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The Role of Gene Therapy in Neurodegenerative Disease Treatment

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Introduction

Neurodegenerative diseases affect millions of Americans each year, and there are currently very few treatments available. Neurodegenerative diseases cause selective loss of neurons and can affect multiple systems of the body (Kovacs 2014). Examples of neurodegenerative diseases include Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis, more commonly known as ALS or Lou Gehrig's disease. Age is the most common risk factor for developing neurodegeneration. As the elderly population has increased in recent years, the prevalence of neurodegenerative disease has also increased greatly over the last decade (Alzheimer's Association 2017). An estimated 40 million people worldwide suffer from some form of dementia due to neurodegenerative disease (Prince et al. 2013). Additionally, this figure is projected to double every 20 years until at least 2050, making this a global health crisis (Scheltens et al. 2016).

Neurodegenerative diseases are notoriously difficult to treat because they are commonly caused by a combination of both genetic and environmental factors. The inability to pinpoint one specific cause for many of these diseases makes it difficult to develop treatments; researchers are unsure which parts of the brain should be targeted by treatments. Due to the complicated nature of these diseases, there are currently no cures and only a few symptomatic treatments of varying effectiveness available for any of the more common neurodegenerative diseases (Heemels 2016).

Given the complex nature of these diseases, researchers have been considering new approaches to treatment. One such approach is gene therapy, "the use of nucleic acids (DNA or RNA) for the treatment, cure, or prevention of human disorders" (Kaufmann et al. 2013). Gene

therapy can be used to treat genetic diseases by replacing a mutated gene with a functional copy. However, gene therapy can also be used to treat diseases that do not have a genetic cause. For example, the first gene therapy in the United States was recently approved by the FDA to treat a rare form of pediatric leukemia. The treatment, known as CAR-T cell therapy, genetically alters the patient's own immune cells to express a new protein in order to better equip them to destroy cancer cells (Collins 2017). These modified cells are then re-infused into the patient's body to fight the disease.

Although gene therapy can be used to add a new gene to compensate for a non-functional one, there are also many cases where precisely modifying an existing gene would be useful in treating a disease. This approach is known as gene editing, the manipulation of the human genome to achieve a therapeutic effect (Maeder and Gersbach 2016). The current approach to gene editing is the use of programmable nucleases. These are nucleases that recognize a specific target DNA sequence and generate a double stranded break in the DNA at that location. There are three major types of programmable nucleases: zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR-Cas9 (Gaj et al. 2016). The basic approach utilizes a cellular mechanism known as non-homologous end joining (NHEJ). The creation of a double stranded break in the DNA triggers the cell's repair machinery to rejoin the broken ends of the DNA. This occurs in a highly-error prone manner that often leads to the insertion or deletion of one or more base pairs (Carroll 2011). These changes in the DNA sequence can alter the function of a gene dramatically, potentially creating what is known as a knockout mutation, a non-functional mutation in the protein-coding region of a gene. These mutations cause a loss of function in that gene, allowing researchers to study

the phenotypic effects of that gene being lost. A variation of this approach involves introducing a new sequence at the location of the double stranded break. If a homologous sequence is present, homology-directed repair (HDR) can occur, leading to the insertion of a new sequence. Researchers have considered the possibilities of using gene editing to replace mutant sequences with wild-type sequences for diseases where eliminating a defective allele would not be sufficient to restore a normal phenotype, such as hemophilia (Nathwani et al. 2017). All three types of programmable nucleases can be used both to create knockout mutations and to insert new DNA sequences.

The first programmable nucleases developed to edit gene sequences were zinc finger nucleases (ZFNs). ZFNs have two separate domains: a DNA binding domain and a nuclease domain from the *Fok*I restriction enzyme (Carroll 2011). The zinc finger portion of a ZFN consists of an amino acid sequence bound to a zinc atom; this sequence determines the ZFN's specificity for target DNA sequences (Carroll 2011). The *Fok*I domain then cleaves the target DNA, generating a double-stranded break. However, because it is difficult to manipulate and engineer ZFNs, the technique has lost popularity.

A newer technique for gene editing is transcription activator-like effector nucleases, TALENs, which were first used in 2009 (Gaj et al. 2016). TALENs work using a mechanism similar to ZFNs: they bind to a specific sequence in the DNA and generate a double-stranded break at that location, which usually leads to error-prone repair of the sequence, thereby disrupting gene function (Gaj et al. 2016). One of the major reasons that TALENs displaced ZFNs in the field of gene editing is their ease of manipulation. TALENs use a simple, modular DNA recognition code, making them easier to design to recognize a specific sequence (Gaj et al.

2016). However, TALENs are much larger proteins than ZFNs. This makes it very challenging to deliver them into target cells using traditional vectors, such as adeno-associated virus (AAV) and lentivirus vectors (Gaj et al. 2016). For these reasons, there has been a need for a technique that is easier to use, yet is still able to specifically target DNA sequences for editing.

In 2012, a technology now known as CRISPR-Cas9 was discovered, which also allows researchers to make double-stranded breaks in DNA by targeting a specific location with a guide RNA (gRNA) that is bound by a Cas9 nuclease (Jinek et al. 2012). CRISPRs, clustered regularly interspaced short palindromic repeats, are repetitive DNA sequences that are found naturally in bacteria and give bacterial cells immunity against invading viruses by using the Cas9 nuclease to cleave the genetic material of the virus (Jinek et al. 2012). Like TALENs and ZFNs, CRISPR-Cas9 can also be used to generate knockout mutations and insert new DNA sequences. To insert a new DNA sequence, all that researchers have to do is insert a copy of the desired DNA sequence along with the Cas9 nuclease and gRNA. Once Cas9 generates a break in the DNA, the cell's homology-directed repair (HDR) system can insert the desired DNA into the space left by the double-stranded break (Dance 2015). The inserted DNA sequence can be anywhere from a few to thousands of base pairs in size, providing an extremely wide range of possibilities for gene editing.

While conceptually similar to the previously discovered ZFNs and TALENs, CRISPR-Cas9 allows researchers to much more simply and efficiently target specific locations in a gene (Jinek et al. 2012). CRISPR-Cas9 works on the principle of complementary base pair interactions between nucleic acids, while ZFNs and TALENs are based on protein/nucleic acid interactions. The ease of developing a nucleotide sequence that can recognize another nucleotide sequence

makes CRISPR-Cas9 much simpler to use than other techniques. However, all three techniques present the risk of off-target effects (Gaj et al. 2016). If the programmable nuclease binds to the incorrect DNA sequence, it could result in a mutation in a non-target gene. Due to its relative simplicity and current popularity, I will focus on using CRISPR-Cas9 as a form of gene therapy.

While the main application of CRISPR-Cas9 with respect to neurodegenerative disease has been the creation of animal models, great potential exists for its use in human gene therapy. In this thesis, I will review how gene therapy could potentially be used to treat neurodegenerative disease. Specifically, I will evaluate the use of gene therapy in treatment of Huntington's disease, a classic Mendelian disease, and Alzheimer's disease, a neurodegenerative disease that involves both genetic and environmental factors. I will also discuss the potential for using the recently discovered gene editing technology, CRISPR-Cas9, to treat these diseases.

Huntington's Disease

One well-studied neurodegenerative disease is Huntington's disease (HD), which affects about 30,000 people in the U.S. each year (Huntington's Disease Society of America 2017). HD causes progressive deterioration of nerve cells in the brain, leading to loss of motor control, balance, and in most cases, decreased cognitive function (Roos 2010). The disease has a delayed onset, with symptoms typically not appearing until patients are 30 to 50 years old (Roos 2010). Death occurs in most patients 15-20 years after the onset of symptoms with these years marked by increasingly debilitating symptoms (Ross and Tabrizi 2011). Some of the main symptoms of the disease that affect mobility include bradykinesia (slowed movements), chorea (involuntary muscle movements), and rigidity (Phillips et al. 2008). Cognitively, Huntington's

disease affects judgment and planning skills and working memory function, and the disease causes various psychiatric disturbances including irritability and aggression (Phillips et al. 2008). Many patients also experience depression and suicidal thoughts as the disease progresses (Wyant et al. 2017).

Huntington's disease displays an autosomal dominant pattern of inheritance, meaning that if only one mutant copy of the *huntingtin* (HTT) gene is inherited, that individual is at very high risk of developing Huntington's disease later in life. The wild-type form of the huntingtin protein contains several evolutionarily conserved regions found in both vertebrates and invertebrates, suggesting that the protein has a critical function. The huntingtin protein is expressed widely throughout the mammalian brain, with the highest concentration of huntingtin-rich neurons found in the cerebral cortex and the hippocampus. In addition, huntingtin is essential for normal brain development (Reiner et al. 2011). A study investigating the effects of a knockout of the HTT gene only in the brains of mice mice showed that decreased HTT expression led to decreased brain size at three months of age, and significant neurodegeneration was observed by four to six months (Liu and Zeitlin 2017). Although the exact function of the huntingtin protein is still unknown, there are a wide variety of proposed functions including neurogenesis, synaptogenesis, and cell signaling (Liu and Zeitlin 2017).

A pathogenic mutation within the HTT gene of people with HD causes production of a defective huntingtin protein, which accumulates within the brain, leading to impaired function of neurons and eventually death. This toxic gain-of-function mutation leads to an expansion of a polyglutamine (polyQ) tract encoded in the HTT gene (Yang et al. 2017). In the wild-type huntingtin gene, the CAG sequence that encodes glutamine is repeated 9-35 times, but in

disease-causing alleles, it is repeated more than 35 times (Saudou and Humbert 2016). While the exact mechanism of pathogenesis is unknown, there are numerous ways that the mutant huntingtin protein might produce its toxic effects, including mitochondrial dysfunction, disruption of transcription, and dominant negative interactions between the wild-type and mutant forms of huntingtin (Jimenez-Sanchez et al. 2017).

There have been many clinical trials of proposed treatments for Huntington's disease, including drug and gene therapies, but treatments are primarily aimed at symptom management (Wyant et al. 2017). The current drug therapies focus on treating the motor and psychiatric symptoms of the disease. One drug that is commonly used for symptomatic treatment of chorea is tetrabenazine, which is one of the only FDA-approved drugs designed to suppress the involuntary movements associated with the disease (Frank 2014). However, this drug has no impact on disease progression and there are many potential negative side effects (Frank 2014). Other drugs focus on preservation of neurons, with treatments focusing on the neurotransmitters dopamine, glutamate, and gamma-aminobutyric acid (Frank 2014). However, the effectiveness of these treatments to date has been limited, with no impact on disease progression or onset, leading to an increased need for more effective treatments (Frank 2014).

One potential treatment route that has been considered for Huntington's disease is gene editing. Recently, a promising study was performed in a mouse model of HD. Researchers first created a knock-in model of HD in mice, replacing the wild-type HTT gene with the mutant form (Menalled et al. 2003). This led to expression of the mutant huntingtin protein (mHTT) in the mice, with significant mutant huntingtin accumulation found in the neurons of 9-10 month old mice (Yang et al. 2017). The researchers then injected the brains of these 9-10 month old

mice with two AAV vectors: one expressing the gRNA (AAV-HTT-gRNA) and one expressing the Cas9 protein (AAV-CMV-Cas9) (Yang et al. 2017). They found that three months after treatment, expression of mHTT in striatal neuronal cells was significantly decreased in mice treated with the gRNA and Cas9 vectors, but not in those mice treated with a control gRNA (Yang et al. 2017). Additionally, the researchers saw that there was a substantial decrease in the aggregates of defective huntingtin protein in the brain, which led to improved motor function (Yang et al. 2017). While this treatment has not yet been tested in human patients with HD, if similar results were seen in humans then CRISPR-Cas9 could become a breakthrough treatment for HD.

Another form of gene therapy that has potential for use in treatment of HD is gene silencing. This group of techniques, which includes RNA interference (RNAi), microRNAs (miRNAs), and antisense oligonucleotides (ASOs), can degrade target mRNA or prevent translation of a target mRNA into protein (Wyant et al. 2017). RNAi works via short interfering RNAs (siRNAs) that bind to target mRNA and activate the RNA-induced silencing complex, leading to degradation of the mRNA. This greatly decreases the amount of target mRNA that is available to be translated into protein (Wyant et al. 2017). miRNAs work by a related mechanism; they are short RNAs that can block translation or degrade target mRNA (Keiser et al. 2016). A study performed in a mouse model of HD used an AAV vector containing miRNAs targeting mutant HTT mRNA to examine the effects on mutant huntingtin expression. They found that there was an approximately 50% decrease in both mutant HTT mRNA and huntingtin protein production in the striatum of treated mice (Stanek et al. 2014). The treated mice also

demonstrated improved motor function after RNAi was performed, making this a potential treatment for HD (Stanek et al. 2014).

An alternative approach uses ASOs, (short, single-stranded nucleic acids that can bind to complementary mRNAs) which trigger degradation of transcripts as well as prevent translation of the mRNA into protein (Wyant et al. 2017). ASOs can bind pre-mRNAs in the nucleus, triggering recruitment of an enzyme that degrades the mRNA before it is mature and transported to the cytoplasm for translation (Skotte et al. 2014). These molecules can also bind to mRNAs in the cytoplasm, interfering with translation (Skotte et al. 2014). To target HD, ASOs that are capable of specifically targeting only the mutant HTT mRNA must be designed. In the case of Huntington's disease, these ASOs would lead to reduced production of the mutant protein, potentially slowing disease progression or even preventing disease onset altogether (Wyant et al. 2017). While this technique has only been tested in cultured cells, it shows potential as a form of treatment for HD.

One major challenge facing researchers is the delivery of gene silencing molecules into cells (Wild and Tabrizi 2017). In the case of RNAi or miRNAs, a vector is required for delivery. However, in many cases, vector size limits the length of the DNA sequence that can be introduced (Wild and Tabrizi 2017). For this reason, researchers have begun to search for other methods of gene silencing that do not require a vector, such as ASOs (Liu et al. 2017). Unlike RNAi or miRNAs, ASOs can be delivered directly to cells without a vector (Kaczmarek et al. 2017). In previous studies, researchers have been able to successfully deliver ASOs to the brain by directly injecting them into the cerebrospinal fluid (DeVos and Miller 2013). When using this method of delivery, the researchers found that the ASOs were evenly distributed throughout

tissues, rather than being concentrated in one area of the brain. This indicated that the ASOs were being taken up by cells via active transport, although the exact mechanism through which uptake occurs remains largely unknown (DeVos and Miller 2013).

Once gene silencing molecules have been successfully delivered into cells, researchers have to be concerned with the degradation of these molecules. siRNAs and miRNAs are broken down much more quickly than ASOs, meaning that frequent treatments might be required in order to achieve the desired effects (Wyant et al. 2017). ASOs, on the other hand, are degraded at a slower rate compared with other small RNA molecules (Evers et al. 2015). This slower degradation rate is due to a chemically modified backbone found in ASOs. There are no enzymes present in the cell that can recognize and cleave these backbones, and so the ASOs may remain active for an extended period of time (Evers et al. 2015). Due to this longer lifespan within the cell, treatments may not be required as frequently, making ASOs a promising treatment for HD.

In addition to the challenges of delivery and degradation, off-target effects are an issue with ASOs, RNAi, and miRNAs. In many cases, the specificity of these nucleotide sequences is not high enough to guarantee that only the mutant mRNA will be targeted for degradation, yet function of the wild-type allele must be preserved while simultaneously eliminating function of the mutant allele (Wyant et al. 2017). Additionally, wild-type mRNAs from unrelated genes with sequences similar to the sequence of the mutant HTT allele may be targeted by the ASOs, producing an off-target effect and blocking translation of non-HTT mRNAs (Wyant et al. 2017). While off-target effects have been observed with ASOs, there is some evidence that they have higher sequence specificity and produce fewer off-target effects than other gene silencing

mechanisms (Wild and Tabrizi 2017). Due to this specificity, there is less risk of the ASO targeting wild-type HTT mRNAs in addition to mutant mRNAs, significantly increasing its appeal as a treatment for HD (Wyant et al. 2017).

Parkinson's Disease

Parkinson's disease (PD) is another neurodegenerative disease that has the potential to be treated with gene therapy. PD is the second most common neurodegenerative disorder after Alzheimer's disease, affecting 2-3% of the population over the age of 65 (Kalia and Lang 2015; Williams-Gray and Worth 2016). Given the growing size of the elderly population in the United States, Parkinson's disease is a risk for many. Similar to Huntington's disease, Parkinson's disease has both physical and psychiatric effects. Some of the physical symptoms seen in those with the disease include bradykinesia, tremors while at rest, or rigidity of the limbs (Williams-Gray and Worth 2016). Mood and sleep disorders, hallucinations, and depression are just a few of the psychiatric symptoms seen in many patients as the disease progresses (Kalia and Lang 2015).

While researchers know that development of Parkinson's disease involves both genetic and environmental factors, the pathology of PD is not entirely understood. As a result, researchers have been unable to develop a highly effective treatment, let alone a cure, for the disease. Treatment options currently available include drugs that focus on treating the symptoms of the disease, such as motor dysfunction and mood disorders (Williams-Gray and Worth 2016). Similar to Huntington's disease, these treatments are often ineffective or, at best, mildly effective for a short period of time; they have no effect on disease progression.

One of the most common drug therapies used to treat Parkinson's disease involves increasing the production of dopamine in the brain (Coune et al. 2012). As PD progresses, neurons in the brain that are responsible for producing dopamine, known as dopaminergic neurons, degenerate and eventually die. The death of these neurons leads to a dopamine deficiency, causing some of the motor symptoms associated with the disease (Kalia and Lang 2015). One type of drug therapy, L-dopa therapy, has been used to treat symptoms of Parkinson's disease since 1969; it works by increasing production of dopamine in the brain's remaining dopaminergic neurons (Coune et al. 2012). However, there are many disadvantages to this treatment. L-dopa therapy requires frequent administrations in high doses in order to achieve any improvement in symptom severity; such high doses can cause adverse side effects (Coune et al. 2012). Additionally, over long periods of time, treatment can become ineffective at controlling motor symptoms in many patients (Lin et al. 2017).

Like HD, the relatively low effectiveness of available drug therapies for PD has led researchers to consider gene therapy as an option. However, in contrast to HD, where a single mutation in a specific gene results in the HD phenotype, development of PD involves numerous genetic and environmental factors. While some specific genetic variants have been shown to be associated with the disease, environmental factors still play a significant role. This suggests that targeting a specific genetic variant would not be an effective approach to disease treatment, as it is with HD. An additional challenge when treating PD is that most cases of the disease are sporadic, with only 2-3% of cases being familial (Williams-Gray and Worth 2016). Because the majority of cases are sporadic, I will be focusing on the use of gene therapy to treat this form of the disease.

When considering gene therapy for PD, researchers must consider the overall goal of treatment. In HD, gene therapy aims to eliminate expression of the toxic huntingtin protein by preventing expression of a specific gene. However, in PD, the goal of gene therapy would be to promote the expression of proteins that protect neurons and stimulate neuronal growth. For this reason, techniques such as ASOs or miRNA that prevent gene expression would not be the right approach for treatment. Instead, researchers have considered using gene therapy to express neurotrophic factors in the brain to protect neurons. Gene therapy using two neurotrophic factors, glial-cell derived neurotrophic factors (GDNF) and neurturin (NTN) are of particular interest to researchers. These factors are known to play a role in maintenance of the nervous system and potentially in survival of dopaminergic neurons (Lin et al. 2017). Utilizing these factors in treatment could be a way to prevent the death of dopaminergic neurons seen in both familial and sporadic forms of Parkinson's disease.

By the end of 2017, three clinical trials testing the safety and efficacy of gene therapy as a form of treatment for Parkinson's disease had been completed. One of these trials involved the surgical insertion of an AAV2 vector containing the gene for NTN into the putamen of patients diagnosed with PD (Olanow et al. 2015). AAV2 is a viral vector that is one of the best characterized vectors used for gene delivery (Olanow et al. 2015). The treatment, known as CERE-120, was performed in 24 patients who were then examined every three months for a total of 15 months. The treatment was well-tolerated by patients, and in the second part of the study, the researchers evaluated patients' motor function (Olanow et al. 2015). Over the course of the 15-month study, no significant improvements in motor function were seen in the patients who received CERE-120 compared to control subjects (Olanow et al. 2015). However,

all patients that participated in the trial were in the late stages of PD, so it is possible that treatment may need to be administered earlier in disease development to be effective (Olanow et al. 2015). Researchers also hypothesized that the ineffectiveness of the treatment could also be attributed to impaired transport of the NTN gene from the surgical site to other areas of the brain (Olanow et al. 2015). However, follow-up studies testing effectiveness of the treatment when administered to other sites also failed to show any differences in efficacy (Olanow et al. 2015).

While this study was not successful at treating symptoms of PD, it did demonstrate the safety of using viral vectors as a delivery method for gene therapy. No significant side effects were found to be associated with the delivery of the AAV2-NTN vector, and weight loss was not seen as a side effect in any of the treated patients (Olanow et al. 2015). This is particularly important with a disease like Parkinson's disease, where significant weight loss can impact disease progression and increase severity of disease symptoms such as dyskinesia (Ma et al. 2018).

Gene therapy to promote expression of GDNF has also been pursued as a possible treatment for PD. One study involved injection of an AAV vector directly into the brain of a rat model of PD. This vector was linked to a promoter that could induce increased expression of GDNF (Tereshchenko et al. 2014). The researchers found that increased GDNF expression levels improved motor control and had a protective effect on dopaminergic neurons in treated rats (Tereshchenko et al. 2014). However, within three weeks of treatment, levels of GDNF had decreased back to baseline levels. This indicates that frequent treatments would be required in order to maintain increased levels of GDNF in the brain. Additionally, while significant weight

loss was not observed in treated rats in this study, other studies have observed severe weight loss as a side effect of GDNF gene therapy (Manfreddson et al. 2009). This indicates that increasing levels of GDNF may be a less advantageous form of gene therapy compared to NTN-based gene therapy.

One of the major benefits of using gene therapy to deliver neurotrophic factors is that fewer treatments may be required, especially in the case of NTN. Additionally, delivery of viral vectors to a specific site has become better understood in recent years, making direct delivery of genes expressing neurotrophic factors a possibility for treatment (Coune et al. 2012). While L-dopa therapy still remains the standard treatment for PD, it only treats the symptoms of the disease and requires frequent treatments.

Conclusion

Neurodegenerative diseases affect millions of Americans every year with limited treatment options available. Those treatments that are available are often minimally effective and have no impact on disease progression. My thesis has focused on the use of gene therapy as a possible treatment for some of these devastating diseases, including Huntington's disease and Parkinson's disease. However, there are several different challenges that must be overcome before gene therapy can be used to treat neurodegenerative disease.

One of the major issues that must be resolved is delivery. Gene editing molecules must be delivered directly to cells, either through injection or the use of a vector. Vectors limit the size of the DNA sequence that can be delivered to the cell, indicating that using a molecule that does not require a vector, such as ASOs, may be more practical for treatment of neurodegenerative disease. Another challenge facing researchers is specificity. When using a

technique that targets a specific DNA sequence for editing, there is always the possibility that the gene editing molecule will target similar sequences and edit those as well. This is a major problem when considering the use of gene therapy for Huntington's disease treatment; any gene silencing molecule that targets the mutant HTT gene or mRNA could also target wild-type HTT, leading to its degradation. To effectively treat a disease using gene therapy, researchers must be able to ensure that off-target effects are not occurring.

A final problem to consider is the degradation of gene editing molecules. Even after successful delivery to the target cells, siRNAs or miRNAs are degraded by the cell at a rapid rate. More frequent treatments may be required in order to achieve the desired effects, placing a greater burden on both patients and researchers. In contrast, ASOs have a chemically modified backbone, that protects them from degradation by RNases (Schoch and Miller 2017). Because of this increased resistance to degradation, is believed that they may remain active within the cell for a longer period of time; this may indicate that fewer treatments would be required.

Compared to other methods of gene therapy, ASOs are easier to deliver to cells and they may remain active within the target cells for an extended period of time. While significant improvements still need to be made in specificity before any clinical trials can be performed in humans, ASOs have been shown to be successfully delivered to the brain via injection into the cerebrospinal fluid of mice. Additionally, the ASOs were evenly distributed throughout the tissues. The decreased rate of degradation within the cell could also indicate that less frequent treatments may be required, alleviating some of the stress placed on patients. For these reasons, I believe that ASOs could be the most feasible method of gene therapy for Huntington's disease.

Given the multitude of genetic and environmental factors involved in development of PD, treatment using gene therapy will not be as straightforward as with HD. However, promising clinical trials have been completed. The CERE-120 study demonstrated the safety of using a vector to deliver neurotrophic factors directly to the brain with very limited adverse effects. On the basis of side effects, NTN seems to be a better treatment option for PD because it does not appear to cause severe weight loss, a major side effect that PD researchers want to avoid. Significant weight loss has been observed in GDNF-based gene therapy; however, results have varied, and more studies will need to be completed before one neurotrophic factor can be considered more beneficial for treatment of PD.

Looking forward, there are still many aspects of gene therapy that need to be improved upon before it can be utilized to treat neurodegenerative diseases. While delivery is one of the primary issues facing researchers, both specificity and maintenance of gene editing molecules must be improved. Based on the current literature available, I believe that ASOs are one of the more promising techniques that could be used in treating HD. While there have been fewer clinical trials examining the use of gene therapy for PD, treatment that increases levels of NTN seem to have fewer side effects than GDNF gene therapy. More studies demonstrating safety and efficacy of gene therapy for neurodegenerative diseases will be required before completing additional trials in humans, but gene therapy remains one of the most promising potential treatment routes available to researchers today.

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