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Review article

CRISPR/Cas9 novel therapeutic road for the treatment of neurodegenerative diseases

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ABSTRACT

CRISPR (clustered regularly interspaced short palindromic Repeats)/Cas9 is a new genetic editing technology that can be a beneficial method to advance gene therapy. CRISPR technology is a defense system of some bacteria against invading viruses. Genome editing based on the CRISPR/Cas9 system is an efficient and potential technology that can be a viable alternative to traditional methods. This system is a compound of a short guide RNAs (gRNAs) for identifying the target DNA sequence and Cas9 protein as nuclease for breaking and cutting of DNA. In this review, recent advances in the CRISPR/Cas9-mediated genome editing tools are presented as well as their use in gene therapy strategies for the treatment of neurological disorders including Parkinson's disease, Alzheimer's disease, and Huntington's disease.

1. Introduction

Recent years have seen an enormous growth of interest in gene therapy. Disease treatment by genetic alterations or even achieving the ability to accurately and purposefully edit any part of the organism's genome has long been a dream for scientists [1]. There are various forms of high-precision gene editing to treat different diseases, including genetic disorders and some kinds of cancer [2]. Gene editing as

Abbreviations: AD, Alzheimer's disease; AP, amyloid plaque; APP, amyloid precursor protein; APOE4, apolipoprotein E4; Bace1, beta-secretase 1; BM, bone-marrow; CRISPR/Cas9, clustered regulatory interspaced short palindromic repeats associated RNA-guided Cas9; CNS, central nervous system; COX2, cyclooxygenase 2; dCas9, dead Cas9; dRCas9, dead RNA-targeting Cas9; DSB, double-strand break; ESC, embryonic stem cell; ER, endoplasmic reticulum; FACE, FACS-assisted CRISPR/Cas9 editing; gRNA, guide RNA; GMF, glia maturation factor; GSAP, γ-secretase activating protein; HO-1, Hemeoxygenase1; HR, homologous recombination; HTT, huntingtin; hESC, human embryonic stem cell; hiPSC, human iPSC; INDELs, insertions and deletions; InsP3R1, inositol 1,4,5-triphosphate receptor type 1; iPSC, induced pluripotent stem cell; LRRK2, leucine-rich repeat kinase 2; mHTT, mutant HTT; MSC, mesenchymal stem cell; MSN, medium spiny neuron; nCas9, Cas9 nickase; nSOC, neuronal store-operated calcium; NHEJ, nonhomologous end joining; NRF2, erythroid 2-related factor 2; NOS2, nitric oxide synthase 2; PAM, protospacer adjacent motif; PD, Parkinson's disease; PK2, Prokineticin 2; PKCδ, protein kinase C-delta; PFF, pre-formed fibrils; RCas9, RNA-targeting Cas9; R7L10, 7-arginine 10-leucine; ROS, reactive oxygen species; SCNT, somatic cell nuclear transfer; pS129-αSyn, serine-129 of α-synuclein; SN, substantia nigra; SSB, single-strand break; ssRNA, single-strand RNA; sgRNA, single guide RNA; SNP, single nucleotide polymorphism; TALENs, transcription activator-like effector nucleases; tracrRNA, trans-activating CRISPR RNA; TH, tyrosine hydroxylase; TRPC1, transient receptor potential canonical 1; uORF, upstream open reading frame; UTR, untranslated region; wt-CRISPR/Cas9, wild-type CRISPR/Cas9; ZFNs, zinc-finger nucleases

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an alternative approach in the treatment of many diseases has made significant progress, as well as significantly developing the ability to accurately and purposefully edit the eukaryotic cell genome [3,4]. Genome editing breaks the specific double-stranded DNA sequence at the desired place in the genome and utilizes the different pathways for DNA repair of cells. Four nuclease classes are employed for cleavage in specific DNA sequences, including meganucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regulatory interspaced short palindromic repeats associated RNA-guided Cas9 (CRISPR/Cas9) [5,6]. Genome editing based on the CRISPR/Cas9 system is an efficient tool that is an excellent alternative to the traditional methods and can benefit gene therapy [4]. CRISPR technology is a defense system of some bacteria against invading viruses, which is used to respond to invading agents such as viruses and is considered as an immune system [7,8]. In this review article, we will present current improvements in the different techniques widely used in gene editing and described therapeutic Advantages and challenges on CRISPR/Cas9 Applications. We will also discuss the development of the CRISPR system's gene therapy strategy for the treatment of neurological disorders, including Parkinson's disease (PD), Alzheimer's disease (AD), and Huntington's disease (HD).

2. The CRISPR/Cas9 system

The CRISPR/Cas9 system is a gene editing and engineering tool for the regulation of gene expression or repair that has developed in medicine and displays a wide perspective of the human genome [9–12]. This system is a combination of a single-guide RNA (sgRNA) for identifying the target location and a Cas9 protein as nuclease for cutting DNA. CRISPR/Cas9 has several types; one type is wild-type-(wt-) CRISPR/Cas9 and Cas9 nickase (nCas9) that can function beyond the editing of the genome. Wt-CRISPR/Cas9 system consists of a nuclease called Cas9 which generates a DNA double-strand break (DSB) and a sgRNA. HNH and RuvC are the catalytic domain of wt-CRISPR/Cas9 and these two parts are responsible for DSBs in the target gene. However, by reprogramming and artificial inactivation either of these two-part, wt-Cas9 turns into nCas9 and leads to single-strand breaks (SSBs), pair of nCas9 can be applied to generate two nicks to reduce off-target cleavage [13]. The duplex RNA used in the CRISPR/Cas9 system is a shortened one and linked together to form a stem-loop structure to attach to the target location [11]. By guiding sgRNA that consists of CRISPR-derived RNA and *trans*-activating CRISPR RNA (tracrRNA) that form the two-RNA structure of sgRNA, Cas9 can exactly knock-out/in marked target genes [12,14]. The sgRNA has a simple and straight structure that is easily designed. The Cas9-based structure can easily connect to almost any position of the genome that the sgRNA is selected complementary [15]. The CRISPR/Cas9 system targets every 20 bp genomic DNA sequence that followed by the 5′-NGG-3′ protospacer adjacent motif (PAM) sequence and the Cas9 nuclease, cleaves 3 bp upstream from the PAM sequence inside the target gene and created DSB in nonhomologous end-joining (NHEJ) or homologous recombination (HR) forms [14,15]. The specificity and efficiency of the CRISPR/Cas9 system are largely dictated by the PAM sequence and the 17–20 nucleotide sequence at the 5′ end of gRNAs [15] (Fig. 1).

The genetic changes applied by the CRISPR/Cas9 are limited to the specific target site and are away from the off-target sequence, which expresses the specificity of the CRISPR/Cas9 system [10]. If both HNH and RuvC domains are inactivated, wt-CRISPR/Cas9 system is converted to dead Cas9 (dCas9), which then can act as a DNA binding tool with high specificity and sensitivity and without endonuclease activity. This system can be conjugated with multiple modifiers like epigenetic effectors for modulation of gene expression [16]. Also, single-strand RNA (ssRNA) can be targeted by designing new Cas/sgRNA to recognize and cleavage target ssRNA. This new tools named RNA-targeting Cas9 (RCas9) and artificial inactivation of Cas9 into dCas9 in this new system leads to the creation of dead RNA-targeting Cas9 (dRCas9) that affect RNA function like splicing, translation, and RNA editing by its fused effectors [17]. With the advancement in target location detection methods in the CRISPR/Cas9 system, and by identifying modified Cas9 enzymes, the risk of off-target activities in the CRISPR/Cas9 system has decreased [10] also CRISPR/Cas9 system can identify multiple target locations easily by using of multiple gRNAs [15].

Despite the great features mentioned for this genome editing system, this system has disadvantages such as binding to untargeted genes which results in the creation of mutations, mismatches, or deletions outside the target sites, and ultimately causes cancer or other genetic conditions [12].

3. CRISPR/Cas9 in Alzheimer's, Parkinson's, and Huntington's diseases

Elderly individuals are more prone to suffer from neurodegenerative diseases because of the existence and accumulation of atypical proteins or peptides such as β-amyloid peptides and phosphorylated tau proteins in AD, α-synuclein in Parkinson's disease, and mutant huntingtin in Huntington's diseases. Generally, aging is the major risk factor for these disorders, in which patients' ability to detoxify or activate self-healing mechanisms is attenuated. Due to the lack of effective treatments, a new approach called CRISPR is drawing a lot of attention nowadays. This approach is one of the new progressively developing strategies and we have fully discussed in the following sections about recent advances of gene therapy in the treatment of neurodegenerative diseases.

3.1. Alzheimer's disease

A growing number of the elderly population suffered from a progressive mental disorder known as Alzheimer disease (AD) which has features such as age-related diffuse loss of cholinergic neurons and accumulation of amyloid-beta (Aβ) peptides in the central nervous system (CNS) soon afterward the accumulated hyperphosphorylated tau protein in intraneuronal neurofibrillary tangles [18]. Since the neuropathological hallmark of AD is the deposition of Aβ, therefore most of the gene therapy strategies for AD have focused on inhibition of the accumulation of neurotoxic species of Aβ via inducing overexpression of either anti-Aβ antibodies to reach passive immunization or Aβ degrading enzymes. In this regard, Hanseul Park and colleagues research lead to efficient gene targeting in post-mitotic neurons of the adult mouse brain by developing novel CRISPR/Cas9 nanocomplexes. Their study showed that adding sgRNA to the CRISPR/Cas9 nano complex could target genes in post-mitotic neurons in the body. Besides, in these nanoparticles, amphiphilic R7L10 peptide (composed of 7-arginine and 10-leucine) was added to Cas9-sgRNA to enhance the efficacy and produce constant R7L10-Cas9-sgRNA nano-complex. Because beta-secretase 1 (Bace1) is needed to produce Aβ peptides, targeting Bace1 is a potential treatment strategy for treating Alzheimer's disease. Accordingly, this team succeeded to target the Bace1 gene, and by suppressing the expression of this gene, inhibits Aβ-associated pathologies in two mouse models of AD by employing this system [19].

Another target of gene therapy in AD is a large intramembrane protein complex known as γ-secretase protease which is regulated by γ-secretase activating protein (GSAP) and evidence showed that reduction in GSAP expression decreases Aβ levels significantly. Nonetheless, evaluations showed that Aβ burden decreases in GSAP RNAi mice crossed with double transgenic AD model mice, so it means that GSAP is a new therapeutic target for the treatment of AD [20]. To further examine the function of GSAP in γ-secretase regulation, the first CRISPR/Cas9 was applied to knockout GSAP gene in HEK293 cell lines that normally express amyloid precursor protein (APP) and then the survey was conducted by Wong. E. et al. and found that lack of GSAP in cells directly decreases γ-secretase activity and so Aβ generation. These findings pave the way for the study of γ-secretase modulation and, eventually, to the improvement of therapeutic tools for AD [21].

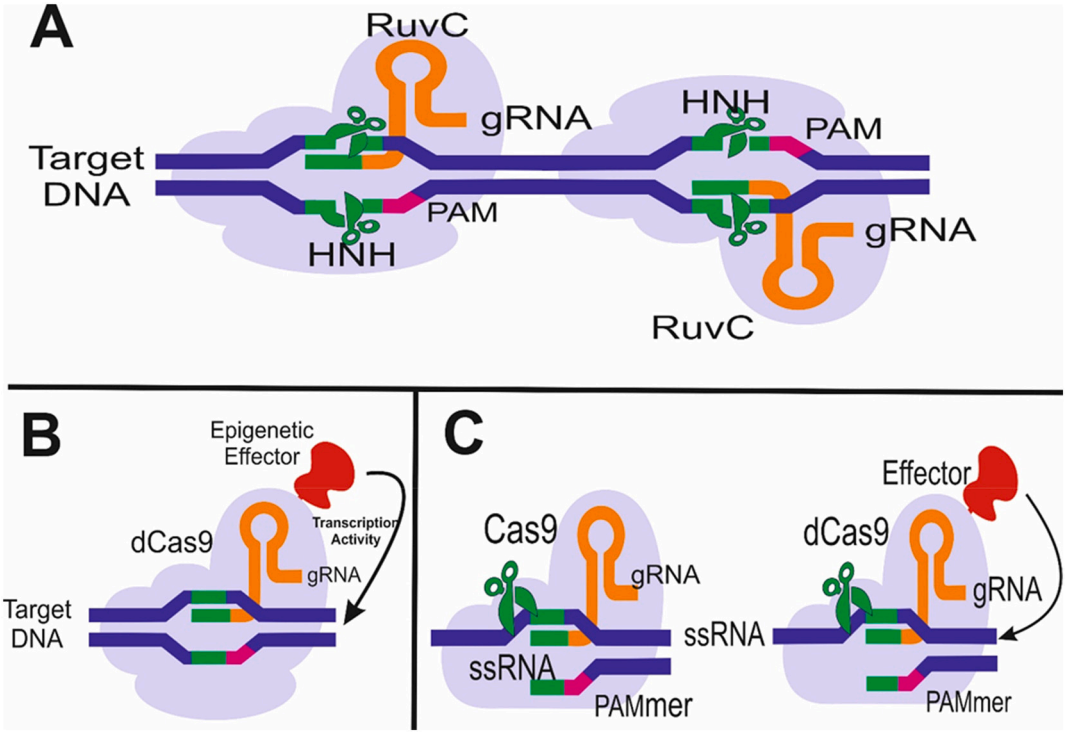

Fig. 1. The schematic representation show CRISPR/Cas9 system functions in gene editing and beyond genome editing. (A) wt-Cas9 consist of two catalytic domains (RuvC and HNH) that cleaving target DNA strand and generating double strand breaks (DSBs). (B) Inactivation of these two nuclease domains, Cas9 turned into dCas9 and dCas9 can be fused with epigenetic effectors. (C) CRISPR/Cas9 can target single strand RNA in this new system PAMmer is essential element that recognize RNA, dead Cas9 (dCas9) can fused with multiple effectors and mediate diverse function on RNA.

Given the above-mentioned therapeutic role of gene editing tools, György. B. et al., to confirm these findings decisively, performed a new study. They investigate the *APPswe* (*Swedish*) mutation in the *APP* gene which is the main reason for inherited AD. Their results indicated that the amount of pathogenic Aβ reduced when the *APPswe* allele disrupted selectively by applying CRISPR/Cas9 from *Streptococcus pyogenes* both in-vivo and ex-vivo. Therefore, this system may pave the way for the development of gene therapy against AD caused by *APPswe* and other mutations associated with elevated Aβ [22].

Moreover, the potential protective effect of a deletion mutation in the 3′-UTR of the *APP* gene has been evaluated by Nagata. K. et al., who edited the *APP* gene via CRISPR/Cas9 in mouse zygotes. Then, they measured quantitatively Aβ and *APP* expression in the edited mouse model and obtained data explain that accumulation of Aβ in the brain mitigated because the expression levels of *APP* were reduced by deletion of the *APP* 3′-UTR [23].

Additionally, one of the definite genetic risk for sporadic AD is the apolipoprotein E4 (APOE4) Allele and varies from the risk-neutral E3 allele via a single nucleotide polymorphism (SNP). The most prevalent SNPs in the APOE gene as a risk factor for sporadic AD are the rs7412 and rs429358, and APOE-ε2, APOE-ε3, and APOE-ε4 (The most common risk factor for Alzheimer's disease). Induced pluripotent stem cells (iPSCs) are an excellent outlook to model AD since these cells have the potential to differentiate in any required cell type. CRISPR/Cas9 technology could be employed on iPSCs from a healthy individual with an APOE-ε3/ε4 genotype with the aim of gaining isogenic APOE-ε2/ε2, APOE-ε3/ε3, and APOE-ε4/ε4 lines along with APOE-knockout line [24]. Besides, in another study from patients who suffered AD and carrying the E4 allele, iPSCs were obtained and altered to the E3/E3 genotype with the CRISPR/Cas9 tool and differentiated into pure cultures of forebrain excitatory neurons. Genetically edited E3 neurons were less sensitive to ionomycin-induced cytotoxicity in comparison with unedited E4 neurons. Consequently, it has been revealed that by using this method E4 neurons produce high levels of phosphorylated tau extracellularly through a heparin sulfate proteoglycan-dependent mechanism in an isoform-dependent manner [25].

In respect to an important role of chronic neuroinflammation in the AD pathogenesis, a proinflammatory molecule called glia maturation factor (GMF) is considerably up-regulated in different parts of AD brains. In addition, reactive glial cells surrounding the amyloid plaques (APs) in the AD brain are the central location of GMF expression. Inflammatory factors such as GMF and proinflammatory cytokines are mainly secreted by microglia. The activation of the microglial cells (brain tissue macrophage cells) initiates a cascade of events that ultimately lead to neurodegeneration and AD pathophysiology. The researchers conducted a study to confirm the hypothesis that CRISPR/Cas9-mediated GMF gene editing directs to the elimination of microglial activation, in which the results were notable. It suggested that GMF gene editing causes inhibition of the inflammatory signaling pathway with suppression of MAPK which was revealed to be upregulated in AD patients [26]. Generally, GMF gene editing by CRISPR/Cas9 considered being a new therapeutic strategy for AD [27] (Table 1).

3.2. Parkinson's disease

Parkinson's disease (PD) is a progressive, age-related, and multifactorial neurodegenerative disorder and one of the most common illnesses of the nervous system characterized by non-motor and motor indications [28]. Since the existence of misfolded proteins which called Lewy bodies and their chief component, α-synuclein is the usual pathology of PD, and it can be postulated that one of the main genes related to PD is the SCNA gene which encodes α-synuclein.

Scientists thought to be a correlation among SNP variants of SNCA and incidence of Parkinson's disease. Furthermore, gradual degeneration of dopaminergic neurons in the substantia nigra (SN) considered as a key pathological feature in patients who suffered PD. However, the complex interactions between environmental factors and human genetics that may cause PD, and the basic mechanisms of pathways and

Table 1
The novel designed CRISPR/Cas9 for treatment of Alzheimer disease.

Target genes	Main findings	In-vitro or in-vivo	Delivery system	Ref
Tyrosine hydroxylase (*Th*), beta-secretase 1 (*Bace1*)	Bace1↓ Th1↓ Aβ↓ Memory impairment↓	Two animal mouse model, 5XFAD as Alzheimer mouse model and wild-type mice	Micelle	[19]
γ-Secretase activating protein (GSAP)	GSAP↓ γ-Secretase activity↓ Aβ↓	HEK-APP cell lines	Plasmid	[21]
Amyloid precursor protein (APP)	APP↓ Aβ↓	Tg2576 mice as mutant models of APP familiar form of Alzheimer	Adeno-associated viral (AAV)-1 vectors	[22]
3′-UTR APP	APP↓ Aβ↓	C57BL/6 mice	px330 plasmid	[23]
APOE-ε3/ε4 APOE	APOE-ε3/ε4 (high risk for AD)↓ APOE-ε2/ε2 (low risk for AD)↑ Turning APOE4 to APOE3↑ Hyper-phosphorylation Tau protein↓ Deposition of amyloid ↓	Induced pluripotent stem cells (iPSCs)	Electroporation with three episomal plasmids	[25]
Glia maturation factor (GMF)	GMF↓ p38 MAPK ↓	BV2 microglial cell line	AAVpro	[56]

proteins associated with that can be identified by CRISPR/Cas9 [29]. Soldner et al. analyzed SNP variants in the SCNA gene by using CRISPR/Cas9 and have documented that the expression level of SCNA enhanced due to the presence of the common variant in a non-coding distal enhancer element which performs through affecting the sequence-dependent binding of transcription factors [30].

Since the genome editing tools could contribute to analyzing PD phenotypes, accordingly, suggested that CRISPR/Cas9 is a useful tool that allows researchers in PD studies to generate isogenic cell lines for PD modeling. In this respect, Arias-Fuenzalida et al. apply CRISPR/Cas9 and fluorescent markers to define a procedure to achieve biallelic genome-edited cell populations. They term this method FACS-assisted CRISPR/Cas9 editing (FACE) and utilize it to derive a set of isogenic cell lines comprising Parkinson's-disease-associated mutations in α-synuclein [31].

Furthermore, the most common genetic cause of sporadic and familial PD is the existence of mutation in the leucine-rich repeat kinase 2 (LRRK2) that induce toxicity in the dopaminergic neuron. The amount of neurite complexity in dopaminergic neurons reduces as a result of using the CRISPR/Cas9 tools and editing of mutated LRRK2 by CRISPR/Cas9 that reduced the incidence of the familial and sporadic form of PD [32].

Among the mutations in the LRRK2 gene, the most common is p.G2019S. Qing et al. used the CRISPR/Cas9 system nuclease-mediated gene editing with the LRRK2-G2019S mutation in patient-derived human iPSC (hiPSCs) and piggyBac transposase technologies. Their observations showed that the percent of tyrosine hydroxylase (TH) positive neurons displayed a significant decrease in LRRK2-G2019S dopaminergic neurons [32].

CRISPR has also provided a broader perception of different PD gene interplays, as well as the recognition of new apoptotic pathways linked with neurodegenerative processes in PD. In a previous study, THAP11 one of the regulators of the Parkin gene, sustained more validation in CRISPR/Cas9 knockout research utilizing various cell types, to find previously undiscovered regulatory networks [33].

Moreover, CRISPR/Cas9 has been employed to investigate neuroinflammatory pathways implicated in PD pathogenesis, for instance, the PKCδ signaling pathway, a main intracellular signaling mechanism operating Mn-induced apoptotic cell death involves activation of the protein kinase C δ. to better understand the PKCδ downstream pathway driving to apoptosis, the CRISPR/Cas9 system was applied to knockdown PKCδ expression in DA neurons (dopaminergic neuronal cells), revealing that downregulation of PKCδ remarkably obstructed Mn-induced DNA fragmentation [34].

Recently Gordon et al. showed that elimination of chemokine termed Prokineticin 2 (PK2) that performs via the Prokineticin receptor 2, with CRISPR/Cas9, enhanced neuronal vulnerability to neurotoxicant-stimulated cell death which was similar to other neuroinflammatory pathways in Parkinson's disease. They also confirmed that PK2 signaling was raised in PD post-mortem brains, and served as a protective compensatory response versus neurodegeneration in PD animal models and cell culture [35].

Although CRISPR/Cas9 has been widely used for creating related cellular or animal models of human diseases, developing the CRISPR/Cas9 technique as a therapeutic tool for gene editing can pave the way for the treatment of human disorders. It can be postulated that the CRISPR/Cas9 system could correct or inactivate mutations caused by both monogenic recessive and dominant-negative disorders to reach therapeutic advantages. Inactivation and depletion of the mutated gene in dopaminergic neurons of PD animal models that express mutant α-synuclein achieved by designing CRISPR/Cas9 which mediated through NHEJ [36]. In this context, the SNCA gene in human embryonic stem cells (hESCs) deleted using CRISPR/Cas9 by Chen et al. these cells were differentiated (into dopaminergic neurons), and then challenged with recombinant α-synuclein pre-formed fibrils (PFFs) to seed the formation for Lewy-like pathology as measured by phosphorylation of serine-129 of α-synuclein (pS129-αSyn). Wild-type neurons were entirely susceptible to pS129-αSyn-positive protein gathering, a pathological sign of Lewy body synucleinopathies, while SNCA + / − and SNCA − / − neurons showed notable resistance to the development of this pathological result [37].

There is a kind of specific cells in the brain called Microglial cells which act as professional phagocytic immune cells and play a vital role in the inflammation-mediated neurodegeneration, particularly in PD. In the brain, a neuroinflammatory protein called GMF is expressed abundantly and revealed that the expression level of this protein upregulated in the SN of PD brains [38].

The impact of GMF editing by applying the CRISPR/Cas9 technique in microglial cells on oxidative stress condition and nuclear factor erythroid 2-related factor 2 (NRF2)/Hemeoxygenase1 (HO-1)-dependent ferritin activation, was investigated by Selvakumar, Ahmed et al. Knockout of GMF in microglial cells weakened oxidative stress by lowering calcium flux and ROS production. Moreover, the absence of GMF decreased nuclear translocation of NRF2, which regulates ferritin activation and HO-1, nitric oxide synthase 2 (NOS2), and cyclooxygenase 2 (COX2) expression in normal microglial cells, and ultimately prevented microglial activation and reduced pro-inflammatory mediators in the brain [39] (Table 2).

Table 2
Overview of the studies on CRISPR/Cas9 editing strategies and Parkinson's disease.

Name of locus	Target genes	In-vitro or in-vivo	Mechanism	Main findings	Ref
PARK7 PARK2 PARK6	DJ-1 Parkin PINK1	In vivo	Co-injection of Cas9 mRNA and multiplexing sgRNAs into in vivo derived pronuclear embryos.	DJ-1 protein↓ Parkin protein↓ PINK1 protein↓	[57]
PARK8	LRRK2	In vivo	The Cre-LoxP recombination system has been used to modify the LRRK2-G2019S mutation in the hiPSCsto produce isogenetic controls.	Production of a footprint-free LRRK2-G2019S isogenic hiPS cell line edited.	[32]
PARK1/4	SNCA	In vitro	Derive biallelic genome-editing via fluorescent markers.	Investigate a set of isogenic lines comprising Parkinson's-disease-associated mutations in a-synuclein.	[31]
PARK2/PINK1	Parkin SNCA	In vivo	CRISPR/Cas9-mediated gene targeting in PFF cells.	The use of gene-targeted somatic cells as a donor for somatic cell nuclear transfer (SCNT) to generate gene-targeted animals with single and identical mutations.	[58]
PINK1	SNCA	In vitro	CRISPR-based deletion of SNCA in hESCs.	SNCA alleles↓	[59]

3.3. Huntington's disease

Huntington's disease is an autosomal neurodegenerative disorder caused by CAG repeat expansions in the huntingtin (HTT) gene which produces an abnormal HTT protein. HTT protein is longer than a normal version of protein due to the addition of several repeats of CAG segments to its gene, which results in the addition of glutamine residue to the protein. Repeats of more than 50 glutamine residues can be reached in some cases. Although there are several therapeutic options for the HD there is no certain cure for it yet [40].

Gene therapy is a promising approach to treat HD disease that currently is under heavy investigation and targets DNA transcription aiming to reduce the level of the abnormal HTT protein or utilizes non-coding RNAs to decrease RNA translation. CRISPR/Cas9 and zinc finger proteins are such two gene therapy approaches that suppress the expression of mutant HTT gene through direct interaction with DNA [41]. Research is underway to develop the application of CRISPR/Cas9 to cure Huntington's disease. It has been shown that CRISPR/Cas9 -mediated inactivation of endogenous mutant HTT (mHTT) expression in the striatum of mHTT-expressing mice can reduce the production of mHTT effectively [19]. In another study, recognized SNPs that have an either causative or destroying effect on PAM motifs are critical in the selection of one allele for CRISPR editing vs. the other to enhancing the efficiency of Cas9 nuclease and applying CRSPR strategy to therapeutic purposes of HD [42]. It has been revealed that the presence of the mutation in the HTT gene leads to the sensitization of type 1 inositol 1,4,5-triphosphate receptor (InsP3R1) and consequently, calcium outflow from the endoplasmic reticulum (ER) and a compensatory elevation in neuronal store-operated calcium (nSOC) entry occurred. Ultimately it resulted in the synaptic damages of striatal MSNs in the HD animal model [43]. Recently, scientists investigated the potential role of Transient receptor potential canonical 1 (TRPC1) inhibition (which is one of the nSOC components) in the HD treatment by using CRISPR/Cas9 technique and the results showed improvement in motor performance and salvage of MSN spines in vitro and in vivo after TRPC1 gene knockout, so inhibition of TRPC1 by CRISPR/Cas9 may serve as a neuroprotective tactic treating of these patients [44].

Recent studies suggested that the 5′ untranslated region (UTR) is important for regulating the synthesis of the HTT protein. 5′ UTR contains an upstream open reading frame (uORF) that encodes a potential polypeptide of 12 amino acids that uORF within 5′ UTR can influence the translation of the downstream ORF. The presence of this uORF has been shown to have a negative effect on expression huntingtin mRNA [45]. Based on this fact, other studies have shown that disruption of uORF in 5′UTR of mRNA via CRISPR-Cas9 could lead to a reduction in the product translation of mutant huntingtin gene mutations in mesenchymal stem cells (MSCs) derived from the HD mice models. Kolli N, et al. investigated inhibiting of mHTT by using two kinds of CRISPR/Cas9 system that one nicks the DNA at untranslated region uORF, and the other system nicks the DNA at exon1-intron boundary. In this study, Kolli N, et al. used plasmid into mesenchymal stem cells (MSCs) extracted from the bone-marrow of YAC128 mice, which carries the human mHTT transgene for HD mice models. Their finding was shown that CRISPR/Cas9 system-mediated silencing of mHTT considerably decreases the production of mHTT in the bone-marrow-derived (BM) MSCs and disrupts the exon1-intron boundary, that influences the translation of the mHTT [46].

In addition to the therapeutic application of CRISPR/Cas9, it has been considered to be an accurate diagnostic method. PCR amplification of the repeat element is the Huntington Disease typical detection method which recognized to have essential limitations in situations that guanine-cytosine content is extremely high in regions associated with HD. So with the aim of improving the detection method of HD, scientists used the CRISPR/Cas9 system to develop a novel protocol. In this respect, using an amplification-free targeted enrichment method enabling to study of the trinucleotide repeat in HTT in clinical HD cases

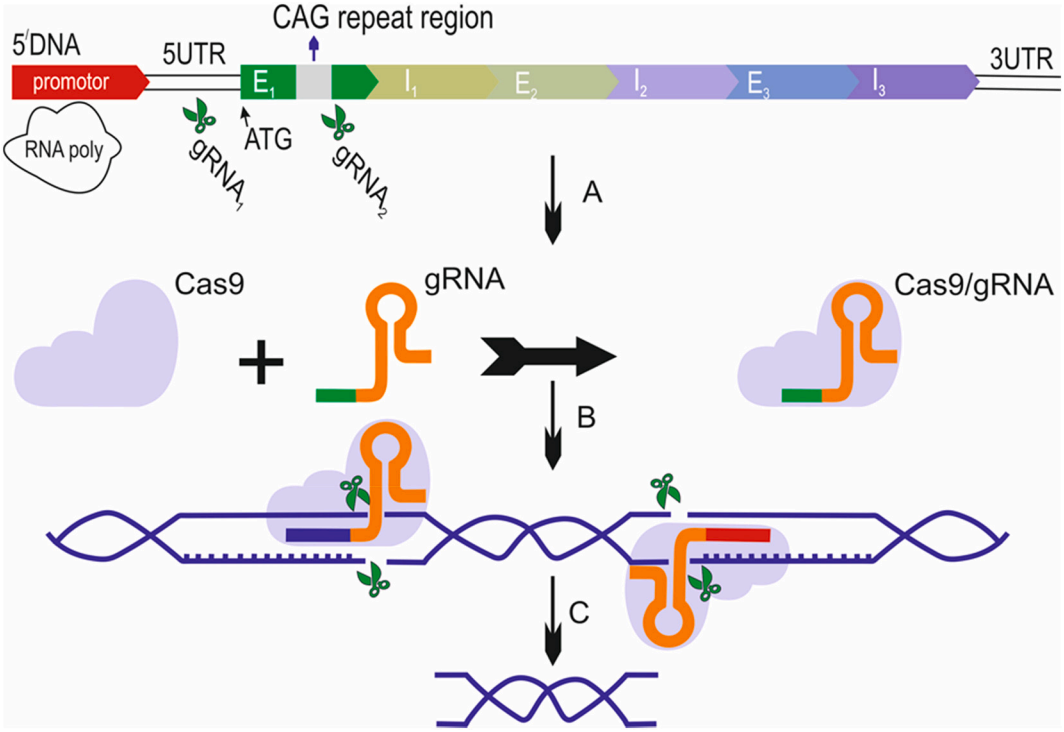

Fig. 2. Schematic diagram of genome editing with CRISPR/Cas9 system. (A) Two different gRNA guide cas9 to designed (guideRNA1 and guideRNA2) induce DNA cleavage at exon1-intron boundary the mutant huntingtin gene (mHTT). (B) CRISPR/Cas9 system. (C) Inhibiting the open reading frame region of the mHTT gene by using the CRISPR/Cas9 system lead to blocking the transcription and decreases the production of mHT.

[47] (Fig. 2) (Table 3).

4. Benefits and challenges of CRISPR/Cas9 applications

Today, the genome editing by CRISPR/Cas9 system is a routine method for all plants and animal models. The unique advantage of the CRISPR/Cas9 system over earlier methods is multiplexable targeting by co-expressing Cas9 with multiple sgRNAs simultaneously [48]. The CRISPR/Cas9 system's main advantages are simple-to-design, easy-to-use, capable of editing multiple genes, cost-effective, and convenient technique for various applications like gene therapy and therapeutic purposes [49]. The potential advantages of CRISPR/Cas9 system are the ability to multiplex genome editing by using multiple sgRNA in parallel to target multiple sites simultaneously in the same cell, although the ZFNs and TALENs by simultaneous using ZFN or TALEN pairs can also function somewhat like CRISPR/Cas9 system [50]. As well as the main advantages of the CRISPR/Cas9 system in comparison with the ZFNs and TALENs are that the ZFNs and TALENs require to build of proteins by using huge DNA fragment 500–1500 bp for each new target site, while the CRISPR/Cas9 is easily adapted to any target sequence of the genome by changing the protospacer of guide RNA (20 bp) [50] and another potential advantage of the CRISPR/Cas9 system is that the only factor that needs to be designed specifically and very accurately is the sgRNA, which significantly reduces time and cost of the experiment and finally the multiplex genome editing process will be simple and entire [51,52].

The main concern of the CRISPR/Cas9 system is the problem of off-target effects, which cause genomic toxicity, carcinogenesis, genome instability, gene functional disruptions, and epigenetic alterations [53]. It has recently been reported that although each nucleotide in the protospacer plays a key role in the specificity of Cas9 and its binding to the target site, however, single mismatches are often tolerated, and if there are multiple mismatches, sometimes can be tolerated and this is depending on their locations in protospacer, their numbers, and their nucleotides [50,53]. Given that genomic changes caused by the CRISPR/Cas9 system are permanent, the effects of off-target should be carefully identified [49,53]. Several methods have been used to reduce off-target effects: the rate of off-target effects is affected by the composition and structure of the sgRNA, so that the sgRNA that represent unique sequences in the genome and a shorter sequence, which has a decreased mismatch tolerance, would suffice [49,54]. Select a target site that has no homology throughout the whole genome is an effective way to reduce off-target effects. Another way that reduces the off-target effects is shortened sgRNA by 2 to 3 nucleotides (protospacer portion) because shortened sgRNA reduce the tolerance of the created mismatch and hence reduce the off-target effects and then can represent the on-target effect [49,50]. Another important factor in decreasing the off-target effects is the control of the CRISPR/Cas9 dosage, which should be carefully considered [53]. The tools used to insert sgRNA and Cas9 into cells also affect the amount of gene-editing activity, and rate of off-target effects. The delivery tools include cell-penetrating-peptide-mediated delivery tool, adenovirus or lentivirus vectors and nonviral physical methods (DNA or RNA injection system), that cause higher on-to off-target effect. For viral vector immune responses is an important challenge of CRISPR/Cas9 System [49,50,53]. Another concern of CRISPR/Cas9 system is the unwanted insertions and deletions (INDELs) (< 20 bp) that rarely occur and if INDELs is too long (up to 600 bp or 1.5 kb) it can lead to pathological defects [10]. Moreover, the size of the Cas9 protein is a key disadvantage, which is larger than a TALEN monomer and much larger than a ZFN monomer and causes Cas9 delivery by viral vectors to be challenging [50].

Finally, with the advancement of CRISPR/Cas9 technology, researchers find the opportunity to carry out complicated gene modifications like point mutations, tag insertions, double-knockouts, and large deletions. To enhance the effectiveness of the CRISPR/Cas9 system, it is essential to understand the details of this system, such as analyzing the crystal structure of the Cas9 protein [55].

Table 3
Overview of investigation on CRISPR/Cas9-mediated gene editing and Huntington's diseases.

Target genes	In-vitro or in-vivo	Delivery system	Mechanism	Main findings	Ref
5′-DNA at the uORF region/exon1-intron region of the mHTT	The use of plasmids in mesenchymal stem cells (MSC) extracted from the bone marrow of YAC128 mice, which carries the transgene for HD.	Plasmid	CRISPR-Cas9 mediated silencing of the mHTT gene in vitro	Production of mHTT↓	[46]
The SNPs at upstream and downstream (Intron1) of HTT exon-1	Fibroblast and HEK293 cell	pX330 plasmid/AAV	CRISPR/SpCas9 system	SNPs are identified that either cause or destroy PAM motifs critical for CRISPR-selective editing of one allele versus the other in cells from HD patients and in a transgenic HD model harboring human allele.	[42]
Transient receptor potential canonical 1 (TRPC1)	YAC128 HD mice/HEK293T cell line	Plasmid	CRISPR/Cas9 technique	Inhibition of TRPC1 by CRISPR/Cas9 may serve as a neuroprotective tactic in the treatment of these patients.	[44]
SNP sites	Fibroblasts cells	Plasmid	Allele-specific CRISPR/Cas9	Permanent inactivation of Huntington's mutation allele by specific CRISPR/Cas9 alleles.	[60]
The polyglutamine repeat in the Huntingtin gene (HTT)	Mouse striatum	AAV	CRISPR/SpCas9 system	Permanent elimination of polyQ domain of mHTT using non–allele-specific CRISPR/Cas9 mediated neuronal toxicity in the adult brain.	[61]

5. Conclusion

Aging and age-related disease by disturbing homeostasis over time increase the risk of many chronic disorders and degenerative conditions, eventually ending with death. Also, epigenetic changes have a key role in age-related disease. In another hand, the accumulation of abnormal proteins and peptides in CNS is one of the most important characters in neurodegenerative diseases such as Alzheimer and Parkinson. Thus, gene therapy by correcting gene sequences, and therefore the proteins involved in these diseases is a suitable strategy for the treatment of neurodegenerative diseases. Studies have shown that the CRISPR/Cas9 system could be a potential tool for genome editing in targeting age-related diseases. It is essential to develop and improve the efficiency of in vivo CRISPR/Cas9 gene-editing before it can be used clinically. As a result, the CRISPR/Cas9 modification tool has several advantages over other methods and tools for improving and treating cell lines or animal models for neurodegenerative disease, infectious diseases, monogenic diseases, cancer, and age-related diseases.

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Author contributions

Ansar Karimian, Negar Gorjizadeh and Forough Alemi participated in writing article. Maryam Majidinia, Zatollah Asemi and Khalil Azizian participated in the data collecting. Niloufar Targhazeh and Faezeh Malakouti involved in draw figures. Bahman Yousefi and Jafar Soleimanpour participated in the study design, manuscript drafting and revising.

Declaration of competing interest

None.

Ethical approval

This article does not contain any studies with human participants.

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