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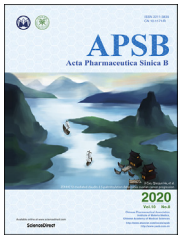

REVIEW

Gene therapy for neurodegenerative disorders: advances, insights and prospects

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Abstract Gene therapy is rapidly emerging as a powerful therapeutic strategy for a wide range of neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). Some early clinical trials have failed to achieve satisfactory therapeutic effects. Efforts to enhance effectiveness are now concentrating on three major fields: identification of new vectors, novel therapeutic targets, and reliable of delivery routes for transgenes. These approaches are being assessed closely in preclinical and clinical trials, which may ultimately provide powerful treatments for patients. Here, we discuss advances and challenges of gene therapy for neurodegenerative disorders, highlighting promising technologies, targets, and future prospects.

Abbreviations: AADC, aromatic-L-amino-acid; AAVs, adeno-associated viruses; AD, Alzheimer's disease; Adv, adenovirus; ARSA, arylsulfatase A; ASOs, antisense oligonucleotides; ASPA, aspartoacylase; BBB, blood−brain barrier; BCSFB, blood−cerebrospinal fluid barrier; Bip, glucose regulated protein 78; BRB, blood−retina barrier; CHOP, CCAAT/enhancer binding homologous protein; CLN6, ceroidlipofuscinosis neuronal protein 6; CNS, central nervous system; CSF, cerebrospinal fluid; ER, endoplasmic reticulum; FDA, U.S. Food and Drug Administration; GAA, lysosomal acid α-glucosidase; GAD, glutamic acid decarboxylase; GDNF, glial derived neurotrophic factor; HD, Huntington's disease; HSPGs, heparin sulfate proteoglycans; HTT, mutant huntingtin; IDS, iduronate 2-sulfatase; Lamp2a, lysosomal-associated membrane protein 2a; LVs, retrovirus/lentivirus; mTOR, mammalian target of rapamycin; NGF, nerve growth factor; PD, Parkinson's disease; PGRN, Progranulin; PINK1, putative kinase 1; PTEN, phosphatase and tensin homolog; RGCs, retinal ganglion cells; RNAi, RNA interference; RPE, retinal pigmented epithelial; SGSH, lysosomal heparan-*N*-sulfamidase gene; siRNA, small interfering RNA; SMN, survival motor neuron; SOD, superoxide dismutase; SUMF, sulfatase-modifying factor; TFEB, transcription factor EB; TPP1, tripeptidyl peptidase 1; TREM2, triggering receptor expressed on myeloid cells 2; UPR, unfolded protein response; ZFPs, zinc finger proteins.

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1. Introduction

Over the past few decades, gene therapy for neurodegenerative disorders has made straightforward progress. Growing understanding of the pathogenetic mechanisms of these diseases has enabled numerous advances in key technologies to converge including identification of novel therapeutic targets and new vectors[1]. This increased knowledge has led to remarkable targeting by multiple genetic interventions of the root causes of neurodegenerative disorders with both single-gene and complex etiologies. The sustained, even permanent therapeutic effects of gene therapy are especially appealing for compartmentalized organs such as eye, cochlea or central nervous system (CNS), which structures are difficult to treat because most agents cannot breach the physiological barriers such as the blood−cerebrospinal fluid barrier (BCSFB), blood−retina barrier (BRB), and blood−brain barrier (BBB)[2−4]. Furthermore, some genetic targets that are refractory to treatment with traditional agents are potentially manageable by gene therapy, which is capable of both gene silencing to handle gain of function mutations and gene overexpression to handle loss of function mutations.

Viral and non-viral vectors can successfully direct transgenes that express therapeutic proteins, antibodies, Cas9/gRNA for gene editing, microRNAs, and small interfering RNA (siRNA) to diseased tissues in human and animals. For neurodegenerative disorders, the most commonly applied vector is one of the adeno-associated viruses (AAVs)[5]. Additionally, a large number of capsids can be employed across species to favorably target multiple tissues and cells within the CNS, including oligodendrocytes, astrocytes and neurons[6−13].

It is critical that there have been significant advances in developing effective delivery routes, especially for the CNS and eye. Preclinical studies have demonstrated that numerous routes of gene delivery, including subpial, intracerebroventricular, intrathecal, intraparenchymal, intravitreal, and subretinal injection, can attain sufficient gene quantities in diseased tissues[14−17]. Intravenous injection has the advantage of being noninvasive, but the BBB and BRB are troublesome obstacles to the passage of agents into CNS or optic nerve[18]. Intramuscular injection provides an effective route for the delivery and production of vaccines and antibodies, and has the theoretical capacity to supply a source of antibodies that permit treatments to cross these physiological barriers in sufficient levels for clinical benefits[19]. It is noteworthy that recently reported vectors have an unequaled capability to transfer genes to the CNS after systemic injection. These vectors are an obvious improvement over AAV9 and potentially enhance our ability to treat additional neurological disorders[20,21].

There have been a considerable number of clinical trials of gene therapy for neurodegenerative disorders (Table 1). Some early clinical trials failed to achieve satisfactory therapeutic effects, perhaps due to insufficient biodistribution within their intended tissues. With improvement in AAVs and non-viral delivery systems, gene therapies have shown wider transgene expression and therapeutic safety. Importantly, there have been recent reports of excellent functional outcomes in experimental models of numerous neurodegenerative disorders, including Alzheimer's disease (AD), Huntington's disease (HD), aromatic-L-amino-acid decarboxylase (AADC) deficiency, and Parkinson's disease (PD)[5,22−25]. Here we review clinical and preclinical studies to describe recent advances in gene therapy for neurodegenerative disorders. We focus on the critical properties that efficient treatment by gene therapy requires, including vector design and selection of transgene strategy, target, and delivery route. We also discuss the challenges and future prospects of gene therapy, and share our own insights and experience.

2. Transgene strategies

Transgene strategies have been designed to deliver any nucleic acid as a genomic cargo, including siRNA, cDNA (gene addition or augmentation), microRNA, guide RNA (gene editing), RNA or DNA editing enzyme, docking site for a DNA binding protein, antisense oligonucleotide, or shRNA[2,20,26−28]. Importantly, none of these genomic cargos should be larger than 4.7 kb for AAV-based gene therapy, the size of the AAV genome[2]. Gene addition or augmentation has been assessed as a treatment strategy for several neurodegenerative disorders, including PD, Canavan disease, spinal muscular atrophy, and AD. This approach has been evaluated for targeted delivery of cDNA for AADC, survival motor neuron (SMN), human aspartoacylase (ASPA), and nerve growth factor (NGF), and has been reported to be effective and well tolerated with reduced clinical stabilization over a long-term follow-up research[2,29,30].

Engineered transcriptional regulators and gene editing targeted to specific genes are also being investigated as novel therapeutic applications for neurodegenerative diseases. Zinc finger proteins (ZFPs) are appealing experimental substances from a clinical perspective due to the similarity of rodent and human proteins and their relatively short genomes. However, although a clinical trial of inserting the iduronate 2-sulfatase (IDS) gene into albumin loci to treat mucopolysaccharidosis II has been carried out, successful gene editing will not be easy to achieve because of the off-target effects[31]. The recent suggestion that CRISPR/Cas9 nanocomplexes targeting BACE1 could suppress cognitive deficits and amyloid β-associated pathologies in AD highlights the huge application potential of non-viral vectors or viral vectors based CRISPR/Cas9 gene editing for neurodegenerative disorders. However, there are still many hurdlers need to be overcome when applying this approach to treat human neurodegenerative diseases, especially safety concerns[31,32].

Another promising transgene strategy is gene silencing. RNA interference (RNAi) is a widespread biological process in which siRNAs decrease synthesis of specific targeted proteins by degrading their corresponding mRNAs. Multiple clinical trials have suggested that artificial siRNAs can be utilized in humans to inhibit targeted proteins or genes and are commonly well tolerated (*e.g.*, NCT01559077 and NCT01437059)[33−35]. For example, recent clinical study demonstrated that HD patients showed dose-dependent reductions in concentrations of mutant huntingtin (HTT) after intrathecal injection of an antisense oligonucleotide (IONIS-HTTRx), suggesting this agent maybe a promising therapeutic[36]. Notably, because synthetic shRNAs or microRNA produced from a single injection of AAV can generate a more lasting gene silencing than artificial siRNAs, these substances provide superior gene therapy approaches for neurodegenerative disorders. For instance, the preclinical research indicated that one-dose administration of gene therapy candidate VY-HTT01 (Voyager Therapeutics) could effectively reduce the levels of HTT responsible for HD in critical brain areas of nonhuman primates. Moreover, studies demonstrating that synthetic primary-microRNA cassettes or AAV5 expressing a microRNA targeting HTT (AAV5-miHTT, UniQure) can generate efficacious, safe

Table 1 Ongoing gene therapy clinical trials for neurodegenerative disorders.

Disorders	Trial code	Delivery route	Gene therapy	Phase
Alzheimer's disease	NCT00876863	Direct basal forebrain injection	AAV2-NGF	Phase II
Huntington's disease	NCT02519036	Intrathecal injection	ASOs to HTT messenger RNA	Phase III
Huntington's disease	NCT03225833 NCT03225846	Intrathecal injection	ASOs to HTT mutant pre-messenger RNA	Phase I
Pompe's disease	NCT02240407	Intramuscular injection	AAV9-GAA	No results
Pompe's disease	NCT00976352	Intramuscular injection	AAV1-GAA	Phase II
Parkinson's disease	NCT03065192 NCT01793543	Intraputaminal injection	AAV2-AADC	Phase I
Parkinson's disease	NCT01621581	Intraputaminal injection	AAV2-GDNF	Phase I
Parkinson's disease	NCT02418598	Intraputaminal injection	AAV2-AADC	Phase II
Parkinson's disease	NCT00400634 NCT00985517	Intraputaminal injection	AAV2-neurturin	Phase II
Parkinson's disease	NCT00627588	Intraputaminal injection	Lentivirus-AADC	Phase I
Parkinson's disease	NCT00643890	Injection into the subthalamic nucleus	AAV2-GAD	Phase II
Metachromic leukodystrophy	NCT01801709	Intracerebral injection	AAVrh10-ARSA	No results
Spinal muscular atrophy	NCT02122952	Intravenous injection	AAV9-SMN	Phase I
Spinal muscular atrophy	NCT02292537	Intrathecal injection	ASOs targeting SMN2 splicing	Phase III
Amyotrophic lateral sclerosis	NCT01041222	Intrathecal injection	ASOs to SOD1	Phase I
Mucopolysaccharidosis type III A	NCT01474343	Intracerebral injection	AAVrh10-SGSH	Phase I
Mucopolysaccharidosis type III A	NCT02053064	Intracerebral injection	AAVrh10-SUMF1	Phase II
Mucopolysaccharidosis type II	NCT03041324	Intravenous injection	AAV6-IDS	Phase II
Batten	NCT02725580	Intrathecal injection	AAV9-CLN6	Phase I/II
Batten	NCT01414985	Intracranial injection	AAVrh10-TPP1	Phase I/II
Batten	NCT01161576	Intracranial injection	AAVrh10-TPP1	Phase I
Canavan	NA	Intraparenchymal injection	AAV2-ASPA	Phase I

NA, not applicable.

production of mature-microRNAs targeting ataxin-1 and HTT in mouse models of spinocerebellar ataxia type-1 and HD, respectively, provide proof-of-concept support for these strategies to utilize RNAi[36–39]. Overall, the rational for transgene strategies selection would be determined by multiple factors including safety concerns, insertional mutagenesis and genotoxicity as well as different pathological conditions[2].

3. Vectors: viral and non-viral based gene therapy

3.1. *Viral vectors*

AAV based vectors have been applied almost exclusively in clinical trials of gene therapy for neurodegenerative diseases. AAV serotypes are the major determinant of several crucial characteristics of successful AAV-based gene therapy, including biodistribution, tissue tropism, and susceptibility to neutralizing antibody generated *in vivo*. Discovering how the specific serotypes distribute gene cargos to their intended tissues for vector delivery is vital for developing a reliable and predictable gene therapy strategy[6,40–43]. More than one hundred AAV variants consisting of 13 serotypes (AAV1−13) have been identified from humans and nonhuman primates[44–48]. Because of its relative safety profile and its sustained expression in neurons, AAV2 has been used in numerous clinical trials and is currently considered a satisfactory vector for gene therapy of neurodegenerative disorders[48–51]. Specifically, researchers indicated that intracerebral administration of AAV2-NGF is well tolerated and shows evidences of therapeutic effect on cognitive decline in AD-related dementia[50]. Interestingly, after administration near or into cerebral ventricles, AAV4 has a predilection to transfect ependymal cells, which constitute the epithelial lining of neuroblasts and the lateral ventricles[52,53]. Because the BBB is an important barrier hindering delivery of most vectors to the CNS, the ability of AAV9 and AAVrh.10 to penetrate this obstacle is also consequential[21,54,55]. Interestingly, reports that AAV-PHP.B, a recently engineered AAV capsid, can transduce more than 50% of astrocytes and neurons and deliver far more AAV genomes (forty fold greater) into the CNS after intravenous injection than other capsids, highlight the critical importance of capsid engineering[20,56]. Indeed cell-type specific screening of different AAV capsid libraries has identified increasing numbers of bioengineered AAV capsids with specific tropisms[57].

Adenovirus (Adv) is an icosahedral capsid virus with size ranging from 70 to 100 nm. Adv cannot insert its gene into the host genome, which leads to relative transient transgene expression but an excellent safety profile. The innate immune responses against Adv restricts Adv's therapeutic potential efficacy for CNS gene therapy[58,59]. Although few studies use Adv as gene therapy vectors to treat neurodegenerative disorders, it should be pointed out, however, that Adv is well tolerated with little side effects in these researches[59]. Unlike AAV and Adv capsids, retrovirus/lentivirus (LVs) could fully integrate DNA into the host genome through reverse transcription, thus providing more stable and longer transgene expression *in vivo*. Of note, these insertions should be controlled under strict conditions to avoid genotoxicity and insertional mutagenesis. The one important clinical trial to date is the use of a lentiviral vector, which can deliver larger DNA cargos for PD[60]. Their data indicated that ProSavin, a lentiviral

vector-based gene therapy aimed at restoring dopamine production, improved motor behavior and demonstrated safe in all patients with advanced PD[60]. Further investigations into Adv and LVs-mediated gene therapy of neurodegenerative disorders are desperately needed given the limited clinical data thus far.

3.2. *Non-viral vectors*

Although most clinical trials have used viral vectors such as AAVs, lentivirus, Adv, and retroviruses to carry therapeutic genes, these vectors have numerous drawbacks, including broad tropism, limited loading capacity, difficulty in vector production, and host inflammatory responses[61–66]. Gene therapies based on non-viral vectors have the potential to avoid several of these drawbacks, especially those related to safety[67–69]. Moreover, although few of these strategies have been used in the clinic, it is extremely important to exploit novel kinds of vectors, particularly nanoparticles and liposomes. Based on the composition of the carriers' material, non-viral delivery vectors can be sorted into lipid-based vectors and polymeric vectors. The most extensively applied non-viral gene carriers are lipid-based vectors[70]. Neutral lipids, like cholesterol, DOPE, and DSPE, have served as the 'helper lipid' among liposomal components to improve liposome stability and transfection capacity[71]. The prominent features of cationic lipids, such as DOTAP, DODAP, DOTMA, and DC-cholesterol, which have been used for gene therapy, include three major domains: hydrophobic tails, linking groups, and cationic cap groups[69,72]. The main shortcomings of cationic lipids are their unsatisfactory pharmacokinetic biodistribution due to nonspecific binding and rapid clearance, and their cytotoxicity[69,73]. To overcome these drawbacks, optimized cationic lipids with appropriate p*K*a values have been developed[68,70]. Lipidoids (lipid-like materials), magnetic nanoparticles, and exosomes have also shown promise as gene delivery carriers for neurodegenerative disorders[74–76]. For instance, recent studies indicated that magnetic Fe_3O_4 nanoparticles coated with *N*-isopropylacrylamide derivatives and oleic acid molecules carrying shRNA-*α-syn* can significantly alleviate PD in mice[74]. Cationic polymers provide another kind of non-viral vector that is extremely attractive for gene therapy due to their capacity for endosomal/lysosome escape, which is the result of their sponge-proton effect, fine spherical architecture, and tremendous chemical diversity[70,77–79]. Overall, therefore, non-viral gene therapy has improved substantially in recent decades. Additional insights into the relationship between structure and function of gene delivery material and fuller understanding of the critical factors that restrict effective gene delivery are likely to advance the clinical treatment of neurodegenerative disorders. We summarize the types, specific characteristics, advantages and disadvantages of viral and non-viral vectors in this section in Table 2.

4. Target selection for neurodegenerative disorders

Neurodegenerative disorders are characterized by progressive dysfunction of neurons in specific regions of CNS, eventually leading to disability and death. The growing number of recently identified targets enlarges the range of potential clinical applications. However, as shown in Table 1, many therapeutic agents and their related targets offer nothing beyond symptomatic relief and do not address the underlying pathology[80]. It is therefore urgently necessary to identify promising pathogenic targets for gene therapy of neurodegenerative disorders, as indicated in Table 3[81–100].

4.1. *Endoplasmic reticulum stress and unfolded protein response*

Almost all neurodegenerative disorders share the same pathological characteristic: abnormal accumulation of misfolded proteins[101,102]. The negative consequences of aggregating misfolded proteins include generation of endoplasmic reticulum (ER) stress and ER-associated degradation[103]. Misfolded proteins, such as amyloid β oligomers and α-synuclein, which aggregate in the ER-lumen, destabilize ER calcium homeostasis and distort unfolded protein response (UPR) signaling intended to restore cellular proteostasis, but instead resulting in proapoptotic responses and neuron death[104–106]. Importantly, investigators, including ourselves, have suggested that gene therapies to reduce ER stress by targeting UPR signaling to enhance protein folding are more likely to provide long-term, local therapeutic effects than antibodies and small molecules. AAV delivered to the mouse retina to downregulate CCAAT/enhancer binding homologous protein (CHOP) or activate XBP-1 prevents the optic nerve degeneration and apoptotic death of retinal ganglion cells (RGCs) that is triggered by glaucoma, optic neuritis, and traumatic optic nerve injury[81,83,107]. Similarly, AAVs-XBP-1 administered locally to the striatum or substantia nigra block neurodegeneration induced by neurotoxins that experimentally model HD and PD[82,86,108,109]. Moreover, gene therapy consisting of overexpression of BiP (glucose regulated protein 78) to treat experimental PD has been reported to reduce dopaminergic neuron apoptosis, enhance motor performance, and delay disease progression[84]. The same strategy

Table 2 Comparison of the different gene vectors for neurodegenerative disorders.

Vector type	Specific characteristics	Advantage	Disadvantage
AAVs	Numerous AAV serotypes; lack of targeting and site-specific; relative safety profile	Relative stable transgene expression; nonpathogenic; various serotypes available	Immune responses; limited gene packaging capacity
Adv	Adv cannot introduce its gene into the host genome	Lower genotoxicity and insertional mutagenesis	Immune responses; relative transient transgene expression; re-administration; requires receptors for cell uptake
LVs	Retroviruses can integrate DNA payloads into the host genome	Lower frequency of administration; stable and long-term transgene expression	Immune responses; genotoxicity; insertional mutagenesis
Polymer- and lipid-based vectors	Non-viral vectors can be altered to impart desired functionalities	Large-scale production; controlled release; large gene packaging capacity; lower immunogenicity	Cytotoxicity; nonspecific binding and rapid clearance

has excellent effects in a mouse model of amyotrophic lateral sclerosis (ALS); intracerebral delivery of AAV6-SIL1 restores ER homeostasis and prolongs survival[85]. Because upregulation of UPR signaling has been reported to sustain the proliferation and invasion of glioblastoma, however, safety assessment in long-term follow-up studies will be required before this approach can be considered for the clinic[110,111].

4.2. *mTOR signaling*

Signaling transductions of mammalian target of rapamycin (mTOR) have been reported to play a pathogenic role in neurodegenerative disorders with diverse clinical characteristics, such as AD, PD, HD, and traumatic brain and optic nerve injury[112,113]. Abnormal mTOR signaling is likely to have distinct effects in different neural cells, such as those in the substantial nigra, caudate nucleus, retina, and entorhinal cortex, but degeneration is the common fate of all of these cells because they are unable to clear toxic protein accumulation[114]. Our work has previously demonstrated that delivery to retina by AAV of positive regulators or effectors of mTOR signaling (such as AAV2-AKT and AAV2-S6K1) or AAV-mediated deletion from retina of negative regulators of mTOR signaling (such as PTEN) can prevent death of RGCs and promote CNS axon regeneration following traumatic optic nerve injury[87–89,115]. Other investigators report that AAV-based overexpression of S6K1 or AKT also has therapeutic effects in a mouse model of PD, indicating that activation of mTOR signaling may provide a new treatment option for PD and traumatic nerve injury[90,91]. Of note, multiple studies have suggested that mTOR signaling is hyperactivated in HD and AD, and that reinstating aberrant mTORC1 activity can rescue neurodegeneration. Future studies should therefore focus on the cellular and molecular mechanisms that relate mTOR signaling and neurodegenerative disorders[92,116–119].

4.3. *Mitochondrial function*

Mitochondrial respiratory dysfunction has been shown to contribute to numerous neurodegeneration disorders, such as AD, PD, HD, glaucoma, ALS, and lysosomal storage diseases[120–122]. These disorders exhibit many characteristics of mitochondrial respiratory dysfunction, including limited regulation of mitochondrial quality, oxidative damage, NAD^+ depletion, disrupted ATP synthesis, protein aggregates, and unbalanced mitochondrial calcium homeostasis[120–124]. Therapeutic agents that inhibit mitochondrial damage or promote mitochondrial biogenesis, among them CoQ_{10}, Bendavia, MitoQ, and NAM, mitigate neurodegeneration in mouse models[121,125–128]. Moreover, gene therapy that overexpresses regulators of mitochondrial oxidative stress and dynamics, such as PGC-1α, HSP70, TFEB, can reduce neurotoxicity in experimental PD and HD, suggesting that these strategies may be significant therapeutic approaches for other neurodegeneration diseases[93,129]. However, clinical translations of mitochondrial treatments have been unsatisfactory, which may be because patients enter clinical trials when their neurodegenerative disorders are too advanced for effective intervention. Optimism about mitochondrial-based gene therapy for neurodegenerative disorders continues to be warranted, particularly for those with obvious mitochondrial dysfunctions[130].

4.4. *Epigenetic regulation*

Epigenetic regulatory mechanisms, such as chromatin remodeling, DNA methylation, histone variant, and histone post-translational

Table 3 Representative promising therapeutic targets for gene therapy of neurodegenerative disorders.

Target selection	Disorder	Gene therapy	Delivery route	Ref.
ER stress and UPR	Traumatic optic nerve injury	AAV2-XBP-1	Intravitreal injection	81
	Parkinson's disease	AAV2-XBP-1	Unilateral brain injection	82
	Optic neuritis and encephalomyelitis	AAV2-CHOP shRNA	Intravitreal injection	83
	Parkinson's disease	AAV5-Bip	Intracerebral injection	84
	Amyotrophic lateral sclerosis	AAV6-SIL1	Unilateral brain injection	85
	Huntington's disease	AAV2-XBP-1	Intrastriatal injection	86
mTOR signaling	Optic nerve injury	AAV2-AKT	Intravitreal injection	87
	Optic nerve injury	AAV2-S6K1	Intravitreal injection	88
	Optic nerve injury	AAV2-PTEN	Intravitreal injection	89
	Parkinson's disease	AAV1-AKT	Intrastriatal injection	90
	Alzheimer's disease and Parkinson's disease	AAV1-AKT	Intrastriatal injection	91
	Huntington's disease	AAV1-caRheb	Unilateral brain injection	92
Mitochondrial function	Parkinson's disease	AAV2-HSP70	Substantial nigra dense area injection	93
	Alzheimer's disease	AAV2-PINK1	Intrahippocampal injection	94
Epigenetic regulation	Alzheimer's disease	AAV2-PSD95-6ZF-VP64	Intrahippocampal injection	95
Autophagy	Alzheimer's disease	AAV2-PINK1	Intrahippocampal injection	94
	Parkinson's disease	AAV6-Lamp2a	Intrastriatal injection	96
	Parkinson's disease	AAV2-TFEB	Unilateral brain injection	97
	Amyotrophic lateral sclerosis	AAV9-snapin	Intravenous injection	98
Microglial and astrocyte function	Alzheimer's disease	AAV2/8-sTREM2	Intracerebral injection	99
	Alzheimer's disease	Lentivirus-PGRN	Unilateral brain injection	100

modification, have been suggested to regulate numerous aspects of axonal development and neuronal survival[131]. One study presented evidence that changes in H3K27ac or H3K4me3 occurred in connection with genetic variants in AD, suggesting an important function for immune-associated enhancers and promoter proteins in determining AD susceptibility[132]. Another study demonstrated that H4K16ac, a histone associated with DNA repair and neurodegenerative disorders, is significantly reduced in the cortex of AD patients, suggesting that the aged brain of these individuals is incapable of upregulating H4K16ac[133]. It is also noteworthy that multiple reports have associated loss of H3K4me3, a protein related to gene activation, with the deterioration found in PD and HD, but that overexpression of H3K4me3 can accelerate A−T mutation that mitigates behavioral impairments and neurodegeneration[134−136]. Additionally, HDAC inhibitors can prevent neurodegeneration in models of glaucoma, AD, and HD, despite the differences in pathogenesis[137−140]. These reports demonstrate that epigenetic profiles are regulated in neurodegenerative diseases and suggest that better understanding of these mechanisms could provide the foundation for developing more precisely targeted epigenome therapies. For example, recent work suggesting that epigenetic editing of the post-synaptic density protein 95 gene can improve cognition in AD highlights the potential of epigenetic regulation-based gene therapy for neurodegenerative disorders[95].

4.5. *Autophagy*

Autophagy, the process by which evolutionarily-conserved intracellular machinery degrades dysfunctional organelles and denatured proteins in lysosomes, has been demonstrated to be associated with the severity of such neurodegenerative disorders as AD, PD, HD, glaucoma and ALS[141,142]. Neuroprotection by autophagy is mainly due to its elimination of misfolded proteins, including tau, HTT, and α-synuclein[142,143]. Previous work has indicated that PTEN-induced overexpression of putative kinase 1 (PINK1) mediated by AAV2 promotes autophagy that facilitates clearance of dysfunctional mitochondria, which in turn ameliorates the loss of mitochondrial functions, cognitive decline and synapses induced by amyloid β oligomers in experimental AD[94]. Similarly, overexpression of the transcription factor EB (TFEB) or lysosome-associated membrane protein 2a *via* intracerebral injection of AAV vectors can effectively alleviate α-synuclein-induced neurodegeneration in PD by enhancing axon regeneration and neuron survival; these benefits have been attributed to induction of lysosome biogenesis and chaperone-mediated autophagy[96,97]. Additionally, overexpressing snapin (AAV9-snapin) rescues defects in retrograde transport, which reverse impairments of autophagy/lysosomes, improve mitochondrial fitness, enhance motor neuron survival, and mitigate disease phenotypes in mouse ALS[98]. Although multiple lines of evidence therefore support the potential of gene therapies to treat neurodegenerative disorders by regulating autophagy, the approach is challenged by difficulties in target selection and limited understanding of underlying mechanisms.

4.6. *Microglial and astrocyte function*

Microglia, as the major neuro-immune cells, execute numerous critical tasks: housekeeping functions that maintain neuronal wellbeing and neuronal networks, sentinel functions associated with constant perception of environmental changes, and defensive functions essential for neuroprotection[144]. Neuronal damage in AD, PD, HD, ALS, glaucoma, and the degeneration associated with chronic and acute trauma stems from disruption of these microglial functions and neuroinflammation. Preventing dysregulation of these functions therefore represents a potential mode of treatment. Specifically, variants of microglial surface innate-immune receptors, such as complement receptor 1 (CR1), CD33, and triggering receptor expressed on myeloid cells 2 (TREM2), have been genetically associated with the risk for AD[145−147]. Moreover, overexpression of soluble TREM2 mediated by AAVs improves microglial migration, proliferation, and degradation of amyloid β protein, which reduces amyloid plaque deposition and rescues dysfunctional spatial memory in a model of AD[99]. Additionally, by modulating microglial function, lentivirus-mediated haploinsufficiency of progranulin overexpression inhibits neuronal loss and spatial memory deficits in AD mice[100].

Astrocytes fulfill lots of interactive and homeostatic functions in the CNS: regulating extracellular neurotransmitters and ions; providing energy metabolites; promoting neurogenesis; and controlling synaptic activity[148]. The complexity and diversity of these performances clearly suggest that the correct activities of astrocytes are very important to physiological functioning of the CNS, and their dysfunction can promote the progression of multiple neurodegenerative diseases[149]. A precise and effective approach to modulate astrocyte signaling pathways involves boosting or inhibiting genes in specific manners. Importantly, studies have demonstrated that AAV capsids AAV9P1 and Anc80L65 are promising tools for gene delivery to astrocytes, which could facilitate activation or inactivation of persisting dysfunction genes[150]. Moreover, pseudotyping lentiviruses with glycoproteins were found to selectively transfect astrocytes after intraparenchymal administration[150,151]. Nanoparticles functionalized with bradykinin B2 receptor antibodies, transferrin receptor or apolipoprotein E have also been indicated to successfully deliver siRNA and mRNA to astrocytes[152,153]. For example, optimized branched poly (β-amino ester)s are applied to deliver NGF expression DNA to astrocytes, and high transfection efficiency is achieved, which provides a viable gene therapy approach for neurodegenerative disorders[154]. Collectively, these studies indicate that targeting microglia or astrocyte may be a promising therapeutic strategy for neurodegenerative diseases.

4.7. *Neuronal progenitors or stem cell therapy*

The therapeutic strategies for neurodegenerative diseases may have a revolutionized change *via* transplanting neuronal progenitors or stem cells. However, better controlling of their proliferation and ameliorating their engraftment as well as improving their differentiation and survival are very important. To modulate stem cell function, delicate regulations of gene expression *via* gene therapy approaches are emerging as safe methods[155]. For example, Jakobsson et al.[155] have used LVs-based CRISPR-Cas9 tool to knockout *DNMT1* in neural progenitor cells which results in feasible, proliferating cells and further implicating a novel gene therapy in human brain disease and development[156]. As another example, Biffi et al.[157] have applied LVs to introduce functional genes into hematopoietic stem cells *ex vivo* and indicated that transplantation of these engineered HSCs inhibited and alleviated the symptoms of metachromatic leukodystrophy. Although neuronal progenitors or stem cell therapy have previously

demonstrated clinical benefit, these therapeutic strategies are often restricted, especially for disorder conditions owing to cell autonomous defects.

5. Delivery routes: a major determinant of efficacy and safety

Gene delivery to sensory organs or CNS, including eye, spinal cord, and brain, is a challenging undertaking. Attaining a proper balance between treatment efficiency and compliance and safety is largely decided by the judicious combination of delivery routes and vectors. Fig. 1 lists many of the preclinical studies and clinical trials and their multiple delivery routes and vectors that will be mentioned in the following section.

5.1. *Intravenous injection*

The physiological barriers that compartmentalize sensory and CNS tissues, such as BBB, present serious obstacles to therapeutic gene access. AAV9 is one agent that effectively penetrates these barriers after intravenous injection. This feature enables widespread expression in CNS to treat multifocal disorders and acts as a stimulus for further technological exploration and development[158–160]. Moreover, capsid engineering research has discovered AAV variants that seem to be superior to AAV9, such as AAV-AS, AAV-B1, and AAV.PHP.B, which represents a significant breakthrough for intravenous delivery[20,161,162]. This delivery route could also result in delivery of the gene cargos to most body tissues which has potential drawbacks but in some cases also potential advantages. Even though intravenous delivery of AAVs is noninvasive and technically feasible, major obstacles continue to complicate clinical applications, including the large doses necessary, generation of antibodies against AAVs, and ongoing safety concerns.

5.2. *Intraparenchymal injection*

Local delivery of vectors has obvious advantages over systemic administration. Intraparenchymal injection is tolerated well and delivers therapeutic genes directly to the neurons and brain region of interest, with little biodistribution to peripheral organs[5,24,163]. Vectors that do not bind heparin sulfate proteoglycans (HSPGs), like AAV1, AAV8, AAV9, and AAVrh.10, diffuse over larger regions after intraparenchymal injection than vectors that bind HSPGs, such as AAV2, AAV-DJ88, and AAV6[164]. For disorders such as Canavan disease, which has been treated with AAV2-ASPA, and PD, which has been treated with AAV2-AADC, AAV2 provides an appropriate vehicle to limit diffusion while obtaining satisfactory delivery[29].

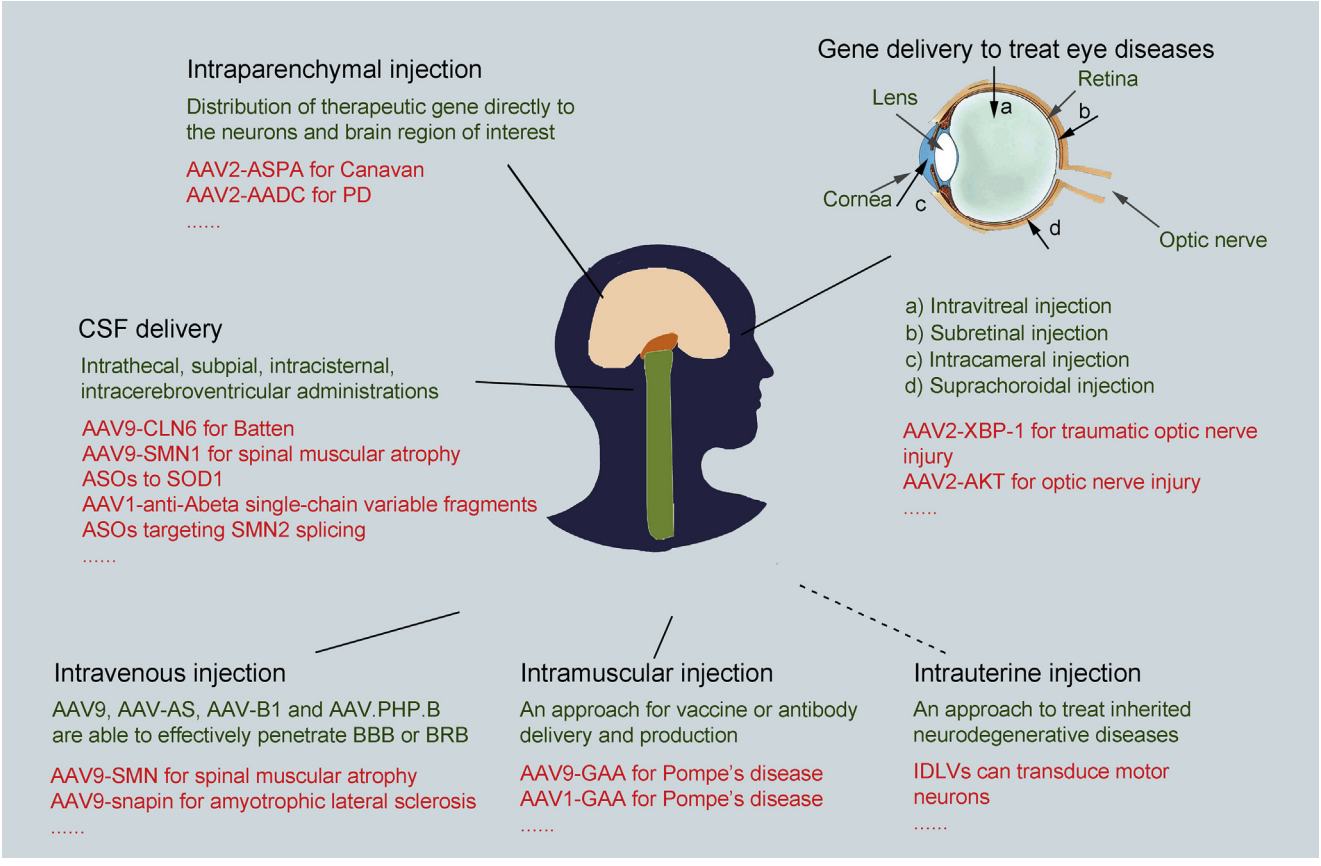

Figure 1 Delivery routes of gene therapy for neurodegenerative disorders. Although intravenous or cerebrospinal fluid (intrathecal, intracerebroventricular, and subpial routes) administration can effectively treat multifocal disorders, intraparenchymal injection is the most frequently applied delivery route for brain diseases. Local gene delivery is preferable for diseases of the eye, because of its relatively straightforward surgical and instrumental accessibility. Intramuscular injection provides a strategy for vaccine and antibody delivery and production, and intrauterine injection may provide an approach to treat inherited neurodegenerative diseases.

5.3. *Intrathecal, subpial, intracisternal, intracerebroventricular and intrauterine injection*

Other delivery routes include administration into various cerebrospinal fluid (CSF) compartments. Intrathecal injection of AAVs is especially suitable for delivering vectors to sensory neurons in dorsal root ganglia or motor neurons and has been well tolerated in numerous preclinical studies[12,165,166]. Interestingly, AAVs, such as AAVrh.10 and AAV9, primarily target spinal cord motor neurons following intrathecal injection in nonhuman primates[167]. Because subpial administration has only been investigated in the laboratory to date, translation of this dosing method for gene therapy of neurodegenerative disorders requires additional studies[15]. Preclinical studies have shown that intracisternal and intracerebroventricular administration also produces effective expression of transgenes in spinal cord and cerebral tissues that has alleviated symptoms in models of numerous neurodegenerative disorders, including AD, ALS, and spinal muscular atrophy[168−170]. Additionally, integration-deficient lentiviral vectors (IDLVs) have recently been reported to transduce motor neurons efficiently and permanently after intrauterine injection, indicating the potential for IDLVs to become effective tools to treat inherited neurodegenerative diseases[171].

5.4. *Gene therapy in eye diseases*

The eye is especially suitable for local injection of AAVs. U.S. Food and Drug Administration (FDA) approval of Luxturna (AAV2-RPE65) to treat Leber's hereditary optic neuropathy signals the arrival of the gene therapy era[172]. Local administration presents multiple advantages for treating ophthalmic diseases, largely because the relatively easy surgical and instrumental accessibility enables practical interventions and rapid examinations, and because the compartmental characteristics of the eye prevent systemic dispersion of the vectors. Intracameral administration provides delivery to the anterior chamber of the eye, which can be targeted to the cornea or the trabecular meshwork[173]. Notably, AAV-based gene therapies for ophthalmic diseases have mostly concentrated on retinal diseases, including the neurodegenerative retinal disorders, and have efficiently targeted the inner retina through intravitreal administration and the outer retina through subretinal administration[2]. These AAVs accumulate between the neural retina and the retinal pigmented epithelial (RPE) cell layer after subretinal administration[174]. Clinically, intravitreal administration has been used to produce AAV-based therapeutic protein expression or to target retinal ganglion cells[175]. However, this delivery route needs high doses of AAVs, which can increase the risk of inflammatory responses remarkably in comparison with subretinal administration[176,177].

Taken together, the selection of delivery routes is a key one yet needs a balancing of numerous factors along a risk/benefit equation. Local delivery route of administration has been acknowledged to be the preferential choice for neurodegenerative disorders as it maximizes delivery while minimizing the safety concerns. In order to target more broadly than what local administration routes can achieve and avoid the invasiveness of the local injection procedures, new techniques were urgently required that achieve meaningful levels of gene transfer through these strategies. These delivery routes almost always need higher doses, putting burden on drug manufacturing and raising the risk of toxicity. In some circumstances, a variety of routes of injection are combined when certain disorders require many organs to be treated[178].

6. Clinical challenges

Numerous preclinical and clinical studies of gene therapy strategies for preventing or treating a wide range of neurodegenerative diseases have been carried out in recent decades[2]. However, safety concerns remain one of the biggest barriers to successful clinical application. Gene therapy may cause severe toxicity due to overexpression of the transgene in targeted tissues or expression in off target cells. Toxic effects have included impaired ambulation, ataxia, damaged dorsal root ganglia, elevated transaminases, and proprioceptive deficits[2,179]. Host responses can also affect the duration and safety of every gene therapy strategy. Patients with adaptive immune responses can produce corresponding neutralizing antibodies, which may prevent the vectors from reaching their intended tissues or cells[180,181]. Insertional mutagenesis and genotoxicity are probably also concerns when certain transgenes are injected with high-dose vectors[182−184]. Potential gene-based therapeutic strategies to treat neurodegenerative disorders should therefore be carefully scrutinized for clinical development, including evaluation of available safety profiles and pharmacological effects, and identification of individuals who can benefit.

7. Concluding remarks and future prospects

Gene therapy is an important emerging strategy for treating neurodegenerative disorders, which is especially suited for well-validated genetic targets that are not amenable to traditional therapies. It has been well tolerated and shown long-lasting efficacy in clinical trials for various human neurodegenerative diseases, including PD, AD, HD, and AADC deficiency[5,65]. Moreover, improvements in delivery, such as direct administration into the CNS, and carriers, such as AAV9 and liposomes, are being vigorously investigated and refined. Although non-viral vectors-based gene therapy has yet to be approved as therapeutics for neurodegenerative disorders, recent advances in the clinical trials have generated great excitement[32−36]. Better understanding of the onset and progression of the neurodegenerative disorders will facilitate prompt diagnosis and target selection, which should allow early treatment for certain of these diseases. As progress continues in optimizing transgene design, delivery, and vectors, the prospects of gene therapy for neurodegenerative disorders will undoubtedly become even brighter.

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Author contributions

Wei Chen and Yang Hu wrote the manuscript. Yang Hu and Dianwen Ju designed structures and supervised the work. The final version of the paper has been approved by all authors.

Conflicts of interest

All authors declare no competing interests.

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