

# Clinical Applications of Adeno-Associated Virus Gene Therapy in Rare Diseases

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**Abstract.** This paper provides a comprehensive overview of adeno-associated virus (AAV) gene therapy, a revolutionary approach that has shown promise in treating various genetic disorders. The therapy uses a harmless virus to deliver a functional copy of a defective gene to patient cells, thereby correcting the underlying congenital defect. AAV gene therapy is first discussed in the context of three specific diseases: Duchenne muscular dystrophy, Spinal Muscular Atrophy, and Huntington's disease. AAV gene therapy has been demonstrated to improve disease progression and patient quality of life in some cases, based on existing findings and arguments. In addition, we acknowledge the high cost of these therapies, which can range from \$850,000 to \$3,500,000, which limits their wide application. Although there are high costs associated with AAV gene therapy, the benefits, such as improved patient survival and quality of life, often outweigh them. Based on a study on the cost-effectiveness of AAV-mediated gene therapy for severe haemophilia B, the therapy was found to be more cost-effective than alternatives in most cases. Despite its potential for treating genetic disorders, AAV gene therapy poses a significant financial challenge due to its high cost. To make the therapy more accessible, future research should focus on reducing these costs. AAV gene therapy's long-term effects and safety require further investigation, as do its long-term safety concerns.

**Keywords:** Rare disease; clinical application; AADC; SMA, Haemophilia.

## 1. Introduction

Gene therapy is an experimental technique for treating and preventing disease by modifying human genes. It has demonstrated significant clinical potential in treating hereditary diseases, tumours and certain viral infections. Rare diseases are a group of diseases that affect a relatively small number of people but, because of their variety, a large number of people overall. These diseases often cause significant suffering to patients because the cause of the disease is unknown, or treatment options are limited [1].

AAV in the 1960s marked the beginning of a long process of understanding and application of these viruses. AAV was initially thought to be a contamination of adenoviral cultures; however, as research progressed, it was discovered that AAV was only capable of replicating when it was present in the presence of adenoviruses or herpes simplex viruses type one (HSV-1), thus establishing an association between adenoviruses and AAVs. After determining that AAV could transform mammalian cells, researchers began to produce recombinant AAV (rAAV). This discovery advanced the field of gene therapy and opened up new possibilities for AAV gene therapy, which has brought new possibilities and hope for treating rare diseases [2, 3].

The potential of AAV gene therapy lies in its ability to directly repair or replace the genes responsible for the disease, addressing the root cause. Viral vectors do not have the pathogenicity of wild viruses and can be modified to carry typical human gene sequences. These vectors can infect human cells like a virus and carry normal human genes into the cell nucleus, causing the "infected" cell to produce functional proteins that may ultimately alleviate or even cure the disease [4].

This paper will explore AAV gene therapy's specific applications and potential in treating rare diseases. This paper will analyze in detail some of the AAV gene therapies that have demonstrated significant efficacy in clinical trials and how these therapies have changed the lives of patients with rare diseases. In addition, this paper discusses some of the challenges of AAV gene therapy, including safety issues, the complexity of manufacturing and distribution, and the high cost of treatment. Finally,

this paper looks at future developments in AAV gene therapy, including new therapy development, strategies to improve existing therapies, and how to overcome existing challenges.

## 2. AAV Technology

AAVs are small, non-enveloped viruses with a single-stranded DNA genome. They belong to the Parvoviridae family and were first discovered in the kidney cell cultures of rhesus monkeys. The AAV genome is approximately 4700 base pairs long and consists of two open reading frames (ORFs) flanked by two inverted terminal repeats (ITRs), each composed of 145 nucleotides [5]. They possess the following distinct characteristics and results.

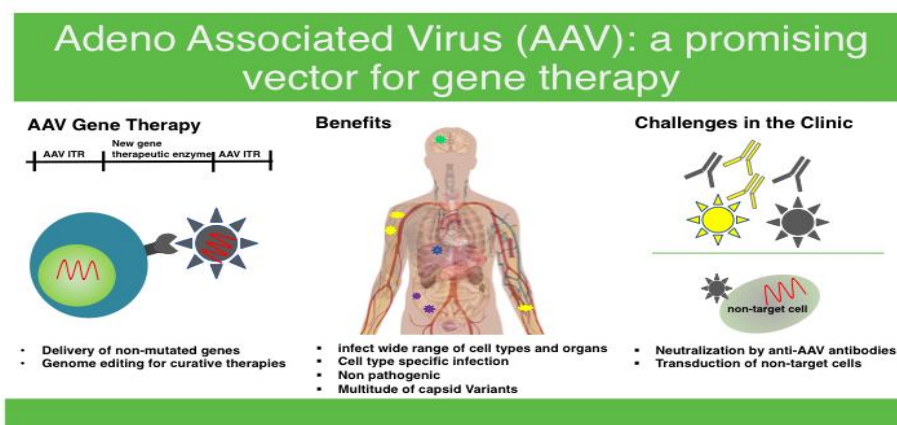
Firstly, AAVs are unable to replicate on their own because they are replication defective. Instead, they require co-infection with a helper virus like an adenovirus or herpes virus to reproduce. Due to their wide host range, safety, low immunogenicity, stable expression, and physical properties, AAVs are commonly utilized in clinical trials and basic research. As a result, they have become one of the most frequently used gene therapy vectors globally.

AAVs are replication-defective, meaning they cannot replicate independently. To reproduce, they require co-infection with a helper virus, such as an adenovirus or a herpes virus. This feature and their broad host range, high safety profile, low immunogenicity, stable expression, and stable physical properties make AAVs widely used in basic research and clinical trials. They have become one of the most commonly used gene therapy vectors worldwide.

Secondly, The AAV vector technology delivers a specific DNA sequence to target cells. The DNA sequence of interest is packaged into the AAV vector, replacing the viral genes. This results in a recombinant AAV (rAAV) that can deliver the DNA to the target cells but cannot replicate or cause disease. The rAAV is then introduced into the patient's body, entering the target cells and delivering the DNA sequence. The cell's machinery can then use the DNA sequence to produce the desired protein, potentially treating the disease.

To visualise this, imagine the AAV vector as a delivery truck. The truck (the AAV vector) is loaded with a package (the DNA sequence) at the factory (the laboratory). The truck then delivers the package to the delivery address (the target cells in the patient's body). The recipient (the cell) then uses the contents of the package (the DNA sequence) to produce the desired product (the protein).

Thirdly, regarding safety, the National Institutes of Health (NIH) has classified AAVs as Risk Group 1 (RG1), the safest level. In contrast, adenovirus vectors are classified as RG2, and retrovirus vectors are classified as RG3. To date, no pathogenicity has been associated with AAVs. Clinical trials for diseases such as haemophilia A and B, retinal diseases, and spinal muscular atrophy (SMA) have demonstrated the safety and effectiveness of AAVs as therapeutic tools. Fig. 1 is the workings of the AAV Vector are displayed [4].



**Fig. 1** The workings of the AAV Vector are displayed. <https://blog.addgene.org/adeno-associated-virus-aav-for-cell-and-gene-therapy>

### 3. AAV in treating rare diseases

As shown in Table 1, there are the Characteristics and Treatment methods of Aromatic L-Amino Acid Decarboxylase Deficiency (AADC), Spinal Muscular Atrophy (SMA), Huntington's disease (HD), and Hemophilia A&B.

**Table 1** Display of Characteristics and Treatment methods of four Rare diseases.

Disease	Characteristic	Treatment	AAV Gene Therapy
AADC	AADC disease is a rare inherited neurotransmitter disorder with significant symptoms, including muscle hypotonia, movement disorders, autonomic dysfunction, and developmental delays.	Current treatment is mainly pharmacological, including the use of dopamine receptor agonists and monoamine oxidase inhibitors.	AAV gene therapy is a new treatment that restores neurotransmitter production by inserting the normal AADC gene into a patient's brain.
SMA	SMA is a hereditary disease characterised by muscle atrophy and weakness.	Current treatment mainly includes physiotherapy, respiratory, and nutritional support	AAV gene therapy is a new treatment that restores muscle function by inserting the normal SMN1 gene into the patient's body.
HD	Huntington's disease is an inherited neurodegenerative disorder with significant symptoms, including movement disorders, cognitive disorders and psychiatric symptoms.	Current treatment focuses on drug therapy and rehabilitation to relieve symptoms and improve quality of life.	AAV gene therapy is a new treatment that slows the neurodegenerative process by inserting the normal Huntingtin gene into a patient's brain.
Hemophilia A&B	Haemophilia A and B is an inherited blood clotting disorders whose primary symptom is a tendency to bleed, including bleeding from joints, muscles, and internal bleeding.	Current treatment focuses on replacing deficient clotting factors to prevent and treat bleeding events.	AAV gene therapy restores clotting by inserting normal clotting factor genes into the patient's body.

#### 3.1. AADC

AADC disease, also known as AADC deficiency, is a rare neurometabolic disorder. Mutations or defects in the AADC gene, which encodes the AADC enzyme, can cause the disease. This enzyme is vital in the nervous system as it converts L-dopa into dopamine, an essential neurotransmitter [6].

AAV gene therapy works by embedding the typical AADC gene sequence into an AAV vector and introducing it into the patient's cells. Once the AAV vector enters the patient's cells, it releases the AADC gene and helps the cells begin to produce the normal AADC enzyme. This approach aims to restore the AADC enzyme's function by repairing or replacing the defective gene, thereby alleviating the symptoms of AADC disease.

Early clinical studies and animal model studies have shown the potential effectiveness of AAV gene therapy in AADC disease. Researchers have observed significant increases in AADC enzyme production with AAV gene therapy and improved motor function and quality of life in patients.

### 3.2. SMA

SMA is a rare neuromuscular disorder that primarily affects neurons in the spinal cord. SMA is caused by mutations or deletions in the SMN1 gene, which encodes motor neuron protein (SMN), which plays a vital role in the nervous system [7].

The severity of SMA disease varies from individual to individual, but the standard features are muscle weakness and muscle atrophy. This disease leads to a decline in muscle function, including motor dysfunction, abnormal muscle tone, and effects on breathing and swallowing. Severe types of SMA may lead to respiratory failure and premature death.

The number of copies of the SMN2 gene largely determines the clinical severity between SMA types, with other genetic or environmental factors playing only a minor role. The greater the number of SMN2 copy mutations, the greater the likelihood of developing a severe phenotype. Therefore, the greater the number of SMN2 types, the greater the clinical severity. If left untreated, it results in severe limitations on motor function, including the inability to walk; a high risk of respiratory complications requiring some level of respiratory support; and a high risk of orthopaedic complications, such as painful contractures and scoliosis, which are shared; as well as a shortened life expectancy. Between 45 per cent and 60 per cent of all cases of SMA are SMA type I, making it the most common form of SMA.

Clinical trial results have shown that AAV gene therapy can significantly improve disease progression and quality of life for certain SMA patients. In particular, for SMA type 1 patients, early intervention and AAV gene therapy can slow disease progression and improve patients' motor function and survival. For SMA type 2 patients, AAV gene therapy can improve muscle strength and function [8].

Zolgensma, also called Onasemnogene abeparvovec, is a newly approved gene therapy by the U.S. Food and Drug Administration. It is used to treat patients with SMA under two years old. Zolgensma is a sterile, preservative-free, intravenously infused, non-replicating, self-replicating adeno-associated virus 9 (AAV9) that can cross the blood-brain barrier. The active substance of Zolgensma contains a functional copy of the SMN1 gene controlled by the cell proliferating virus (CMV) enhancer/chicken-beta-actin-mixed promoter (CB). The transgene is a double-stranded structure that is ready for transcription, which is formed by modifying one of two adeno-associated viruses (AAV) inverted terminal repeats (ITRs) to promote intramolecular annealing. The therapy restores the normal SMN protein, which regulates cellular homeostatic pathways and influences the state of motor neurons.

### 3.3. HD

HD is an inherited neurological disorder primarily affecting the brain. The disease is caused by a repeat amplification of the CAG trinucleotide in the HTT gene (Huntington's protein gene), which leads to an abnormal accumulation of Huntington's protein and degeneration of nerve cells.

Symptoms of HD usually appear in middle-aged adults, although the age of onset can vary in children and older adults. Symptoms include movement disorders (such as involuntary twisting movements and muscle rigidity), cognitive deficits (such as memory loss and thought disorders), and behavioural and emotional problems (such as depression and anxiety). These symptoms worsen as the disease progresses and seriously impact the patient's daily life [9].

AAV gene therapy has been studied to treat HD disorders and reduce symptoms and disease progression. Researchers have typically used an approach known as RNA interference to suppress the expression of HTT genes. They have developed AAV vectors carrying molecules capable of producing specific RNA interference molecules (siRNAs or shRNAs) that selectively target the

mRNA of the HTT gene, inhibiting its production of the Huntington protein. This reduces the accumulation of abnormal proteins and attenuates nerve cell degeneration.

### 3.4. Haemophilia

Haemophilia is a group of inherited bleeding disorders that primarily affect males and are majorly classified as haemophilia A (coagulation factor VIII deficiency) and haemophilia B (coagulation factor IX deficiency). Due to the genetic abnormality of these two factors, they cannot be translated to produce normal F8/F9 proteins, resulting in the patient's difficulty in blood clotting, which will not stop once bleeding occurs. Clinical manifestations include spontaneous bleeding in joints, muscles, internal organs, and deep tissues or bleeding that is difficult to control after minor wounds [10]. The symptoms often start in childhood. Moreover, repeated bleeding in joints will lead to progressive joint mobility disorders and disability in children. Current treatments can effectively reduce the disability rate and improve the quality of life, but they cannot completely cure the disease.

The history of haemophilia is deeply intertwined with the scientific community's evolving understanding of blood clotting mechanisms. The earliest recorded instance of a bleeding disorder can be traced back to approximately 200 A.D. However, it was in the early 1820s that French surgeon Jean-Louis Petit made a significant breakthrough. His discovery that hemostasis following an amputation was due to the formation of blood clots in the vessels marked the first association between blood coagulation and hemostasis.

The most common treatment for haemophilia medication is Enzyme replacement therapy (ERT). Since haemophilia A is Factor VIII-deficient and type B is Factor IX-deficient, the patient's clotting ability can be strengthened with intravenous injections of F8 or F9 protein, and injections of plasma containing Factor VIII or Factor IX will also work [11]. However, regular intravenous injections and prophylactic medication (sometimes daily) are a great financial burden.

However, gene therapy is currently the most cutting-edge therapy for haemophilia. I'm taking the example of BioMarin Pharmaceutical's haemophilia A therapy BMN270 to show how gene therapy can help haemophilia A.

AAV is a virus that many people carry or have carried. When infected, AAV's DNA is free from the cell's nuclear genome, with a low chance of integration, and is relatively safe with few symptoms after infection. Haemophilia A is a mutation in the Factor VIII gene at the DNA level, resulting in the inability to produce the typical Factor VIII protein. Simply expressing the normal Factor VIII protein in the body's cells and secreting it into the plasma improves systemic clotting. Therefore, BMN270 delivers Factor VIII DNA to the patient's liver via intravenous infusion, replacing its faulty version of the DNA to achieve a cure. Similar gene therapies for haemophilia B, such as Spark Therapeutic's SPK-9001, improve coagulation by expressing the F9 variant, specifically in liver cells. Gene therapy drugs are costly to develop and produce (for example, the cost, and for rare diseases, there are very few patients, making gene therapy drugs relatively expensive).

## 4. Challenges and Future Perspectives

### 4.1. Challenges and Limitations of AAV Gene Therapy in Treating Rare Diseases

Although AAV gene therapy has shown great potential in treating rare diseases, many challenges remain. Firstly, producing and purifying AAV vectors is a complex and expensive process, which may limit their application in the clinic. Secondly, the safety and efficacy of AAV vectors still require further research and validation. For example, AAV vectors may elicit an immune response from the host, affecting their effectiveness. In addition, the targeting of AAV vectors is also an important issue, and there is a need to ensure that AAV vectors can accurately deliver genes into target cells.

Another significant challenge in AAV gene therapy is how to improve the targeting of viral vectors. Although AAV viruses effectively deliver genes to specific cells or tissues, ensuring that the viral vectors only infect the target cells and do not affect other healthy cells remains a problem that needs

to be solved. To address this problem, researchers are developing new viral vectors that can deliver genes more precisely into target cells.

#### 4.2. Regulatory Challenges

The industry lacks sufficient experience in supplying viral vector gene therapies on a commercial scale and therefore requires enhanced guidance on quality and chemistry, manufacturing and controls (CMC). Regulators are continuously issuing specific guidance for cell and gene therapies but tend to be cautious due to the limited availability of long-term safety data and the potential risk of unintended changes to patient genomes. As a result, regulators may request adjustments to CMC controls, Good Manufacturing Practices (GMP)-certified materials, and potency assays, leading to delays in the approval process.

**Improving delivery efficiency:** Although AAV has shown a favourable safety profile in gene therapy, its delivery efficiency remains challenging. Researchers are continually working to enhance AAV vectors to increase their delivery efficiency to target cells for more efficient delivery of genetic material.

**Expanding vector capacity:** AAV can only carry more minor gene sequences due to its limited gene loading capacity. Future developments will focus on technological innovations to expand the ability of AAV vectors to carry more extensive gene sequences, making them suitable for more types of gene therapy.

**Precise targeting for specific cell types:** Different diseases and therapeutic targets often involve different types of cells. Therefore, researchers are committed to developing AAV vectors that target particular cell types precisely. This allows for better delivery of genetic material to the target cells, improving therapeutic efficacy and reducing potential side effects.

**Improving long-term expression stability:** Long-term gene expression is critical for treating many diseases. The stability and durability of AAV vectors is a crucial issue. Future research will focus on improving the long-term presentation of AAV to ensure that therapeutic effects are durable and stable over time.

**Combination with other therapies:** Combining AAV gene therapy with different therapeutic approaches, like drug therapy or gene editing techniques, can be beneficial. This combined treatment strategy may provide additional efficacy and broaden the field of application of AAV in the treatment of multiple diseases.

For the past few years, for AAV gene therapy, pre-existing anti-AAV neutralising antibodies have posed a complex problem, as they have greatly limited the population of patients who might benefit from AAV gene therapy. However, significant advances have been made against this formidable obstacle, addressing these issues and providing additional benefits through engineered AAV. With the emergence of clinically approved products on the global market and an increasing number of successful ongoing clinical trials, AAV is at the forefront of gene therapy. While further technological advances and additional clinical trials are needed to validate its effectiveness, AAV has shown great promise for the future of gene therapy.

It is still possible to treat haemophilia using a wide variety of treatments at present. The disease cannot be completely cured, but patients can still live like ordinary people if they cooperate actively. In their daily life, patients need to pay more attention to themselves and try to prevent trauma. Otherwise, it will cause the disease to relapse and significantly impact the body. At the same time, many better gene therapies have been expensive for many haemophiliacs, which also requires the government's cooperation. In addition, viral vector manufacturing must evolve rapidly because of the increasing demand for viral vectors. Broader applications of viral vector gene therapy (e.g., for more common diseases) require higher yields and lower costs of goods. However, this rapid influx of funding and new technologies has not yet addressed the bottlenecks and challenges of viral vector manufacturing.

Despite the challenges described above, the outlook for the future is promising as scientific research continues to advance our understanding of AAV gene therapy. As new research methods

and technologies continue to evolve, we will likely find more effective ways to improve the targeting and safety of AAV gene therapy. In addition, as our understanding of the human immune system continues to grow, we may find ways to overcome the immune responses that patients may experience. Although AAV gene therapy faces many challenges, as scientific research continues to progress, AAV gene therapy will play a critical role in future medical treatments.

## 5. Conclusion

This paper explores AAV gene therapy in treating genetic disorders such as Duchenne muscular dystrophy, spinal muscular atrophy, and Huntington's disease. AAV gene therapy corrects underlying congenital defects by delivering functional copies of the genes into the patient's cells using a harmless virus. However, the high cost of this therapy and concerns about its long-term effects and safety constitute significant challenges to its application. Nonetheless, the potential of AAV gene therapy to improve disease progression and enhance patients' quality of life makes it a promising avenue for future genetic disease treatment. In treating diabetic cardiomyopathy, we need to gain a deeper understanding of the pathogenesis of the disease and find new therapeutic targets. Even though some drugs have been shown to reduce diabetes-related cardiovascular risk, lowering blood glucose on its own is often insufficient to prevent the development of diabetic cardiomyopathy. In various disease settings, adeno-associated virus (AAV) gene therapy is highly effective. It effectively targets specific cells or tissues with a low host immune response. We must consider multiple factors to target the virus to the heart, including AAV serotype, promoter, etc. Although phase 2b clinical trials of AAV gene therapy did not show significant improvements in disease recurrence and ultimate events (death), the results of both its phase 1 and 2 clinical trials were favourable, with few or no side effects in some patient populations. In an encouraging development, a phase 3 clinical trial has been planned to use adenylyl cyclase-6 gene therapy to treat heart failure. There is evidence from these studies that therapeutic gene approaches are being used to treat heart-related diseases and are showing promise, at least in the preclinical stages.

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