Chapter 9

Induced Pluripotent Stem Cells (iPSCs) and Neurological Diseases

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A. Introduction

A cell is the smallest unit of an organism. The cell was first discovered by Robert Hooke in 1665. All cells in the human body come from a single-cell zygote, and although there are about 220 types of cells, the mechanisms of their work are synergistic with each other. Some of them work independently, like blood cells, while others form tissues, like synapses from the brain to the ends of the body. Stem cells play an important role in this developmental process. Stem cells are the term for groups of undifferentiated cells (Ohnuki & Takahashi, 2015).

The ability to self-replicate and differentiate into different cells with specific functions are two main characteristics of stem cells. Stem cell hierarchies are based on their differentiation potential and

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classified into totipotent stem cells, pluripotent stem cells, multipotent stem cells, and unipotent stem cells. The highest hierarchy is totipotent stem cells, which means that cells have the ability to produce a whole organism. In the development of the human body, this is only present until the morula phase. The lower the ability to differentiate, for example a unipotent stem cell, can produce only one type of cell (Hochedlinger & Plath, 2009).

The Waddington epigenetic landscape explain process of stem cell differentiation as a mole seed rolling down from a hill to one of several valleys, where the potential for stem cell development decreases. Stem cells do not have the ability to choose a different type of route when passing through their crossroads (biffurcatio point) (Counce, 1958).

In certain situations, nuclear reprogramming occurs when differentiated cells can return to their previous condition. For example, embryonic stem cells (ESCs) are made from B lymphocytes or neurons using somatic cell nuclear transfer (SCNTs), giving a group of transcription factors that transform mature cells into pluripotences (producing induced pluripotent stem cells; iPSCs). Induced pluripotent stem (iPSCs) are a type of pluripotent stem cell derived from adult somatic cells that have been genetically reprogrammed to an embryonic stem (ES) cell-like state through the forced expression of genes and factors important for maintaining the defining properties of ES cells (González et al., 2011; Takahashi et al., 2007).

Induced pluripotent stem cells (iPSCs), which remarkably resemble ESCs, developed as the two scientific streams. Although the reprogramming procedure used to create iPSCs is still a mystery, the products show promise in several fields, including drug discovery, pathological research, toxicology, the assessment of side effects from drugs, and regenerative medicine (Wang et al., 2020). Soon after the conversion of human somatic cells into iPSCs, the modelling of neurodevelopmental and neurodegenerative diseases offers new insights into the biology of diseases and the potential of new therapies.

This article will discuss induced pluripotent stem cells (iPSCs) from development to utilization, applications in neurology and

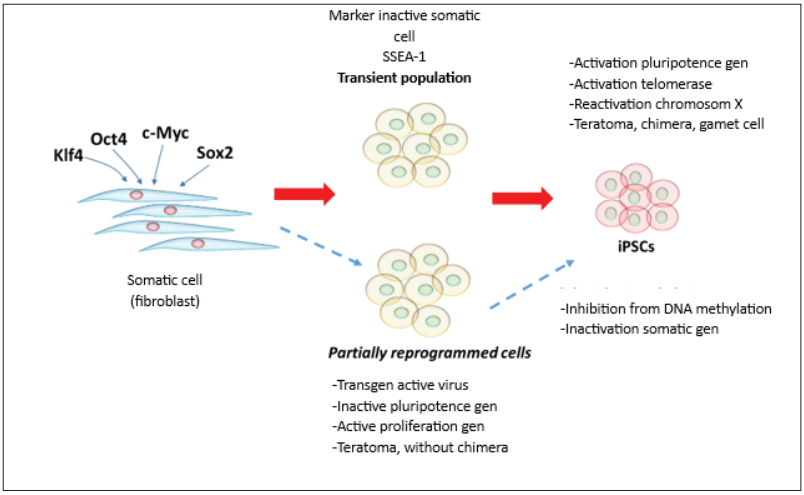
neuropsychiatry, potential advantages, and challenges that will be faced in the future. iPSCs are considered a valuable resource for regenerative medicine because they can be generated from any healthy person or patient.

Development of Pluripotent Cells

The first method of converting mature cells into pluripotent cells is known as nuclear transfer. Experiments conducted on amphibians, mammals, and humans showed that the genome of each adult cell, after undergoing terminal differentiation, can still produce live cloning. This suggests that the boundaries of development on the genome cannot be removed (Kumar et al., 2015).

Using a combination of four retrovirally transcripting factors from 24 candidate genes, Sox-2, Klf-4, Oct-4, and c-Myc, Kazutoshi Takahashi and Shinya Yamanaka managed to transform adult human cells (fibroblasts) into iPSCs5 (Takahashi et al., 2014). Initially, iPSCs were isolated by drug selection against the expression of the ESC marker (Fbx15), which was specific but not essential to ESCs, so that the first generation of iPScs was similar but not identical with ESCs (Halevy & Urbach, 2014). The transcription and epigenetic patterns of the first generation of iPSC indicate partial reprogramming conditions from fibroblasts to ESCs; these cells cannot produce chimera when injected into the blastocyst or do not contribute to the formation of gametocytes, indicating the status of mass reprogramming (Hochedlinger & Jaenisch, 2015).

In-depth studies replace iPSC selection with promoters of genes important for pluripotence, such as Oct-4 or Nanog (Olariu et al., 2016). These genes are thought to be more selective in cells that have undergone complete reprogramming. While Fbx15 is activated in virus-infected cells, so many cells that only undergo partial reprogramming are selected (Figure 9.1)(Okita et al., 2007). On a molecular level, the next generation of iPSCs showed patterns of transcription, DNA demethylation, and histone methylation (histone trimetylation H3 lysin 4 (K4) and lysin 27 (K27)) similar to the ESC pattern (Hanna et al., 2008).



Notes: This figure shows direct reprogramming of somatic cells into pluripotence cells, while partial reprogrammation produces iPSCs that require DNA demethylation agents and somatic gene inactivation.

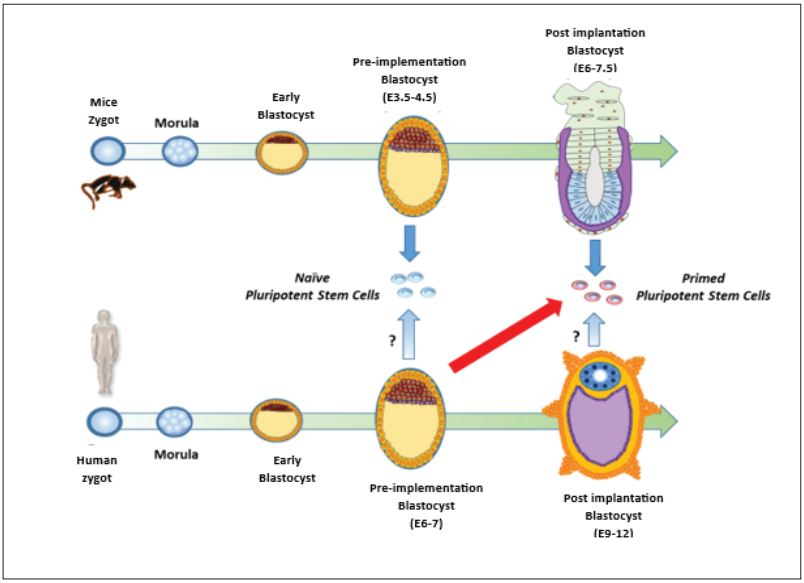
Source: Okita et al. (2007)

Figure 9.1 Direct Reprogramming of Somatic Cells into iPSCs

iPSCs have been successfully made from a wide range of cells on scatter since their invention by Kazutoshi Takahashi and Shinya Yamanaka. The technology can also be used on a variety of species, such as humans, monkeys, naked mole rats, and others (Aoi et al., 2008). This suggests that with the correct combination of transcription factors, somatic cells can be converted into pluripotent cells.

B. Relevance of Pluripotent Cells for Human Embryological Development

Conventional human pluripotency cell cultures cannot maintain cells in pre-implantation status, as most ESCs and iPSCs have characteristics similar to epiblast-derived stem cells (EpiSCs). Human pluripotent cells are identical to post-implant epiblasts, according to the transcription examination of primate embryos at the various



Notes: This figure shows the status of human pluripotence and scit. The characteristics of the human ESC and the scabies are very different, although they both originate from pre-implanted blastocysts. Human ESC is molecularly and functionally similar to post-implant epiblast tissue or comparable to epiSC mice.

Source: Smith (2017)

Figure 9.2 The Status of Human Pluripotence and Scit

phases of embryonic development (Boroviak & Nichols, 2017). Furthermore, there are differences in gene and epigenetic expression between pluripotential cells and pre-implantable epiblastic cells in humans. Therefore, experts agree that cells produced from the cell mass in the human body are more similar to primed epiblast stem cells (EpiSc humans; naive ESCs. (Figure 9.2) (Nichols & Smith, 2012; Smith, 2017).

The difference in species characteristics in pluripotency status is an interesting topic in the study of naive human pluripotent cells, which shows differences in the process of human embryonic development. Success in determining naive pluripotency status in

human cells is crucial for the study of embryonic development and human pluripotence and is expected to provide further benefits in clinical applications such as stable cell expansion and efficiency.

Understanding the evolution of the human body is the biggest challenge in biology. This is caused by the desire to learn about life and the pragmatic need to find a cure for a variety of human diseases. With their ability to proliferate and differentiate, human pluripotent cells are highly promising as a subject of human biological research. The long process has resulted in many discoveries and knowledge about human pluripotent cells. This allows research into the disease to understand the cause of the disease and find its cure. With current technological advances, human cells can be produced in large quantities with high quality. This allows cell therapy to replace damaged body cells (Bai, 2020).

C. Development of the Central Nervous System

Often, there is no cure for diseases or brain abnormalities. In addition, difficulties in obtaining human brain tissue for research and unrepresentative measurement models hinder progress in the science and therapy of neurological diseases. The development of human pluripotent cell technology, especially iPSCs, has excellent prospects for neurological disease research. The ability of iPSCs to produce certain patient cells or tissues connects clinical and animal studies. To make certain cells or tissues in the patient, a good understanding of the development of the brain and nerve tissue is required (Xie & Tang, 2016).

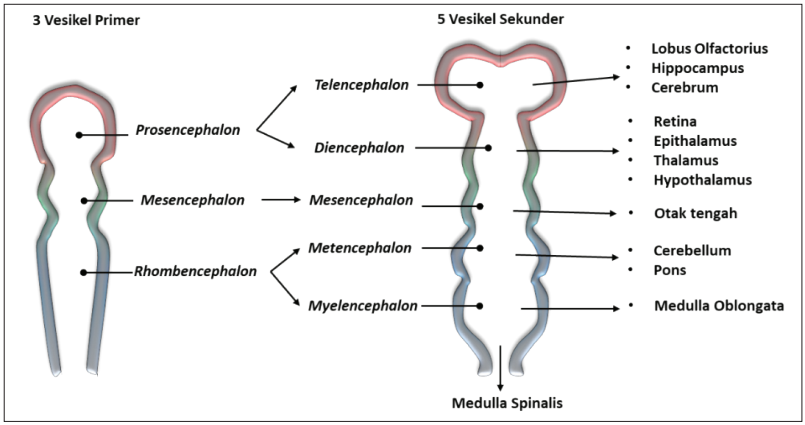
The embryonic phase in humans begins with conception and continues until the eighth week of gestation (GW), when each layer of the embryo forms a large number of specific organ tissues (organogenesis). The main structures of the brain and central nervous system have grown at the end of the embryonal phase. These include segmentation of neural tubes, gastrulations, and primary and secondary neurulations (Kostović & Jovanov-Milošević, 2006).

During the development of the human embryo, gastrulation is the beginning of the birth of the central nervous system. The reorganization of embryonic disc structures (embryonic bilaminary networks) into layered structural tissues is a sign of gastrula, an important process that prepares the embryo for organogenesis, the formation of more complex tissues (Kostović & Jovanov-Milošević, 2006).

The formation of neuralis tubes, which will form the brain and medulla spinalis, is known as neurulation. This process in humans begins at the 3rd GW, which is shown by the formation and fusion of the neuralistic pathway in the middle line of the embryo. The notochord pushes the ectodermal cells on it to form neurulation plates, which starts the neuralization process. The ectodermal layer naturally tends to be pro-neural rather than epidermal because signals from primitive nodes stop the formation of the epidermis. The apico-basal thickening process of the ectodermal layer, known as placode, is caused by the suppression of bone morphogenetic protein (BMP) and signals from the WNT pathway. At the ends of the skull, the placode nerve tends to be wider than in the caudal (Kostović & Jovanov-Milošević, 2006).

At first, the human neural tube had a straight tube structure. However, before the closure of the posterior neuropore, the anterior portion of the tube underwent significant changes. Three primary vesicles are composed of neural tubes in this area: the anterior cerebral vesicle (prosencephalon), the mid-brain vesicules (mesencephalone), and the posterior cerebral vesicles (rhombencephalon). When the posterior neural tube is closed, the optical vesicle, which is a secondary protrusion, appears from the lateral side of each of the frontal brain vesicles (Figure 9.3) (Elshazzly et al., 2023).

The neuralistic body has a polarization in its dorsal-ventral focus. For example, the regio dorsal medulla spinalis is where spinal neurons receive input from sensory neurons, and the regio ventral is where the spinal motor neurons are located. Many interneurons in the mid-medulla spinalis process information between neurons. Signals from



Notes: The first structure of the human brain three primary cerebral vesicles will develop, producing five secondary vesicles that help in the derivation of adult brain tissue.

Source: Moore (1993)

Figure 9.3 The First Structure of the Human Brain Three Primary Cerebral Vesicles

the environment affect this polarization. The epidermal membrane and roof plates affect the dorsal regio, while the notochord and floor plates impact the ventral regio (Elshazzly et al., 2023).

D. Neural and Human Pluripotent Cells

Neurons and glia form complex but orderly human brain tissue. Many types of cells in the mice (1) and human (2) brains were found through single cell profiling research (Molnar & Gair, 2015). However, the underlying mechanisms and processes of this cellular diversity are still not fully understood, especially in humans. Embryonal stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are interesting models for the study of the specifications of human neural subtypes (Tao & Zhang, 2016).

In the early stages, most neurological diseases tend to attack certain neural subtypes. The cerebrocentral dopaminergic neurons, mainly those that regulate motor function (A9-dopamine neurons), are damaged in Parkinson's disease. On the other hand, in patients

with Huntington's disease, the spiny medium neuron (GABAergic) in the striatum has primary nerve damage. Otherwise, with many decay systems, oligodendrosites are usually subjected to degenerative processes. Why only certain types of neurons or glia are sensitive or resistant to damage remains a mystery or question mark. The ability to direct human pluripotent cells to a specific neural subtype is expected to reveal the susceptibility of neuronal subtypes and the mechanisms responsible for neurological diseases (Xiao et al., 2016).

This organized differentiation naturally occurs gradually and usually takes three to four times longer than measuring, resulting in expensive operating costs and the risk of microbial contamination. By using a combination of external transcription factors to change cell phenotypes, the concept of direct reprogramming offers a technical solution to this problem. This concept developed after the invention of iPSCs and stems from the overexpression of MyoD in fibroblasts that produce muscle cells (Nogami et al., 2018).

The combination of ISL1, LHX3, and NGN2 with the Sendai virus managed to convert iPSCs into HB9+ cells, which are immature motor neurons, accounting for more than 90% of the total cells in just 14 days. A practical illustration of obtaining a particular neuron. Interestingly, these three combinations are not enough to convert fibroblast cells into motor spinal neurons; additional transcription factors such as ASCL1, BRN2, NEUROD1, and MYT1L are needed to assist the conversion process. This suggests that iPSCs and fibroblasts have different epigenetic resistances. However, research has shown that, compared to iPSCs, direct conversions from fibroblasts do not undergo complicated processes of chromatin remodelling, which makes them better suited to the patient's genetic background (Akter & Ding, 2022).

E. Modeling of Neurological Disorders Using IPSc-Derived Neural Cells

iPSC-based disease modelling has become increasingly popular for the study of neurological diseases due to its advantages. Reprogrammed iPSCs of human somatic cells originate from humans, thus avoiding

concerns about species differences associated with using animal models.

Most importantly, reprogrammed iPSCs of the patient's somatic cells retain their original genomic characteristics, such as gene mutations and chromosomal abnormalities. These genomic characteristics can be retained after differentiation, so they can be used to study the effects of certain genomic defects on cellular function. This is especially beneficial for drug development. Liu et al. showed the advantages of using an iPSC-based system for drug development by suggesting that treating neurons derived from iPSC with potential drugs for Alzheimer's disease could better reflect biomarker changes in real patients (Liu et al., 2014). With recent advances in genomic editing technologies, notably TALEN and CRISPR/Cas9 iPSCs can be manipulated from single nucleotide changes from one gene of interest to the removal of specific fragments on chromosomes related to disease (De Masi et al., 2020). The flexibility of genomic editing in iPSCs allows us to compare molecular and cellular phenotypes on the same genetic background, leading to more relevant conclusions about the mechanisms of disease (Gaj et al., 2016).

Using NSC-derived iPSC, a variety of neurological diseases have been studied, including monogenic and complex nerve disorders. Using small molecules including CHIR99021, SB431542, dorsomorphin, and compound E, Liu et al. produce NSCs from iPSCs derived from Parkinson's disease patients, which carry LRRK2 mutations (Liu et al., 2012).

The study found a new phenotype in iPSC patients derived from NSCs, which indicated nuclear architectural defects associated with LRRK2 mutations and increased proteasomal stress. However, since cells derived from iPSC have been shown to have characteristics similar to fetal cells, the role of NSCs in aging diseases needs to be carefully evaluated in iPSC-based modelling systems. Aging-inducing compounds, for example progerin, MG132, and concanamycin A, have been identified to facilitate the aging of nerve cells derived from

iPSCs, which may benefit the modelling of age-related neurological diseases (Weykopf et al., 2019).

Neurons, as the basic work unit in the brain, are affected by most neurological diseases. Neurons derived from iPSC have attracted great interest in the modelling of neurodegenerative diseases. To model the disease using iPSCs, the first challenge is to produce neuronal subtypes that are relevant to the disease. The diversity of neuronal subtypes is determined by complex genetic and environmental factors. During embryogenesis, the morphogenesis of the neuroectoderm is determined by a combination of morphogens along two axes: the rostro-dorsal axis by WNT, fibroblast growth factors (FGF), and retinoic acid (RA); and the dorso-ventral axis by WNT, BMP, and Sonic hedgehog protein (SHH). With this knowledge from evolutionary biology, the researchers have used morphogens and growth factors to produce specific neurons in the subtype and region of iPSC (Li et al., 2018).

Astrocytes are the most numerous cell type in the brain but are largely overlooked compared to their extensive focus on neurons to date. With increasing knowledge of astrocyte biology, its role in neurological diseases is now increasing. Similar to neurons, astrocytes are modelled by morphogenic gradients along the rostro-dorsal and dorsal-ventral axes and show heterogeneity in terms of subtype and regionality (Chiareli et al., 2021).

Neurological disease models using astrocytes derived from iPSC can be traced back to 2012. Jouperri et al. observed emptiness in Huntington astrocyte-iPSC-derived patients as well as peripheral lymphocyte-derived patients, suggesting a new phenomenon of Huntington's disease. Astrocytes derived from ALS iPSC patients carrying transactive DNA protein-binding reactions (TDP-43) mutations showed cell autonomic defects, including TDP-43 proteinopathies and cell death, but no adverse effects on motor neurons were observed (Juopperi et al., 2012).

F. Modelling Neuropsychiatric Disorders with Human Pluripotent Cell Technology

The scientific world still faces a huge challenge in understanding the biological foundations of nervous system diseases. This is mainly due to the complexity of the human brain, which consists of many types of cells with a variety of complex functions and relationships. In addition, the symptoms and severity of neuropsychiatric disorders vary from one person to another due to genetic, environmental, psychosocial, and developmental history factors affected. As a result, neurological disorders remain difficult to treat and have a negative impact on the health of individuals and communities.

Recent biotechnological developments can accelerate mechanistic research on nervous system diseases and drive new therapies. First, the genomic revolution allowed to discover specific gene variants and copy numbers that potentially cause neurological abnormalities. Second, rapid, accurate, and easy-to-do genetic modification through genetic engineering techniques with CRISPR-Cas9 enables the study of genetic function in human cells, squirrels, and primates (Knott & Doudna, 2018).

The discovery of iPSCs allowed the transformation of a patient's somatic cells into pluripotency cells that could distinguish different cell types. Cellular reprogramming can now be used to obtain neurons and other brain cells from patients with different genetic abnormalities. Furthermore, advances in bioengineering, known as 3D culture or organoid, show very small brain structures within a culture cup that have a design similar to the biological structures found in vivo.

The latest biotechnology development of new therapies can be assisted by these advanced technologies, which can speed up mechanical research on nervous system diseases. iPSC technology has a major advantage in exploring unusual genetic diseases by using patient genetic data; this increases specificity and approaches research to clinical applications. The most promising diseases to study are those identified by genetic mutations.

In most cases, there is a causal relationship that can be observed in the neural derivatives of the patient's iPSCs. Neural derivatives of monolayed-cultured (2D) iPSCs have been used to investigate various mechanisms responsible for neuropsychiatric disorders. For example, there is a decrease in the number of glutamatergic synapses in iPSC monolayer neurons in patients with Rett syndrome. The administration of IGF-1, a peptide often used in clinical trials of neurodevelopmental disorders, can alter this phenotype. In addition, the neurons of patients with Timothy syndrome (mutations in the Ca2+ channel type-L) showed changes in dendritic retraction and production of neurotransmitters (Centeno et al., 2018).

Furthermore, research on psychiatric disorders brought on by ancestors, but not caused by the disease is very beneficial with iPSC technologies. In one study, the glutamatergic neuron culture of schizophrenia patients showed a decrease in connectivity and number of neurons; anti-psychotic drugs could restore some of the molecular and cellular phenotypes of patients' neurons. There are mitochondrial abnormalities and neuron hyperexcitability in the hippocampus of patients with bipolar disorder, according to studies conducted on their hypocampal neurons. Lithium can recover this neuronal hyperexcitability, but it only occurs in patients' neurons with bipolar symptoms that indicate a response to lithium. This suggests that iPSC technology can be used to predict drug therapeutic responses (Osete et al., 2023).

In addition to successfully recapitulating synaptic and neurodevelopmental disorders, neural derivatives of iPSC patients can also be used to investigate neurodegenerative diseases or proteinopathies. These diseases are usually more characterized by neurotoxicity due to oxidative stress and proteasome disorders than synapses. Nguyen et al. (2011), for example, found that the gene expression of dopaminergic neurons associated with oxidative stress and -synuclein proteins increased in Parkinson's patients with the LRRK2 mutation, or leucine-rich repeat kinase-2. As a result, compared to control neurons, these mutant neurons are more

prone to cell death due to exposure to H2O2, MG-132 (proteasome inhibitors), and 6-hydroxydopamine (neurotoxic) (Weykopf et al., 2019; Xiao et al., 2016). The findings about the susceptibility to stress in patients' neuron derivatives provide a new understanding of the mechanisms of disease and form the basis for drug screening. Soon after the conversion of human somatic cells into iPSCs, the modelling of neurodevelopmental and neurodegenerative diseases (see Table 9.1 for a summary of neurological disease modelling with iPSC) offers new insights into the biology of diseases and the potential of new therapies.

Neural culture with a single-layer system has produced a lot of results, but the system does not have features like the human brain, so it cannot be used to model some disease phenomena. Intercellular and ligand-receptor interactions lead to neurobiological development and synaptic connectivity. When neurons are cultured monolayer, this signalling dynamics is unclear. The development of in vitro models

Table 9.1 Summary of Neurological Disease Modelling with iPSC

Disease	Gen	Neurological Symptom	Phenotypes in Neural Derivatives	Testing Therapy	Ref.
Adrenoleu-kodystrophy	*ABCD1*	Demyelination and loss function system nerve center and edge in a manner progressive	Increasing *very long chain fatty acids* (VLCFA) in oligodendrocytes	Lovastin. 4-phenylbu-tyrate degrades VLCFA levels	Jang et al. (2011)
Alzheimer`s disease	*PS1*, *PS2*, *APPs*, Sporadic	Disturbance cognition and memory as well as progressive disorientation	Increasing secretion of amyloid β (Aβ), *phospho-tau* (Thrc231) and *glycogen synthase kinase-3β* (aGSK-3β) in neurons	γ-secretase inhibitors decrease Aβ secretion β-secretase inhibitors decrease phosphotau (Thrc231) and aGSK-3β levels The combination of Bromocrip-tine, Cromolyn and Topimarate lowering Aβ _	Yagi et al. (2011)

Disease	Gen	Neurological Symptom	Phenotypes in Neural Derivatives	Testing Therapy	Ref.
Amyotrophic lateral sclerosis (ALS)	*SOD1*,*VAP*, *TDP43*	degeneration and loss *upper* and *lower motor neurons* in a manner progressive	VAPB: decline VAPB levels in motor neurons. TDP43: motor neuron mutant own enhancement dissolved and *detergent-resistant* TDP-43 protein levels, decreased neuron survival and increased vulnerability to antagonist PI3K pathway	*Anacardic acid* lower mutant TDP43 protein levels Bosutinib promotes autophagy and decreases amount *protein misfolding* Ropinirole prevents death cells, abnormal protein aggregation, and production molecule oxygen-radicals	Kondo et al. (2017)
Huntington's disease	CAG repetitions in the Huntingtin gene (HTT)	Chorea and progressive dementia be marked with loss *medium spiny neurons* striatal and *cortical neurons*	NSCs-HD shows stress susceptibility to decline BDNF levels, increased death cells, and disorders bioenergetic mitochondria Formation inclusion protein aggregates after administration of *proteasome* inhibitors (MG132) Disturbance *corticogenesis* Vacuolization of astrocytes increasing activity lysosomes in iPSCs	Correct genetics *Pyruvate* increase rate ATP and activity bioenergetic mitochondria	Mehta et al. (2018)
Familial dysautonomia (FD)	*IKBKAP*	Degeneration of sensory and autonomic neurons	Decline gene expression related to neurogenesis and neuronal differentiation Disturbance migration *neural crest*	Kinetin repair phenotype	Lee et al. (2009)
Parkinson's disease (PD)	*LRRK2*, *PINK1*, *SNCA* and *parkin*	Degeneration of dopaminergic neurons substantia nigra	PINK1: Interference function mitochondria in dopaminergic neurons LRRK2 and SNCA: sensitivity against oxidative stress	Inh2 reduces degeneration of dopaminergic neurons with LRRK2 mutation	Weykopf et al. (2019)

Disease	Gen	Neurological Symptom	Phenotypes in Neural Derivatives	Testing Therapy	Ref.
Syndrome Rett (RTT)	*MeCP2 CDKL5* _	Disturbance function motor, regression skill, hypotonia, seizures. *Atypical Rett syndromes*: disabilities intellectual, epilepsy, and autism.	MeCP2: neuronal maturation deficit, decreased amount synapse and *dendritic spines*, larger soma size small, increasing LINE1 retrotransposition CDKL5: aberrant dendritic spines	IGF-1 repairs phenotype *in vitro*	Ricciardi et al. (2012)
Schizophrenia	Multi-factorial	Hallucination, delusions, disturbances talk. Abnormality neurotrans-mitter, decrease arborization dendritic, and disorder myelination	Lost neuronal connectivity, Decline amount neurites, PSD95, and receptors glutamate Abnormality migration and myelination oligodendrocytes	Loxapine, *valproic AC* ID repair phenotype *in vitro*	Windrem et al. (2017)
Timothy's syndrome	*CACNA1C*	Syndrome *Long-QT*, deficit neurological characteristics autistic	Decline gene expression in layers cortex lower and callosal projection neurons Increased production of norepinephrine and dopamine abnormality in retraction dendritic	Roscovitine restore phenotype *in vitro*	Paşca et al. (2011)

that can measure the structural and functional complexity of the human brain is motivated by the limitations of monolayer culture. Human pluripotenic cells (iPSCs and ESCs) can produce human organoid brains that have structural characteristics similar to fetal brains by using the intrinsic ability of cellular aggregation (Lancaster & Knoblich, 2014).

All efforts to create experimental models for neuropsychiatric disorders will only succeed if the models can describe the processes that occur in the human brain. The monolayer (2D) culture system has advantages mainly in scalability and homogeneity, which facilitate the screening of genetic and pharmacological systems that have

many tests. However, brain organoid culture is more related to the characteristics of cellular interactions and the structural organization of the human brain. Basically, the choice of a cultural system depends on the purpose of the investigation and the design of the experiment. Current biotechnological advances have expanded our understanding of a variety of neuropsychiatric conditions. Therapeutic innovations are also expected to improve the quality of life of patients.

G. Prospects of iPSCs-Based Therapy in Neurological Diseases

The brain and medulla spinalis have been the subject of cell therapy studies since the emergence of stem cell biology. Due to the numerous diseases associated with ineffective cell replacement therapy, the central nervous system is attracted to cell substitution therapy. Nevertheless, because the brain is an organ that has many structural classifications and complex intercellular interactions, cell therapy cannot always help the brain regenerate structurally after damage.

This problem is becoming increasingly complex due to the limitations of the repair capacity of the adult human brain. Although neural stem cells exist in the human brain, their role in structurally repairing brain tissue remains a mystery. According to early stem cell biology, it is expected that donor cells (also known as NSCs or pluripotenic cells) may vary depending on the situation after the transplant. However, further research found that spontaneous differences in donor cells may not produce the expected cell type. At present, it is known that when the neuronal or glial subtypes suffer degeneration replacement should be done with the same cell subtype or their forefathers in order to achieve proper structural repair. Luckily, many nervous system diseases only attack certain types of cells. This means neuronal or glial cell therapy can be used for many neurological diseases (Li et al., 2018).

In this regard, glial progenitor cells, which can produce astrocytes and oligodendrosites (similar to oligodendrocyte progenitor cells, or

OPCs), have been thoroughly studied as a potential drug to repair myelin in the spinal cord and the human fetal brain. These cells can be extracted from human pluripotent cells (both ESCs and iPSCs). In trial animals, transplanted OPCs tend to spread and migrate along the neural focus, becoming myelinating oligodendrosites of the dismielinated locus. Human perinatal OPC transplantation into a hypomyelinated shiverer scab, which usually dies at the age of twenty weeks, prolongs the scab life locus. stores myelinated and neurological phenotypes. This is a demonstration of proof of concept for remyelination therapy with OPC on various white skin disorders.

The very common post-transplant OPC characteristics allow the treatment of dysmyelination diseases caused by enzyme deficiency or disorders of lysosome storage such as mucopolysaccharidoses, Krabbe's disease, and metachromatic leukodystrophy. In the future, autologous cell therapy is highly likely to be used in patients with leukodystrophy abnormalities. Hopefully, the transplanted cells could prove effective in remyelinating and correcting the patient's metabolic abnormalities. This can be achieved through the integration of genetic engineering technology to repair mutations and iPSC technology.

In the 1980s, a Swedish scientist, A. Bjorklund, had an idea of treating patients with Parkinson's disease by transplantation; they tried to replace the dopaminergic neurons in the substantia nigra of an aborted fetus with the middle brain tissue. This discovery promoted the development of cell therapy for Parkinson's disease. Technically speaking, about 2–3 fetuses are needed for one patient, which makes its implementation difficult due to donor issues and ethical issues. However, with advances in human pluripotency cell technology, dopaminergic neurons can be produced efficiently from ESCs or iPSCs in large quantities (Björklund & Lindvall, 2017).

Although its clinical trials are promising, there are still some problems with unresolved cell transplants. If fetal midbrain tissue is implanted into the striatum of a Parkinson's patient, the results are inconsistent, ineffective, and produce many side effects (graft-induced dyskinesia). In addition, the nigral dopaminergic cells are located at

a heterotopic location in the putamen or striatum. Donor cells do not have normal afferent connectivity and are exposed to different neural circuits. Furthermore, it is not known whether intrastriatal dopaminergic neuron transplantation can improve the normal basal function of the ganglia in Parkinson's patients. Additionally, some studies show that the -synuclein and Lewy body aggregates are transmitted from the recipient cell to the donor cell, so that the donor neurons are involved in the disease process (Björklund & Lindvall, 2017).

At least, there was caution in the use of these strategies in clinics due to confusion about the spread of the disease from the recipient to the donor, the need for immunosuppression, and the ideal dosage. Overall, the benefits and risks of this approach should be compared to the availability of effective pharmacotherapy for Parkinson's disease and deep brain stimulation to reduce the severity of the disease. Whether dopaminergic cell transplants are beneficial and whether the cost is comparable to the benefits or risks is still debated. Patients who were refractory to current medical choices and did not experience cognitive impairment were a small group.

H. Problems in Cell Therapy Applications

To ensure that cell therapy is safe and effective, a variety of issues need to be studied or considered before being applied clinically. Some problems with cell replacement therapy with stem cells are as follows (Dodson & Levine, 2015; Mousaei Ghasroldasht et al., 2022). Somatic cell reprogramming is not a perfect process. There are imperfections in somatic cell reprogramming, epigenetic marker retention, and new mutations that can cause tumors.

However, this does not indicate that the ESC is better. There is evidence that long-term culture processes increase the likelihood of genetic erosion or genetic mutation in human pluripotent cells (ESCs and iPSCs). To ensure the safety of cell derivatives before clinical use, a thorough examination of iPSCs and ESCs is required.

If the test design is inappropriate, many potentially effective agents fail in clinical trials. For optimal and objective results, clinical trial designs should understand pathogenesis, disease course, and disease heterogeneity.

One should not expect too much from the therapeutic ability of cells to cure diseases because animals cannot recapture all the pathological conditions that exist in human diseases. To ensure that human pluripotent cell derivatives are safe, examinations of the potential for tumor genesis, immune reactions, heterotopic differentiation, and microbial transmission should be carried out before implementing cell therapy in clinical conditions.

I. Conclusion

Induced pluripotent stem cells (iPSCs) have opened new avenues for stem cell research and unique opportunities in the pharmaceutical industry and clinical practice. Reprogramming technology has also made it possible to study cell fate decision mechanisms and model human diseases. This has greatly increased the chances of discovering new drugs. iPSCs show promise in some fields, including drug discovery, pathological research, toxicology, the assessment of side effects from drugs, and regenerative medicine. Soon, after the conversion of human somatic cells into iPSCs, the modelling of neurodevelopmental and neurodegenerative diseases will offer new insights into the biology of diseases and the potential of new therapies. However, like many other fields, reprogramming technology has several challenges, such as clinical trial design, risk, and benefit considerations.

References

Akter, M., & Ding, B. (2022). Modeling movement disorders via generation of hipsc-derived motor neurons. *Cells*, *11*(23), Article 3796. https://doi.org/10.3390/CELLS11233796

Aoi, T., Yae, K., Nakagawa, M., Ichisaka, T., Okita, K., Takahashi, K., Chiba, T., & Yamanaka, S. (2008). Generation of pluripotent stem cells from adult mouse liver and stomach cells. *Science*, *321*, 699–702. https://doi.org/10.1126/SCIENCE.1154884

Bai, X. (2020). Stem cell-based disease modeling and cell therapy. *Cells*, *9*(10), Article 2193. https://doi.org/10.3390/CELLS9102193

Björklund, A., & Lindvall, O. (2017). Replacing dopamine neurons in Parkinson's disease: How did it happen? *Journal of Parkinson's Disease*, *7*(Suppl 1), 21–31. https://doi.org/10.3233/JPD-179002

Boroviak, T., & Nichols, J. (2017). Primate embryogenesis predicts the hallmarks of human naïve pluripotency. *Development*, *144*(2), 175–186. https://doi.org/10.1242/DEV.145177

Centeno, E. G. Z., Cimarosti, H., & Bithell, A. (2018). 2D versus 3D human induced pluripotent stem cell-derived cultures for neurodegenerative disease modelling. *Molecular Neurodegeneration*, *13*(1), Article 27. https://doi.org/10.1186/S13024-018-0258-4

Chiareli, R. A., Carvalho, G. A., Marques, B. L., Mota, L. S., Oliveira-Lima, O. C., Gomes, R. M., Birbrair, A., Gomez, R. S., Simão, F., Klempin, F., Leist, M., & Pinto, M. C. X. (2021). The role of astrocytes in the neurorepair process. *Frontiers in Cell and Developmental Biology*, *9*:,Article 665795. https://doi.org/10.3389/FCELL.2021.665795/BIBTEX

Counce, S. J. (1958). The strategy of the genes. *The Yale Journal of Biology and Medicine*, *30*(6), 470–471. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2603894/

De Masi, C., Spitalieri, P., Murdocca, M., Novelli, G., & Sangiuolo, F. (2020). Application of CRISPR/Cas9 to human-induced pluripotent stem cells: From gene editing to drug discovery. *Human Genomics*, *14*(1), 1–12. https://doi.org/10.1186/S40246-020-00276-2/TABLES/2

Dodson, B. P., & Levine, A. D. (2015). Challenges in the translation and commercialization of cell therapies. *BMC Biotechnology*, *15*(1), Article 70. https://doi.org/10.1186/S12896-015-0190-4

Elshazzly, M., Lopez, M. J., Reddy, V., & Caban, O. (2023). Embryology, central nervous system. *StatPearls*. https://www.ncbi.nlm.nih.gov/books/NBK526024/

Gaj, T., Sirk, S. J., Shui, S. L., & Liu, J. (2016). Genome-editing technologies: Principles and applications. *Cold Spring Harbor Perspectives in Biology*, *8*(12), Article a023754. https://doi.org/10.1101/CSHPERSPECT.A023754

González, F., Boué, S., & Belmonte, J. C. I. (2011). Methods for making induced pluripotent stem cells: Reprogramming à la carte. *Nature Reviews Genetics*, *12*(4), 231–242. https://doi.org/10.1038/nrg2937

Halevy, T., & Urbach, A. (2014). Comparing ESC and iPSC—Based models for human genetic disorders. *Journal of Clinical Medicine*, *3*(4), 1146–1162. https://doi.org/10.3390/JCM3041146

Hanna, J., Markoulaki, S., Schorderet, P., Carey, B. W., Beard, C., Wernig, M., Creyghton, M. P. P., Steine, E. J., Cassady, J. P., Foreman, R., Lengner, C. J., Dausman, J. A. A., & Jaenisch, R. (2008). Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. *Cell*, *133*(2), 250–264. https://doi.org/10.1016/J.CELL.2008.03.028

Hochedlinger, K., & Jaenisch, R. (2015). Induced pluripotency and epigenetic reprogramming. *Cold Spring Harbor Perspectives in Biology*, *7*(12), Article a019448. https://doi.org/10.1101/CSHPERSPECT.A019448

Hochedlinger, K., & Plath, K. (2009). Epigenetic reprogramming and induced pluripotency. *Development*, *136*(4), 509–523. https://doi.org/10.1242/DEV.020867

Jang, J., Kang, H. C., Kim, H. S., Kim, J. Y., Huh, Y. J., Kim, D. S., Yoo, J. E., Lee, J. A., Lim, B., Lee, J., Yoon, T. M., Park, I. H., Hwang, D. Y., Daley, G. Q., & Kim, D. W. (2011). Induced pluripotent stem cell models from X-linked adrenoleukodystrophy patients. *Annals of Neurology*, *70*(3), 402–409. https://doi.org/10.1002/ANA.22486

Juopperi, T. A., Kim, W. R., Chiang, C. H., Yu, H., Margolis, R. L., Ross, C. A., Ming, G. L., & Song, H. (2012). Astrocytes generated from patient induced pluripotent stem cells recapitulate features of Huntington's disease patient cells. *Molecular Brain*, *5*(1), Article 17. https://doi.org/10.1186/1756-6606-5-17

Knott, G. J., & Doudna, J. A. (2018). CRISPR-Cas guides the future of genetic engineering. *Science*, *361*, 866–869. https://doi.org/10.1126/SCIENCE.AAT5011

Kondo, T., Imamura, K., Funayama, M., Tsukita, K., Miyake, M., Ohta, A., Woltjen, K., Nakagawa, M., Asada, T., Arai, T., Kawakatsu, S., Izumi, Y., Kaji, R., Iwata, N., & Inoue, H. (2017). iPSC-based compound screening and in vitro trials identify a synergistic anti-amyloid β combination for alzheimer's disease. *Cell Reports*, *21*(8), 2304–2312. https://doi.org/10.1016/J.CELREP.2017.10.109

Kostović, I., & Jovanov-Milošević, N. (2006). The development of cerebral connections during the first 20-45 weeks' gestation. *Seminars in Fetal & Neonatal Medicine*, *11*(6), 415–422. https://doi.org/10.1016/J.SINY.2006.07.001

Kumar, D., Talluri, T. R., Anand, T., & Kues, W. A. (2015). Induced pluripotent stem cells: Mechanisms, achievements and perspectives in farm animals. *World Journal of Stem Cells*, *7*(2), 315–328. https://doi.org/10.4252/WJSC.V7.I2.315

Lancaster, M. A., & Knoblich, J. A. (2014). Organogenesis in a dish: modeling development and disease using organoid technologies. *Science (New York, N.Y.)*, *345*(6194). https://doi.org/10.1126/SCIENCE.1247125

Lee, G., Papapetrou, E. P., Kim, H., Chambers, S. M., Tomishima, M. J., Fasano, C. A., Ganat, Y. M., Menon, J., Shimizu, F., Viale, A., Tabar, V., Sadelain, M., & Studer, L. (2009). Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. *Nature*, *461*, 402–406. https://doi.org/10.1038/NATURE08320

Li, L., Chao, J., & Shi, Y. (2018). Modeling neurological diseases using iPSC-derived neural cells: iPSC modeling of neurological diseases. *Cell and Tissue Research*, *371*(1), 143–151. https://doi.org/10.1007/S00441-017-2713-X

Liu, G. H,, Qu, J., Suzuki, K., Nivet, E., Li, M., Montserrat, N., Yi, F., Xu, X., Ruiz, S., Zhang, W., Wagner, U., Kim, A., Ren, B., Li, Y., Goebl, A., Kim, J., Soligalla, R.D., Dubova, I., Thompson, J., … Belmonte, J. C. I. (2012). Progressive degeneration of human neural stem cells caused by pathogenic LRRK2. *Nature*, *491*, 603–607. https://doi.org/10.1038/nature11557

Mehta, S. R., Tom, C. M., Wang, Y., Bresee, C., Rushton, D., Mathkar, P. P., Tang, J., & Mattis, V. B. (2018). Human huntington's disease iPSC-derived cortical neurons display altered transcriptomics, morphology, and maturation. *Cell Reports*, *25*(4), 1081–1096.e6. https://doi.org/10.1016/J.CELREP.2018.09.076

Molnar, C., & Gair, J. (2015). Neurons and glial cells. In C. Molnar & J. Gair, *Concept of biology-1st Canadian edition Molnar class* (Chapter 16.1). BCcampus.

Moore, K. L. (1993). *Before we are born: Essentials of embryology and birth defects*. Saunders.

Mousaei Ghasroldasht, M., Seok, J., Park, H. S., Liakath Ali, F. B., & Al-Hendy, A. (2022). Stem cell therapy: From idea to clinical practice. *International Journal of Molecular Sciences*, *23*(5), Article 2850. https://doi.org/10.3390/IJMS23052850

Nguyen, H. N., Byers, B., Cord, B., Shcheglovitov, A., Byrne, J., Gujar, P., Kee, K., Schüle, B., Dolmetsch, R. E., Langston, W., Palmer, T. D. & Pera, R. R. (2011). LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. *Cell Stem Cell*, *8*(3), 267–280. https://doi.org/10.1016/j.stem.2011.01.013

Nichols, J., & Smith, A. (2012). Pluripotency in the embryo and in culture. *Cold Spring Harbor Perspectives in Biology*, *4*(8), Article a008128. https://doi.org/10.1101/CSHPERSPECT.A008128

Nogami, K., Blanc, M., Takemura, F., Shin'ichi Takeda, & Miyagoe-Suzuki, Y. (2018). Making skeletal muscle from human pluripotent stem cells. In K. Sakuma (Ed.), *Muscle cell and tissue - Current status of research field* (Chapter 7). https://doi.org/10.5772/INTECHOPEN.77263

Ohnuki, M., & Takahashi, K. (2015). Present and future challenges of induced pluripotent stem cells. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1680), Article 20140367. https://doi.org/10.1098/RSTB.2014.0367

Okita, K., Ichisaka, T., & Yamanaka, S. (2007). Generation of germline-competent induced pluripotent stem cells. *Nature*, *448*, 313–317. https://doi.org/10.1038/nature05934

Olariu, V., Lövkvist, C., & Sneppen, K. (2016). Nanog, Oct4 and Tet1 interplay in establishing pluripotency. *Scientific Reports*, *6*, Article 25438. https://doi.org/10.1038/SREP25438

Osete, J. R., Akkouh, I. A., Ievglevskyi, O., Vandenberghe, M., de Assis, D. R., Ueland, T., Kondratskaya, E., Holen, B., Szabo, A., Hughes, T., Smeland, O. B., Steen, V. M., Andreassen, O. A., & Djurovic, S. (2023). Transcriptional and functional effects of lithium in bipolar disorder iPSC-derived cortical spheroids. *Molecular Psychiatry*, *28*, 3033–3043. https://doi.org/10.1038/s41380-023-01944-0

Paşca, S. P., Portmann, T., Voineagu, I., Yazawa, M., Shcheglovitov, A., Paşca, A. M., Cord, B., Palmer, T. D., Chikahisa, S., Nishino, S., Bernstein, J. A., Hallmayer, J., Geschwind, D. H., & Dolmetsch, R. E. (2011). Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. *Nature Medicine*, *17*(12), 1657–1662. https://doi.org/10.1038/nm.2576

Ricciardi, S., Ungaro, F., Hambrock, M., Rademacher, N., Stefanelli, G., Brambilla, D., Sessa, A., Magagnotti, C., Bachi, A., Giarda, E., Verpelli, C., Kilstrup-Nielsen, C., Sala, C., Kalscheuer, V. M., & Broccoli, V. (2012). CDKL5 ensures excitatory synapse stability by reinforcing NGL-1-PSD95 interaction in the postsynaptic compartment and is impaired in patient iPSC-derived neurons. *Nature Cell Biology*, *14*(9), 911–923. https://doi.org/10.1038/NCB2566

Smith, A. (2017). Formative pluripotency: The executive phase in a developmental continuum. *Development*, *144*(3), 365–373. https://doi.org/10.1242/DEV.142679

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., & Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, *131*(5), 861–872. https://doi.org/10.1016/J.CELL.2007.11.019

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Sasaki, A., Yamamoto, M., Nakamura, M., Sutou, K., Osafune, K., & Yamanaka, S. (2014). Induction of pluripotency in human somatic cells via a transient state resembling primitive streak-like mesendoderm. *Nature Communications*, *5*(1), Article 3678. https://doi.org/10.1038/ncomms4678

Tao, Y., & Zhang, S. C. (2016). Neural subtype specification from human pluripotent stem cells. *Cell Stem Cell*, *19*(5), 573–586. https://doi.org/10.1016/J.STEM.2016.10.015

Wang, X., Qu, J., Li, J., He, H., Liu, Z., & Huan, Y. (2020). Epigenetic reprogramming during somatic cell nuclear transfer: Recent progress and future directions. *Frontiers in Genetics*, *11*, Article 501202. https://doi.org/10.3389/FGENE.2020.00205/BIBTEX

Weykopf, B., Haupt, S., Jungverdorben, J., Flitsch, L. J., Hebisch, M., Liu, G. H., Suzuki, K., Belmonte, J. C. I., Peitz, M., Blaess, S., Till, A., & Brüstle, O. (2019). Induced pluripotent stem cell-based modeling of mutant LRRK2-associated Parkinson's disease. *The European Journal of Neuroscience*, *49*(4), 561–589. https://doi.org/10.1111/EJN.14345

Windrem, M. S., Osipovitch, M., Liu, Z., Bates, J., Chandler-Militello, D., Zou, L., Munir, J., Schanz, S., McCoy, K., Miller, R. H., Wang, S., Nedergaard, M., Findling, R. L., Tesar, P. J., & Goldman, S. A. (2017). Human iPSC glial mouse chimeras reveal glial contributions to schizophrenia. *Cell Stem Cell*, *21*(2), 195–208. https://doi.org/10.1016/J.STEM.2017.06.012

Xiao, B., Hui Ng, H., Takahashi, R., & Tan, E. K. (2016). Induced pluripotent stem cells in Parkinson's disease: Scientific and clinical challenges. *Journal of Neurology, Neurosurgery & Psychiatry*, *87*(7), 697–702. https://doi.org/10.1136/JNNP-2015-312036

Xie, N., & Tang, B. (2016). The application of human iPSCs in neurological diseases: From bench to bedside. *Stem Cells International*. https://doi.org/10.1155/2016/6484713

Yagi, T., Ito, D., Okada, Y., Akamatsu, W., Nihei, Y., Yoshizaki, T., Yamanaka, S., Okano, H., & Suzuki, N. (2011). Modeling familial Alzheimer's disease with induced pluripotent stem cells. *Human Molecular Genetics*, *20*(23), 4530–4539. https://doi.org/10.1093/HMG/DDR394