Abstract

Hemophilia is an X-linked recessive genetic disorder in which the body has an inability to clot, leading to an increased risk of mortality for individuals if a bleeding episode were to occur. Traditional hemophilia treatments, such as prophylactic factor replacement therapy of recombinant factor VIII and IX, have been proven to be costly and do not provide long-lasting protection during bleeding episodes. In recent years, the use of Adeno-associated viral vectors (AAV) gene therapy has been explored as a potential alternative due to efficient gene delivery and tissue tropism, overall safety and efficacy, and longer-lasting effects. However, concerns over inhibitor development persist due to the treatment complications. This review article seeks to provide an overview of the current state of AAV-based gene therapy as a treatment for hemophilia.
Introduction

I. Background Information
Hemophilia is a bleeding disorder associated with difficulties in blood clotting. These complications are caused by the low levels of factor VIII, associated with Hemophilia A, or factor IX, associated with Hemophilia B. The amount of clotting factor deficiency determines the severity of hemophilia for each patient\textsuperscript{1-3}. Hemophilia affects over 30,000 individuals in the United States. Over half (around 60%) of that population is diagnosed with severe hemophilia while fewer suffer from moderate and mild hemophilia. Hemophilia A is four times more common than Hemophilia B and is one of the most widely distributed bleeding disorders in the population\textsuperscript{4}. There are three classes of hemophilia: mild, moderate, and severe. The severity of hemophilia is defined by the percentage of clotting factor deficiency. A healthy range of factor VIII and IX is between 50-150% factor activity. Individuals with mild hemophilia have 5-40% factor activity where bleeding proceeds injury, trauma, or surgery. Moderate hemophilia has 1-5% factor activity where minor injuries or spontaneous bleeding occurs. Severe hemophilia, the most common, is classified by <1% factor activity where frequent exterior or interior bleeds may physically disable the individual\textsuperscript{5}. Current non-gene therapy includes methods such as direct infusion of the exogenous proteins\textsuperscript{6}. These specific methods that are non-gene therapeutic do not provide long-lasting effects due to inhibitors and antibodies. Replacement therapy is effective until an alloantibody is formed against the exogenous clotting factors\textsuperscript{7}.

II. Emerging Technologies
When researching gene therapies for hemophilia, there were an abundant amount of other gene therapies shown to be effective and helpful. Some studies used the method by adding more precursor proteins to make the factors FVIII and FIX, the liver will be able to produce thrombin and platelets for hemophilic patients. In other words, these studies experimented by adding to the protein pathway rather than the genes of the factors; they added factors to help build the precursors of the factors or necessary stabilizers for the factors. One of these methods is called transgene therapies. There are many transgene therapies that evolved for various diseases as one
study suggests. One of the transgene therapies is the FIX-Pauseda and robinhood gene therapy which in short is the transfer of FIX from a patient who has an abundance of FIX, FIX-Pauseda proband, to a patient with a low abundance, HB patients. This will help hemophilia patients regain their FIX levels. The limitation to this technique is the safety issue of immunogenicity and thrombogenicity as the doses were of concern.

Including other gene therapies, there are additional useful therapies not using the Another method explored was activating more of the existing Factor VIII if they are turned off in presence of Thrombosis, such as an inducible vector. Factor VIII is a cofactor in the coagulation cascade and is primarily produced by endothelial cells. A study by Alam et. al. observed that because thrombin regenerates itself with the increased amount of platelets and itself in the blood in the coagulation cascade it is important to have thrombin in the body. By endothelial injury the release of thrombin will amplify the activation of Factor VIII by re-detachment of Factor VIII from the von Willebrand factor and Factor VIII complex. Two patients who had undergone both arterial and venous thrombotic events before observing an increase in Factor VIII. Von Willebrand Factor (vWF) is a major contributing factor to the availability of Factor VIII (FVIII) as vWF is a specific carrier protein that protects FVIII of proteolytic enzymes and from degradation. This insight of the activation of Factor VIII suggests that there might be an activation method to make the Factor VIII protein more readily available to the coagulation cascade and help with blood clotting. This activating more of the existing Factor VIII if they are turned off in presence of thrombosis can prove to be a consideration in hemophiliac studies. Another study also included alternating the amino acids on Factor VIII will make it more readily activated. A study by Nogami et.al. conducted an in vivo experiment which included altering the 372 amino acid position of an Arginine protein on the factor VIII protein to a Histidine protein to make it more detachable from the vWF-FVIII complex. Although this is a novel in vivo approach, the study saw little to no change in the cleavage rate with the amino acid change suggesting that there may be other properties associated with the thrombin and the vWF-FVIII complex cleavage mechanism. This inducible mechanism can also be used in the AAV vector that is an emerging technology. These mechanisms are SIN
vectors which contain an inducible package of proteins that can turn the expression of the vector on and off determined by a protein presence. Particularly the protein would function as an antibiotic such as doxycycline or a small molecule\textsuperscript{45}.

Furthermore, another method that was proven to be effective is site-specific bioconjugation which alters the activation of Factor VII which triggers the whole cascade of coagulation as suggested by Lieser et. al\textsuperscript{12}. Another type of therapy that was proven successful by clinical trials of different companies as suggested by a review article by Sadiki et. al.. Site-specific bioconjugation is the concept of aiding the specific proteins needed for delivery of drug carriers and moieties of proteins within the body. This technology increases protein circulation, activity, target specificity, and protein properties as a whole. There are many site-specific bioconjugations which include unnatural amino acid incorporation, Sortase-mediated ligation, and SpyCatcher/SpyTag bioconjugation. All of these methods make alterations to the amino acids present in a particular drug carrier making the permeability of that drug easier in the body. This method is favorable because it changes the way particular factors and proteins are delivered to the body without recombinant engineering\textsuperscript{13}. Jivi is an antihemophilic factor (recombinant) gene or a factor VIII concentrate. This study focused on reducing the amount of times patients need to have routine prophylaxis by making an on-demand treatment to control the bleeding. They used recombinant DNA technology in Baby Hamster Kidney (BHK) cells to generate the recombinant B-domain deleted human coagulation Factor VIII (BDD-rFVIII) to obtain the activated molecule. Due to its on-demand property, it can also be used as a perioperative treatment plan in the means of managing bleeding before surgery based on the patient’s needs. In terms of Hemophilia, this study and product took into consideration the intensity of bleeding of patients with hemophilia and treated people with severe hemophilia (two or more bleeds per week) two times weekly 30-40 IU/kg. Patients with a low bleeding tendency were treated every 5 days or twice weekly just as the high intensity patients as weekly treatments did not show efficiency. At the end of the study, there was a decrease of bleeds per week in patients in total\textsuperscript{14}. In research studies, intensity or severity of hemophilia is defined by measuring FVIII or FIX activity as suggested by a review paper.
by Samelson-Jones et. al.. This is again based on the potency of protein factor products and monitored post-infusion processes. Additionally, two methods to test (if this information is going to be included this information, it would be helpful to elaborate on what these tests look like) out this are run through One-stage clotting assays (OSAs) or Chromogenic substrate assays (CSAs) which can help define or identify the severity of hemophilia in the patient15.

Lastly, there are a number of non-viral ways to transduct genes. Some of those methods include electroporation, cationic proteins, cell-penetrating peptides, nanoparticles, CRISPR editing genes, and virus-like particles (VLPs)45.

III. AAV Gene Therapy

The most popular and most successful emerging therapy to treat hemophilia relies on the use of an Adeno-Associated Viral Vector (AAV) as a vehicle for gene delivery of factor VIII and IX. AAV-gene therapy is popular due to its efficacy, relatively low invasiveness, minor side effects in animal models, and its ability to provide long-lasting expression of factor VIII and IX. Despite these advantages, however, the presence of neutralizing antibodies from previous exposure to AAV remains a challenge that obstructs this therapy from being widely accepted. With minor concerns over inhibitor development, AAV-gene therapy is proving to be a potential treatment for hemophilia patients.

AAV Vector Advantages and Uses

A particular gene therapy that is a gene therapy model that is being explored right now and very successful in various clinical trials. Adeno-associated virus (AAV) vector is a new technology that is emerging into the scientific community as an effective method to amend the existing molecular DNA sequence of cells, without incorporating the virus’s DNA into the host’s DNA sequence16. There are many advantages to AAV which are used for a plethora of diseases within the body including neurodegenerative diseases, cancer, and genetic diseases as a whole. This gene therapy model is being explored currently in various methods and research papers to observe new
DNA appending techniques\textsuperscript{17}. This gene therapy is nonpathogenic and has many uses to express genes of interest.

Additionally, there are new genetic sequencing methods that allow for the design of vectors/plasmids and target specific parts of the body. Because of its great amendable characteristics to genetic engineering and repurposing makes it easy to design relative to previous gene therapy techniques suggested by a review by Andari et. al.\textsuperscript{18}. This not only makes it easy to design but increases the cell specificity, cell or organ targeting, and transduction efficiency. AAV has shown to be non-pathogenic and induces a minimal inflammatory response in mouse models and early human clinical trials. Furthermore, AAV has fewer biosafety hazards, unlike other gene therapies suggested by a review by Aschauer et.al., while having a low immunogenicity and limiting the risk of insertional mutagenesis or other mutational changes when replicating\textsuperscript{17}.

![Figure 1: Structure of an AAV vector. GOI, the gene of interest, target sequence that is to be inserted into the vector.](image)

Other gene therapies focused on providing the proteins necessary for transduction while we wanted to focus on the core protein that is stopping transduction and see what was lacking in the pathway. The coagulation cascade is a concept that is still being explored as many elements play a role in the triggering events. A general outlook on the cascade is started with a stimulus from the external environment such as a cut or broken tissue increasing the amount of the protein thrombin to form the extrinsic pathway. A set of triggering events will then increase the von Willebrand factor and factor VIII to interact with factor IX. Factor VIII and IX are the main factors necessary for transduction which will trigger factor 10 to convert prothrombin to thrombin. Thrombin activates factors V, VIII, IX
and XI, to promote its own generation. Thrombin then will then activate soluble fibrinogen to convert into an insoluble version of itself called fibrin to make the webbing of the blood clot in the location of the cut or tissue breakage. There is a plethora of gene therapy methods explored that focus on providing the proteins necessary for transduction, and the main protein factors being factors VIII and IX. AAV gene therapy focuses on promoting factors VIII and IX which stop transduction and make up hemophilia\textsuperscript{19, 20}.

![Figure 2: Blood Coagulation Cascades -- the Intrinsic and Extrinsic Pathways. By Joe D - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=1983833](image)

**Vector and Plasmid Design**

From various clinical trials, we will compose a collection of effective AAV vectors that can transduce the Factors 8/9 into the hepatocytes. AAV is a very broad type of vector that has many versions of itself that exist in nature\textsuperscript{21}. The key differences between such vectors can include the length of gene it can hold, various repair enzymes, and the type of capsid proteins present for replication. All of these elements make up the specific AAV vectors’ characteristics of replication speed and the size of the vectors. Additionally, the size of the target sequence of the factor needed to insert into the is important to consider\textsuperscript{42}. There is more research done on Hemophilia B than hemophilia A due to the smaller size of factor 9 (c. 1.6 kb) and factor 8 (c. 7 kb). The whole size of AAV vectors (c. 4.7 kb) limits
the packaging capacity so this is another limitation of clinical studies. A review paper by Neinhus et. al. suggested that the recombinant AAV2 serotype was used in initial studies because it was the first AAV vector serotype to be characterized. This serotype was a single-stranded vector and was 7 kb in length. This length included 145 base pairs of inverted terminal repeats (ITRs) which are the regions of the vector that must be preserved for the vector to allow packaging and further sequencing. This virus also included adeno-viral proteins in the form of capsid proteins for packaging and to form viral vectors. Another comprehensive study by Sands et. al. showed the various types of AAV vectors’ serotypes tested through liver-mediated infusion. It was known that the AAV2 serotype was very versatile and could pack many genes with other supporting capsid proteins. As AAV2 was kept as one type of control, changing the serotype of the vector greatly changed its tropism and effectiveness to help the body. Vector serotypes 1, 5, and 4 were tested to be more efficient in transducing cells in the murine nervous system or within the mouse model. Because the liver is shown to have more affinity to particular capsid proteins of different serotypes, vectors 8 and 9 had more affinity to the liver greater than that of serotype AAV 2. AAV vector 8 was very readily transduced regardless of the method it was transferred through; it was equally efficient in both the intraportal vein injection and by intravenous (tail vein) injection. Additionally, when comparing the efficiency of the rAAV-8 of FIX administered through the portal vein injection and tail vein injection in vivo experiments in mice, there was an equal amount of expression of the FIX factor in the plasma throughout 20 weeks of observation time. The factor peaked during the 4 weeks mark and gradually decreased its expression by 75% as by the end of the 20 weeks. The vectors were prepared with the help of a native liver cell from the mouse with the double stranded vector and 10 μg of the factor plasmid. In conclusion, this study has resulted in the type of capsid proteins for each serotype making the AAV vector serotype have a different affinity, transduction efficiency, and tropisms in the body.

**Limiting Factor**

However, the transduction of AAV particles into the liver is limited by the ability for ssDNA to be converted into dsDNA, as the viral mechanism is dependent on host machinery. To solve this problem, a study by Nathwani
et. al. devised a liver-restricted mini-human factor IX (hFIX) expression cassette that allows AAV DNA to be packaged as dimers\textsuperscript{24}. As a result, hFIX expression in mice models produced a 20-fold increase compared to similar single-stranded AAV vectors (ssAAV). Advantage of AAV technology is that different serotypes have the capability of targeting specific tissues. AAV2 serotype has been shown to minimize tissue tropism as its primary transduction comes from hepatocytes. This also was beneficial in that it allowed researchers to use fewer AAV particles in hemophilia patients, thus lowering cytotoxicity.

![Figure 3: Stages in AAV vector delivery and transgene expression, listed in combination with some resulting proteins involved in the process.](image)

**Gene Target Sequence**

After designing and analyzing which AAV vector to use for such an experiment, a good gene target sequence is necessary to insert into the AAV vector to express the needed vectors. Many pharmaceutical companies such as BioMarin Pharmaceuticals, Spark Therapeutics, Pfizer, and UniQure\textsuperscript{25}. The following companies have already designed and inserted the target FVIII or FIX gene sequence gene therapy products into the vectors and are in clinical trials evaluated in phase III studies. A particular review paper by Doshi et. al.\textsuperscript{26} discussed clinical trials that suggested broadening the gene
therapy vector applicability with patients who had pre-existing neutralizing alloantibodies to the vector or in other words the clotting factors. Here the vector genome is manipulated so that the needed gene target sequence can replace the vector sequence under a tissue-specific promoter to make a recombinant AAV vector (rAVV). In totality, the completed modified AAV vector in order included the ITR, promoter, inhibitor, transgene, polyA, and ITR. The results varied based on the way the AAV vector was given via skeletal muscle, liver-directed, and intramuscular trials.

**AAV Administration**

Refining administration of AAV gene therapy so as to optimize “uptake” of vectors, induce expression, provide longer-lasting effects, and reduce liver damage/minimize immune system effects. The site of infection can greatly increase or decrease the efficiency of the transduction of AAV in the body. Intraportal vein injection includes an infusion directly to the portal vein of the liver\(^{21}\). According to a study by Nienhuis et. al. from the University of London, rAAV2 was shown to have the same efficiency as the portal vein method and the peripheral vein infusion method. Initially, it was thought that a portal vein injection was needed to secure the liver transduction in the mice but this was not true. The transduction was largely affected by the types of capsid proteins present in the AAV vector serotype. Based on the study by Doshi et. al. suggesting that patients who already had existing neutralizing antibodies (NAbs) in the liver—directed gene therapy showed that the AAV transduction efficiency was hindered by pre-existing NAbs.\(^{26}\) Although NAbs can be present in 30-70% of the population, immunosuppressors were used in subsequent trials involving the liver-directed transduction. Contrastingly, the intramuscular trial did not have this effect. Another method of vector infusion is direct intraparenchymal injection. A study by Sands et. al. suggested that a direct injection of an AAV2 vector into the liver parenchyma which results in a relatively widespread transduction throughout the liver\(^{22}\). This method also retains the AAV gene expression for a longer period of time in the body.
Dosage
Scientists must determine the correct dose of AAV vector particles injected into the patients, as a low concentration may not produce a robust amount of expression, while a dosage that is too high may cause an immune response and liver damage. Scientists have sought to address this issue by increasing the transduction of the vector into cells such that fewer vector particles are needed and thus limiting host immunity.

Minimizing Immunogenicity
There are two ways to decrease immunogenicity; one such measure refers to a process known as Site Directed Mutagenesis. Two vector serotypes currently being used in clinical studies, rAAV-DJ and rAAV-LK03 are chimeric receptors of natural AAV serotypes. A study by Ran et. al. (2020) showed that site-directed mutagenesis (rAAV-DJ-S269T) yielded higher transduction efficiency compared to wild-type AAV vectors, likely due to evasion of the host immune response. The second method of refining the AAV vector for administration is the use of directed evolution, where a wild-type AAV vector is used to generate large mutant capsid libraries and AAV2 variants with enhanced properties, such as immune evasion, non-infection of resistant cell types, and tissue transport.

Inhibitors
The immune system develops antibodies to defend against foreign agents. Patients with hemophilia sometimes develop antibodies to FVIII in response to treatments and medication. Polyclonal high-affinity
immunoglobulin G (IgG), or an inhibitor, prohibits clotting factor activity that further promotes bleeding without clot formation. Type I and Type II inhibitors vary based upon the extent of inhibition of clotting factors. The development of inhibitors is a widely understood phenomenon that continues to burden treatment efficiency. Its effects are being studied through both genetic and environmental factors that contribute to formation. The detection and quantification of FVIII inhibitors include the Bethesda assay and the Nijmegen-modified Bethesda assay.\textsuperscript{29, 30}

Inhibitor formation contains treatment-related risk factors that include age and intensity at first exposure, prophylaxis, and the type of treatment (recombinant or plasma derived). The effect of age was observed in a population of hemophilia A patients with the onset of FVIII therapy within 1 year of age. It was found that patients that started therapy earlier in life are more prone to inhibitor development. However, there is a lack of knowledge on the effects of delayed treatment initiation due to the tendency for patients to require therapy in early age.\textsuperscript{31} The intensity of treatment measured by scheduled gaps between series of exposures showed that shorter gaps were reflective of increased risk of inhibitor development. Prophylaxis is a treatment that exposes patients to antigens to minimize the possibility of immune response to additional treatment. It was found that prophylaxis reduces probability for the development of inhibitors.\textsuperscript{32}

Pre-existing immunity from neutralizing antibodies or inhibitors can be overcome by creating alternate AAV serotypes.\textsuperscript{33} The success of serotype-switching is contingent on tropism similarities of the new serotype and the lack of crossreactivity between the serotypes. The criteria has been proven difficult upon the high rate of crossreactivity in trials that prohibit successful efforts.\textsuperscript{34}

Immune tolerance induction (ITI) is currently the only proven treatment to eliminate inhibitor development. Despite a 60-80% success rate, the high cost creates financial difficulties for patients in need of this treatment. There is potential for gene therapy to serve as an ITI-type therapy to eradicate the need for ITI due to the continual production of clotting factors.\textsuperscript{35}
Alternatives to Gene Therapies

While AAV-gene therapy has made the most progress in the future treatment for hemophilia, new research has come out in an effort to bypass the concerns of AAV explained previously. A recent study published by Song et. al. (2022) shows a promising alternative to AAV gene therapy known as Ultrasound Mediated Gene Delivery (UMGD), where the plasmid containing the functional Factor VIII gene is injected directly into Hemophilia A patients. UMGD of reporter plasmids targeting mice livers achieved high levels of transgene expression predominantly in hepatocytes, while maintaining minimal transaminase levels following injection. However, these results have yet to be proven in human clinical trials and are still years away. Similar to AAV vectors, lentiviral vectors do not contain viral genes and promote long-term gene expression which make both vectors strong candidates for gene therapy. Despite increased stability upon genomic integration, lentiviral vectors activate the innate immune response to limit its capabilities in increasing clotting factor levels. The adaptive immune system, however, is unresponsive upon cell transduction. Hemostatic agents have been engineered to overcome FVIII and FIX activity in hemophilic patients as an alternate protein therapy. The hyperactive FVIIa variant has undergone experimentation as a transgene for AAV-based liver-directed gene therapy. This resulted in the usage of a lower vector dose while maintaining a similar response to hemostatic conditions. Although clinical trials have proceeded into late stages, it has halted due to immunogenicity speculation. If continued in the future, the FVIIa transgene may be able to lower the vector dose for patients. Protein-engineered bypassing agents have prolonged the half-life of coagulation factors downstream of clotting factors that are designed to resist inactivation via conformational changes to protein structures. This treatment is undergoing early-stage clinical trials and has proven efficacy as a protein therapy for acute hemorrhagic conditions.
Table 1: Comparison between AAV, lentivirus, and adenovirus vectors.

<table>
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<tr>
<th></th>
<th>AAV</th>
<th>Lentivirus</th>
<th>Adenovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genome Size (nm)</td>
<td>approx. 25</td>
<td>80 - 120</td>
<td>approx. 95</td>
</tr>
<tr>
<td>Genome Type</td>
<td>ssRNA</td>
<td>ssRNA</td>
<td>dsRNA</td>
</tr>
<tr>
<td>Packaging Capacity (kb)</td>
<td>4.6</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Presence of Envelope</td>
<td>Not Enveloped</td>
<td>Enveloped</td>
<td>Not Enveloped</td>
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<tr>
<td>Integration</td>
<td>Non-integrating</td>
<td>Integrating</td>
<td>Non-integrating</td>
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<tr>
<td>Expression</td>
<td>Persistent</td>
<td>Persistent</td>
<td>Transient</td>
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<tr>
<td>Immunogenicity</td>
<td>Medium to Low</td>
<td>Low</td>
<td>High</td>
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</tbody>
</table>

Inhibitors

Additionally, there are structural, functional, and expression differences between the AAV vector, lentivirus, and adenoviral vectors to account for. AAV is a smaller vector consisting of approximately 25 nm, whereas lentivirus is 80 nm to 120 nm, and adenovector is approximately 95 nm. AAV alongside lentiviruses carry single-stranded DNA, whereas adenoviral vectors carry double-stranded DNA. Double-stranded AAV vectors were also formulated by mutation of the inverted terminal repeat (ITR). These vectors were shown to have better transduction in many other cell lines including in vivo experiments with hepatocytes. In comparison to the ssAAV, the dsAAV gene helped maintain better transduction and better DNA stability when evaluated 6 months after the initial in vivo experiment. AAV has a lower packaging capacity consisting of approximately 4.7 kb while both lentiviral and adenoviral vectors have approximately 8 kb. This may be a limitation due to the large gene target sequence of factors 8 and 9. Adenoviruses and AAV vectors are not enveloped which may cause more efficient transduction of viral DNA. The lentiviral vector is enveloped which keeps it well protected, but concurrently if the envelope is compromised, the virus can disintegrate. Additionally, the lentivirus is double-stranded and embeds itself well into the host’s DNA. The integration of the viral DNA into the host’s DNA will prelude long-term transgene expression. The major disadvantage of this mechanism is that it can disrupt the host’s genome by insertional mutagenesis. AAV and adenovirus cannot integrate themselves into the host cell’s DNA. The adenoviral vector is mostly used for short term uses as it is effective in transient expression unlike the persistent gene expression of the AAV and
lentiviral vectors. Lastly, immunogenicity decreases of each vector adenovirus, AAV, and lentivirus respectively40.

**Conclusion**

The purpose of this review article is to provide an outlook on the current state of gene therapy as a treatment for Hemophilia. Results of recent clinical trials have proven to be effective in restoring clotting ability in hemophilic patients. By identifying key concerns with gene therapy, scientists are taking steps to account for these problems by utilizing the following collection of sources and information to make an impact on their research. The findings that we make will help other researchers improve upon this research to have a collection and a review composed of AAV vectors to use in their clinical studies. Results influence researchers to use the AAV gene therapy because of its effectiveness in transducing genes into hepatocytes. Just as in hemophilia, AAV gene therapy is a useful technique applicable to many other diseases, which can lead to more breakthroughs and for other studies. This results in longer lasting and consistent treatment plans after insertion due to the limited amount of insertional mutability17. Predicting or compiling effective AAV vectors and their key qualities can advance further studies that want to use such gene manipulating technology that is not as biohazardous and feasible scheduled usage as it is effective for months17. With a comprehensive collection of data from various clinical trials, researchers in the future will also have a good understanding of challenges of such treatment that requires deliberation before pursuing treatment about options with the type of AAV vectors to use and understanding the best way to infuse into the body18. Our research can also advise a potential gene sequence for further research to use when developing the precise gene sequence to use in future studies.
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