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Chapter 14

# Modelling Neurodegenerative Diseases Using Human Pluripotent Stem Cells

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/63521

**Abstract**

Neurodegenerative diseases are being modelled in-vitro using human patient-specific, induced pluripotent stem cells and transgenic embryonic stem cells to determine more about disease mechanisms, as well as to discover new treatments for patients. Current research in modelling Alzheimer's disease, frontotemporal dementia and Parkinson's disease using pluripotent stem cells is described, along with the advent of gene-editing, which has been the complimentary tool for the field. Current methods used to model these diseases are predominantly dependent on 2D cell culture methods. Outcomes reveal that only some of the phenotype can be observed in-vitro, but these phenotypes, when compared to the patient, correlate extremely well. Many studies have found novel molecular mechanisms involved in the disease and therefore elucidate new potential targets for reversing the phenotype. Future research that includes studying more complex 3D cell cultures, as well as accelerating aging of the neurons, may help to yield stronger phenotypes in the cultured cells. Thus, the use and application of pluripotent stem cells for modelling disease have already shown to be a powerful approach for discovering more about these diseases, but will lead to even more findings in the future as gene and cell culture technology continues to develop.

**Keywords:** Disease modelling, Alzheimer's disease, frontotemporal dementia, Parkinson's disease, pluripotent stem cells

## 1. Introduction

The ability for researchers to model diseases in a dish has accelerated during the past decade, thanks to the discovery of a new stem cell type, the induced pluripotent stem cell (iPSC). This is an artificially created cell that recapitulates all the features of embryonic stem cells (ESCs)

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isolated from the early pre-implantation embryo. The production of this cell type in 2006 was a remarkable finding which led its founder, Shinya Yamanaka, to receive the Nobel Prize in Physiology and Medicine, just 6 years after its discovery, in 2012. The prize at that time was also shared with Sir John Gurdon who uncovered the mechanism of reprogramming in the late 1950s. These iPSCs were first produced from mouse fibroblasts by the transduction of four transcription factors, which when overexpressed, could completely change the fibroblast's phenotype into that of an embryonic stem cell-like cell, capable of forming all cell types in the body, upon differentiation [1]. Today, iPSCs are being produced from human cells and other species in many labs across the world, and production of these has been streamlined using more refined reprogramming techniques as well as different combinations of either genes, proteins, small molecules or miRNAs that can replace the function of the transgenes [2]. What makes these cells so useful for studying disease is that they can easily be produced from patients suffering from the disease (patient-specific iPSCs) and be differentiated into the cell type/s affected by the disease, thus modulating and mimicking the disease in a dish.

The ability to produce patient-specific iPSCs has a number of advantages for both learning more about the disease itself and also in improving therapies and treatments. The ability to produce autologous stem cell populations from easy-to-access cells from the patient (e.g. blood cells and skin biopsies) overcomes the ethical conundrum of having to first produce a cloned human embryo using a donor cell from the patient and host de-nucleated oocyte and then having to destroy the cloned embryo, to harvest the pluripotent ESCs within [3]. This in itself is an enormous breakthrough. There are several benefits in being able to have autologous cells from the patient. In patients that have degenerative diseases (e.g. diabetes, heart disease, osteoporosis, atherosclerosis and varying neurodegenerative diseases), the potential opportunity to have healthy cells transplanted back into the site affected is particularly appealing. The patient's own cells can in fact also be corrected, in cases where genetic mutations induce the disease pathology. Alternately, autologous iPSCs derived from patients can also be used to improve the patient's own medical treatment. In this case, the iPSC-derived cells can be screened in-vitro to determine which drugs prove most beneficial for the patients. This is one aspect of many approaches for developing tailored-specific treatments for patients, known as 'personalized medicine'. The iPSCs, when differentiated into the target cells affected in the disease, can also be used to screen the potential new drugs being developed by Pharma, or potentially even used to discover new biomarkers of the disease. One of the latest developing fields in medical research includes the development of nanoparticles for treating disease, which are particularly attractive for use in brain diseases as they may pass easily through the blood-brain-barrier [4].

Despite the forefront in iPSC research, human ESC research is still in practice today for modelling neurodegenerative disease. Cell lines can be gene-targeted to induce familial-linked mutations, and in this way can be compared to genetically matched, unmodified control cell lines which are similar, if not more stringent controls than isogenic controls produced from iPSCs (see section on gene-editing below). Human ESCs are derived following the culture of the inner cell mass isolated from a pre-implantation embryo [5]. Hundreds of lines have been produced over the years for research purposes for the study of cell pluripotency and regen-

http://dx.doi.org/10.5772/63521

eration, and these can be easily sourced from stem cell banks, registries or commercial companies. Generally, human ESCs are non-autologous, unless derived by somatic cell nuclear transfer from the patient. Only a handful of studies have produced autologous human ESCs from patients with disease [6, 7], and none exist at present for neurodegenerative diseases. Reasons for this are likely due to the ethical dilemmas in producing cloned human embryos and the technical challenges in cloned embryo production, compared to the ease in production of iPSCs.

Neurodegenerative diseases are characterized by progressive dysfunction of the nervous system as a result of loss of neuronal function in either the brain or the spinal cord and include Alzheimer's disease (AD), frontotemporal dementia (FTD), Parkinson's disease (PD), Huntington's disease, amylotrophic lateral sclerosis and multiple sclerosis. Pluripotent stem cells (PSCs) have shown to be of particular promise for studying these diseases, since they can be expanded exponentially, hence providing much cell material for study. This is useful since it is particularly difficult to obtain tissue from the brain from patients suffering from the disease. In this review, we focus on the use of both iPSCs and PSCs in modelling AD, FTD and PD. In order for PSCs to deliver on their promises, it is important that clinical grade and safe cells can be produced for potential cell therapy. It is also important that these cells can modulate the disease accurately in the dish. That is, the cells must show the same pathology linked to the disease. In this review, we focus on how well iPSCs can model disease in a dish. We discuss how far the field has come in correcting the familial forms of AD, FTD and PD and how important the corrected mutations are for these diseases in relation to both the in-vitro studies and the potential for future cell therapy. Finally, we discuss what more is required to improve modelling in a dish, and where the current research is heading.

## 2. Use of gene-editing in modelling disease

Gene-editing involves insertion, deletion or replacement of DNA in the genome of an organism using engineered nucleases. This field has advanced considerably in just over a decade, thanks to the discovery and application of nucleases, combined with the latest molecular technology, which both enhance and improve the editing process. Gene-editing using designer nucleases was first applied to PSCs in 2007 [8]. Since then, its application and use on PSCs have become widespread. It is currently being used by researchers to correct disease-causing mutations (endogenous gene correction) found within patient-specific iPSCs. In the case of PD, corrected autologous iPSCs through gene-editing are particularly promising for future cell transplantation studies, where diseased cells are genetically corrected and transplanted back into the patient's brain. Another application for gene-editing in modelling disease is to produce genotypically matched control cell lines of disease iPSC lines. That is, because comparisons with age-matched healthy control lines from non-related persons are genetically different, which may impede on both phenotype and even differentiation capabilities [9]. This use of gene-editing technology thus enables eloquent comparable studies in-vitro of disease phenotypes, which is directly linked to the mutation *per se* and not to other compounding factors. One other application is to use gene-editing for gene knockout studies [10]. PSCs are especially amenable for gene-editing, since they can be cultured in-vitro for a very long duration without

changing either their genotype or their phenotype. To date, three custom-engineered nuclease technologies have been developed. This technology shares a common background. That is, all introduced engineered nucleases are able to introduce double-strand breaks (DSBs) in the DNA, which trigger DNA repair either via an error-prone non-homologous end joining (NHEJ) or via the preferred route of precise homology-directed repair (HDR) [9].

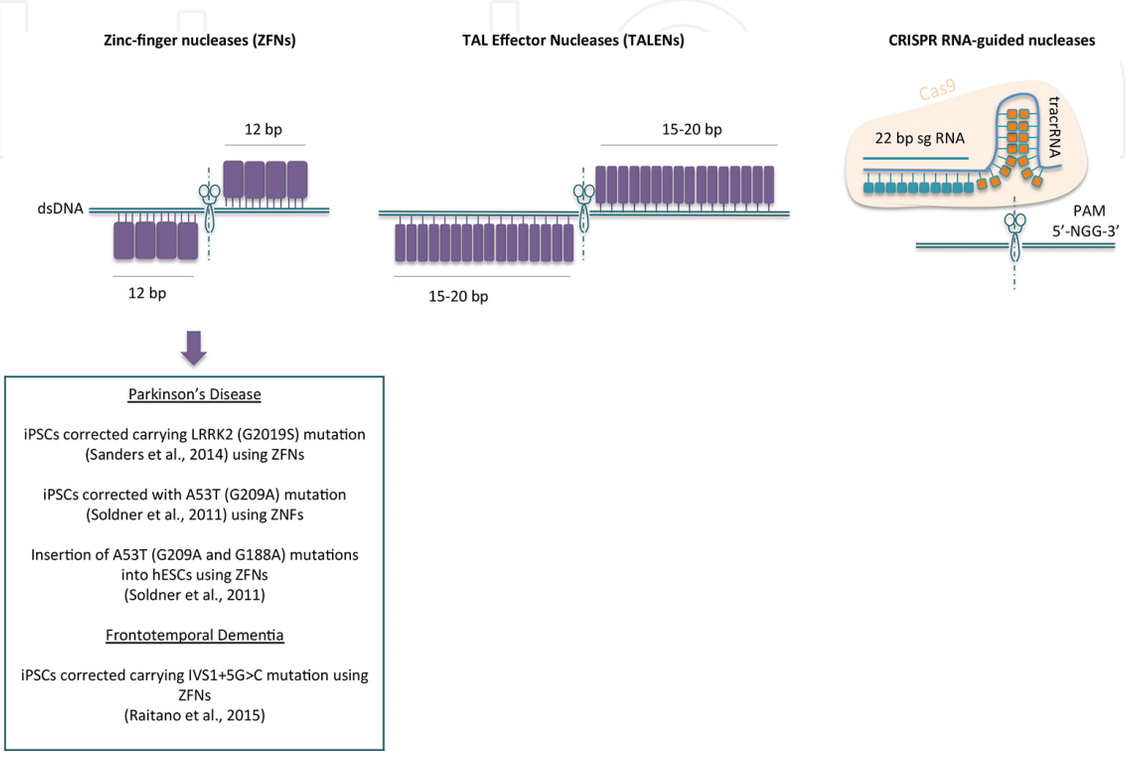



**Figure 1.** Three custom-engineered nuclease technologies exist, including zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENS) and clustered, regularly interspaced, short palindromic repeats (CRISPR) RNA-guided repeats, although only ZFNs have so far been used to correct point mutations in two neurodegenerative diseases.

The first generation of engineered nucleases produced were the zinc-finger nucleases (ZFNs), which were initially developed as chimeric restriction enzymes [11]. These are modular proteins containing a *FokI* endonuclease catalytic domain fused to several $Cys_2$-$His_2$ zinc finger (ZF) DNA-binding motifs [12]. They function as dimers, with each monomer consisting of a non-specific cleavage domain from the *Fok1* endonuclease, fused to a ZF array that is designed to bind to the target sequence of interest [13]. Each ZF domain consists of a 3bp subsite that can be constructed into monomers that recognize up to 24bp of the target site (**Figure 1**) [13]. Several studies have shown that ZFNs can target endogenous genes in both human ESCs and iPSCs with variable efficiency (from >1% up to 94%) [14]. In addition, they have been used to target and insert gene cassettes within the *AAVS1* locus in both human ESCs and iPSCs, which is a commonly targeted locus for long-term stable transgene expression in mammalian cells [14]. ZFNs can be designed and produced using two different methods, including modular assembly (mix-and-match combination of several individual pre-characterized ZFs) and cell-based selection using a public platform called 'OPEN' provided by the ZF consortium [15,

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16]. The length and design of the ZFs, as well as unwanted homodimer binding and cleavage, can result in off-target binding and cleavage of DNA, which can lead to unwanted alterations of the genome elsewhere. Thus, to overcome this, newer strategies have been developed such as 'obligate heterodimers', which include modified *Fok1* nucleases, which only cleave when heterodimers form [16] as well as another manipulated form of *Fok1*, called zinc finger nickases, which stimulate HDR and produce fewer off-target effects [17].

Another gene-editing tool is the transcription activator-like effector nucleases (TALENs). These are composed of a sequence-specific DNA-binding domain and a non-specific DNA cleavage module [18]. The DNA binding domain contains a series of tandem repeats comprising 33–35 amino acids, similar to tandem repeats first discovered in the plant pathogen *Xanthomonas* [19]. The DNA recognition is conferred by the highly variable amino acids at positions 12 and 13 [20]. Like ZFNs, TALENs form dimers on either side of the DNA strand and use the non-specific cleavage effect of the *Fok1* cleavage domain to produce a DSB (**Figure 1**) [20]. TALENS can be designed to almost any sequence due to their simple protein-DNA code. The only requirement is for the presence of thymine at each 5' end of the DNA recognition site [10]. TALENS generally also have fewer off-target cuts due to their longer recognition motifs and they are also less cytotoxic when compared to ZFNs, which is an attractive feature of this technology [10, 20]. To date, TALENs have been used for generating gene reporter lines, biallelic knock-out of genes and repair and introduction of point mutations in human PSCs [9].

The most recently developed method, which is even easier to use than TALENs and ZNFs is clustered, regularly interspaced, short palindromic repeats/Cas9-mediated genome-editing method (CRISPR/Cas9). This method consists of a specialized two-RNA structure containing CRISPR RNA (crRNA) and trans-activating RNA (tracrRNA), which are able to bind as a monomer to DNA strands next to a protospacer adjacent motif (PAM) composed of the sequence, 5'NGG'3 [18]. This chimeric RNA is known as a guide RNA (gRNA) and facilitates a DSB by guiding an endonuclease (first derived from *Streptococcus pyogenes* (Cas9)) to induce cleavage near the PAM site (**Figure 1**). The target recognition site is also typically 22 bp, which is shorter than the recognition site of TALENs. The CRISPR/Cas9 system has already been used successfully in gene-editing PSCs and has been shown to have a higher efficacy when compared to TALENs and ZFNs for gene knock-out studies [21]. It is also comparable to TALENs for HDR-mediated gene-editing of PSCs [22]. Importantly, for precise editing of point mutations (both deletions and insertions), dependence on HDR is required and use of an exogenous DNA template is needed such as single-stranded oligodeoxynucleotides or plasmid DNA templates [9]. An advantage of both TALENs and CRISPRs is that they do not leave a trace in the DNA, following the genome-editing process. Despite these advantages, there are some drawbacks in the case of CRISPRs. Some constraints exist in the target design, due to requirement for a PAM motif in the target site. Another potential disadvantage is the potential increased off-target binding and cleavage compared to TALENs, due to its monomeric action, shorter target sequences and greater chance of binding to identical target sites elsewhere in the genome [9, 23]. Like ZFNs, nickases have been effectively used to prevent off-target binding and cleavage [18].

To date, despite a surge in literature in gene-editing technologies, only three reports have been published that have led to the correction of iPSCs from patients with neurodegenerative diseases or alternately, insertion of disease-causing mutations into healthy PSCs. Two of these have been in the field of PD, and one in the field of FTD and all cases used ZFNs [24, 25] (**Figure 1**). In the case of PD, insertion of point mutations, A53T and G188A located in the gene, $\alpha$-synuclein (*SNCA*), which leads to familial onset of PD, was successfully achieved in ESCs [24]. In the same study, repair of the A53T and point mutation in *SCNA* mutation was also successfully performed in patient-specific iPSCs. Genome-wide analyses did not reveal any off-target effects following the ZFN targeting in all engineered lines. A follow-up study of the repaired A53T iPSCs showed that defects in dopaminergic neurons and mitochondrial dysfunction originally observed in the patient-iPSC lines could be reversed in the corrected iPSCs [26]. This mitochondrial dysfunction, which led to apoptotic death, was induced by nitrosative stress and could be directly linked with impaired functioning of the MEF2C-PGC1$\alpha$ pathway. This research was therefore unable to uncover a novel pathway, which could be used for the development of new drugs. Another study was able to correct the G2019S LRRK2 mutation in PD patient-specific iPSCs and rescued phenotypic and genotypic dysfunction [27]. This study was also able to find a new molecular pathway responsible for the phenotypes found in-vitro. In this case, ERK signaling was dysfunctioning and could be repaired through correction of the mutation. In the field of FTD, one recent study revealed that iPSCs derived from patients carrying a familial inherited mutation in the progranulin gene (*GRN*) were unable to form cortical neurons in-vitro. However, this phenotype could be restored when the mutation was corrected using ZFNs [28]. This helped to verify that the phenotype, which had not been described before, was directly related to the mutation and disease and not due to other unrelated, technical or cell culture factors.

Together, these studies show that gene-editing is a particularly helpful tool for modelling diseases with iPSCs, as well as for helping to determine new molecular pathways involved in the disease by repair of the mutation and assessment for amelioration of the phenotype. It is likely that an increase in similar studies will soon emerge for PD and FTD as well as other neurodegenerative diseases, such as AD in the near future.

## 3. Current approaches to developing neural cells in a dish

Investigations on iPSC-derived neural cells can be performed either in two-dimensional (2D) models or three-dimensional (3D) models. Traditional stem cell research has been performed in 2D, predominantly by culturing cells that adhere to a plastic surface, and which form a flattened monolayer across the plastic surface. The advantage of this technique is that it is low cost and an easy system to use. Stem cells, however, can alternately be cultured in 3D. One popular 3D method is to culture cells in small spheres in suspension within the media, termed either 'embryoid bodies', or to differentiate them into neural progenitor cells that also cluster together in small spheres, termed 'neurospheres'. These can be further differentiated into more mature neural cell types, which are often termed 'engineered neural tissue' or 'organoid' cultures. The cells can be cultured as described in aggregates, but can also be cultured in either

http://dx.doi.org/10.5772/63521

the presence of microcarriers, on alginate microencapsulates, in thermoreversible hydrogel or in scaffolds [29]. It is accepted today that neural stem cells (NSCs) isolated from primary tissue from foetal tissue or brain differ to neurospheres differentiated from EBs, as the former spheres tend to contain radial glial-like stem cells that are unable to form complex neural tissues such as the layered cortical neuroepithelium and complex pattern formations [30]. In contrast, the stem cell-derived neurospheres can be instructed to form specific neural regions of the developing brain when exposed to potent mitogens/morphogens [30]. Alternately, neural cells can also be cultured within artificially produced 3D scaffolds or in microwells formed within the plastic substrate that help to re-create a microenvironmental cue for the cells to form in 3D clusters. In fact, 3D cultures originated in the NSC field in the early 1990s when the first suspension cultures of rodent brain NSCs was performed [31], and have become a standardized way of culturing NSCs in-vitro in labs across the world. Today, there are several types of 3D scaffolds available, including metal, synthetic organic types made from polymers, synthetic inorganic materials, natural organic materials, natural inorganic material types and even nanostructure scaffolds [29]. All of these have their advantages. For example, microcarrier systems allow for good diffusion properties and induce cells of high quality. Also, encapsulated cells in gels allow them to be protected from shear force-induced cell death, and thermoreversible hydrogel allows for rapid expansion of cells [29].

Comparative studies of 2D versus 3D cultures suggest that 3D culturing may improve the quality of the cell expression profile of the cultured cells as cells are influenced by the biochemical, mechanical and physical surface properties of the surrounding matrix in which they normally reside [32]. One such comparative study showed neural-derived ESCs expressed more neural markers and greater neurite outgrowth when cultured in a 3D scaffold than the equivalent neural cells cultured in 2D [33]. Furthermore, timing in differentiation appears to differ between 2D versus 3D cultures. In fact, stem cells appear to differentiate earlier in 2D culture when compared to culture in extracellular matrix gel or as spheres, shown by the earlier upregulation of differentiation markers [34]. Whether this is abnormal or not has not yet been determined. Cell size and proliferation can also be altered by culture in 3D. One study has illustrated human ESCs cultured in 3D within microwells were smaller in size and divided more slowly compared to equivalent cells grown in 2D [35].

There has also been a recent surge in developing 3D models that better recapitulate the 3D complexity of the tissue in the body and which contain several cell types. The recent discovery that a human foetal-like brain could be recapitulated in the dish after culture of neural stem cells for several weeks was a remarkable discovery. This tissue was termed as a 'cerebral organoid' and was formed by embedding neural aggregates into Matrigel® droplets and culturing these in a bioreactor for 75 days [36]. The tissue contained both early-born and late-born cortical neurons, suggesting more complex cortical neural development could be recapitulated in-vitro. It also contained interneurons, suggesting a mix of different progenitor origins were present in the tissue. Other researchers have also produced complex neural tissue with cortical layer patterning in 3D neural cultures, which depict both proliferative cell populations and post-mitotic cortical cell types; however, the complex stratification of the cortical layers has not yet been replicable [30]. The addition of extracellular matrix molecules

to both the substrate of 2D and within 3D culture systems may also be particularly advantageous for the growth and cellular expression of neural cell types, as shown by Lancaster and colleagues where neural aggregates were cultured in Matrigel® droplets [36].

There appears to be improvement in the cellular expression and cell function when cultured in 3D, as well as other physical and changes in size and growth. However, some drawbacks in using 3D scaffolds are the difficulties of performing molecular analyses on the tissue, which are related to problems in extracting the cells from the scaffolds or light refraction that emanate from the scaffold structures and which interfere with fluorescence microscopy. In addition, there are also seeding issues related to the complexity of some scaffold structures. Furthermore, 3D culturing is more labour-intensive and can also be difficult to scale up. Bioreactors help, in part, to solve this issue when cells are grown in spheres or small scaffolds and they also help through their spinning properties to distribute medium evenly throughout the culture. Use of bioreactors, however, requires extensive volumes of media, which can be costly when large volumes of cytokines or growth factors are required in the culture medium.

Despite the given advantages in use of 3D culture over 2D, disease modelling studies for AD, PD and FTD using PSCs have been performed using 2D cultures. One interesting research article relevant for AD demonstrates the advantages of producing 3D cultures. Choi and colleagues showed that transgenic human NSCs overexpressing either *APP* or *PSEN1* could be differentiated into 3D tissue, which contained the classical hallmarks of the disease, including amyloid plaques and aggregates of phosphorylated tau (p-tau) [37]. These hallmark pathologies have not yet been demonstrated in iPSC models of AD. What seems apparent is that 3D modelling may recapitulate the in-vivo environment better. Use of more complex models might be better for modulating and studying the brain and the disadvantages of 3D modelling described are outweighed by the advantages.

## 4. Modelling Alzheimer's disease using pluripotent stem cells

Alzheimer's disease is the most prevalent type of dementia, which in most cases (approximately 90%) arises in patients with no known genetic link. However, some risk factor genes (e.g. presence of the allele ε4 of apolipoprotein E4 (*APOE* ε4) appear to play a role in more than half of these cases [38]. See section under "Sporadic cases with mutations in APOE", for more details. The disease induces loss of memory and impairs cognitive function, but is also known to induce loss in olfaction, hearing and even some motor function [39]. Depression, agitation, apathy, social withdrawal, insomnia, delusion and emotional/physical outbursts are all typical symptoms associated with AD. Ultimately, the disease culminates in loss of respiration function, leading to death. The disease is induced by protein aggregation and has two distinct histopathological signatures, which lead to neural degeneration of the tissue. The first includes the development of extracellular amyloid plaques, which are clusters of overproduced toxic forms of amyloid-beta (Aβ) peptides, deposited outside of the cell. The common theory accepted today is that these plaques develop first, prior to the development of the second signature, the intracellular neurofibrillary tangles (NFTs), which are composed of a predominant protein, tau in a hyperphosphorylated state. The disease spreads throughout the patient's

brain and is present pathologically years before the first symptoms appear. It arises first in the periallococortical transentorhinal region of the temporal mesocortex before spreading to the entorhinal cortex and then the hippocampus before later spreading to many regions of the brain, including the temporal neocortex, the insular cortex, the medial temporal gyrus and superior temporal gyru and then the occipital lobe [40]. Since many parts of the brain are affected, modulating the disease in a dish is a difficult task. In addition, the likelihood of the success of stem cell therapy is very low considering the widespread nature of the pathology. Instead, there is more interest in understanding AD pathogenesis and for developing new and more effective therapies by using PSCs [41]. Most researchers model the cortical tissue, which is affected later on in the disease; however, NSCs derived from PSCs have also been studied. These progenitor cells are attractive as they might reveal early mechanisms of the disease.

### 4.1. Sporadic AD with no known genetic mutations

The main pathology of sporadic AD is typical for the disease with the accumulation of toxic forms of Aβ peptides and tau protein. However, other proteins are also known to accumulate, including *SNCA*, *TDP-43* and *ACTIN* which form Lewy-like bodies and Hirano bodies [42]. In sporadic AD, the symptoms occur later than in the familial forms [42]. However, the complexity and heterogeneity of both symptoms and pathology are widespread in sporadic AD, which may be due to multiple unknown risk factors, mutations in varying genes which have not yet described, but potentially also the diverse effects of environmental factors and potential interaction with genes [42]. To date, three studies have been performed to modulate sporadic AD using patient-specific iPSCs. From these studies, no clear pathology for altered processing APP was observed. The patient iPSC-derived neurons did not reveal a change in the levels of either Aβ40 or Aβ42 [43, 44]. Tau pathology was reported only from one of the studies, with increased p-tau and enlarged endosomes observed in neurons, but only in one of the analysed patient's cell lines [43]. Other pathology, however, was reported. One study found altered WNT signalling and glutamate metabolism in mixed cell cultures from one patient, as well as, gene expression changes related to proteosome function, reactive oxygen species (ROS) and cell death [45]. Another study found elevated oxidative stress-related gene expression and also elevated ROS in the iPSC-derived cortical neurons [44]. Interestingly, this study that consequently found no altered expression in Aβ expression in the neurons found elevated Aβ in iPSC-derived astrocytes.

### 4.2. Sporadic cases with mutations in APOE

The polymorphism of the *APOE* gene is a risk factor for the disease and presence of the ε4 allelle (*APOE4*) has been linked with AD [42]. One copy of this allele increases the risk of AD twofold, whereas two copies increases the risk of AD by 12-fold [46]. The ε4 allele has been shown to be less efficient in transporting cholesterol from neurons [42]. One iPSC study performed on patients with a APOε3/ε4 genotype found elevated Aβ42 in neurons derived from two of the three patients studied. Two of the patients also showed increased cell stress following glutamate-induced excitation [46]. No investigation of cholesterol transport was performed, which could have been useful to confirm this specific phenotype related to *APOE*

dysfunction. There are also no iPSC studies to date performed on patients with the Apoε4/ε4 genotype.

## 5. Familial AD

Pathology common to all familial mutations includes an earlier onset of the disease and increased amyloid plaque formation when compared to sporadic AD in many, but not all cases [47]. Plaques tend to predominantly contain Aβ42 with often no increase in Aβ40 observed, contrasting that seen in sporadic AD [47]. A summary of the PSC-derived neural cell pathology and comparative pathology known in familial AD patients is summarized in **Table 1**.

| Gene | Mutation | Pathology in Patients | Study | Pathology in PSCs | Study |
|---|---|---|---|---|---|
| ***PSEN1*** | A246E (ex7) | Unknown | | Increased Aβ42:Aβ40<br>No increase in Aβ42 | Yagi et al., 2011; Liu et al., 2014; Mahairaki et al., 2014<br>Duan et al., 2014 |
| | D385N | Unknown | | Decreased Aβ40 | Koch et al., 2012 |
| | L166P (ex6) | Amyloid plaques<br>Cotton wool plaques | Moehlmann et al., 2002 | Decreased Aβ40 | Koch et al., 2012 |
| | H163R (ex6) | Increased Aβ42<br>Lewy bodies<br>Atrophy of substantia nigra and cerebral cortex<br>Cotton wool plaques<br>Corticospinal degeneration<br>SNCA deposits<br>Neurofibrillary tangles<br>Amyloid plaques | Ishikawa et al., 2005,84 | Increased Aβ42:Aβ40 | Liu et al., 2014 |
| | M146L (ex5) | Amyloid plaques<br>Pick bodies | Halliday et al., 2005 | Increased Aβ42:Aβ40 | Liu et al., 2014 |
| ***PSEN2*** | N141I | Increased Aβ42:Aβ40<br>*SNCA*deposits<br>Amyloid Plaques<br>Neurofibrillary tangles<br>Some cases with Lewy body pathology in frontal cortex and amydala | Levy-Lahad et al., 1995; Rogaev et al., 1995<br>Jayadev et al., 2010 | Increased Aβ42:Aβ40 | Yagi et al., 2011 |

| Gene | Mutation | Pathology in Patients | Study | Pathology in PSCs | Study |
|---|---|---|---|---|---|
| ***APP*** | duplication | Intercerebral haemorrhage,<br>Diffuse brain atrophy<br>Cerebral ventricular dilation,<br>Intraneural Aβ40<br>Cerebellar purkinje cell atrophy<br>Amyloid plaques | Cabrejo et al., 2006 | Increased Aβ40<br>Increased p-tau (Thr231) | Israel et al., 2012<br>Israel et al., 2012 |
| | E693Δ | Early stages—limited brain atrophy and little accumulation of Aβ.<br>Cerebellar ataxia<br>Aβ oligomers<br>No plaques | Shimada et al., 2011 | Decreased Aβ40 and Aβ42<br>Elevated Aβ oligomers<br>Oxidative stress | Kondo et al., 2013 |
| | V717L | Unknown | | Increased Aβ42:Aβ40<br>Oxidative stress | Kondo et al., 2013 |
| | V717I | Tau pathology<br>Neurofibrillary tangles<br>Plaque neurites<br>Neuropil threads<br>Cortical and subcortical Lewy bodies | Lantos et al., 1992 | Increased Aβ42:Aβ40<br>Increased Aβ38<br>Increased total tau<br>Increased p-tau (S262) | Muratore et al., 2014 |

Abbreviations: Aβ - amyloid beta; ex - exon; p-tau - phosphorylated tau; *SNCA* – alpha synuclein gene

**Table 1.** Pathology in patients with familial Alzheimer's disease patients and respective pluripotent stem cell (PSC) studies.

### 5.1. PSEN1 mutations

Over 170 mutations in *PSEN1* have been described, making this the most common cause of autosomal dominant early onset AD [48, 49]. Patients with mutations in the *PSEN1* locus have the earliest age of onset (AOO). These patients often have seizures, myoclonus, paraparesis and cerebeller signs [50]. For all *PSEN1* cases, classic hallmark pathologies are observed, including amyloid plaques, NFTs, tissue atrophy, neuronal loss and inflammation [47]. However, pathological differences do exist depending on the gene affected, and even depending on the location of the mutation within the gene. For example, Lewy bodies and other parkinsonian pathologies have been described in *PSEN1* H163R carriers, and pick bodies have been described in M146L carriers (see **Table 1**). Pathological mechanisms common to both *PSEN1* and *PSEN2* are altered Aβ peptide metabolism induced by disrupted γ-secretase cleavage, which results in increased Aβ42 production. In some *PSEN1* mutations, severe neurodegeneration has been described without any Aβ pathology, which contradicts the hypothesis that amyloid pathology arises prior to tau pathology. Other pathological differences described include some plaques containing predominantly Aβ40. These Aβ40 plaques have

been observed in the cortex of some *PSEN1* mutation patients [47]. Studies have also highlighted that soluble and insoluble levels of Aβ42 is higher in familial AD brain tissue compared to sporadic AD [47]. It is thus apparent that given the diversities in the patient's pathology, a similar diversity in iPSC-derived neurons might also be evident.

The majority of iPSC lines modelling AD have in fact been produced from patients carrying mutations in *PSEN1*. These studies have revealed that most of the neurons generated from *PSEN1* AD patients also have increased Aβ42 [51–54], although decreases in Aβ40 have also been described in two particular mutations [55]. However, little other pathology has been described. In one study, no tau accumulation or tangle formation was observed [51]. One other study has reported small changes in gene function in *PSEN1* mutant iPSC-derived neurons, including increased *NLRP2*, *ASB9* and *NDP* [53]. In addition, overexpression of *PSEN1* in human ESCs led to increased Aβ42/Aβ40 and Aβ43/Aβ40 ratios as well as synaptic dysfunction in neurons expressing NeuN and BIII-tubulin [56].

### 5.2. PSEN2 mutations

There are 23 known DNA variants reported in the *PSEN2* gene. Patients with mutations in *PSEN2* have a delayed AOO, suffer from disorientation and endure a long duration of the disease [50]. Pathologically, similar to *PSEN1*, mutations affect Aβ peptide metabolism by γ-secretase cleavage which results in Aβ42 production.

To date, only one study has investigated the pathology from neurons derived from a patient carrying the N141I mutation in *PSEN2*. This study reported increased Abβ2:Aβ40, but no tau accumulation or NFT formation [51]. Interestingly, some patients with this mutation do also have Lewy body pathology and SNCA deposition within neurons [49]. Clearly, more research is required to investigate Aβ metabolism, γ-secretase function and whether neural atrophy, inflammation, tau, NFT and even parkinsonism pathology can be observed in-vitro from patient cells carrying this mutation.

### 5.3. APP mutations

Patients with *APP* mutations frequently are more aggressive and those with an *APP* duplication frequently have apraxia [50], cerebellar dysfunction and some cases have cerebral haemorrhaging [57]. Different *APP* mutations induce neural death by different mechanisms. For example, some mutations induce an increased production of AICD and other C-terminal APP fragments directly regulate apoptosis [47]. Other mutations affect intracellular mechanisms which increase oxidative stress and death [47]. Studies have also highlighted that soluble and insoluble levels of Aβ42 are reportedly also higher in *APP* mutation cases compared to sporadic AD cases.

Three studies have investigated the pathology in neurons derived from patients carrying mutations in *APP*. These studies have conflicting results in relation to *APP* processing, likely related to diverse pathology dependent on the mutation. One study found elevated Aβ40 [43], another study found one patient had increased Aβ42 whilst a second patient had decreased Aβ40 [44], and a third study revealed a patient with both increased Aβ42 and Aβ38 [58]. Two

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of these studies showed good correlation with known patient pathology, whilst the study reporting increased Aβ42:Aβ40 levels cannot be validated to the patient pathology since this mutation has not been well characterized. One study also reported an increase in Aβ oligomers in the analysed neural cells and astrocytes [44], which again correlates well with the patient's phenotype [59]. Increased p-tau has been observed in patients from two different studies [43, 58] and total tau has also been reported [58]. Increased αGSK-3β has also been reported [43]. No studies have looked at AICD function; however, oxidative stress has been partially investigated in one study, which revealed elevated ROS and oxidative stress-related genes in the cortical neurons and also elevated ROS in astrocytes [44]. No pathology related to cerebellar dysfunction or parkinsonism-related pathology described in some of these mutations has been reported.

## 6. Other models of AD

### 6.1. Trisomy 21

Trisomy 21 (also known as Down's syndrome) results in the duplication of the APP gene, which is located on chromosome 21. People with this syndrome develop symptoms and pathology early in life, which are strikingly similar to AD [60]. The extra copy of APP is

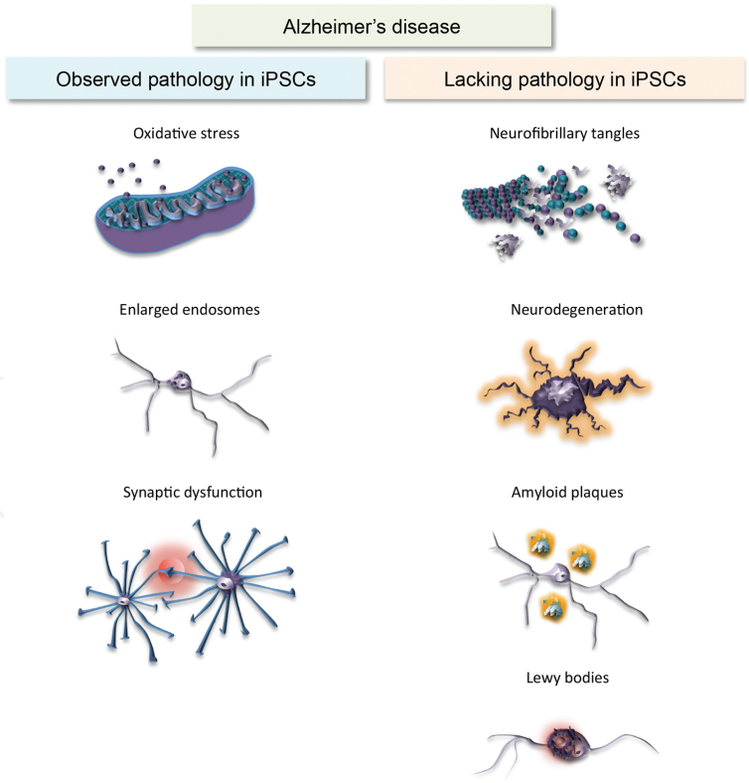



**Figure 2.** A summary of pathology observed in published induced pluripotent stem cell (iPSC) models of Alzheimer's disease (AD) and pathology found in AD patients but currently lacking in the iPSC models.

considered the major factor in the AD-like symptoms and, in addition, duplication of Dyrk1A kinase (which is also located on chromosome 21), which phosphorylates tau, may also contribute to the pathology and symptoms [60]. Increased Aβ peptides can be observed in early childhood which are the main candidate thought to induce the early onset of dementia [60]. Since duplications of APP are observed in AD patients, trisomy 21 has also been used to model AD in many studies. One study has produced iPSCs from patients with trisomy 21 and neurons derived from the iPSCs showed perturbed Aβ processing, including increased Aβ40 and Aβ42 in long-term cultured neurons, as well as Aβ42 intracellular and extracellular aggregates [60]. Furthermore, this study also reported increased p-tau and total tau, as well as increased cell death [60]. This is the only study to date which reports cell death in an iPSC model of AD, which suggests this may be a relevant and worthy model of APP duplication and study of AD-like dementia.

To conclude, iPSCs from AD models tend to show early features of the disease in the dish, rather than distinct histopathological hallmarks (**Figure 2**). The most common observations include altered expression levels of Aβ and increased levels of tau. It might be that the main pathological hallmarks only develop after many years of protein aggregation and build-up in the cell.

## 7. Modelling frontotemporal dementia using pluripotent stem cells

Frontotemporal dementia accounts for a large proportion (50% of dementia cases that arise before the age of 60, and is the second most common early-onset dementia). This disease is characterized by the progressive loss and degeneration of the cortical neuron population, in the frontal and temporal lobes of the brain. Common symptoms include altered behaviour and deterioration in both speech and cognition [61, 62]. This disease has a much stronger genetic link than AD and PD. Approximately 40% of the cases are attributed to mutations in one of three genes, including microtubule-associated protein tau (*MAPT*), progranulin (*GRN*) and *C9ORF72* [28]. However, many other genes carrying mutations have also been linked to the disease, including, valosin-containing protein (*VCP*), charged multivesicular body protein 2B (*CHMP2B*), ubiquilin 2, transactive response DNA-binding protein (*TDP-43*), dynactin (*DCTN1*) and fused in sarcoma (*FUS*) [63]. The large genetic diversity is reflected by diverse symptoms and pathology amongst the patients that differ from the common symptoms. Hence, the disease is stratified into a behavioural variant of FTD (bvFTD), two language variants (semantic dementia and progressive nonfluent aphasia (PNFA)) and an overlap of these with atypical parkinsonian disorders corticobasal syndrome (CBS) and progressive supranuclear palsy (PSP) [63]. In addition, in some cases of FTD, shared pathological features with motor neuron disease (MND)/amyotrophic lateral sclerosis (ALS) are observed, including the accumulation of proteins TDP-43 and FUS [63]. Thus, another variant (FTD-MND/ALS) is described and is due to mutations in *VCP* and *CHMP2B, C9ORF72* and *UBQLN2*, which can lead to the development of either FTD or ALS [64]. A summary of the familial FTD studies modelled using PSCs and respective known pathology from the patients is shown in **Table 2**.

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| Gene | Mutation | Pathology in Patients | Study | Pathology in PSCs | Study |
|---|---|---|---|---|---|
| ***C90RF72RF72*** | expansion repeat | Brain atrophy<br>Hippocampal sclerosis<br>TDP-43 accumulation<br>Argyrophilic grain disease in limbic areas and orbital frontal cortex<br>Tau pathology<br>Neurofibrillary tangles<br>Atrophy also in substantia nigra, brain stem and spinal cord<br>Lewy bodies<br>RNA foci | Shinagawa et al., 2014<br>DeJesus-Hernandez et al., 2011 | Cellular stress<br>RNA foci<br>Glutamate excitotoxicity | Almeida et al., 2013<br>Almeida et al., 2013; Donnelly et al., 2013<br>Donnelly et al., 2013 |
| ***MAPTT*** | ex10 N279K FTDP-17-1 | Hyperphosphorylated tau in DA neurons and glia and in brain stem and temporal cortex<br>Neurofibrillary tangles<br>Increased 4R tau isoform | Ehrlich et al., 2015; Wren et al., 2015 | Increased expression of 4R tau isoform, increased tau fragmentation<br>Neurite shortening<br>Oxidative stress<br>Cellular stress<br>Enlarged vesicles<br>Early maturation<br>Altered axonal mitochondrial transport | Ehrlich et al., 2015: Iovino et al., 2015<br>Ehrlich et al., 2015<br>Wren et al., 2015<br>Iovino et al., 2015 |
| | Ex12 V337M FTDP-17-2 | Frontotemporal atrophy<br>Moderate parietal cortical atrophy<br>Hippocampal atrophy<br>Astrogliosis<br>Atrophy of substantia nigra<br>Tau pathology | Domoto-Reilly et al., 2016 | Increased tau fragmentation<br>Neurite shortening<br>Oxidative stress | Ehrlich et al., 2015 |
| | P301L | Frontotemporal atrophy<br>Astrogliosis<br>Atrophy of substantia nigra<br>Tau pathology<br>Pick bodies | Spillantini et al., 1998 | Early maturation<br>Altered axonal mitochondrial transport<br>*SNCA* deposition<br>4R tau | Iovino et al., 2015 |
| ***GRN*** | IVS1+5G>C | Frontotemporal atrophy<br>Caudate nucleus atrophy<br>Substantia nigra atrophy<br>Gliosis | Brouwers et al., 2007 | Impaired corticogenesis<br>Impaired WNT signalling | Raitano et al., 2015 |

| Gene | Mutation | Pathology in Patients | Study | Pathology in PSCs | Study |
|---|---|---|---|---|---|
| | | Ubiquitin inclusions containing TDP-43<br>Lewy bodies<br>Amyloid plaques | | | |
| | S116X | Unknown | | Cellular stress | Almeida et al., 2012 |
| *TARDBP* | A90V | Unknown | | Staurosporine-induced cellular stress | Zhang et al., 2013 |
| | M337V | TDP-43 accumulation | Tamaoka et al., 2010 | Decreased survival | Bilican et al., 2012 |

Abbreviations: *SNCA* – alpha synuclein gene; TDP-43 – TAR DNA-binding protein 43 gene

**Table 2.** Pathology in familial frontotemporal dementia patients and respective pluripotent stem cell (PSC) studies.

### 7.1. Sporadic FTD

Sporadic FTD has been modulated in-vitro by two independent studies to date [65, 66]. Brain atrophy is greater in the anterior cingulate compared to familial FTD cases [67]. In one study, no characterization of the phenotype was performed. Another study also characterized patient iPSCs from a sporadic case of FTD and the cultured neurons showed greater cellular stress and oxidative stress compared to control cells [66]. Whether all cases of sporadic FTD are in fact undiscovered genetic mutations is a distinct possibility, since there have been many recent discoveries of gene mutations lying behind the disease [68].

### 7.2. C90RF72 mutations

A GGGGCC repeat expansion in the noncoding region of *C9ORF72* is a common mutation for both FTD and ALS, representing approximately 40% of FTD cases and familial ALS [69]. This mutation leads to specific pathology including non-ATG-initiated translation of RAN peptides [69] and formation of nuclear RNA foci which lead to bvFTD with diverse phenotypes, even within the same family [68]. The AOO appears to be earlier in carriers of this mutation and have higher prevalence of delusional psychotic symptoms and hallucinations [70].

Two studies have produced patient-specific iPSCs from patients carrying this mutation. Characterization of the neurons derived from the iPSCs revealed they were more susceptible to cellular stress compared to control neurons [64]. In addition, RNA foci were observed in the in-vitro produced neurons [69], but there is controversial evidence that suggests patient neurons have these [64]. Both studies also showed cytoplasmic expression of RAN. Of interest was one other study that used patient-specific iPSCs to discover potential binding partners, e.g. ADARB2 to the expanded repeat region in an attempt to discover more about the mechanisms that lead to disease onset with this mutation [69] and to find out more about the gene's actual function.

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### 7.3. MAPT mutations

Mutations in *MAPT* account for approximately half of the familial cases of FTD and tend to present symptoms typical of FTD, but may also include Parkinson's disease-like symptoms; therefore, these patients are termed frontotemporal dementia with parkinsonism related to chromosome 17 tau (FTDP-17T). There are at least 50 family kindreds carrying mutations in this gene, which includes nine missense mutations, one deletion mutation, two transition mutations within exons 9, 10, 12 and 13 and five intronic mutations leading to alternate splicing of exon 10 [71]. The missense mutations give rise to pathology very similar to AD and include the formation of NFTs, whereas those that lead to alternatively spliced exon 10 show progressive PSP, corticobasal degeneration and Pick's disease [71]. The N279K substitution is an intronic mutation and one of the most common types of FTDP-17T mutations, along with the mutation *P301L* [72]. The N279K mutation leads to overproduction of tau protein isoforms containing four tandem microtubule-binding domain repeats (4R-tau) which induces disease pathogenesis, including accumulation of neurotoxic tau aggregates and NFTs in both neurons and glia [73]. In addition, MAPT mutations (in particular the N279K mutation) are also associated with degeneration of the basal ganglia and depigmentation of the substantia nigra [71].

Two eloquent studies have generated iPSCs from patients carrying the N279K mutation and compared the in-vitro pathology directly to pathology in the deceased patient brain. Here, the cultured neurons had increased expression of the 4R tau isoform and fragmentation of tau [74], which helped to confirm the phenotype; however, neurite shortening, oxidative stress [74], cellular stress and enlarged vesicles [72] were also observed in the cultured neurons. The NFTs, however, were not able to be recapitulated in-vitro. Another study reported similar pathology in iPSC-derived neurons also carrying the N279K mutation [75]. In addition, this same study also compared the N279K iPSCs to iPSCs carrying the *MAPT* mutation P301L and found that they shared some cellular phenotypes and differed in others [75]. Specifically, neurons from both mutation backgrounds matured earlier compared to controls and had altered axonal mitochondrial transport, whereas the P301L iPSCs showed SNCA and 4R tau deposition in varicosity-like structures in the neurons.

### 7.4. GRN mutations

Patients carrying mutations in *GRN* tend to display Parkinson-like symptoms. Haploinsufficiency of GRN induces the disease and typically does not present with tau pathology, but instead, patients have cytoplasmic ubiquitin inclusions and intranuclear inclusions, comprising of TDP-43 both in neurons and in microglia [76]. They are often characterized as frontotemporal lobar degeneration (FTLD-TDP) and generally increased expression of *GRN* is associated with pathogenesis. However, patients tend to have varying expression of *GRN* in the brain at late stages of disease [77]. In another study, neurons were characterized from a patient carrying a novel nonsense mutation in *GRN* (S116X) [66]. The patient had Parkinson-like symptoms. The neurons were found to be more susceptible to cellular stress and oxidative stress compared to control neurons, which could be reversed upon induced expression of *GRN*

[66]. A follow-up study on the same neurons used these neurons to test different approaches of rescuing *GRN* expression as a way of finding new tools to treat the disease [78].

### 7.5. TARDBP mutations

Mutations in the *TARDBP* gene tend to result in aggregation and mislocalization of its protein TDP-43 to the cytoplasm [79] and many cases can be characterized as semantic dementia [80]. One study has produced patient-specific iPSCs carrying a M337V *TARDBP* mutation, and found during long-term culture that these cells had decreased cell survival [81]. A later study on these same cell lines found that application of two different autophagy-inducing molecules (which help to clear the accumulating TDP-43) could help to increase cell survival in these patient iPSCs [82]. Another study produced iPSCs from patients carrying the A90V mutation, and here cellular stress could be detected in the cells following exposure to staurosporine [83]. No other pathology was reported.

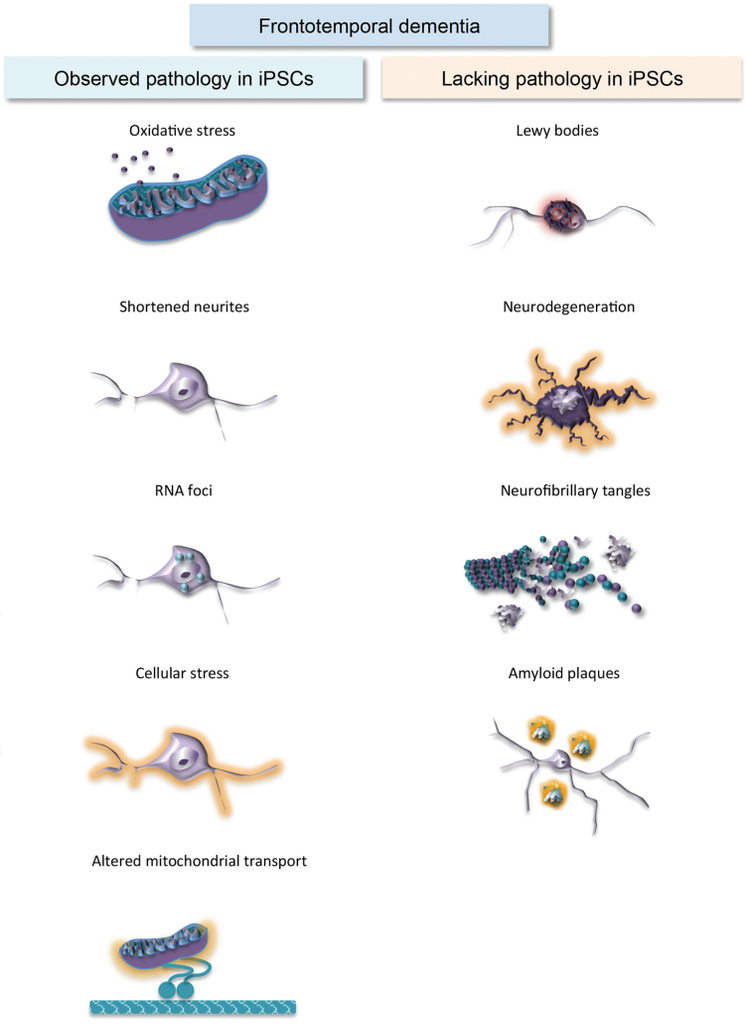



**Figure 3.** A summary of pathology observed in published induced pluripotent stem cell (iPSC) models of frontotemporal dementia (FTD) and pathology found in FTD patients but currently lacking in the iPSC models.

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To date, only a fraction of the familial FTD mutations have been modelled using PSCs. Given the expanse of different mutations that exist as well as the broad pathology from each of the FTD variants, as well as within the variants themselves, it is important that the in-vitro studies can be correlated to the known pathology in the patients. Many different phenotypes can be observed in the dish, but the classical hallmarks appear to be missing in modelling the disease using iPSC-derived cells (**Figure 3**). How this might be improved upon is discussed more at the end of the chapter in the section under "Current limitations in modelling neurodegenerative disease using pluripotent stem cells".

## 8. Modelling Parkinson's disease using pluripotent stem cells

Parkinson's disease is the second most common neurodegenerative disease, which is both sporadic and monogenic in form. The inherited monogenic form accounts for the minority of cases with approximately 5–10% of presented cases. The genetic contribution to Parkinson's disease has been firmly characterized in the past few years to be directly induced by over 15 mutations in PARK loci, which are located within six genes: *SNCA*, *LRRK2*, *PARK2*, *PINK1*, *PARK7* and *ATP13A2*. Furthermore, several genetic risk factors are linked to the onset of the disease [84]. Dependent on the gene affected, the disease may initiate in juveniles, early in adult life or in late adult life. Some of these genes are autosomal dominant (*SNCA* and *LRRK2*), whilst the others are autosomal recessive (*PARK2*, *PINK1*, *PARK7* and *ATP13A2*).

The common idiopathic features of the disease are motor disturbances including resting tremor, rigidity and bradykinesia, as well as non-motor symptoms such as cognitive impairment, autonomic dysregulation, sleep deterioration and neuropyschiatric symptoms [84]. These symptoms and disturbances arise due to the loss of nigrostriatal dopaminergic neurons in the substantia nigra pars compacta and the development of Lewy bodies in surviving neurons. This makes PD a particularly easy disease to modulate in a dish, as one predominant neuron type is affected. Despite this, there still lacks perfect differentiation protocols that result in the A9 type dopaminergic neuron in high proportion. Given the simplicity in the tissue affected by the disease, it has been considered that PSC-derived nigrostriatal dopaminergic neurons from healthy donors or genetically corrected iPSCs could be used for transplantation either into the striatum where they migrate to or in the substantia nigra where the cell bodies lie. In this case, many studies have attempted to improve the production and numbers of nigrostriatal dopaminergic neurons from PSCs [85]. A summary of the PSC studies that model familial PD in a dish are shown in **Table 3** along with known pathology in the patients.

| Gene | Mutation | Pathology in Patients | Study | Pathology in PSCs | Study |
|---|---|---|---|---|---|
| ***SNCAA*** | A53T (G209A) | Lewy body pathology | Golbe et al., 1990 | Oxidative stress<br>Mitochondrial dysfunction | Ryan et al., 2013 |

| Gene | Mutation | Pathology in Patients | Study | Pathology in PSCs | Study |
|---|---|---|---|---|---|
| | | | | Cell death | |
| | A53T (G188A) | | | | Soldner et al., 2011 |
| | Triplication | Lewy body pathology, hippocampal neuronal loss, temporal lobe vacuolation | Farrer et al., 2004; Muenter et al., 1998 | Increased $\alpha$-syn<br>Oxidative stress | Devine et al., 2011<br>Byers et al., 2011<br>Byers et al., 2011 |
| ***LRRK2*** | G2019S | Lewy bodies in brain stem<br>Lewy body + AD pathology<br>Transitional Lewy body disease<br>SN neuronal loss, tau pathology, + AD pathology<br>SN neuronal loss<br>Lewy body pathology | Giasson et al., 2006; Ross et al., 2006<br>Giasson et al ., 2006<br>Ross et al., 2006<br>Rajput et al., 2006<br>Gaig et al., 2007<br>Gilks et al., 2005 | Oxidative stress<br>Altered ERK signalling<br>Neurite shortening<br>Increased sensitivity to toxins<br>Increased expression of MAPT and p-tau<br>Mitochondrial DNA damage<br>Increased autophagy<br>Dysregulated epigenome | Nguyen et al., 2011<br>Reinhardt et al., 2013<br>Reinhardt et al., 2013; Sanchez-Danes et al., 2012<br>Reinhardt et al., 2013; Cooper et al., 2012<br>Reinhardt et al., 2013<br>Sanders et al., 2014<br>Sanches-Danes et al., 2012<br>Fernandez-Santiago et al., 2015 |
| | R1441C substitution | Not known | | Increased sensitivity to toxins | Cooper et al., 2012 |
| ***PARK2K2*** | del_40bp ex3/del ex4+5 | Not known | | Oxidative stress | Aboud et al., 2012 |
| | del_40bp ex3 | Lewy body pathology | Farrer et al., 2001 | | |
| | del_ex7 | Lewy body pathology | Pramstaller et al., 2005 | | |
| | del ex4 | SN neuronal loss, no Lewy bodies | Mori et al., 1998<br>Hayashi et al., 2000 | | |

| Gene | Mutation | Pathology in Patients | Study | Pathology in PSCs | Study |
|---|---|---|---|---|---|
| | del ex5 | Only clinical features known | | Proteosome dysfunction<br>Oxidative stress<br>Increased *SNCA* | Chang et al., 2016 |
| ***PINK1*** | del_ex7/c.1488+1 G>A | Lewy body pathology | Samaranch et al. 2010 | | |
| | c.1366C>T;p.Q456X | Only clinical features known | Hedrich, et al., 2006 | Oxidative stress | Seibler et al.,2011 |
| | c.509T>G;pV170G | | | | |
| | Q456X homozygote | | | Increased sensitivity to toxins<br>Oxidative stress | Cooper et al., 2012 |
| | D525N/W577R compound | | | | Cooper et al., 2012 |

Abbreviations: AD – Alzheimer's disease; del – deletion; ex – exon; p-tau – phosphorylated tau; *SNCA* – alpha synuclein gene; SN – substantia nigra

**Table 3.** Pathology in familial Parkinson's disease patients and respective pluripotent stem cell (PSC) studies.

### 8.1. Sporadic PD

One study that produced iPSC lines from seven patients with idiopathic PD revealed that dopaminergic neurons produced in-vitro had reduced numbers of neurites, neurite arbourisation and increased autophagy [86]. Another study observed methylation alterations in sporadic PD iPSC-derived dopaminergic neurons [87], which was the first to describe epigenetic dysregulation in the disease.

### 8.2. SNCA mutations

There are at least five mutations characterized within *SNCA*, which induce PD which also include either duplication or triplication of the gene. Mutations in *SNCA* lead to an early-onset parkinsonism with or without the development of dementia. The pathology generally contains the presence of Lewy bodies, and tau pathology has also been observed some of the mutations.

Induced PSC lines have been derived from PD patients carrying either a triplication of *SNCA* [88–90] or a mutation in A53T [24]. In two articles, triplication of *SCNA* led to increased production of *SNCA* [88, 89]. Oxidative stress has also been reported in iPSC-derived neurons containing triplication of *SCNA* [89] and A53T mutations [26]. This has been shown to contribute to mitochondrial dysfunction and apoptotic cell death [26].

### 8.3. LRRK2 mutations

There have been five identified mutations in the *LRRK2* gene and many of these have either brainstem-predominant Lewy body pathology, diffuse Lewy body disease or no Lewy body pathology. Three research groups have produced iPSCs from patients carrying two of the known mutations. One study found increased oxidative stress gene expression and increased production of SNCA protein [91]. One research group was able to uncover altered cell signalling of several genes, some of which were involved in ERK signalling; and repression of ERK signalling could reverse certain pathology in neurons including prevention of neurodegeneration, more cell robustness when treated with oxidative stress and a reversal in the shortening of neurites. Genetically corrected iPSCs also revealed a reversal in neurite shortening and sensitivity to neurotoxins that were observed in the patient iPSC-derived neurons [27]. Furthermore, this same study also found increased expression of *MAPT* expression and p-tau[181]. The *LRRK2* lines have also been used to more closely evaluate mitochondrial function. One study found increased mitochondrial DNA damage in neural cells from patients, which was reversed when the mutation was genetically corrected [25]. Similar to sporadic PD iPSC-derived dopaminergic neurons, it has been shown that epigenetic dysregulation also occurs in *LRRK2*-associated PD patients [87]. Interestingly, the commonly described Lewy body pathology has yet to be observed in the iPSC-derived neurons.

### 8.4. PARK2 mutations

Mutations in *PARK2* lead to an early-onset parkinsonism. Seven different mutations have been identified, and general pathology in most of the mutations does not include Lewy bodies. One study has evaluated the effects of manganese exposure on neural progenitor cells derived from *PARK2* mutation PD iPSCs and discovered increased ROS generation upon exposure in the patient cells compared to healthy controls [92]. Another study which evaluated iPSCs produced from patients with a deleted exon 5 in *PARK2* showed the iPSC-derived neurons had proteasome dysfunction, oxidative stress and increased expression of *SNCA* [93].

### 8.5. PINK1 mutations

A mutation in *PINK1* leads to early-onset parkinsonism. Here, Lewy body pathology has been identified. Two studies have produced patient-specific iPSCs harbouring mutations in *PINK1*. In one study, mitochondrial function was analysed and revealed increased mitochondrial copy numbers and increased expression of PGC-1$\alpha$ in the patients' in-vitro-produced dopaminergic neurons [94]. Another research article found *PINK1* mutation iPSCs were more vulnerable to chemical toxins. Mitochondrial perturbation was also discovered, with a lower basal oxygen consumption rate and an increased bidirectional motility of neurons in the proximal axon of neurons [95].

Together, the literature reveals a vast array of disease modelling studies using PSCs to model PD and a number of phenotypes have emerged in the dish although the classic hallmark, Lewy bodies and neurodegeneration, remain absent in the in-vitro cultures (**Figure 4**). Oxidative stress is a clear phenotype observed in many of the different studies and appears common for

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many of the mutations, but it is clear that the disease pathology is diverse and therefore the pathology in the iPSC-derived cells would also expect to be diverse.

## 9. Current limitations in modelling neurodegenerative disease using pluripotent stem cells

Modelling AD and FTD is definitely more challenging than modelling PD, since many different cell types and regions within the brain are affected by these diseases compared to PD.

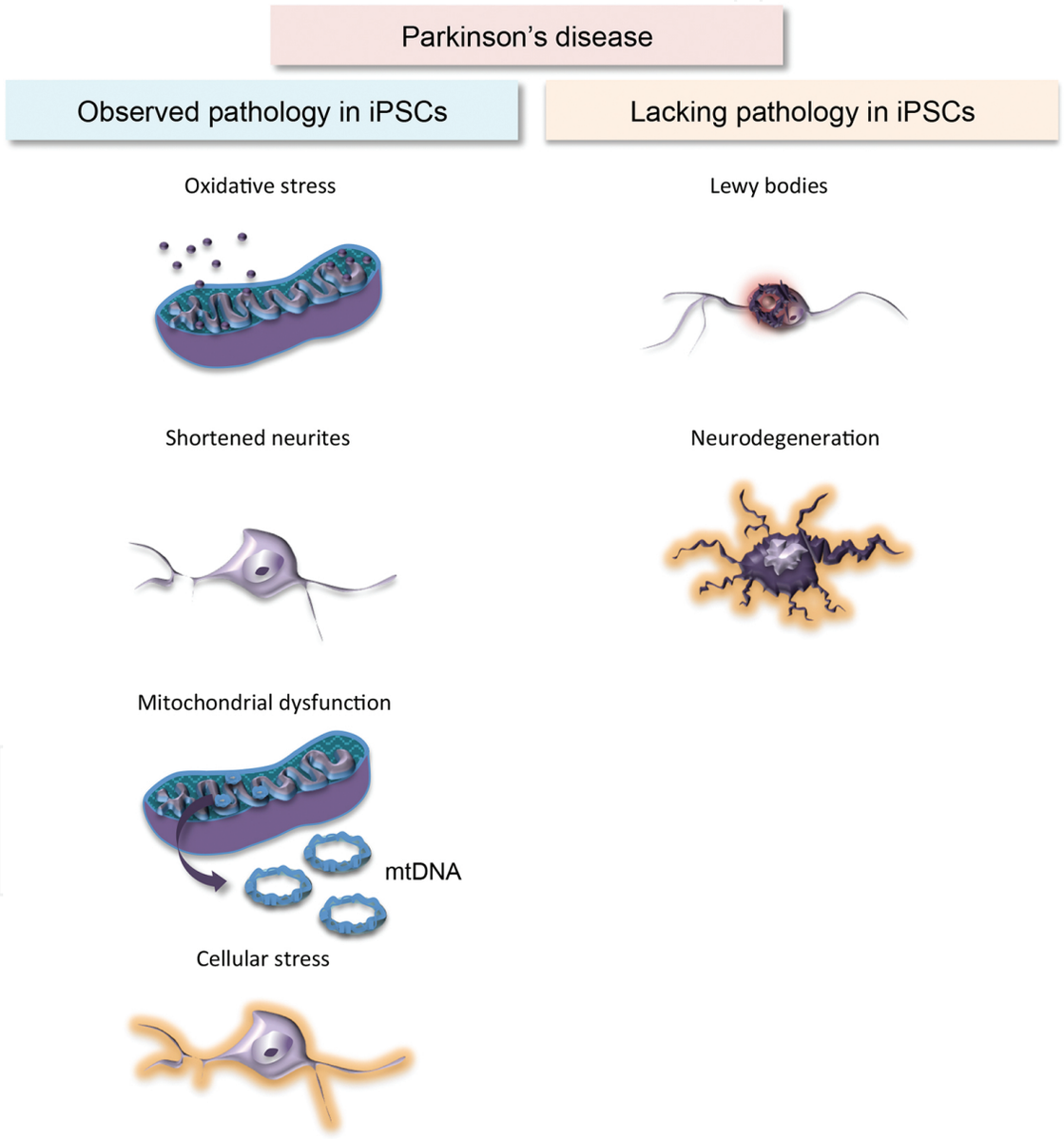



**Figure 4.** A summary of pathology observed in published induced pluripotent stem cell (iPSC) models of Parkinson's disease (PD) and pathology found in PD patients but currently lacking in the iPSC models.

Therefore, these former diseases are considered less attractive for cell therapy compared to PD. In fact, there is much discussion in the scientific community on the embarkment of clinical trials for PD using human PSCs and it is therefore only a matter of time before these trials begin to emerge. However, the majority of the studies to date have been performed to understand more about the mechanisms of the disease and to find new targets that could be used for discovering new and more effective medicines for braking the disease and for improving symptoms and quality of the patient's life.

One of the limitations observed to date in modelling the diseases is producing the right type of cells that are affected by the disease following differentiation of the iPSCs. In the case of AD and FTD, most studies have focused on evaluation of MAP2/TUJ1-positive neurons, cortical neurons and neural progenitors, which are often derived in heterogenic cultures. Whether these are the best cell types to examine in the dish is debatable. MAP2/TUJ1 expression is relatively unspecific and can label a vast number of different neuron subtypes, so it might be more important to use more specific antibodies to identify the specific neuron subtype that is being analysed. Cortical neurons are also numerous and some research, but not all, has identified the cortical subtype that has been produced in-vitro. In the case of AD, for example, the superficial cortical neurons are affected earlier on in the disease and the deeper cortical neurons are affected later [96]. Therefore, knowing which cortical neuron subtype is produced in the dish will help to understand better the disease mechanisms and whether the pathology being observed is related to early or late stages of the disease. Some researchers are pursuing the development of more complex laminated cortical layer cultures in-vitro [97], which could be a great source of tissue for performing further studies on. The study of neural progenitors might be more relevant for looking at potential early mechanisms of the disease and to evaluate regions of the brain where progenitors exist, as well as the effect of the disease on the cell cycle. In the case of PD, protocols for the generation of dopaminergic neurons have been refined over recent years allowing for relatively high proportions of the correct ventral-mesencephalon type following differentiation [85]. However, it is easy to forget the other cell types that can be affected in PD and the role they may also have in the disease. Therefore, the identity of the cell types produced needs to be more carefully defined in studies to help reveal more details about how the disease affects that particular cell type. More complex in-vitro models could also help to mimic the in-vivo environment better, which might help to reveal more phenotypes associated with the disease.

It is clear from the studies that the phenotypes, and in particular, the classic hallmark pathologies, are not represented in-vitro. The reasons for this are not really clear, but may relate to the fact that neural subtypes may be relatively young in the dish compared to the neural cells found in the patients. The evidence so far reveals that the iPSC-derived cells can model early changes related to the disease and therefore might prove useful for finding ways to reverse the disease or slow it down in the early or pre-symptomatic stages. If we are to evaluate some of the classic hallmarks, which are generally missing in culture (i.e. neurodegeneration, amyloid plaques, NFTs and Lewy bodies), then it might be needed to accelerate the aging of neurons in-vitro using artificial methods. One approach successfully used to age neurons in a dish was demonstrated using iPSCs from PD patients [98]. Overexpression of progerin (a gene

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linked to a disease of accelerated aging, progeria) was performed, which resulted in a pronounced PD phenotype of the dopaminergic neurons. The neurons were able to show much more pathology compared to non-aged neurons, including dendrite degeneration, loss of tyrosine hydroxylase expression (a typical marker of dopaminergic neurons) and Lewy-body-precursor inclusions within the neurons. This has not yet been applied to AD or FTD, but is an intriguing tool that could help to elaborate more of the classic pathologies/phenotypes in the dish.

## 10. Conclusions

To conclude, disease modelling of neurodegenerative diseases using PSCs has developed dramatically over a short period of time (a space of about 5 years). Already new mechanisms related to AD, FTD and PD have been discovered and these will likely lead to the development and trial of new medicines for the disease. There are many reported phenotypes that have been linked to the disease that can be reversed when familial mutations are genetically corrected using gene-editing technology. However, not all phenotypes have been reported so far, which may be linked to the cell types evaluated, the relatively simple systems used and also the relatively young neural cell types analysed. New studies developing 3D cultures and more complex tissue types may help move the field forward. In addition, new technologies that accelerate aging in the dish are also likely to help overcome these limitations. Thus, iPSCs have already been useful for uncovering some of the mysteries surrounding neurodegenerative disease and the future will likely lead to uncovering more about the disease mechanisms and how we can repair and treat the dysfunctioning cells before they are lost in the patient.

## Acknowledgements

The author's current research and activities are currently funded from the People Programme (Marie Curie Actions) of the European Union Seventh Framework Program FP7/2007-2013/ under REA grant agreement n°PIAPP-GA-2012-324451 (STEMMAD), as well as the Innovation Fund Denmark supported project, BrainStem (a Stem Cell Center of Excellence in Neurology).

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