, and further elevated.	The 3D culture matrix provides a model that better represents a "brain-like" environment and may also prevent extracellular Aβ diffusion that in 2D models.

are not related to typical FAD mutations (Duan et al., 2014; Hossini et al., 2015).

Further limiting AD models is the curious neglect to date of key processes such as apoptosis, synaptic loss and synaptic dysfunction. Altered electrophysiological activity and susceptibility to cell death have been reported (Duan et al., 2014; Honda et al., 2016), but lack of connection with Aβ abnormality or tauopathy suggests these are more likely due to cellular immaturity (Israel et al., 2012; Muratore et al., 2014).

Given the abundance of research in FAD (e.g., DIAN project (Cairns et al., 2015; Su et al., 2016), in the near future it may be interesting to apply gene editing technology on patient-specific iNs or iPSC-derived neurons to examine whether rescue of point mutations can ameliorate disease phenotype or improve cellular function.

Neuronal cells models of PD

Parkinson's disease (PD) is a chronic and progressive movement disorder caused by dysfunction and eventual cell death of dopaminergic neurons in the substantia nigra and beyond. The mechanism driving cellular degeneration remains elusive; increasingly, studies point to a confluence of mechanisms rather than a single pathway. These include mitochondrial dysfunction, accumulation of toxic α-synuclein, mitophagy and oxidative stress. Several genes implicated in familial PD have been successfully recapitulated in iPSC-derived dopaminergic neurons, including the SNCA (α-synuclein), *LRRK2* (leucine-rich repeat kinase 2) and *PINK1* (PTEN induced putative kinase 1) mutations (Table 2).

As seen in Table 2, *in vitro* models of PD have focused so far solely on familial cases of PD, with a limited number of studies demonstrating disease-specific phenotypes and features of dopaminergic degeneration (Byers et al., 2011; Fernández-Santiago et al., 2015). Nevertheless, these studies have provided valuable insights into potential mechanisms of cellular dysfunction driven by a discrete genetic mutation. Environmental factors such as oxidative damage and neuroinflammation certainly have a significant role in disease pathogenesis, and future studies should begin to incorporate these factors into their models. Where possible, novel disease-related phenotypes expressed by iPSCs should be cross-validated by isogenic *post mortem* neuropathology (Hu et al., 2015b). However, this is clearly not always possible in a manageable time frame and so cross validation by neuropathology in non-isogenically matched individuals is justifiable.

Neuronal cells models of ALS

ALS is an adult-onset progressive neurodegenerative disease marked by degeneration of upper and lower motor neurons (Pasinelli and Brown, 2006). 90% of ALS is of unknown genetic etiology (Pasinelli and Brown, 2006) and there is no animal model of sporadic ALS. This is in contrast to several transgenic rodent models of familial ALS, particularly the SOD1 gene mutations. While animal models can recapitulate some aspects of the disease phenotype, including degeneration of spinal motor neurons (Gurney et al., 1994; Bruijn et al., 1997), these have yet to yield an effective treatment. Thus, model systems that allow validation of results from animal models in adult human motor neurons derived from sporadic ALS cases could be critical in bridging this gap. Notable studies in this area are summarized in Table 3.

Early successes in generating patient-specific cellular models of familial ALS may allow researchers to gain a deeper understanding of disease processes in the large majority without familial disease. However, ALS models suffer from many of the same limitations that apply for other NDDs. While modeled cells express markers of neural stem cells and motor neurons, they lack disease-specific phenotypes and do not show age-dependent degenerative features. Promisingly, a study by Liu et al. (2016) showed that induced motor neurons expressed some features of neurodegeneration, suggesting that more representative models are possible. Also, while motor neurons are the main cell type affected in ALS, neuroimaging and pathological studies have implicated non-motor brain areas in ALS, with some patients showing cognitive and behavioral impairment similar to frontotemporal dementia (Strong and Yang, 2011) along with corticospinal tract abnormalities (Mascalchi et al., 1995). Future studies should aim to address this by looking at multi-cellular culture systems, combining wild type motor neurons with ALS-derived non-motor neurons or glial cells (and vice versa), to further explore non-cell autonomous disease pathways.

Recommendations for evaluating NDD models

Evidence is mixed for new mechanistic insights or novel therapeutic targets based on models of NDD. AD models (either familial or sporadic) have to date been disappointing in terms of mainly recapitulating known pathology. On the other hand, a model of PD that includes neurodegeneration found that epigenetic modification of the *LRRK2* gene may generalize from the familial to sporadic context (Fernández-Santiago et al.(2015), while another model confirmed at *post mortem* that AKT/GSK-3 beta signaling pathway may be dysregulated, as is the case in AD, further supporting linkages across classes of NDDs. Similarly, a model of ALS has suggested that a previously known pathogenic mechanism in familial disease (TDP-43 proteinopathy) may be relevant in a small subset of sporadic cases (Burkhardt et al., 2013).

Cellular models of NDD therefore have potential but a critical approach is recommended. Based on this review and

Table 2 Key studies of human iPSC-derived dopaminergic neurons (DAn), generated from healthy control and PD cases, for *in vitro* modeling of PD.

Study	Cell type. Generation and differentiation methods	Efficiency	Disease phenotypes	Key findings and significance
Soldner et al. (2009)	iPSCs generated from 5 **sporadic PD** cases using a modified **doxycycline (DOX)-lentiviruses for the first time**. iPSCs generated neural precursors when incubated with fibroblast growth factor 2 (FGF), FGF8, and sonic hedgehog (SHH). Terminal differentiation to Dan induced by growth factor withdrawal for 8 days.	Factor-free iPSCs were morphologically indistinguishable from iPSCs carrying the transgene and could be maintained *in vitro* in the absence of DOX for more than 30 passages. Factor-free iPSCs showed a global gene expression profile more closely related to ESCs.	No.	The first study to generate iPSCs from sporadic PD patients. DOX-lentivirus improved the efficiency of reprogramming and allowed the removal of c-MYC which significantly reduces the risk of tumorgenesis while maintaining similar kinetics and dopaminergic differentiation efficiency.
Seibler et al. (2011)	PD patient with *PINK1* mutation and genetically matched (family member) healthy control. iPSC induction using conventional lentivirus carrying OCT4, SOX2, c-MYC, and KLF4. DAn differentiation induced using SMAD-inhibitor noggin and culture on Matrigel.	Patient-derived iPSCs could be generated and differentiated to DAn with efficiency comparable to the control iPSC lines.	Demonstrated mitochondrial translocation of *Parkin* in the control but not in the *PINK1* mutant neurons, suggesting a disease-relevant phenotype.	The first DAn model aimed to study the role of the *PINK1* mutation in PD.
Byers et al. (2011)	One PD patient with a triplicated α-synuclein gene (SNCA) and his healthy sibling as the control. iPSC induction using conventional l entivirus carrying OCT4, SOX2, KLF4, and c-MYC. iPSCs subsequently cultured as neural rosettes to generate neural progenitors, then passaged onto laminin and patterned with FGF8 prior to terminal differentiation in the presence of transforming growth factor β-3.	Multiple iPSC clones from both PD and healthy donor lines were similar to native H9 human ESCs with respect to morphology, genetic profile and generation efficiency.	PD-derived DAn exhibited accumulation of α-synuclein aggregates, overexpression of oxidative stress markers and increased sensitivity to peroxide-induced oxidative stress.	These findings suggest that the SNCA-triplication mutation can affect dopamine cellular function and could corroborate a cell-autonomous etiology for PD.
Fernández-Santiago et al. (2015)	Four monogenic *LRRK2* PD, six sporadic PD and healthy controls. iPSCs generated using retroviral delivery of OCT4, KLF4, and SOX2. DA differentiation induced via lentiviral - mediated expression of the DAn determinant *LMX1A* and patterning factors culture with mouse PA6 feeding cells.	PD and control-derived iPSC and DAn show similar morphological and functional properties, as well as similar DAn maturation state.	Consistent with features of late onset PD, patient-derived DAn cells developed specific neurodegenerative phenotypes upon long - term culture (75 days) including impaired axonal outgrowth, deficient autophagic vacuole clearance, and accumulation of α - synuclein (SNCA).	First evidence suggesting that epigenetic deregulation of iPSC - derived DAn in PD is associated with both mutant *LRRK2* and sporadic cases. Furthermore, PD-related DNA methylation changes in iPSC-derived DAn are partially associated with gene expression and correlate with the RNA and protein downregulation of a network of transcription factors.

(*Continued*)

Study	Cell type. Generation and differentiation methods	Efficiency	Disease phenotypes	Key findings and significance
Hu et al. (2015b)	Two healthy controls and two PD patients with *Parkin* mutation. iPSCs generated using DOX-inducible lentiviruses expressing Oct4, Sox2, Klf4, c-MYC and Nanog, cocultured with mouse embryonic fibroblast feeder cells. Neural induction via SHH and ascorbic acid to generate midbrain DAn.	Patient-specific iPSCs exhibit cell doubling time and clonal efficiency similar to that of mouse ESCs.	PD-derived DAn exhibited the phenotypes associated with *Parkin* mutations: increased MAO-A and MAO-B mRNA expression associated with elevated oxidative stress, increased spontaneous DA release and decreased specific DA reuptake.	Confirmed that patient-specific iPSC-derived DAn can be used as an appropriate model for the *Parkin* mutations. Isogenic iPSC models eliminate the confounding influence of different genetic backgrounds. High-efficiency gene targeting using engineered site-specific nucleases such as TALEN suggests this model would be useful for the study of specific gene mutations.
Ohta et al. (2015)	Two PD patients with I2020T *LRRK2* mutations in the Sagamihara family and two healthy controls. iPSCs generated via retrovirus induction carrying Oct4, Sox2, Klf4 and c-MYC genes. Neural induction via culture of iPSC as embryoid bodies and neurospheres. Terminal dopaminergic differentiation on poly-L-ornithine- and fibronectin-coated coverslips.	Low differentiation efficiency (5-20% of total iPSCs).	Mutant *LRRK2* iPSC-derived neurons released less dopamine, had a lower phospho-AKT level and increased incidence of apoptosis relative to controls. *LRRK2*–derived iPSCs showed glycogen synthase kinase-3 beta activation and high Tau phosphorylation. Post-mortem brain analysis from the same patient also showed neurofibrillary tangles and increased Tau phosphorylation in DAn.	**First study to compare disease phenotype of iPSCs with post mortem findings from the same patients**. PD-generated iPSCs-derived DAn exhibit increased Tau phosphorylation, possibly through the AKT/GSK-3 beta signaling pathway. I2020T *LRRK2*-iPSC could be a new tool to study the role of the I2020T mutation in PD pathology.

In all studies, dermal fibroblasts were the somatic cell of choice for iPSC induction

Table 3 Significant published in vitro studies in ALS using human induced pluripotent stem cell technology and directly induced motor neurons (iMNs)

Study	Cell type. Generation and differentiation methods	Efficiency	Disease phenotype	Key findings and significance
Boulting et al. (2011)	7 healthy controls, 2 familial ALS with SOD1 mutations and human ESCs. All iPSC lines were derived by retroviral transduction of skin fibroblasts, using either only three factors (OCT4, KLF4, SOX2) or with c-MYC for comparison. Differentiation to spinal MNs were performed using standard retinoic acid and sonic hedgehog pathway protocol.	Demonstrated comparable differentiation efficiency into MNs between patient-specific iPSCs, healthy control iPSCs and standard ESC.	No	The first study to perform extensive characterization of patient-derived MNs with several control lines, and demonstrated similar differentiation capacity between ALS-derived iPSC, healthy control iPSC and ESC. The differentiation protocol was reproducible in two separate laboratories, which is important for large-scale systematic studies.
Burkhardt et al. (2013)	Fibroblasts generated from healthy subjects, sporadic ALS, familial ALS patient with TAR DNA binding protein 43 (TDP-43) mutations or SOD1 and FUS mutations. iPSCs induced using retroviruses expressing four transcription factors OCT4, SOX2, KLF4, and c-MYC. Differentiation into MNs via incubation with SMAD inhibitors and subsequent growth factors.	A total of 92 iPSC clones from 34 patients were screened to confirm stem cell characteristics and identify TDP-43 pathology expression.	Only a subset of MNs derived from sporadic cases (3/16) show spontaneous intranuclear and hyperphosphorylated TDP-43 aggregations that recapitulate pathology in post mortem tissue from the same patient from which the iPSCs were derived.	The only study thus far to generate a cellular model for TDP-43 proteinopathy in sporadic ALS and directly validate the disease phenotype of iPSC-derived neurons with post mortem neurons from the same patient (isogenic control).
Liu et al. (2016)	Fibroblasts from healthy controls and ALS patients with FUS mutations were directly converted to iMNs via incubation with lentivirus expressing NEUROG2-IRES-GFP-T2A-SOX11, NEUROG2-IRES-SOX11, and ISL1-T2A-LHX3on, followed by culture on Matrigel.	Rapid and efficient ($>60\%$ transduced fibroblasts) generation of iMNs. Mature iMNs exhibit cytological and electrophysiological features of spinal MNs and form functional neuromuscular junctions (NMJs) with skeletal muscles.	**ALS-derived iMNs show disease-specific degeneration**, manifested through reduced cell survival, soma shrinkage, hypoactivity, and an inability to form NMJs.	**The only report of ALS-derived iMNs exhibiting some of the known disease phenotypes.** A direct and efficient protocol to generate patient-specific MNs for study of familial ALS with FUS mutations. Using this cellular model, a pilot drug screen identified a small molecule capable of rescuing key deficits in these diseased MNs.
Lim et al. (2016)	Fibroblasts from healthy controls and patients with FUS mutations were directly converted to induced neurons by repressing a polypyrimidine-tract binding protein.	Effective generation of induced neurons from patient-specific fibroblasts, albeit lack of specific cellular characterisations.	ALS-derived induced neurons exhibit relevant disease phenotype: cytoplasmic aggregation of mutant FUS and decreased nuclear distribution.	Demonstrated feasibility of generating a patient-specific cellular model of ALS which bypasses genetic integrations. Directly converted patient-specific induced neurons mirror the neuropathology of mutant FUS in clinical ALS.

1. **Pathology should emerge in developmentally mature neuronal cells and increase in severity with cellular age.** As far as we know, there is no neurological deficit or dysfunction at birth or even in early life across the NDDs. Therefore, phenotypes should emerge after the offset of immature neuronal markers and increase in severity with cellular age post differentiation or in response to mimicking environmental stressors.
2. **Neurodegeneration is mandatory.** Most NDD models so far have not observed neuronal degeneration of any kind. Clearly, this is not satisfactory. Neuronal death or structural damage, loss, degradation or dysfunction should be a core feature of any model, acknowledging that high levels of apoptosis is experimentally counter-productive.
3. **Multiple disease phenotypes are realistic.** Multiple within-class and across-class NDD pathologies increase the fidelity of any model. Absence of pathology in clinically "disease-free" controls should not be seen as validation; rather, phenotype expression is on a spectrum.

Box 1 Principles for evaluating cellular models of neurodegenerative disease. Proposed for the purpose of initiating discussion rather than prescriptive.

the core pathological features of human NDDs introduced at the outset, Box 1 proposes three criteria when evaluating models of neurodegenerative disease.

Future directions

Neuronal modeling of sporadic NDDs is the greatest challenge for the field. Here, because of the absence of any "ground-truth" studies will need to strive for more complex models, perhaps mixing cell types (e.g., tripartite neuronal-glia-vascular interactions), applying enviromimetics (e.g., oxidative stressors) or incorporating 3D architecture (e.g., cerebral organoids). Eventually, models will need to move from considering just single-cell or univariate phenotypic features to multivariate characteristics (e.g., using multi-electrode arrays).

External validation of NDD models will be essential prior to use in pharmacological screening. This can include rescue of known familial defects or comparison against isogenic or non-isogenic *post mortem* histopathology. Modeling of not only risk factors but also resilience or protective factors is unchartered territory, but likely to produce fascinating outcomes.

Underlying all such advances is production of modeled cells under a systematic, efficient and low variance protocol. If only a small subset of cells express a given phenotype, in a small subset of cell lines, and these are the subject of publications, then the field will flounder. On the other hand, the rate of advance in reprogramming technology is remarkable. If the field can converge on a set of core methods for producing NDD models then it is more likely to thrive.

Compliance with ethics guidelines

An Truong, Emily Si, Thomas Duncan and Michael Valenzuela declare that they have no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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