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Review

Modeling ALS and FTD with iPSC-derived neurons

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ABSTRACT

Recent advances in genetics and neuropathology support the idea that amyotrophic lateral sclerosis (ALS) and frontotemporal lobar dementia (FTD) are two ends of a disease spectrum. Although several animal models have been developed to investigate the pathogenesis and disease progression in ALS and FTD, there are significant limitations that hamper our ability to connect these models with the neurodegenerative processes in human diseases. With the technical breakthrough in reprogramming biology, it is now possible to generate patient-specific induced pluripotent stem cells (iPSCs) and disease-relevant neuron subtypes. This review provides a comprehensive summary of studies that use iPSC-derived neurons to model ALS and FTD. We discuss the unique capabilities of iPSC-derived neurons that capture some key features of ALS and FTD, and underscore their potential roles in drug discovery. There are, however, several critical caveats that require improvements before iPSC-derived neurons can become highly effective disease models.

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Contents

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1. The expanding landscape of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD)

1.1. *ALS and FTD: two ends of a disease spectrum*

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is an adult-onset neurodegenerative disease that affects upper and lower motor neurons. As initially described by Jean-Martin Charcot more than 140 years ago, the key clinical features in ALS patients include muscle wasting, and progressive loss of spinal motor neurons and upper motor neurons and their axons in the lateral columns of the spinal cord. This century-old classic definition, however, has been under scrutiny due to the emerging appreciations that some ALS patients who exhibit deficits in higher cognitive functions at the early stage of their clinical course eventually develop behavioral variant frontotemporal dementia (bvFTD). Indeed, it has been estimated that ~15% of FTD patients develop features of ALS (Lomen-Hoerth et al., 2002; Ringholz et al., 2005), and up to 50% of ALS patients show abnormal neuropsychological testing indicative of frontal lobe dysfunctions (Lillo et al., 2011; Lomen-Hoerth et al., 2003). Furthermore, patients with FTD–ALS usually have shortened life span compared to patients with pure FTD or ALS (Olney et al., 2005). Together, these studies provide a new framework to re-evaluate the diagnostic criteria for ALS (Strong et al., 2009), and a new clinical paradigm in which ALS and FTD are linked within a disease spectrum (Fig. 1).

1.2. *Unraveling the genetics and neuropathology of ALS and FTD*

The idea that FTD and ALS are a disease spectrum is further supported by genetic evidence that patients with familial FTD–ALS and ALS often carry mutations in the same genes (Ling et al., 2013). In fact, of all the different subtypes of FTD, FTD–ALS cases have such a high propensity (~30%) of familial inheritance that genetic counseling is now considered as the standard of care for these patients (Goldman et al., 2005). Among a growing number of genes implicated in familial ALS and FTD–ALS (Fig. 1), mutations in three genes account for the majority of cases. These mutations include missense mutations in genes encoding two RNA/DNA binding proteins, *TDP-43* (*TARDBP* or TAR-DNA-binding protein-43) and *FUS/TLS* (*fused in sarcoma/translocation in liposarcoma* or *FUS*) and the GGGGCC hexanucleotide expansions in *C9ORF72* gene (Lee et al., 2012; Ling et al., 2013). The discovery of TDP-43 as a component in the ubiquitin-positive, tau-negative insoluble protein aggregates in neurons and glia represents a major breakthrough in FTD research (Arai et al., 2006; Neumann et al., 2006). Moreover, the impact of this discovery

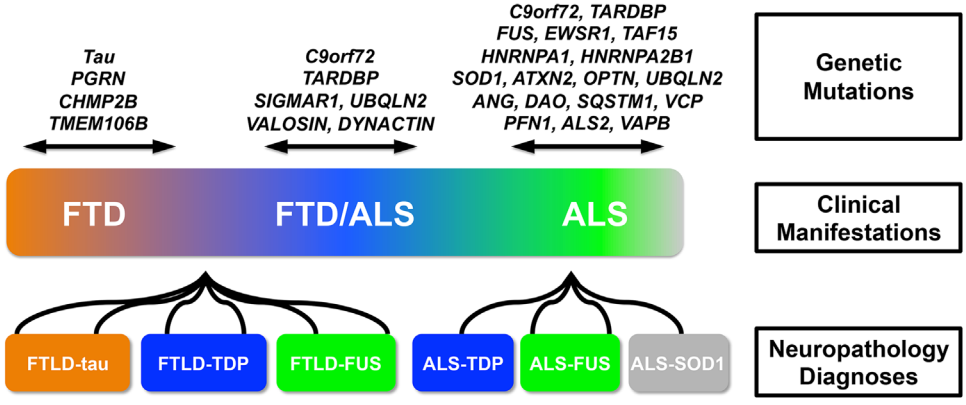

Fig. 1 – A schematic diagram illustrating the disease spectrum, genetics and proteinopathy of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Clinical manifestations indicate that ALS and FTD are two ends of a disease spectrum. It is estimated that ~15% of FTD patients develop ALS and up to 50% of ALS patients show impairments in frontal executive functions. This new clinical paradigm is further supported by the findings that patients with FTD–ALS and ALS share similar genetic mutations, in particular mutations that involve the GGGGCC hexanucleotide expansion in the non-coding region of the *C9ORF72* gene and two RNA/DNA binding protein TDP-43 and FUS. FTD–ALS and ALS patients with mutations in the same gene (*C9ORF72*, *TARDBP* or *FUS*) often exhibit similar neuropathological features with neurons containing distinct protein aggregates of TDP-43 or FUS proteins. Therefore, the neuropathology of these patients can be categorized by the nature of the proteinopathy into FTLD (frontotemporal lobar degeneration)-tau (FTLD-tau), FTLD-TDP, FTLD-FUS, ALS-TDP, ALS-FUS or ALS-SOD1.

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goes beyond the identification of a single disease gene and essentially ushers in a new era of research that focuses on the potential contributions of transcription, RNA splicing and RNA metabolism on neurodegenerative diseases.

TDP-43 is originally identified to bind to the TAR DNA sequence in HIV-1 genome to regulate viral gene expression (Ou et al., 1995). Under physiological conditions, TDP-43 is a ubiquitous nuclear protein. In FTD patients, however, TDP-43 proteins form aggregates that are found predominantly in neuronal cytoplasm and dystrophic neuronal processes (Arai et al., 2006; Neumann et al., 2006). This distinctive feature, also known as TDP-43 proteinopathy, defines a major neuropathological diagnosis entity in frontotemporal lobar degeneration (FTLD-TDP) and in sporadic ALS (ALS-TDP) (Fig. 1) (Mackenzie et al., 2011, 2010). Several subsequent studies show that dominant mutations in the *TARDBP* gene can also be identified in familial ALS and FTD patients (Lattante et al., 2013). The identification of autosomal dominant mutations in the *FUS* gene in large kindred of familial ALS (FALS) further expanded the genetic and neuropathological landscape of ALS (Kwiatkowski et al., 2009; Vance et al., 2009). Similar to TDP-43, FUS proteins reside primarily in the neuronal nuclei, but in ALS-FUS patients FUS proteins form large aggregates in the cytoplasm. The morphology of FUS proteinopathy in FALS ranges from diffuse and dense cytoplasmic aggregate present in late onset cases, to basophilic inclusions commonly found in juvenile FALS with FUS-P525L mutation. Finally, in 2011 two groups independently reported the GGGGCC hexanucleotide repeat expansions in the noncoding region of the *C9ORF72* gene as causal links to ALS and FTD (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Although TDP-43 proteinopathy can be detected in FTLD and ALS patients with *C9ORF72* mutations, the neuropathological features in these cases are quite heterogeneous and also include prominent ubiquitin and p62 positive, but TDP-43 negative intracytoplasmic and intranuclear inclusions (Bigio, 2012; Mackenzie et al., 2014). Interestingly, TDP-43 proteinopathy has also been identified in protein aggregate myopathies, such as inclusion body myositis (Olive et al., 2009). Together, these results raise the possibility that TDP-43 proteinopathy may not be specific for FTLD or ALS.

It is estimated that mutations in *TARDBP* and *FUS* each account for ~5% of FALS, whereas the GGGGCC expansion mutations in *C9ORF72* account for 20–40% of familial ALS and FTD–ALS cases, depending on the population studied. Given the large number of cases with *C9ORF72* mutations, there have been tremendous interests in understanding the underlying mechanisms. While several mechanisms, including RNA toxicity and dipeptide accumulation (Ash et al., 2013; Donnelly et al., 2013; Mori et al., 2013), have been proposed for *C9ORF72* mutations, the rapidly progressing research on this subject will definitively bring many more surprises in the future. One important feature noted in a recent study indicates that the age of disease onset for FALS caused by *FUS*, *TARDBP* and *C9ORF72* mutations differ quite drastically in that mutations in *FUS* account for ~35% of FALS in patients younger than 40 years old, whereas mutations in *C9ORF72* are much more common in patients older than 50 years of age (Millecamps et al., 2012). Indeed, meta-analyses of 154 ALS cases with *FUS* mutations (including FALS and SALS with *de novo FUS* mutations) show an average disease onset of 43.8±17.4 years (Deng et al., 2014; Lattante et al., 2013). More than 60% of cases with *FUS* mutations show disease onset before 45 years of age, with many juvenile ALS cases presenting with disease onset in late teens and early 20's (Baumer et al., 2010; Huang et al., 2010). These findings are similar to those from another study using smaller sample size, and show that the average disease onset for *FUS*, *SOD1* or *TARDBP* mutations is 43.6±15.8, 47.7±13.0 and 54.7±15.3, respectively (Yan et al., 2010). Kaplan–Meier survival analysis shows statistically significant differences in the trend of age of onset among these three genes. This distinctive feature of FUS mutations raises the intriguing hypothesis that, despite the similarities between TDP-43 and FUS proteins, mutations in FUS may target divergent mechanisms that perturb the development and maintenance of synaptic homeostasis in the nervous system in early postnatal life and during the aging process (Qiu et al., 2014; Sephton et al., 2014).

2. Induced pluripotent stem cells (iPSCs) as models for ALS

Recent advances in stem cell biology have provided exciting and unprecedented opportunities to develop disease-specific cell types that allow us to understand and explore mechanisms that contribute to pathogenesis of disease. In particular, the ability to reprogram somatic cells into induced pluripotent stem cells (iPSCs) provide obvious advantages to generate patient-specific iPSCs that carry the exact same genetic makeup, including mutations that may contribute to the disease process (Fig. 2) (Takahashi et al., 2007; Takahashi and Yamanaka, 2006). These iPSCs can be differentiated into cell types that provide strong correlations to clinical benchmarks and pathology (Han et al., 2011). In addition, patient-specific iPSCs may also serve as powerful resources for personalized medicine, including drug discovery, genetic testing, and ultimately cell replacement therapy (Fig. 2). Despite these unique advantages, one important caveat with the iPSC models is that a myriad of epigenetic changes due to reprogramming and the lengthy process of cell selection and *in vitro* cultures might contribute to the variations in this system. Here we focus on the recent progress on using induced pluripotent stem cell (iPSC)-derived neurons as the new models to investigate disease mechanisms for ALS and FTD. The salient features of these studies are summarized in Table 1.

2.1. *Modeling SOD1 mutations*

Autosomal dominant mutations in the *superoxide dismutase 1* (*SOD1*) are the first to be causally linked to familial ALS. Following this discovery, several transgenic rodent models overexpressing SOD1 mutant proteins become widely used to reveal a plethora of disease mechanisms, including axonal transport defects, oxidative stress, protein misfolding, mitochondrial dysfunction and excitotoxicity. In addition, non-cell autonomous pathogenic mechanisms involving astrocytes, microglia and NG2 cells, have also been identified to contribute to neurodegeneration caused by mutant SOD1 overexpression. The availability of iPSC-derived motor neurons from patients with *SOD1* mutations provide unique opportunities to capture salient neurodegenerative features of ALS-SOD1 at more realistic

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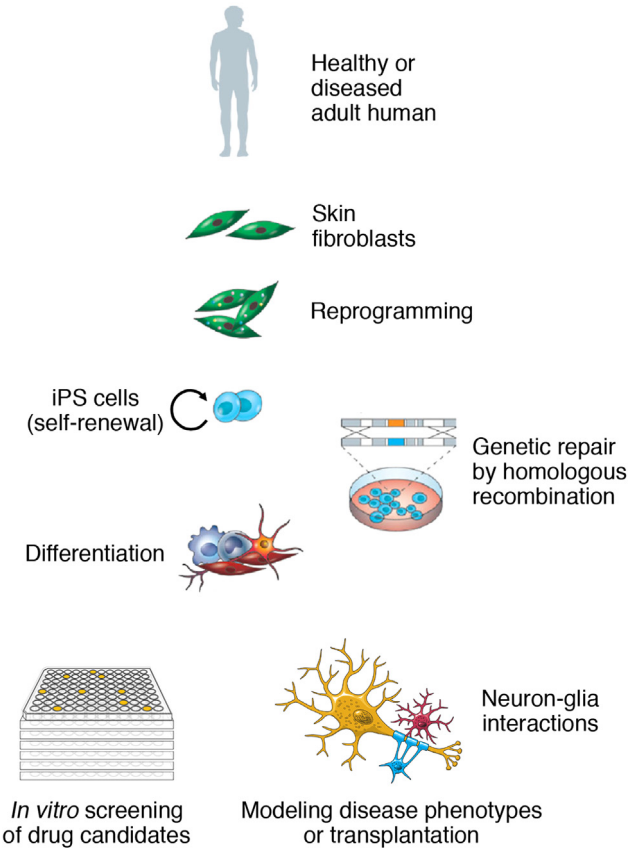

Fig. 2 – A schematic diagram illustrating the generation of disease-specific induced pluripotent stem cells (iPSCs) and its applications in modeling disease phenotypes and in identifying therapeutic targets.

expression levels and to re-evaluate disease mechanism(s) that may lead to novel therapeutic insights. By reprogramming skin fibroblasts from two patients with *SOD1*^{A4V} mutation (*SOD1*$^{A4V/+}$), Kiskinis et al. (2014) showed that iPSC-derived ISL+/HB9+ *SOD1*$^{A4V/+}$ motor neurons exhibit a higher propensity to undergo apoptosis, similar to those observed in the *SOD1*^{G93A} mouse model. In addition, iPSC-derived *SOD1*$^{A4V/+}$ motor neurons show significant reductions in soma size, and fewer and shorter neuronal processes. These pathological features appear to be specific to motor neurons because ISL- *SOD1*$^{A4V/+}$ neurons in the same culture conditions do not show similar defects. To further demonstrate the specificity of these phenotypes, the authors used zinc finger nuclease (ZFN)-mediated gene targeting to correct the *SOD1*$^{A4V/+}$ mutation and showed that motor neurons with corrected mutation (*SOD1*$^{+/+}$) show significant rescues in both survival and soma size.

Genome-wide transcriptome analyses in FACS-purified *SOD1*$^{A4V/+}$ motor neurons using RNA-seq further reveal altered expression in genes related to cytoskeleton organization. Consistent with these findings, another study shows that iPSC-derived motor neurons carrying *SOD1*^{D90A} mutation progressively develop neurofilament (NF) inclusions such that by Day 31 in cultures >60% of *SOD1*^{D90A} motor neuron cell body and 25% of neurites contain NF inclusions (Chen et al., 2014). The development of NF inclusions in *SOD1*^{D90A} motor neurons correlates with degeneration of neurites. The underlying mechanism for the formation of NF inclusion in *SOD1*^{D90A} motor neurons appears to be caused by mutant SOD1 proteins that bind to the 3′UTR of *NF-L* mRNA, destabilize *NF-L* mRNA and thereby change the proportion of NF subunit composition. In addition to the defects in cytoskeleton organization, gene expression profiling in *SOD1*$^{A4V/+}$ motor neurons also reveals defects in mitochondrial function and structure. In support of these findings, ultrastructural analyses show distortion of mitochondrial morphology in *SOD1*$^{A4V/+}$ motor neurons. In addition, live cell imaging shows that mitochondrial movement is reduced in the axons of *SOD1*$^{A4V/+}$ neurons (Chen et al., 2014).

Another major group of genes misregulated in *SOD1*$^{A4V/+}$ motor neurons are those related to ER stress and unfolded protein response (UPR). While the exact cause(s) leading to the activation of ER stress and the UPR pathways remain unclear, at least two mechanisms may contribute to these processes. First, the progressive accumulation of misfolded mutant SOD1 proteins may trigger ER stress and UPR in *SOD1*$^{A4V/+}$ motor neurons. In support of this idea, *SOD1*$^{A4V/+}$ motor neurons show a significant increase in the insoluble mutant SOD1 proteins when the neurons are treated with proteasome inhibitor MG-132. Second, additional evidence indicates that spinal cord motor neurons appear to have inherently higher level of ER stress, which may be interconnected with the unique electrophysiological properties endowed in motor neurons. Interestingly, compared to control motor neurons, *SOD1*$^{A4V/+}$ motor neurons have reduced delayed-rectifier potassium current amplitudes, which may contribute to their hyperexcitability properties (Wainger et al., 2014). Remarkably, the Kv7 channel activator retigabine blocks the hyperexcitability in *SOD1*$^{A4V/+}$ motor neurons and improves their survival *in vitro*. These results support the idea that the hyperexcitable properties of *SOD1*$^{A4V/+}$ motor neurons may directly contribute to neurodegeneration in ALS. Intriguingly, similar up-regulation of ER stress-related genes and hyperexcitability can also be detected in iPSC-derived motor neurons that carry *C9ORF72* or *FUS* mutations, suggesting that protein misfolding and the ensuring consequences of ER stress and UPR may have broader pathogenic roles in different subtypes of ALS (Wainger et al., 2014). Finally, one recent study tests the utility of iPSC-derived motor neurons with *SOD1* mutations in drug screening (Yang et al., 2013). The results from this study show that blocking HGK (hepatocyte progenitor kinase like or MAP4K4) with inhibitor kenpaullone can promote the survival of *SOD1*^{L144F} human iPSC-derived motor neurons as well as mouse ESC-derived *SOD1*^{G93A} motor neurons. These encouraging results support the idea that iPSC-derived motor neurons can be used as effective tools to identify novel therapeutic targets for ALS.

2.2. *Modeling C9ORF72 mutations*

At least three mechanisms, including the formation of RNA foci, repeat-associated non-ATG-initiated (RAN) translation products, and loss-of-function in *C9ORF72* gene product, have been postulated to contribute to the neurodegeneration in *C9ORF72* hexanucleotide repeat mutations (Gendron et al., 2014). Several groups have generated iPSC-derived neurons and developed RNA fluorescent *in situ* hybridization (FISH) to show that ~20–35% of iPSC-derived neurons contain intranuclear GGGGCC RNA foci (Almeida et al., 2013; Donnelly et al.,

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Table 1 – Summary of the results from iPSC-derived neurons as disease models for ALS and FTD.

Genetic mutation	Neuron types	Analyses	Phenotypes and mechanisms	Gene targeted corrections, drug screening or other interventions	References
$SOD1^{D90A}$	Motor neurons	Cell biology and immune-gold EM	• Neurofilament aggregates and inclusions • Mutant SOD1 binds to 3′UTR of *NF-L* mRNA and destabilizes *NF-L* mRNA	TALEN-mediated homologous recombination	Chen et al. (2014)
$SOD1^{A4V}$ *C9ORF72*	Motor neurons	RNA-seq gene expression profiling	• Increased apoptosis, reduced soma size and shorter and fewer processes • Defects in mitochondrial morphology and motility • Unfolded protein response (UPR) and ER stress	Zinc finger nuclease (ZFN) correction of $SOD1^{A4V}$ mutation improves survival and soma size	Kiskinis et al. (2014)
$SOD1^{G93A}$, $SOD1^{L144F}$	Motor neurons	MN survival assay, Electrophysiology	• Improves motor neuron survival via cell-autonomous mechanisms by Inhibition of GSK3α/β andTak1-MKK4-JNK-c-Jun	Small molecule screening	Yang et al. (2013)
$SOD1^{A4V}$ $SOD1^{D90A}$ $SOD1^{G85S}$ *C9ORF72* FUS^{M511FS} FUS^{H517Q}	Motor neurons	Extracellular multielectrode array recording (MEAs) and whole-cell patch clamp	• Hyperexcitability that can be blocked by Retigabe	n.d.	Wainger et al. (2014)
$TDP\text{-}43^{M337V}$	Motor neurons and astrocytes	Selective cellular vulnerability by longitudinal fluorescent microscopy	• $TDP\text{-}43^{M337V}$ neurons show selective cellular vulnerability to PI3K inhibitors • $TDP\text{-}43^{M337V}$ neurons show increase in detergent-insoluble TDP-43 proteins • $TDP\text{-}43^{M337V}$ astrocytes show increase in TDP-43, but no non-cell autonomous cyto-toxicity to iPSC neurons	$TDP\text{-}43^{M337V}$ allele-specific siRNA knock-down reduces cytosolic TDP-43	Bilican et al. (2012) Serio et al. (2013) Nishimura et al. (2014)
$TDP\text{-}43^{Q343R}$ $TDP\text{-}43^{M337V}$ $TDP\text{-}43^{G298S}$	Motor neurons	Cell biology, gene expression profiling and drug screening	• Reduced NFM and NFL expression • Increase in detergent-insoluble TDP-43 in ALS neurons • Increased TNFα/NFκB signaling pathway and sensitivity to arsenite-induced cell death • Gene expression profiling reveals increases in RNA metabolism-related genes in ALS neurons	Candidate drug screenings identify HAT inhibitor Anacardic acid to decrease TDP-43 mRNA, reduce insoluble TDP-43 and increase NFM/NFL expression	Egawa et al. (2012)
$TDP\text{-}43^{A90V}$ $TDP\text{-}43^{M337V}$	Tuj1+ neurons	Electrophysiology, cellular response to stress and microRNA expression	• Cytoplasmic localization of TDP-43 in $TDP\text{-}43^{A90V}$ neurons • Reduced total TDP-43 protein level in $TDP\text{-}43^{A90V}$ neurons • Decrease in miR-9 and precursor	n.d.	Zhang et al. (2013)
$TDP\text{-}43^{M337V}$ $TDP\text{-}43^{G298S}$ $TDP\text{-}43^{A315T}$	Motor neurons	Axonal transport of RNA beacons, live imaging of RNA transport	• Axonal transport of target mRNAs	n.d.	Alami et al. (2014)
C9ORF72	Mixed neuron-glia cultures	FISH for RNA foci, RAN translation pathology, identification of RNA binding	• RNA foci and RAN translation products detected	ASOs targeting *C9ORF72* rescues glutamate cytotoxicity and reverses disease-specific transcriptional changes	Donnelly et al. (2013)

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Table 1 – (*continued*)

		proteins for GGGGCC RNA and ASO treatment	• Screening for RNA binding proteins that bind GGGGCC and characterize ADARB2 • Gene expression profiling • Increase in glutamate cytotoxicity		
C9ORF72	Motor neurons	Southern blot analyses for GGGGCC expansion, FISH for RNA foci, gene expression profiling and ASO treatment	• RNA foci detected, but not RAN translation products • Support gain-of-function properties in RNA foci to sequester RNA binding proteins and affect splicing and transcription • Gene expression profiling reveals enrichment in genes related to cell adhesion, synaptic transmission and neural differentiation • C9-ALS neurons show reduced excitability upon depolarization	ASOs targeting *C9ORF72* reverses disease-specific transcriptional changes	Sareen et al. (2013)
C9ORF72	Telencephalon neurons (FOXG1-expressing)(80% MAP2+, 30% VGlut1+, 10% GABA+)	Southern blot analyses for GGGGCC expansion, RNA foci and stress	• RNA foci and RAN translation products detected • Reduced cell viability in the presence of autophagy inhibitors	n.d.	Almeida et al. (2013)
C9ORF72 *TDP-43*^{M337V}	Motor neurons	Whole-cell patch clamping recordings	• ALS motor neurons display initial hyperexcitability, followed by a progressive loss in action potential and synaptic activity	n.d.	Devlin et al. (2015)
Sporadic ALS	Motor neurons	Cell biology, drug screening and comparison with postmortem neuropathology	• Validation of TDP-43 protein aggregates in iPSC-derived motor neurons from SALS cases.	High content screening for TDP-43 aggregation inhibitors	Burkhardt et al. (2013)
MAPT^{A152T}	MAP2+ neurons	Isogenic iPSC using zinc-finger nuclease-mediated gene editing	• Tau protein fragmentation, Tau protein phosphorylation, Degeneration of neuronal process	Zinc finger nuclease mediated gene editing to correct the mutation	Fong et al. (2013)
Grn^{S116X} and *Sporadic FTD*	Neurons (80% MAP2+) and microglia	Electrophysiology, PGRN level, sensitivity to cellular stress, and gene expression profiling	• *Grn*^{S116X} neurons show PGRN haploinsufficiency • *Grn*^{S116X} neurons show increased sensitivity to ER stress, staurosporine and kinase inhibitors, and down-regulation of S6K2 • Pharmacological suppression of sortilin 1 (SORT1) increases extracellular PGRN in *Grn*^{S116X} neurons	Lentivirus-mediated expression of PGRN rescues cellular sensitivity to stress	Almeida et al. (2012) Lee et al. (2014)
Grn$^{IVS1+5G>C}$	Cortical neurons (CTIP2, FOXP2, TBR1+)	Genome-wide transcriptomes analyses	• Aberrantly activated Wnt signaling pathway	Genetic correction using homologous recombination with zinc finger nucleases (ZFNs)	Raitano et al. (2014)

Notes: n.d., not done.

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2013; Sareen et al., 2013). The presence of RNA foci in *C9ORF72* iPSC-derived neurons recapitulates a key neuropathological feature of *C9ORF72* mutations, and further allows characterization of how the intranuclear RNA foci might affect RNA metabolism. For instance, several RNA binding proteins, including ADARB2, hnRNPA1, hnRNPA1B2, Pur-α, FUS, and TDP-43, have been shown to potentially interact with GGGGCC RNA repeats (Donnelly et al., 2013; Sareen et al., 2013). However, so far only ADARB2 co-localizes with intranuclear RNA foci, suggesting that such association might have functional consequences (Donnelly et al., 2013). It is important to note that the various *C9ORF72* iPSC models have implicated different sets of RNA binding proteins in the development of RNA foci. This variability may reflect the inherent clonal variations in the iPSC models, differences in the GGGGCC repeat lengths or other unknown factors (Table 1). Finally, despite the attention to RNA foci, it remains unclear whether these foci are toxic to neurons or simply reflect the disease state.

In addition to the intranuclear RNA foci, a higher percentage of *C9ORF72* iPSC-derived neurons contain cytoplasmic RNA foci, suggesting that RAN translation may lead to accumulation of high molecular weight cytoplasmic dipeptide protein products, including poly-(Gly-Ala), poly-(Gly-Pro) and poly-(Gly-Arg) (Ash et al., 2013; Mori et al., 2013). In support of this idea, immunohistochemical staining confirms the presence of poly-(Gly-Pro) dipeptide protein products in *C9ORF72* iPSC-derived neurons (Almeida et al., 2013; Donnelly et al., 2013). However, despite the presence of RNA foci and RAN translation protein products in iPSC-derived neurons, how these phenotypes directly contribute to human disease remain unclear. For instance, the variations in the quantity of RNA foci per cell in different lines of iPSC-derived neurons suggest that the formation of RNA foci may not correlate directly with the number of GGGGCC repeat, but perhaps may be influenced by other genetic or epigenetic factors (Almeida et al., 2013). Similarly, the levels of RAN translation protein products appear to be independent of the GGGGCC repeat length. In at least one study, the RAN translation protein products and aberrant increase of proteasome marker p62 are not consistently detected (Sareen et al., 2013). These results have also been further extended by another recent study (Gomez-Deza et al., 2015), which shows a distinct lack of RAN translation protein products in spinal motor neurons in FALS patients with *C9ORF72* mutations. In the future, it will be important to use the iPSC models to determine how RAN proteins induce p62 pathology and whether p62 pathology is causally linked to neuronal degeneration and functional deficits.

The *C9ORF72* iPSC-derived neurons provide a convenient and effective tool to further interrogate the pathogenesis of ALS. There are several directions to achieve this goal. First, transcriptome analyses in iPSC-derived motor neurons reveal misregulation of genes that are involved in cell adhesion, synaptic transmission and neural differentiation (Donnelly et al., 2013; Sareen et al., 2013). Interestingly, however, direct comparison of the transcriptomes of *C9ORF72* iPSC-derived neurons and motor cortex of *C9ORF72* ALS patients shows very limited overlapping. This difference may be due to the contribution of the glial cells in the motor cortex. Alternatively, it is possible that the difference may reflect that the iPSC-derived neurons represent a much earlier stage in the disease manifestations. Second, *C9ORF72* iPSC-derived neurons exhibit elevated sensitivity to stress-mediated cytotoxicity. For instance, *C9ORF72* iPSC-derived neurons (mixed population with 30–40% HB9+ neurons) exhibit more vulnerability to glutamate-mediated cytotoxicity in a dose-dependent manner (Donnelly et al., 2013). Similarly, another study indicated that *C9ORF72* iPSC-derived neurons show reduced viability when exposed to chloroquine, an inhibitor that blocks the autophagy pathway (Almeida et al., 2013). Finally, *C9ORF72* iPSC-derived neurons should also provide a convenient tool to investigate the potential loss-of-function effect of C9ORF72 gene product in the regulation of endosomal trafficking and in the pathogenesis of TDP-43 proteinopathy, which are common neuropathological features in these cases (Farg et al., 2014; Gomez-Deza et al., 2015).

In addition to revealing disease mechanisms, *C9ORF72* iPSC-derived neurons provide an efficient tool to identify potential therapeutics. Based on the success of using antisense oligonucleotides (ASOs) approach to reverse RNA toxicity due to myotonic dystrophy (MD1) (Mulders et al., 2009), two studies showed that ASOs that targets the GGGGCC repeat containing transcripts can reduce RNA foci, normalize disease-specific transcriptional changes and rescue the increased vulnerability to glutamate toxicity, without affecting the viability of iPSC-derived neurons (Donnelly et al., 2013; Sareen et al., 2013). These results confirm that RNA toxicity, rather than loss-of-function in *C9ORF72*, is likely to be the major contributing factor to the pathogenesis of C9 ALS and provide a promising blueprint for future development of therapeutics.

2.3. *Modeling TDP-43 mutations*

The identification of TDP-43 proteinopathy as a key neuropathological feature in sporadic ALS and FTLD represents a major advance in neurodegenerative disease research (Arai et al., 2006; Neumann et al., 2006). Subsequent studies show that autosomal dominant mutations in the TDP-43 gene *TARDBP* can indeed be identified in both sporadic and familial ALS patients (Lagier-Tourenne and Cleveland, 2010), though the incidence of these mutations is quite rare. To further understand how mutations in TARDBP might alter the subcellular localization of TDP-43 proteins, several groups have generated iPSC-derived motor neurons that carry TDP-43 mutations, including *TDP-43*^{M337V}, *TDP-43*^{Q343R}, *TDP-43*^{G298S}, *TDP-43*^{A90V} or *TDP-43*^{A315T}. These iPSC lines provide unique resources to determine whether these mutations directly contribute to the pathogenesis of disease, or merely represent risk factors. For instance, in control motor neurons wild type TDP-43 proteins are present predominantly in the soluble fraction. In contrast, *TDP-43*^{M337V} and *TDP-43*^{Q343R} motor neurons show increases of mutant TDP-43 proteins in the detergent-resistant insoluble fractions (Bilican et al., 2012; Egawa et al., 2012). These results suggest that the increases in mutant TDP-43 proteins are probably due to post-translational modifications rather than transcriptional mechanisms. Although the exact cause(s) that promote the accumulation of TDP-43 in the insoluble fractions remain unclear, there is evidence that mutant TDP-43 can form more stable complex with splicesome factor SNRPB2 (Egawa et al., 2012), suggesting that mutant TDP-43 may disrupt the splicing machinery through gain-of-function properties. Interestingly, automated live microscopy shows that *TDP-43*^{M337V}

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motor neurons have increased relative risks to undergo cell death. Together, these results are in agreement with previously reported cellular and transgenic TDP-43 models of elevated levels of cytoplasmic, but not nuclear, TDP-43 correlates with cellular toxicity (Barmada et al., 2010). The accumulation of cytoplasmic TDP-43 proteins in *TDP-43*^{M337V} motor neurons shows a significant reduction when these neurons are treated with allele-specific siRNA to knock-down the mutant *TDP-43*^{M337V} allele (Nishimura et al., 2014). These results not only provide definitive evidence that the accumulation of mutant TDP-43 proteins in cytoplasm is specific, they also support the idea that RNA interference has the potential to become an effective therapeutic tool for ALS.

Several studies further take advantage of the iPSC-derived motor neurons to interrogate the roles of mutant TDP-43 in cell autonomous and non-cell autonomous mechanisms that cause motor neuron dysfunctions. For instance, *TDP-43*^{A90V} and *TDP-43*^{M337V} iPSC-derived neurons exhibit increased vulnerability to stress-induced cytotoxicity and reduced microRNA-9 expression (Zhang et al., 2013). The findings in *TDP-43*^{A90V} iPSCs are quite intriguing because *TDP-43*^{A90V} has been considered as a risk factor for ALS. Hence, results from this study suggest that other additional factors, such as genetic background or environmental factors, may have contributed to the initiation and progression of disease. With respect to *TDP-43*^{M337V} mutation, gene expression profiling in the purified *TDP-43*^{M337V} iPSC-derived motor neurons further reveals misregulation of genes that are implicated in RNA metabolism and cytoskeleton functions (Egawa et al., 2012). In particular, the expression of neurofilament-medium (NEFM) and neurofilament-light (NEFL) chain is significantly reduced in *TDP-43*^{M337V} iPSC-derived motor neurons. These results are similar to those reported in *SOD1*^{D90V} motor neurons (Chen et al., 2014), suggesting that different genetic mutations may share the same target genes leading to ALS phenotype. In another study that combines three model systems, namely *Drosophila* motor neurons, mouse cortical neurons and human iPSC-derived motor neurons, Alami and colleagues provide compelling evidence that wild type TDP-43 proteins form cytoplasmic mRNP granules and facilitate the bidirectional, microtubule-dependent transport of these mRNP granules in axons. In contrast, mutant TDP-43 proteins, either TDP-43^{M337V} or TDP-43^{A315T}, consistently cause impairments in the transport of *NEFL* (neurofilament-L chain) mRNP granules in axons (Alami et al., 2014). Given the pluripotent nature of iPSCs, these cells provide rather convenient tools to determine whether glial cells expressing mutant TDP-43 proteins might cause cytotoxicity to motor neurons. Indeed, neural precursors from *TDP-43*^{M337V} iPSCs are treated with epidermal growth factor (EGF) and leukemia inhibitory factor (LIF) for 4–6 weeks, followed by 14 days treatment of ciliary neurotrophic factor (CNTF) to promote differentiation to astrocytes. Using motor neuron-astrocyte co-cultures, Serio and colleagues show that *TDP-43*^{M337V} iPSC-derived astrocytes do not exhibit non-cell autonomous cytotoxic effects to motor neurons (Serio et al., 2013).

Finally, *TDP-43*^{M337V} iPSC-derived motor neurons also provide a unique tool to identify potential therapeutic targets that may mitigate the ALS phenotypes. To this end, Egawa and colleagues show that when exposed to arsenite to induce oxidative stress, *TDP-43*^{M337V} iPSC-derived motor neurons show increase in insoluble TDP-43 and reduced survival. This arsenite-induced motor neuron death assay provides a convenient tool to identify anacardic acid that can promote the survival of these neurons. Anacardic acid reportedly reduces TDP-43 mRNA expression in *TDP-43*^{M337V} iPSC-derived motor neurons by 147-fold compared to untreated motor neurons, and reduces the amount of insoluble TDP-43, but not those in the soluble fractions. In addition, anacardic acid also increases the neurite length, increases the expression of NEFM in treated motor neurons, down-regulates the RNA metabolism-related genes, and reverses the changes in TNFα/NFκB signaling pathway (Egawa et al., 2012).

2.4. *Modeling sporadic ALS*

Although TDP-43 proteinopathy is a common feature in sporadic ALS, the cause-effect relationship between TDP-43 protein aggregates and the pathogenesis of ALS remains unclear. This is complicated by the observations that mutations in TDP-43 gene *TARDBP* are quite rare in sporadic ALS cases, and that TDP-43 proteinopathy can also be identified in other neurodegenerative diseases, such as Alzheimer's disease (Amador-Ortiz et al., 2007). Moreover, none of the rodent models that express wild type or mutant TDP-43 show definitive TDP-43 proteinopathy that recapitulates human pathology. Given these limitations, the iPSC-derived motor neurons provide an ideal and highly relevant model to address this important question. Currently, only one study reports the isolation and characterization of iPSC-derived motor neurons from sporadic ALS cases. While the results require further verifications, this study shows that under basal culture conditions, motor neurons in sporadic ALS patients inherently have a higher propensity to develop intranuclear TDP-43 aggregates even without the presence of stress (Burkhardt et al., 2013). Although these findings are encouraging, these inclusions only capture one feature of TDP-43 proteinopathy in sporadic ALS. There is no evidence that TDP-43 aggregates are identified in the cytoplasm or neuronal processes in sporadic ALS iPSC-derived motor neurons. It is also unclear if these intranuclear TDP-43 aggregates are ubiquitinated or hyperphosphorylated just like those identified in human tissues. Using intranuclear TDP-43 aggregates as readouts, this study further identifies several compounds that potentially may inhibit the formation of TDP-43 protein aggregates.

2.5. *Modeling progranulin mutations*

Autosomal dominant mutations in human progranulin (*GRN*) gene have been causally linked to FTD. Although it is well-established that non-sense-mediated decay of mutant progranulin mRNA contributes to haploinsufficiency, the exact mechanisms of neurodegeneration caused by PGRN deficiency are not entirely clear. Patient-specific iPSC-derived neurons that carry *GRN*^{S116X} mutation show about 50% loss of PGRN mRNA, as well as secreted and intracellular PGRN proteins. *GRN*^{S116X} iPSC-derived neurons exhibit increased sensitivity to a number of cellular stress conditions, including treatment with ER stress inducer tunicamycin and inhibitors for PI3 kinase (PI3K), AKT, and ERK/MAPK signaling pathways (Almeida et al., 2012). Interestingly, lentivirus-mediated expression of PGRN in *GRN*^{S116X} iPSC-derived neurons rescues the elevated sensitivity to cellular stress conditions. In

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addition, gene expression profiling show that Grn^{S116X} iPSC-derived neurons have reduced expression of ribosomal protein S6 kinase beta-2 (RPS6KB2), a member of the S6 kinase family that has been implicated in both PI3K/AKT and MEK/MAPK signaling pathways (Fenton and Gout, 2011).

Although GRN^{S116X} iPSC-derived neurons have no problem with neuronal differentiation, iPSCs derived from another mutation $GRN^{IVS1+5G>C}$ show reduced differentiation efficiency into CTIP2-, FOXP2- or TBR1-positive cortical neurons (Raitano et al., 2015). This defect can be corrected by zinc finger nuclease-mediated rescue of *GRN* cDNA. Interestingly, transcriptome analyses in $GRN^{IVS1+5G>C}$ iPSC-derived cortical neurons reveal that genes in the Wnt signaling pathway are significantly misregulated (Rosen et al., 2011), and treatment of $GRN^{IVS1+5G>C}$ iPSCs with Wnt inhibitors can restore the neuronal differentiation phenotype.

2.6. *Modeling MAPT mutations*

Mutations in human *MAPT* gene are a major contributing factor to the pathogenesis of familial FTD (Wolfe, 2009). The neuropathology features in these patients are characterized by the profound tau protein aggregates in the cytoplasm and processes of neurons and glia (frontotemporal lobar degeneration-tau or FTLD-tau). The large number of tau protein isoforms, alternatively spliced products, and various post-translational modifications make it quite challenging to decipher the mechanisms of tau protein aggregate formation and its contribution to disease process. The majority of *MAPT* mutations are missense mutations that cluster around the microtubule binding domain, suggesting that these mutations most likely perturb the ability of tau to bind to microtubules and cause neuronal dysfunction or even death. Several groups have reported the isolation of iPSC-derived neurons, including one with $MAPT^{A152T}$ mutation from a patient with progressive supranuclear palsy (PSP), one with $MAPT^{N279K}$ and $MAPT^{V337M}$ from FTDP-17 patients, and one with 10+16 splice site mutation in *MAPT* gene (Ehrlich et al., 2015; Fong et al., 2013; Sposito et al., 2015). The study by Fong et al. (2013) showed that $MAPT^{A152T}$ neurons exhibit distinct degenerative features, characterized by breaks, bends and bulges along neuronal processes and reduced neuronal survival. Interestingly, $MAPT^{A152T}$ iPSC-derived neurons show phosphorylated tau protein aggregates in cytoplasm and neuronal processes, though no abnormal tau protein aggregates are identified in astrocytes derived from $MAPT^{A152T}$ iPSCs. Using zinc finger nuclease-mediated gene editing, the authors showed that the phenotypes in $MAPT^{A152T}$ iPSC-derived neurons can be further aggravated when the mutation is present in both allele ($MAPT^{152T/T}$). Conversely, genetic correction of the $MAPT^{A152T}$ allele to wild type ($MAPT^{152A/A}$) eliminates the neuronal phenotypes in isogenic iPSC-derived neurons. Furthermore, $MAPT^{152A/T}$ and $MAPT^{152T/T}$ iPSC-derived neurons show very low percentage of dopaminergic neurons, which can be reversed by genetic correction. Together, these results suggest that the dopaminergic neurons may be more vulnerable to neurotoxicity caused by $MAPT^{A152T}$ mutation. These interesting findings support the idea that $MAPT^{A152T}$ is a disease risk factor that affects the development and maintenance of neuronal processes.

The effects of *MAPT* mutations in the development of neuronal processes are further confirmed in neurite outgrowth assays using iPSC-derived neurons with $MAPT^{N279K}$ or $MAPT^{V337M}$ mutation (Ehrlich et al., 2015). In addition, iPSC-derived neurons with $MAPT^{N279K}$ or $MAPT^{V337M}$ mutation are much more vulnerable to oxidative stress-induced toxicity, which is supported by an increase in unfolded protein response (UPR) and distinct "disease-specific" gene expression profiles. Finally, it is well-recognized that different isoforms of tau can be generated via alternative splicing. Using iPSCs from controls and two patients with 10+16 splice-site mutation in *MAPT*, Sposito and colleagues show that control iPSC-derived neurons express 3R tau only during the first 100 days in culture (Sposito et al., 2015). Interestingly, prolonged culturing of control neurons switches the tau expression from 3R tau to a diverse complement of tau isoforms. In contrast, iPSC-derived neurons with 10+16 splice site mutation in *MAPT* express both 3R and 4R tau during the first 100 days in culture. These results suggest that FTD-related splice site mutation in *MAPT* can override the developmental program that determines splicing-mediated generation of tau isoforms. Given the robust post-translational modifications of tau, it will be useful to take advantage of patient-specific iPSCs to investigate the signaling mechanisms that regulate phosphorylation and acetylation of tau, and how perturbations to these mechanisms contribute to disease.

3. Concluding remarks and future directions

3.1. *Conclusions and potential caveats*

The large numbers of studies using iPSCs derived from ALS and FTD patients have led to several important conclusions. First, it is highly feasible to generate patient-specific iPSCs and harness the genetic editing technology to correct mutations. Second, most iPSC-derived neurons that carry disease-causing mutations can recapitulate certain key features of human disease, including proteinopathy and RNA toxicity. Third, the phenotypes observed in iPSC-derived neurons provide confidence that they can be quite effective models for ALS and FTD. Finally, these studies also support the feasibility of using iPSC-derived neurons as platforms for the discovery of disease-specific therapeutic targets.

Despite these promising results, however, there are several caveats that deserve our attention before entering the next phase of research using iPSC-derived neurons. For example, there is sufficient evidence that phenotypic variations do exist in iPSC-derived neurons. In the case of *C9ORF72* mutations, at least two studies have reported distinct differences in the electrophysiological properties in iPSC-derived neurons (Devlin et al., 2015; Sareen et al., 2013). Despite using the same reagents to characterize C9ORF72 iPSC-derived neurons, not all the studies are able to identify RAN translation protein products (Sareen et al., 2013). Furthermore, although all disease-specific iPSC-derived neurons exhibit increased vulnerability to stress conditions, it is unclear if these phenotypes are disease-specific, nor do we know the underlying mechanisms that contribute to these phenotypes. Looking ahead, as we improve and refine iPSC-derived neurons as bona fide

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disease models, it will be important to compare and contrast the similarities and differences with human neuropathological features. Furthermore, by expanding the repertoire of neuron subtypes from iPSCs, we can begin to determine the molecular mechanisms that define "selective vulnerability" that is a consistent feature in neurodegenerative disease.

3.2. *Future directions*

The diverse phenotypes reported in iPSC-derived neurons that carry pathogenic mutations causally linked to ALS or FTD pose challenges for future efforts to identify therapeutic targets. While the ASO-mediated knock down of RNA foci caused by *C9ORF72* GGGGCC expansions provide promising therapeutic strategies, it could be a daunting task to restore the hundreds, or thousands, of disrupted RNA processing events that may occur following sequestration of RNA processing factors by repeat expansion RNAs. For example, many proteins involved in RNA metabolism, RNA transport and splicing have been isolated in the interactomes of GGGGCC RNA repeats, but it remains unclear whether ASO-based therapeutic approaches will be sufficient to mitigate the gain-of-function properties of RNA repeats (Lagier-Tourenne et al., 2013; Wheeler et al., 2012). Another challenge for ASO-based approach is to develop highly efficient delivery of ASO into brain and spinal cord. Considering these obstacles, small molecule inhibitors that aim at disrupting specific RNA-protein interactions might circumvent the technical challenges confronting ASO-based therapy and be more effective in releasing endogenous proteins from the gain-of-function properties of RNA repeats (Arambula et al., 2009; Jahromi et al., 2013; Warf et al., 2009). In this context, *C9ORF72* iPSC-derived neurons should serve as effective tools to screen for small molecule inhibitors. Given the variations and diversity in human disease, it might be more prudent to develop patient-specific iPSC-derived neurons for the purpose of screening for the best and most effective ASO and small molecule candidates.

Another therapeutic potential is to use iPSC-derived neurons in cell-based therapy to replace disease-damaged neurons and to restore the normal function of specific neural circuits. Several studies in rodents and in chicks have provided proof-of-principle evidence that iPSC-derived neurons can indeed be transplanted into cerebral cortex or spinal cord, and demonstrate functional integration into the existing neural circuits (Espuny-Camacho et al., 2013; Sareen et al., 2014; Toma et al., 2015). Given the available technology to correct the genetic mutations, it is possible that patient-specific iPSC-derived neurons might be able to reduce immune response and improve the chance of graft survival.

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