Induced Pluripotent Stem Cells: A Novel Tool for Modelling and Treating Parkinson's Disease

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

School of Pharmacy

Brac University

March, 2022

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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Approval

The thesis/project titled "Induced Pluripotent Stem Cells: A Novel Tool for Modelling and Treating Parkinson's Disease" Submitted by Nahid Sultana Tanin (18146069) of Spring, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on 1 March, 2022.

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Ethics Statement

This study does not involve any human or animal trial.

Abstract

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons primarily affecting the substantia nigra pars compacta, leading to a deficiency of dopamine in the striatum. Currently, existing treatments of PD only provide symptomatic relief and a permanent cure is yet to be discovered. Although animal models have provided valuable insights into the pathophysiology underlying PD, they are unable to recapitulate the full range of symptoms of human PD mainly due to species differences. These factors highlight an important clinical unmet need for developing cellular models of PD to study pathogenic mechanisms in-depth and identify potential drug targets. iPSCs provide a unique platform to model certain human diseases in vitro and offer the potential to develop cell-transplantation therapies as an innovative treatment strategy for PD. This comprehensive review discusses the utilization of patient-specific iPSCs to study disease mechanisms at a molecular level and discusses the challenges and potential solutions to overcome them.

Keywords: Parkinson’s disease; dopaminergic neurons; α-synuclein aggregation; mitochondrial dysfunction; induced pluripotent stem cells; 3D organoids; gene editing tools; cell reprogramming; cell transplantation therapy.

Dedication

Dedicated to My Parents

Acknowledgment

Firstly, I am grateful to almighty Allah for making me able to choose this field and study Pharmacy. Without His blessings, I would not be able to continue this project paper and submit it for passing my Bachelor's degree in Pharmacy.

This study would not have been completed without the support of many people who are gratefully acknowledged here. First of all, I would like to express my deepest and sincere gratitude to my project supervisor Kazi Fatema Rahman, Lecturer, School of Pharmacy, Brac University. Without her helpful guidance, sincere encouragement, motivational directions, and mentoring capacity I would not be able to carry out my project work. In terms of monitoring and instruction, she continually and persuasively expressed sincerity which encouraged me to complete this project. I am deeply grateful to her and I feel very fortunate to have had the opportunity to have her as my supervisor.

Furthermore, I give my gratitude to all the faculty members of the School of Pharmacy for their contribution which has enriched me with all the knowledge and technical support. It has also allowed me to run the research in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

Last but not the least, I am also thankful to my family for their continuous support and motivation in every step of my life. I also would like to thank my fellow mates and friends for their cooperation and mental support which I needed to complete my project work.

List of Contents

List of Tables

List of Figures

List of Acronyms

PD	Parkinson's Disease
iPSC	Induced Pluripotent Stem Cell
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
HSPs	Heat Shock Proteins
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
HLA	Human Leukocyte Antigen
LRRK2	Leucine-Rich Repeat Kinase 2
PINK1	PTEN-Induced Putative Kinase 1
DBS	Deep Brain Stimulation
mDA	Midbrain Dopaminergic Neuron
SNPs	Single nucleotide polymorphism
NAD+	Nicotinamide Adenine Dinucleotide

Chapter 1

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease (AD). It affects around 2% of the global population over the age of 60. The disease lasts for an average of 15 years from diagnosis to death, with a mortality rate of 2:1 (Avazzadeh et al., 2021). In 1817, James Parkinson published the “Essay on the shaking palsy”, a famous monograph in which he first described a neurological illness termed as Parkinson's disease along with tremor at rest and motor complaint (Torrent et al., 2015). Ageing is the most important risk factor underlying PD. The incidence of PD rises by 5-10 fold beyond the age of 60 (Poewe et al., 2017). In most populations, men are twice as likely to develop the disease compared to women (Jankovic & Tan, 2020).With the progressive rise in the ageing population and better life expectancy, age-related neurological disorders have become the leading cause of disability worldwide and PD seems to be the most rapidly growing one (Poewe et al., 2017). There are around ten million people worldwide who suffer from PD (Hayes, 2019). According to the most recent WHO data, PD death rate in Bangladesh reached 1,363 in 2018 (“Parkinson disease in Bangladesh-world life expectancy”, 2018)

The primary motor symptoms of PD include resting tremor, bradykinesia, rigidity, and postural instability. Besides the classic motor symptoms, PD is also accompanied by a number of non-motor symptoms such as rapid eye movement sleep disorder, depression, loss of smell, constipation and autonomic dysfunction which seem to appear during the prodromal stage of the disease (Jankovic & Tan, 2020) . The hallmark pathological features of PD include the presence of α-synuclein-containing protein aggregates in the cell cytoplasm called Lewy bodies and

substantial loss of dopaminergic neurons in the substantia nigra culminating in a significant lack of dopamine in the striatum, which is crucial for motor movements. The gradual loss of dopaminergic neurons and abnormal accumulation of α-synuclein also leads to significant cognitive impairment in more advanced stages of the disease (Jankovic & Tan, 2020). Apart from the loss of dopaminergic neurons, studies have also reported the involvement of glutamatergic, noradrenergic, cholinergic and serotonergic neural pathways in other brain regions, indicating the complex multifactorial nature of the disease. Malfunction in these additional brain regions are thought to contribute to the non-motor symptoms of PD (Giguère et al., 2018).

Misfolding and aggregation of α-synuclein, abnormal proteostasis, mitochondrial dysfunction, oxidative stress, and neuroinflammation have all been implicated in the pathogenesis of PD (Tysnes & Storstein, 2017). Multiple genes underlying the pathogenesis of monogenic PD have been identified in early studies. Recently, with the advent of improved genetic techniques and large-scale GWAS studies, 90 independent risk-associated genes have been revealed for PD. Monogenic forms of PD, which are often familial, account for 5-10% of all cases whilst 95% of cases are sporadic. However, studies have revealed significant overlaps between the neuropathology of certain monogenic forms and sporadic cases of PD, indicating the possibility of shared disease mechanisms(Nalls et al., 2019). Several environmental factors have also been associated with a higher risk of PD (Tysnes & Storstein, 2017).

The existing treatment options mainly include dopamine replacement therapy and some remedies to relieve the non-motor symptoms. Whilst these therapies provide symptomatic relief, they do not treat the underlying cause or slow down the progression of the disease. Moreover, a subset of patients do not respond to these therapies. This is perhaps due to the lack of a complete understanding of the underlying causes of PD (Torrent et al., 2015). To overcome these obstacles

researchers are currently seeking to develop appropriate disease models that closely resemble PD's phenotypic features in order to better understand the pathophysiological mechanisms (Stoddard-Bennett & Reijo Pera, 2019).In 2006, Yamanaka, a Japanese scientist, made a remarkable scientific and medical breakthrough when he discovered that inducing the activation of a subset of pluripotency transcription factors could reprogram mouse somatic cells back to an embryonic-like state. This opened up a new frontier in human disease modeling. OCT4, SOX2, KLF4, and MYC- are the four factors designated as the "Yamanaka factors" and the stem cells they produced are termed as induced pluripotent stem cells (iPSCs) (Shi et al., 2017).

Following this discovery, research involving the use of iPSCs have grown dramatically for disease modelling, drug discovery and regenerative medicine. iPSCs are currently being employed in PD research, with an emphasis on understanding new phenotypes of the disease using iPSC-derived DA neurons (Sison et al., 2018). Furthermore, the first human trial for PD iPSC transplantation was recently launched in Japan. This form of cell transplantation therapy holds great potential to delay or prevent the progression of PD (Sison et al., 2018 ;Stoddard-Bennett & Reijo Pera, 2019).

This review will begin with a comprehensive overview of PD, its symptoms, overall treatment management, and genetic mutations associated with the disease. It will particularly focus on the application of iPSCs to model PD and the progress that has been made in the field following their discovery. Additionally, it will shed light on the recent advancements in gene editing and 3D organoid technologies that have enhanced the power of iPSC-based platforms. Lastly, the paper will discuss about the key limitations associated with the use of iPSCs and the potential solutions, to overcome them.

1.1 What is Parkinson's Disease:

Parkinson's disease is a neurological condition that causes both motor and non-motor symptoms. It is characterized by the presence of protein aggregates Lewy bodies in the midbrain as well as reduction of dopaminergic neuron activity , particularly in the substantia nigra (Bandres-Ciga et al., 2020). The hallmark symptoms of PD include bradykinesia, resting tremor ,rigidity , and imbalance or uncontrolled movement (Reich & Savitt, 2019). Figure 1 below shows a diagram which represents the pathway that extend from substantia nigra pars compacta leading to deficiency of dopamine in the striatum which is the main cause of developing PD.

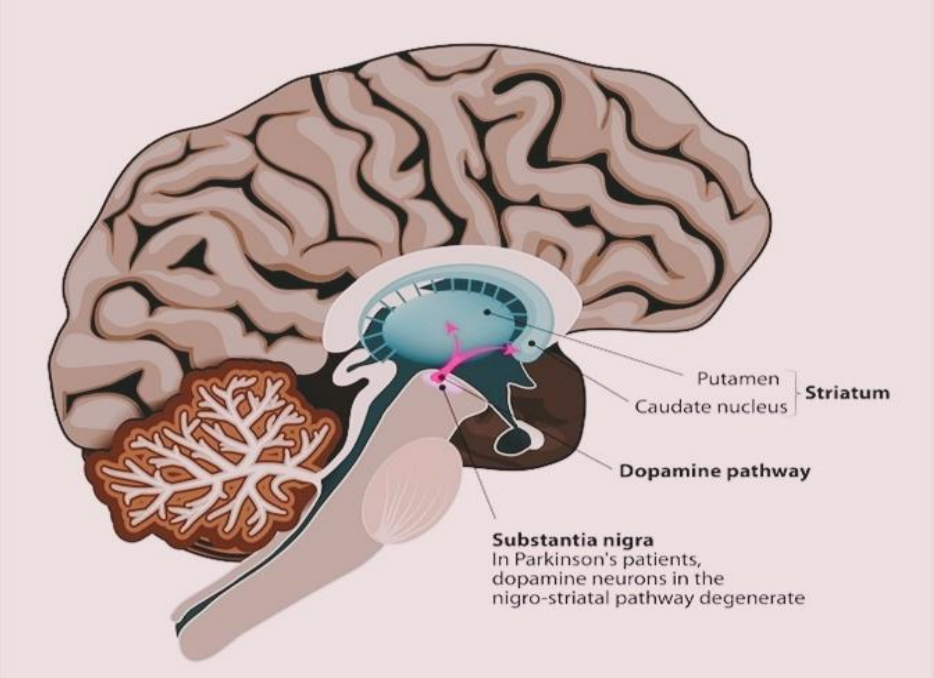

Figure 1: Brain image indicates the pathways that can be responsible for the development of Parkinson's disease [Diagram is adapted from (Parkinson's Disease Pathophysiology, 2019)]

1.2 Clinical incidence rate of Parkinson Disease

Around 10 million people worldwide who suffer from PD. Approximately 60,000 Americans are clinically diagnosed with Parkinson disease each year. It is more common as people get older, and it is rarely observed in those under the age of 40. This neurological disorder affects approximately 3% of the community over the age of 80, according to estimates (Hayes, 2019).

Table 1 shows data published by WHO which indicates that the death rate of PD in Bangladesh reached 1,363 in 2018. ("Parkinson disease in Bangladesh-world life expectancy",2018)

Table 1: *The rate of Parkinson Disease in Bangladesh*

Death	Percentage	Rate	World Rank
1363	0.18	1.31	159

1.3 Clinical Features

As mentioned earlier, tremor, stiffness, bradykinesia, and akinesia are some of the most common motor symptoms of PD. Besides these types of symptoms, there are a variety of non-motor symptoms shown in this condition such as, cognitive collapse, depression, anxiety, sleep disturbances, and dysautonomia (Hayes, 2019).

1.3.1 Resting tremor:

Around 70% of PD patients usually exhibit resting tremor as the first symptom of PD. However, tremor is asymmetric at the start of the disease and gets worse with anxiety and ambulation. (Samii

et al., 2004). In the initial stages, the tremor starts in one extremity (limb of the human body and foot) and often only on the thumb or a finger (Hayes, 2019)

1.3.2 Bradykinesia:

Bradykinesia is the most debilitating symptom in the early stages of PD. It manifests itself in challenges with complex motor tasks such as writing, as well as a restricted arm swing while walking (Samii et al., 2004). In addition, there is decreased voluntary movement, and reduced blink rate. Furthermore, the facial muscles become less active, and speech becomes softer. The mechanics of swallowing are impaired, and sialorrhea can be developed (Hayes, 2019).

1.3.3 Rigidity

Rigidity is characterized by elevated resistance during passive joint movement. Contralateral motor activity can be increased by this condition (Samii et al., 2004).

1.3.4 Dementia

Besides motor symptoms, PD can also be accompanied by cognitive decline. About 40% of PD patients develop dementia. Men between the ages of 60 and 80 have been found to have higher rates of dementia. Commonly Lewy body dementia which is caused by abnormal deposits of alpha-synuclein protein aggregates in the brain is seen in PD. In this stage, patients are unable to think properly (Hayes, 2019).

Here, Figure 2 illustrates the motor and non-motor symptoms of Parkinson's disease.

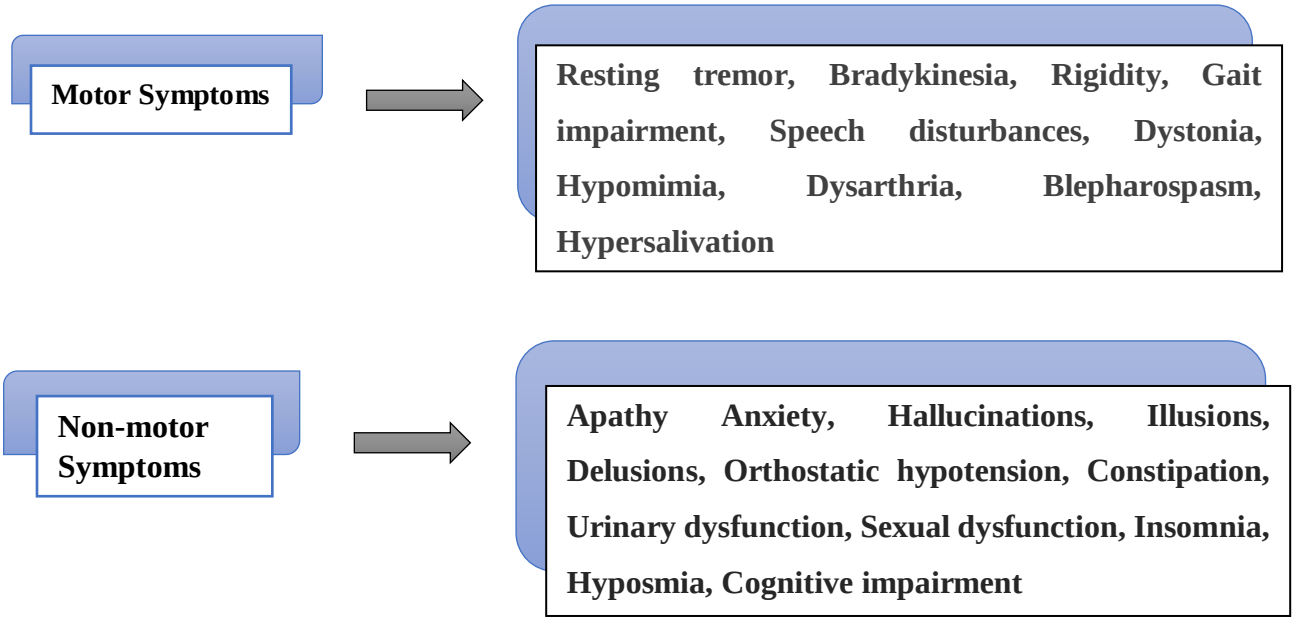

Figure 2: Motor and Non-motor symptoms of Parkinson's disease (Schapira et al., 2017 **;** Sveinbjornsdottir, 2016)**.**

Chapter 2

2.1 Pathogenesis factors of Parkinson Disease

Parkinson's disease has many pathogenetic factors. Figure 3 illustrates the underlying pathogenetic factors implicated in PD-

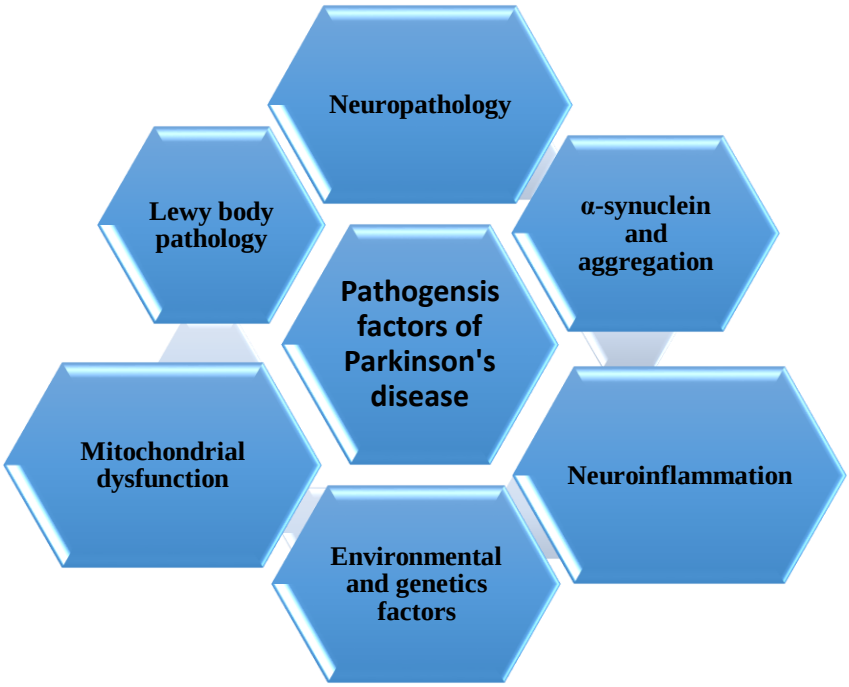

Figure 3: Pathogenesis factors of Parkinson's Disease

2.1.1 Neuropathology

The primary reason of PD is a substantial loss of dopaminergic neurons in the substantia nigra pars compacta of the brain which causes a significant reduction of dopamine in this region. Dopamine is a neurotransmitter that mediates feelings of pleasure or reward and enables the motor function. It acts as a chemical mediator between the parts of the brain and nervous system. Dopamine has many important functions in the neurological and physiological sectors such as motor function,

mood changing, making the decision and controlling and balancing body movements (Kouli et al., 2018).

The nigrostriatal pathway facilitates motor movements. As mentioned previously, the substantia nigra contains dopaminergic neurons and approximately 75 % of dopamine in the brain is found here. So, a lack of dopamine in this pathway can disruption in neurotransmission and motor impairment. In addition to dopaminergic neuron the cholinergic, glutamatergic, GABAergic, noradrenergic, and serotonergic are nondopaminergic neurotransmitter systems affected in PD which are most likely responsible for producing the non-motor symptoms (Kouli et al., 2018 ; Dickson, 2012).

Additionally, various mechanisms were suggested to understand the etiology of this disease including Lewy body pathology, alpha-synuclein aggregation, mitochondrial dysfunction, neuroinflammation also has some environmental factors for developing the risk of PD such as cigarette smoking , drug induced parkinsonism , caffeine , pesticides, herbicides, and heavy metals (Schapira, 2009).

2.1.2 Lewy body pathology:

A pathological characteristic of PD can be developed by the existence of abnormal cytoplasmic deposits within neuronal cell bodies that are immunoreactive for the protein α-synuclein. The Lewy bodies indicate are abnormal protein aggregates (LBs). Several proteins are found in an LB, like as α -synuclein, ubiquitin, parkin, heat shock proteins (HSPs), oxidized proteins, cytoskeletal proteins. The fundamental structural component of LBs is the protein filamentous α -synuclein. So in PD patients it becomes inappropriately aggregated and phosphorylated (Kouli et al., 2018).

2.1.3 α-synuclein aggregation

α -synuclein is found in robust tetramers that resist aggregation in aqueous solutions thus it is commonly unfolded. α -synuclein develops an amyloid-like, β sheet-rich structure that is liable to aggregate in PD. Some pathways for the structural alterations that lead to α-synuclein aggregation have been postulated, such as serine phosphorylation, ubiquitination, and C-terminal truncation. As a consequence, numerous α-synuclein species, notably unfolded monomers, soluble oligomers, protofibrils, are found in the PD brain (Kouli et al., 2018 ; Bartels et al., 2011).

2.1.4 Mitochondrial dysfunction

Idiopathic and familial PD have mitochondrial dysfunction as a primary etiologic component. The mitochondrial complex-I is a prominent component of the electron transport chain, according to a recent postmortem analysis of the SNpc in PD brains. Moreover, abuse of MPTP substances can cause PD and studies have shown that uptake of oxidized MPTP by dopamine neurons leads to complex -1 inhibition. Mutations of PINK1 and PARKIN genes impaired the mitochondrial function and cause autosomal recessive PD. Furthermore, α -synuclein binds to the mitochondrial membrane and the activity of complex-I is damaged by this. Eventually, this is responsible for mitochondrial malfunction and induces oxidative stress (Kouli et al., 2018 ; Hattori & Mizuno, 2002).

2.1.5 Neuroinflammation:

Postmortem brain investigations of PD patients' SNpc and striatum indicated microglial (responsible for degradation of dopaminergic cell) and complement activation, as well as T-lymphocyte infiltration compared to healthy people. Recently. Genetic studies consider that there is a strong relationship between the HLA class II region which is a major component of the immune system and responsible for developing PD (Kouli et al., 2018 ; Hirsch & Hunot, 2009).

2.1.6 Drug-induced parkinsonism:

Exposure to neuroleptics is the most prevalent trigger of drug-induced parkinsonism. Antiemetic and promotility agents such as promethazine, metoclopramide, and prochlorperazine as well as reserpine, several calcium-channel blockers (flunarizine and cinnarizine, tetrabenazine) can also produce parkinsonism. It should be mentioned that drug-induced parkinsonism clears up after the medicine is stopped, but it can take weeks or months (Samii et al., 2004).

2.2 Genetics of Parkinson disease

Parkinson's disease is divided into two groups based on genetic factors namely familial and sporadic PD. PD was once thought to be a sporadic disorder with no hereditary cause. The classification of familial and sporadic PD is given in Figure 4.

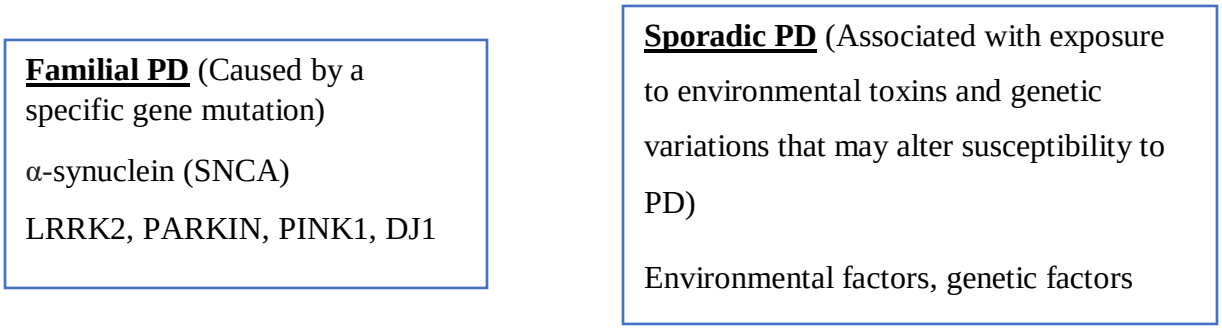

Figure 4: Classification of Parkinson Disease.

The first PD gene was discovered is SNCA with autosomal dominant inheritance. The enormous number of PD is caused due to a complicated interaction between genetics and the environment. So, it should be stated that mutations in the SNCA, Parkin, PINK1, DJ-1, LRRK2and ATP13A2 genes cause monogenic types of PD. (Kumar et al., 2011).

Here, Figure 5 represents the classification of Familial PD and Figure 6 the classification of genetic Parkinson's disease.

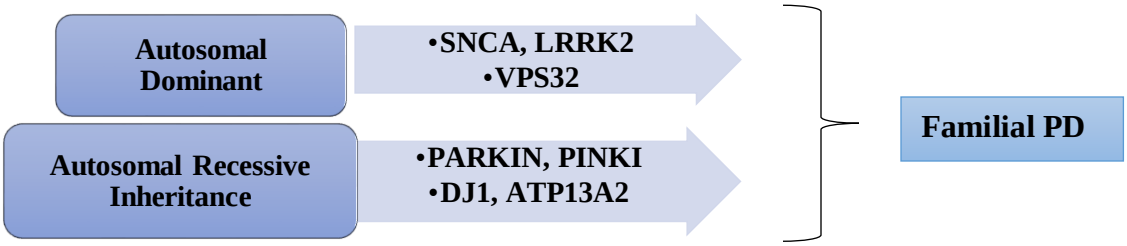

Figure 5: Classification of Familial PD.

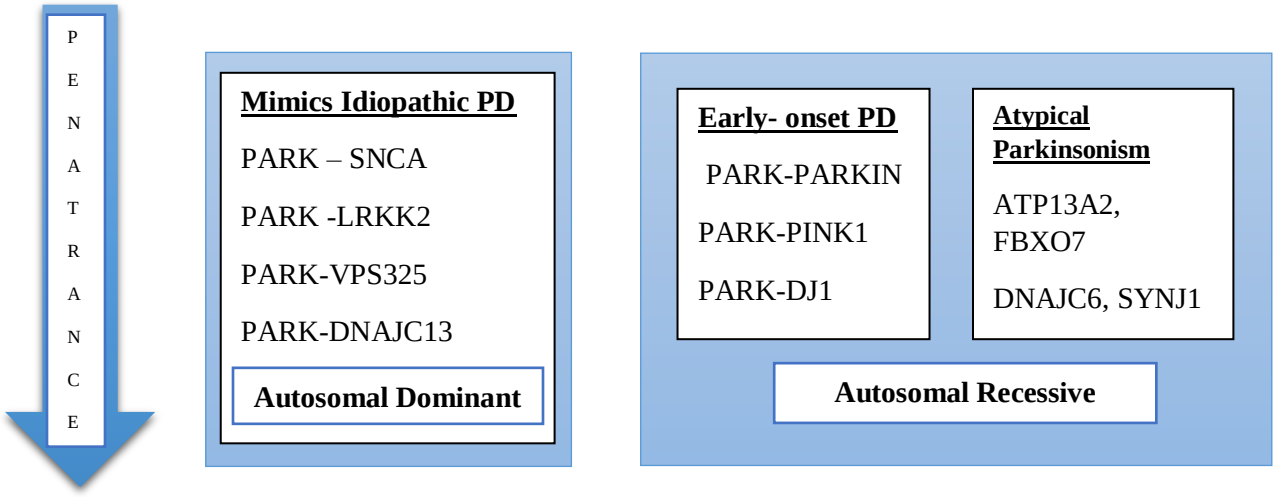

Figure 6: Atypical parkinsonian syndromes and genetic Parkinson disease [Idea is adapted from (Domingo & Klein, 2018)]

2.2.1 α-synuclein (SNCA):

α-synuclein (SNCA) mutations are a rare causative gene of PD, and this mutation commonly causes early-onset PD (age of onset below 50 years) (Kumar et al., 2011).However, duplications and triplications of the whole gene have been associated with familial PD. An increased number of SNCA copies has been linked to primary onset, a severe phenotype, and a rapid course of the disease. The symptoms of PD which are occurred by the mutations in α -synuclein (SNCA) are-

- orthostatic hypotension,
- strong myoclonus,

- central hypoventilation

Moreover, there are so many pathologic changes that occur due to these mutations and that are primarily susceptible to levodopa (Kumar et al., 2011 ; Shulman et al., 2011).

2.2.2 LRRK2 (PARK8)

The most prevalent mutations of the LRRK2 gene, as well as the phenotype of LRRK2 p.G2019S are linked to autosomal dominant PD. According to studies, and pathogenic LRRK2 mutations (such as p.G2019S) may enhance autophosphorylation or kinase activity. Eventually, this creates the chance of LRRK2 kinase inhibitors being employed as neuroprotective medicines in PD.

So far studies have been proven that the most common symptoms of PD are associated with these mutations are tremor (Kumar et al., 2011 ; Domingo & Klein, 2018).

2.2.3 PARKIN (PARK2):

Parkin mutations are usually caused of early-onset autosomal recessive PD. When compared with other cases of the early beginning of PD, parkin mutation carriers are more prone to have dystonia at onset and hyperreflexia. According to postmortem examination studies, these mutations have been identified the neuronal loss in the substantia nigra and LBs (Kumar et al., 2011). Moreover, it is important for mitochondrial function. Mutations in Parkin or PINK1 causes-

- Impair mitophagy,
- The mutations of PARKIN 1 may contribute to the neurodegenerative process by causing the accumulation of abnormal mitochondria (Kumar et al., 2011).

2.2.4 PINK1(PARK6)

Mutations in the PINK1 gene are the second leading cause of autosomal recessive early-onset PD. When the Drosophila PINK1 homolog is abolished, mitochondrial morphological abnormalities,

apoptotic muscle degeneration and increased vulnerability to oxidative stress can be occur (Kumar et al., 2011).

2.2.5 DJ-1 (PARK7)

This gene is very important for mitochondrial function. Furthermore, mutant proteins of DJ-1 are misfolded, and destroyed quickly by the proteasome. According to the reports , DJ-1-dependent mitochondrial abnormalities might generate oxidative stress, which can lead to cell death sensitivity. (Domingo & Klein, 2018) (Kumar et al., 2011). The main clinical features of DJ-1 are-

- A slow progression of the disease,
- Levodopa responsiveness
- Psychological friction can be perceived (Kumar et al., 2011).

2.2.6 ATP13A2 (PARK9)

ATP13A2 (PARK9) mutations result in unstable proteins that are eventually destroyed by the proteasome. In addition, excessive mutant ATP13A2 may cause proteasome malfunction and toxic aggregation that resulting KRS (a rare autosomal recessive form of PD that causes parkinsonism, cognitive impairment etc.). Furthermore, the deficiency of this gene can cause lysosomal malfunction, which can lead to insufficient lysosomal protein degradation (Kumar et al., 2011).

2.2.7 PARK-VPS35

PARK-VPS35 is a very rare gene that is related to develop PD. This gene exhibits a phenotype that is quite comparable to typical PD, such as significant levodopa response, and motor difficulties, and is dominantly inherited with a lower penetrance. (Domingo & Klein, 2018).

Chapter 3

3.1 Treatment strategy of Parkinson's disease

The most effective and widely used treatment for motor symptoms of PD is dopamine replacement therapies. Initial therapy at the beginning of treatments includes levodopa preparations, dopamine agonists, and monoamine oxidase-B (MAO-B) inhibitors and at present these are the mainstays of PD treatment. Anticholinergics drugs can help young on set PD-patients to reduce tremors, but they should be cautiously due to the risk of side effects. The best way to start treating PD is to decide with the patient and consider the benefits and risk ratio. Certain types of exercises may help with various motor symptoms of PD. Moreover, physiotherapy, and speech therapy (for speech and swallowing) can also be beneficial. Gait and balance training, treadmill activity, strength training, and other therapy approaches can preventative maintenance for motor symptoms, Deep brain stimulation (DBS), MRI-guided targeted ultrasound, and levodopa-carbidopa enteral suspension therapy are all current treatments for motor symptoms. Depression associated with PD can be treated with selective serotonin reuptake inhibitors, selective serotonin-norepinephrine reuptake inhibitors, and tricyclic antidepressants in non-motor symptoms of PD (Armstrong & Okun, 2020).

For patients whose symptoms are not well treated by oral drugs alone, surgical and other promising treatment options could be considered. Surgical procedures have revolutionized Parkinson's disease care (PD). Moreover , Deep brain stimulation (DBS), lesioning techniques (pallidotomy, thalamotomy, subthalamotomy), and dopaminergic drug infusion devices are all alternatives for treating motor complications of PD and dramatically revolutionized Parkinson's disease care(Sharma et al., 2020).

3.2 Current Drugs of Parkinson's Disease

There are currently many drugs are available for PD. Table 2 summarizes different types of dopaminergic medications, their mechanism of action, advantages, and limitations.

3.2.1 Dopaminergic medications

Table 2: *Dopaminergic medications*

Drug Name	M/O	Advantages	Limitations	Reference
Levodopa [Gold standard therapy] Levodopa - Carbidopa combination therapy [enabling the conversion of levodopa to active dopamine and crosses the BBB]	The loss of dopaminergic neurons in the SNpc causes striatal dopamine depletion, which causes severe motor symptoms in PD. For patients with advanced PD, levodopa-carbidopa intestinal gel (LCIG) is an approved therapy. It minimizes changes in L-dopa plasma levels, lowering the risk of motor problems.	-Useful options of initial therapies of PD. -Provides the most symptomatic relief. -Highly effective in bradykinesia and rigidity -Rapid onset of action -Potent medication.	-Short half life -Motor fluctuations and dyskinesias are the major complications of long-term levodopa use. -Nausea.	(Radhakrishnan & Goyal, 2018) (Hayes, 2019) (Reich & Savitt, 2019)
Dopamine Agonist [Pramipexole, Ropinirole, Injected Apomorphine.	The D2 receptor is usually targeted by dopamine receptors. Dopamine agonists imitate dopamine's impact on the dopamine receptor. When administered as initial monotherapy, dopamine agonists is more effective to minimize the symptom of motor complications.	-Used as initial treatment in people who are particularly risk of dyskinesia -Longer duration effect -May help to decrease the side effects of levodopa.	-Less potent -Dizziness -Insomnia -Orthostatic hypotension -Loss of appetite	

MAO B inhibitors [Selegiline, Rasagiline, Safinamide, Zonisamide]	Inhibition of MAO B results in an increase in synaptic dopamine levels as well as symptomatic efficacy. -Rasagiline is an irreversible MAO B inhibitor which act as an add-on-therapy for patients with motor fluctuations. -Safinamide is an anti-glutaminergic MAOB inhibitor that is reversible and more effective to improving control of motor symptoms.	-Levodopa needs are reduced as well as lowering the risk of dyskinesias. -Well tolerated on long-term usage -The incidence of the 'wearing off' phenomena was minimized.	-Nausea -Dizziness -Insomnia -Orthostatic -hypotension -Loss of appetite.	
Catechol-O-methyl transferase (COMT) inhibitors [Entacapone, Opicapone, Tolcapone]	The metabolism of both levodopa and dopamine is inhibited by this kind of drug. These medications increase the bioavailability of the previous medicament.	-Inhibition of the COMT pathway will enhance levodopa's bioavailability and half-life, which will benefit patients with motor fluctuations.	- Entocapone is less efficacious. - sleep disorders - Nausea - Orthostatic - hypotension - GIT disturbance	

3.2.2 Non -dopaminergic Medications:

Late-stage PD symptoms do not acknowledge well to dopaminergic therapy. Non-dopaminergic drugs are used to treat symptoms such as motor levodopa-induced dyskinesias, motor fluctuations, and treatment-resistant tremor (Radhakrishnan & Goyal, 2018).

Here, Table 3 able describe about the Non-dopaminergic Medications, their mechanism of action, advantages, and limitations.

Table 3: *Non-dopaminergic Medications*

Drug Name	Indication	Reference
Cholinesterase inhibitor [Rivastigmine]	Degeneration of cholinergic neurons causes acetylcholine insufficiency, which causes dementia, gait problems, and falls. Rivastigmine, a cholinesterase inhibitor, is used to treat dementia caused by PD.	
Antidepressant medications	Depression in patients with PD responds to a variety of antidepressants. Clozapine works well for psychotic symptoms in Parkinson's disease. According to the latest studies the 5hydroxytryptamine 2A (HT2A) inverse agonist substantially confirm clozapine's serotonergic impact in the treatment of psychosis.	(Radhakrishnan & Goyal, 2018)
N-methyl-D-aspartate (NMDA) receptor antagonist [Amantadine]	Amantadine has a significant role to decrease levodopa-induced dyskinesia.	(Armstrong & Okun, 2020)
Adrenergic agents [Midodrine and Etilefrine]		
The noradrenaline precursor [Droxidopa]	These drugs are usually used to treat autonomic dysfunctions of in the late stage of PD. To treat orthostatic hypotension the noradrenaline precursor is used. Anti-muscarinic agents are used to treat urinary urgency or incontinence. Prokinetic medicines are used to treat constipation.	
Anti-muscarinic [Oxybutynin, Tolterodine]		

3.2.3 Surgical options for the treatment of Parkinson's disease

In PD, dopaminergic and nondopaminergic medications, are the basis of treatment. Patients may develop motor fluctuations and dyskinesia as their disease progresses and they continue to utilize dopaminergic treatments. Concerning fact is that repeated use of dopaminergic medications can develop various side effects which can impair the therapeutic effectiveness of some patients,

especially starting therapy. For patients whose symptoms are not well managed by oral drugs alone, than alternative treatment options, such as surgery can be the best (Sharma et al., 2020). Here, Table 4 illustrates the surgical options for the treatment of PD, it's mechanism of action, advantage and limitations.

Table 4: *Surgical options for the treatment of PD*

Approaches	Indications	Advantages	Limitation	Reference
DBS (Deep Brain Stimulations): Electric currents delivered by surgically implanted electrodes attached to a neurostimulator are used to modulate neuronal networks in DBS therapy.	-Motor fluctuations and dyskinesia - Medication-refractory tremor	-Reversible -Treatment options for tremor that hasn't responded to medicines	- Patients with dementia are not recommended to take this medication. - Poor axial symptom control - Invasive therapy	(Sharma et al., 2020) (Reich & Savitt, 2019)
Lesioning surgeries: In lesioning surgeries (LS) a particular brain tissue volume is eliminate to terminate maladaptive neuronal networks. There are 3 techniques are available for this surgery.	-Motor fluctuations and dyskinesia -Medication-refractory tremor	- There is less postoperative care. - Follow-ups are less frequent.	-Lesion is irreversible -As the condition advances, it is no longer modifiable. -Bilaterally, it is not advised.	(Sharma et al., 2020) (Reich & Savitt, 2019)

Chapter 4

4.1 Induced Pluripotent stem cell (iPSCs)

Over than 50 years ago, in 1961, Drs. James A. Till and Ernest A. McCulloch of the University of Toronto in Canada originally characterized stem cells (Liu et al., 2020).

A stem cell is a type of cell which has the potential to differentiate into many different cell types within the body. Stem cells contribute to the body's growth by creating new cells and replacing damaged cell. Stem cell could potentially be used in the future to replace cells and tissues that have been damaged or misplaced due to the ailments. One of the main characteristics of stem cells can make it unique and advanced is capability of diving and producing new cell endlessly.

Stem cell can be divided by 5 basic categories –

I.	Embryonic stem cells (ESCs)
II.	Very Small Embryonic-like Stem Cells (VSELs)
III.	Induced pluripotent stem cells (iPSCs)
IV.	Nuclear transfer stem cells (NTSCs)
V.	Adult stem cells (ASCs)

Pluripotent stem cells are cells that can develop into cells from all three germ layers endoderm, mesoderm and ectoderm and thus can give rise to all cells in the adult body. They have the capacity to self-renew, meaning they can divide and multiply indefinitely (Yamanaka, 2020).

The classification of Human Pluripotent Stem Cells is given (Figure 7) below-

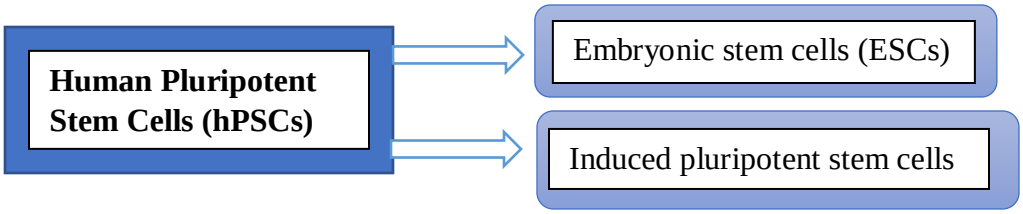

Figure 7: Classification of Human Pluripotent Stem Cells.

The concept of induced pluripotent stem cell technology was first introduced in 2006 by Shinya Yamanaka a Japanese Novel prize - winning stem cell researcher. This discovery appeared to be groundbreaking because it allowed researchers to convert any somatic cell into a stem cell. iPSCs were made by taking a somatic cell from the patient's body usually skin cells and treating the cell with specific transcription factors - Oct3/4, Sox2, Klf4, c-Myc. These transcription factors are also known as Yamanaka Transcription factors (Rowe & Daley, 2019 ; Liu et al., 2020).

In a subsequent study in 2007 by Yamanaka and colleagues Human iPSCs were first created. The accumulated knowledge of hESC allowed the rapid from mouse-derived to human - derived iPSCs. Human-cell derived iPSCs were produced in the lab by reprogramming normal adult cells, such as skin or blood cells. Many groups are attempted to administer iPSCs to patients as well as cell regenerative cell therapy since then, and some of them are currently being studied in clinical trials. Furthermore, in order to obtain a better knowledge of PD, iPSCs can be used to investigate genetic and environmental variables that contribute to the pathogenesis of PD.

iPSC stem cells are preferred over human embryonic stem cells because (hESC) there are some controversial and ethical issues are present. In the hESC process, human embryos reach the blastocytes stage, inner cell mass has been removed in order to harvest ESC in the lab. So, it is

involved the direct destruction of embryos for research purposes. iPSC is not involved with these types of ethical issue. Moreover, hESC is more difficult to access than iPSC also it has the ability to self -renew and obtain an unlimited supply of cells for research (De Wert & Mummery, 2003; Z. Jiang et al., 2014).

Clinical trials for stem cell and Parkinson's disease are ongoing and there is still a long way to go before these treatments are approved. If these stem cell therapies are fully approved for PD, they could alleviate movement symptoms including tremors, stiffness, and slowness, as well as drug requirements(Yamanaka, 2020). Here, Figure 8 represents cell therapies for some particular diseases that can be done by using iPSC.

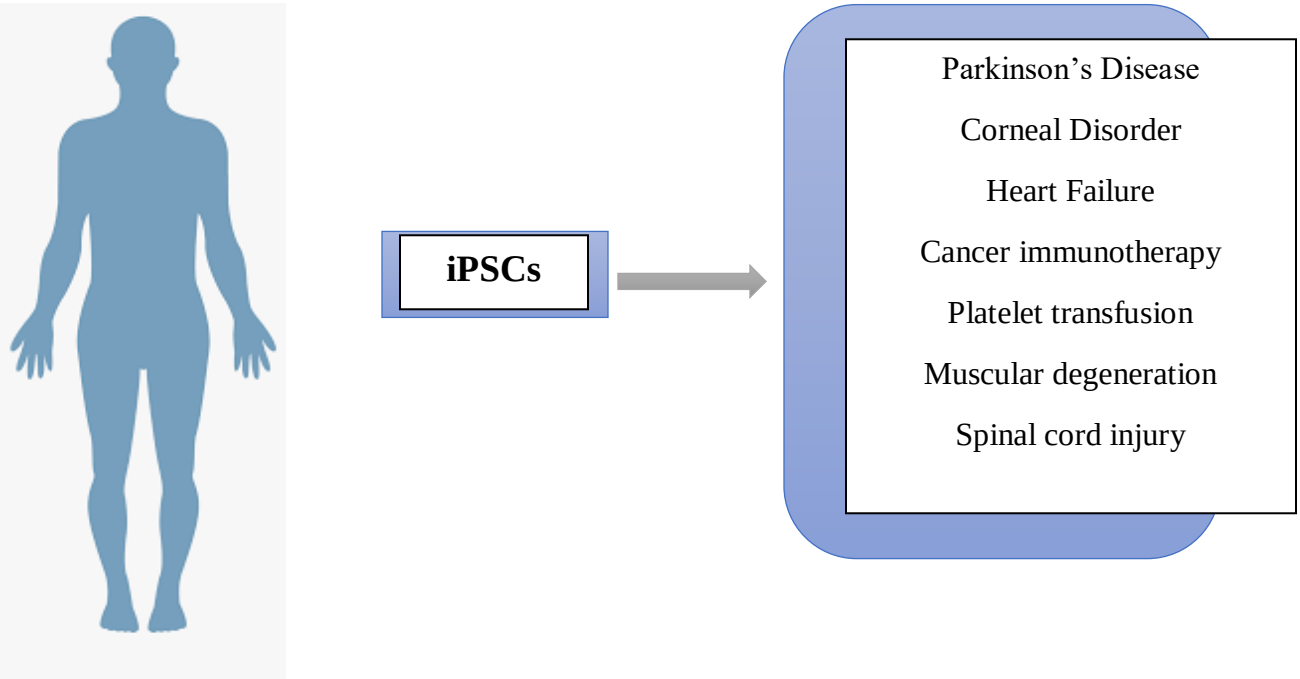

Figure 8 : Cell Therapies of some particular diseases Using iPSCs (Yamanaka, 2020).

4.2 Potential benefits of iPSC over currently available treatment strategies:

The newest tool for modeling PD is induced pluripotent stem cells (iPSCs). This can be used as both a treatment option and a research tool for novel drug discovery. If we look into the advantages of iPSCs, researchers can study patient's specific cells in iPSC cultures, which can reveal a great deal of information about various genetics subtypes of the disease. It is already mentioned that patients with PD have had their fibroblasts reprogrammed into iPSCs, which can then be differentiated into cell types. As it has been already known, dopaminergic neurons in the substantia nigra pars compacta are lost in PD patients. iPSC-derived midbrain dopaminergic neurons is useful to investigate pathogenic pathways as a manner of modeling PD (Beevers et al., 2013).

Although PD iPSCs have been used to evaluate a variety of disease-relevant characteristics in PD iPSC-derived neurons, such as DA release, mitochondrial dysfunction (pathological reason for PD which are across genetic backgrounds), oxidative stress, ER stress, and buildup of alpha-synuclein. More advanced cultural techniques are being developed such as directed reprogramming and midbrain organoids which provide novel approaches to studying intraneuronal causes of PD pathogenesis. iPSCs produced from PD patients are a growing resource for understanding disease pathophysiology and identifying treatment targets (Sison et al., 2018).

4.3 iPSC as disease model of Parkinson's Disease

Patient-specific iPSC-derived dopaminergic neurons have enabled researchers to investigate a variety of PD characteristics in a dish (Avazzadeh et al., 2021). Patients with mutations in SNCA, LRRK2, PINK1, PARK2 (encodes parkin), GBA (-glucocerebrosidase) have all been investigated using patient derived iPS cells (Beevers et al., 2013).

4.4 General working process of iPSC disease model

Shinya Yamanaka and Sir John B. Gurdon in 2012 for their revolutionary work on reversing the in vitro process which allows virtually any terminally differentiated cell to be reprogrammed to a pluripotent state and in this way the iPSC was born. He used retroviral delivery of four transcription factors named **Oct-3/4, Sox2, Klf4, and c-Myc** to revert mouse and human fibroblasts cells to a state of pluripotency (Beevers et al., 2013).Yamanaka factors are thought to play the following functions in cell reprogramming: Oct4 and Sox2 are essential for pluripotency to be established. Oct4 promotes ES-like cell fate , c-Myc may create immortal and active chromatin features in pluripotent stem cells, and Klf4 is involved in cell death, senescence, and pluripotency maintenance. (W. Chen et al., 2012). In addition with this, in this model process, human adult somatic cells might be reprogrammed back to an embryonic-like state by forcing the expression of a subset of pluripotency transcription factors (Sison et al., 2018).

Firstly, fibroblasts (inherent characteristics of self-renewal) from a person with PD can be obtained, reprogrammed to a pluripotent state using those transcriptional factors, and then differentiated into the PD-affected DAns in the midbrain. The process of converting iPSC lines to the DA neuronal destiny of the SNpc is quite challenging. However, the main advantage lies in the fact that this model preserves genetic susceptibility, native molecular machinery, and distinct transcriptional pathways, all of which are crucial to accurately model such a complicated multifactorial disease (Stoddard-Bennett & Pera, 2020). In PD models, mutations of consequence may be collected in iPSC lines and steered to a DAn fate by small molecules and this whole process is done within a dish in laboratory. CRISPR/Cas9 editing, which reduces genetic and clonal variation, could help isolate genetic influences even more (Stoddard-Bennett & Reijo Pera, 2019).

The overall process of the iPSC disease modelling has been summarized in Figure 9.

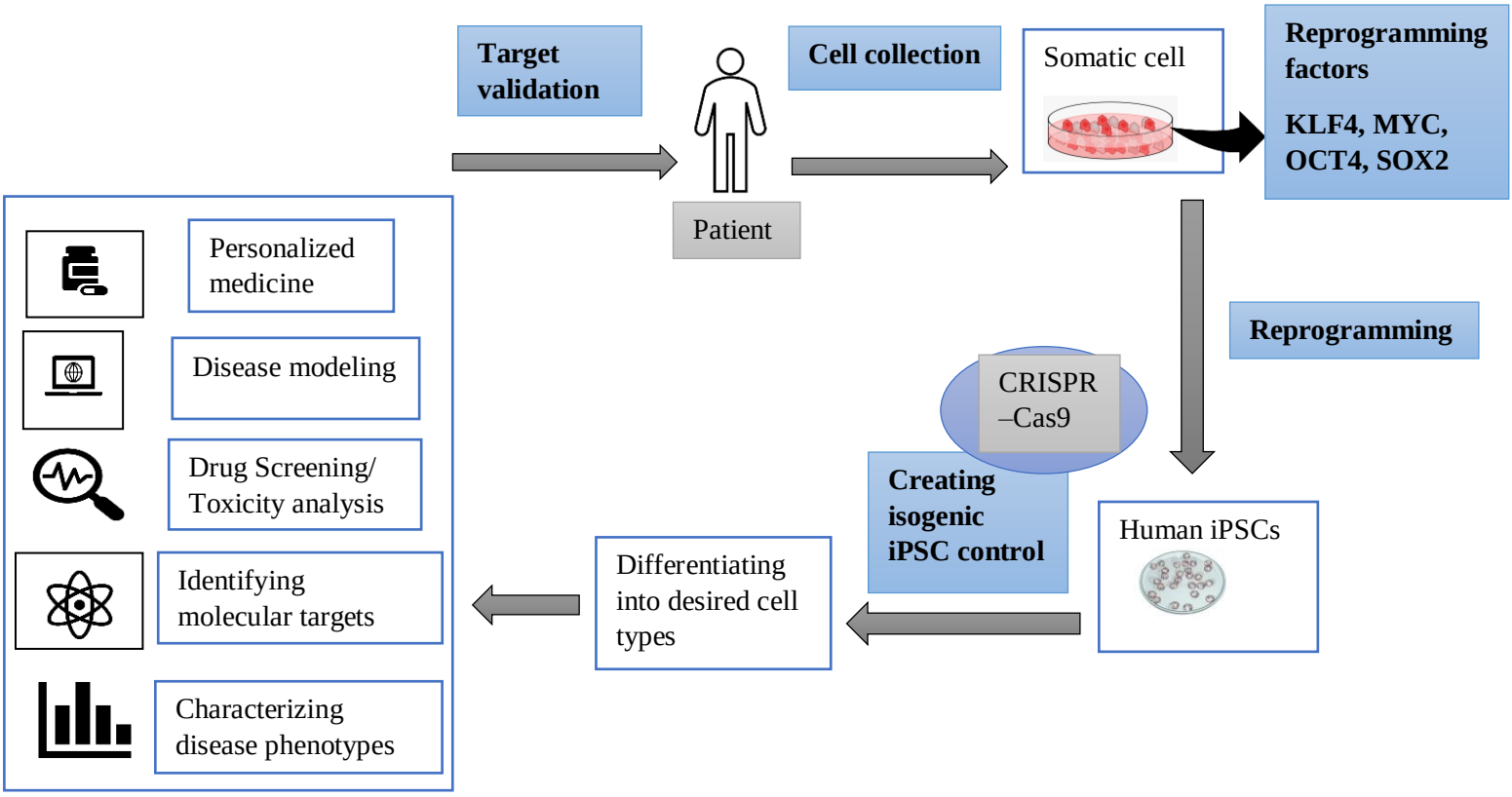

Figure 9: A diagram for disease modelling using human iPSCs. First, individual patients' iPSCs are derived and then they can be reprogramed using transcription factors. In the next step, CRISPR–Cas9 gene-editing tools are used to build isogenic controls. After that, the iPSCs are differentiated into specific cell types, and the resulting cells are studied to find disease-specific phenotypes. The investigation of these phenotypes can lead to the discovery of new pathological mechanisms, toxicity testing, as well as contribute to give the opportunity in the field of drug discovery and personalized medicine [Figure adapted from (Shi et al., 2017;Rowe & Daley, 2019)].

4.4.1 Isogenic control of iPSC model

Isogenic refers to a group of people who have nearly identical genes. There are procedures for modifying the DNA of cells, which can subsequently be utilized to create a disease model. One of the primary potential disadvantages of iPSC- based studies is the variability amongst iPSC lines and lack of appropriate controls. Variability among cell lines can introduce a lot of cofounding making it difficult to distinguish pathology between diseased and unaffected cells. Previous studies have used cells from similarly-aged healthy family members as controls. However, these controls were insufficient as they were not genetically identical (Wang et al., 2014). To get reliable data, it is necessary to confirm the study in a larger number of replicates with the same genetic background which will help to minimize variability. Genome editing methods can be utilized to establish point mutations into the genome, resulting in isogenic clonal cell lines that differ solely at the changed base (s) (Beevers et al., 2013)**.**

4.4.2 Neuronal Co-culture:

An in-vitro culture that includes many types of cells is usually known as a neuronal co-culture due to the co- existence with other types of cells in the CNS. Cells like microglia and astrocytes are present in the neuronal co-culture. In the process of iPSC model, co-culture can imitate the complex interaction between cells and has the greatest benefits for study and analysis of neural function and disease progression of neurodegenerative disease. Microglia, the brain's resident immune cells, have been linked to a variety of neurodegenerative and neurodevelopmental disorders. Microglia are activated by a range of stimuli, which causes the release of anti-inflammatory cytokines, as well as reactive oxygen species, which help to modulate neuroinflammation and oxidative stress. The in vitro procedures give a set of protocols for isolating and plating primary cerebellar granule neurons from a mixed glia culture, as well as ways for co-

culturing both cell types. These techniques enable researchers to investigate how microglia and the substances they secrete in this shared environment mediate toxicant effects on neuronal function. The design of these technique is flexible and useful for the investigation of a wide range of toxicological endpoints and neuroprotective measures (Roqué & Costa, 2017).

4.4.3 3D Organoids

The creation of organoids from iPSCs was a significant step forward in disease modeling with iPSCs. 3D organoids are developed from stem cells and self-organize to resemble the structural properties and cell–cell interactions of mature tissues. It produces a new possibility to scrutinize disease pathogens in the brain. Human iPSC-derived organoids have become a valuable research tool as they permit the exploration of cell–cell interactions in a biological setting that closely resembles human physiology and development. Moreover, it has been used to evaluate medicinal chemicals and do cell transplantation. It allows for the modeling of pharmacological responses at the organ level rather than individual cells (Lee et al., 2017; Wray, 2021). Moreover, the 3D organoid platform is more efficient and reproducible than typical 2D cultures because of its ability to self-organize and reproduce embryonic and tissue development in vitro (Ho et al., 2018).

4.4.4 Gene Editing Tools

One of the common examples (other examples are given in figure 12) of gene - editing tools is CRISPR. The Cas9 nuclease from Streptococcus pyrogens is a widely used gene-editing tool based on a bacterial (CRISPR)-associated protein 9 (Cas9) nuclease. Recently, the CRISPR–Cas9 system has gained a lot of popularity and is being used in gene editing of human ESCs and iPSCs. Researchers can utilize this gene-editing method to introduce disease-causing mutations into wild type iPSCs and subsequently delete those mutations from patient to provide isogenic controls for disease modeling utilizing iPSCs. This helps to create isogenic control cell lines which have the

same genetic background but differ only at the specific mutation site (Shi et al., 2017). Figure 10 illustrates the examples of gene editing tools.

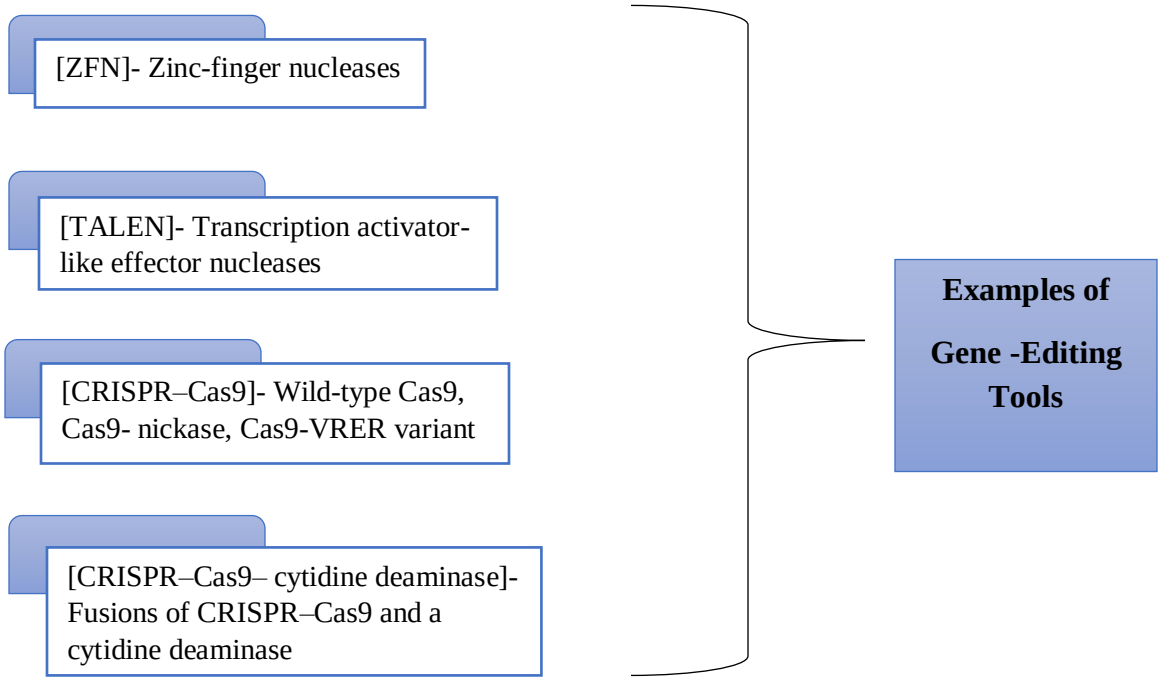

Figure 10: Examples of Gene-Editing Tools. In human iPSCs, these techniques result in DNA double-stranded breaks at the gene modification site and significantly improved gene editing efficiency [Adapted from- (Shi et al., 2017)].

4.5 Modeling Sporadic and Familial PD Using iPSC

Many researchers have described the production of iPSC from individuals with sporadic and genetic types of Parkinson's disease in recent years. Researchers created PD-specific iPSC from a sporadic PD patient in 2008 (Torrent et al., 2015).

In order to understand the phenotypes of PD, researchers have attempted to investigate certain underlying PD-related mutations using patient-specific iPSC-derived DA neurons carrying specific mutations (Avazzadeh et al., 2021). The following are the current findings, which are based only on human iPSC-derived neuronal models and examine specific mutation-associated characteristics of this disease:

4.5.1 iPSC Modelling of SNCA Mutation

A30P, G51D, E46K, A53T, and A53E are the 5 particular missense mutations linked to SNCA-related PD pathophysiology. The number of SNCA copies deletion is proportional to the severity of PD symptoms. Alterations in α -synuclein physiology can cause some cellular changes, most of which are caused by mitochondrial malfunction and oxidative stress and these are responsible for neuronal death as well as alterations of neuronal regeneration (Avazzadeh et al., 2021).

- A triplication of SNCA is carried by iPSC-derived DA neurons and this triplication functions in α -synuclein protein as a coding gene. When these cells are exposed to oxidative-stress inducers, it showed elevated α -synuclein mRNA and protein levels and causing cell death (Torrent et al., 2015).
- SNCA-triplication iPSC-derived neurons exposed to low concentrations of serotonin laser-induced ROS, resulting in more susceptibility to the generation of PTP (permeability transition holes). Moreover, In SNCA-A53T iPSC-derived neurons, upregulation of Mirol (essential protein in mitochondrial transport) , has been found to cause a delay in mitophagy (Ludtmann et al., 2018).
- SNCA-mutated neurons are more susceptible to mitochondrial toxin-induced oxidative stress. Moreover, the uttering levels of neuroprotective oxidative stress markers such as DNAJA1, HMOX2, UCHL1, and HSPB1 are considerably dysregulated. As a potential

response to oxidative stress, endogenous antioxidant mechanisms are boosted through increased activity of catalase or PGC-1. After being exposed to modest amounts of agrichemicals, SNCA-A53T iPSC-derived neurons produce more nitrous oxide (NO), which degrades microtubules (Byers et al., 2011).

- Physiological changes α-synuclein can cause cellular abnormalities. Most of which are mediated by the mitochondrial breakdown and oxidative stress. These alterations along with α -synuclein aggregation, not only cause neuronal death but also appear to hinder neuronal regeneration (Avazzadeh et al., 2021).
- Normal Mitochondrial Function in iPSC-Derived Neurons is disrupted by alteration in the SNCA gene. Mitochondrial dysfunction is prevalent in all SNCA-affected iPSC-derived neurons. It manifests itself in SNCA-triplication iPSC-derived neural progenitors (NPCs) as altered energy consumption and decreased ATP generation (Avazzadeh et al., 2021).

SNCA gene related phenotypes have been summarized in the part of 4.5.1.1(Table 5) -

4.5.1.1 iPSC-derived neuronal phenotypes with SNCA mutations

Table 5: *iPSC-derived neuronal phenotypes with SNCA mutations*

Number of groups	Types of mutation	Cell types	Phenotype observed	Reference
1 PD line vs. 1 control line	Triplication Autosomal dominant	iPSC-derived Dopamine neuron (DA neurons)	-Increased aggregation of α -synuclein -Oxidative stress is on the rise.	(Byers et al., 2011)
1 PD line vs. 1 control line	Triplication Autosomal - dominant	Cortical neurons generated from iPSC	-Increased α -synuclein -Oxidative stress is on the rise.	(Attached et al., 2005)
1 PD line vs. 1 isogenic control line	Autosomal dominant A53T	iPSC-derived A9 DA neurons (Dopamine neuron)	-Mitochondrial dysfunction. -Oxidative stress is on the rise. -Increased apoptosis and cell death -Impaired neuronal maturation	(Ambasudhan et al., 2013)
1 PD line vs. 1 control line vs. 1 isogenic control line	Triplication Autosomal - dominant	iPSC-derived cortical neurons	-Higher level of α - synuclein -Mitochondrial dysfunction	(Ludtmann et al., 2018)
2 PD line vs. 1 control line	Autosomal dominant A53T	DA, GABAergic, and glutaminergic neurons generated from iPSCs	-Synaptic activity changes -Increase the aggregation of -synuclein -Neuronal Developmental Defects	(Kouroupi et al., 2017)

4.5.2 iPSC modelling of LRRK2 mutations:

LRRK2 mutations have shown to be the most common genetic defects that lead to familial PD and some of the clinical features of this form of PD overlap idiopathic PD (Torrent et al., 2015). The

LRRK2 G2019S, I2020T, Y1699C, and R1441C are all missense mutations of LRRK2. Among this G2019S is the most common genetic determinant of familial PD (Avazzadeh et al., 2021).

- **In iPSC-Derived Neurons, LRRK2 mutation promotes α -synuclein aggregate formation:**

  - The G2019S mutation causes the LRRK2 kinase domain to become more hyperactive. The G2019S LRRK2-PD iPSC model closely mimics the classic PD pathophysiology, including the accumulation of α -synuclein, accelerated neuronal death, an increase in genes involved in oxidative stress, and increased vulnerability to hydrogen peroxide, which is demonstrated by caspase-3 activation (Torrent et al., 2015).
  - In addition, LRRK2 mutations in iPSC-derived astrocytes cause higher α-synuclein aggregation, which leads to cell death. Moreover, studies have demonstrated that endocytosis is also disrupted in iPSC ventral midbrain neurons as a result of G2019S mutations (Pan et al., 2017 ; Avazzadeh et al., 2021).

- **In iPSC-Derived Neurons, LRRK2 mutation is responsible for Mitochondrial Dysfunction:**

  - Defective mitochondria assemble in the axons of LRRK2 mutant iPSC-derived DA neurons for the disturbance in mitophagy. In addition, compared to control neurons, LRRK2 R1441C iPSC-derived neurons have a higher level of mitochondrial DNA. Moreover, in LRRK2 G2019S human neuroepithelial stem cells (NESCs), mitochondrial dysfunction has been seen that implying a faulty mechanism at the primary stage in neuronal development (Walter et al.,2019). Furthermore, nicotinamide adenine dinucleotide (NAD+) is protective towards neurons. So lack of NAD+ in LRRK2-mutated

iPSC generated neurons causes abnormalities in mitochondrial biogenesis and energetics (Schwab et al., 2017).

LRRK2 gene related phenotypes have been summarized in the part of 4.5.2.1(Table 6) –

4.5.2.1 iPSC-derived neuronal phenotypes with LRRK2 mutations

Table 6: *iPSC-derived neuronal phenotypes with LRRK2 mutations*

Number of groups	Types of mutation	Cell types	Phenotype observed	Reference
2 PD lines vs. 4 control lines	G2019S	iPSC-derived Dopamine neuron (DA neurons)	-Neuronal development is hampered -Reduced phosphorylation of α - synuclein	(Reinhardt et al., 2013)
12 PD lines vs. 3 control lines	G2019S R1441C	iPSC-derived Dopamine neuron (DA neurons)	-Aggregation of Tau and α - synuclein is increased. Neuronal development is hampered	(Sanders et al., 2014)
3 PD and 2 isogenic KO lines vs. 4 controls and isogenic lines	G2019S	Neural stem cells generated from iPSCs	-Mitochondrial dysfunction. -Dysfunction of the dopaminergic system -The rate of cell death is higher.	(Walter et al., 2019)
2 PD lines vs. 3 control lines vs. 1 isogenic control line	G2019S	iPSC-derived Dopamine neuron (DA neurons)	-The rate of neuronal degeneration is higher	(di Domenico et al., 2019)
4 PD lines vs. 4 control lines	G2019S	iPSC-derived Dopamine neuron (DA neurons)	-Increase the aggregation of - α synuclein - The rate of neuronal degeneration is higher -A boost in autophagy	(Fernandes et al., 2016)

4.5.3 iPSC modelling of PARK 2 mutations:

Parkin mutations are linked to 50% of all PD cases in people below the age of 45. This mutations range from single nucleotide deletions to massive deletions spanning hundreds of nucleotides (Avazzadeh et al., 2021).

- **Mitochondrial Dysfunction and Oxidative Stress in iPSC-Derived Neurons Are Caused by PARKIN Mutations:**

  - Parkin iPSC-derived DA neurons have included some features which are responsible for developing PD. Those are mitochondrial malfunction, aberrant shape, and impaired mitochondrial homeostasis. The inner mitochondrial membrane (IMM) of these neurons has inflated cristae and a condensed matrix, with aberrant mitochondrial morphology directly influencing function and an increase in the number of expanded mitochondria (Imaizumi et al., 2012 ; Bogetofte et al., 2019).
  - iPSC models with PARK2 mutations demonstrated an increase in oxidative stress. Studies showed that iPSC from patients with PARK2 mutations increased monoamine oxidase transcription, spontaneous dopamine release, and dramatically reduced dopamine absorption, increasing vulnerability to reactive oxygen species (H. Jiang et al., 2012; Torrent et al., 2015).

- Moreover, Monoamine Oxidases (MAO) A and B are limited under normal physiological conditions. But in parkin mutant iPSC - derived DA neurons, MAO-A and B levels were found to be considerably higher and resulting increase in dopamine-induced oxidative stress (H. Jiang et al., 2006).

- Oxidative stress-inducing environment can be resulting from the lower level of dopamine absorption and a higher level of dopamine release that can be seen in iPSC-derived DA neurons with parkin mutations (H. Jiang et al., 2012).

PARK2 gene related phenotypes have been summarized in the part of 4.5.3.1(Table 7) -

4.5.3.1 iPSC-derived neuronal phenotypes with PARK2 mutations

Table 7: *iPSC-derived neuronal phenotypes with PARK2 mutations*

Number of groups	Types of mutation	Cell types	Phenotype observed	Reference
2 PD patient lines vs. 2 control lines	Exon 2–4 or Exon 6–7 deletions	iPSC-derived Dopamine neuron (DA neurons)	-Oxidative stress is on the rise. -Mitochondrial dysfunction -Increase the aggregation of α -synuclein	(Imaizumi et al., 2012)
2 PD lines vs. 2 control lines	Exon 4 deletion	iPSC-derived Dopamine neuron (DA neurons)	-Dysregulation of dopamine -Oxidative stress is on the rise.	(H. Jiang et al., 2006)
1 PD line vs. 1 control line	Exon 5 deletion	iPSC-derived Dopamine neuron (DA neurons)	Increase the aggregation of α -synuclein Antioxidant proteins level is decreased.	(Chang et al., 2016)
2 Isogenic mutated PD lines vs. 1 control line	Exon 2 deletion	iPSC-derived Dopamine neuron (DA neurons)	Mitochondrial dysfunction	(Bogetofte et al., 2019)
3 PD patient lines vs. 3 control lines	Exon 3–5 or R42P deletions	iPSC-derived Dopamine neuron (DA neurons)	- Dysregulation of dopamine	(Zhong et al., 2017)

4.5.4 iPSC modelling of PINK1 mutations:

PINK1 has been demonstrated to be required for mitochondrial function, mitophagy, and protein misfolding. Commonly, (PINK1) mutations resulting in an autosomal recessive familial form of PD (McWilliams & Muqit, 2017).

- **In iPSC-Derived Neurons, PINK1 Mutations Cause Mitochondrial Dysfunction and Increase Reactive Oxygen Species Generation-**
  - In PD patients, pathogenic mtDNA mutations are common, leading to mitochondrial malfunction (Seibler et al., 2011). Mitochondrial damage stimulates PINK1 kinase activity under normal physiological conditions, and activates PINK1 phosphorylates ubiquitin at a conserved Ser65 position. Moreover, Parkin works along with PINK1 to phosphorylate damaged mitochondria, preparing them for lysosomal and proteasomal destruction (Avazzadeh et al., 2021).
  - Furthermore, Due to ubiquitination pathway failure, iPSC-derived neurons with PINK1 mutations have considerably lower levels of endogenous parkin and are unable to promote mitophagy (Rakovic et al., 2013).
- Mitochondrial dysfunction and oxidative stress can result the cell damage and these are caused by the generation of reactive oxygen species (ROS). In iPSC-derived DA neurons, PINK1 loss causes higher basal ROS in both the mitochondria and the cytoplasm, leading to enhanced oxidative stress (Wood-Kaczmar et al., 2008).

PINK1 gene related phenotypes have been summarized in the part of 4.5.4.1(Table 8) –

4.5.4.1 iPSC-derived neuronal phenotypes with PINK1 mutations

Table 8: *iPSC-derived neuronal phenotypes with PINK1 mutations*

Number of Groups	Types of mutation	Cell types	Phenotype observed	Reference
1 PD line vs. 1 control line	V170G	iPSC-derived Dopamine neuron (DA neurons)	-Mitophagy dysfunction	(Rakovic et al., 2013)
7 PD lines vs. 5 control lines	Exon 4 or 7 deletion	iPSC-derived Dopamine neuron (DA neurons)	-LRKK2 level dysregulation -Mitochondrial dysfunction.	(Azkona et al., 2018)

4.5.5 iPSC modelling of GBA and DJ-1 mutations:

4.5.5.1 GBA Mutations:

The lysosomal glucocerebrosidase enzyme (GCase) is made by a mutated GBA gene found on chromosome 1, hydrolyzes glucosylceramide (GlcCer) into ceramide and glucose (Straniero et al., 2017).

- **Mitochondrial activation is disrupted by GBA Mutations in iPSC-Derived Neurons:**

Mitochondrial morphology and function have been shown to be disrupt in all pN370S, pL444P GBA iPSC-derived neurons, resulting in abnormalities in mitochondrial dynamics (Schöndorf et al., 2018).

- **ES Stress in iPSC-Derived Neurons Is Caused by GBA Mutations:**

Increased ER stress, α-synuclein aggregation (evidenced by autophagic/lysosome pathway abnormalities) lysosomal malfunction, and are all consequences of GBA mutations of a neuron. Studies using iPSC-derived DA neurons with GBA mutations have demonstrated the buildup of misfolded GBA in the ER culminating in ER stress and eventually UPR activation, which is a mechanism to cope with ER stress (Fernandes et al., 2016).

4.5.5.2 DJ1 Mutations:

- Dj-1 is a 189-amino-acid protein that forms homodimers. It's function is anti-oxidant activity and preventing α -synuclein aggregation (Wilson, 2011).
- Increased dopamine oxidation was discovered in iPSC-derived DA neurons, resulting in mitochondrial oxidative stress and glucocerebrosidase inactivation. Moreover, this inactivation impairs lysosomal degradation processes, that can increase in the quantity of α -synuclein (Burbulla et al., 2017).

Chapter 5

5.1 Therapeutic potential of iPSC Disease modelling and Cell therapy

The therapeutic potential of iPSC disease modelling and cell therapy in PD is shown in Figure 11-

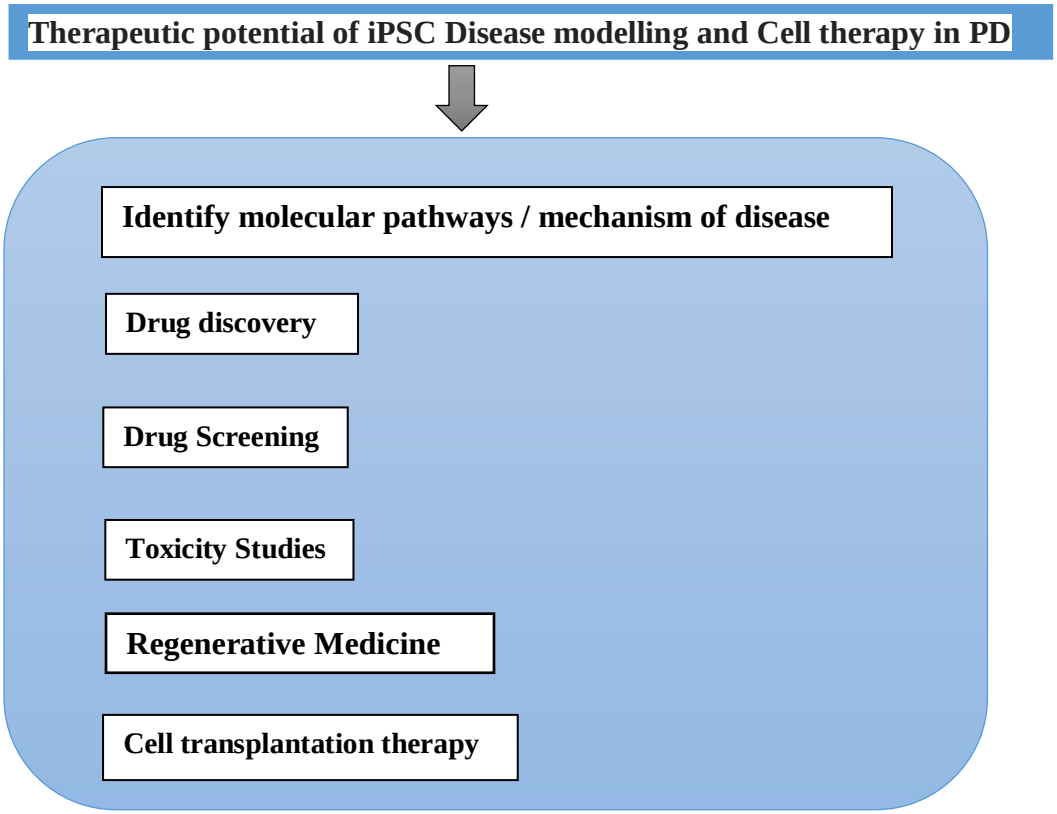

Figure 11: Therapeutic potential of iPSC Disease modelling and Cell therapy.

5.1.1 Cell transplantation therapy

iPSCs are stem cells obtained from skin or blood cells that have been reprogrammed into an embryonic-like pluripotent state, allowing for the expansion of an infinite number of different types of human cells for therapeutic applications. In 2010, PD patient iPSCs were differentiated to functional DA neurons. After that, in 2015 researchers successfully transplanted human iPSC in mice (Stoddard-Bennett & Pera, 2020). Finally, in 2018, under the supervision of Takahashi and his team in Japan was first to introduce a human clinical trial of iPSC-generated DAn transplantation to treat PD at Kyoto University Hospital. A total of seven patients participated in

this trial and they were observed for 2 years (Stoddard-Bennett & Reijo Pera, 2019). Cell transplantation therapy is a significant step in the therapeutic application of hiPSCs in PD that shows actual DA neurons can be produced from hiPSC (Zeng & Couture, 2013).

First, fibroblasts from a patient with familial PD are collected. To create a mutant iPSC line, researchers expressed important reprogramming transcription factors. The major mutation is repaired by employing ZNF/TALEN or CRISPR/Cas9 technologies. In xeno-free circumstances, the line is then differentiated into mature DA neurons. After stringent quality control measures, the differentiated cells can be used in cell therapy (Stoddard-Bennett & Reijo Pera, 2019). Although cell transplantation therapy offers a "personalized medicine" approach to treat neurodegenerative disease such as PD, rigorous quality control steps must be employed to create clinical-grade iPSCs. Before transplantation, cultured cells for replacement therapy must be tested for their safety and efficacy which include checking for the presence of undifferentiated cells, irrelevant or contaminating cell types, oncogenic mutations, epigenetic memory, and genetic instability such as chromosomal abnormalities. Furthermore, microbiological sterility, viability must also be well-defined before administering these therapies into patients (Sullivan et al., 2018).

The diagram below (Figure 12) illustrates the overall process of cell transplantation therapy by using iPSC.

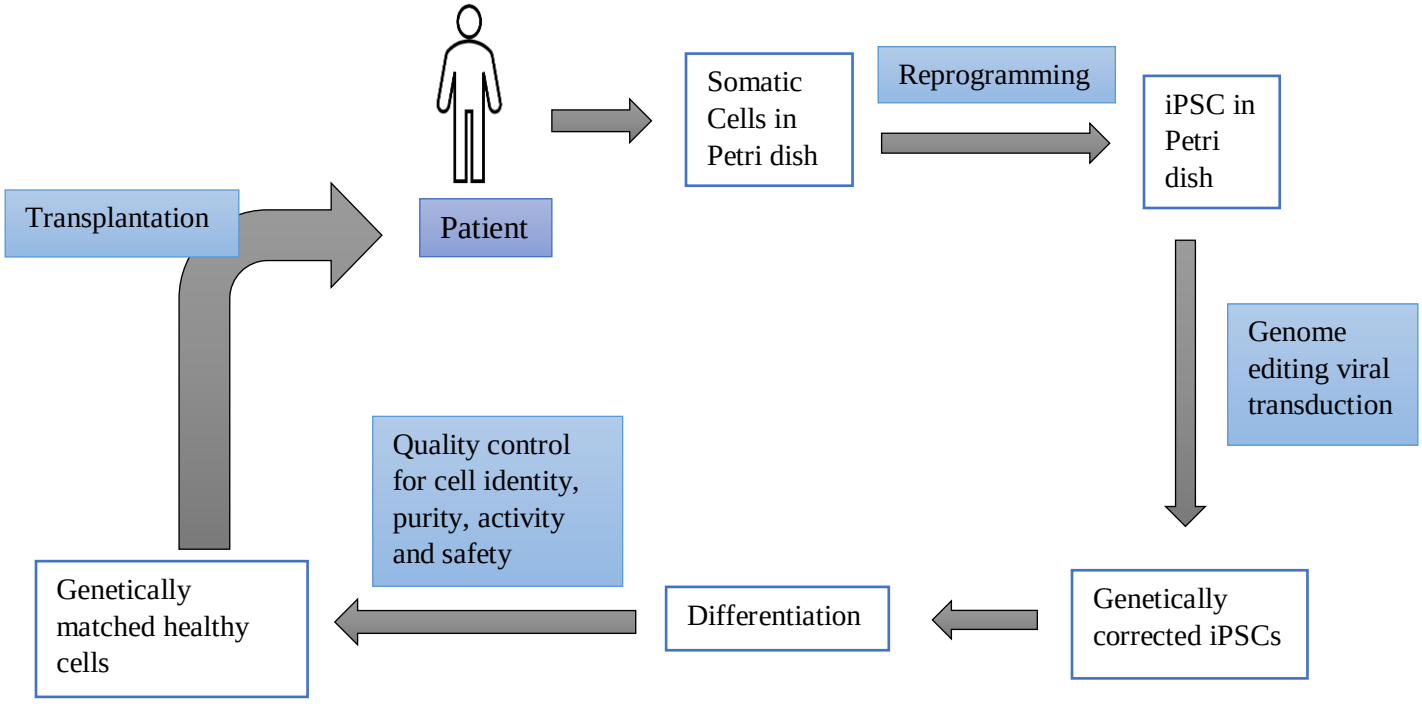

Figure 12: First, affected patients' somatic cells are harvested and cultivated. Then somatic cells from the patient are converted into iPSCs. In the next stage, the patient-derived iPSCs are genetically corrected by employing genome editing technology. After that, the corrected iPSCs are differentiated into appropriate cell types so that they can be used as healthy donor cells that are genetically matched. In the next step, quality control tests can be done for cell identification, purity, activity, and safety. Finally, patients acquire the cell treatment after receiving genetically matched healthy cells [Adapted from (Shi et al., 2017)].

5.1.2 Disease modeling and drug screening

For disease modeling, a variety of animal models have been used, including rats, mice, monkeys, dogs, and primates. In case of disease modeling of PD, the use of animal models is limited due to species differences. Animal models of PD have provided invaluable evidence regarding the pathophysiology of the disease. However, the main drawback is the limited ability of these animal models to mimic the full-spectrum of the human disease because the genetic makeup of different

species varies evidence (Singh et al., 2015). Many patient-specific iPSC lines have been created and are being used to model disease, especially for rare, monogenic disorders. Disease phenotypes could be reproduced using patient-derived iPSCs, also could be used for drug screening and repurposing (Ohnuki & Takahashi, 2015). Moreover, iPSC disease models can serve as in vitro models and can be compared with in vivo animal models to obtain a more in-depth understanding of the disease. More than 1,000 compounds have been evaluated using iPSC-based drug screening for a variety of diseases (Shi et al., 2017). Here, Figure 13 shows some advantages of iPSC technology as a disease.

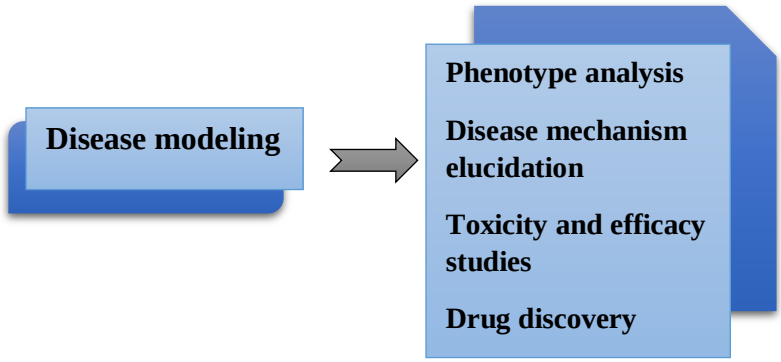

Figure 13: Advantages of iPSC as a disease model.

5.1.3 Regenerative Medicines

In regenerative medicine, damaged or degenerated tissues are repaired by generating them in labs using iPSCs and then transplanting them to the site of injury. iPSC-based gene therapy has been widely used for the treatment of degenerative diseases as well as benefits for improving the function of degenerated organs (Singh et al., 2015).In 2014, Japan launched the first clinical trial to treat age-related macular degeneration (AMD), and based on these trial now Japan is working

on the commercialization of iPSC-based regenerative medicines as long as they are proved to be safe (Doss & Sachinidis, 2019).

5.1.4 Toxicological Screening

Toxicological Screening can be done by using iPSC derived neurons. A chemical substance may be hazardous to one animal but not to another and animal models are inefficient testing models for drug toxicity. That is why, before being approved, a newly discovered drug or therapy must be tested on human cells. Similarly, iPSC-derived neurons can be used to validate potential targets discovered through screening. (Avazzadeh et al., 2021).

5.2 Limitations of Current iPSC Studies

- Despite the numerous benefits of iPSCs, there are a number of drawbacks that limit their use in various experimental settings. Several aspects of PD pathology have yet to be adequately modeled by current iPSC studies. For example, LB development has not been detected by this model. Although neuroinflammation has been implicated in the pathogenesis of PD, very few studies have been able to model this particular disease mechanism in iPSC derived microglia, which are the innate immune cells of the brain that mediate neuroinflammation. (Sison et al., 2018).
- Immune rejection might be a concern in case of allogeneic iPSC transplants, however studies have found that fully differentiated patient-derived iPSCs as well as autologous iPSCs do not pose this problem (Doss & Sachinidis, 2019).
- There is another concerning limitation that has emerged from recent studies. PD is a neurodegenerative disorder but iPSCs are reprogrammed cells and most likely do not retain senescent features or aging markers of the cell. So, modeling late onset PD is difficult using iPSCs. (Miller et al., 2013).

- The quality attributes required for iPSC transplantation have not been yet properly defined. A well-designed rigorous quality control protocol is imperative to maintain the safety and efficacy of cell transplantation therapy. A further limitation is that the quality control procedures for production of clinical-grade iPS cells are very complex expensive (Doss & Sachinidis, 2019).
- Certain diseases have been linked to changes in the expression of basal reprogramming factors. Studies have found that Oct4 overexpression may result in epithelial cell dysplasia. Mucinous colon carcinoma has been linked to an abnormal expression of Sox2. Klf4 is involved in the development of breast tumors. cMyc is involved in the development of approximately 70% of human cancers (Singh et al., 2015). Moreover, iPS cells have tumorigenic potential They can divide indefinitely and may become cancerous. The development of both teratomas and malignant tumors can be possible and increasing the risk of tumorigenicity if transplanted cells are indefinitely divided (Doss & Sachinidis, 2019).
- Another limitation regarding iPSCs is genetic instability. Because iPSCs are kept in -vitro culture for prolonged durations, they can accrue chromosomal defects and copy number variation and lose their heterozygosity (Doss & Sachinidis, 2019).
- CRISPR-cas-9 is a great tool but it has several off-target effects which are undesirable and may introduce confounding factors to iPSC-based experiments (Doss & Sachinidis, 2019).

5.3 Challenges and Future Direction

To create better disease models and gain a better understanding of pathogenic mechanisms ,3D organoids have been introduced that allow researchers to study the pathogenesis of neurodegenerative diseases in conditions that mimic the human brain microenvironment. 3D cultures enable cell-cell interactions in a three-dimensional space, providing a better insight into disease pathology at the organ level. Such disease models could not be generated using traditional 2D cultures (Antonov & Novosadova, 2021).

Undifferentiated iPSCs are responsible for enhancing the risk of potential tumorigenicity. In order to eliminate this risk, various differentiation protocols and purification methods have been developed. These protocols include flow cytometry-magnetic bead-based sorting that help to identify undifferentiated cell populations and small chemical molecules that induce undifferentiated cells to die (Doss & Sachinidis, 2019).

To advance the field of cell transplantation therapy, current cell transplantation clinical trials, as well as, the development of drug screening and disease repositioning methods should be prioritized to refine and speed up the development of iPSC-based therapy (Ohnuki & Takahashi, 2015).

Last but not the least, in the future to improve and develop this stem cell therapy, advanced technologies for example, next- generation sequencing, gene-editing tools, artificial intelligence n and microRNA switches, etc., can be integrated with iPSCs. So that the process of disease modeling and cell therapy goes can be advanced one step further (Doss & Sachinidis, 2019).

Chapter 6

Conclusion

Parkinson's disease is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons along with the accumulation of cytoplasmic aggregates known as Lewy bodies. Pathogenic α-synuclein protein aggregates, mitochondrial dysfunction, oxidative stress and neuroinflammation have all been commonly implicated as etiological factors of PD. For the management of this neurodegenerative disease, it is necessary to be aware of the disease symptoms, treatment options, and the disease's long-term progression (Radhakrishnan & Goyal, 2018).

In this new era, human PD-derived iPSCs are an advanced technology for effective understanding of PD pathology, revealing disease phenotypes, identifying gene-linked PD biomarkers, and analyzing the main framework for DA degeneration and loss which sets up a new window for early diagnosis and therapeutic options for the disease. Moreover, iPSC technology offers a unique opportunity to learn not only about the pathology of PD but also provides a platform to develop personalized cell-based therapies through a dish-to-clinic approach. The use of iPSC-based disease modelling is still a work in progress to precisely imitate PD phenotypes in humans. Advanced 3D organoid systems and CRISPR genome editing technologies have highly strengthened iPSC research and have set the stage for the discovery of innovative therapeutic approaches (Xiao et al., 2016). However, there are factors that limit their use both in disease modeling and routine clinical use. Therefore, a lot of improvements and refinements are yet to be made to this excellent technology to enhance its utility further. Currently, research is underway to overcome these shortcomings and the scientific community is optimistic that iPSCs would revolutionize the treatment of debilitating neurodegenerative disorders like PD in the near future.

Bibliography

Ambasudhan, R., Ryan, S. D., Dolatabadi, N., Chan, S. F., Zhang, X., Akhtar, M. W., Parker, J., Soldner, F., Sunico, C. R., Nagar, S., Talantova, M., Lee, B., Lopez, K., Nutter, A., Shan, B., Molokanova, E., Zhang, Y., Han, X., Nakamura, T., … Lipton, S. A. (2013). Isogenic Human iPSC Parkinson's Model Shows Nitrosative Stress-Induced Dysfunction in MEF2-PGC1α Transcription. *Cell*, *155*(6), 1351. https://doi.org/10.1016/j.cell.2013.11.009

Antonov, S. A., & Novosadova, E. V. (2021). Current state-of-the-art and unresolved problems in using human induced pluripotent stem cell-derived dopamine neurons for parkinson's disease drug development. *International Journal of Molecular Sciences*, *22*(7). https://doi.org/10.3390/ijms22073381

Armstrong, M. J., & Okun, M. S. (2020). Diagnosis and Treatment of Parkinson Disease: A Review. *JAMA - Journal of the American Medical Association*, *323*(6), 548–560. https://doi.org/10.1001/jama.2019.22360

Attached, W., Summary, P., Search, Y., Identifier, C., & Text, C. (2005). *Page 1 of 54. 14*, 1–54.

Avazzadeh, S., Baena, J. M., Keighron, C., Feller-Sanchez, Y., & Quinlan, L. R. (2021). Modelling parkinson's disease: Ipscs towards better understanding of human pathology. *Brain Sciences*, *11*(3). https://doi.org/10.3390/brainsci11030373

Azkona, G., Maturana, R. L. de, Del Rio, P., Sousa, A., Vazquez, N., Zubiarrain, A., Jimenez-Blasco, D., Bolaños, J. P., Morales, B., Auburger, G., Arbelo, J. M., & Sánchez-Pernaute, R. (2018). LRRK2 expression is deregulated in fibroblasts and neurons from parkinson patients with mutations in PINK1. *Molecular Neurobiology*, *55*(1), 506–516. https://doi.org/10.1007/s12035-016-0303-7

Bandres-Ciga, S., Diez-Fairen, M., Kim, J. J., & Singleton, A. B. (2020). Genetics of Parkinson's disease: An introspection of its journey towards precision medicine. *Neurobiology of Disease*, *137*, 104782. https://doi.org/10.1016/j.nbd.2020.104782

Bartels, T., Choi, J. G., & Selkoe, D. J. (2011). α-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation. *Nature*, *477*(7362), 107–111.

https://doi.org/10.1038/nature10324

Beevers, J. E., Caffrey, T. M., & Wade-Martins, R. (2013). Induced pluripotent stem cell (iPSC)-derived dopaminergic models of Parkinson's disease. *Biochemical Society Transactions*, *41*(6), 1503–1508. https://doi.org/10.1042/BST20130194

Bogetofte, H., Jensen, P., Ryding, M., Schmidt, S. I., Okarmus, J., Ritter, L., Worm, C. S., Hohnholt, M. C., Azevedo, C., Roybon, L., Bak, L. K., Waagepetersen, H., Ryan, B. J., Wade-Martins, R., Larsen, M. R., & Meyer, M. (2019). PARK2 mutation causes metabolic disturbances and impaired survival of human iPSC-derived neurons. *Frontiers in Cellular Neuroscience*, *13*(July), 1–14. https://doi.org/10.3389/fncel.2019.00297

Burbulla, L. F., Song, P., Mazzulli, J. R., Zampese, E., Wong, Y. C., Jeon, S., Santos, D. P., Blanz, J., Obermaier, C. D., Strojny, C., Savas, J. N., Kiskinis, E., Zhuang, X., Krüger, R., Surmeier, D. J., & Krainc, D. (2017). Dopamine oxidation mediates mitochondrial and lysosomal dysfunction in Parkinson's disease. *Science*, *357*(6357), 1255–1261. https://doi.org/10.1126/science.aam9080

Byers, B., Cord, B., Nguyen, H. N., Schüle, B., Fenno, L., Lee, P. C., Deisseroth, K., Langston, J. W., Pera, R. R., & Palmer, T. D. (2011). SNCA triplication parkinson's patient's iPSC-Derived DA neurons accumulate α-Synuclein and are susceptible to oxidative stress. *PLoS ONE*, *6*(11). https://doi.org/10.1371/journal.pone.0026159

Chang, K. H., Lee-Chen, G. J., Wu, Y. R., Chen, Y. J., Lin, J. L., Li, M., Chen, I. C., Lo, Y. S., Wu, H. C., & Chen, C. M. (2016). Impairment of proteasome and anti-oxidative pathways in the induced pluripotent stem cell model for sporadic Parkinson's disease. *Parkinsonism and Related Disorders*, *24*, 81–88. https://doi.org/10.1016/j.parkreldis.2016.01.001

De Wert, G., & Mummery, C. (2003). Human embryonic stem cells: Research, ethics and policy. *Human Reproduction*, *18*(4), 672–682. https://doi.org/10.1093/humrep/deg143

di Domenico, A., Carola, G., Calatayud, C., Pons-Espinal, M., Muñoz, J. P., Richaud-Patin, Y., Fernandez-Carasa, I., Gut, M., Faella, A., Parameswaran, J., Soriano, J., Ferrer, I., Tolosa, E., Zorzano, A., Cuervo, A. M., Raya, A., & Consiglio, A. (2019). Patient-Specific iPSC-Derived Astrocytes Contribute to Non-Cell-Autonomous Neurodegeneration in Parkinson's Disease.

Stem Cell Reports, *12*(2), 213–229. https://doi.org/10.1016/j.stemcr.2018.12.011

Dickson, D. W. (2012). Parkinson's disease and parkinsonism: Neuropathology. *Cold Spring Harbor Perspectives in Medicine*, *2*(8). https://doi.org/10.1101/cshperspect.a009258

Domingo, A., & Klein, C. (2018). Genetics of Parkinson disease. In *Handbook of Clinical Neurology* (1st ed., Vol. 147). Elsevier B.V. https://doi.org/10.1016/B978-0-444-63233-3.00014-2

Doss, M. X., & Sachinidis, A. (2019). Current Challenges of iPSC-Based Disease Modeling and Therapeutic Implications. *Cells*, *8*(5), 403. https://doi.org/10.3390/CELLS8050403

Fernandes, H. J. R., Hartfield, E. M., Christian, H. C., Emmanoulidou, E., Zheng, Y., Booth, H., Bogetofte, H., Lang, C., Ryan, B. J., Sardi, S. P., Badger, J., Vowles, J., Evetts, S., Tofaris, G. K., Vekrellis, K., Talbot, K., Hu, M. T., James, W., Cowley, S. A., & Wade-Martins, R. (2016). ER Stress and Autophagic Perturbations Lead to Elevated Extracellular α-Synuclein in GBA-N370S Parkinson's iPSC-Derived Dopamine Neurons. *Stem Cell Reports*, *6*(3), 342–356. https://doi.org/10.1016/j.stemcr.2016.01.013

Giguère, N., Nanni, S. B., & Trudeau, L. E. (2018). On cell loss and selective vulnerability of neuronal populations in Parkinson's disease. *Frontiers in Neurology*, *9*(JUN). https://doi.org/10.3389/fneur.2018.00455

Hattori, N., & Mizuno, Y. (2002). Mitochondrial dysfunction in Parkinson's disease. *Nippon Rinsho. Japanese Journal of Clinical Medicine*, *60 Suppl 4*(2), 406–411. https://doi.org/10.5607/en.2015.24.2.103

Hayes, M. T. (2019). Parkinson's Disease and Parkinsonism. *American Journal of Medicine*, *132*(7), 802–807. https://doi.org/10.1016/j.amjmed.2019.03.001

Hirsch, E. C., & Hunot, S. (2009). Neuroinflammation in Parkinson's disease: a target for neuroprotection? *The Lancet Neurology*, *8*(4), 382–397. https://doi.org/10.1016/S1474-4422(09)70062-6

Ho, B. X., Pek, N. M. Q., & Soh, B. S. (2018). Disease modeling using 3D organoids derived from

human induced pluripotent stem cells. *International Journal of Molecular Sciences*, *19*(4). https://doi.org/10.3390/ijms19040936

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., … Okano, H. (2012). Mitochondrial dysfunction associated with increased oxidative stress and α-synuclein accumulation in PARK2 iPSC-derived neurons and postmortem brain tissue. *Molecular Brain*, *5*(1), 1–13. https://doi.org/10.1186/1756-6606-5-35

Jankovic, J., & Tan, E. K. (2020). Parkinson's disease: Etiopathogenesis and treatment. *Journal of Neurology, Neurosurgery and Psychiatry*, *91*(8), 795–808. https://doi.org/10.1136/jnnp-2019-322338

Jiang, H., Jiang, Q., Liu, W., & Feng, J. (2006). Parkin suppresses the expression of monoamine oxidases. *Journal of Biological Chemistry*, *281*(13), 8591–8599. https://doi.org/10.1074/jbc.M510926200

Jiang, H., Ren, Y., Yuen, E. Y., 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. *Nature Communications*, *3*, 668–669. https://doi.org/10.1038/ncomms1669

Jiang, Z., Han, Y., & Cao, X. (2014). Induced pluripotent stem cell (iPSCs) and their application in immunotherapy. *Cellular and Molecular Immunology*, *December 2013*, 17–24. https://doi.org/10.1038/cmi.2013.62

Kouli, A., Torsney, K. M., & Kuan, W.-L. (2018). Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis. *Parkinson's Disease: Pathogenesis and Clinical Aspects, December*, 3–26. https://doi.org/10.15586/codonpublications.parkinsonsdisease.2018.ch1

Kouroupi, G., Taoufik, E., Vlachos, I. S., Tsioras, K., Antoniou, N., Papastefanaki, F., Chroni-Tzartou, D., Wrasidlo, W., Bohl, D., Stellas, D., Politis, P. K., Vekrellis, K., Papadimitriou, D., Stefanis, L., Bregestovski, P., Hatzigeorgiou, A. G., Masliah, E., & Matsas, R. (2017). Defective synaptic connectivity and axonal neuropathology in a human iPSC-based model of

familial Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(18), E3679–E3688. https://doi.org/10.1073/pnas.1617259114

Kumar, K. R., Djarmati-Westenberger, A., & Grünewald, A. (2011). Genetics of Parkinson's disease. *Seminars in Neurology*, *31*(5), 433–440. https://doi.org/10.1055/S-0031-1299782

Lee, C. T., Bendriem, R. M., Wu, W. W., & Shen, R. F. (2017). 3D brain Organoids derived from pluripotent stem cells: Promising experimental models for brain development and neurodegenerative disorders Julie Y.H. Chan. *Journal of Biomedical Science*, *24*(1), 1–12. https://doi.org/10.1186/s12929-017-0362-8

Liu, G., David, B. T., Trawczynski, M., & Fessler, R. G. (2020). Advances in Pluripotent Stem Cells: History, Mechanisms, Technologies, and Applications. *Stem Cell Reviews and Reports*, *16*(1), 3–32. https://doi.org/10.1007/s12015-019-09935-x

Ludtmann, M. H. R., Angelova, P. R., Horrocks, M. H., Choi, M. L., Rodrigues, M., Baev, A. Y., Berezhnov, A. V., Yao, Z., Little, D., Banushi, B., Al-Menhali, A. S., Ranasinghe, R. T., Whiten, D. R., Yapom, R., Dolt, K. S., Devine, M. J., Gissen, P., Kunath, T., Jaganjac, M., … Gandhi, S. (2018). α-synuclein oligomers interact with ATP synthase and open the permeability transition pore in Parkinson's disease. *Nature Communications*, *9*(1). https://doi.org/10.1038/s41467-018-04422-2

McWilliams, T. G., & Muqit, M. M. (2017). PINK1 and Parkin: emerging themes in mitochondrial homeostasis. *Current Opinion in Cell Biology*, *45*, 83–91. https://doi.org/10.1016/j.ceb.2017.03.013

Miller, J. D., Ganat, Y. M., Kishinevsky, S., Bowman, R. L., Liu, B., Tu, E. Y., Mandal, P. K., Vera, E., Shim, J. W., Kriks, S., Taldone, T., Fusaki, N., Tomishima, M. J., Krainc, D., Milner, T. A., Rossi, D. J., & Studer, L. (2013). Human iPSC-based modeling of late-onset disease via progerin-induced aging. *Cell Stem Cell*, *13*(6), 691–705. https://doi.org/10.1016/j.stem.2013.11.006

Nalls, M. A., Blauwendraat, C., Vallerga, C. L., Heilbron, K., Bandres-Ciga, S., Chang, D., Tan, M., Kia, D. A., Noyce, A. J., Xue, A., Bras, J., Young, E., von Coelln, R., Simón-Sánchez, J., Schulte, C., Sharma, M., Krohn, L., Pihlstrøm, L., Siitonen, A., … Zhang, F. (2019).

Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. *The Lancet Neurology*, *18*(12), 1091–1102. https://doi.org/10.1016/S1474-4422(19)30320-5

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

Pan, P. Y., Li, X., Wang, J., Powell, J., Wang, Q., Zhang, Y., Chen, Z., Wicinski, B., Hof, P., Ryan, T. A., & Yue, Z. (2017). Parkinson's disease-associated LRRK2 hyperactive kinase mutant disrupts synaptic vesicle trafficking in ventral midbrain neurons. *Journal of Neuroscience*, *37*(47), 11366–11376. https://doi.org/10.1523/JNEUROSCI.0964-17.2017

Poewe, W., Seppi, K., Tanner, C., Halliday, G. M., Brundin, P., Volkmann, J., Schrag, A. E., & Lang, A. E. (2017). Fibromyalgia. Nature Reviews Disease Primers. *Nature Reviews Disease Primers*, *3*(17014), 1–53. http://sci-hub.tw/

Radhakrishnan, D. M., & Goyal, V. (2018). Parkinson's disease: A review. *Neurology India*, *66*(7), S26–S35. https://doi.org/10.4103/0028-3886.226451

Rakovic, A., Shurkewitsch, K., Seibler, P., Grünewald, 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. *Journal of Biological Chemistry*, *288*(4), 2223–2237. https://doi.org/10.1074/jbc.M112.391680

Reich, S. G., & Savitt, J. M. (2019). Parkinson's Disease. *Medical Clinics of North America*, *103*(2), 337–350. https://doi.org/10.1016/j.mcna.2018.10.014

Reinhardt, P., Schmid, B., Burbulla, L. F., Schöndorf, D. C., Wagner, L., Glatza, M., Höing, S., Hargus, G., Heck, S. A., Dhingra, A., Wu, G., Müller, S., Brockmann, K., Kluba, T., Maisel, M., Krüger, R., Berg, D., Tsytsyura, Y., Thiel, C. S., … 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. https://doi.org/10.1016/j.stem.2013.01.008

Roqué, P. J., & Costa, L. G. (2017). Co-Culture of Neurons and Microglia. *Current Protocols in Toxicology*, *74*(1), 11.24.1-11.24.17. https://doi.org/10.1002/cptx.32

Rowe, R. G., & Daley, G. Q. (2019). Induced pluripotent stem cells in disease modelling and drug discovery. *Nature Reviews Genetics*, *20*(7), 377–388. https://doi.org/10.1038/s41576-019-0100-z

Samii, A., Nutt, J. G., & Ransom, B. R. (2004). Parkinson's disease. *The Lancet*, *363*(9423), 1783–1793. https://doi.org/10.1016/S0140-6736(04)16305-8

Sanders, L. H., Laganière, J., Cooper, O., Mak, S. K., Vu, B. J., Huang, Y. A., Paschon, D. E., Vangipuram, M., Sundararajan, R., Urnov, F. D., Langston, J. W., Gregory, P. D., Zhang, H. S., Greenamyre, J. T., Isacson, O., & Schüle, B. (2014). LRRK2 mutations cause mitochondrial DNA damage in iPSC-derived neural cells from Parkinson's disease patients: Reversal by gene correction. *Neurobiology of Disease*, *62*, 381–386. https://doi.org/10.1016/j.nbd.2013.10.013

Schapira, A. H. V. (2009). Etiology and Pathogenesis of Parkinson Disease. *Neurologic Clinics*, *27*(3), 583–603. https://doi.org/10.1016/j.ncl.2009.04.004

Schapira, A. H. V., Chaudhuri, K. R., & Jenner, P. (2017). Non-motor features of Parkinson disease. *Nature Reviews Neuroscience*, *18*(7), 435–450. https://doi.org/10.1038/nrn.2017.62

Schöndorf, D. C., Ivanyuk, D., Baden, P., Sanchez-Martinez, A., De Cicco, S., Yu, C., Giunta, I., Schwarz, L. K., Di Napoli, G., Panagiotakopoulou, V., Nestel, S., Keatinge, M., Pruszak, J., Bandmann, O., Heimrich, B., Gasser, T., Whitworth, A. J., & Deleidi, M. (2018). The NAD+ Precursor Nicotinamide Riboside Rescues Mitochondrial Defects and Neuronal Loss in iPSC and Fly Models of Parkinson's Disease. *Cell Reports*, *23*(10), 2976–2988. https://doi.org/10.1016/j.celrep.2018.05.009

Schwab, A. J., Sison, S. L., Meade, M. R., Broniowska, K. A., Corbett, J. A., & Ebert, A. D. (2017). Decreased Sirtuin Deacetylase Activity in LRRK2 G2019S iPSC-Derived Dopaminergic Neurons. *Stem Cell Reports*, *9*(6), 1839–1852. https://doi.org/10.1016/j.stemcr.2017.10.010

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. *Journal of Neuroscience*, *31*(16), 5970–5976. https://doi.org/10.1523/JNEUROSCI.4441-10.2011

Sharma, V. D., Patel, M., & Miocinovic, S. (2020). Surgical Treatment of Parkinson's Disease: Devices and Lesion Approaches. *Neurotherapeutics*, *17*(4), 1525–1538. https://doi.org/10.1007/s13311-020-00939-x

Shi, Y., Inoue, H., Wu, J. C., & Yamanaka, S. (2017). Induced pluripotent stem cell technology: A decade of progress. *Nature Reviews Drug Discovery*, *16*(2), 115–130. https://doi.org/10.1038/nrd.2016.245

Shulman, J. M., De Jager, P. L., & Feany, M. B. (2011). Parkinson's disease: Genetics and pathogenesis. *Annual Review of Pathology: Mechanisms of Disease*, *6*, 193–222. https://doi.org/10.1146/annurev-pathol-011110-130242

Singh, V. K., Kalsan, M., Kumar, N., Saini, A., & Chandra, R. (2015). Induced pluripotent stem cells: Applications in regenerative medicine, disease modeling, and drug discovery. *Frontiers in Cell and Developmental Biology*, *3*(FEB), 1–18. https://doi.org/10.3389/fcell.2015.00002

Sison, S. L., Vermilyea, S. C., Emborg, M. E., & Ebert, A. D. (2018). Using Patient-Derived Induced Pluripotent Stem Cells to Identify Parkinson's Disease-Relevant Phenotypes. *Current Neurology and Neuroscience Reports*, *18*(12). https://doi.org/10.1007/s11910-018-0893-8

Stoddard-Bennett, T., & Pera, R. (2020). Stem cell therapy for Parkinson's disease: safety and modeling. *Neural Regeneration Research*, *15*(1), 36. https://doi.org/10.4103/1673-5374.264446

Stoddard-Bennett, T., & Reijo Pera, R. (2019). Treatment of Parkinson's Disease through Personalized Medicine and Induced Pluripotent Stem Cells. *Cells*, *8*(1), 26. https://doi.org/10.3390/cells8010026

Straniero, L., Rimoldi, V., Samarani, M., Goldwurm, S., Di Fonzo, A., Krüger, R., Deleidi, M., Aureli, M., Soldà, G., Duga, S., & Asselta, R. (2017). The GBAP1 pseudogene acts as a ceRNA for the glucocerebrosidase gene GBA by sponging miR-22-3p. *Scientific Reports*,

7(1), 1–13. https://doi.org/10.1038/s41598-017-12973-5

Sullivan, S., Stacey, G. N., Akazawa, C., Aoyama, N., Baptista, R., Bedford, P., Bennaceur Griscelli, A., Chandra, A., Elwood, N., Girard, M., Kawamata, S., Hanatani, T., Latsis, T., Lin, S., Ludwig, T. E., Malygina, T., Mack, A., Mountford, J. C., Noggle, S., … Song, J. (2018). Quality control guidelines for clinical-grade human induced pluripotent stem cell lines. *Regenerative Medicine*, *13*(7), 859–866. https://doi.org/10.2217/rme-2018-0095

Sveinbjornsdottir, S. (2016). The clinical symptoms of Parkinson's disease. *Journal of Neurochemistry*, 318–324. https://doi.org/10.1111/jnc.13691

Torrent, R., De Angelis Rigotti, F., Dell'Era, P., Memo, M., Raya, A., & Consiglio, A. (2015). Using iPS cells toward the understanding of parkinson's disease. *Journal of Clinical Medicine*, *4*(4), 548–566. https://doi.org/10.3390/jcm4040548

Tysnes, O. B., & Storstein, A. (2017). Epidemiology of Parkinson's disease. *Journal of Neural Transmission*, *124*(8), 901–905. https://doi.org/10.1007/s00702-017-1686-y

W. Chen, L., Kuang, F., C. Wei, L., X. Ding, Y., K.L. Yung, K., & S. Chan, Y. (2012). Potential Application of Induced Pluripotent Stem Cells in Cell Replacement Therapy for Parkinsons Disease. *CNS & Neurological Disorders - Drug Targets*, *10*(4), 449–458. https://doi.org/10.2174/187152711795563994

Walter, J., Bolognin, S., Antony, P. M. A., Nickels, S. L., Poovathingal, S. K., Salamanca, L., Magni, S., Perfeito, R., Hoel, F., Qing, X., Jarazo, J., Arias-Fuenzalida, J., Ignac, T., Monzel, A. S., Gonzalez-Cano, L., Pereira de Almeida, L., Skupin, A., Tronstad, K. J., & Schwamborn, J. C. (2019). Neural Stem Cells of Parkinson's Disease Patients Exhibit Aberrant Mitochondrial Morphology and Functionality. *Stem Cell Reports*, *12*(5), 878–889. https://doi.org/10.1016/j.stemcr.2019.03.004

Wang, Y., Liang, P., Lan, F., Wu, H., Lisowski, L., Gu, M., Hu, S., Kay, M. A., Urnov, F. D., Shinnawi, R., Gold, J. D., Gepstein, L., & Wu, J. C. (2014). Genome editing of isogenic human induced pluripotent stem cells recapitulates long QT phenotype for drug testing. *Journal of the American College of Cardiology*, *64*(5), 451–459. https://doi.org/10.1016/j.jacc.2014.04.057

Wilson, M. A. (2011). The role of cysteine oxidation in DJ-1 function and dysfunction. *Antioxidants and Redox Signaling*, *15*(1), 111–122. https://doi.org/10.1089/ars.2010.3481

Wood-Kaczmar, A., Gandhi, S., Yao, Z., Abramov, A. S. Y., Miljan, E. A., Keen, G., Stanyer, L., Hargreaves, I., Klupsch, K., Deas, E., Downward, J., Mansfield, L., Jat, P., Taylor, J., Heales, S., Duchen, M. R., Latchman, D., Tabrizi, S. J., & Wood, N. W. (2008). PINK1 is necessary for long term survival and mitochondrial function in human dopaminergic neurons. *PLoS ONE*, *3*(6). https://doi.org/10.1371/JOURNAL.PONE.0002455

Wray, S. (2021). Modelling neurodegenerative disease using brain organoids. *Seminars in Cell and Developmental Biology*, *111*(May), 60–66. https://doi.org/10.1016/j.semcdb.2020.05.012

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

Yamanaka, S. (2020). Pluripotent Stem Cell-Based Cell Therapy—Promise and Challenges. *Cell Stem Cell*, *27*(4), 523–531. https://doi.org/10.1016/j.stem.2020.09.014

Zeng, X., & Couture, L. A. (2013). Pluripotent stem cells for Parkinson's disease: Progress and challenges. *Stem Cell Research and Therapy*, *4*(2). https://doi.org/10.1186/scrt173

Zhong, P., Hu, Z., Jiang, H., Yan, Z., & Feng, J. (2017). Dopamine Induces Oscillatory Activities in Human Midbrain Neurons with Parkin Mutations. *Cell Reports*, *19*(5), 1033–1044. https://doi.org/10.1016/j.celrep.2017.04.023