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REVIEW ARTICLE

Gene Therapy for Huntington's Disease: The Final Strategy for a Cure?

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ABSTRACT

Huntington's disease (HD) has become a target of the first clinical trials for gene therapy among movement disorders with a genetic origin. More than 100 clinical trials regarding HD have been tried, but all failed, although there were some improvements limited to symptomatic support. Compared to other neurogenetic disorders, HD is known to have a single genetic target. Thus, this is an advantage and its cure is more feasible than any other movement disorder with heterogeneous genetic causes. In this review paper, the authors attempt to cover the characteristics of HD itself while providing an overview of the gene transfer methods currently being researched, and will introduce an experimental trial with a preclinical model of HD followed by an update on the ongoing clinical trials for patients with HD.

Keywords Gene transfer; Huntington's disease; Movement disorder.

INTRODUCTION

More than half of human genes are expressed in the nervous system.[1] Perhaps this is the reason why human genetic disorders frequently manifest with neurological problems. Many neurodegenerative disorders have been explored, and one of the most common causes is defects in gene structures. Usually, genes that affect most movement disorders are heterogeneous. As a result, one success in a genetic trial is difficult to apply to another disorder, despite rigorous new drug development.

However, Huntington's disease (HD) is a well-known disorder that could be an ideal model, as it is an autosomal dominant progressive neurodegenerative movement disorder with a single genetic target. The defect is present in a trinucleotide repeat (CAG) expansion of the *IT15* gene, leading to a polyglutamine strand at the N-terminus. Under the system of central dogma, the DNA genetic code determines the RNA that will be transcribed in complement to the DNA; then, this RNA will go on to be translated into a protein that has a certain function in cells. The CAG repeats present in the gene will result in the translated protein Huntingtin (Htt) containing repeated stretches of glutamine that make it prone to misfolding and eventually cause the aggregation of the protein[2,3] that then loses its original function. This mutant protein causes clinical symptoms such as chorea, dystonia, incoordination, cognitive decline, and behavioral difficulties.

The cellular functions of Htt are still not fully understood. Htt shuttles into the nucleus, but it is usually active in the cytoplasm, and it plays a role in vesicle transport and can regulate gene expression. When an abnormal conformation arises in the Htt protein, it gains toxic functions that affect the central nervous system (CNS), directly causing massive striatal neuronal death within the brain. There can be up to 95% loss of γ-aminobutyric acid–releasing (GABAergic) medium spiny projection neurons, with selective sparing of some large interneurons that project to the

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globus pallidus and the substantia nigra.[3]

With technical advancements, gene transfer has been tried for several neurogenetic disorders, and now, gene therapy such as antisense oligonucleotides (ASOs) for spinal muscular atrophy has already been Food and Drug Administration (FDA) approved for clinical use. Hopefully, HD will be soon approved for treatment with ASOs or other new advances for its gene therapy. So far, the list of FDA-approved gene therapy products is quite short, showing that the approaches to gene therapy research are experimental. This review addresses the gene therapy methods for HD that are under development.

SEVERAL STRATEGIES TO DELIVER GENES

The main task of gene therapy is to selectively replace, repair, or control the expression of mutant genes by the transfer of genetic material to the target cells that are responsible for each disease. Vectors for delivering genes in gene therapy can be classified as viral and nonviral.[4,5]

To date, most clinical trials have used viral vectors because viruses such as adeno-associated virus (AAV), lentivirus (LV), adenovirus (Adv), and retroviruses can express full-length genes, thus allowing for gene replacement.[5] Viruses can easily be utilized to develop more targeted treatments because they have tropism for specific tissues and cell types.[6] Because there are no adverse effects reported for humans, AAV has become the most common type of virus utilized as a vector in human gene therapy. Adv is an icosahedral capsid virus with a size of 70 to 100 nm, and few studies used Adv as a vector for gene therapy because it has features that limit its gene transfer. Adv induces transient expression of transgenes because they are unable to insert their gene into the host genome.[7,8] In addition, humans have an innate immune response to Adv, which limits the therapeutic potential of Adv for CNS gene therapy.[7,9] However, it has an excellent safety profile.[8] Unlike Adv, retroviruses and LVs can provide more stable and longer transgene expression by reverse transcription, which enables them to insert their DNA into the host genome. However, it should be noted that genotoxicity and insertional mutagenesis must be considered carefully before the adoption of retroviruses and LVs for gene therapy.[8]

Although viral vectors are commonly used for gene therapy, broad tropism, limited loading capacity, difficulty in vector production, and host inflammatory responses are critical drawbacks that limit the adoption of viral vectors in gene therapy.[10-12] Nonviral vectors can be an alternative for transferring genes by overcoming some of these issues because they are relatively free from safety issues.[13-15] Nonviral vectors, including nanoparticles, liposomes, exosomes, etc., can be classified into lipid-based vectors and polymeric vectors depending on their composition.[8]

Lipid-based vectors are the most common type of nonviral vectors[16] and they can be sorted into neutral lipids such as cholesterol, dioleoyl phosphoethanolamine (DOPE), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) and cationic lipids such as 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), 1,2-dioleyl-3-dimethylammonium propane (DODAP), N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyammonium chloride (DOTMA), and 3b(N-(N',N'-dimethylaminoethane) carbarmoyl)-cholesterol (DC-cholesterol). Neutral lipids help liposomal components improve the stability and capacity of transfection,[17] and cationic lipids have interesting characteristics that make them promising vectors for gene therapy: hydrophobic tails, linking groups, and cationic cap groups.[13,18] However, the unsatisfactory pharmacokinetic biodistribution and cytotoxicity due to nonspecific binding and rapid clearance of cationic lipids limit their capability of gene transfer.[13,19] Thus, optimizing the pKa values of cationic lipids has been studied.[14,16] Another type of nonviral vector, cationic polymers, has a sponge-proton effect, fine spherical architecture, and a large variety of functional groups. The high degree of freedom in designing functional groups such as hydrophobic modifications or the addition of naphthalimide units or aneN$_3$ moieties to cationic lipids enables them to have a large capacity for endosomal/lysosomal escape, making them promising candidates for vectors of gene therapy.[16,20-22] Lipidoids (lipid-like materials) and exosomes also have the potential to be vectors of gene therapy for neurodegenerative diseases.[23,24]

Exosomes are the smallest membranous vesicles (40−1,100 nm) that have cargo ability for intercellular matter exchange.[25-27] Exosomes are generated via the inward budding of endosomes to form multivesicular bodies that fuse with membranes to release exosomes into the surrounding environment.[28,29] Exosomes, depending on their parental origin, contain a variety of proteins, lipids, noncoding RNAs, mRNA, and miRNA, collectively termed "cargo." Due to their cargo ability, exosomes represent a novel form of intracellular communication among cells that does not require cell-to-cell direct contact. Exosomes are selectively taken up by the surrounding or distal cells and can reprogram the recipient cells due to their active cargo content.[30,31]

Recently, it has been reported that clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 nanocomplexes targeting β-site amyloid precursor protein cleaving enzyme 1 (BACE1) could modulate the pathologies of Alzheimer's disease, including cognitive deficits and amyloid β accumulation. Therefore, developing vectors carrying the CRISPR/Cas9 complex is a promising strategy for neurodegenerative disorders. However, many obstacles remain when targeting CRISPR/Cas9 to treat neurodegenerative diseases in humans.[8] In addition to safety issues, since it cannot be applied to nondividing cells, neurological dis-

orders that do not have mitotic potential in the neurons themselves is a major limitation of the technology.[32,33] Further understanding of the characteristics, morphology, and function of vectors for gene transfer and the important factors limiting effective gene transfer must be achieved before it will be possible to use gene therapy in the clinical treatment of neurodegenerative diseases.

HISTORY OF GENE TRANSFER IN THE EXPERIMENTAL MODEL OF HD

Most neurodegenerative disorders are not yet fully understood in terms of their genetic bases.[34] However, since the primary cause of HD is a single genetic target, that would bind to the Htt gene and reduce its expression. Therefore, many treatments that were conducted for HD aimed to target this specific gene in different forms of gene therapy. One of the methods used for gene therapy was the discovery of RNA interference in 1998. This revolutionized gene therapy as it worked to silence genes at the posttranscriptional level. The study showed[33] the design of small interfering, short hairpins, and microRNAs that would bind to the Htt gene and reduce its expression. Viral vectors are usually used as the delivery method, especially AAV vectors. It was successful in delivering the RNA to mice and contributed to suppressing mutant Htt aggregation formation. Another study[34] also used similar methods for HD treatment, as it was based on the use of recombinant AAV serotype-5 vectors to deliver the anti-mutant huntingtin protein (mHTT) short hairpin RNA molecule into a transgenic HD mouse model.[35]

Other studies used gene therapy as a treatment of HD, but not all treatments aimed to target the Htt gene directly. In some studies,[36] the PA28γ gene was injected via LVs, as it is assumed that this gene transfer improves the proteasome function and motor behavior in HD mouse models. Their results proved that this gene transfer did indeed help with motor improvements in HD mouse models.[36]

A recent approach is the use of ASOs. ASOs are short, synthetic DNA molecules that bind to target RNA and silence the targeted gene. The CAG region of the Htt gene is associated with single nucleotide polymorphisms and it can be targeted with ASOs to silence the Htt gene. These could be injected directly into the intracerebroventricular space without a vector.[37] However, they can also bind nonspecifically to mRNA as well as premRNA.[37]

DEVELOPMENT OF GENE TREATMENTS FOR NEURODEGENERATIVE DISEASE

Examples of gene therapy for the treatment of neurogenetic disease are listed in Table 1.[5] One of those described above is now commercially available, i.e., spinal muscle atrophy (SMA). The expense of gene therapy targeting SMA is 1.4 M USD per patient. In 2017, the FDA approved gene therapy for a type of Leber's congenital amaurosis that applied AAV to transfer genes, and in 2019, the FDA also approved gene therapy for SMA that is based on the onasemnogene transfer by AAV.

Currently, many trials are being developed for other neurodegenerative and genetic diseases. For example, a study on the efficacy of nusinersen was conducted using a double-blind, placebo-controlled method for 126 patients with spinal muscular atrophy type2 (SMA2). They were aged from two to twelve years, and their symptoms started after six months of age. They were able to sit without assistance, had no history of independent walking, and had Hammersmith Functional Motor Scale-Expanded (HFMSE) scores of 10 to 54. Among these patients, 100 completed the 15-month treatment regimen, with 66 allocated to the Nusinersen arm and 34 to the sham-treatment arm. Of the 66 patients treated, seven were over 6 years of age, and 59 were under 6 years. Only one patient (14%) older than 6 years of age at baseline was considered a responder based on an improvement of 3 points or more on the HFMSE, whereas 38 of the 59 patients (64%) under 6 years of age at treatment initiation improved by 3 points or more.[38]

Table 1. Methods of gene therapy depending on the genetic makeup of the disease

Disease	Gene	Method	Administration
Spinal muscle atrophy	SMN1	AAV, ASO	Intravenous, intrathecal
Duchenne muscular dystrophy	DMD	AAV, ASO	Intravenous
Batten disease	CLN2	AAV	Intraparenchymal, intrathecal
Charcot–Marie–Tooth disease	NTF3	AAV	Intramuscular
Alzheimer's disease	APOE	AAV	Intracisternal
Parkinson's disease	GDNF	AAV	Neurosurgical
Leber's congenital amaurosis	RPE65, G11778A mitochondrial	AAV	Intraocular
Retinitis pigmentosa	USH2A	ASO	Intraocular
Huntington's disease	HTT	ASO	Intrathecal

SMN, survival motor neuron; AAV, adeno-associated virus; ASO, antisense oligonucleotide; DMD, Duchenne muscular dystrophy; CLN, ceroid-lipofuscinosis; NTF, neurotrophin; APOE, apolipoprotein E; GDNF, glial cell derived neurotrophic factor; RPE, retinal pigment epithelium; USH2A, usherin; HTT, huntingtin. Adapted from Renthal W.[5] Pract Neurol 2019;18:88-91.

CURRENT ADVANCES IN Htt-LOWERING GENE TRANSFER IN PATIENTS WITH HD

The Htt-lowering strategy using ASOs is the state-of-art strategy. ASOs are short, single-stranded nucleic acids that bind to the complementary mRNA strand through Watson-Crick base pairing. Depending on the target sequence of the mRNA, they can prevent protein biosynthesis, block splicing, or inhibit the binding of proteins.[39] However, they can only transiently downregulate the expression level of Htt. Therefore, to keep the Htt expression level low, repetitive application is essential. This is a great disadvantage compared to gene therapy using viral vectors such as AAV[6] because they can regulate gene expression permanently by introducing RNA interfering structures into brain regions.[39] Other than approaches using ASOs,[40] single-stranded RNA molecules (ssRNAs), small interfering RNAs (siRNAs)[41] and short hairpin RNA (shRNAs)[42] or artificial micro RNA (miRNAs)[43] were investigated to confirm the effect of lowering Htt levels by interfering with transcription in the modification of the disease. All of them interfere with transcription at the RNA level and downregulate Htt levels in vitro and in vivo.

Roche's ASO RO7234292 (RG6042) was developed by IONIS Pharmaceuticals Inc. (Carlsbad, CA, USA) (ISIS443139; IONIS-HTTRx), and it is currently being tested in Phase III clinical trials based on the results in a Phase I/IIa study.[43] This technology has been used successfully in other rare, inherited conditions. ASO is a chemically modified synthetic oligomer in the second generation that is complementary to a 20-nucleotide portion of Htt mRNA. Tabrizi et al.[44] demonstrated an approximately 40% reduction in mutant Htt protein in the cerebrospinal fluid (CSF) through 4 intrathecal injections of 120 mg HTTRx (later 267 RG6042) by lumbar puncture every 4 weeks. This effect was maintained during the follow-up period of two months, satisfying the safety requirements for consecutive multicenter Phase III testing. The expansion of open labels for the Phase I/IIa trial is currently in progress. The progressing Phase III clinical trial (called Generation HD 1, NCT03761849, Sponsor: Hoffmann-La Roche) is the first to investigate ASO in HD to demonstrate the potential of modulating the progression of the disease by a global study conducted in 101 regions. This randomized, double-blind, placebo-controlled trial will have registered 804 participants. In the 3-arm study, participants received either RG6042 at 120 mg or RG6042 at 120 mg plus placebo every 8 weeks or constantly received placebo for 2 years.[39] The purpose of the Phase III trial was to determine whether Tominersen was effective in reducing the expression of Htt protein, leading to improved clinical signs of HD in patients already showing symptoms.[45]

However, Roche recently announced that they are stopping the Phase III trial of the ASO HD drug Tominerson.[45] The decision was based on the potential risk/benefit profile, and data need to be further analyzed by Roche, but until then, the Phase III trial is not fully closed. Some factors that researchers might need to review are whether blocking the normal Htt protein affects patients and whether Tominersen's injection into the spinal column method is not effective enough.[45] The information is still very limited, but the Phase III trial is not yet canceled, as there are no reported new poor medical outcomes, and second, the benefit/risk profile could have changed. The future of ASO research in HD treatment depends on the decision about the continued use of Tominersen.

In parallel to Generation HD1, studies using ASO to downregulate the Htt level are in progress: Precision-HD1 (NCT03225833, Sponsor: Wave Life Sciences Ltd. [Cambridge, MA, USA]) and Precision-HD2 (NCT03225846). The criteria that target mutant Htt mRNA, while not affecting the translation of the normal allele, are achieved in the ASOs WVE-120101 and WVE-120102 by the recognition of 2 single nucleotide polymorphisms (SNPs) in the extended Htt gene.[46] The first SNP (RS362037) and the second SNP (RS362331) are present in approximately 50% and 40% of HD patients, respectively. Clinical Phase I/II is in progress to investigate the safety and target selectivity of WVE-12010 and WVE-120102. Phase I of Precision-HD1 and Precision-HD2 started in 2017, and preliminary data for the Precision-HD2 study were published in a press release in 2019 by WAVE Life Sciences Ltd. It was demonstrated that mutated Htt in CSF was downregulated by 12% through WVE-120102, for which the regulation is rather inferior as compared to RG6042 but it is not completely ineffective.[39]

AAV-based gene therapy is being developed in Uniqure, called AMT-130 therapy, which delivers adeno-associated viral vector serotype 5 (AAV5) by one-shot brain surgery, resulting in permanently reduced Htt expression. AVV5 is injected into the brain parenchyma using MRI technology. The miRNA will be loaded into the nucleus of the cell and bind to the Htt mRNA, marking it for degradation. In theory, the procedure is thought to be irreversible. It is a nonselective miRNA targeted for people in the early HD stages.[47] In vivo studies conducted in Q175 mouse models utilizing the surgical method of injecting AVV5 described above revealed a significant dose-dependent average decrease in mHTT protein of up to 39% in the striatum and up to 13% in the cortex.[48] The current AMT-130−01 study is researching its effect in humans as well as its safety. This study was conducted under double-blind conditions with a sham group. It is currently being investigated at the Phase I/II level.[47] Enrollment is currently ongoing in the US, and 26 patients will receive therapy by brain surgery and will be monitored at specialist HD clinics around the world.

SUGGESTIONS AND CONCLUSION

HD can be a potential and ideal model neurogenetic disorder with a single unique target. We have learned certain lessons from HD therapeutic advancement that modulating the mHTT level through gene transfer is one of the most important strategies to ameliorate the disease. This strategy is versatile and can be applied to various other neurodegenerative diseases, such as targeting amyloid-beta in Alzheimer's disease by gene transfer. Several independent clinical trials for HD gene therapy are ongoing in Phase III. Other than HD, there are patients with triplet repeat disorders with similar pathogenic mechanisms, waiting for a new therapeutic strategy to cure their disorders. In conclusion, the impact of the outcome of gene targeting therapy seems to be an initial step toward overcoming the tragedy of suffering from neurological genetic disorders.

Conflicts of Interest

The authors have no financial conflicts of interest.

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