The Role of Gene Editing in Neurodegenerative Diseases

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Abstract

Neurodegenerative diseases (NDs), at least including Alzheimer’s, Huntington’s, and Parkinson’s diseases, have become the most dreaded maladies because there are no precise diagnostic tools or definite treatments for these debilitating diseases. The increased prevalence and a substantial impact on the social–economic and medical care of NDs propel governments to develop policies to counteract the impact. Although the etiologies of NDs are still unknown, growing evidence suggests that genetic, cellular, and circuit alternations may cause the generation of abnormal misfolded proteins, which uncontrollably accumulate to damage and eventually overwhelm the protein-disposal mechanisms of these neurons, leading to a common pathological feature of NDs. If the functions and the connectivity can be restored, alterations and accumulated damages may improve. The gene-editing tools including zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats–associated nucleases (CRISPR/CAS) have emerged as a novel tool not only for generating specific ND animal models for interrogating the mechanisms and screening potential drugs against NDs but also for the editing sequence-specific genes to help patients with NDs to regain function and connectivity. This review introduces the clinical manifestations of three distinct NDs and the applications of the gene-editing technology on these debilitating diseases.

Keywords

neurodegenerative diseases (NDs), Alzheimer’s disease (AD), Huntington’s disease (HD), Parkinson’s diseases (PD), zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeats–associated nucleases (CRISPR/CAS)

Introduction

Neurodegeneration is the overarching term for medical conditions with progressive failure of neuronal networks and eventually the death of neurons participating in motor, sensory, and cognitive functions. Neurodegenerative disorders (NDs), at least including Parkinson’s disease (PD)1, Huntington’s disease (HD)2, Alzheimer’s disease (AD)3, and so on, are complex and multifactorial diseases that threaten human health and have no specific diagnostic tests or effective therapies. Since the number of cases is rapidly growing worldwide and the World Health Organization predicts that NDs will overtake cancer in the rank of top causes of death by 20504, the pressure on social–economic and the financial burden of medical care system propel governments to develop policies to counteract the impact. Pathophysiologically, there are several mechanisms underlying NDs, including an excessive abnormal structural aggregation-prone proteins accumulation5; impaired ubiquitin–proteasome and/or autophagy–lysosomal pathways6; apoptosis and 1 Department of Pediatrics, Tungs’ Taichung Metroharbor Hospital, Taichung, Taiwan
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autophagy; glutamate transporters; calcium, free radicals, and mitochondria; and so on. These versatile mechanisms suggest that NDs are caused by a complex interplay of many genetic factors, each of which acts individually or symptomatically to lead to clinical features. Disrupting gene expression is a common approach to investigating the functions of these genes. Classical strategies to assess the functions of these genes include RNA interference (RNAi) and homologous recombination (HR). RNAi is a rapid, inexpensive, and high-throughput method to knock down a specific gene. This technique has been applied to cell lines, primary cultures, or animal models. RNAi was the “gold standard” for gene silencing and studying gene function in vitro and in vivo in the past. However, several drawbacks regarding this technique include the following: (1) this technique is difficult to transfet multiple genes to a cell or animal in vitro or a gene to an adult animal in vivo; (2) effects of the mutant-selective RNAi targeting single nucleotide may be variable, incomplete, and temporary in different experiments and laboratories; (3) RNAi cannot generate stable gene knockouts or site-specific epigenetic modifications; and (4) this technique may produce unpredictable off-target effects. These defects may restrict the use of RNAi in the clinical practice. HR in mouse embryonic stem cells is a common and popular method of building up genetically modified animals for modeling human diseases. However, the drawbacks of this technique include the following: (1) HR is time- and labor-consuming, (2) HR is of low efficiency, and (3) HR has the potential for unwanted mutagenic effects. Recent advances in gene-editing techniques including zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats–associated nucleases (CRISPR/CAS) can accelerate the pace of biological research, generate sets of gene-related disease models, and provide potential therapies targeting the incurable diseases. This review will focus on the clinical features of three distinct NDs: PD, HD, and AD, and the applications of gene-editing technology on these three debilitating diseases.

General Neurodegenerative Diseases

PD. PD is an age-related, progressive, disabling, and motor neuron degenerative disorder. Prevalence is estimated to be more than 1% of 60-y-old individuals with PD and 4% of those ages over 80. The prevalence of PD in North America and Europe is 100 to 250/100,000, Japan 118.7/100,000, and Taiwan 84.8/100,000. An increased trend in the annual prevalence rates of PD was reported in Asia and Europe. The initial presentations are subtle because at least 70% of neurons in PD patients are degenerative damaged or completely lost before the onset of typical symptoms including tremor, rigidity, and hypokinesia. As PD progresses, the disease course spawns and neuronal loss becomes more widespread, leading to dementia and hallucination. Pathological investigations have revealed that in 80% to 90% of the cases, the clinical diagnosis of PD was confirmed at autopsy. Common PD pathology includes degeneration and loss of the neurons in the substantia nigra of the midbrain, and at least 13 loci and 9 genes linking to PD, both suggesting that PD is a genetic disease. Familial PD accounts for 5% to 10% of all PD cases and can be divided into an autosomal dominant (AD) or an autosomal recessive pattern. Six genes are connected to Mendelian patterns of PD. Alpha synuclein (SNCA) and leucine-rich-repeat kinase 2 (LRRK2) are associated with ADPD, while Parkin, phosphatase and tensin homolog–induced kinase 1 (PINK1), DJ-1, and ATPase type 13A2 (ATP13A2) are linked to autosomal recessive Parkinson’s disease (ARPD). SNCA proteins aggregating to form Lewy bodies and Lewy neuritis and causing numerous detrimental consequences on eurons are the major pathological characteristics of PD. Although a combination of pharmacological treatment with nonpharmacological interventions may temporally alleviate symptoms, none of them can completely cure this disease.

HD. HD is a rare, autosomal dominant, and progressive neurodegenerative disease. The prevalence of HD in Europe is 0.1 to 0.8/100,000, United States and Canada 0.3 to 0.69/100,000, Japan 0.65/100,000, and Taiwan 0.08 to 0.42/100,000. Mean age at onset of symptoms is 30 to 50 y. Typical features of HD include the development of chorea, dystonia, bradykinesia, motor incoordination, and behavioral or psychiatric features such as personality changes, poor attention, cognitive decline, irritability, and dementia. The progression of the disease results in a complete dependency in daily life and requiring full-time care and finally death approximately 15 to 20 y after disease onset. With the help of the advanced molecular biology, the diagnosis of HD can be made by a DNA test, which demonstrates a polyglutamine expansion in the Huntingtin gene (HTT) with more than 40 copies within the amino-terminal region of the Huntingtin protein (HTT), and the mutant HTT (muHTT) is efficient for diagnosis. Animal studies showed that ablation of HTT gene in mice led to death when they were embryonic day 7.5 old because of aberrant brain development, suggesting the essential role of HTT gene in the cell development and survival. So far, there is no treatment for HD.

AD. AD is a chronic and progressive neurodegenerative disorder and is the most common cause of dementia. The onset of AD occurs predominantly in elderly subjects in their 60s. Studies that examined age consistently found that prevalence and incidence of AD increased with age and an estimated 5% of individuals 65 y of age may develop dementia due to AD as well as 30% of individuals 85 y of age or older. The prevalence of AD in Europe is 3% to 4%. United States 7%, Japan 2%, China 5%, and Taiwan 4%. The World Health Organization estimated 47.5 million people with dementia worldwide, and these numbers may double by 2030 and triple by 2050. The degenerative process in the
central nervous system (CNS) is related to (i) genes; (ii) amyloid β (Aβ), which is processed from amyloid precursor protein (APP) through the sequential cleavage by β-secretase and γ-secretase; (iii) neurofibrillary tangles, which is hyperphosphorylated microtubule-associated protein tau (MAPT) and other pathological changes associated with this neurodegenerative disorder; (iv) inflammation; (v) gliosis; (vi) oxidative stress; (vii) neuronal dystrophy; (viii) neuronal loss; (ix) synapse loss; (x) altered levels of neurotransmitter; (xi) cell cycle; and (xii) Apolipoprotein E (apoE)32. These pathological changes are correlated with the progressive deficit in memory, predominantly short-term memory loss in early stages33. As AD progresses, cognitive function deteriorates, and the brain is globally atrophic34,40. There is no curative treatment for AD35.

**Principles of Gene-Editing Tools**

Gene editing is a chimera of specific DNA-binding domains (DBDs) and nonspecific DNA cleavage domains (DCDs). DBDs enable efficient and precise-targeting sequence binding. DCDs, like genomic scissors, cleave the targeted DNA site to produce a double-strand break (DSB), which consequently stimulates the cellular DNA repair mechanisms including error-prone nonhomologous end joining (NHEJ) and homology-directed repair (HDR)36. The HDR searches for homology between the damaged DNA sequence and the sister chromatids, homologous DNA strands, or other related DNA as templates and copies the sequence of the fragment between the 2 broken ends of the damaged sequence fragments to restore the original DNA sequence at DSB sites, regardless of whether the fragment contains the original sequence37. Based on the machinery, the designed DNA can then be inserted into the targeted cleavage site and NHEJ directly connects the end of the broken strands. The repair process can be error-prone, resulting in small insertions, deletions, and/or rearrangements41. NHEJ can also cause frameshifts in the coding sequence of a gene to produce premature truncations, leading to an effective gene knock-out. The status of cycle phase in the target cells determines which DNA repair mechanisms will be initiated. HDR initiates in the synthesis (S) and the premitotic (G2) because sister chromatids are available at these phases42. NHEJ activates in the growth 1 (G1) and the mitotic (M) phases43. DSB repair mechanism in mammals is mainly through NHEJ44. The repair systems are crucial in the maintenance of genomic integrity and the generation of genetic variability.

**ZFNs.** ZF domain is one of the most common conserved structures in the human beings45. ZF was initially discovered as a DNA-binding motif in transcription factor IIIA (TFIIIA) in the *Xenopus laevis*46. Functions of ZF include DNA recognition, lipid binding, mRNA trafficking, transcriptional activation, chromatin remodeling, protein folding and assembly, regulation of apoptosis, zinc sensing, cytoskeleton organization, epithelial development, and cell adhesion47. The structure of ZF is composed of 2 cysteines and 2 histidines (Cys2His2), which tetrahedrally binds a zinc ion to form a compact structure48 (Fig. 1A). A single ZF domain cannot be used to bind a specific DNA sequence49, and therefore, the modular feature of the ZFs enables them to assemble into a linear array to target focused sequences50,51 (Fig. 1B).

ZFNs, the first genome-editing tool used in zebra fish52,53, can generate gene point mutations, deletions, insertions, inversions, duplications, and translocations in a complex genome. ZFNs have been applied to cell and animal biotechnology and with potential therapeutics54. Structurally, ZFNs are a chimeric fusion between a customer-designed ZFDBD and a nonspecific DCD. ZFDBD typically contains between 3 and 6 repeated ZFs (Fig. 1C). Each ZF can recognize approximately 3 bp of DNA55. It is difficult to design effective and efficient ZFs because the binding site of ZF and the neighboring ZF moiety may affect the binding affinity. Several tools can assist users including the selection methods56, the module assembly (MA)57, the oligomerized pool engineering (OPEN) program58, the bacterial one-hybrid52, and the context-dependent assembly (CoDA) program59. However, the selection-based methods are labor-intensive and time-consuming57. The MA uses hundreds of assembled ZFNs to target dozens of genomic sites. Theoretically, the MA is simple and fast, but it is reported to have a very low probability to successfully generate active ZFNs60. The OPEN program uses multiple and parallel low-stringency selections for binding of randomized ZFs to each triplet in the targeted sequence and ZFs from these pools are linked, and the products are selected at high stringency for binding to the final target. The OPEN has a higher success rate than the MA; however, complex, time-consuming, and labor-intensive processes and expertise required to screen combinatorial libraries have restricted its broad applications56. The bacterial one-hybrid is similar to OPEN but a different strategy for the construction of the library55. For each target triplet, a library is assembled that randomizes only a subset of residues at the ZF-DNA interface. At the remaining positions, specificity is achieved by the chosen residues to contact each other well52. The CoDA is a publicly available platform of reagents and software that is simple to practice and requires no specialized expertise. Multi-ZF arrays can be constructed in 1 to 2 wk. The ZF design of the CoDA is based on the OPEN program59. Together, with the approach of these programs, a customized array of individual ZF domains assembled into ZFNs can be designed to target a larger DNA sequence.

DCD consists of the type II restriction enzyme FokI, with the molecular weight of 65.4 kDa and is found in *Flavobacterium okeanokoites*61. FokI exists as an inactive monomer and turns into genomic scissors to cleave the targeted DNA site to produce a DSB when it becomes an active dimer62. Therefore, a pair of ZFNs was needed to bind opposite strands of DNA with their C-termini a certain distance apart and the cleavage domain requires the 5′ edge of each binding
site to be separated by 5 to 7 bp. When ZFNs and donor DNA are ready, viral vectors, liposome transfection, or electroporation have been used to deliver them into the target cells. Once ZFNs are bound to the duplex DNA at the 5′-GGATG-3′ recognition site, the FokI endonuclease, which is a non-specific DCD, will cleave at the first strand 9 nucleotides downstream and the second strand 13 nucleotides upstream of the nearest nucleotide of the recognition to generate DSBs at specified loci36 (Fig. 1C). Exonuclease can be introduced to digest the ends of the strands generated at DSBs64. The generated DNA defects need DSB repair systems to yield new DNA mutations. Also, FokI variants requiring heterodimerization have been developed to increase ZFNs and target DNA sequences65.

TALENs. Like ZFNs, TALENs can be customized to recognize specific DNA sequences. Structurally, TALENs contain a domain that activates the target gene transcription (transcription activator-like effector [TALE]), a DCD, and a nuclear localization signal66. TALENs generate DSB at specific loci, which initiates the DNA repair machinery, thereby that these mutations are transmitted through the germ line67. Although previous reports showed that the efficiency of TALENs was similar to that of ZFNs68, recent comments on this technique concluded that the easier usage and higher targeting capacity and successful rate of targeting DNA sequences are higher than ZFNs69,70.

The discovery of the DNA-binding element, Xanthomonas, was a breakthrough of the gene-editing technology. Xanthomonas, which are bacteria and may cause serious damage to plants such as rice, pepper, and tomato, secrete virulence factors (TALEs) that bind to the genomic DNA of the plant. TALEs act as transcriptional activators to upregulate or downregulate the expression of target genes, thereby facilitating pathogenic bacterial colonization71. The TALEs contain a DBD composed of highly conserved 33 to 35 amino acid tandem repeats that are largely identical72 (Fig. 2). The 12th and 13th amino acid positions, referred to as the repeat variable diresidue (RVD), show high variabilities and have a specific DNA recognition72. TALENs can be customized to target specific DNA sequences by designing appropriate RVDs. Crystal analysis of 3 dimensional structures of TALE-DNA complexes revealed that the amino acid 8 and 12 within the same repeat contacting with each other may

Fig. 1. Schematic diagram of zinc-finger nucleases (ZFNs). Table indicates references regarding applications of ZFNs in neurodegenerative diseases (NDs) including Parkinson’s disease (PD), Huntington’s disease (HD), and Alzheimer’s disease (AD). (A) Structure of zinc finger (ZF). The paired cysteines (Cys) and histidines (His), (Cys2His2) tetrahedrally bind a zinc ion to form a compact structure. (B) Typical arrangement of C2H2 ZF motifs. The modular feature of the ZFs enables them to be assembled into a linear array to target DNA. (C) Structure of ZFNs. ZFNs consist of 2 functional domains, including a ZF DNA-binding domain includes a chain of 3 finger modules (ZF1 to ZF3). Each ZF can recognize a 3 bp of DNA; a DNA cleavage domain is comprised of the nuclease domain of the FokI. However, this technique requires 2 ZFNs to bind at or near the cleavage site because the FokI needs to form a dimer, and then it will function properly. Also, the target sequences must be separated by 5 to 7 base pairs to allow formation of the catalytically active FokI dimer, causing a double-strand break at a specific sequence to trigger the cell DNA repair machinery including homology-directed repair and nonhomologous end joining to repair the defects resulting in targeted gene disruptions or gene integration.
stabilize the structure of the domain. The amino acid 13 mediates specific recognition of the sense-strand DNA base. Before the 5'-end of a sequence bound by a TALE monomer, the target DNA molecule always contains the same nucleotide, thymidine (T), and the indole ring of tryptophan 232 of the N-terminal of the DBD, which may interact with 5'-T. These 2 nucleotides may affect the efficiency of TALENs binding to the target site. The DCD of TALENs contains the FokI endonuclease. As mentioned above, the FokI needs to form a dimer to function properly. The design of TALENs should consider that the DCD requires 2 constructs with unique DBDs in proper orientation and spacing, and then the TALENs can function well. Also, the type of the FokI endonuclease may affect the cleavage specificity and activity, and the mutated FokI endonucleases are even better than the wild-type one. Both the number of amino acid residues between the TALE DBDs and the DCDs and the number of bases between the 2 individual TALEN-binding sites may affect the results of the reactions.

CRISPR-CAS, a revolutionary gene-editing technology. CRISPR, an array of short repeated sequences separated by spacers with unique sequences, is a common feature of prokaryotes. These repetitions were first noted by Ishino and his colleagues in Escherichia coli. The sequence in the exogenous nucleic acid element corresponding to a CRISPR spacer was defined as a protospacer (Fig. 3, green coil). The protospacer is usually flanked by a highly conserved aN base-Guanosine-Guanosine (NGG) motif-protospacer adjacent motif (PAM; Fig. 3, red-marginated rectangle). Most PAMs contain 2 to 5 highly conserved nucleotides. PAM is an unique and critical component of the invading DNA because CRISPR/CAS needs it to identify and destroy the foreign DNA. The functions of the CRISPR/CAS were further clarified by the dairy industry. Bacteriophage contamination has been a serious problem for the dairy product business in which lactic acid bacterium, such as Streptococcus Thermophilus, is used to ferment milk into an array of products (e.g., cheese and yogurt). Once the bacteriophages infect the S. thermophile, the dairy processes are significantly impaired. To overcome this infection, the dairy industry sequenced the bacteriophage-insensitive strains and accidentally found some short, partially palindromic DNA repeats (CRISPR repeats; Fig. 3, black rectangle) in the bacteria. CRISPR/CAS is an innate immune system to eliminate invading DNA or RNA.

The functions of CRISPR/CAS systems can be divided into 3 steps, including adaptation, expression, and interference. First, “adaptation” involves recognition and integration of foreign DNA as a new spacer (Fig. 3, green diamond) within the CRISPR locus. The protospacer contains a short stretch (2–5 bp) of conserved nucleotides (PAMs) that act as a recognition motif. The insertion of a single copy of spacer of approximately 30 bp occurs at the leader side of the CRISPR array and is followed by its duplication. Any mutations in the PAMs of the viral genome can interfere with the activation of CRISPR-mediated immunity against pathogen attacks. In the “expression,” the CRISPR array(s), including repeat and spacer sequences, is transcribed to precursor of CRISPR RNAs (pre-crRNAs) that undergo maturation to generate crRNA with the help of CAS proteins (CAS1, CAS2, CAS9, and CAS4) and the trans-activating crRNA (tracrRNA) molecule. crRNA is composed of a repeat portion and an invader DNA (protospacer) portion. The tracrRNA also participates in the processing of pre-crRNA. The tracrRNA is combined with crRNA via base complementarity to form a tracrRNA-crRNA complex. TracrRNA facilitates the processing of pre-crRNA into mature crRNA. The processed crRNAs enter the CRISPR-associated complex for antiviral defense and help to recognize a specific target region of the foreign DNA. In the “interference” process, crRNA guide CAS proteins to cleave foreign nucleic acid at sites complementary to the crRNA spacer sequence to process foreign genetic elements into small DNA fragments (Fig. 3). The location of small clusters of cas genes is closed to CRISPR repeat–spacer arrays. There are more than 45 cas gene families. Functions of CAS proteins included nucleases, RNase and/or DNase activity, helicases, and RNA-binding proteins. CAS1 protein is a basic and metal-dependent
DNase and involved in the integration of spacer DNA into the CRISPR locus. CAS3 is a part of the cascade complex. CAS1 and CAS2 proteins are involved in adaptation. Cas4, a RecB-like exonuclease, is involved in spacer acquisition. CAS5, CAS6, and CAS7 are possibly related to repeat-associated mysterious proteins (RAMPs), which...
Table 1. Different Classes of the CRISPR/CAS System and Cas Proteins.

| Class | Type | CAS Proteins | Target |
|-------|------|--------------|--------|
| 1     | Type I | CAS1, CAS2, CAS3, CAS5, CAS6, and CAS7 | DNA |
| 2     | Type II | CAS1, CAS2, CAS3, and CAS9 | DNA |
| 1     | Type III | CAS1, CAS2, CAS6, CAS10, and RAMPs | DNA/RNA |
| 1     | Type IV | CAS1, CAS2 | |
| 2     | Type V | ? | ? |

are involved in crRNA processing. CAS9 involves in crRNA processing and cleaves the target DNA. CAS9 protein shows helicase and contains 2 endonuclease domains: the HNH (an endonuclease domain named for characteristic histidine and asparagine residues) and the RuvC (an endonuclease domain named for an E. coli protein involved in DNA repair). Each domain cleaves one strand of double-stranded DNA and induces DSBs, which initiate cellular DNA repair machinery. The CAS10 protein is associated with crRNA processing and targeting DNA cleavage.

The CRISPR/CAS system is divided into 2 major classes incorporating 5 types of systems, and each system uses distinct molecular mechanisms to achieve nucleic acid recognition and cleavage, and diversity of CAS proteins may be linked (Table 1). Class 1 is divided into types I, III, and IV; class 2 is divided into types II and V. Among these 5 types, only 3 of them had been studied in detail. In common, the types I, II, and III of CRISPR/CAS all have CAS1 and CAS2 proteins. The type I system, which is found in both bacteria and archaea, uses a complex of multiple CAS proteins, such as CAS3, to degrade foreign nucleic acids. All type I systems encode a cascade-like complex, which binds crRNA and locates the target. Type I and II systems target DNA, and type III systems target DNA and/or RNA. The type II CRISPR/CAS has been found in bacteria. It contains CAS1, CAS2, and CAS9. Additionally, the presence of PAMs is characteristic of the type II system. Synthetic type II system requires a single protein for RNA-guided DNA (gRNA) recognition and cleavage. gRNA is a chimeric RNA containing all essential crRNA and tracrRNA components. CRISPR from Prevotella and Francisella (Cpf1), a single RNA-guided nuclease, can be used for genome editing without tracrRNA. Specificity of the gRNA is established through a 20 nucleotide homology to the target region that is followed by a 5′-NGG PAM (Fig. 3). The type II system is becoming the most popular system for eukaryotic genome engineering applications for its convenience and good cost effect. The type III system contains CAS10 and CAS6 proteins in addition to the RAMPs. The type IV system does not have CRISPR, cas1, or cas2 and is guided by protein-DNA complex, not by crRNA. The type V (class 2) systems share common features with the type I system, but detailed mechanisms are not clear.

The Status of Disease Model Production and Application

ZFNs. ZFNs have been used in plants, animal, and stem cell models, a mouse model of hemophilia, and a potential treatment of HIV/AIDS in phase 2 clinical trials, and modifying disease-causing alleles in triplet repeat disorders. In PD, the missense mutation of SNCA gene can be genetically corrected by ZFNs in vitro. The level of neurite length in the corrected induced pluripotent stem cell (iPSC) by ZFNs was greater in the mutated cells with genetic correction than the mutated cells without correction. Additionally, the levels of α-synuclein and MAPT expression were increased in the mutated cells but not in the genetically corrected ones. Patient-derived human iPSCs harbor the genetic information of the donor, enabling to generate several neurological disorders, including PD, HD, and AD. It is reported that PD iPSCs with the A53T mutation in the SNCA gene can be genetically repaired by ZFNs without affecting other part of genomes in the stem cells. The neural cells of PD iPSCs with the G2019S and R1441C mutations in the LRRK2 gene were vulnerable to mitochondria-associated stress. After ZFNs correction, the mtDNA damage was no longer detected in differentiated neuroprogenitor and neural cells from iPSCs. After genetically corrected stem cells may be able to infuse safely to the patient to reverse abnormal phenotypes, leading to a promising iPSC-based cell replacement therapy. In HD, in vitro studies showed that the expression levels of the mutant gene were significantly decreased at both the protein and mRNA levels through ZFNs, and in vivo study showed that ZFNs via adeno-associated virus (AAV) delivery injected into the striatum of the HD mice substantially repressed the mutant HTT in the brain and improved their functions. Sangamo Bioscience, USA designed a ZFN drug to target the mutant DNA sequence. The treatment repressed the expression of the HTT gene in a mouse HD model and improved HD-related symptoms. The treatment also decreased the expression of the mutant HTT in the human fibroblasts and embryonic-derived neurons. In AD, mouse fibroblast cells could overexpress APP by ZFNs. Loss-of-function mutations of β-secretase have been successfully introduced into the zebra fish genome by using ZFNs, suggesting that this novel technology may hold promise in the treatment of genetic disorders.

TALENs. TALENs can improve food crops and modify or knock out endogenous genes in Caenorhabditis elegans, zebra fish, rat, human stem cells, and iPS cells. Several disease models, such as tuberculosis-resistant cattle, a familial hypercholesterolemia rat model, or T cells with resistance to chemotherapeutic drugs, are generated with this novel technique. TALENs are applied to treat human genetic diseases such as sickle cell disease, xeroderma pigmentosum, and epidermolysis bullosa. The first human trial with this...
technique was genetically engineered T cells in an 11-mo-old girl with refractory leukemia in the UK, and the results were good without any significant toxicity. In PD, a heterozygous glucocerebrosidase 1 mutation (GBA1$^{+/−}$) was a risk factor for PD. A PD model is generated when the genome of zebrafish was inserted with a mutated GBA1 via TALENs. In HD, the HTT exon 1 in human iPSCs derived from HD patient fibroblasts (HD-iPSCs) was corrected by TALENs. The treatment normalized dysregulated cadherin, transforming growth factor-β, BDNF pathways, caspase activation, and reversed HD-iPSCs phenotypes including susceptibility to cell death and altered mitochondrial bioenergetics in neural stem cells. In AD, since mutations in the gene-encoding APP have been linked with the progression of AD, A673V variant, near the APP β-secretase cleavage site, contributed to AD pathology by increasing the Aβ and enhancing aggregation and toxicity. A673T variant showed protection against AD. A673V and A673T were introduced into normal iPS cells through TALENs. These cells then differentiated to develop cortical neurons, which showed variant levels of AD-related biomarkers. Although TALENs are powerful and have numerous advantages, however, the size of DNA or mRNA affecting the efficiency of the delivery and the need of expertise to design gene-editing targeting multiple site-specific proteins propel researchers to develop easier and simpler approaches for gene manipulation.

**CRISPR/CAS**

CRISPR/CAS enables efficient and precise point mutations and modification of gene, gene knockins/knockouts, repression or activation of specific genes, epigenomes, and targeting multiple loci simultaneously. This technique has been applied in C. elegans, yeast, mice, zebra fish, and pig; in pathogenic bacteria such as Mycobacterium and Salmonella; Yersinia; Corynebacterium diphtheriae; and also in the normal human stem cells, stem cells from a patient with cystic fibrosis, thalassemia, and Hemophilia A; myoblasts from Duchenne muscular dystrophy and from Myotonic dystrophy; and rats expressing HIV, expressing hepatitis B DNA, and with liver cirrhosis. In PD, double mutants including PARK and PINK1 and triple mutants including parkin, PINK1, and DJ1 were both successfully generated through CRISPR/CAS9 and delivered into the substantia nigra of the PD pig model. As human pluripotent stem cells (hPSCs) are highly expandable in vitro and can be directed to form any cell type of the body, hPSCs may be an extensive source for maintaining cell numbers in the growth of embryonic development and in injury, and disease and transplantation of hPSC-derived dopaminergic neurons may therefore be a “complete cure” in PD. However, transplanted cells do not substantially integrate into the host circuitry and cause undesired outcomes. To overcome this defect, Chen et al. transplanted the hPSC-derived human midbrain dopaminergic neurons (hPSC-mDA) into a PD mouse. hPSC-mDA were previously knocked in the designer receptors exclusively activated by designer drugs (DREADDs) by the CRISPR/CAS9, like a functional switch. The switch was activated by clozapine-N-oxide, leading to enhanced or improved motor functions. To investigate the relationship between the enhancer sequence variation, allele-specific differences, and the enhancer activity in PD, Soldner et al. identified a PD-associated risk variant in a noncoding distal enhancer element that regulates the expression of SNCA and used CRISPR/CAS9 to delete the putative enhancer elements in human ES cells and restore the regions with known variants from PD. After differentiating these cells into neural precursors, they found that the transcriptional deregulation of SNCA is associated with sequence-dependent binding of the transcription factors. In HD, Merienne et al. used the CRISPR/CAS9 system, including the gRNA recognizing the mutant HTT gene and the CAS9 protein generating DSBs to activate the cell DNA repair machinery to permanently halt the mutant HTT gene expression in HEK 293T cells and in the mouse cortical neurons and human iPSC-derived neurons, leading to the alleviation of the HD’s neuropathology. This technique has been proposed in constructing a pig model for NDs, including HD and PD. In AD, Paquet et al. used an approach, including a single-stranded oligo DNA nucleotide, CRISPR/CAS-blocking mutations, the distance to control zygosity, and the consecutive reguide or re-CASs steps to erase CRISPR/CAS-blocked targets (CORRECT) method to generate the human iPS cells with heterozygous and homozygous dominant early onset AD-causing mutations in APP and presenilin 1 and derived cortical neurons, and the results showed that the levels of Aβ were higher in the homozygous than in the heterozygous, suggesting that this technique can successfully and efficiently introduce specific sequence changes to the iPS cells for studying genotype-dependent human diseases. Despite several advantages of CRISPR/CAS, the drawbacks of the CRISPR/CAS include the need for a PAM adjacent to the target, variable efficiencies of delivery methods, including injection of the plasmids expressing cas and gRNA or CRISPR components as RNA or the off-target effects, which may lead to cell death and transformation.

**Conclusion**

NDs, at least including PD, HD, and AD, are common in age-related, progressive, disabling, and neuron degenerative diseases. Advances in medical science and technology have extended our life span, which has increased the prevalence of NDs. Our understanding of the pathogenesis of NDs has been hindered by a lack of precise diagnostic tools and effective treatments. Growing evidence suggests that accumulated abnormal misfolded protein complex is a common pathological feature of NDs. If the misfolded protein complex–associated genes can be fixed, the damaged networks and dysfunctions of the neurons may be restored. The gene-editing tools, including ZFNs, TALENs, and CRISPR/CAS,
can efficiently snip out or add specific segments of DNA to precisely edit the sequence-specific gene in several organisms. With these techniques, a specific ND animal model can be generated for understanding of human diseases and large-scale screening potential drugs. Moreover, these techniques can potentially provide effective treatments against many genetic human diseases which were previously thought untreatable. Among these techniques, ZFNs and TALENs are based on the protein-guided DNA cleavage, which need expertise and time-consuming protein design, assembly and selection, and validation.\(^{178,179}\). CRISPR/CAS, a simple and easy method with a lower price, can simultaneously modify genes at multiple independent sites. These advantages have made it to be employed more frequently than ZENs and TALENs in investigating functions of genes, generating transgenic animals with multiple gene mutations, and correcting gene defects in diseases.\(^{180}\). Even with numerous advantages, CRISPR/CAS was reported to have a lower knock in rate and a relative high risk of off-target mutations in human cells.\(^{181}\). Ultimately, it is hoped that by using a better method of gene editing to maximize benefits and minimize drawbacks, it will not only become feasible to unravel the puzzles of NDs but to counter the accumulated cellular abnormalities that cause neurodegeneration.

### Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Paul R. Sanberg (PRS) is the coeditor in chief of Cell Transplantation. Neither PRS nor any of his colleagues were involved in the peer-review process or decision for this manuscript.

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