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■ REVIEW ARTICLE

Advances in the Research of Common Neurodegenerative Diseases Using CRISPR-Cas9 System and hiPSCs

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ABSTRACT: The CRISPR (Clustered Regulatory Interspaced Short Palindromic Repeats) – Cas9 (CRISPR Associated) system has become the most widely used gene editing tool due to its simplicity and efficiency. Advances in iPSC (induced pluripotent stem cell) technology have resulted in the development of hiPSCs (human iPSC) that can be differentiated into any cell of choice while ensuring that the modified cells retain patient-specific genetic information. The CRISPR-Cas system and hiPSCs have opened new avenues for gene therapy and treatment of previously incurable diseases by transplanting or replacing damaged or mutated cells with patient-specific, healthy, normally functioning cells. However, the success of these techniques has been limited in the case of neurodegenerative diseases, as recovery entails the restoration of motor skills and cognitive abilities, including the patient's memory. This paper discusses the symptoms and genetic causes of three neurodegenerative diseases, namely Alzheimer's, Huntington's, and Parkinson's diseases, along with the latest advances in applying the CRISPR-Cas9 system and hiPSCs in identifying their genetic causes, modeling, and validating them while also briefly touching upon the challenges in the application of gene-editing techniques to brain cells and the introduction of corrected healthy differentiated cells derived from hiPSCs into the brain leading to relatively slow progress in finding permanent cures of neurodegenerative diseases compared to diseases affecting other organs.

KEYWORDS: Biomedical and Health Sciences; Alzheimer's; CRISPR-Cas9 system; hiPSCs; Huntington's; Parkinson's.

■ Introduction

Humans have been afflicted with various neurodegenerative diseases since ancient times, but their causes started to be thoroughly researched in the early 20th century. It is only in the last few decades, with progress in genetics and cell therapy, that the root causes of many neurodegenerative diseases and their effects on the brain have become more apparent. Finding cures for diseases like Alzheimer's,[1] Huntington's,[2] and Parkinson's[3] that onset with age is especially challenging even with today's technologies as a seemingly healthy individual becomes incurable.

Neurodegenerative diseases affect motor skills and cognitive abilities, including memory.[1-5] Research in these neurodegenerative diseases is incredibly demanding as many genes that cause these diseases are absent or manifest naturally in experimental animals like mice.[6] Though motor skills and rudimentary cognitive abilities can be studied to an extent in mice, species closer to humans in evolution, like non-human primates, provide a modicum of semblance to human behavior and a better platform for research in these diseases. However, their use results in increased research costs while raising ethical questions.

Recent advances in gene editing technologies like ZFN (Zinc Finger Nucleases),[7] TALEN (Transcription Activator-Like Effector Nucleases),[8] and CRISPR (Clustered Regulatory Interspaced Short Palindromic Repeats)[9,10] have opened avenues to edit specific genes to correct genetic sequences responsible for hereditary diseases and stem their inheritance. However, this doesn't cure individuals already afflicted with these diseases. Human induced pluripotent stem cells (hiPSCs)[11,12] are human cells that possess self-renewal and differentiation capabilities and can differentiate into any other human cell type. They have found increasing use in cell replacement in adult humans and recently shown instances of providing cures for diseases like leukemia by replacing diseased cells with gene-edited healthy cells. However, applying hiPSC cell replacement to treat neurodegenerative diseases is still out of reach. The gene-edited healthy cells that replace the damaged neural cells should connect with other neural cells and restore the patient's motors skills and cognitive abilities, including memory, for a cure. However, hiPSC cells have provided a platform to model neural cells affected by neurodegenerative diseases and study the effects of applying gene editing technologies to treat them.

This paper discusses three common neurodegenerative diseases, namely Alzheimer's, Huntington's, and Parkinson's diseases, briefly touching upon their genetic causes, the most recent and promising gene-editing technique CRISPR-Cas9,[13-15] followed by the advances in hiPSC technology. Recent advances in the application of the CRISPR-Cas9 system along with hiPSC in identifying genetic causes of the mentioned neurodegenerative diseases, modeling of the mutations, and their corrections in the context of a future potential to treat the diseases are discussed.

■ Symptoms and Causes Three Common Neurodegenerative Diseases

Alzheimer's Disease:

Alzheimer's disease (AD), described first by Alois Alzheimer in 1907, is the sixth leading cause of death in the United States.[1,4] About 6.7 million Americans 65 years and older have Alzheimer's disease. AD is an incurable and fatal neural disease that steadily leads to a decline in memory and cognitive

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abilities coupled with the loss of bodily functions like bodily movement, locomotion, and speech. It eventually results in dementia and loss of even elementary involuntary functions like breathing, culminating in the death of the patient.[16-21]

AD is classified into familial AD (FAD) and sporadic AD (SAD) based on its underlying causes. FAD is inherited through an autosome from one generation to the next, and patients start to show AD symptoms in middle age. SAD occurs much later in life, typically after retirement age, but its trigger is not fully understood and is attributed to environmental factors.[22]

Both FAD and SAD are characterized by steady and progressive neural loss and degeneration, leading to brain atrophy. The pathological changes in the brain are caused by the extracellular deposits of amyloid beta (Aβ) peptides in senile plaques and intracellular inclusions rich in hyperphosphorylated tau protein called neurofibrillary tangles (NFTs) within the walls of cerebral vessels.[6,22] Proteolytic processing of the β-amyloid precursor protein (APP) causes extracellular accumulations of amyloid plaques called β-amyloid (Aβ) peptides. Of the many variants of Aβ, its more virulent forms, Aβ42 and Aβ43 result in cellular toxicity and dysfunction, while Aβ40 is present in abundance in both healthy and AD-affected brains.[23-26] Overproduction of Aβ, especially the Aβ42 variant and the increase in the Aβ42:Aβ40 ratio in FAD patients, and the lack of Aβ clearing mechanisms resulting in its accumulation in SAD patients have been observed. Hyperphosphorylated tau are susceptible to coalescing, causing cell death. Both Aβ and NFTs in AD patients lead to the inflammation of the neurons and activation of innate immune cells, causing synaptic and neuron loss.[22]

Inherited AD in FAD is linked to mutations in three genes, namely presenilin 1 (PSEN1), presenilin 2 (PSEN2), and amyloid precursor protein (APP) encoder gene. Amyloidogenic cleavage of APP is performed by gamma (γ) secretase. PSEN1 and PSEN2 regulate the encoding of γ-secretase. γ-secretase is responsible for the final cleavage step of APP in the amyloidogenic pathway, resulting in the release of varying lengths of Aβ peptides. Ala246Glu (A246E) mutation in PSEN1 located on autosome 14 is commonly found in FAD patients, resulting in Aβ overproduction.[27-29] Thus, the key to eliminating FAD is correcting the pertinent mutations in gametes to prevent their transmission between generations and neutralizing or replacing the mutated cells with healthy cells in an afflicted individual.

Huntington's Disease:

Huntington's disease (HD) is an inherited neural disease that initially manifests itself through involuntary movements of the body, mainly legs, and difficulty in focusing with lapses of memory. This gradually progresses into full cognitive decline, resulting in a significant loss in locomotion and memory, comprehension, and ability to perform regular bodily functions like breathing, eating, and speaking. HD patients exhibit depression, mood swings, personality changes, and other emotional instabilities.[2,5] Thus, HD is a neural degenerative disease that ultimately results in the death of the patient after about two decades of manifestation of involuntary motor movements. In the United States, its prevalence is between 3 to 5 cases per 100,000.

In the brain, HD results in shrinkage with neuron degeneration in the putamen, caudate nucleus, and cerebral cortex. There is a loss of efferent medium spiny neurons (MSNs) in the caudate and putamen.[30-33] Substantial neural loss in the brain's cortical, thalamic, and hypothalamic regions is eventually observed.[34]

HD is a hereditary disease triggered by the inheritance of a single dominant mutant Huntington (mHTT) gene.[35] HD is caused when the regular Huntington (HTT) gene that codes for huntingtin protein has dozens of CAG trinucleotide repeats in the first exon.[36] HD typically manifests if the CAG repeats are greater than 42, with a lower severity of the disease manifesting itself if the repeats are between 35 and 41. HD results in young adults if the CAG repeats are greater than 60. [37] The higher the number of CAG repeats, the greater and earlier in life the likelihood of a person developing HD.[38] The mHTT gene results in the production of mutant Huntingtin (mHTT) protein. The repeated CAG sequences in mHTT result in an enlarged polyglutamine (polyQ) tract in the protein's N-terminal fragment, causing it to fold and accumulate in the neural cells.[39,40] However, the normal or healthy Huntington (HTT) gene encodes healthy huntingtin 350 kDa protein, essential for normal human development, including in embryos, and maintenance of neural health and functions.[41,42] Huntingtin is essential for forming cortical and striatal excitatory synapses and maintaining synaptic vesicles in the presynaptic terminal and postsynaptic density.[43-45] Any gene therapy must ensure that the regular HTT gene remains unaffected and only the mutant mHTT gene is altered. Removing the additional CAG repeats is key to preventing the transmission of Huntington's disease between generations, and their removal in afflicted individuals is key to its cure.

Parkinson's Disease:

Parkinson's disease (PD) is a neurodegenerative disease that is characterized by tremors in hands, arms, and limbs, muscle stiffness, slowed movement, loss of balance, and impaired coordination. It is the second most common neural disease after Alzheimer's, affecting 1% of the population over 65 years of age. PD cases in the United States are estimated to be between half a million and over a million. Many patients develop cognitive decline, depression, anxiety, psychosis, hallucinations, and delusions much before the motor dysfunctions start to appear.[3] Dopamine deficiency is attributed to mood and psychological changes, while changes in levels of serotonin and noradrenaline are attributed to motor dysfunctions.[46,47]

Only 10-15% of PD cases are familial and passed from generation to generation. The causes of the remaining 85% sporadic PD cases have not been identified. Autosomal dominant PD is attributed to Synuclein Alpha (SNCA) and leucine-rich repeat kinase 2 (LRRK2) mutations. Autosomal recessive PD is attributed to mutations in phosphate and tensin homolog (PTEN) - induced putative kinase 1 (PINK1), Parkin RBR E3 Ubiquitin Protein Ligase (PRKN), Parkinsonism-associated deglaze (PARK7), PARK9 (ATP13A2), and DaisukeJunko1 (DJ-1) genes.[48-51]

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Two point mutations, G51D and H50Q, and three missense mutations, E46K, A30P, and A53T, in SCNA have been discovered to cause PD.[52] Repeated regions have been found in SNCA. A person's susceptibility to developing PD is higher when carrying microsatellite repeat regions Rep1-261 or Rep1-263 compared to Rep1-257 or Rep1-259 in the upstream region of SNCA, as these repeat region(s) enhance SNCA expression.[53]

Mutations in LRRK2 account for 4-5% of familial and 2% of sporadic autosomal dominant PD cases.[54,55] G2019S is a common mutation in LRRK2 and accounts for 4% of familial and 1% of sporadic PD.[52,56] R1441C is another common LRRK2 mutation.[57]

Under the influence of PINK1, PRKN encodes Parkin E3 ubiquitin ligase, which regulates the quality of mitochondria and mitophagy.[58] 19% of early-onset PD cases result from mutations on PRKN. The PARK7 gene, which causes an autosomal recessive form of PD, encodes the DJ-1 protein.[59] Mutations in DJ-1 result in autosomal recessive early onset of PD in less than 1% of the patients.

In both types of PD, there is a significant loss of ventral midbrain dopaminergic neurons (vmDAns) in the substantia nigra pars compacta (SNpc), accompanied by the development of Lewy bodies.[60,61] Lewy bodies are insoluble high molecular weight protofibrils aggregations.[62] Misfolded alpha-synuclein (α-syn) accumulation is found inside the Lewy bodies.[63] It has been postulated that dopaminergic transmission through neurons is inhibited due to α-syn accumulation, resulting in reduced dopamine transporter levels. Loss of dopaminergic neuron (DAn) cells of SNpc disrupts the basal ganglia's motor control network, resulting in disrupted motor functions.[64] Mutations in LRRK2 and PARK9 result in pathological interaction between astrocytes and neurons. Dysfunctional astrocytes also contribute to the increase in α-syn levels in DAn cells, resulting in their degeneration. About 50% of the dopaminergic neurons are lost when the patient shows PD symptoms. Neural loss in the striatum, hippocampus, and neocortex results in cognitive disabilities.[65] PD patients also exhibit neuroinflammation and mitochondrial dysfunction.[66] Thus, rectifying or neutralizing the underlying mutation(s) in a Parkinson's patient is indispensable for curing the patient.

Gene Editing Technique CRISPR-Cas System:

Among the latest and most prolific gene editing and gene splicing techniques is Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-Cas9 system. Cas stands for 'CRISPR-associated' protein. Its simplicity and ease of use have revolutionized the field of gene editing.

History:

CRISPR was first discovered in *Escherichia coli* in 1987 while analyzing a gene responsible for isozyme conversion of alkaline phosphate.[9] Sequences containing five homologous sequences of 29 nucleotides separated by spacers of 32 nucleotides were found. CRISPRs were subsequently found in 85.2% of archaea and 42.3% of bacteria, though the number of sequences and nucleotides varied.[67] The occurrence of genetic sequences of bacteriophages, archaeal viruses, and plasmids in the spacers of bacteria led to the discovery of CRISPR being used as a natural adaptive immune or defense mechanism by prokaryotic host organisms while providing a mechanism to store memory of past infections by incorporating part of the infecting viral DNA in the genetic matter of the host organism. Spanish microbiologist Mojica coined the term CRISPR in the early 2000s.[10] The 2020 Nobel Prize in Chemistry was awarded to E. Charpentier and J. A. Doudna for developing the CRISPR-Cas9 system for gene editing.

Structure and Function:

A CRISPR system generally consists of short, inverted nucleotide sequences that are repeated but separated by different non-repeating and typically longer nucleotide sequences referred to as spacers. The name CRISPR reflects the repeating inverted nucleotide sequences. The repeat nucleotide sequences vary between 21 and 47 bp, while the spacers vary between 20 and 72 bp, as shown in Figure 1 below.[68] In addition to the repeat and spacer nucleotide sequences, CRISPR is accompanied by Cas nucleases, which assist in incorporating parts of the DNA sequences of the attacker into spacer(s) of a CRISPR nuclease in the host through Cas proteins and protecting the host prokaryotic cells against such attacks in the future.[69] Once DNA sequences of the attacking entity, like viruses, have been incorporated into the CRISPR of the host, subsequent attacks by the entity are neutralized by the host.[70] Thus, the changes to the host genome are typically irreversible and need to occur only once for a unique set of nucleotide sequences in a spacer. Ninety-three different Cas nucleases identified to date, ranging from Cas1 to Cas13, are classified into 35 other families based on nucleotide sequences.[71]

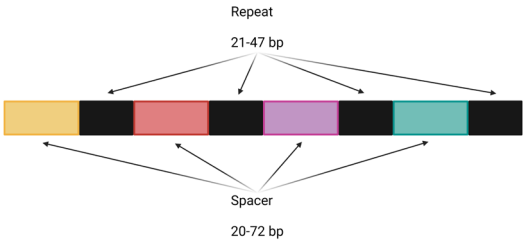

Figure 1: CRISPR Array is showing spacers and repeats.

A typical leader sequence of hundreds of base pairs is located on one side of the repeat sequences and is associated with adaptation and transcription. Together, CRISPR and Cas are referred to as CRISPR-Cas system (CCS).

Functional Steps:

Adaptation, Expression, and Interference are the three significant functional steps of the CRISPR-Cas system, as shown in Figure 2 below. Adaptation is the process by which the foreign DNA from an invading entity is incorporated into a new spacer(s) of the CRISPR nuclease.

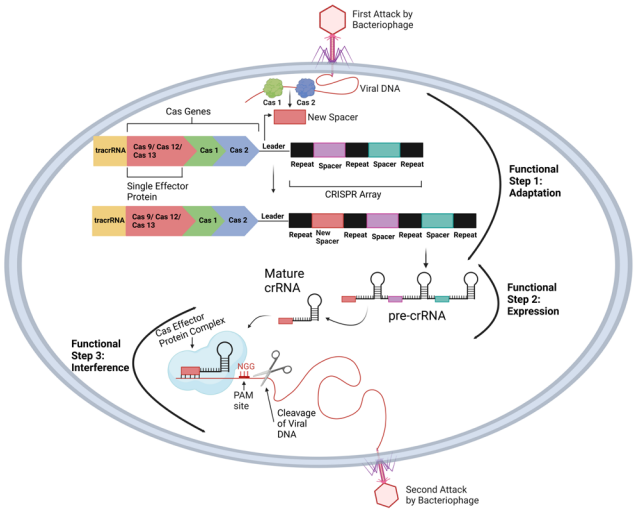

Figure 2: Functional steps of CRISPR- Adaptation, Expression, and Interference, Created with Biorender.com.

The protein complex of Cas1 and Cas2 integrates a new spacer into the leader sequence of the CRISPR array. Thus, Cas1 and Cas2 genes are preserved in CRISPR-Cas systems and are indispensable for inserting new spacers into the CRISPR arrays.[72] Expression or genesis of CRISPR RNA (crRNA) processing is the transcription of a CRISPR array into a precursor CRISPR RNA (pre-crRNA).[73] Cas endonucleases process the pre-crRNA into mature crRNAs.

Interference forms an effector protein consisting of one or more Cas proteins and crRNAs. This effector protein helps the host to identify a similar or identical genetic sequence in the invading virus or plasmid. Once the invading genetic sequence is determined, it is cleaved or split by the protein complex and neutralized.[74]

Classification of CCS:

Based on the differences in the number, type, and sequence of Cas proteins, the CRISPR-Cas system has been broadly classified into Class 1 and Class 2.[75] The two classes are further divided into 6 types and 33 sub-types.[67]

The Class 1 CRISPR-Cas system uses a multi-subunit effector complex between 4 and 7 Cas proteins in the Interference stage. The Class 1 system is further divided into Type I, Type III, and Type IV systems. This class 1 CCS is prevalent in bacteria and archaea, accounting for 90% of all currently identified CRISPR-Cas systems.

The Type I system comprises seven subtypes: I-A, I-C, I-D, I-E, I-F, and I-G. The cleavage of pre-crRNA in the Type I system is mediated by Cas5 (I-C) or Cas6 proteins. Different combinations of Cas3, Cas5, Cas7, and Cas8 proteins are present in the effector complex in the Interference step. The use of Cas3 protein for cleavage of the viral DNA sequence is a characteristic of the Type 1 system.[67,71]

The Type III system comprises six subtypes: III-A, III-B, III-C, III-D, III-E, and III-F. The presence of the Cas10 protein characterizes Type III. The Cas6 protein is responsible for the cleavage of pre-crRNA. The effector complex consists of Cmr/ Cas proteins and crRNA.

The Type IV system comprises three subtypes: IV-A, IV-B, and IV-C. Type IV doesn't contain Cas1 and Cas2 proteins, so it can't perform the Adaptation step. It is primarily used to cleave the invading genome during the Interference step. Type IV system contains Cas5, Cas7, and Csf1 proteins.[76]

The Class 2 CRISPR-Cas system uses a large single multidomain Cas protein in the effector complex bonded with crRNA. The Class 2 system is further divided into Type II, Type V, and Type VI systems. Class 2 is less common and currently accounts for about 10% of all currently identified CRISPR-Cas systems.

The Type II system comprises three subtypes: II-A, II-B, and II-C. Cas9, tracrRNA, and a non-Cas protein RNase III are used to process the pre-crRNA. The use of Cas9 protein in the Interference step is a characteristic of the Type II system and is used to cleavage a strand of the targeted genetic sequence.

The Type V system comprises ten subtypes: V-A, V-B, V-C, V-D, V-E, V-F, V-G, V-H, V-I, and V-K. The use of Cas12 protein in the Interference step is a characteristic of the Type V system.

The Type VI system comprises four subtypes: VI-A, VI-B, VI-C, and VI-D. The use of Cas13 protein in the Interference step is a characteristic of the Type VI system.[77]

Genome editing using CRISPR-Cas9 System:

The Class 2 Type II CRISPR-Cas9 system is primarily employed for gene editing in laboratories. It consists of a single guide RNA (sgRNA) and Cas9 endonuclease. sgRNA is an engineered fusion of CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA). sgRNA directs the Cas9 to the target DNA by matching the base pairs, as shown in Figure 3 below.[13] Cas9 is used to cleave the invading DNA. Cas9 consists of a recognition (REC) lobe and a nuclease (NUC) lobe. The REC lobe performs nucleic acid recognition. The NUC lobe contains two unrelated nuclease domains, RuvC and histidine-asparagine-histidine (HNH), and a C-terminal region containing a protospacer adjacent motif (PAM) - interacting (PI) domain.[14] RuvC domain cleaves the target DNA stand, while the HNH domain cleaves the non-target DNA stand of the invading DNA. This forms a duplex with crRNA and the other DNA strands, resulting in a double-strand break (DSB) in the target DNA.

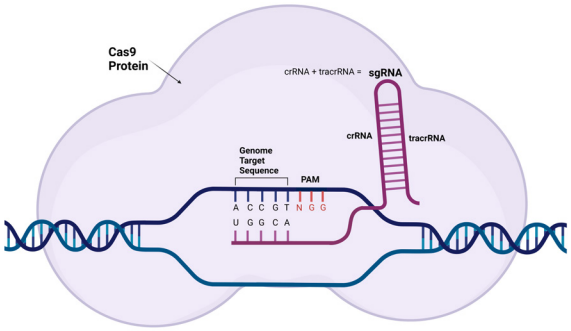

Figure 3: Details of CRISPR Cas9 Cleavage using sgRNA.

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A PAM sequence of nucleotides on the target DNA is indispensable for initiating Cas9 recognition and binding.[15] For human gene editing, Streptococcus pyogenes Cas9 (SpCas9) is primarily used as it has a PAM of NGG, which is present in 12.5% of the human genome. N could be any DNA nucleotide A, T, C, or G. However, Cas9 from other species has different sequences and numbers of nucleotides, allowing PAM to be tailored for specific applications.[78]

After the DSB of the target DNA, cells initiate cell repair mechanisms or pathways to repair the DSB. The two major repair pathways are non-homologous end joining (NHEJ) and homology-directed recombination (HDR). NHEJ functions in all stages of the cell life cycle but may cause gene disruption and reduced protein expression called frameshift mutations because of the introduction of small insertions and deletions around the break. HDR pathway repairs the DSB ideally and can insert the required genetic material into the target, but it requires the cell to be in the dividing stage. This makes HDR less usable compared to NHEJ.[79]

Thus, a combination of Cas9 protein bound to a sgRNA can be used as a programmable nuclease to cleavage specific genetic sequences and remove mutation(s) from a genome. If a template is provided to the repair pathways, the required DNA sequences can be introduced in the desired DNA sequences. This can correct or deactivate disease-causing mutations to non-disease-causing DNA sequences.

■ hiPSCs and Its Use in Research of Neurodegenerative Diseases

Challenges in Using Animals for Neurodegenerative Disease Research:

Due to genetic differences between humans and lab research animals, the results obtained using animals in the lab can't be exactly replicated or applied to human cells.[6] This can also be attributed to metabolic differences between species and the number of isoforms expressed in key molecules.[80-82] In the case of neurodegenerative diseases that manifest in humans as they age, studying or simulating them in lab animals with much shorter life spans doesn't lead to the correct translation of the disease pathology and symptoms. Moreover, cognitive decline and memory loss are multi-faceted, typically involving higher orders of intelligence, and can't be studied in detail in animals like mice. Studying them in the nearest relatives of humans, like non-human primates, is expensive due to their dwindling numbers while raising ethical questions about such experiments.[83] Thus, using human cells, specifically a patient's cells with the desired characteristics, is the best option to study and find cures for diseases affecting an individual.

Human Induced Pluripotent Stem Cell (hiPSC):

Stem cells possess self-renewal and differentiation capabilities and can be classified into totipotent, pluripotent, multipotent, oligopotent, and unipotent stem cells based on their potency. Pluripotent cells can form cells of ectoderm, mesoderm, and endoderm but can't create the extra-embryonic cells of the placenta.[84-86] Embryonic stem cells (ESCs) possess all these capabilities, but their limited availability and ethical concerns about their usage have limited the use of ESCs in research.

Shinya Yamanaka first developed induced pluripotent stem cells (iPSC) by converting mouse fibroblast cells into pluripotent stem cells in the year 2006 by introducing four genetic transcription factors octamer-binding transcription factor-3/4 (OCT-3/4), sex-determining region Y- box transcription factor 2 (SOX2), c-Myc, and kruppel-like factor 4 (KLF4) into human fibroblasts.[11,12] The iPSCs were like ESCs in characteristics and appearance and could form the three germ layers *in vivo* and *in vitro*. Stable human iPSCs were generated from human somatic cells by introducing OCT-4, SOX2, Nanog, and LIN-28.[87]

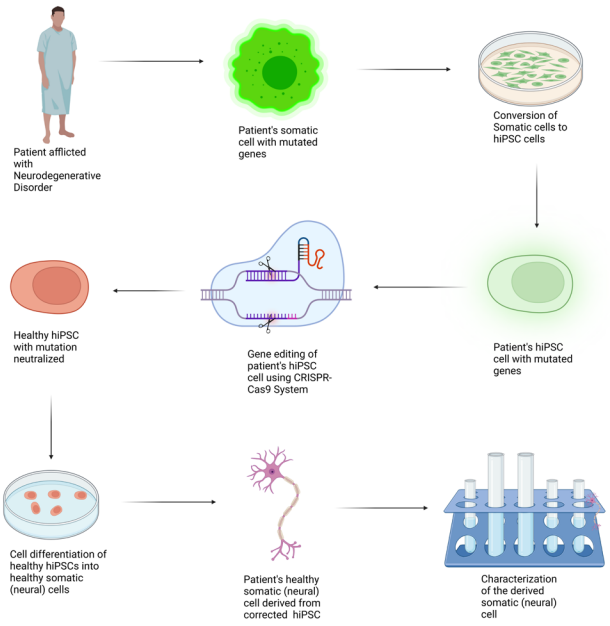

Figure 4: Steps in deriving healthy somatic (neural) cells from a patient's mutated somatic cells using CRISPR-Cas9 System and hiPSCs, Created with Biorender.com.

Using iPSCs overcame the limitations of species specificity encountered when using animal models and genetic overexpression when other methods to simulate the disease in cells that didn't accurately reflect the human disease pathology were used. iPSCs derived from other human cells and not from non-embryonic stem cells are called hiPSCs. hiPSCs allowed researchers to consider the individual genetic variations and mutations of a patient or subject under study for pathogenesis studies, cell-based therapeutic measures, and tailor therapeutic medications and agents. It has also broadened the study of diseases by creating different cell lines and taking genetic information from other individuals. In the last decade, hiPSCs have been used extensively *in vitro* for drug screening, disease modeling, studying pathophysiological effects of human diseases, and cell therapy.

For studying and modeling neurodegenerative diseases, hiPSCs have been differentiated into different kinds of neurons and glia to replicate the disease-induced transformations in the patients. The hiPSC cells are first differentiated into specific neuronal subtypes manifested or affected by the disease by induction of neuroectoderm, followed by patterning the regional progenitors and, ultimately, maturation of the post-mitotic neurons.[88-90]

■ Advances in the usage of CRISPR-Cas9 System and hiPSCs in Research of Common Neurodegenerative Diseases

Alzheimer's Disease Research :

Gene editing using the CRISPR-Cas9 system on hiPSC has successfully disrupted or neutralized Alzheimer's mutations *in vitro*. Thirty-plus APP genes and 300 plus PSEN1 mutations have been discovered in FAD patients that cause increased Aβ levels. A few of these mutations, which were targeted by the CRISPR-Cas9 system, have been briefly described in the previous sections above to highlight the usage of CRISPR-Cas9 in conjunction with hiPSC to model and treat genetic mutations of FAD.

The KM670/671NL mutation in APP, referred to as APPswe, is an APP gene mutation indigenous to Sweden. Its mutant allele is denoted as APPSW and referred to as the Swedish APP allele. This mutation results in increased Aβ levels in the brain, leading to Alzheimer's disease. CRISPR-Cas9 was used to target the mutant allele caused by the APPswe mutation in fibroblasts from FAD patients. APPswe is a double nucleotide mutation located immediately adjacent to an NGG PAM site in exon 16 of APP and hence can be targeted by the *Streptococcus pyogenes* Cas9 (SpCas9). Three different guide RNAs (gRNAs) with lengths of 20 (SW1), 19 (SW2), and 17 (SW3) nucleotides against the APPswe were created. gRNAs composed of 20 and 19 nucleotides disrupted the APPswe mutation. NHEJ pathway was used to heal the resulting DSB. However, this also led to insertions and deletions (indels), a drawback of the CRISPR-Cas9 system, resulting in frameshifts of the DNA sequence. However, the CRISPR-Cas9 system successfully disrupted the APPswe mutation *in vitro* as there was a 50-60% decrease in $A\beta_{40}$ and $A\beta_{42}$ levels in the cell lines of the disrupted APPswe mutation.[91]

Other mutations cause FAD. The M146L mutation in the PSEN1 gene results in autosomal dominant FAD, causing an increase in the $A\beta_{42}$:$A\beta_{40}$ ratio. Its mutant allele is denoted as PSEN1M146L. A 20bp long gRNA resulted in 67.73% disruption in the PSEN1M146L allele, leading to a significant drop in the $A\beta_{42}$:$A\beta_{40}$ ratio in the fibroblasts containing this allele. However, the NHEJ pathway caused random indel formation, resulting in frameshift mutations.[92]

A heterozygous single point mutation (c.449C>T) in exon 6 of the PSEN1 gene results in the replacement of leucine (L) by proline (P) in the 150th position of the amino acid sequence.[93] This mutation is commonly called the L150P mutation of PSEN1, resulting in increased Aβ peptide production. hiPSCs were created from fibroblasts of a patient with the L150P mutation after introducing the human transcription factors OCT4, SOX2, KLF4, L-Myc, and LIN-28 along with a short hairpin against tumor protein P53 (TP53).[94] The CRISPR-Cas9 system was used with single-stranded oligonucleotides (ssODNs) to create an isogenic gene-corrected control L150P-GC-hiPSC. The ssODN containing the "T" nucleotide replaced the "C" nucleotide at the location of the mutation. The sgRNA, along with Cas9, was successfully used to engineer the desired replacement at the site of the mutation, resulting in the elimination of the mutation.[95] The resulting hiPSC was referred to as L150P-GC-hiPSC and retained all its pluripotency characteristics after the point mutation.

Another heterozygous mutation (c.236 C>T) in exon 4 of the PSEN1 gene changes amino acid A79V. This mutation is commonly called the 79V mutation of PSEN1, resulting in the overproduction of Aβ peptides. As in the L150P mutation above, hiPSCs were successfully created from fibroblasts of a patient with the A79V mutation after introducing the human transcription factors OCT4, SOX2, KLF4, L-MYC, and LIN-28 along with a short hairpin against TP53.[94,96] CRISPR-Cas9 corrected the A79V-hiPSC by replacing the "T" nucleotide with the "C" nucleotide in exon 4 of PSEN1. No indel or frameshift was generated, and the successful new gene-corrected hiPSC was referred to as A79V-GC-hiPSC. No other mutation was detected, and the corrected hiPSC maintained its pluripotency after gene editing.[97-99]

Huntington Disease Research:

Huntington's disease is caused by the presence of 35 or more CAG repeats in the Huntington gene, referred to as the mutated HTT gene (mHTT), creating a mutated huntingtin protein. Correcting this mutation involves possible deletion of the additional CAG repeats or replacing the repeated CAG repeats with a benign sequence.

In one of the earliest attempts to correct the mHTT gene using hiPSCs, fibroblasts from a patient afflicted with HD having 72 CAG repeats in the mHTT gene were used. These patient fibroblasts were used to create hiPSCs. A normal HTT gene with 21 CAG repeats was created on longer homologous arms of a bacterial artificial chromosome (BAC). Instead of CRISPR-Cas9, homologous recombination (HR) was used to target the hiPSC using a targeting vector comprising of a 4.5Kb short arm, a 10Kb long arm flanking the CAG repeat region, and a 1.5 kb upstream sequence of HTT to remove the exon1, the CAG repeat region, and the 1.5kb upstream sequence. This resulted in the hiPSCs being corrected to having normal 21 CAG repeats in the HTT gene and not losing their pluripotency.[100] The cell lines of the corrected hiPSCs showed a reversal of HD phenotypes. The same researchers later used CRISPR-Cas9 to perform homogenous recombination to generate HD isogenic allele cells with 21, 72, and 97 CAG repeats. gRNA sequences were used, which resulted in cleavage within 100 bp of the CAG region of exon 1 of the mHTT gene.[101]

The CRISPR-Cas9 system was used to correct the CAG repeats in exon 1 of HTT in hiPSCs, along with a PiggyBac transposon-based selection system.[102] PiggyBac transposon (PB) selection cassette-based homologous recombination (HR) donor was used to establish isogenic controls for the HD hiPSCs by removing the selected cassette from the target locus. A pair of sgRNAs, consisting of sgRNA-a and sgRNA-b, using Cas9 nickase (Cas9n), was used to cleavage at the HTT locus to reduce off-target (OT) activity and enhance homology-dependent repair efficiency.[103] CRISPR-Cas9 D10A nickase was shown to introduce DNA gaps that resulted in contractions, thereby reducing the number of CAG repeats.[104] mHTT hiPSCs were transfected with the HR donor plasmid

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and the sgRNA-a and sgRNA-b Cas9n-expressing plasmids. The isogenic cell lines of the corrected hiPSCs retained their pluripotency. Neurons with active synapses were differentiated from the corrected hiPSCs. These neural cells showed reduced neural rosetta formation, a blossom arrangement of elongated neuroepithelial cells, but increased cell death due to growth factor withdrawal.[105] Increased neural cell death when differentiated from hiPSCs has also been observed in other experiments.[100]

CRISPR-Cas9 disrupted the human mHTT gene on exon 1 in R6/2 mice carrying 115 to 150 CAG repeats *in vivo* delivery in transgenic mice model of HD.[106] sgRNA was used to target CAG repeat sequences upstream or downstream on exon 1 of the human HTT gene. Instead of using SpCas9, Cas9 nuclease from Staphylococcus aureus (SaCas9) was used because it is about 1 kb smaller than SpCas9 and fits into a single adeno-associated virus (AAV) that acts as the vehicle along with the sgRNA. The indels caused by the CRISPR-Cas9 system altered the mHTT and reduced the production of the HTT protein. When the AAV1-SaCas9-HTT was infused into the R6/2 mice, 40% lower mHTT proteins were generated. The mice exhibited better motor function and survived 15% longer than the mice without this treatment. This experiment demonstrated that the mHTT gene could be disrupted using the CRISPR-Cas9 system. When the resultant nuclease was infused *in vivo*, reduced HD symptoms and improved life expectancy were observed in mice models of HD.[79]

Parkinson Disease Research:

CRISPR-mediated knockout (KO) was used to create isogenic models of mutations in PRKN, PARK9, and DJ-1 to simulate the effects of PD, namely, neural degeneration, neuroinflammation, and mitochondrial dysfunction.[52,107] CRISPR-Cas9 system was used on hiPSCs with PD mutations to develop three different isogenic models after disrupting the PRKN, DJ-1, and ATP13A2 autosomal recessive genes. DAn cells were subsequently differentiated from these genetically mutated models.[108] DAn cells with disrupted PRKN gene didn't manifest the PARKIN protein. Depletion in mitochondrial proteins was observed in all three DAn cell lines.[109]

To check if PD-related mutations can be replicated in hiPSCs, 1 bp was deleted from PARK7 in hiPSCs derived from a PD patient's fibroblasts with uniparental disomy and was matched with CRISPR-Cas9 induced PARK7 mutation in the Gibco Human Episomal hiPSC line.[110]

CRISPR-Cas9 deleted repeated microsatellite regions Rep1-257, Rep1-259, Rep1-261, and Rep1-263 of SNCA and replaced them with representative alleles in hiPSCs. The cis-regulatory effects of repeat regions that differentiated from the modified hiPSCs were not seen in neurons.[111]

Neurons differentiated from iPSCs containing SNCA triplication were used to demonstrate the aggregation of α-syn. The α-syn interacted with the ATP synthase to induce permeability transition pore (PTP) opening in mitochondria. This causes the mitochondria to swell, resulting in cell death.[112] This proves that an SNCA mutation causes PD-related mitochondria dysfunction. To demonstrate the reverse, cell lines derived from modified iPSCs in which two alleles of SNCA triplication were removed showed a lower generation of α-syn levels.[112]

The three known missense mutations E46K, H50Q, and G51D in SCNA that cause PD were induced using CRISPR-Cas9 in Guangxi Bama minipig cells (not hiPSCs) to show symptoms like α-syn aggregation and loss of DAn.[113]

To prove that in PD astrocytes lead to neurodegeneration of vmDAn and transfer of α-syn from astrocytes to the neurons, hiPSC astrocytes derived from patients carrying the G2019S mutation in LRRK2 gene were co-cultured with controlled healthy vmDAns. CRISPR-Cas9 was used to tag the astrocytes derived from PD patients with a FLAG peptide to track the α-syn produced. After several weeks, the α-syn with the FLAG tag was found to accumulate on the neurons. However, the healthy vmDAns developed shortened neurites, leading to neural degeneration and decreased survival rates.[66]

Conclusions

Neurodegenerative diseases, like Alzheimer's, Huntington's, and Parkinson's diseases, start in the brain but gradually impact motor skills, body functions, and cognitive abilities, including memory, ultimately resulting in the patient's death. The underlying physiological and chemical changes manifesting in each disease are also different, though the epicenter and genesis of these diseases is in the brain. All these diseases have underlying genetic causes, which can be corrected with gene editing techniques like the CRISPR-Cas9 system.

With the advances in the development of hiPSCs, possibilities of treating previously incurable diseases through gene editing of hiPSCs using the CRISPR-Cas9 system have emerged. hiPSCs have enabled the use of the patient's cells for the treatment, eliminating the possibility of rejection of the modified cells by the patient's immune system, leading to higher success rates while ensuring effective treatment. However, the application of this technique for treating neurodegenerative diseases is still in its infancy, as a true cure would involve not only reversing the disease but also restoring the patient's full cognitive abilities and motor skills.

Nevertheless, applying the CRISPR-Cas9 system and hiPSCs has allowed researchers to identify and validate the different causes of neurodegenerative diseases.

In the case of Alzheimer's disease, the APPswe, M146L, and PSEN1 mutations on exons 4 and 6 were targeted using the CRISPR-Cas9 system to validate and correct the disease *in vitro*. hiPSC-corrected cells resulted in at least a 50% reduction in the $A\beta_{42}$:$A\beta_{40}$ ratio, indicating that loss of these mutations results in the normal functioning of a patient's cells. Similarly, the CRISPR-Cas9 system and hiPSCs successfully demonstrated the ability to correct point mutations 79V and L150P in exons 4 and 6, respectively.[92,94,96]

In the case of Parkinson's disease, in which mitochondrial dysfunction causes cell death, DAn cells differentiated from the isogenic models of hiPSCs in which PD causing mutations in PRKN, DJ-1, and ATP13A2 autosomal recessive genes were disrupted using CRISPR-Cas9 system didn't manifest the PARKIN protein and showed depletion in mitochondrial proteins. Neurons that differentiated from the hiPSCs in which the CRISPR-Cas9 system was used to delete repeated

microsatellite regions Rep1-257, Rep1-259, Rep1-261, and Rep1-263 of SNCA and replaced with representative alleles didn't show the cis-regulatory effects of repeat regions. SNCA triplication that results in α-syn aggregation causing cell death due to swelling of the mitochondria was demonstrated using iPSCs. Conversely, by removing two alleles of SNCA triplication, a reduction in α-syn aggregation was validated with iPSCs.[111-113]

In the case of Huntington's disease, different CAG repeats in exon 1 of the mHTT gene were successfully deleted using the CRISPR-Cas9 system. In one study, genetically engineered mice carrying between 115 and 150 CAG repeats of the human mHTT gene showed a 40% reduction in the production of mHTT proteins when CRISPR with SaCas9 was used to edit the mutation by removing the CAG repeats. This highlights the efficacy of the CRISPR-Cas9 system in editing not only a single nucleotide but also longer genetic sequences of different lengths.[106]

In summary, the CRISPR-Cas9 system and hiPSCs have enabled scientists to study the causes and effects of the various mutations causing neurodegenerative diseases, primarily *in vitro*. Research to introduce healthy hiPSCs with corrected or neutralized mutations of neurodegenerative diseases back into the patient's brain is still challenging. The corrected cells need to be healthy, position themselves at the right places in the brain's neural network, and work seamlessly with the rest of the brain cells. Since the brain controls all cognitive and bodily functions, any aberration caused by these cells, either physically or functionally, may lead to debilitating side effects in other parts of the body or bodily functions. Moreover, the neural cells differentiated from hiPSCs have low survival rates that are measurable in days, impeding their transplantation into humans. Researchers should attempt to implant these neural cells in animal models, including rodents and non-human primates, to perfect this treatment method before trying them on humans. For a true and complete cure, the modified hiPSCs introduced into the patient in sufficient quantities should be able to replace the damaged or dead cells and regenerate the neural network, restoring the patient's motor skills and cognitive abilities, including memory. It is hoped that this may become a reality in the coming decades.

References

1. Hippius, H.; Neundörfer, G. The Discovery of Alzheimer's Disease. *Dialogues Clin. Neurosci*. **2003**, *5* (1), 101–108.
2. Ross, C. A.; Aylward, E. H.; Wild, E. J.; Langbehn, D. R.; Long, J. D.; Warner, J. H.; Scahill, R. I.; Leavitt, B. R.; Stout, J. C.; Paulsen, J. S.; Reilmann, R.; Unschuld, P. G.; Wexler, A.; Margolis, R. L.; Tabrizi, S. J. Huntington Disease: Natural History, Biomarkers and Prospects for Therapeutics. *Nat. Rev. Neurol.* **2014**, *10* (4), 204–216. https://doi.org/10.1038/nrneurol.2014.24.
3. Blažeković, A.; Borovečki, F. Psychiatric Comorbidities in Parkinson's Disease Seen through the Prism of Genomics and Epigenetics. *Psychiatr. Danub*. **2021**, *33* (Suppl 4), 456–462.
4. Graeber, M. B.; Kösel, S.; Egensperger, R.; Banati, R. B.; Müller, U.; Bise, K.; Hoff, P.; Möller, H. J.; Fujisawa, K.; Mehraein, P. Rediscovery of the Case Described by Alois Alzheimer in 1911: Historical, Histological and Molecular Genetic Analysis. *Neurogenetics* 1997, 1 (1), 73–80. https://doi.org/10.1007/s100480050011.
5. Klempír, J.; Klempírová, O.; Stochl, J.; Spacková, N.; Roth, J. The Relationship between Impairment of Voluntary Movements and Cognitive Impairment in Huntington's Disease. *J. Neurol*. **2009**, *256* (10), 1629–1633. https://doi.org/10.1007/s00415-009-5164-9.
6. Sukoff Rizzo, S. J.; Homanics, G.; Schaeffer, D. J.; Schaeffer, L.; Park, J. E.; Oluoch, J.; Zhang, T.; Haber, A.; Seyfried, N. T.; Paten, B.; Greenwood, A.; Murai, T.; Choi, S. H.; Huhe, H.; Kofler, J.; Strick, P. L.; Carter, G. W.; Silva, A. C. Bridging the Rodent to Human Translational Gap: Marmosets as Model Systems for the Study of Alzheimer's Disease. *Alzheimers Dement. Transl. Res. Clin. Interv.* 2023, 9 (3), e12417. https://doi.org/10.1002/trc2.12417.
7. Carroll, D. Genome Engineering With Zinc-Finger Nucleases. *Genetics* **2011**, *188* (4), 773–782. https://doi.org/10.1534/genetics.111.131433.
8. Joung, J. K.; Sander, J. D. TALENs: A Widely Applicable Technology for Targeted Genome Editing. *Nat. Rev. Mol. Cell Biol*. **2013**, *14* (1), 49–55. https://doi.org/10.1038/nrm3486.
9. Ishino, Y.; Shinagawa, H.; Makino, K.; Amemura, M.; Nakata, A. Nucleotide Sequence of the Iap Gene, Responsible for Alkaline Phosphatase Isozyme Conversion in Escherichia Coli, and Identification of the Gene Product. *J. Bacteriol.* **1987**, *169* (12), 5429–5433. https://doi.org/10.1128/jb.169.12.5429-5433.1987.
10. Mojica, F. J. M.; Díez-Villaseñor, C.; García-Martínez, J.; Soria, E. Intervening Sequences of Regularly Spaced Prokaryotic Repeats Derive from Foreign Genetic Elements. *J. Mol. Evol.* **2005**, *60* (2), 174–182. https://doi.org/10.1007/s00239-004-0046-3.
11. Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K.; Yamanaka, S. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. *Cell* **2007**, *131* (5), 861–872. https://doi.org/10.1016/j.cell.2007.11.019.
12. Takahashi, K.; Yamanaka, S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell* **2006**, *126* (4), 663–676. https://doi.org/10.1016/j.cell.2006.07.024.
13. Jinek, M.; Chylinski, K.; Fonfara, I.; Hauer, M.; Doudna, J. A.; Charpentier, E. A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity. *Science* **2012**, *337* (6096), 816–821. https://doi.org/10.1126/science.1225829.
14. Ishino, Y.; Krupovic, M.; Forterre, P. History of CRISPR-Cas from Encounter with a Mysterious Repeated Sequence to Genome Editing Technology. *J. Bacteriol.* **2018**, *200* (7), e00580-17. https://doi.org/10.1128/JB.00580-17.
15. Qiu, H.-Y.; Ji, R.-J.; Zhang, Y. Current Advances of CRISPR-Cas Technology in Cell Therapy. *Cell Insight* **2022**, *1* (6), 100067. https://doi.org/10.1016/j.cellin.2022.100067.
16. 2023 Alzheimer's Disease Facts and Figures. Alzheimers Dement. *J. Alzheimers Assoc*. **2023**, *19* (4), 1598–1695. https://doi.org/10.1002/alz.13016.
17. Sperling, R. A.; Aisen, P. S.; Beckett, L. A.; Bennett, D. A.; Craft, S.; Fagan, A. M.; Iwatsubo, T.; Jack, C. R.; Kaye, J.; Montine, T. J.; Park, D. C.; Reiman, E. M.; Rowe, C. C.; Siemers, E.; Stern, Y.; Yaffe, K.; Carrillo, M. C.; Thies, B.; Morrison-Bogorad, M.; Wagster, M. V.; Phelps, C. H. Toward Defining the Preclinical Stages of Alzheimer's Disease: Recommendations from the National Institute on Aging-Alzheimer's Association Workgroups on Diagnostic Guidelines for Alzheimer's Disease. Alzheimers Dement. *J. Alzheimers Assoc.* **2011**, *7* (3), 280–292. https://doi.org/10.1016/j.jalz.2011.03.003.
18. Albert, M. S.; DeKosky, S. T.; Dickson, D.; Dubois, B.; Feldman, H. H.; Fox, N. C.; Gamst, A.; Holtzman, D. M.; Jagust, W. J.; Petersen, R. C.; Snyder, P. J.; Carrillo, M. C.; Thies, B.; Phelps, C. H. The Diagnosis of Mild Cognitive Impairment Due to Alzheimer's Disease: Recommendations from the National Institute on Aging-Alzheimer's Association Workgroups on Diagnostic Guidelines

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References

1. Hippius, H.; Neundörfer, G. The Discovery of Alzheimer's Disease. *Dialogues Clin. Neurosci*. **2003**, *5* (1), 101–108.
2. Ross, C. A.; Aylward, E. H.; Wild, E. J.; Langbehn, D. R.; Long, J. D.; Warner, J. H.; Scahill, R. I.; Leavitt, B. R.; Stout, J. C.; Paulsen, J. S.; Reilmann, R.; Unschuld, P. G.; Wexler, A.; Margolis, R. L.; Tabrizi, S. J. Huntington Disease: Natural History, Biomarkers and Prospects for Therapeutics. *Nat. Rev. Neurol.* **2014**, *10* (4), 204–216. https://doi.org/10.1038/nrneurol.2014.24.
3. Blažeković, A.; Borovečki, F. Psychiatric Comorbidities in Parkinson's Disease Seen through the Prism of Genomics and Epigenetics. *Psychiatr. Danub*. **2021**, *33* (Suppl 4), 456–462.
4. Graeber, M. B.; Kösel, S.; Egensperger, R.; Banati, R. B.; Müller, U.; Bise, K.; Hoff, P.; Möller, H. J.; Fujisawa, K.; Mehraein, P. Rediscovery of the Case Described by Alois Alzheimer in 1911: Historical, Histological and Molecular Genetic Analysis. *Neurogenetics* 1997, 1 (1), 73–80. https://doi.org/10.1007/s100480050011.
5. Klempír, J.; Klempírová, O.; Stochl, J.; Spacková, N.; Roth, J. The Relationship between Impairment of Voluntary Movements and Cognitive Impairment in Huntington's Disease. *J. Neurol*. **2009**, *256* (10), 1629–1633. https://doi.org/10.1007/s00415-009-5164-9.
6. Sukoff Rizzo, S. J.; Homanics, G.; Schaeffer, D. J.; Schaeffer, L.; Park, J. E.; Oluoch, J.; Zhang, T.; Haber, A.; Seyfried, N. T.; Paten, B.; Greenwood, A.; Murai, T.; Choi, S. H.; Huhe, H.; Kofler, J.; Strick, P. L.; Carter, G. W.; Silva, A. C. Bridging the Rodent to Human Translational Gap: Marmosets as Model Systems for the Study of Alzheimer's Disease. *Alzheimers Dement. Transl. Res. Clin. Interv.* 2023, 9 (3), e12417. https://doi.org/10.1002/trc2.12417.
7. Carroll, D. Genome Engineering With Zinc-Finger Nucleases. *Genetics* **2011**, *188* (4), 773–782. https://doi.org/10.1534/genetics.111.131433.
8. Joung, J. K.; Sander, J. D. TALENs: A Widely Applicable Technology for Targeted Genome Editing. *Nat. Rev. Mol. Cell Biol*. **2013**, *14* (1), 49–55. https://doi.org/10.1038/nrm3486.
9. Ishino, Y.; Shinagawa, H.; Makino, K.; Amemura, M.; Nakata, A. Nucleotide Sequence of the Iap Gene, Responsible for Alkaline Phosphatase Isozyme Conversion in Escherichia Coli, and Identification of the Gene Product. *J. Bacteriol.* **1987**, *169* (12), 5429–5433. https://doi.org/10.1128/jb.169.12.5429-5433.1987.
10. Mojica, F. J. M.; Díez-Villaseñor, C.; García-Martínez, J.; Soria, E. Intervening Sequences of Regularly Spaced Prokaryotic Repeats Derive from Foreign Genetic Elements. *J. Mol. Evol.* **2005**, *60* (2), 174–182. https://doi.org/10.1007/s00239-004-0046-3.
11. Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K.; Yamanaka, S. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. *Cell* **2007**, *131* (5), 861–872. https://doi.org/10.1016/j.cell.2007.11.019.
12. Takahashi, K.; Yamanaka, S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell* **2006**, *126* (4), 663–676. https://doi.org/10.1016/j.cell.2006.07.024.
13. Jinek, M.; Chylinski, K.; Fonfara, I.; Hauer, M.; Doudna, J. A.; Charpentier, E. A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity. *Science* **2012**, *337* (6096), 816–821. https://doi.org/10.1126/science.1225829.
14. Ishino, Y.; Krupovic, M.; Forterre, P. History of CRISPR-Cas from Encounter with a Mysterious Repeated Sequence to Genome Editing Technology. *J. Bacteriol.* **2018**, *200* (7), e00580-17. https://doi.org/10.1128/JB.00580-17.
15. Qiu, H.-Y.; Ji, R.-J.; Zhang, Y. Current Advances of CRISPR-Cas Technology in Cell Therapy. *Cell Insight* **2022**, *1* (6), 100067. https://doi.org/10.1016/j.cellin.2022.100067.
16. 2023 Alzheimer's Disease Facts and Figures. Alzheimers Dement. *J. Alzheimers Assoc*. **2023**, *19* (4), 1598–1695. https://doi.org/10.1002/alz.13016.
17. Sperling, R. A.; Aisen, P. S.; Beckett, L. A.; Bennett, D. A.; Craft, S.; Fagan, A. M.; Iwatsubo, T.; Jack, C. R.; Kaye, J.; Montine, T. J.; Park, D. C.; Reiman, E. M.; Rowe, C. C.; Siemers, E.; Stern, Y.; Yaffe, K.; Carrillo, M. C.; Thies, B.; Morrison-Bogorad, M.; Wagster, M. V.; Phelps, C. H. Toward Defining the Preclinical Stages of Alzheimer's Disease: Recommendations from the National Institute on Aging-Alzheimer's Association Workgroups on Diagnostic Guidelines for Alzheimer's Disease. Alzheimers Dement. *J. Alzheimers Assoc.* **2011**, *7* (3), 280–292. https://doi.org/10.1016/j.jalz.2011.03.003.
18. Albert, M. S.; DeKosky, S. T.; Dickson, D.; Dubois, B.; Feldman, H. H.; Fox, N. C.; Gamst, A.; Holtzman, D. M.; Jagust, W. J.; Petersen, R. C.; Snyder, P. J.; Carrillo, M. C.; Thies, B.; Phelps, C. H. The Diagnosis of Mild Cognitive Impairment Due to Alzheimer's Disease: Recommendations from the National Institute on Aging-Alzheimer's Association Workgroups on Diagnostic Guidelines

J. M.; Zeisler, J.; Borowski, A.; Marth, J. D.; Phillips, A. G.; Hayden, M. R. Targeted Disruption of the Huntington's Disease Gene Results in Embryonic Lethality and Behavioral and Morphological Changes in Heterozygotes. *Cell* **1995**, *81* (5), 811–823. https://doi.org/10.1016/0092-8674(95)90542-1.

42. McKinstry, S. U.; Karadeniz, Y. B.; Worthington, A. K.; Hayrapetyan, V. Y.; Ozlu, M. I.; Serafin-Molina, K.; Risher, W. C.; Ustunkaya, T.; Dragatsis, I.; Zeitlin, S.; Yin, H. H.; Eroglu, C. Huntingtin Is Required for Normal Excitatory Synapse Development in Cortical and Striatal Circuits. J. Neurosci. Off. *J. Soc. Neurosci.* **2014**, *34* (28), 9455–9472. https://doi.org/10.1523/JNEUROSCI.4699-13.2014.
43. Molina-Calavita, M.; Barnat, M.; Elias, S.; Aparicio, E.; Piel, M.; Humbert, S. Mutant Huntingtin Affects Cortical Progenitor Cell Division and Development of the Mouse Neocortex. *J. Neurosci. Off. J. Soc. Neurosci*. 2014, 34 (30), 10034–10040. https://doi.org/10.1523/JNEUROSCI.0715-14.2014.
44. DiFiglia, M.; Sapp, E.; Chase, K. O.; Davies, S. W.; Bates, G. P.; Vonsattel, J. P.; Aronin, N. Aggregation of Huntingtin in Neuronal Intranuclear Inclusions and Dystrophic Neurites in Brain. *Science* **1997**, *277* (5334), 1990–1993. https://doi.org/10.1126/science.277.5334.1990.
45. Marcora, E.; Kennedy, M. B. The Huntington's Disease Mutation Impairs Huntingtin's Role in the Transport of NF-κB from the Synapse to the Nucleus. *Hum. Mol. Genet*. **2010**, *19* (22), 4373–4384. https://doi.org/10.1093/hmg/ddq358.
46. Politis, M.; Wu, K.; Loane, C.; Quinn, N. P.; Brooks, D. J.; Oertel, W. H.; Björklund, A.; Lindvall, O.; Piccini, P. Serotonin Neuron Loss and Nonmotor Symptoms Continue in Parkinson's Patients Treated with Dopamine Grafts. *Sci. Transl. Med.* **2012**, *4* (128), 128a41. https://doi.org/10.1126/scitranslmed.3003391.
47. Delaville, C.; Deurwaerdère, P. D.; Benazzouz, A. Noradrenaline and Parkinson's Disease. *Front. Syst. Neurosci.* **2011**, *5*, 31. https://doi.org/10.3389/fnsys.2011.00031.
48. Tan, Y.; Wu, L.; Zhao, Z.; Wang, Y.; Xiao, Q.; Liu, J.; Wang, G.; Ma, J.; Chen, S. Methylation of α-Synuclein and Leucine-Rich Repeat Kinase 2 in Leukocyte DNA of Parkinson's Disease Patients. *Parkinsonism Relat. Disord*. **2014**, *20* (3), 308–313. https://doi.org/10.1016/j.parkreldis.2013.12.002.
49. Verstraeten, A.; Theuns, J.; Van Broeckhoven, C. Progress in Unraveling the Genetic Etiology of Parkinson Disease in a Genomic Era. *Trends Genet. TIG* **2015**, *31* (3), 140–149. https://doi.org/10.1016/j.tig.2015.01.004.
50. Lill, C. M. Genetics of Parkinson's Disease. *Mol. Cell. Probes* 2016, 30 (6), 386–396. https://doi.org/10.1016/j.mcp.2016.11.001.
51. Safari, F.; Hatam, G.; Behbahani, A. B.; Rezaei, V.; Barekati-Mowahed, M.; Petramfar, P.; Khademi, F. CRISPR System: A High-Throughput Toolbox for Research and Treatment of Parkinsons's Disease. *Cell. Mol. Neurobiol*. **2020**, *40* (4), 477–493. https://doi.org/10.1007/s10571-019-00761-w.
52. Mehmood, A.; Ali, W.; Din, Z. U.; Song, S.; Sohail, M.; Shah, W.; Guo, J.; Guo, R.-Y.; Ilahi, I.; Shah, S.; Al-Shaebi, F.; Zeb, L.; Asiamah, E. A.; Al-Dhamin, Z.; Bilal, H.; Li, B. Clustered Regularly Interspaced Short Palindromic Repeats as an Advanced Treatment for Parkinson's Disease. *Brain Behav*. **2021**, 11 (8), e2280. https://doi.org/10.1002/brb3.2280.
53. Farrer, M.; Maraganore, D. M.; Lockhart, P.; Singleton, A.; Lesnick, T. G.; de Andrade, M.; West, A.; de Silva, R.; Hardy, J.; Hernandez, D. Alpha-Synuclein Gene Haplotypes Are Associated with Parkinson's Disease. *Hum. Mol. Genet.* **2001**, *10* (17), 1847–1851. https://doi.org/10.1093/hmg/10.17.1847.
54. Gilks, W. P.; Abou-Sleiman, P. M.; Gandhi, S.; Jain, S.; Singleton, A.; Lees, A. J.; Shaw, K.; Bhatia, K. P.; Bonifati, V.; Quinn, N. P.; Lynch, J.; Healy, D. G.; Holton, J. L.; Revesz, T.; Wood, N. W. A Common LRRK2 Mutation in Idiopathic Parkinson's Disease. *The Lancet* 2005, 365 (9457), 415–416. https://doi.org/10.1016/S0140-6736(05)17830-1.
55. Nichols, W. C.; Pankratz, N.; Hernandez, D.; Paisán-Ruíz, C.; Jain, S.; Halter, C. A.; Michaels, V. E.; Reed, T.; Rudolph, A.; Shults, C. W.; Singleton, A.; Foroud, T. Genetic Screening for a Single Common LRRK2 Mutation in Familial Parkinson's Disease. *The Lancet* **2005**, *365* (9457), 410–412. https://doi.org/10.1016/S0140-6736(05)17828-3.
56. Healy, D. G.; Falchi, M.; O'Sullivan, S. S.; Bonifati, V.; Durr, A.; Bressman, S.; Brice, A.; Aasly, J.; Zabetian, C. P.; Goldwurm, S.; Ferreira, J. J.; Tolosa, E.; Kay, D. M.; Klein, C.; Williams, D. R.; Marras, C.; Lang, A. E.; Wszolek, Z. K.; Berciano, J.; Schapira, A. H. V.; Lynch, T.; Bhatia, K. P.; Gasser, T.; Lees, A. J.; Wood, N. W.; International LRRK2 Consortium. Phenotype, Genotype, and Worldwide Genetic Penetrance of LRRK2-Associated Parkinson's Disease: A Case-Control Study. *Lancet Neurol.* **2008**, *7* (7), 583–590. https://doi.org/10.1016/S1474-4422(08)70117-0.
57. Luo, J.; Padhi, P.; Jin, H.; Anantharam, V.; Zenitsky, G.; Wang, Q.; Willette, A. A.; Kanthasamy, A.; Kanthasamy, A. G. Utilization of the CRISPR-Cas9 Gene Editing System to Dissect Neuroin flammatory and Neuropharmacological Mechanisms in Parkinson's Disease. *J. Neuroimmune Pharmacol. Off. J. Soc. NeuroImmune Pharmacol*. 2019, 14 (4), 595–607. https://doi.org/10.1007/s11481-019-09844-3.
58. Lazarou, M.; Sliter, D. A.; Kane, L. A.; Sarraf, S. A.; Wang, C.; Burman, J. L.; Sideris, D. P.; Fogel, A. I.; Youle, R. J. The Ubiquitin Kinase PINK1 Recruits Autophagy Receptors to Induce Mitophagy. *Nature* **2015**, *524* (7565), 309–314. https://doi.org/10.1038/nature14893.
59. Bonifati, V.; Rizzu, P.; van Baren, M. J.; Schaap, O.; Breedveld, G. J.; Krieger, E.; Dekker, M. C. J.; Squitieri, F.; Ibanez, P.; Joosse, M.; van Dongen, J. W.; Vanacore, N.; van Swieten, J. C.; Brice, A.; Meco, G.; van Duijn, C. M.; Oostra, B. A.; Heutink, P. Mutations in the DJ-1 Gene Associated with Autosomal Recessive Early-Onset Parkinsonism. *Science* **2003**, *299* (5604), 256–259. https://doi.org/10.1126/science.1077209.
60. Kaplitt, M. G.; Feigin, A.; Tang, C.; Fitzsimons, H. L.; Mattis, P.; Lawlor, P. A.; Bland, R. J.; Young, D.; Strybing, K.; Eidelberg, D.; During, M. J. Safety and Tolerability of Gene Therapy with an Adeno-Associated Virus (AAV) Borne GAD Gene for Parkinson's Disease: An Open Label, Phase I Trial. *Lancet Lond. Engl.* **2007**, *369* (9579), 2097–2105. https://doi.org/10.1016/S0140-6736(07)60982-9.
61. Michel, P. P.; Hirsch, E. C.; Hunot, S. Understanding Dopaminergic Cell Death Pathways in Parkinson Disease. *Neuron* **2016**, *90* (4), 675–691. https://doi.org/10.1016/j.neuron.2016.03.038.
62. Cookson, M. R. Mechanisms of Mutant LRRK2 Neurodegeneration. *Adv. Neurobiol*. **2017**, *14*, 227–239. https://doi.org/10.1007/978-3-319-49969-7_12.
63. Stoddard-Bennett, T.; Reijo Pera, R. Treatment of Parkinson's Disease through Personalized Medicine and Induced Pluripotent Stem Cells. *Cells* **2019**, *8* (1), 26. https://doi.org/10.3390/cells8010026.
64. McKeith, I. Dementia with Lewy Bodies. Dialogues Clin. Neurosci. 2004, 6 (3), 333–341. https://doi.org/10.31887/DCNS.2004.6.3/imckeith.
65. Di Maio, R.; Barrett, P. J.; Hoffman, E. K.; Barrett, C. W.; Zharikov, A.; Borah, A.; Hu, X.; McCoy, J.; Chu, C. T.; Burton, E. A.; Hastings, T. G.; Greenamyre, J. T. α-Synuclein Binds to TOM20 and Inhibits Mitochondrial Protein Import in Parkinson's Disease. *Sci. Transl. Med.* 2016, 8 (342), 342ra78. https://doi.org/10.1126/scitranslmed.aaf3634.
66. di Domenico, A.; Carola, G.; Calatayud, C.; Pons-Espinal, M.;

DOI: 10.36838/v6i5.17

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. Patient-Specific iPSC-Derived Astrocytes Contribute to Non-Cell-Autonomous Neurodegeneration in Parkinson's Disease. Stem Cell Rep. 2019, 12 (2), 213–229. https://doi.org/10.1016/j.stemcr.2018.12.011.

67. Makarova, K. S.; Wolf, Y. I.; Iranzo, J.; Shmakov, S. A.; Alkhnbashi, O. S.; Brouns, S. J. J.; Charpentier, E.; Cheng, D.; Haft, D. H.; Horvath, P.; Moineau, S.; Mojica, F. J. M.; Scott, D.; Shah, S. A.; Siksnys, V.; Terns, M. P.; Venclovas, Č.; White, M. F.; Yakunin, A. F.; Yan, W.; Zhang, F.; Garrett, R. A.; Backofen, R.; van der Oost, J.; Barrangou, R.; Koonin, E. V. Evolutionary Classification of CRISPR-Cas Systems: A Burst of Class 2 and Derived Variants. *Nat. Rev. Microbiol*. **2020**, *18* (2), 67–83. https://doi.org/10.1038/s41579-019-0299-x.
68. Karginov, F. V.; Hannon, G. J. The CRISPR System: Small RNA-Guided Defense in Bacteria and Archaea. *Mol. Cell* **2010**, *37* (1), 7. https://doi.org/10.1016/j.molcel.2009.12.033.
69. Barrangou, R.; Fremaux, C.; Deveau, H.; Richards, M.; Boyaval, P.; Moineau, S.; Romero, D. A.; Horvath, P. CRISPR Provides Acquired Resistance against Viruses in Prokaryotes. *Science* **2007**, *315* (5819), 1709–1712. https://doi.org/10.1126/science.1138140.
70. Pourcel, C.; Salvignol, G.; Vergnaud, G. CRISPR Elements in Yersinia Pestis Acquire New Repeats by Preferential Uptake of Bacteriophage DNA, and Provide Additional Tools for Evolutionary Studies. *Microbiol. Read. Engl.* **2005**, *151* (Pt 3), 653–663. https://doi.org/10.1099/mic.0.27437-0.
71. Makarova, K. S.; Wolf, Y. I.; Alkhnbashi, O. S.; Costa, F.; Shah, S. A.; Saunders, S. J.; Barrangou, R.; Brouns, S. J. J.; Charpentier, E.; Haft, D. H.; Horvath, P.; Moineau, S.; Mojica, F. J. M.; Terns, R. M.; Terns, M. P.; White, M. F.; Yakunin, A. F.; Garrett, R. A.; van der Oost, J.; Backofen, R.; Koonin, E. V. An Updated Evolutionary Classification of CRISPR-Cas Systems. *Nat. Rev. Microbiol.* **2015**,*13* (11), 722–736. https://doi.org/10.1038/nrmicro3569.
72. McGinn, J.; Marraffini, L. A. Molecular Mechanisms of CRISPR-Cas Spacer Acquisition. *Nat. Rev. Microbiol.* **2019,** *17* (1), 7–12. https://doi.org/10.1038/s41579-018-0071-7.
73. Hochstrasser, M. L.; Doudna, J. A. Cutting It Close: CRISPR-Associated Endoribonuclease Structure and Function. *Trends Biochem. Sci*. 2015, 40 (1), 58–66. https://doi.org/10.1016/j. tibs.2014.10.007.
74. Gleditzsch, D.; Pausch, P.; Müller-Esparza, H.; Özcan, A.; Guo, X.; Bange, G.; Randau, L. PAM Identification by CRISPR-Cas Effector Complexes: Diversified Mechanisms and Structures. *RNA Biol.* **2018**, *16* (4), 504–517. https://doi.org/10.1080/15476286.2018.1504546.
75. Shmakov, S.; Smargon, A.; Scott, D.; Cox, D.; Pyzocha, N.; Yan, W.; Abudayyeh, O. O.; Gootenberg, J. S.; Makarova, K. S.; Wolf, Y. I.; Severinov, K.; Zhang, F.; Koonin, E. V. Diversity and Evolution of Class 2 CRISPR-Cas Systems. *Nat. Rev. Microbiol*. **2017**, *15* (3), 169–182. https://doi.org/10.1038/nrmicro.2016.184.
76. Charpentier, E.; Richter, H.; van der Oost, J.; White, M. F. Biogenesis Pathways of RNA Guides in Archaeal and Bacterial CRISPR-Cas Adaptive Immunity. *FEMS Microbiol. Rev.* **2015**, *39* (3), 428–441. https://doi.org/10.1093/femsre/fuv023.
77. Koonin, E. V.; Makarova, K. S.; Zhang, F. Diversity, Classification and Evolution of CRISPR-Cas Systems. *Curr. Opin. Microbiol*. **2017**, *37*, 67–78. https://doi.org/10.1016/j.mib.2017.05.008.
78. Nidhi, S.; Anand, U.; Oleksak, P.; Tripathi, P.; Lal, J. A.; Thomas, G.; Kuca, K.; Tripathi, V. Novel CRISPR-Cas Systems: An Updated Review of the Current Achievements, Applications, and Future Research Perspectives. *Int. J. Mol. Sci.* **2021**, *22* (7), 3327. https://doi.org/10.3390/ijms22073327.
79. Shin, J. W.; Lee, J.-M. The Prospects of CRISPR-Based Genome Engineering in the Treatment of Neurodegenerative Disorders. *Thr. Adv. Neurol. Disord*. **2017**, *11*, 1756285617741837. https://doi.org/10.1177/1756285617741837.
80. Sasaguri, H.; Nilsson, P.; Hashimoto, S.; Nagata, K.; Saito, T.; De Strooper, B.; Hardy, J.; Vassar, R.; Winblad, B.; Saido, T. C. APP Mouse Models for Alzheimer's Disease Preclinical Studies. *EMBO J.* **2017**, *36* (17), 2473–2487. https://doi.org/10.15252/embj.201797397.
81. Li, H.; Jiang, H.; Zhang, B.; Feng, J. Modeling Parkinson's Disease Using Patient-Specific Induced Pluripotent Stem Cells. *J. Park. Dis*. **2018**, *8* (4), 479–493. https://doi.org/10.3233/JPD-181353.
82. 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. Dopamine Oxidation Mediates Mitochondrial and Lysosomal Dysfunction in Parkinson's Disease. *Science* **2017**, *357* (6357), 1255–1261. https://doi.org/10.1126/science.aam9080.
83. Feng, G.; Jensen, F. E.; Greely, H. T.; Okano, H.; Treue, S.; Roberts, A. C.; Fox, J. G.; Caddick, S.; Poo, M.; Newsome, W. T.; Morrison, J. H. Opportunities and Limitations of Genetically Modified Nonhuman Primate Models for Neuroscience Research. *Proc. Natl. Acad. Sci*. **2020**, *117* (39), 24022–24031. https://doi.org/10.1073/pnas.2006515117.
84. Amit, M.; Shariki, C.; Margulets, V.; Itskovitz-Eldor, J. Feeder Layer- and Serum-Free Culture of Human Embryonic Stem Cells. *Biol. Reprod.* **2004**, *70* (3), 837–845. https://doi.org/10.1095/biolreprod.103.021147.
85. Kolios, G.; Moodley, Y. Introduction to Stem Cells and Regenerative Medicine. Respir. *Int. Rev. Thorac. Dis*. **2013**, *85* (1), 3–10. https://doi.org/10.1159/000345615.
86. Surani, M. A.; Durcova-Hills, G.; Hajkova, P.; Hayashi, K.; Tee, W. W. Germ Line, Stem Cells, and Epigenetic Reprogramming. *Cold Spring Harb. Symp. Quant. Biol.* **2008**, *73*, 9–15. https://doi.org/10.1101/sqb.2008.73.015.
87. Yu, J.; Vodyanik, M. A.; Smuga-Otto, K.; Antosiewicz-Bourget, J.; Frane, J. L.; Tian, S.; Nie, J.; Jonsdottir, G. A.; Ruotti, V.; Stewart, R.; Slukvin, I. I.; Thomson, J. A. Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells. *Science* **2007**, *318* (5858), 1917–1920. https://doi.org/10.1126/science.1151526.
88. Tao, Y.; Zhang, S.-C. Neural Subtype Specification from Human Pluripotent Stem Cells. *Cell Stem Cell* **2016**, *19* (5), 573–586. https://doi.org/10.1016/j.stem.2016.10.015.
89. Karpe, Y.; Chen, Z.; Li, X.-J. Stem Cell Models and Gene Targeting for Human Motor Neuron Diseases. *Pharm. Basel Switz.* **2021**, *14* (6), 565. https://doi.org/10.3390/ph14060565.
90. Paolini Sguazzi, G.; Muto, V.; Tartaglia, M.; Bertini, E.; Compagnucci, C. Induced Pluripotent Stem Cells (iPSCs) and Gene Therapy: A New Era for the Treatment of Neurological Diseases. *Int. J. Mol. Sci.* **2021**, *22* (24), 13674. https://doi.org/10.3390/ijms222413674.
91. György, B.; Lööv, C.; Zaborowski, M. P.; Takeda, S.; Kleinstiver, B. P.; Commins, C.; Kastanenka, K.; Mu, D.; Volak, A.; Giedraitis, V.; Lannfelt, L.; Maguire, C. A.; Joung, J. K.; Hyman, B. T.; Breakefield, X. O.; Ingelsson, M. CRISPR/Cas9 Mediated Disruption of the Swedish APP Allele as a Therapeutic Approach for Early-Onset Alzheimer's Disease. Mol. *Ther. Nucleic Acids* **2018**, *11*, 429–440. https://doi.org/10.1016/j.omtn.2018.03.007.
92. Konstantinidis, E.; Molisak, A.; Perrin, F.; Streubel-Gallasch, L.; Fayad, S.; Kim, D. Y.; Petri, K.; Aryee, M. J.; Aguilar, X.; György, B.; Giedraitis, V.; Joung, J. K.; Pattanayak, V.; Essand, M.; Erlandsson, A.; Berezovska, O.; Ingelsson, M. CRISPR-Cas9 Treatment Partially Restores Amyloid-β 42/40 in Human Fibroblasts with th

e Alzheimer's Disease PSEN 1 M146L Mutation. Mol. Ther. Nucleic Acids 2022, 28, 450–461. https://doi.org/10.1016/j.omtn.2022.03.022.
93. Tubsuwan, A.; Pires, C.; Rasmussen, M. A.; Schmid, B.; Nielsen, J. E.; Hjermind, L. E.; Hall, V.; Nielsen, T. T.; Waldemar, G.; Hyttel, P.; Clausen, C.; Kitiyanant, N.; Freude, K. K.; Holst, B. Generation of Induced Pluripotent Stem Cells (iPSCs) from an Alzheimer's Disease Patient Carrying a L150P Mutation in PSEN-1. Stem Cell Res. 2016, 16 (1), 110–112. https://doi.org/10.1016/j.scr.2015.12.015.
94. Okita, K.; Matsumura, Y.; Sato, Y.; Okada, A.; Morizane, A.; Okamoto, S.; Hong, H.; Nakagawa, M.; Tanabe, K.; Tezuka, K.; Shibata, T.; Kunisada, T.; Takahashi, M.; Takahashi, J.; Saji, H.; Yamanaka, S. A More Efficient Method to Generate Integration-Free Human iPS Cells. *Nat. Methods* **2011**, *8* (5), 409–412. https://doi.org/10.1038/nmeth.1591.
95. Poon, A.; Schmid, B.; Pires, C.; Nielsen, T. T.; Hjermind, L. E.; Nielsen, J. E.; Holst, B.; Hyttel, P.; Freude, K. K. Generation of a Gene-Corrected Isogenic Control hiPSC Line Derived from a Familial Alzheimer's Disease Patient Carrying a L150P Mutation in Presenilin 1. *Stem Cell Res.* **2016**, *17* (3), 466–469. https://doi.org/10.1016/j.scr.2016.09.018.
96. Li, T.; Pires, C.; Nielsen, T. T.; Waldemar, G.; Hjermind, L. E.; Nielsen, J. E.; Dinnyes, A.; Hyttel, P.; Freude, K. K. Generation of Induced Pluripotent Stem Cells (iPSCs) from an Alzheimer's Disease Patient Carrying an A79V Mutation in PSEN1. *Stem Cell Res.***2016**, *16* (2), 229–232. https://doi.org/10.1016/j.scr.2016.01.002.
97. Pires, C.; Schmid, B.; Petræus, C.; Poon, A.; Nimsanor, N.; Nielsen, T. T.; Waldemar, G.; Hjermind, L. E.; Nielsen, J. E.; Hyttel, P.; Freude, K. K. Generation of a Gene-Corrected Isogenic Control Cell Line from an Alzheimer's Disease Patient iPSC Line Carrying a A79V Mutation in PSEN1. *Stem Cell Res.* **2016**, *17* (2), 285-288.https://doi.org/10.1016/j.scr.2016.08.002.
98. Sharma, G.; Sharma, A. R.; Bhattacharya, M.; Lee, S.-S.; Chakraborty, C. CRISPR-Cas9: A Preclinical and Clinical Perspective for the Treatment of Human Diseases. *Mol. Ther. J. Am. Soc. Gene Ther.* **2021**, *29* (2), 571–586. https://doi.org/10.1016/j.ymthe.2020.09.028.
99. Khlidj, Y. What Did CRISPR-Cas9 Accomplish in Its First 10 Years? Biochem. *Medica* **2023**, *33* (3), 030601. https://doi. org/10.11613/BM.2023.030601.
100. An, M. C.; Zhang, N.; Scott, G.; Montoro, D.; Wittkop, T.; Mooney, S.; Melov, S.; Ellerby, L. M. Genetic Correction of Huntington's Disease Phenotypes in Induced Pluripotent Stem Cells. *Cell Stem Cell* **2012**, *11* (2), 253–263. https://doi.org/10.1016/j.stem.2012.04.026.
101. An, M. C.; O'Brien, R. N.; Zhang, N.; Patra, B. N.; De La Cruz, M.; Ray, A.; Ellerby, L. M. Polyglutamine Disease Modeling: Epitope Based Screen for Homologous Recombination Using CRISPR/Cas9 System. PLoS Curr. 2014, 6, ecurrents.hd.0242d2e7ad72225efa72f6964589369a. https://doi.org/10.1371/currents.hd.0242d2e7ad72225efa72f6964589369a.
102. Yusa, K. Seamless Genome Editing in Human Pluripotent Stem Cells Using Custom Endonuclease-Based Gene Targeting and the piggyBac Transposon. Nat. Protoc. 2013, 8 (10), 2061–2078. https://doi.org/10.1038/nprot.2013.126.
103. Ran, F. A.; Hsu, P. D.; Lin, C.-Y.; Gootenberg, J. S.; Konermann, S.; Trevino, A. E.; Scott, D. A.; Inoue, A.; Matoba, S.; Zhang, Y.; Zhang, F. Double Nicking by RNA-Guided CRISPR Cas9 for Enhanced Genome Editing Specificity. *Cell* **2013**, *154* (6), 1380–1389. https://doi.org/10.1016/j.cell.2013.08.021.
104. Cinesi, C.; Aeschbach, L.; Yang, B.; Dion, V. Contracting CAG/CTG Repeats Using the CRISPR-Cas9 Nickase. *Nat. Commun.* **2016**, *7* (1), 13272. https://doi.org/10.1038/ncomms13272.
105. Xu, X.; Tay, Y.; Sim, B.; Yoon, S.-I.; Huang, Y.; Ooi, J.; Utami, K. H.; Ziaei, A.; Ng, B.; Radulescu, C.; Low, D.; Ng, A. Y. J.; Loh, M.; Venkatesh, B.; Ginhoux, F.; Augustine, G. J.; Pouladi, M. A. Reversal of Phenotypic Abnormalities by CRISPR/Cas9-Mediated Gene Correction in Huntington Disease Patient-Derived Induced Pluripotent Stem Cells. *Stem Cell Rep.* **2017**, *8* (3), 619–633. https://doi.org/10.1016/j.stemcr.2017.01.022.
106. Ekman, F. K.; Ojala, D. S.; Adil, M. M.; Lopez, P. A.; Schaffer,D. V.; Gaj, T. CRISPR-Cas9-Mediated Genome Editing Increases Lifespan and Improves Motor Deficits in a Huntington's Disease Mouse Model. Mol. Ther. *Nucleic Acids* **2019**, *17*, 829–839. https://doi.org/10.1016/j.omtn.2019.07.009.
107. Coccia, E.; Ahfeldt, T. Towards Physiologically Relevant Human Pluripotent Stem Cell (hPSC) Models of Parkinson's Disease. *Stem Cell Res. Ther.* **2021**, *12* (1), 253. https://doi.org/10.1186/s13287-021-02326-5.
108. Pinjala, P.; Tryphena, K. P.; Prasad, R.; Khatri, D. K.; Sun, W.; Singh, S. B.; Gugulothu, D.; Srivastava, S.; Vora, L. CRISPR/Cas9 Assisted Stem Cell Therapy in Parkinson's Disease. Biomater. *Res.* **2023**, *27* (1), 46. https://doi.org/10.1186/s40824-023-00381-y.
109. Ahfeldt, T.; Ordureau, A.; Bell, C.; Sarrafha, L.; Sun, C.; Piccinotti, S.; Grass, T.; Parfitt, G. M.; Paulo, J. A.; Yanagawa, F.; Uozumi, T.; Kiyota, Y.; Harper, J. W.; Rubin, L. L. Pathogenic Pathways in Early-Onset Autosomal Recessive Parkinson's Disease Discovered Using Isogenic Human Dopaminergic Neurons. *Stem Cell Rep.* **2020**, *14* (1), 75–90. https://doi.org/10.1016/j.stemcr.2019.12.005.
110. Mazza, M. C.; Beilina, A.; Roosen, D. A.; Hauser, D.; Cookson, M. R. Generation of iPSC Line from a Parkinson Patient with PARK7 Mutation and CRISPR-Edited Gibco Human Episomal iPSC Line to Mimic PARK7 Mutation. *Stem Cell Res.* **2021**, *55*, 102506. https://doi.org/10.1016/j.scr.2021.102506.
111. Soldner, F.; Laganière, J.; Cheng, A. W.; Hockemeyer, D.; Gao, Q.; Alagappan, R.; Khurana, V.; Golbe, L. I.; Myers, R. H.; Lindquist, S.; Zhang, L.; Guschin, D.; Fong, L. K.; Vu, B. J.; Meng, X.; Urnov, F. D.; Rebar, E. J.; Gregory, P. D.; Zhang, H. S.; Jaenisch, R. Generation of Isogenic Pluripotent Stem Cells Differing Exclusively at Two Early Onset Parkinson Point Mutations. *Cell* **2011**, *146* (2), 318–331. https://doi.org/10.1016/j.cell.2011.06.019.
112. 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.; Pavlov, E. V.; Klenerman, D.; Abramov, A. Y.; Gandhi, S. α-Synuclein Oligomers Interact with ATP Synthase and Open the Permeability Transition Pore in Parkinson's Disease. *Nat. Commun.* **2018**, *9* (1), 2293. https://doi.org/10.1038/s41467-018-04422-2.
113. Zhu, X.-X.; Zhong, Y.-Z.; Ge, Y.-W.; Lu, K.-H.; Lu, S.-S. CRISPR/Cas9-Mediated Generation of Guangxi Bama Minipigs Harboring Three Mutations in α-Synuclein Causing Parkinson's Disease. *Sci. Rep.* **2018**, *8* (1), 12420. https://doi.org/10.1038/s41598-018-30436-3.

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