

# Gene Therapy for Hemophilia

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## Abstract

Current treatment for hemophilic bleeding episodes is limited to the intravenous infusion of intravenous purified and recombinant factor protein. While this is generally effective, it is transient due to the brief lifespan of the proteins. Aside from this, factor protein treatment is costly, constrains the prophylactic use of factors and can potentially lead to degenerative joint disease as well as susceptibility to infectious agents, including HIV virus [1]. Hence, for many years now, attention has been focused on gene therapy as a potential cure for hemophilia. Nevertheless, clinical trials that have been undertaken in this direction have shown that this cure will not be easily achieved. This paper discusses the progress done in the field of research in the context of gene therapy for hemophilia, as well as the challenges that have emerged and how these could possibly be resolved based on research.

**Keywords:** Gene Therapy, Hemophilia, Gene Disease

## 1 Introduction

Hemophilia is a monogenic hereditary disease for which there is no known cure yet, and which affects the blood's ability to clot. Haemophilia A and B are x-linked hereditary diseases that are caused by different mutations in the FVIII (F8) and FIX (F9), which are genes for pro-coagulant factors VIII (FVIII) and IX (FIX), respectively. These mutations result in lower FVIII or FIX protein expression or activity. In lay man's terms, this means that hemophiliacs do not have essential clotting factors in their blood that, when combined with platelets, turn the blood sticky. This also means to say that when hemophiliacs cut themselves, their bleeding tends to last longer compared to others who do not have the condition, sometimes to the point when the bleeding episode can become potentially life-threatening. Thus, people with hemophilia are not ideal candidates for major surgical procedures, unless they are under surgeons who have advanced knowledge in gene therapy, because this can lead to serious blood loss.

Hemophilia is not a common disease, with type A occurring in one out of 5,000 males

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worldwide [2]. Hemophilia B is much rarer as it is five times less frequent than hemophilia A.

### 1.1 Hemophilia and Its Genetic Qualities

Hemophilia, a deficiency of clotting factors FVIII or FIX, is a rare, X-linked congenital disorder that leads loss of normal hemostasis and manifests through “prolonged, spontaneous, potentially life-threatening bleeding” [3]. Repeated bleeding episodes, in most cases in muscles and joints, can lead to long-term, disabling complications. As mentioned earlier, there remains no cure for hemophilia but it may be managed by prompt treatment of bleeding episodes with infusions of FVIII for hemophilia A or FIX for hemophilia B. There are some cases wherein desmopressin or anti fibrinolytics may be given to the hemophilic [3].

Normal hemostasis is regulated by a sequence of enzymatic reactions collectively known as the “coagulation cascade” [3]. This cascade involves several proteins and enzymes, each of which plays a critical role in hemostasis. Years ago, hemostasis was explained as having two individual pathways so that coagulation may take place; intrinsic and extrinsic. However, according to recent studies, hemostasis is achieved through the release of tissue factor, a part of the extrinsic pathway, following vascular surgery [3]. Consequently, tissue factors “complexes with VII, with subsequent conversion of factor, to activated factor VII (factor VIIa) [3]. This process takes place at the site of injury so that other factors are activated, including FVIII and FIX. As a result, a thrombin burst occurs. This small quantity of thrombin activates platelets as well as other factors within the intrinsic pathway, including FVIII and FIX. At this point, coagulation depends on the actions of FVIII and FIX for the purpose of producing more thrombin. With adequate thrombin and other coagulation factors, fibrinogen is converted to fibrin thereby forming a stable clot at the site of injury. To note, hemostasis disorders may be manifested through excessive bleeding or excessive blood coagulation. To note, excessive bleeding happens when proteins or other elements crucial to coagulation are missing because of either qualitative or quantitative deficiencies. For hemophilia A, the deficiency is of coagulation FVIII and for hemophilia B, it is FIX. Both of these factors are needed for blot to clot. Meanwhile, as mentioned earlier, hemophilia A and B are both X-linked recessive disorders caused by mutations in either F8 or F9 genes found in the X chromosome [3].

The reason why there is a wide gap in the prevalence of hemophilia A and B is the size of the genes. F8 gene is larger; thereby there are more chances for mutation. The most common type of mutations in F8 and F9 are point mutations, “but other chromosomal aberrations” have been identified, including “deletions, insertions, and rearrangements or inversions” [3]. To note, male and female offspring of a hemophilia gene mutation has a 50% chance of inheriting the mutated gene [3]. Nevertheless, female carriers of the gene mutation are typically not bleeders but they tend to have lower levels of clotting factor and could be prone to excessive bleeding following trauma.

Diagnosis of hemophilia is based on factor assays, bleeding patterns as well as pattern history. However, almost 30% of those diagnosed have not inherited the disease [3]. Hemophilia may be mild, moderate or severe. Those with severe factor deficiency experience bleeding even without trauma. Moderate hemophilia is characterized do not bleed spontaneously but could bleed excessively even due to minor trauma, surgery or other invasive procedures. Mild hemophilia is characterized by excessive bleeding only after significant trauma [3].

## 2 Current Treatments for Hemophilia

Before the 1940s, the only treatment for hemophilia was the transfusion of whole blood or fresh plasma, aside from supportive care [4]. However, neither of these can provide sufficient levels of FVIII or FIX, which is why hemophiliacs continued to suffer from major bleeding and long-term morbidity. At best, blood transfusion can only provide short-term help to patients without exactly prolonging the life. Hence, the average life span for hemophiliacs during that time was 27 years old [4]. As transfusion medicine improved, lifespan increased to about 40 years old, which is still short when compared with healthy people and others who are also suffering from chronic illnesses [4].

It was in the 1970s that dramatic improvements took place in the development and availability of plasma-derived clotting factors [4]. These were available in freeze-dried concentrations of FVIII and FIX, usually obtained from a pool of 20,000 donors. As a result, home therapy became possible and for the first time, hemophiliacs were able to travel, attend school and gain employment. Combined with holistic care, lifespan eventually increased to about 60 years old in the 1980s. This was a big milestone in treating hemophilia because people with this disorder can already live normal lives, provided they are careful about their activities. Although there were risks that the hemophiliac may acquire hepatitis B and C through the blood transfusions, it was calculated that the risks were acceptable compared to the vast improvement in quality of life. Blood screenings can also be done to ensure that donors do not have the hepatitis virus in the blood being given to the hemophiliac [4].

However, screenings for other blood-borne diseases were not yet in effect. In particular, there was no strict screening for HIV as it was not yet a well-known disease during that time. It existed but was not common, and could not be detected through the usual blood screening protocols. As such, hemophiliacs were in great danger of receiving HIV-infected blood. In 1982, the first hemophiliacs succumbing to AIDS made the realization that transfusion of plasma-derived factor concentrates “was the vector of this unidentified infectious moiety” [4]. By 1984, the HIV virus was isolated and it became standard practice to heat factor concentrate to kill the virus. Strict screening of donor blood was also implemented.

Current hemophilia treatments are the following:

### 1. Factor VIII Products

In 1984, cloning and sequencing of FVIII was achieved thereby leading to the development of recombinant FVIII, or rFVIII [4]. The scale-up of production and purification processes led to the “commercial production of full-sequence-length rFVIII products” [4]. In 199s, rFVIII was marketed for clinical use. Technically, 1 unit of FVIII per kilogram bodyweight increases factor activity in the recipient’s plasma by 1.5-2.0 IU/dL [4]. rFVIII products have been proven to generate excellent hemostatic efficacy in both previously untreated as well as treated hemophilia A patients.

### 2. Factor IX Products

With the cloning of F9 in 1982, the expression of human rFIX was expressed in Chinese hamster ovary cells [4]. Structurally, rFIX is similar to plasma-derived FIX although there are “minor differences in the post-translational sulfation and phosphorylation of rFIX” such that there is a 30% difference (lower) in recovery especially among children below 15 years old [4]. Clinical trials have evidenced the effectiveness of rFIX for the treatment of hemorrhages as well as in the prophylactic

and surgical settings in previously treated and untreated patients with hemophilia B. In general, 1 unit of FIX per kilogram bodyweight increases factor activity in the recipient's plasma by 0.7-1.4IU/dL [4].

### 3. Replacement Strategies

The treatment goal in hemophilia is to replace missing coagulation factor from external sources. Episodic or "in demand" replace is the conventional approach in which the missing factor is immediately replaced as soon as bleeding starts [4]. Recently, it has been recommended that prophylactic administration of clotting factor concentrate for the prevention of bleeding be done as a standard of care. Indeed, more evidence has shown that prophylactic dosing of factor must start even before joint damage occurs in order to promote joint integrity. Patients with pre-existing joint diseases and who have "frequent acute haemarthroses" can be treated by "periodic use of factor concentrates for a short or long period of time to curtail bleeding recurrence" [4].

### 4. Treatment of Patients with Inhibitors

Unfortunately, there are hemophiliacs who have formed antibodies against FVIII or FIX, thereby neutralizing the activities of these clotting factors. It has been found that recombinant factor VII a (rFVIIa), is almost identical to native FVII. Therefore, this agent is widely licensed as an effective treatment for bleeding in hemophilia A or B that is complicated by inhibitory antibodies [4].

## 3 Overview of Gene Therapy

Albeit somewhat ambitious, the objective of gene therapy is to "edit a defective gene sequence *in situ* to achieve complete reversion of a disease phenotype for the lifetime of the patient" [5]. Despite recent successes with in site-specific correction of defective gene sequences, gene therapy strategies tend to focus on gene addition instead of gene replacement. Specifically, the key goals of gene therapy for genetic disease are, (i) longterm expression, and (ii) "expression at levels high enough to ameliorate or cure the clinical phenotype of the disease" [6]. In short, gene therapy aims to replace the defective genes with healthy ones so that the person is ultimately cured from hemophilia. The objective is not merely to produce coagulation when the patient needs it; instead, coagulation should become normal so that the patient does not have to worry about bleeding conditions.

There are two strategies for attaining long-term expression. First, an integrating vector is used to transduce a stem cell. All daughter cells subsequently carry the donated gene in order to provide long-lasting expression. The second strategy is "transducing long-lived post-mitotic cells, such as cells in the central nervous system, skeletal muscle, cardiac muscle, or even (less long-lived) hepatocytes" [6]. Under this situation, provided that the donated DNA may be stabilized, then expression will last for the lifetime of the cell.

Current research shows that gene therapy in the form of lentiviral and adeno-associated vectors can be applied to "adult stem cells, autologous fibroblasts, platelets and hematopoietic stem cells; by means of non-viral vectors; or through the repair of mutations by chimeric oligonucleotides" [7]. Hemophiliacs mostly receive adult cells meant to correct the defect in coagulation factors.

## 4 Progress on Gene Therapy for Hemophilia

Hemophilia is considered as an ideal disease for gene therapy for several reasons. For instance, current therapies are episodic, expensive as well as inconvenient. Small and large animal models of both hemophilia A and B are available for preclinical testing and just as importantly, treatment of the disease may be quantitatively measured by means of well-defined coagulation assays thereby eradicating an issue hounds gene therapy efforts for other diseases. Hemophilia is also amenable to the more advanced cell therapies because it is a monogenic disease that does not require high expression levels for coagulants to take effect [5].

Another essential factor for treatment of hemophilia by gene transfer is that the threshold for success is relatively low. In the event that long-term expression of the “defective coagulation factor at 2–3% of wild-type levels could be achieved,” then it is also anticipated that a significant decrease in the clinical manifestations of the disease would occur [5]. At the other end of the spectrum, expression beyond “30% of the wild-type level of the defective coagulation factor would result in a phenotypically normal patient” under most circumstances [5].

Between 1998 and 2001, researchers have conducted five Phase I clinical trials on possible treatment of hemophilia through gene transfer [5]. Different types of gene delivery systems were utilized in these studies, including a retroviral vector, an adenoviral vector, two different adeno-associated viral vectors, and a nonviral gene delivery method [5].

Although these trials were generally safe and no adverse events were associated with these trials, none of them really resulted in long-term expression of clotting factor at therapeutic levels [8]. For example, it was discovered that retroviral vectors need a dividing target cell because vector will not be able to reach the nucleus until the nuclear membrane disassembles during mitosis. Therefore, it appeared that intravenous infusion of a retroviral vector would result in transduction of “rapidly dividing cells such as haematopoietic cells and cells lining the gastrointestinal tract, if indeed the vector survived intact in the circulation” [5]. Moreover, it was also discovered that that intravenous infusion of retroviral vectors into neonatal animals could lead to the transduction of the liver, because hepatocytes in a growing animal are rapidly dividing [5]. Currently, clinical trials are focusing on adenovirus vectors as a way of obtaining long-term expression of both FVIII and FIX [5]. New discoveries in the field of retroviral vectors are believed to be more promising for application in the treatment of hematological disorders. One such important innovation has been the development of lentiviral vectors, which are considered as more advantageous than the first-generation retroviral vectors in several ways. First, they have the capability to transduce non-dividing cells, so that they are more appropriate for transduction of, for instance, hepatocytes and haematopoietic stem cells. Second, whereas retroviral vectors “preferentially integrate their genomes near transcriptional start sites,” lentiviral vectors have been found to have “a random integration pattern into the open-reading frames of genes” [5]. Experts believe that this shows positive outlook for gene therapy in hemophiliacs.

Continued developments are also achieved in animal models of “AAV vector-mediated gene transfer for treatment of haemophilia” [5]. There have also been significant improvements in transgene expression levels by using liver-specific promoters and portal vein administration. Once the safety of these therapies for human applications has been ascertained, hemophiliacs can have additional treatment options.

## **5 Challenges in Gene Therapy for Hemophilia**

The most important challenge in gene therapy for hemophilia is that in spite of several studies that have successfully achieved safe, long-term expression of clotting factors, these studies have been conducted on animal models only using multiple gene transfer strategies. These studies have yet to be translated into success for patients. This is problematic considering that it may not be ethical to conduct human testing without being certain of its success. Researchers need to find ways in order to transfer their success from the laboratory setting into actual hospital settings. This may require consent from patients who know that they are part of an untested study.

Moreover, in spite the utility for delivery of large sequences of DNA with extremely high efficiency, “therapeutic transgene engineering, adenovirus vectors are highly immunogenic,” such that their use may be constrained by pre-existing antibodies because of endemic infections in mammals [2]. Therefore, there is continuing search for less-immunogenic viral vectors that may be used for gene transfer as treatment for hemophilia.

## **6 Future Areas for Research**

While it seems that gene therapy as cure for hemophilia will not be realized in a number of years yet, future research efforts need to continue especially in terms of improving hemophilia management by means of prolonging the half-life of factor concentrates using different technologies. Some of the more recent paths researchers have taken regarding this include sustained delivery, chemical modification as well as genetic mutation or fusion [4].

## **7 Conclusion**

Hemophilia is an X-linked inheritable disease wherein mutations in the F8 and the F9 lead to deficiencies in clotting factors FVIII and FIX, respectively. Due to these deficiencies, a hemophiliac may suffer from prolonged and more profuse bleeding compared to other people, with or without trauma. As such, people with this disorder need to be careful about wounds because simple ones may not clot as easily as those in other people. This condition can lead to blood loss and more serious problems.

There is no cure for hemophilia, and the current treatment that is commonly used is replacement therapy through the infusion of FVIII for those with hemophilia A, and FIX for those with hemophilia B. Although these treatments are generally effective, it must be noted that they are also expensive, episodic and convenient. This makes hemophilia an attractive disease to study when it comes to gene therapy. Currently, research has been focusing on lentiviral and adenovirus vectors as a method for obtaining long-term expression of both FVIII and FIX.

Treatments normally applied to hemophiliacs involve the introduction of health cells that are meant to correct the factor expression in coagulation. Future researches can include testing the other identified advanced therapies to provide more options to people suffering from hemophilia.

Overall, the disease remains rare worldwide. However, the future does not look dim for those with this problem because advanced gene therapies show promise for resolving the

coagulation factor expression on a permanent basis. There are continuing studies on this disorder involving gene therapy, which can be its ultimate solution.

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