Chapter 2
Induced Pluripotent Stem Cell Therapy and Safety Concerns in Age-Related Chronic Neurodegenerative Diseases

Erden Eren, Erdogan Pekcan Erkan, Sermin Genc, and Kemal Kursad Genc

Abbreviations

6-OHDA	6-Hydroxydopamine
AD	Alzheimer's disease
AKT	RAC-alpha serine/threonine-protein kinase
APOE	Apolipoprotein E
APP	Amyloid precursor protein
BBB	Blood-brain barrier
BiP	Binding immunoglobulin protein
BSI	β-secretase inhibitor
ChAT	Choline acetyltransferase
c-Myc	Cytoplasmic Myc protein
CNS	Central nervous system
CNV	Copy number variation
Crispr/Cas9	Clustered regularly interspaced short palindromic repeat/CAS9 RNA-guided nucleases
DHA	Docosahexaenoic acid
DJ-1	PARK7
ECM	Extracellular matrix

E. Eren • E.P. Erkan • S. Genc
Department of Neuroscience, Institute of Health Sciences, Dokuz Eylul University Health Campus, Inciralti, 35340 Izmir, Turkey

Izmir Biomedicine and Genome Center, Dokuz Eylul University Health Campus, Inciralti, 35340 Izmir, Turkey

K.K. Genc (✉)
Department of Neuroscience, Institute of Health Sciences, Dokuz Eylul University Health Campus, Inciralti, 35340 Izmir, Turkey
e-mail: kkursadgenc@hotmail.com

P.V. Pham, A. Rosemann (eds.), *Safety, Ethics and Regulations*, Stem Cells in Clinical Applications, DOI 10.1007/978-3-319-59165-0_2

ER	Endoplasmic reticulum
ES	Embryonic stem cells
FAD	Familial form of Alzheimer's disease
FDA	Food and Drug Administration
GABA	Gamma-aminobutyric acid
G-CSF	Granulocyte colony-stimulating factor
GSK-3	Activated glycogen synthase kinase 3
GSK-3	Glycogen synthase kinase 3
GWAS	Genome-wide associated studies
hES	Human embryonic stem cells
hiPSCs	Human-induced pluripotent stem cells
HLA	Human leukocyte antigen
HUVEC	Human umbilical vein endothelial cells
IFN-γ	Interferon gamma
IKBKAP	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein
iNSC	Induced neural stem cells
iPSC	Induced pluripotent stem cells
Klf4	Kruppel-like factor 4
LRRK2	Leucine-rich repeat kinase 2
LV	Lentivirus
MAO	Monoamine oxidase
MEFs	Mouse embryonic fibroblasts
MMP	Mitochondrial membrane permeabilization
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mtDNA	Mitochondrial DNA
NaB	Sodium butyrate
NAB2	*N*-aryl benzimidazole
NHP	Nonhuman primate
NMDA	*N*-methyl-D-aspartate
NOS	Nitric acid synthase
NPC	Neural progenitor cells
Nrf2	Nuclear factor (erythroid-derived 2)-like 2
NTFs	Neurofibrillary tangles
Oct3/4	Octamer 3/4
PCR	Polymerase chain reaction
PD	Parkinson's disease
PDAPP	Promoter-driven amyloid precursor protein
PDGF	Platelet-derived growth factor
PINK1	PTEN-induced putative kinase 1
PLG	Polylactide-co-glycolide
PSEN1	Presenilin 1
PSEN2	Presenilin 2
ROS	Reactive oxygen species
SAD	Sporadic form of Alzheimer's disease

SNCA	Synuclein, Alpha
Sox2	SRY-box containing gene 2
TALEN	Transcription activator-like effector nucleases
VEGF	Vascular endothelial growth factor
VPA	Valproic acid
VPS35	Vacuolar protein sorting-associated protein 35
VSV-G	Vesicular stomatitis virus G
ZFN	Zinc finger nucleases

2.1 Overview of Age-Related Chronic Neurodegenerative Diseases

2.1.1 Alzheimer's Disease

Alzheimer's disease (AD) was identified more than 100 years ago, and it is considered as the most common type of dementia (Alzheimer's Association 2015). AD is known as a progressive disease that affects primarily memory and cognitive and functional abilities (Nussbaum and Ellis 2003). Approximately 8 million new cases are recorded each year, and it is estimated that this number will reach 115 million by the end of 2050 (Jindal et al. 2014). Additionally, family members and other unpaid caregivers provided 17.9 billion hours of care in the USA (Alzheimer's Association 2015). However, the prevalence of AD around the world is changing depending on diagnostic criteria and other factors such as ethnicity, age, etc. (Hendrie et al. 2001; Hy and Keller 2000). Yet, definite diagnosis can only be done post-mortem; there are several studies, which are being studied to identify novel biomarkers for earlier diagnosis.

The pathological hallmarks of AD are loss of neurons in the hippocampus and extracellular senile plaques consisting of β-amyloid peptides and neurofibrillary tangles (NFTs), which are composed of hyperphosphorylated form of microtubule protein tau (De-Paula et al. 2012).

β-Amyloid is produced by cleavage of amyloid precursor protein (APP) with α-, β-, and γ-secretases. Normally, the cleavage of APP with first α-secretase and then γ-secretase occurs in the non-amyloidogenic pathway. However, involvement of β-secretase results in the formation of longer C-terminal fragment (C99), which contains amyloidogenic amino acid sequence. Further cleavage with γ-secretase yields β-amyloid peptides. β-Amyloid $_{(1–42)}$ is the most toxic form of amyloid oligomers, and it can aggressively accumulate in the extracellular niche, leading to neuronal cell death.

Another hallmark of AD is hyperphosphorylation of tau protein. Tau is a microtubule-associated protein, which binds to α- and β-tubulins for their stabilization. Additionally, its phosphorylation state is important in stabilization. Yet, its abnormal phosphorylation leads inability to bind tubulins that result in destabilization of microtubules and finally cell death.

AD have two types: sporadic form (SAD) and familial form (FAD). Mutations in three different genes result in familial AD. These genes are APP, which encodes amyloid precursor protein, and presenilin 1 and 2 (*PSEN1* and *PSEN2*), which encode parts of gamma-secretase family proteins. Individuals with mutations in one of those genes are likely to develop AD. However, familial AD constitutes only 1–2% of all AD cases.

SAD constitutes the vast majority of the disease. Since it is considered late onset, it develops in individuals >65 years old. Younger individuals can also develop AD before the age of 65, but this is rare. There are certain risk genes that may cause to develop AD. Apolipoprotein E (APOE) gene is the most validated risk gene, which has three different alleles. If an individual has at least one copy of apoε4 allele, the risk for developing AD is 3- to12-folds higher (Alzheimer's Association 2015).

2.1.2 Parkinson's Disease

Parkinson's disease (PD) is an idiopathic and chronic neurodegenerative disease that primarily affects motor functions. It is the second most common neurodegenerative disease, which was named in 1800s in the honor of James Parkinson. PD is not common in younger adults aged below 40 (Beitz 2014). The prevalence of the disease varies with increasing age. Additionally, several factors affect the prevalence of PD; these includes geographical location, sex, and age (Pringsheim et al. 2014). Moreover, several studies have shown that exposure to exogenous toxins, genetic background, inflammation, and their combinations can increase the chance of developing PD (Bartels and Leenders 2009).

PD is characterized by the loss of dopaminergic neurons in the substantia nigra and the formation of Lewy bodies. Together with the formation of cellular inclusions, these findings represent the hallmarks of pathophysiology of PD (Davie 2008). Lewy bodies contain neurofilamentous proteins along with the proteins that are responsible for proteolysis including ubiquitin, a heat shock protein. Mutations in α-synuclein are responsible for familial PD. However, mutations in *parkin* gene can cause parkinsonism, without the formation of Lewy bodies. Furthermore, *LRRK* 2 gene is known to cause sporadic, idiopathic, or familial PD (Davie 2008). Genome-wide association studies (GWAS) have revealed that mutations in different genes may cause PD development. Those include *SNCA*, *VPS35*, *PINK1*, and *DJ-1* in addition to genes that are mentioned above. Mutations in *SNCA*, *LRRK2*, and *VPS35* genes are known as an autosomal dominant cause of PD. Furthermore, mutations in *parkin*, *DJ-1*, and *PINK1* genes are an autosomal recessive form of PD and accounted for early-onset parkinsonism (Bonifati 2014).

Clinical diagnosis is based on some physical changes and requires accurate anamnesis. Those characteristics include rest tremor, rigidity, bradykinesia, and postural instability. Additionally, some other clinical signs are worth to give attention such as problems in handwriting and reduced facial expression (Hughes et al. 1992). Since, it's shown that Lewy bodies first accumulate in the olfactory bulb, reduced sense of smell cannot be ruled out for more accurate diagnosis (Hawkes 1995).

2.2 Induced Pluripotent Stem Cells Technology

Embryonic stem (ES) cells are capable of differentiating into cells of all three germ layers. They are able to proliferate indefinitely, while preserving their pluripotency. Furthermore, they hold great promise to treat neurodegenerative diseases, such as AD and PD. However, ethical concerns have emerged about the use of ES cells, since they are found in the inner mast of the blastocysts in addition to tissue rejection problems (Vazin and Freed 2010).

To overcome those issues regarding ES cells, new ways have to be found to produce stem cells while maintaining their pluripotency and self-renewal capabilities. In 2006, Yamanaka and his coworkers found a new way to obtain from somatic cells, and his work was granted the Nobel Prize in Physiology or Medicine 2012. They found that using four transcription factors known as "Yamanaka factors" can reprogram mouse embryonic and adult fibroblasts into pluripotent stem cells. These factors are octamer 3/4 (Oct3/4), SRY-box containing gene 2 (Sox2), cytoplasmic Myc protein (c-Myc), and Kruppel-like factor 4 (Klf4) (Takahashi and Yamanaka 2006).

In 2007, Yamanaka and his coworkers move a step to further their work and use adult human fibroblasts to produce induced pluripotent stem cells (iPSCs) by using the same defined transcription factors (Takahashi et al. 2007). Since then, a great number of studies have been done to develop iPSCs technology. Furthermore, human somatic cells were reprogrammed with Oct4, Sox2, Nanog, and LIN28 (Yu et al. 2007). For this purpose, different reprogramming factors, small compounds, mRNAs, and proteins are being used to enhance efficiency for the generation of iPSCs. Moreover, different delivery methods and sources are being examined.

Apart from fibroblasts, various cell types are being used to generate iPSCs, since reprogramming the efficiency and quality of iPSCs differs among different cells. To date, different cells have been used as source for iPSCs, such as primary hepatocytes, exfoliated renal epithelial cells, umbilical cord and peripheral blood cells, keratinocytes (Raab et al. 2014), pancreatic β cells (Stadtfeld et al. 2008), melanocytes (Utikal et al. 2009), neural cells (Kim et al. 2008), and adipose tissue cells (Sugii et al. 2010). Furthermore, human umbilical vein endothelial cells (HUVEC) are reprogrammed into iPSCs and differentiated to astrocytes and neurons (Haile et al. 2015). The choice of cell origin for reprogramming depends on several factors including reprogramming efficiency, availability, invasiveness, and methods to be used (Durnaoglu et al. 2011). Fibroblasts are still the first choice for iPSCs reprogramming studies. There are some disadvantages to start with fibroblasts. First, fibroblasts are obtained from the skin by punch biopsy. This procedure is very painful and has some risks such as bleeding and infection. Other disadvantages are longtime period and efficiency. The whole reprogramming takes a long time (5 weeks), and the efficiency is quite low compared with keratinocytes. Peripheral blood is another source for iPSCs generation. The donor should be prepared with G-CSF injection and then $CD34^+$ cells isolated via 4 h of apheresis. The convenient alternative source is keratinocyte. It is possible to obtain keratinocytes easily from scalp hair. In addition, these cells can be reprogrammed faster, and the method has higher efficiency.

2.2.1 Reprogramming Methods

There are several methods to deliver reprogramming factors into the cells. These methods can be classified according to the vector type: viral vector based, naked DNA based, and non-DNA based (de Lazaro et al. 2014).

2.2.1.1 Viral Vector-Based Methods

Retroviruses are the most used and known method for reprogramming. This method contains higher risk of immunogenicity, and the integration of reprogramming factors into genome can be a problem for further applications of iPSCs. Moreover, lentiviruses (LV) are used for low-efficiency problems of retroviruses, since they can only transduce dividing cells, and they also have enhanced tropism owing to vesicular stomatitis virus G (VSV-G) pseudotyping. Integration can still be an issue for LV transduction (Hu 2014). However, the use of excisable transgenes with LV vectors may overcome this problem (Sommer et al. 2010). Another method involves adenoviruses, which make transgene-free iPSCs possible; yet it has a low expression of reprogramming factors and higher integration frequency than naked plasmid DNA. *Sendai virus* (SeV)-based vectors are DNA-free vectors, and a single vector can contain all four reprogramming factors. So, efficiency is much higher compared to using four different vectors. In addition, genomic integration does not occur, and removal of virus particles is much easier. Thus, safety and immunogenicity can be established. Furthermore, alphaviruses are used to deliver RNA replicons. However, integration can occur due to cDNA conversion in the target cells.

2.2.1.2 Naked DNA-Based Methods

Other method involves the use of naked DNA. For this purpose PiggyBac transposons, plasmids, and episomal plasmids are used. Using plasmids for the delivery of reprogramming factors requires repeated transfection steps, and also current transfection methods are inadequate for reprogramming. Nucleofection can be used, but this method requires a relatively high number of cells, as it results in significant cell death. Nevertheless, some random integration can occur. Using episomal plasmids can be delivered by using *Epstein-Barr virus*, which can replicate in human cells. With this method, the quality of iPSCs is high and has lower immunogenicity. However, efficiency is very low, and additional reprogramming factors are required. Furthermore, polycistronic sequences can also be used. By this way, integration into genome can be reduced, and every infected cell receives all four factors, but packing into viral particles is hard due to a larger size of plasmid. In addition to polycistronic sequences, Lox sequences can be added using 2A sequences. Using Cre recombinase, excision of integrated sequences can be easy. The PiggyBac transposon system is a more advanced version of plasmid system, since it provides excision without any genomic alteration and lower immunogenicity. Yet, an extra excision step is required, and imperfect excision may occur (Hu 2014).

2.2.1.3 Non-DNA-Based Methods

Non-DNA-based methods include the use of synthetic mRNAs, miRNA mimics, and small compounds. mRNA transfection can induce innate immune response through toll-like receptors, which leads to severe cytotoxicity. However, synthetic mRNAs can bypass innate responses and allow the generation of transgene-free iPSCs. This method has higher efficiency and low toxicity. Additionally, functionality of mRNAs is higher due to translation in the cytoplasm and proper posttranslational modifications. The main disadvantage of this system is that expression time is low (about 2–3 days), and repeated transfection is needed.

miRNAs are a class of short, noncoding RNAs, which regulate their target mRNAs by binding to the 3′ untranslated regions (UTRs), 5′ UTRs, or open reading frames (ORFs). miRNAs have key regulatory functions, starting from the embryonic development and extending to cellular differentiation and growth. Thus, it is not surprising that miRNAs are associated with pluripotency of stem cells. Earlier studies have demonstrated the requirement for ES cell-specific miRNA signatures for self-renewal and differentiation of ES cells (Kanellopoulou et al. 2005; Jia et al. 2013). Afterwards, several studies have shown that miRNAs can be used as reprogramming factors. Advantages of this system include the ease of their synthesis, non-integrating nature of miRNAs, and controllable administration. Furthermore, miRNA expression in the cytoplasm is relatively longer, and less transfection is needed (Hu 2014).

iPSCs generation can also be done using proteins of four reprogramming factors. Thus, there is no need for any exogenous genetic material that can cause integration into genome. However, this technique requires permeabilization of cell membranes prior to the delivery of proteins. So, there are a few techniques to achieve this problem. One of them is the usage of cell-penetrating peptides, which contain high amount of basic amino acids. These peptides can be linked with C-terminus of four reprogramming factors. These fusion proteins can be produced in *E. coli* or HEK293 cell line. Furthermore, nuclear localization signal peptide can be fused to reprogramming factors, and this provides minimalization of lysosomal degradation of proteins (Li et al. 2014).

Small compounds are also used to generate iPSCs. These molecules enhance the efficiency of iPSCs generation. For this purpose, histone deacetylation, demethylation, and methyltransferase inhibitors are used. Furthermore, signaling pathway inhibitors (e.g., glycogen synthase kinase 3, GSK-3) and epigenetic modulators can also be used. The purpose is to increase efficiency and generate iPSCs without using genetic materials. One of these compounds is the valproic acid (VPA), which inhibits histone deacetylation. It enhances iPSC generation and can be used as replacement for c-Myc. Furthermore, this chemical can improve efficiency as far as 1000-fold. Another chemical used for this purpose is sodium butyrate (NaB). It can be used throughout the whole process, and reprogramming efficiency is increased (Revilla et al. 2015). Moreover, lithium also has increased the efficiency of reprogramming of both mouse endothelial fibroblasts (MEFs) and HUVECs (Wang et al. 2011; Wu et al. 2013; Masuda et al. 2013). In addition, vitamin C can reduce senescence state partially. Sodium chloride can reduce nearly all demethylation

levels via hyperosmosis. Ascorbic acid and GSK3-β inhibitor can facilitate reprogramming as well (Revilla et al. 2015). Besides, Hou et al. showed that VPA, CHIR99021, 616,452, tranylcypromine, forskolin, 3-deazaneplanocin A, 2-methyl-5-hydroxytryptamine hydrochloride, and D4476 are used to generate iPSCs from mouse somatic cells (Hou et al. 2013).

2.3 Applications of iPSCs

Animal models are used to understand the mechanisms of neurodegenerative diseases and to screen potential drugs and seeking therapeutic strategies. However, generating models that accurately mimic the disease as in human physiology is a problem, since there are differences in species, cell-line specificity, and lack of brain complexity (Wan et al. 2014). Furthermore, there are no models for rare diseases, and using animal models to observe disease progression remains difficult and raises some ethical issues regarding using too much animals. Moreover, screening for new drugs and performing toxicity tests for available drugs are time-consuming. An additional challenge is to obtain cells (e.g., neurons) from living individuals. In this context, Daley's group developed disease-specific iPSC models for the first time. They use fibroblasts and bone marrow mesenchymal cells to generate disease-specific iPSCs including PD, Huntington's disease, and Down syndrome (Park et al. 2008).

Using iPSCs technology to establish disease models has its own advantages. This technology allows us to model diseases more accurately. Therefore, it can provide insight into the mechanistic basis of the diseases and leads to discovery of new effective treatment strategies. Furthermore, high-throughput chemical screening with iPSCs allows predicting more accurate drug-induced toxicity. Additionally, cell replacement therapies with patient-specific iPSCs are the ultimate goal, and it can develop our current personalized medicine strategies for various diseases.

2.3.1 Disease Modeling

There are inherent differences between the nervous systems of rodents and humans, and difference in life spans of those species may also cause inability to serve as appropriate AD and PD models. Modeling sporadic and familial AD with iPSCs provides understanding the mechanisms of AD pathology and establishing new drug testing platforms. Recent studies revealed that human-generated iPSCs could be used for disease modeling. Initial studies were focused on familial AD mutations, since these are more homogenized and well characterized (Doege and Abeliovich 2014).

First AD-specific iPSCs were produced from the skin fibroblast of familial AD patients with *PSEN1* and *PSEN2* mutations (Yagi et al. 2011). Then iPSC-derived neurons from different familial AD mutations (*APP*, *PSEN1*, and *PSEN2*) have been

generated to study the pathogenesis of the disease (Table 2.1). Woodruff and colleagues used transcription activator-like effector nucleases (TALENs) to introduce ΔE9 PSEN1 mutations, whether the mutation reduced γ-secretase activity in iPSC-derived neural cells (Woodruff et al. 2013).

Additionally, tau phosphorylation was also increased in iPSC-derived neurons from patients with familial AD (Israel et al. 2012). In another study, iPSCs, which are reprogrammed from fibroblasts of one daughter and father, carrying APP London mutation (V717I), differentiated into forebrain neurons, and they showed AD-like phenotypes and increased levels of $A\beta_{(42)}$ and $A\beta_{(38)}$ and t-tau and p-tau. Interestingly, they found an alteration in γ-secretase cleavage site (Muratore et al. 2014). Heterogenic phenotypes were seen in iPSC-derived neurons. For example, increased phosphorylated tau levels were not seen in the neurons carrying *PSEN1* or *PSEN2* mutation (Yagi et al. 2011).

In addition to AD phenotypes, differential gene expression changes were seen in iPSC-derived neurons from patients with familial AD. iPSC-derived neuron with different PSEN1 mutations have shown that ten different genes have been upregulated, and four genes have been downregulated along with increased generation of $A\beta_{(42)}/A\beta_{(40)}$ (Yagi et al. 2011; Sproul et al. 2014; Liu et al. 2014). Furthermore, in one study, iPSC-derived cortical neurons have increased the endoplasmic reticulum and oxidative stress, and also accumulated Aβ oligomers are prone to proteolysis (Kondo et al. 2013).

Apart from these models, iPSC-derived neurons can be obtained from sporadic AD patients who carry the risk gene allele Apoε4 and others (Table 2.1). iPSC-derived cholinergic neurons carrying Apoε4 allele showed elevated $A\beta_{(42)}/A\beta_{(40)}$ ratio, increased calcium levels within the cytoplasm upon glutamate exposure, and sensitivity for neurotoxic stimuli (Duan et al. 2014). iPSC-derived neurons generated from sporadic form of AD showed increased levels of $A\beta_{(1–40)}$ and phosphor-$tau_{(Thr231)}$ levels along with activated GSK-3β. Yet, one of them has increased Aβ levels in neurons and increased ER and oxidative stress also (Israel et al. 2012; Kondo et al. 2013).

PD is the other common neurodegenerative disease. There are many studies which are used in iPSC-derived dopaminergic neurons to model PD. Despite of familial PD cases compromising 5–10% of total PD cases (Nishimura and Takahashi 2013), most of the iPSCs are derived from fibroblasts of patients who have familial, while a few studies use iPSCs from sporadic PD patients. Patient-derived iPSC, which have different mutations in *LRRK2*, *PINK1*, *SCNA*, and *PARK2*, can differentiate into dopaminergic neurons as control iPSCs. These iPSC-derived dopaminergic neurons show various phenotypes including increased oxidative stress, increased α-synuclein expression and elevated mitochondrial gene expressions, etc. (Table 2.2). These findings are consistent with non-iPSCs models and brain autopsies (Lee et al. 2012b). Moreover, other studies have revealed novel phenotypes, which are worth to investigate. For instance, increased monoamine oxidase (MAO) activity was observed in *PARK2* mutant iPSC-derived dopaminergic neurons, and cells showed increased dopamine release and decreased uptake (Jiang et al. 2012). Furthermore, Ryan et al. reported that A53T α-synuclein mutant

Table 2.1 Disease modeling studies with patient-specific iPSCs in Alzheimer's disease

Gene	Genomic alteration	Reprogramming delivery methods	iPSC-derived cell types	Phenotype	Reference
APP	Duplication	Retroviral (*OCT4*, *SOX2*, *KLF4*, *c-MYC*, and ± *EGFP*)	Neuron (glutamergic and GABAergic)	Increased Aβ40, p-tau(Thr 231) and aGSK-3β	Israel et al. (2012)
	E693Δ	Episomal vector (*SOX2*, *KLF4*, *OCT4*, *L-MYC*, *LIN28*, and small hairpin RNA for p53)	Cortical neuron, astrocyte	Aβ oligomers accumulated in iPSC-derived neurons, increased ER, and oxidative stress	Kondo et al. (2013)
	V717 L	Episomal vector (*SOX2*, *KLF4*, *OCT4*, *L-MYC*, *LIN28*, and small hairpin RNA for p53)	Cortical neuron, astrocyte	Intracellular Aβ oligomers were not detected, increased extracellular Aβ42/Aβ40 ratio	Kondo et al. (2013)
	V717I	Lentiviruses (Oct4, SOX2, cMYC, and KLF4)	Forebrain neurons	Alteration in initial cleavage site of γ-secretase, increased Aβ42 and Aβ38, increased total and p-tau	Muratore et al. (2014)
	Trisomy 21	Retroviral (*OCT4*, *SOX2*, *cMYC*, *KLF4*, and *NANOG*)	Neuron	**Increased Aβ peptide production and aggregation,** hyperphosphorylation, and redistribution of tau (localized to cell bodies and dendrites)	Shi et al. (2012)

PS 1	A246E	Retroviral (OCT4, SOX2, KLF4, LIN28, and NANOG)	Neuron	Increased Aβ secretion, no tau accumulation	Yagi et al. (2011)
		Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Neuron	Elevated levels of Aβ42, increased Aβ42/Aβ40 ratio	Liu et al. (2014)
		Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Basal forebrain cholinergic	Not increased Aβ42/Aβ40 ratio	Duan et al. (2014)
		Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Neuron	Increased generation of Aβ42/40, ten upregulated genes (*ASB9*, *BIK*, *C7orf16*, *NDP*, *NLRP2*, *PLP1*, *SLC45A2*, *TBX2*, *TUBB4*, *ZNF300*), and four downregulated genes (*ADM2*, *FLJ35024*, *MT2A*, *PTGS2*)	Sproul et al. (2014)
		Episomal vector (OCT4, SOX2, NANOG, LIN28), (OCT4, SOX2, SV40LT, KLF4), or (OCT4, SOX2, MYC, KLF4)	Neuron	Elevated levels of Aβ42, increased Aβ42/Aβ40 ratio	Mahairaki et al. (2014)
	M146 L	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Neuron	Increased generation of Aβ42/40, ten upregulated genes (*ASB9*, *BIK*, *C7orf16*, *NDP*, *NLRP2*, *PLP1*, *SLC45A2*, *TBX2*, *TUBB4*, *ZNF300*), and four downregulated genes (*ADM2*, *FLJ35024*, *MT2A*, *PTGS2*)	Sproul et al. (2014)
		Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Neuron	Elevated levels of Aβ42, increased Aβ42/Aβ40 ratio	Liu et al. (2014)
	H163R	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Neuron	Elevated levels of Aβ42, increased Aβ42/Aβ40 ratio	Liu et al. (2014)
	ΔE9	Retroviral from normal human fibroblast (*OCT4*, *SOX2*, *KLF4*, *c-MYC*, and ± *EGFP*), mutation generated with TALENs	Neuron	Decreased Aβ40, increased Aβ42/Aβ40 ratio	Woodruff et al. (2013)
	L166P	Retroviral	Neuron	Partial loss of γ-secretase function, decreased production of endogenous Aβ40, and an increased Aβ42/β40 ratio	Koch et al. (2012)

(continued)

Table 2.1 (continued)

Gene	Genomic alteration	Reprogramming delivery methods	iPSC-derived cell types	Phenotype	Reference
PS2	N1411	Retroviral (OCT4, SOX2, KLF4, LIN28, and NANOG)	Neuron	Increased Aβ secretion, no tau accumulation	Yagi et al. (2011)
Sporadic	*ApoE3/E4* genotypes	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Basal forebrain cholinergic neurons	Increased Aβ42/40 ratios, increased sensitivity to neurotoxic stimuli, increased cytoplasmic calcium levels upon glutamate exposure	Duan et al. (2014)
	None	Retroviral (*OCT4*, *SOX2*, *KLF4*, *c-MYC*, and ± *EGFP)*	Neuron	One patient iPSC shows increased Aβ40, p-tau(Thr 231), and active glycogen synthase kinase-3β, one patient iPSC is normal	Israel et al. (2012)
	None	Episomal vector (*SOX2*, *KLF4*, *OCT4*, *L-MYC*, *LIN28*, and small hairpin RNA for p53)	Cortical neuron, astrocyte	Aβ oligomers accumulated in iPSC-derived neurons, increased ER and oxidative stress	Kondo et al. (2013)
	None	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Neuron	N/A	Yagi et al. (2011)
	None	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Neuron	Positive for neuronal cell markers (PAX6, NESTIN, and b-TUBULIN III), expressed p-tau and GSK3B, down-regulation of AD-related genes	Hossini et al. (2015)

Table 2.2 Disease modeling studies with patient-specific iPSCs in Parkinson's disease

Gene	Genomic alteration	Initial cell	Reprogramming methods	iPSC-derived cell types	Phenotype	Genetic correction	Reference
SNCA	Triplication	Fibroblast	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Dopaminergic neuron	Increased α-synuclein expression, gene expression profile is similar with hES cell	N/A	Devine et al. (2011)
					Increased α-synuclein expression, overexpression of markers of oxidative stress (DNAJA1, HMOX2, UCHL1, HSPB1, and MAO-A), and sensitivity to peroxide-induced oxidative stress	N/A	Byers et al. (2011)
					Reduced proliferative capacity in NPCs, altered cellular energy balance and decreased mitochondrial function, delayed protein import and increased protein aggregation, increased cellular stress and reactive oxygen species	Lentiviral SNCA gene knockdown	Flierl et al. (2014)
					Impaired mitochondrial integrity and permeability transition, Caspase activation and apoptosis	N/A	Chung et al. (2013)
	A53T (G209A)	Fibroblast	Lentiviral vectors (Oct4, KLF4, SOX2 c-Myc, and Nanog)	Dopaminergic neuron	Higher number of CNVs, ZFN correction leads loss of expression of the mutated A53T (G209A) transcript, the genetic repair did not affect differentiation capacity of iPSc	ZFN	Soldner et al. (2011)
			Lentiviral vectors (Oct4, KLF4, SOX2, and c-Myc)	Dopaminergic neuron	α-synuclein aggregation, increased oxidative and nitrosative stress, increased toxin mediated apoptosis	ZFN	Ryan et al. (2013)
			Lentiviral vectors (Oct4, KLF4, SOX2, and c-Myc)	Dopaminergic neuron	Endoplasmic reticulum form of glucoserebrosidase and nicastin accumulated	ZFN	Chung et al. (2013)

(continued)

Table 2.2 (continued)

Gene	Genomic alteration	Initial cell	Reprogramming methods	iPSC-derived cell types	Phenotype	Genetic correction	Reference
PARK2	Heterozygous exon 3 and 5 deletion	Fibroblast	Lentiviral vectors (Oct4, KLF4, c-Myc, and Nanog)	Dopaminergic neuron	Increased dopamine release, decreased dopamine uptake, elevated oxidative stress, increased MAO transcript	Lentiviral overexpression of parkin	Jiang et al. (2012)
					Reduce the length and complexity of neuronal processes, reduce microtubule stability	Lentiviral overexpression of parkin	Ren et al. (2015)
	Exon 3 deletion	Fibroblast	Lentiviral vectors (Oct4, KLF4, c-Myc, and Nanog)	Dopaminergic neuron	Increased dopamine release, decreased dopamine uptake, elevated oxidative stress, increased MAO transcript	Lentiviral overexpression of parkin	Jiang et al. (2012)
					Reduce the length and complexity of neuronal processes, reduce microtubule stability	Lentiviral overexpression of parkin	Ren et al. (2015)
	Exon 2–4 deletion	Fibroblast	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Dopaminergic neuron	Increased oxidative stress and enhanced activity of Nrf2, abnormal mitochondrial morphology	N/A	Imaizumi et al. (2012)
	Exon 6–7 deletion						
	V324A	Fibroblast	*Sendai virus* (KLF4, SOX2, and c-Myc)	Dopaminergic neuron	Progerin overexpression causes increased apoptosis, enhanced dendrite shortening, and reduced AKT activation	N/A	Miller et al. (2013)
	Heterozygous R275W				Increased in condensed nuclei and caspase-3, progerin overexpression leads loss of dendrite length	N/A	Miller et al. (2013)
	40 bp deletion in exon 3 of one allele and exons 5 and 6 deletion in the other allele	Fibroblast	Retroviral (Oct4, KLF4, SOX2, and c-Myc) and episomal (OCT3/4, SOX2, KLF4, L-MYC, LIN28, and shRNA for TP53)	NPC	Mn exposure was associated with significantly higher reactive oxygen species	N/A	Aboud et al. (2012)

	R42P and exon 3 deletion	Fibroblast	*Sendai virus* (Oct4, KLF4, SOX2, and c-Myc)	Dopaminergic neuron	Impaired dopaminergic differentiation on the percentage of TH+ neurons was decreased, reduced mitochondrial volume fraction, increased expression of mitochondrial genes and cell death genes	N/A	Shaltouki et al. (2015)
	Exons 3–4 deletion 1-bp deletion 255A						
	R275W						
	R42P						
PINK1	Q456X	Fibroblast	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Dopaminergic neuron	Impaired stress induced mitochondrial translocation of parkin, increased mitochondrial copy number, and upregulation of PGC-1α	Lentiviral overexpression of PINK1	Seibler et al. (2011)
			Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Dopaminergic neuron	Vulnerability to cell stressors, oxidative stress	N/A	Cooper et al. (2012)
			Sendai virus (KLF4, SOX2, and c-Myc)	Dopaminergic neuron	With progerin overexpression causes increased apoptosis, enlarged mitochondria	N/A	Miller et al. (2013)
	V170G	Fibroblast	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Dopaminergic neuron	Impaired recruitment of lentivirally expressed parkin to mitochondria, increased mitochondrial copy number, and upregulation of PGC-1α	Lentiviral overexpression of PINK1	Seibler et al. (2011)
					Detectable mitophagy when parkin gene is overexpressed in fibroblast but not iPSC derived neurons	Lentiviral overexpression of PINK1	Rakovic et al. (2013)

(continued)

Table 2.2 (continued)

Gene	Genomic alteration	Initial cell	Reprogramming methods	iPSC-derived cell types	Phenotype	Genetic correction	Reference
LRRK2	G2019S	Fibroblast, keratinocytes	Retroviral (Oct4, KLF4, SOX2, and c-Myc) or (Oct4, KLF4, and SOX2)	Dopaminergic neuron	Increased expression of key oxidative stress-response genes and a-synuclein protein; increased sensitivity to caspase-3 activation and cell death caused by exposure to stress agents (hydrogen peroxide, MG-132, and 6-hydroxydopamine), decreased tau expression, and increased α-synuclein, 6-OHDA sensitivity	N/A	Nguyen et al. (2011)
		Fibroblast	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Neural cells	Decreased oxygen consumption rate, dysfunctional mitochondrial mobility, sensitive to valinomycin and concanamycin A	N/A	Cooper et al. (2012)
		Fibroblast	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Dopaminergic neuron	LMX1A overexpression efficient way for generate dopaminergic neurons	N/A	Sanchez-Danes et al. (2012a)
		Fibroblast, keratinocytes	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Dopaminergic neuron	Reduced numbers of neurites, impaired autophagy	N/A	Sanchez-Danes et al. (2012b)
		Fibroblast	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	NPCs	Increased susceptibility to proteasomal stress and passage-dependent deficiencies in nuclear-envelope organization, clonal expansion, and neuronal differentiation	Gene targeting with HDAdV	Liu et al. (2012)
		Fibroblast	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Dopaminergic neuron	Impaired autophagy, increased α-synuclein accumulation	N/A	Orenstein et al. (2013)

		Fibroblast	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Dopaminergic neuron	Neurite shortening and sensitivity to neurotoxins (rotenone, 6-OHDA), tau upregulation, dysregulation of CPNE8, MAP7, UHRF2, ANXA1, and CADPS2, correction decreased sensitivity of neurons to rotenone	ZFN	Reinhardt et al. (2013)
		Fibroblast	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Dopaminergic neuron	Altered mitochondrial morphology, less mitochondria loss of MMP, increased mitoROS, and decreased ATP levels	N/A	Su and Qi (2013)
		Fibroblast	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	NPC, mature neural cell	Increased mtDNA damage	ZFN	Sanders et al. (2014)
	R1441C	Fibroblast	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Neural cells	Decreased oxygen consumption rate, dysfunctional mitochondrial mobility, sensitive to valinomycin and concanamycin A	N/A	Cooper et al. (2012)
		Fibroblast	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	NPC, mature neural cell	Increased mtDNA damage	ZFN	Sanders et al. (2014)

(continued)

Table 2.2 (continued)

Gene	Genomic alteration	Initial cell	Reprogramming methods	iPSC-derived cell types	Phenotype	Genetic correction	Reference
GBA	N370S/84GG insertion	Fibroblast	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Dopaminergic neuron	Lysosomal protein degradation, causes accumulation of α-synuclein and results in neurotoxicity	N/A	Mazzulli et al. (2011)
	L444P N370S	Fibroblast	lentiviral vectors (Oct4, KLF4, SOX2, and c-Myc)	Dopaminergic neuron, astrocyte, oligodendrocyte	Very low GC enzymatic activity	N/A	Panicker et al. (2012)
Sporadic	None	Fibroblast	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	N/A	N/A	N/A	Park et al. (2008)
	None	Fibroblast, keratinocytes	Retroviral (Oct4, KLF4, SOX2, and c-Myc)	Dopaminergic neuron	Reduced numbers of neurites, impaired autophagy	N/A	Sanchez-Danes et al. (2012b)
	None	Fibroblast	lentiviral vectors (Oct4, KLF4, SOX2, and c-Myc) or (Oct4, KLF4, and SOX2)	Dopaminergic neuron	Differentiation efficiency similar with control. TH+ and TUJ + neurons generated	N/A	Soldner et al. (2009)

iPSC-derived cells nitrosative/oxidative stress resulted in S-nitrosylation of myocyte enhancer factor 2C (MEF2C) (Ryan et al. 2013). Neural progenitor cells (NPC) differentiated from both healthy and PARK2 mutation-carrying individuals without any PD manifestations showed that manganese (Mn) treatment did not result in any difference between groups. Yet, Mn treatments caused increased reactive oxygen species (ROS) levels in mutated iPSC-derived NPCs (Aboud et al. 2012). Ren et al. have shown that iPSC-derived neurons carrying parkin mutation have decreased microtubule stability and shorter neurite length. Further, overexpression of parkin gene restores microtubule stability and complexity of neural processes (Ren et al. 2015).

Not all studies are related with familial PD mutations. iPSC-derived dopaminergic neurons generated from sporadic PD patient cells have revealed that their phenotype is similar to those found in familial PD. However, dopaminergic neurons generated from sporadic PD-derived iPSCs need to be cultured for a long time in cell culture to be able to show PD-related phenotype (Sanchez-Danes et al. 2012b).

Taken together, these findings are accelerating the research on neurodegenerative disease and lead to new translational approaches such as high-throughput drug screening. In spite of new developments, there are still major concerns to overcome before using iPSC technology.

2.3.2 Drug Screening and Testing

The current drug discovery methods are time-consuming and expensive, as well as failure rate is higher due to serious side effects such as cardiotoxicity and hepatotoxicity. Approximately 90% of the drugs are not able to reach the market. Additionally, 30% of the drugs are given up due to side effects and lack of efficiency in clinical trials (Singh et al. 2015). Furthermore, safety data come from animal models, and interpreting the results is not efficient due to species-specific differences. Using human cell-based toxicity test can overcome these problems, since organ-specific cells can be used for high-throughput toxicity screening, while ethical concerns and time-consuming procedures of animal usage are emerging (Heilker et al. 2014).

Current treatments for AD include cholinesterase inhibitors, which are used to treat mild to moderate AD, and *N*-methyl-D-aspartate (NMDA) receptor antagonists which are used to treat moderate to severe AD. However, these treatment strategies rely on only improving symptoms. To date, there are no drugs that can reverse neuronal loss and stop the cognitive decline in AD. Several experimental therapy options exist; these include immunotherapies, which target to enhance Aβ clearance such as bapineuzumab, solanezumab, and intravenous immunoglobulins. Moreover, gamma-secretase inhibitors and modulators have been also tested in clinical trials. However, these studies have failed to pass phase II and III trials. Herbal supplements such as docosahexaenoic acid (DHA) have the potential as drugs in the treatment of AD, and they need to be investigated further to provide as symptomatic treatment option. Conclusively, there are also significant amount of potential drug failures in

late-stage clinical trials, yet these failures may alter the future of novel therapy options (Berk and Sabbagh 2013).

Despite the advancements in PD treatment, there is no drug that can cure PD completely. The major challenge for this problem is that molecular mechanism of PD pathology remains unknown and primary cause of dopaminergic neuron loss is also not known. Several drugs have been found to be effective in animal models; however, they failed in clinical trials due to the aforementioned problems related to animal models. Also, doses of drugs used in clinical trials may not be effective. Furthermore, there is no drug used for neuroprotection. Coenzyme Q, green tea, creatine, and minocycline have no effect on disease progression. Currently used drugs focus on the improvement of symptoms as in AD treatment. Levodopa (best known antiparkinsonian drug), dopamine agonists, glutamate antagonists, MAO B inhibitors, and catechol-O-methyltransferase (COMT) inhibitors are used to improve symptoms such as dyskinesia (Stocchi 2014).

Currently, experimental drugs are being evaluated using iPSC-derived neurons and dopaminergic neurons in both AD and PD models (Table 2.3). Kondo and colleagues used *β*-secretase inhibitor (BSI), DHA, NSC23766 (Rac1 inhibitor), and dibenzoylmethane (DBM14–26) to examine the effects on familial and sporadic AD iPSC-derived neurons. The authors found no change in the levels of Aβ oligomers; on the other hand, they found that DHA reduced ROS generation as well as cleaved caspase-4 and peroxiredoxin-4 in neurons. Furthermore, high dose of DBM14–26, NSC23766, or DHA treatment elevated binding immunoglobulin protein (BiP) levels. In familial AD mutant iPSC-derived neurons, long-term DHA treatment increased cell viability. However, the same treatment did not alter cell survival of sporadic AD iPSC-derived neurons (Kondo et al. 2013). Furthermore, Israel et al. used β and γ-secretase inhibitors to examine the relationship among amyloid-β, p-tau, and GSK-3β. Both inhibitors reduced $A\beta_{(1–40)}$. However, only *β*-secretase inhibitors (βSi-II and OM99–2) significantly reduced aGSK-3β and p-tau/total tau (Israel et al. 2012). Moreover, compound E, a γ-secretase inhibitor, was used to examine its effects on a different mutation carrying iPSC-derived neurons in AD model. Compound E reduced both $A\beta_{(42)}$ and $A\beta_{(40)}$ levels in both PSEN1 and PSEN2 mutations carrying iPSC-derived neurons, and they further used compound W (selective Aβ lowering agent) and found reduced $A\beta_{(42)}/A\beta_{(40)}$ ratio in iPSC-derived neurons (Yagi et al. 2011). Another study has shown that compound E treatment reduces p-tau levels in AD-iPSC-derived neurons (Hossini et al. 2015).

Experimental drugs are also being evaluated in iPSC-based PD models. Cooper et al. have used coenzyme Q_{10}, rapamycin, and LRRK2 inhibitor (GW 5074) in iPSC-derived neurons and found that coenzyme Q_{10} reduced cell vulnerability to valinomycin and concanamycin. However, rapamycin did not change cell death induced by concanamycin. Furthermore, GW 5074 reduced cell death by valinomycin but not concanamycin (Cooper et al. 2012). Moreover, *N*-arylbenzimidazole (NAB2) reversed the PD phenotype such as mitochondrial dysfunction in A53T mutation carrying iPSC-derived neurons (Chung et al. 2013). In another study, they used L-NAME, a nitric oxide synthase (NOS) inhibitor, to prevent S-nitrosylation for the enhancement of MEF2C-PGC1α pathway. They found that L-NAME pretreatment partially recovered pesticide-induced cell death (Ryan et al. 2013).

Table 2.3 Drug testing using iPSCs derived from in Alzheimer's disease and Parkinson's disease patients

Disease	Molecules	Genetic of iPS	Results	Reference
Alzheimer's disease	*β*-secretase inhibitor (BSI)	APP (E693Δ)	Intracellular Aβ oligomers decreased, inhibited secretion of Aβ40 from control neural cells, decreased the amount of binding immunoglobulin protein, and cleaved caspase-4	Kondo et al. (2013)
	β-secretase inhibitor II	APP duplication Sporadic AD	Decreased Aβ40, aGSK-3β, p-tau/total tau	Israel et al. (2012)
	OM99–22 (*β*-secretase inhibitor)	APP duplication Sporadic AD	Decreased Aβ40, aGSK-3β, p-tau/total tau	Israel et al. (2012)
	Docosahexaenoic acid (DHA)	APP (E693Δ)	Decreased binding immunoglobulin protein, cleaved caspase-4, peroxiredoxin-4, ROS, not altered Aβ oligomer	Kondo et al. (2013)
	Docosahexaenoic acid (DHA)	Sporadic AD	decreased binding immunoglobulin protein, peroxiredoxin-4	Kondo et al. (2013)
	DBM14–26	APP (E693Δ)	Increased binding immunoglobulin protein	Kondo et al. (2013)
	NSC23766 (Rac 1 inhibitor)	APP (E693Δ)	Increased binding immunoglobulin protein	Kondo et al. (2013)
	DAPT (gamma-secretase inhibitor)	APP (V717I)	Decreased Aβ38, Aβ40, and Aβ42	Muratore et al. (2014)
		Trisomy 21	Decreased Aβ42 and Aβ40	Shi et al. (2012)
		PS1 (A246E) *ApoE3/E4* genotypes	Variable responses (increased secretion Aβ40 at 100 nM and 200 nM one *ApoE3/E4* genotypes iPSC and decreased the other *ApoE3/E4* genotypes iPSC; inhibited Aβ40 production in all iPSC at 400 nM and 800 nM)	Duan et al. (2014)
		PS1 (L166P)	Secretion of both Aβ40 and Aβ 42 was strongly reduced	Koch et al. (2012)
		APP duplication Sporadic AD	Decreased Aβ40	Israel et al. (2012)

(continued)

Table 2.3 (continued)

Disease	Molecules	Genetic of iPS	Results	Reference
	CPD-E (gamma-secretase inhibitor)	APP duplication Sporadic AD	Decreased Aβ40	Israel et al. (2012)
	Compound E (gamma-secretase inhibitor)	PS1 (A246E), PS2 (N1411)	Decreased Aβ42 and Aβ40	Yagi et al. (2011)
		ApoE3/E4 genotypes	Variable responses (200 pm concentration of compound E increased A β40, 400 pm concentration of compound E decreased Aβ40 in other iPSC line)	Duan et al. (2014)
		Sporadic	Reduced p-Tau in one iPSC but did not result in any reduction of p-tau in other iPSC; p-GSK-3β and GSK-3β expression did not alter	Hossini et al. (2015)
	Compound W (selective Abeta1–42 lowering agent)	PS1 (A246E), PS2 (N1411)	Decreased in the ratio Aβ42 to Aβ40 in neurons	Yagi et al. (2011)
	Semagacestat (gamma-secretase inhibitor)	PS1 (A246E)	Decreased Aβ42 and Aβ40, the ratio Aβ42 to Aβ40	Liu et al. (2014)
	Gamma-secretase modulator-4	PS1 (A246E, M146, H163R) PS2 (N1411)	Decreased Aβ42 and Aβ40 not Aβ38 and total Aβ	Liu et al. (2014)
	Ibuprofen	PS1 (L166P)	Reduced the secretion of Aβ42 and Aβ42/Aβ40	Koch et al. (2012)
	Indomethacin	PS1 (L166P)	Reduced the secretion of Aβ42 and Aβ42/Aβ40	Koch et al. (2012)

Parkinson's disease	Coenzyme Q10	PINK1 (Q456X), LRRK2 (G2019S, R1441C)	Reduced cell vulnerability to low-dose valinomycin and concanamycin A	Cooper et al. (2012)
	Rapamycin		Reduced cell death induced by valinomycin but not concanamycin A in LRRK2 mutant cells. PINK1 mutant cells unresponsive to valinomycin	
	GW 5074 (LRRK2 inhibitor)		Reduced cell death induced by valinomycin but not concanamycin A in PINK1 mutant cells; reduced cell death induced both valinomycin and concanamycin A	
	Y-27632 (ROCK inhibitor)	LRRK2 (G2019S)	No effect on hydrogen peroxide, exacerbate cell death induced by MG-132	Nguyen et al. (2011)
	N-arylbenzimidazole (NAB2)	SCNA A53T (G209A)	Reversed pathologic phenotypes	Chung et al. (2013)
	L-NAME (NOS inhibitor)	SCNA A53T	Rescue from paraquat and MANEB-induced death	Ryan et al. (2013)
	P110 (inhibitor of fission dynamin-related protein 1	LRRK2 (G2019S)	Reduced mitochondrial impairment, lysosomal hyperactivity, and neurite shortening	Su and Qi (2013)

Currently, animal models are used for drug screening and toxicity tests. Yet these systems are merely imperfect replicas of human system. Molecules which are found to be toxic in one animal species may not be toxic for another species. Furthermore, newly discovered drugs should be tested on human cells or the human itself; since it is not exactly possible, we need a system to mimic the conditions in human physiology. iPSC-derived cells are ideal sources for drug screening and toxicity testing, and they represent diseases more accurately in vitro and facilitate drug discovery efforts. Developing more robust and reliable differentiation techniques will improve the application of iPSCs to drug development for different diseases. Also, it is possible to assess how an individual may respond to certain drugs. Furthermore, significant risks and costs of early-stage clinical trials can be avoided. Thus, development of new drugs and patient-specific treatments can increase easily (Qi et al. 2014; Ko and Gelb 2014).

Lee et al. used patient-specific iPSC-derived neural crest precursor cells for drug screening. They performed high-throughput assay which consists of 6912 compounds. They found eight candidate compounds to rescue *IKBKAP* expression, and one of them was SKF-86466 which induces *IKBKAP* expression (Lee et al. 2012a). Furthermore, a low-throughput assay composed of 44 compounds was performed by using iPSC-derived dopaminergic neurons in MPP^+ and rotenone toxicity. They found that 16 of 44 compounds showed a neuroprotective effect (Peng et al. 2013). Moreover, 3.313 drugs were screened using iPSC-derived hepatocytes, yielding 263 hit compounds, 42 of which are approved by the Food and Drug Administration (FDA). Further screening of these 42 compounds showed that 5 compounds were found to be consistent by showing a similar effect on four different patient-specific iPSC-derived hepatocytes (Choi et al. 2013).

Therefore, it is possible to say that iPSC-based drug screening provides a safer, cost-effective, and faster way to develop new drugs and testing existing drugs for toxicity and side effects. Additionally, comparing healthy and diseased iPSC-derived mature cells provides valuable information about disease mechanisms, and novel molecular therapeutic targets can be found in a dish (Giri and Bader 2015).

2.3.3 Cell Replacement Therapy

The first transplantation of iPSC-derived cells was used for the treatment of humanized sickle cell anemia mouse model (Hanna et al. 2007). iPSC-derived cell replacement is a new alternative for the treatment of neurodegenerative diseases. Several groups transplanted iPSC-derived cells into the brain in preclinical animal models of neurodegenerative diseases including AD and PD. Transplanted cells can survive in the different brain regions and provide functional recovery (Table 2.4). Human clinical studies using iPSCs in neurodegenerative disease have not started yet.

The important advantages of autologous iPSCs for transplantation therapy are absence of immune rejection risk and ethical problems. On the other hand, a previous study has shown that autologous undifferentiated iPSCs elicit a very strong

Table 2.4 Cell therapy using iPSCs in preclinical models of Alzheimer's disease and Parkinson's disease

	Animal	Model	iPS source	Cell numbers	Route of administration	Evaluation time	Results	Reference
AD	Mice	APP	hiPSC cell lines, 201B7 and 253G1	2×10^5	Hippocampus		Spatial memory improved, choline acetyltransferase (ChAT) positive cholinergic human neurons and vesicle GABA transporter (VGAT) positive GABAergic human neurons detected in the hippocampus	Fujiwara et al. (2013)
PD	Rat	6-OHDA	hiPSC from PD patient	1×10^5	Intrastriatal	16 week	Behavioral recovery, transplanted cell survive and functional	Hargus et al. (2010)
	Rat	6-OHDA	iPSC cell line (IMR90, clone 4)	1×10^6	Intrastriatal	6 week	No behavioral recovery, but hiPSC mDA progenitor cells survive long term and many develop into bona fide mDA neurons	Cai et al. (2010)

(continued)

Table 2.4 (continued)

Animal	Model	iPS source	Cell numbers	Route of administration	Evaluation time	Results	Reference
Rat	6-OHDA	Autologous fibroblast	7.5×10^5	Intrastriatal	8 weeks	Dramatic functional recovery in amphetamine-induced rotation scores, abundant TH+ cells	Rhee et al. (2011)
			2.5×10^5	Intrastriatal	8 weeks	Moderate but significant level of functional recovery The graft size was more appropriate and uniform, without irregular proliferating mass or rosette formation	
			2.5×10^5 (terminal differentiated)	Intrastriatal	8 weeks	No behavioral recovery, no TH+ cells were readily detectable in the graft. Severe cell death was evident from the central area of the grafts	
Rat	6-OHDA	Embryonic mouse fibroblast	1×10^5 with DHA	Intrastriatal	4 months	DHA induces the differentiation of functional dopaminergic precursors and improves the abnormal behavior	Chang et al. (2012)
Rat	6-OHDA	Mice iNSC derived with transdifferentiation from Sertoli cells	8×10^5—Lmx1a	Intrastriatal	10 weeks	Decreased ipsilateral rotations, a few transplanted cells survive	Wu et al. (2015)
Macaca mulatta monkeys	MPTP	Autologous fibroblast	1×10^5	Caudate nucleus, putamen and the substantia nigra	6 months	iPSC-derived neural progenitors survive for up to 6 months and differentiate into neurons, astrocytes, and myelinating oligodendrocytes	Emborg et al. (2013)

immune response with high lymphocytic infiltration and elevated interferon-gamma (IFN-γ), granzyme B, and perforin intragraft (de Almeida et al. 2014). Generation of autologous iPSCs is a time-consuming process and delays iPSC-based cell therapies. Therefore, allogenic iPSC-derived cells provide a useful strategy for transplantation therapy in acute brain disorders such as stroke. It requires well-characterized human leukocyte antigen (HLA)-typed iPSC lines and their biobanking. Even so, small difference in culture conditions alters gene expressions (Newman and Cooper 2010). The differentiation stage of iPSCs is an important point for the successful outcome in transplantation therapy. Differentiated neurons are less immunogenic and tumorigenic and do not require differentiation factors. Due to the ability of NSCs to differentiate into a variety of cell types, including neurons, astrocytes, and oligodendrocytes, they become a favorable cell type in cell replacement therapy. However, undifferentiated iPSCs could cause teratoma formation in the transplanted brain region (Kawai et al. 2010).

The most critical factor affecting the success of stem cell therapy is the route of administration. Intravenous and intraperitoneal routes are the easiest ways; however, the number of cells that reach the brain is limited (Li et al. 2015). Additionally, blood-brain barrier (BBB) also limits iPSCs to cross to the brain. More specific methods should be found for delivering iPSCs into the brain. Direct intrastriatal and intranigral routes have been successfully used for the transplantation of iPSC-derived dopaminergic neurons in animal model of PD (Nishimura and Takahashi 2013). Intracerebral injection could be a more specific way, but it is invasive and carries tissue injury risk throughout the route of administration (Martinez-Morales et al. 2013). Intracerebroventricular route may help widespread distribution of iPSCs into the CNS, but it is also an invasive route for stem cell delivery (Li et al. 2015). Intranasal route is an easy and noninvasive delivery method for stem cell therapy. In addition, it is suitable for repeated administration (Li et al. 2015). This route was used for delivering other types of stem cells, but not for iPSCs delivery, even in animal experiments.

Genome editing methods allow the correction of mutations in iPSCs from individuals carrying mutations. Zinc finger nucleases (ZFNs) are the first used genome editing method for mutation correction. It has some disadvantages such as off-target effects and cell toxicity (Velasco et al. 2014). Similar to ZFNs, TALENs also generate double-strand breaks at target site in the genome (Gupta and Musunuru 2014). The advantages of TALENs include easier design, low levels of off-target effects, and toxicity; however, the size of TALENs limits their use in stem cell therapy. The third genome editing tool is clustered regularly interspaced short palindromic repeat/CAS9 RNA-guided nucleases (CRISPR/CAS9), which has gained attention due to easier design, high success rate, low cost, and side effects (Velasco et al. 2014).

Before proceeding to clinical trials, the benefits and safety of iPSCs-derived cell transplantation should be evaluated in in vivo animal studies. Toxin-based animal models of PD, especially the 6-OHDA model, were used for iPSCs transplantation studies (Table 2.4). Intrastriatal delivery method was preferred due to regional localization of degenerative neurons. Most studies have confirmed long-term survival of transplanted

dopaminergic neurons, which provide behavioral recovery (Hargus et al. 2010; Swistowski et al. 2010; Rhee et al. 2011; Chang et al. 2012; Wu et al. 2015). Although there was a high concentration of iPSC-derived dopaminergic neuron transplantation, functional recovery was not observed in short-term periods (Cai et al. 2010). Additionally, transplantation of terminally differentiated cells results in ineffective engraftment (Rhee et al. 2011). The available iPSCs transplantation methods still need to be improved before clinical trial. For instance, selection of subtypes of neurons using cell sorting may increase transplantation success (Doi et al. 2014). The first in vivo iPSC-derived neuronal precursor cell transplantation study for AD was carried out in platelet-derived growth factor (PDGF) promoter-driven amyloid precursor protein (PDAPP) transgenic mice (Fujiwara et al. 2013). iPSC-derived cholinergic neurons were transplanted into bilateral hippocampus of the 10-week-old PDAPP mice. Transplanted neurons survive and show cholinergic and GABAergic phenotypes in the recipient mouse brain 45 days after transplantation. Additionally, transplantation of the neurons restored spatial memory dysfunction of PDAPP mice.

Nonhuman primates (NHPs) have anatomical and functional similarities compared to humans. In addition, gene expression profile in the brain is also similar between NHPs and humans (Verdier et al. 2015). These similarities make NHPs useful animal models to study neurodegenerative diseases. NHP models enable the monitoring of long-term outcome of the transplanted cells (Qiu et al. 2013). Moreover, these models provide considerable information about aging process in the brain due to display characteristics similar to human aging. AD-related pathological findings, including Aβ accumulation, tau phosphorylation, and atrophy, also naturally occur in NHPs (Verdier et al. 2015). They are also valuable animals for Parkinson's disease studies. While 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) does not show toxicity in rats, it leads to loss of dopaminergic neurons of the substantia nigra with PD symptoms in NHPs (Capitanio and Emborg 2008). In addition to toxin models, mutant A53T α-synuclein over-expressing monkey and transgenic A53T monkey have been generated as NHPs animal models of PD (Eslamboli et al. 2007; Niu et al. 2015). NHPs have been used for iPSCs transplantation studies for PD. Dopaminergic neurons derived from human iPSCs were transplanted to the putamen of MPTP-lesioned cynomolgus monkeys, and transplanted cells survived in the monkey brain for 6 months (Kikuchi et al. 2011). Autologous fibroblast of *Macaca mulatta* monkeys was used for the generation of iPSCs in NHPs model of PD, and iPSC-derived neural progenitors survived 6 months and differentiated into neurons and glial cells (Emborg et al. 2013).

The first human clinical study using iPSC derivatives was started for the treatment of patients suffering from age-related macular degeneration (Okano and Yamanaka 2014). The results of this study have not been reported yet. There are also several planned clinical trials of iPSCs-based therapies (Okano and Yamanaka 2014). But, there is no record on web pages of clinical trials for iPSCs-derived cell replacement therapy (clinicaltrials.gov, Accessed 04 May 2015). Clinical studies should be started with safety and side effects analysis.

2.4 Future Directions: Challenges and Advancements

2.4.1 Limitations in iPSC Generation and Potential Solutions

2.4.1.1 Integration into Genome

One of the major limitations of iPSC technology is low efficiency. The efficiency is as low as 0.001% with current methods. Besides, the current methods are slow and require the overexpression of multiple transcription factors at the same time. Retroviral systems are still the most used methods for generating iPSCs. This system uses transduction of reprogramming factors into host genome, which may result in random integration into genome, karyotype abnormalities, and copy number variations. Further, this problem can also affect differentiation efficiency. Furthermore, major goals in iPSC generation are avoiding genomic integration with developing more efficient non-integrative method. Moreover, the elimination of residual transgene expression and reactivation of reprogramming factors should be achieved (Hu 2014). Another problem is that individual iPSC clones have differences among them even if they are generated from the same individual. This might affect differentiation efficiency. Gender and usage of integration factors may cause this problem. If iPSCs are reprogrammed from cells of a female person, cells have X chromosome inactivation which can lead to altered expression of cognition and brain development-related genes. If integrating method was used, there might be incomplete transgene silencing (Zhao et al. 2014). For avoiding this problem, proper PCR screening, excisable vector, or non-integrative methods can be used that are mentioned in the reprogramming of iPSCs. However, these non-integrating methods are not perfect and have disadvantages such as lower efficiency. There are various ways to excise those integration transgenes, but these methods have their own disadvantages such as micro-deletions in genomic DNA. Lastly, high-quality iPSCs must be met along with high efficient reprogramming.

2.4.1.2 Epigenetic Memory

Epigenetic memory could be a problem in differentiation and reprogramming. A study showed that iPSCs showed significant reprogramming variability independent of reprogramming technique including epigenetic memory of somatic cells and abnormal DNA methylation after reprogramming (Lister et al. 2011). However, reprogramming cells can erase epigenetic signature but imperfectly. This can affect reprogramming and differentiation capacity. Furthermore, disease-related epigenetic signature can be required for showing disease phenotype, so removal of epigenetic signature may result in the loss of disease phenotype (Doege and Abeliovich 2014).

2.4.1.3 Differentiation and Purity

The use of iPSCs in disease modeling has various limitations and problems. Currently, there are no standardized and optimized differentiation protocols for a given cell type. In additionally, the available protocols are time-consuming and inefficient. For instance, iPSC differentiation into dopaminergic neurons takes about 21–70 days, and the efficiency differs with respect to techniques used (Badger et al. 2014).

After differentiation, cell population must be validated. For this purpose, immunochemistry and PCR methods are used for cell-specific markers. In addition to those, in-depth analysis of neuron functionality can be performed via calcium imaging, dopamine release, and electrophysiological properties such as patch clamp method (Badger et al. 2014). Further, differentiation yields heterogeneous population because of maturation at different time points. Hu et al. has shown that regardless of cell origin which iPSCs are derived from, differentiation is highly variable and not efficient. They investigated PAX6+ levels and found that PAX6+ expression is variable among different clones and clones that were generated from iPSCs reprogrammed in the same fibroblasts (Hu et al. 2010). Selecting clones with the same differentiation capacity may eliminate this variation. Furthermore, disease phenotype can be mixed with abnormal phenotypes (Zhao et al. 2014). Moreover, current methods are unable to generate specific cell type in reliable and high amounts. Differentiation results in mixed cell types. For example, differentiation neurons from iPSCs results in cell population composed of neurons and glial cells (Kondo et al. 2013). However, if the cell type of interest is unknown for a given disease, analyzing multiple cell types at once will be an opportunity, since state-of-the-art methods can be used for single cell analysis.

In addition to those problems, most of the studies focused on cell-autonomous models. While this approach is acceptable in first steps, the developing brain is not working in that way. Notably, cell-cell interactions are required for proper modeling, but it is hard to study in differentiation, synapse formation, etc. Studying diseases at the network level may be required for adequately modeling diseases. For this purpose, complex cellular interactions are needed rather than single-cell analysis (REF).

Furthermore, aging is another problem which researchers are faced, since developing AD and PD takes decades in humans. However, current iPSC-dependent models in culture take a couple of months. Fibroblasts taken from elder individuals have shown aging markers, whereas iPSCs reprogrammed from these fibroblasts did not show any of those markers (Miller et al. 2013). Furthermore, progerin, which is associated with premature aging syndrome "progeria," overexpression in iPSCs provided aging-associated marker expression (Miller et al. 2013), and progerin expression may promote degenerative phenotypes in iPSC-derived models. Moreover, it is proposed that environmental factors (toxins, nutrion stress, etc.) may promote aging in culture (Doege and Abeliovich 2014).

2.4.1.4 Control Groups for Research

Selection of control groups in iPSC-derived disease models is another problem. Proper control groups are required for revealing disease mechanism and/or drug screening. They can be generated from the same age, gender, and ethnic group of healthy controls. However, they have different genetic background along with different risk factor exposure (Santostefano et al. 2015). Ideally, isogenic control groups should be used. To be able to obtain such cells, mutations in iPSCs should be corrected via proper gene editing tools which are ZNFs, TALENs, and Crispr/Cas9 system (Xu and Zhong 2013).

2.4.2 Safety Concerns for Clinical Grade iPSCs

2.4.2.1 Tumorigenicity, Immunogenicity, and Genomic Instability

Tumorigenicity is one of the major concerns in the usage of iPSCs, since oncogenes are used for reprogramming somatic cells into iPSC. Furthermore, the potential presence of undifferentiated iPSCs can also cause tumor formation after transplantation. It is shown that reprogramming without oncogenes (c-Myc and Klf4) can reduce tumor formation in mice. Alternative methods can be used to achieve this problem such as non-viral methods mentioned earlier (Sect. 2.2.1).

Genomic aberrations can still occur in iPSCs regardless how they were generated. In most cases, reprogramming does not result in alterations in the karyotype, but in some instances, it is possible to see abnormalities in karyotype. Furthermore, when genomic stability is looked closer, it can be seen that subkaryotypic changes can happen during reprogramming or in prolonged subculture periods. Copy number variation (CNV) can be detected in iPSC lines. After CNV analysis, early passages of iPSCs can have deletions in tumor suppressor genes, and early passages tend to have more deletions than late passages. On the other hand, amplifications in oncogenes tend to occur in late passages. Furthermore, mutations in exons (i.e., protein-coding regions) can occur, and most of these mutations are acquired during reprogramming or in culture of iPSCs. Mutations are maintained during culture of iPSCs. All in all, genomic instability of iPSCs, whether gained during reprogramming or in culture, can affect the quality of iPSCs in clinical use (Martins-Taylor and Xu 2012).

Another safety concern in iPSC-based therapy is the issue of immunogenicity. Generally, autologous cell therapy is generally considered as immune safe. However, Zhao and colleagues showed that iPSC cell therapy can induce immune response in syngeneic recipients. Furthermore, they showed that after iPSC implantation, immune rejection occurred via T-cell infiltration. Moreover, they identified two genes (*Hormad1* and *Zg16*), which were expressed in iPSC-teratoma, and these genes directly contribute immunogenicity of iPSC derivatives (Zhao et al. 2011). Immunogenicity is essential in

iPSC-based cell therapy. Using autologous iPSC-derived cell can bypass immune rejection. However, autologous iPSC confer some practical problems. The most important one is that it is time-consuming. Further, clone selection, differentiation, and characterization require more time. If donors have mutations in their genome, correction of mutated gene or genes is a must, and this requires additional time.

2.4.2.2 Biobanking

iPSC banking is an essential matter in terms of both research and clinical applications. It should assure scientific reproducibility in iPSC researches. Furthermore, iPSC lines can have genomic and epigenetic variations, their quality must be checked carefully, and genotyping is necessary for providing required genotypic iPSC lines for scientific researches (Stacey et al. 2013). Additionally, iPSC biobanks can meet cell demands in cell replacement therapies. Patient-specific iPSCs can be used in cell replacement therapies. However, their generation is time-consuming and expensive. Additionally, such cells can have genetic defects which have to be corrected. Moreover, the quality of such cells can be an issue. iPSC biobanks can provide high quality and low chance of immune rejection iPSCs. Furthermore, iPSC supply of these banks cannot be depleted due to the nature of iPSCs. Biobanks, which are based on HLA matching, can be used to avoid immune rejection problems. HLA is a polymorphic gene inherited with monogenic dominant Mendelian manner. Overall, more than 2558 possible HLA classes (HLA I and II) exist. However, according to one estimate, 150 lines are sufficient to match with 90% of England population, and more diverse population may need more than 150 lines. Therefore, it can be said that the creation of HLA-matched-based banks with sufficient HLA matching which represent different geographical population can ease iPSC-based therapies (Solomon et al. 2015).

2.4.2.3 Clearance of Animal Products

Contamination with animal products is an important issue in terms of producing clinical-grade iPSCs and biobanking. The use of animal products in reprogramming and differentiating iPSCs contains the risk of unknown pathogens, exogenous antigens, etc., because there would be unpredictable risks to humans. Animal product-free culture medium is also important. Furthermore, animal-derived MEFs are used for feeder layer. Alternatives to mouse-derived feeder layer and human-derived feeder layer were developed. However, producing them is time- and effort consuming. Matrigel can be used in human iPSCs generation, yet Matrigel is derived from Engelbreth-Holm-Swarm mouse. Other alternatives including recombinant proteins, CellStart®, and synthetic polymers can be used instead of Matrigel (Seki and Fukuda 2015).

2.4.3 Recent Biological and Biotechnological Advancements

2.4.3.1 Alternative Strategies for Reprogramming

iPSC generation includes nuclear reprogramming. These iPSCs are in a transient pluripotent state which are susceptible to chromosomal aberrations. The generation of iPSC results in almost complete epigenetic memory erasure. Furthermore, methods for generation and differentiation of iPSCs are time-consuming and expensive. There are alternative ways to achieve those problems. Direct reprogramming is one of them. Epigenetic memory is not completely erased after reprogramming. Moreover, iPSC generation is performed in vitro, but direct reprogramming can be performed in both in vitro and in vivo (Amamoto and Arlotta 2013), for instance, overexpression of Pax6 in astrocytes isolated from postnatal cerebral cortex of mice differentiated into neurons (Heins et al. 2002). However, some cell types are not always feasible when human physiology is considered. This problem leads the scientist into more lineage distant cell types such as skin fibroblasts. Vierbuchen et al. showed that mouse tail fibroblast could be directly differentiated into neurons with three distinct transcription factors, which are Brn2, Ascl1, and Myt1l (Vierbuchen et al. 2010). Thus, these methods can provide time-efficient patient-specific cells for both research and clinical applications. Apart from these, in vivo reprogramming is a developing area. The advantages of it include cells residing in their native tissue, having low tumorigenesis risk, and having new cells autologous in origin. Furthermore, delivery of transcription factor into specific cell type requires virus mediated transfer. However, it can have unknown consequences (Heinrich et al. 2015).

2.4.3.2 Three-Dimensional (3D) and Organoid Cultures

iPSCs are capable to receive early developmental signals. Thus, when specific signal is given to iPSCs, different cell type can be differentiated autonomously via interacting each other and the environment. Two-dimensional cultures are limited to deliver full potential of iPSCs. Using 3D culture with functional biomaterials create more relevant physiological environment (Shao et al. 2015). Using human midbrain-derived neural progenitor cells, 3D cell culture has been established and called as 3D neurospheres containing functional dopaminergic neurons, oligodendrocytes, and astrocytes (Simao et al. 2015). Sophisticated 3D organoid cultures can also be used to mimic this differentiation process. Lancaster et al. were able to generate 3D organoid culture with human iPSCs. This system includes cerebral cortex (Lancaster et al. 2013). Additionally, similar systems have been developed by various groups (Dye et al. 2015; Beauchamp et al. 2015).

2.4.3.3 Biotechnological Strategies

Biomaterials

The efficiency of iPSC expansion and differentiation process can be improved by controlling the microenvironment. For this purpose, biomaterials, which are designed to interact with cells, can be used. Nano- or microparticles can control reprogramming factors as well as modulating epigenetic state of iPSCs. Biomaterials can be designed to deliver reprogramming factors more safely and efficiently. Furthermore, they can increase the efficiency of iPSC derivation by controlling the duration of exposure to extracellular matrix (Tong et al. 2015). Moreover, artificial transcription factors can be generated such as NanoScript which was designed as a platform for mimicking transcription factor domains. It contained nuclear localization signal, DNA binding domain, and activation domain (Patel et al. 2014). Additionally, factors can be integrated into scaffolds, and their differential release can be adjusted. For instance, PLGA-based scaffold was used for differential release of both vascular endothelial growth factor (VEGF) and PDGF. VEGF was mixed with polymer for rapid release, and PDGF was pre-encapsulated with polylactide-co-glycolide (PLG) for extended release (Richardson et al. 2001).

Bioprinting

Three-dimensional bioprinting is also biomaterial-based iPSC-derived organ systems. Functional tissues and organs can be produced by using biological elements such as cells via 3D bioprinting. These tissues/organs can further be transplanted. Spatial control of layers can be controlled, and desired biological properties can be obtained. Furthermore, with nanoscale resolution of bioprinting, it is possible to construct closely mimicking physiological properties of a desired tissue/organ. Moreover, this technique diminishes vascularization and innervation problems in 3D culture systems (Tong et al. 2015). Kolesky et al. showed that they printed intricate and heterogeneous tissue construct supplied with vasculature, extracellular matrix (ECM), and multiple cell types by their novel 3D printing method (Kolesky et al. 2014).

2.5 Conclusion

iPSCs technology by Shinya Yamanaka and colleagues in 2006 was a groundbreaking invention. Since then, numerous advancements have been made in the field of iPSC research. This development can provide the treatment of incurable diseases. Consequently, iPSCs can overcome immune rejection problems and problems faced with ES cells such as ethical issues. Although, there are some disadvantages of this technology, numerous studies are being conducted for achieving those obstacles.

Recent technical advances have provided to overcome safety issues in clinical use of iPSCs. However, these methods are still in their infancy. Further investigations will provide much safer and efficient ways to the use of iPSCs in clinical applications.

References

Aboud AA, Tidball AM, Kumar KK, Neely MD, Ess KC, Erikson KM, Bowman AB (2012) Genetic risk for Parkinson's disease correlates with alterations in neuronal manganese sensitivity between two human subjects. Neurotoxicology 33(6):1443–1449. doi:10.1016/j.neuro.2012.10.009

Alzheimer's Association (2015) 2015 Alzheimer's disease facts and figures. Alzheimers Dement 11(3):332–384. doi:10.1016/j.jalz.2015.02.003

Amamoto R, Arlotta P (2013) Reshaping the brain: direct lineage conversion in the nervous system. F1000Prime Rep 5:33. doi:10.12703/P5-33

Badger JL, Cordero-Llana O, Hartfield EM, Wade-Martins R (2014) Parkinson's disease in a dish—using stem cells as a molecular tool. Neuropharmacology 76(Pt A):88–96. doi:10.1016/j.neuropharm.2013.08.035

Bartels AL, Leenders KL (2009) Parkinson's disease: the syndrome, the pathogenesis and pathophysiology. Cortex 45(8):915–921. doi:10.1016/j.cortex.2008.11.010

Beauchamp P, Moritz W, Kelm JM, Ullrich ND, Agarkova I, Anson BD, Suter TM, Zuppinger C (2015) Development and characterization of a scaffold-free 3D spheroid model of induced pluripotent stem cell-derived human cardiomyocytes. Tissue Eng Part C Methods. doi:10.1089/ten.TEC.2014.0376

Beitz JM (2014) Parkinson's disease: a review. Front Biosci 6:65–74

Berk C, Sabbagh MN (2013) Successes and failures for drugs in late-stage development for Alzheimer's disease. Drugs Aging 30(10):783–792. doi:10.1007/s40266-013-0108-6

Bonifati V (2014) Genetics of Parkinson's disease—state of the art, 2013. Parkinsonism Relat Disord 20(Suppl 1):S23–S28. doi:10.1016/S1353-8020(13)70009-9

Byers B, Cord B, Nguyen HN, Schule B, Fenno L, Lee PC, Deisseroth K, Langston JW, Pera RR, Palmer TD (2011) SNCA triplication Parkinson's patient's iPSC-derived DA neurons accumulate alpha-synuclein and are susceptible to oxidative stress. PLoS One 6(11):e26159. doi:10.1371/journal.pone.0026159

Cai J, Yang M, Poremsky E, Kidd S, Schneider JS, Iacovitti L (2010) Dopaminergic neurons derived from human induced pluripotent stem cells survive and integrate into 6-OHDA-lesioned rats. Stem Cells Dev 19(7):1017–1023. doi:10.1089/scd.2009.0319

Capitanio JP, Emborg ME (2008) Contributions of non-human primates to neuroscience research. Lancet 371(9618):1126–1135. doi:10.1016/S0140-6736(08)60489-4

Chang YL, Chen SJ, Kao CL, Hung SC, Ding DC, Yu CC, Chen YJ, Ku HH, Lin CP, Lee KH, Chen YC, Wang JJ, Hsu CC, Chen LK, Li HY, Chiou SH (2012) Docosahexaenoic acid promotes dopaminergic differentiation in induced pluripotent stem cells and inhibits teratoma formation in rats with Parkinson-like pathology. Cell Transplant 21(1):313–332. doi:10.3727/096368911X580572

Choi SM, Kim Y, Shim JS, Park JT, Wang RH, Leach SD, Liu JO, Deng C, Ye Z, Jang YY (2013) Efficient drug screening and gene correction for treating liver disease using patient-specific stem cells. Hepatology 57(6):2458–2468. doi:10.1002/hep.26237

Chung CY, Khurana V, Auluck PK, Tardiff DF, Mazzulli JR, Soldner F, Baru V, Lou Y, Freyzon Y, Cho S, Mungenast AE, Muffat J, Mitalipova M, Pluth MD, Jui NT, Schule B, Lippard SJ, Tsai LH, Krainc D, Buchwald SL, Jaenisch R, Lindquist S (2013) Identification and rescue of

alpha-synuclein toxicity in Parkinson patient-derived neurons. Science 342(6161):983–987. doi:10.1126/science.1245296

Cooper O, Seo H, Andrabi S, Guardia-Laguarta C, Graziotto J, Sundberg M, McLean JR, Carrillo-Reid L, Xie Z, Osborn T, Hargus G, Deleidi M, Lawson T, Bogetofte H, Perez-Torres E, Clark L, Moskowitz C, Mazzulli J, Chen L, Volpicelli-Daley L, Romero N, Jiang H, Uitti RJ, Huang Z, Opala G, Scarffe LA, Dawson VL, Klein C, Feng J, Ross OA, Trojanowski JQ, Lee VM, Marder K, Surmeier DJ, Wszolek ZK, Przedborski S, Krainc D, Dawson TM, Isacson O (2012) Pharmacological rescue of mitochondrial deficits in iPSC-derived neural cells from patients with familial Parkinson's disease. Sci Transl Med 4(141):141ra190. doi:10.1126/scitranslmed.3003985

Davie CA (2008) A review of Parkinson's disease. Br Med Bull 86:109–127. doi:10.1093/bmb/ldn013

de Almeida PE, Meyer EH, Kooreman NG, Diecke S, Dey D, Sanchez-Freire V, Hu S, Ebert A, Odegaard J, Mordwinkin NM, Brouwer TP, Lo D, Montoro DT, Longaker MT, Negrin RS, Wu JC (2014) Transplanted terminally differentiated induced pluripotent stem cells are accepted by immune mechanisms similar to self-tolerance. Nat Commun 5:3903. doi:10.1038/ncomms4903

de Lazaro I, Yilmazer A, Kostarelos K (2014) Induced pluripotent stem (iPS) cells: a new source for cell-based therapeutics? J Control Release 185:37–44. doi:10.1016/j.jconrel.2014.04.011

De-Paula VJ, Radanovic M, Diniz BS, Forlenza OV (2012) Alzheimer's disease. Subcell Biochem 65:329–352. doi:10.1007/978-94-007-5416-4_14

Devine MJ, Ryten M, Vodicka P, Thomson AJ, Burdon T, Houlden H, Cavaleri F, Nagano M, Drummond NJ, Taanman JW, Schapira AH, Gwinn K, Hardy J, Lewis PA, Kunath T (2011) Parkinson's disease induced pluripotent stem cells with triplication of the alpha-synuclein locus. Nat Commun 2:440. doi:10.1038/ncomms1453

Doege CA, Abeliovich A (2014) Dementia in a dish. Biol Psychiatry 75(7):558–564. doi:10.1016/j.biopsych.2014.01.007

Doi D, Samata B, Katsukawa M, Kikuchi T, Morizane A, Ono Y, Sekiguchi K, Nakagawa M, Parmar M, Takahashi J (2014) Isolation of human induced pluripotent stem cell-derived dopaminergic progenitors by cell sorting for successful transplantation. Stem Cell Reports 2(3):337–350. doi:10.1016/j.stemcr.2014.01.013

Duan L, Bhattacharyya BJ, Belmadani A, Pan L, Miller RJ, Kessler JA (2014) Stem cell derived basal forebrain cholinergic neurons from Alzheimer's disease patients are more susceptible to cell death. Mol Neurodegener 9:3. doi:10.1186/1750-1326-9-3

Durnaoglu S, Genc S, Genc K (2011) Patient-specific pluripotent stem cells in neurological diseases. Stem Cells Int 2011:212487. doi:10.4061/2011/212487

Dye BR, Hill DR, Ferguson MA, Tsai YH, Nagy MS, Dyal R, Wells JM, Mayhew CN, Nattiv R, Klein OD, White ES, Deutsch GH, Spence JR (2015) In vitro generation of human pluripotent stem cell derived lung organoids. Elife 4. doi:10.7554/eLife.05098

Emborg ME, Liu Y, Xi J, Zhang X, Yin Y, Lu J, Joers V, Swanson C, Holden JE, Zhang SC (2013) Induced pluripotent stem cell-derived neural cells survive and mature in the nonhuman primate brain. Cell Rep 3(3):646–650. doi:10.1016/j.celrep.2013.02.016

Eslamboli A, Romero-Ramos M, Burger C, Bjorklund T, Muzyczka N, Mandel RJ, Baker H, Ridley RM, Kirik D (2007) Long-term consequences of human alpha-synuclein overexpression in the primate ventral midbrain. Brain 130(Pt 3):799–815. doi:10.1093/brain/awl382

Flierl A, Oliveira LM, Falomir-Lockhart LJ, Mak SK, Hesley J, Soldner F, Arndt-Jovin DJ, Jaenisch R, Langston JW, Jovin TM, Schule B (2014) Higher vulnerability and stress sensitivity of neuronal precursor cells carrying an alpha-synuclein gene triplication. PLoS One 9(11):e112413. doi:10.1371/journal.pone.0112413

Fujiwara N, Shimizu J, Takai K, Arimitsu N, Saito A, Kono T, Umehara T, Ueda Y, Wakisaka S, Suzuki T, Suzuki N (2013) Restoration of spatial memory dysfunction of human APP transgenic mice by transplantation of neuronal precursors derived from human iPS cells. Neurosci Lett 557(Pt B):129–134

Giri S, Bader A (2015) A low-cost, high-quality new drug discovery process using patient-derived induced pluripotent stem cells. Drug Discov Today 20(1):37–49. doi:10.1016/j.drudis.2014.10.011

Gupta RM, Musunuru K (2014) Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. J Clin Invest 124(10):4154–4161. doi:10.1172/JCI72992

Haile Y, Nakhaei-Nejad M, Boakye PA, Baker G, Smith PA, Murray AG, Giuliani F, Jahroudi N (2015) Reprogramming of HUVECs into induced pluripotent stem cells (HiPSCs), generation and characterization of HiPSC-derived neurons and astrocytes. PLoS One 10(3):e0119617. doi:10.1371/journal.pone.0119617

Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, Cassady JP, Beard C, Brambrink T, Wu LC, Townes TM, Jaenisch R (2007) Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science 318(5858):1920–1923. doi:10.1126/science.1152092

Hargus G, Cooper O, Deleidi M, Levy A, Lee K, Marlow E, Yow A, Soldner F, Hockemeyer D, Hallett PJ, Osborn T, Jaenisch R, Isacson O (2010) Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. Proc Natl Acad Sci U S A 107(36):15921–15926. doi:10.1073/pnas.1010209107

Hawkes CM (1995) Diagnosis and treatment of Parkinson's disease. Anosmia is a common finding. BMJ 310(6995):1668

Heilker R, Traub S, Reinhardt P, Scholer HR, Sterneckert J (2014) iPS cell derived neuronal cells for drug discovery. Trends Pharmacol Sci 35(10):510–519. doi:10.1016/j.tips.2014.07.003

Heinrich C, Spagnoli FM, Berninger B (2015) In vivo reprogramming for tissue repair. Nat Cell Biol 17(3):204–211. doi:10.1038/ncb3108

Heins N, Malatesta P, Cecconi F, Nakafuku M, Tucker KL, Hack MA, Chapouton P, Barde YA, Gotz M (2002) Glial cells generate neurons: the role of the transcription factor Pax6. Nat Neurosci 5(4):308–315. doi:10.1038/nn828

Hendrie HC, Ogunniyi A, Hall KS, Baiyewu O, Unverzagt FW, Gureje O, Gao S, Evans RM, Ogunseyinde AO, Adeyinka AO, Musick B, Hui SL (2001) Incidence of dementia and Alzheimer disease in 2 communities: Yoruba residing in Ibadan, Nigeria, and African Americans residing in Indianapolis, Indiana. JAMA 285(6):739–747

Hossini AM, Megges M, Prigione A, Lichtner B, Toliat MR, Wruck W, Schroter F, Nuernberg P, Kroll H, Makrantonaki E, Zouboulis CC, Adjaye J (2015) Induced pluripotent stem cell-derived neuronal cells from a sporadic Alzheimer's disease donor as a model for investigating AD-associated gene regulatory networks. BMC Genomics 16:84. doi:10.1186/s12864-015-1262-5

Hou P, Li Y, Zhang X, Liu C, Guan J, Li H, Zhao T, Ye J, Yang W, Liu K, Ge J, Xu J, Zhang Q, Zhao Y, Deng H (2013) Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. Science 341(6146):651–654. doi:10.1126/science.1239278

Hu BY, Weick JP, Yu J, Ma LX, Zhang XQ, Thomson JA, Zhang SC (2010) Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. Proc Natl Acad Sci U S A 107(9):4335–4340. doi:10.1073/pnas.0910012107

Hu K (2014) All roads lead to induced pluripotent stem cells: the technologies of iPSC generation. Stem Cells Dev 23(12):1285–1300. doi:10.1089/scd.2013.0620

Hughes AJ, Daniel SE, Kilford L, Lees AJ (1992) Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 55(3):181–184

Hy LX, Keller DM (2000) Prevalence of AD among whites: a summary by levels of severity. Neurology 55(2):198–204. doi:10.1212/wnl.55.2.198

Imaizumi Y, Okada Y, Akamatsu W, Koike M, Kuzumaki N, Hayakawa H, Nihira T, Kobayashi T, Ohyama M, Sato S, Takanashi M, Funayama M, Hirayama A, Soga T, Hishiki T, Suematsu M, Yagi T, Ito D, Kosakai A, Hayashi K, Shouji M, Nakanishi A, Suzuki N, Mizuno Y, Mizushima N, Amagai M, Uchiyama Y, Mochizuki H, Hattori N, Okano H (2012) Mitochondrial dysfunction

associated with increased oxidative stress and alpha-synuclein accumulation in PARK2 iPSC-derived neurons and postmortem brain tissue. Mol Brain 5:35. doi:10.1186/1756-6606-5-35

Israel MA, Yuan SH, Bardy C, Reyna SM, Mu Y, Herrera C, Hefferan MP, Van Gorp S, Nazor KL, Boscolo FS, Carson CT, Laurent LC, Marsala M, Gage FH, Remes AM, Koo EH, Goldstein LS (2012) Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. Nature 482(7384):216–220. doi:10.1038/nature10821

Jia W, Chen W, Kang J (2013) The functions of microRNAs and long non-coding RNAs in embryonic and induced pluripotent stem cells. Genomics Proteomics Bioinformatics 11(5):275–283. doi:10.1016/j.gpb.2013.09.004

Jiang H, Ren Y, Yuen EY, Zhong P, Ghaedi M, Hu Z, Azabdaftari G, Nakaso K, Yan Z, Feng J (2012) Parkin controls dopamine utilization in human midbrain dopaminergic neurons derived from induced pluripotent stem cells. Nat Commun 3:668. doi:10.1038/ncomms1669

Jindal H, Bhatt B, Sk S, Singh Malik J (2014) Alzheimer disease immunotherapeutics: then and now. Hum Vaccin Immunother 10(9):2741–2743. doi:10.4161/21645515.2014.970959

Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, Livingston DM, Rajewsky K (2005) Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. Genes Dev 19(4):489–501. doi:10.1101/gad.1248505

Kawai H, Yamashita T, Ohta Y, Deguchi K, Nagotani S, Zhang X, Ikeda Y, Matsuura T, Abe K (2010) Tridermal tumorigenesis of induced pluripotent stem cells transplanted in ischemic brain. J Cereb Blood Flow Metab 30(8):1487–1493. doi:10.1038/jcbfm.2010.32

Kikuchi T, Morizane A, Doi D, Onoe H, Hayashi T, Kawasaki T, Saiki H, Miyamoto S, Takahashi J (2011) Survival of human induced pluripotent stem cell-derived midbrain dopaminergic neurons in the brain of a primate model of Parkinson's disease. J Parkinsons Dis 1(4):395–412. doi:10.3233/JPD-2011-11070

Kim JB, Zaehres H, Wu G, Gentile L, Ko K, Sebastiano V, Arauzo-Bravo MJ, Ruau D, Han DW, Zenke M, Scholer HR (2008) Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. Nature 454(7204):646–650. doi:10.1038/nature07061

Ko HC, Gelb BD (2014) Concise review: drug discovery in the age of the induced pluripotent stem cell. Stem Cells Transl Med 3(4):500–509. doi:10.5966/sctm.2013-0162

Koch P, Tamboli IY, Mertens J, Wunderlich P, Ladewig J, Stuber K, Esselmann H, Wiltfang J, Brustle O, Walter J (2012) Presenilin-1 L166P mutant human pluripotent stem cell-derived neurons exhibit partial loss of gamma-secretase activity in endogenous amyloid-beta generation. Am J Pathol 180(6):2404–2416. doi:10.1016/j.ajpath.2012.02.012

Kolesky DB, Truby RL, Gladman AS, Busbee TA, Homan KA, Lewis JA (2014) 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. Adv Mater 26(19):3124–3130. doi:10.1002/adma.201305506

Kondo T, Asai M, Tsukita K, Kutoku Y, Ohsawa Y, Sunada Y, Imamura K, Egawa N, Yahata N, Okita K, Takahashi K, Asaka I, Aoi T, Watanabe A, Watanabe K, Kadoya C, Nakano R, Watanabe D, Maruyama K, Hori O, Hibino S, Choshi T, Nakahata T, Hioki H, Kaneko T, Naitoh M, Yoshikawa K, Yamawaki S, Suzuki S, Hata R, Ueno S, Seki T, Kobayashi K, Toda T, Murakami K, Irie K, Klein WL, Mori H, Asada T, Takahashi R, Iwata N, Yamanaka S, Inoue H (2013) Modeling Alzheimer's disease with iPSCs reveals stress phenotypes associated with intracellular Abeta and differential drug responsiveness. Cell Stem Cell 12(4):487–496. doi:10.1016/j.stem.2013.01.009

Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, Hurles ME, Homfray T, Penninger JM, Jackson AP, Knoblich JA (2013) Cerebral organoids model human brain development and microcephaly. Nature 501(7467):373–379. doi:10.1038/nature12517

Lee G, Ramirez CN, Kim H, Zeltner N, Liu B, Radu C, Bhinder B, Kim YJ, Choi IY, Mukherjee-Clavin B, Djaballah H, Studer L (2012a) Large-scale screening using familial dysautonomia induced pluripotent stem cells identifies compounds that rescue IKBKAP expression. Nat Biotechnol 30(12):1244–1248. doi:10.1038/nbt.2435

Lee Y, Dawson VL, Dawson TM (2012b) Animal models of Parkinson's disease: vertebrate genetics. Cold Spring Harb Perspect Med 2(10) doi:10.1101/cshperspect.a009324

Li X, Zhang P, Wei C, Zhang Y (2014) Generation of pluripotent stem cells via protein transduction. Int J Dev Biol 58(1):21–27. doi:10.1387/ijdb.140007XL

Li YH, Feng L, Zhang GX, Ma CG (2015) Intranasal delivery of stem cells as therapy for central nervous system disease. Exp Mol Pathol 98(2):145–151. doi:10.1016/j.yexmp.2015.01.016

Lister R, Pelizzola M, Kida YS, Hawkins RD, Nery JR, Hon G, Antosiewicz-Bourget J, O'Malley R, Castanon R, Klugman S, Downes M, Yu R, Stewart R, Ren B, Thomson JA, Evans RM, Ecker JR (2011) Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. Nature 471(7336):68–73. doi:10.1038/nature09798

Liu GH, 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 RD, Dubova I, Thompson J, Yates J 3rd, Esteban CR, Sancho-Martinez I, Izpisua Belmonte JC (2012) Progressive degeneration of human neural stem cells caused by pathogenic LRRK2. Nature 491(7425):603–607. doi:10.1038/nature11557

Liu Q, Waltz S, Woodruff G, Ouyang J, Israel MA, Herrera C, Sarsoza F, Tanzi RE, Koo EH, Ringman JM, Goldstein LS, Wagner SL, Yuan SH (2014) Effect of potent gamma-secretase modulator in human neurons derived from multiple presenilin 1-induced pluripotent stem cell mutant carriers. JAMA Neurol 71(12):1481–1489. doi:10.1001/jamaneurol.2014.2482

Mahairaki V, Ryu J, Peters A, Chang Q, Li T, Park TS, Burridge PW, Talbot CC Jr, Asnaghi L, Martin LJ, Zambidis ET, Koliatsos VE (2014) Induced pluripotent stem cells from familial Alzheimer's disease patients differentiate into mature neurons with amyloidogenic properties. Stem Cells Dev 23(24):2996–3010. doi:10.1089/scd.2013.0511

Martinez-Morales PL, Revilla A, Ocana I, Gonzalez C, Sainz P, McGuire D, Liste I (2013) Progress in stem cell therapy for major human neurological disorders. Stem Cell Rev 9(5):685–699. doi:10.1007/s12015-013-9443-6

Martins-Taylor K, Xu RH (2012) Concise review: genomic stability of human induced pluripotent stem cells. Stem Cells 30(1):22–27. doi:10.1002/stem.705

Masuda S, Wu J, Hishida T, Pandian GN, Sugiyama H, Izpisua Belmonte JC (2013) Chemically induced pluripotent stem cells (CiPSCs): a transgene-free approach. J Mol Cell Biol 5(5):354–355. doi:10.1093/jmcb/mjt034

Mazzulli JR, Xu YH, Sun Y, Knight AL, McLean PJ, Caldwell GA, Sidransky E, Grabowski GA, Krainc D (2011) Gaucher disease glucocerebrosidase and alpha-synuclein form a bidirectional pathogenic loop in synucleinopathies. Cell 146(1):37–52. doi:10.1016/j.cell.2011.06.001

Miller JD, Ganat YM, Kishinevsky S, Bowman RL, Liu B, Tu EY, Mandal PK, Vera E, Shim JW, Kriks S, Taldone T, Fusaki N, Tomishima MJ, Krainc D, Milner TA, Rossi DJ, Studer L (2013) Human iPSC-based modeling of late-onset disease via progerin-induced aging. Cell Stem Cell 13(6):691–705. doi:10.1016/j.stem.2013.11.006

Muratore CR, Rice HC, Srikanth P, Callahan DG, Shin T, Benjamin LN, Walsh DM, Selkoe DJ, Young-Pearse TL (2014) The familial Alzheimer's disease APPV717I mutation alters APP processing and tau expression in iPSC-derived neurons. Hum Mol Genet 23(13):3523–3536. doi:10.1093/hmg/ddu064

Newman AM, Cooper JB (2010) Lab-specific gene expression signatures in pluripotent stem cells. Cell Stem Cell 7(2):258–262. doi:10.1016/j.stem.2010.06.016

Nguyen HN, Byers B, Cord B, Shcheglovitov A, Byrne J, Gujar P, Kee K, Schule B, Dolmetsch RE, Langston W, Palmer TD, Pera RR (2011) LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. Cell Stem Cell 8(3):267–280. doi:10.1016/j.stem.2011.01.013

Nishimura K, Takahashi J (2013) Therapeutic application of stem cell technology toward the treatment of Parkinson's disease. Biol Pharm Bull 36(2):171–175

Niu Y, Guo X, Chen Y, Wang CE, Gao J, Yang W, Kang Y, Si W, Wang H, Yang SH, Li S, Ji W, Li XJ (2015) Early Parkinson's disease symptoms in alpha-synuclein transgenic monkeys. Hum Mol Genet 24(8):2308–2317. doi:10.1093/hmg/ddu748

Nussbaum RL, Ellis CE (2003) Alzheimer's disease and Parkinson's disease. N Engl J Med 348(14):1356–1364. doi:10.1056/NEJM2003ra020003

Okano H, Yamanaka S (2014) iPS cell technologies: significance and applications to CNS regeneration and disease. Mol Brain 7:22. doi:10.1186/1756-6606-7-22

Orenstein SJ, Kuo SH, Tasset I, Arias E, Koga H, Fernandez-Carasa I, Cortes E, Honig LS, Dauer W, Consiglio A, Raya A, Sulzer D, Cuervo AM (2013) Interplay of LRRK2 with chaperone-mediated autophagy. Nat Neurosci 16(4):394–406. doi:10.1038/nn.3350

Panicker LM, Miller D, Park TS, Patel B, Azevedo JL, Awad O, Masood MA, Veenstra TD, Goldin E, Stubblefield BK, Tayebi N, Polumuri SK, Vogel SN, Sidransky E, Zambidis ET, Feldman RA (2012) Induced pluripotent stem cell model recapitulates pathologic hallmarks of Gaucher disease. Proc Natl Acad Sci U S A 109(44):18054–18059. doi:10.1073/pnas.1207889109

Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, Lensch MW, Cowan C, Hochedlinger K, Daley GQ (2008) Disease-specific induced pluripotent stem cells. Cell 134(5):877–886. doi:10.1016/j.cell.2008.07.041

Patel S, Jung D, Yin PT, Carlton P, Yamamoto M, Bando T, Sugiyama H, Lee KB (2014) NanoScript: a nanoparticle-based artificial transcription factor for effective gene regulation. ACS Nano 8(9):8959–8967. doi:10.1021/nn501589f

Peng J, Liu Q, Rao MS, Zeng X (2013) Using human pluripotent stem cell-derived dopaminergic neurons to evaluate candidate Parkinson's disease therapeutic agents in MPP+ and rotenone models. J Biomol Screen 18(5):522–533. doi:10.1177/1087057112474468

Pringsheim T, Jette N, Frolkis A, Steeves TD (2014) The prevalence of Parkinson's disease: a systematic review and meta-analysis. Mov Disord 29(13):1583–1590. doi:10.1002/mds.25945

Qi SD, Smith PD, Choong PF (2014) Nuclear reprogramming and induced pluripotent stem cells: a review for surgeons. ANZ J Surg 84(6):417–423. doi:10.1111/ans.12419

Qiu Z, Farnsworth SL, Mishra A, Hornsby PJ (2013) Patient-specific induced pluripotent stem cells in neurological disease modeling: the importance of nonhuman primate models. Stem Cells Cloning 6:19–29. doi:10.2147/SCCAA.S34798

Raab S, Klingenstein M, Liebau S, Linta L (2014) A comparative view on human somatic cell sources for iPSC generation. Stem Cells Int 2014:768391. doi:10.1155/2014/768391

Rakovic A, Shurkewitsch K, Seibler P, Grunewald A, Zanon A, Hagenah J, Krainc D, Klein C (2013) Phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1)-dependent ubiquitination of endogenous Parkin attenuates mitophagy: study in human primary fibroblasts and induced pluripotent stem cell-derived neurons. J Biol Chem 288(4):2223–2237. doi:10.1074/jbc.M112.391680

Reinhardt P, Schmid B, Burbulla LF, Schondorf DC, Wagner L, Glatza M, Hoing S, Hargus G, Heck SA, Dhingra A, Wu G, Muller S, Brockmann K, Kluba T, Maisel M, Kruger R, Berg D, Tsytsyura Y, Thiel CS, Psathaki OE, Klingauf J, Kuhlmann T, Klewin M, Muller H, Gasser T, Scholer HR, Sterneckert J (2013) Genetic correction of a LRRK2 mutation in human iPSCs links parkinsonian neurodegeneration to ERK-dependent changes in gene expression. Cell Stem Cell 12(3):354–367. doi:10.1016/j.stem.2013.01.008

Ren Y, Jiang H, Hu Z, Fan K, Wang J, Janoschka S, Wang X, Ge S, Feng J (2015) Parkin mutations reduce the complexity of neuronal processes in iPSC-derived human neurons. Stem Cells 33(1):68–78. doi:10.1002/stem.1854

Revilla A, Gonzalez C, Iriondo A, Fernandez B, Prieto C, Marin C, Liste I (2015) Current advances in the generation of human iPS cells: implications in cell-based regenerative medicine. J Tissue Eng Regen Med. doi:10.1002/term.2021

Rhee YH, Ko JY, Chang MY, Yi SH, Kim D, Kim CH, Shim JW, Jo AY, Kim BW, Lee H, Lee SH, Suh W, Park CH, Koh HC, Lee YS, Lanza R, Kim KS, Lee SH (2011) Protein-based human iPS cells efficiently generate functional dopamine neurons and can treat a rat model of Parkinson disease. J Clin Invest 121(6):2326–2335. doi:10.1172/JCI45794

Richardson TP, Peters MC, Ennett AB, Mooney DJ (2001) Polymeric system for dual growth factor delivery. Nat Biotechnol 19(11):1029–1034. doi:10.1038/nbt1101-1029

Ryan SD, Dolatabadi N, Chan SF, Zhang X, Akhtar MW, Parker J, Soldner F, Sunico CR, Nagar S, Talantova M, Lee B, Lopez K, Nutter A, Shan B, Molokanova E, Zhang Y, Han X, Nakamura T, Masliah E, Yates JR 3rd, Nakanishi N, Andreyev AY, Okamoto S, Jaenisch R, Ambasudhan R, Lipton SA (2013) Isogenic human iPSC Parkinson's model shows nitrosative stress-induced

dysfunction in MEF2-PGC1alpha transcription. Cell 155(6):1351–1364. doi:10.1016/j.cell.2013.11.009

Sanchez-Danes A, Consiglio A, Richaud Y, Rodriguez-Piza I, Dehay B, Edel M, Bove J, Memo M, Vila M, Raya A, Izpisua Belmonte JC (2012a) Efficient generation of A9 midbrain dopaminergic neurons by lentiviral delivery of LMX1A in human embryonic stem cells and induced pluripotent stem cells. Hum Gene Ther 23(1):56–69. doi:10.1089/hum.2011.054

Sanchez-Danes A, Richaud-Patin Y, Carballo-Carbajal I, Jimenez-Delgado S, Caig C, Mora S, Di Guglielmo C, Ezquerra M, Patel B, Giralt A, Canals JM, Memo M, Alberch J, Lopez-Barneo J, Vila M, Cuervo AM, Tolosa E, Consiglio A, Raya A (2012b) Disease-specific phenotypes in dopamine neurons from human iPS-based models of genetic and sporadic Parkinson's disease. EMBO Mol Med 4(5):380–395. doi:10.1002/emmm.201200215

Sanders LH, Laganiere J, Cooper O, Mak SK, Vu BJ, Huang YA, Paschon DE, Vangipuram M, Sundararajan R, Urnov FD, Langston JW, Gregory PD, Zhang HS, Greenamyre JT, Isacson O, Schule B (2014) LRRK2 mutations cause mitochondrial DNA damage in iPSC-derived neural cells from Parkinson's disease patients: reversal by gene correction. Neurobiol Dis 62:381–386. doi:10.1016/j.nbd.2013.10.013

Santostefano KE, Hamazaki T, Biel NM, Jin S, Umezawa A, Terada N (2015) A practical guide to induced pluripotent stem cell research using patient samples. Lab Invest 95(1):4–13. doi:10.1038/labinvest.2014.104

Seibler P, Graziotto J, Jeong H, Simunovic F, Klein C, Krainc D (2011) Mitochondrial Parkin recruitment is impaired in neurons derived from mutant PINK1 induced pluripotent stem cells. J Neurosci 31(16):5970–5976. doi:10.1523/JNEUROSCI.4441-10.2011

Seki T, Fukuda K (2015) Methods of induced pluripotent stem cells for clinical application. World J Stem Cells 7(1):116–125. doi:10.4252/wjsc.v7.i1.116

Shaltouki A, Sivapatham R, Pei Y, Gerencser AA, Momcilovic O, Rao MS, Zeng X (2015) Mitochondrial alterations by PARKIN in dopaminergic neurons using PARK2 patient-specific and PARK2 knockout isogenic iPSC lines. Stem Cell Rep. doi:10.1016/j.stemcr.2015.02.019

Shao Y, Sang J, Fu J (2015) On human pluripotent stem cell control: the rise of 3D bioengineering and mechanobiology. Biomaterials 52:26–43. doi:10.1016/j.biomaterials.2015.01.078

Shi Y, Kirwan P, Smith J, MacLean G, Orkin SH, Livesey FJ (2012) A human stem cell model of early Alzheimer's disease pathology in down syndrome. Sci Transl Med 4(124):124ra129. doi:10.1126/scitranslmed.3003771

Simao D, Pinto C, Piersanti S, Weston A, Peddie CJ, Bastos AE, Licursi V, Schwarz SC, Collinson LM, Salinas S, Serra M, Teixeira AP, Saggio I, Lima PA, Kremer EJ, Schiavo G, Brito C, Alves PM (2015) Modeling human neural functionality in vitro: three-dimensional culture for dopaminergic differentiation. Tissue Eng A 21(3–4):654–668. doi:10.1089/ten.TEA.2014.0079

Singh VK, Kalsan M, Kumar N, Saini A, Chandra R (2015) Induced pluripotent stem cells: applications in regenerative medicine, disease modeling, and drug discovery. Front Cell Develop Biol 3:2. doi:10.3389/fcell.2015.00002

Soldner F, Hockemeyer D, Beard C, Gao Q, Bell GW, Cook EG, Hargus G, Blak A, Cooper O, Mitalipova M, Isacson O, Jaenisch R (2009) Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 136(5):964–977. doi:10.1016/j.cell.2009.02.013

Soldner F, Laganiere J, Cheng AW, Hockemeyer D, Gao Q, Alagappan R, Khurana V, Golbe LI, Myers RH, Lindquist S, Zhang L, Guschin D, Fong LK, Vu BJ, Meng X, Urnov FD, Rebar EJ, Gregory PD, Zhang HS, Jaenisch R (2011) Generation of isogenic pluripotent stem cells differing exclusively at two early onset Parkinson point mutations. Cell 146(2):318–331. doi:10.1016/j.cell.2011.06.019

Solomon S, Pitossi F, Rao MS (2015) Banking on iPSC—is it doable and is it worthwhile. Stem Cell Rev 11(1):1–10. doi:10.1007/s12015-014-9574-4

Sommer CA, Sommer AG, Longmire TA, Christodoulou C, Thomas DD, Gostissa M, Alt FW, Murphy GJ, Kotton DN, Mostoslavsky G (2010) Excision of reprogramming transgenes improves the differentiation potential of iPS cells generated with a single excisable vector. Stem Cells 28(1):64–74. doi:10.1002/stem.255

Sproul AA, Jacob S, Pre D, Kim SH, Nestor MW, Navarro-Sobrino M, Santa-Maria I, Zimmer M, Aubry S, Steele JW, Kahler DJ, Dranovsky A, Arancio O, Crary JF, Gandy S, Noggle SA (2014) Characterization and molecular profiling of PSEN1 familial Alzheimer's disease iPSC-derived neural progenitors. PLoS One 9(1):e84547. doi:10.1371/journal.pone.0084547

Stacey GN, Crook JM, Hei D, Ludwig T (2013) Banking human induced pluripotent stem cells: lessons learned from embryonic stem cells? Cell Stem Cell 13(4):385–388. doi:10.1016/j.stem.2013.09.007

Stadtfeld M, Brennand K, Hochedlinger K (2008) Reprogramming of pancreatic beta cells into induced pluripotent stem cells. Curr Biol 18(12):890–894. doi:10.1016/j.cub.2008.05.010

Stocchi F (2014) Therapy for Parkinson's disease: what is in the pipeline? Neurotherapeutics 11(1):24–33. doi:10.1007/s13311-013-0242-1

Su YC, Qi X (2013) Inhibition of excessive mitochondrial fission reduced aberrant autophagy and neuronal damage caused by LRRK2 G2019S mutation. Hum Mol Genet 22(22):4545–4561. doi:10.1093/hmg/ddt301

Sugii S, Kida Y, Kawamura T, Suzuki J, Vassena R, Yin YQ, Lutz MK, Berggren WT, Izpisua Belmonte JC, Evans RM (2010) Human and mouse adipose-derived cells support feeder-independent induction of pluripotent stem cells. Proc Natl Acad Sci U S A 107(8):3558–3563. doi:10.1073/pnas.0910172106

Swistowski A, Peng J, Liu Q, Mali P, Rao MS, Cheng L, Zeng X (2010) Efficient generation of functional dopaminergic neurons from human induced pluripotent stem cells under defined conditions. Stem Cells 28(10):1893–1904. doi:10.1002/stem.499

Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676. doi:10.1016/j.cell.2006.07.024

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. doi:10.1016/j.cell.2007.11.019

Tong Z, Solanki A, Hamilos A, Levy O, Wen K, Yin X, Karp JM (2015) Application of biomaterials to advance induced pluripotent stem cell research and therapy. EMBO J 34(8):987–1008. doi:10.15252/embj.201490756

Utikal J, Polo JM, Stadtfeld M, Maherali N, Kulalert W, Walsh RM, Khalil A, Rheinwald JG, Hochedlinger K (2009) Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. Nature 460(7259):1145–1148. doi:10.1038/nature08285

Vazin T, Freed WJ (2010) Human embryonic stem cells: derivation, culture, and differentiation: a review. Restor Neurol Neurosci 28(4):589–603. doi:10.3233/RNN-2010-0543

Velasco I, Salazar P, Giorgetti A, Ramos-Mejia V, Castano J, Romero-Moya D, Menendez P (2014) Concise review: generation of neurons from somatic cells of healthy individuals and neurological patients through induced pluripotency or direct conversion. Stem Cells 32(11):2811–2817. doi:10.1002/stem.1782

Verdier JM, Acquatella I, Lautier C, Devau G, Trouche S, Lasbleiz C, Mestre-Frances N (2015) Lessons from the analysis of nonhuman primates for understanding human aging and neurodegenerative diseases. Front Neurosci 9:64. doi:10.3389/fnins.2015.00064

Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernig M (2010) Direct conversion of fibroblasts to functional neurons by defined factors. Nature 463(7284):1035–1041. doi:10.1038/nature08797

Wan W, Cao L, Kalionis B, Xia S, Tai X (2015) Applications of induced pluripotent stem cells in studying the neurodegenerative diseases. Stem Cells Int 2015:382530. doi:10.1155/2015/382530

Wang Q, Xu X, Li J, Liu J, Gu H, Zhang R, Chen J, Kuang Y, Fei J, Jiang C, Wang P, Pei D, Ding S, Xie X (2011) Lithium, an anti-psychotic drug, greatly enhances the generation of induced pluripotent stem cells. Cell Res 21(10):1424–1435. doi:10.1038/cr.2011.108

Woodruff G, Young JE, Martinez FJ, Buen F, Gore A, Kinaga J, Li Z, Yuan SH, Zhang K, Goldstein LS (2013) The presenilin-1 DeltaE9 mutation results in reduced gamma-secretase activity, but not total loss of PS1 function, in isogenic human stem cells. Cell Rep 5(4):974–985. doi:10.1016/j.celrep.2013.10.018

Wu J, Sheng C, Liu Z, Jia W, Wang B, Li M, Fu L, Ren Z, An J, Sang L, Song G, Wu Y, Xu Y, Wang S, Chen Z, Zhou Q, Zhang YA (2015) Lmx1a enhances the effect of iNSCs in a PD model. Stem Cell Res 14(1):1–9. doi:10.1016/j.scr.2014.10.004

Wu YL, Pandian GN, Ding YP, Zhang W, Tanaka Y, Sugiyama H (2013) Clinical grade iPS cells: need for versatile small molecules and optimal cell sources. Chem Biol 20(11):1311–1322. doi:10.1016/j.chembiol.2013.09.016

Xu XH, Zhong Z (2013) Disease modeling and drug screening for neurological diseases using human induced pluripotent stem cells. Acta Pharmacol Sin 34(6):755–764. doi:10.1038/aps.2013.63

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. Hum Mol Genet 20(23):4530–4539. doi:10.1093/hmg/ddr394

Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318(5858):1917–1920. doi:10.1126/science.1151526

Zhao P, Luo Z, Tian W, Yang J, Ibanez DP, Huang Z, Tortorella MD, Esteban MA, Fan W (2014) Solving the puzzle of Parkinson's disease using induced pluripotent stem cells. Exp Biol Med 239(11):1421–1432. doi:10.1177/1535370214538588

Zhao T, Zhang ZN, Rong Z, Xu Y (2011) Immunogenicity of induced pluripotent stem cells. Nature 474(7350):212–215. doi:10.1038/nature10135