Hemophilia
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Genetics
Hemophilia is an X-linked recessive disorder that exists in two forms, hemophilia A and hemophilia B. Hemophilia A is characterized specifically by a mutation on the factor VIII gene of the X, whereas hemophilia B is caused by a mutation on the factor IX gene of the X chromosome. Hemophilia A is noted to have a mutation at the chromosomal locus Xq28 and cause an absence of the functional protein made by factor VIII, coagulation factor VIII. Other times the mutation may produce normal levels of a dysfunctional protein. Mutations found in individuals with hemophilia A include gene inversions, nonsense mutations, frameshift mutations, deletions, splice site mutations and missense mutations. Inversions are responsible for 45% of all severe cases of hemophilia A. Nonsense mutations or mutations that result in a new stop codon, as well as frameshift mutations also contribute to the severe phenotype. Deletion mutations are characterized by the deletion of 8-10 consecutive adenosine bases, and gives rise to a moderately severe phenotype. Splice site mutations often contribute to a severe phenotype as well but may be mild. Finally, missense mutations are almost always attributed to mild or moderate phenotypes but sometimes occur in less than 20% of individuals with severe hemophilia A.

Hemophilia B has a mutation at the chromosomal locus Xq27.1-q27.2 which alters the functional protein produced by the factor IX gene, coagulation factor IX. The mutation often results in a lack of functional protein or the production of a dysfunctional protein product. Mutations that are responsible for severe forms of hemophilia B include large gene deletions, nonsense mutations, and most frameshift mutations. Missense mutations can produce hemophilia in a range of severities depending on the specific substitution that occurs as well as the location of the substitution. Both hemophilia A and hemophilia B are completely penetrant and occur in 100% of males and only 10% of female carriers.

Genetic testing is often used for both types of hemophilia to determine which specific mutation is causing the disorder. For hemophilia A, targeted mutation analysis is used for cases of severe hemophilia because they are caused by one of 2 major gene inversion mutations. In other cases, mutation scanning, or sequence analysis is used as a means to pinpoint the exact location of a mutation. In circumstances where a mutation in a family is unidentified, linkage analysis can also be used to determine which mutation is present in all affected family members. To identify a de novo mutation a mutation analysis in addition to linkage analysis is used.

Similar to hemophilia A, sequence analysis is also used for testing hemophilia B. This tool is able to detect mutations in approximately 9% of all individuals with the disorder. Also, linkage analysis is used to identify the specific mutation in a family with multiple affected members.

Biochemistry and Molecular Biology
All of the hemophilias are caused by deficiencies in coagulation of blood. In normal people, blood clotting initially involves the aggregation of platelets to the lesion. Platlets are anuclear blood cells that contain several types of storage granules, notably
alpha granules and dense granules. Alpha granules contain the adhesive factors von Willebrand factor (vWF) and fibrinogen, growth factors, and coagulation factors. Dense granules contain platelet activating molecules that are solvent in blood, including ADP and serotonin. Furthermore, there are surface proteins on platelets that promote surface adhesion and activation.

Upon injury to the endothelial lining of blood vessels, the subendothelial layer of tissue and subendothelial matrix proteins like collagen will be exposed, which activates platelet binding via platelet surface proteins. Activated platelets will release the contents of their alpha granules and dense granules, which recruit more platelets to the lesion, promote the synthesis of more platelets, and help platelets adhere to each other. The activated platelet membranes also undergo structural change that involves releasing negatively charged membrane particles to provide a framework for the coagulation cascade.

Subsequently, the formation of strands of a protein called fibrin across the platelet plug will occur for reinforcement. This is accomplished via the coagulation cascade during which a series of inactive zymogens and their cofactors are cleaved and activated. There are two pathways that can activate the coagulation cascade: the extrinsic or tissue factor pathway and the intrinsic or contact pathway. The extrinsic pathway is activated

![Figure 1](image-url)  
**Figure 1** The coagulation cascade showing both intrinsic and extrinsic activation, inhibitors and feedback activation (dashed lines). HMWK=high molecular-weight kininogen. C1-inh=C1-inhibitor. TF=tissue factor. TFPI=tissue factor pathway inhibitor. PL=phospholipids. Ca=calcium. AT=antithrombin. (Figure reproduced from Norris)
when injury to the endothelial layer of blood vessels causes factor VII in the plasma to be exposed to the membrane-bound protein tissue factor. The intrinsic pathway is activated when factor IX binds to negatively charged surfaces, thus increasing its local concentration, which allows it to activate itself.

Activated factor IX and factor VIII form a complex known as the tenase complex, which is crucial to proper clotting in both the intrinsic and extrinsic pathways. Both blood clotting pathways are illustrated in figure 1. Hemophilia A is caused by a deficiency of factor VII. Hemophilia B, also known as Christmas Disease, is caused by a deficiency of factor IX.

**Diagnosis**

The clinical features of hemophilia A and hemophilia B are indistinguishable and so will be described together. The severity of symptoms and the onset of the first bleeding episode vary greatly with disease severity. This is inversely proportional to the patient’s levels of factor VIII (hemophilia A) or factor IX (hemophilia B). The normal levels of VIII in the population range from 50-200 iu/dl. Mild hemophilia A corresponds to factor levels between 5-40 iu/dl. Severe hemophilia is seen in patients with factor levels of less than 1iu/dl, while moderate disease is in the range of 1-5 iu/dl.

Patients with mild hemophilia and no family history are often not diagnosed well into adulthood, if ever, as they rarely have episodes of spontaneous bleeding. However, they can bleed severely following surgery or intense trauma. Severe hemophilia is rarely diagnosed within the first month of life. In the neonatal period bleeding is rarely seen unless the newborn undergoes a surgical procedure such as circumcision that elicits heavy bleeding. The reason for this is unknown, as trauma associated with vaginal delivery could be expected to induce hematoma formation.

The first bleeding episodes are generally seen in the 6-12 month period. Bleeding from the lower incisor teeth or lower lip and tongue from chewing on toys are often the first clinical sings. Bleeding from the mouth can persist for weeks in intermittent periods. Since the volume of blood lost in each incident may be small, the parents may not be alerted to the problem until the child becomes severely anemic. Severe bruising of the head from bumps received while crawling or on the buttocks from falling while learning to walk can also occur if the disease has not yet been diagnosed or treated. This often leads to hematomas that present a pattern that may look similar to and raise the question of “baby battering.”

Diagnostic testing begins with coagulation screening tests for patients suspected of having any kind of bleeding disorder. Tests to consider include: platelet count and bleeding time or platelet function analysis (PFA closure times), partial thromboplastin time (PTT), activated partial thromboplastin time (APTT), and prothrombin time (PT). For hemophiliacs these screening tests are all normal with the exception that in severe and moderately severe hemophilia, the PTT is prolonged. In mild hemophilia, the PTT is often normal.

Patients with abnormal screening tests or who have a prolonged history of bleeding are referred for coagulation factor assays. If the deficiency in factor VIII or factor IX is found, the patient is diagnosed with hemophilia. The severity of the disease is assigned according to the previously mentioned factor levels. Confirmatory genetic
testing may be indicated, but is generally used only when it is necessary to determine the allele needed for testing of other family members.

**Treatment**

Treatment of hemophilia A and B is predominantly by factor replacement therapy. Since 1964 concentrations of factor VIII and factor IX have been isolated from plasma cryoprecipitate have been the standard infusion treatments. In the early days of this therapy, the viral transmission of hepatitis A, B, and C as well as HIV through these transfusions was high. However high purity concentrates are now available in most developed countries that greatly reduced the risk of transmission. Commercial and non-profit manufacturers generally use two methods of antiviral purification to protect against transmission, but the risk cannot be entirely removed. The development of inhibitors for factor VIII and factor IX produced by patients receiving replacement therapy from human sources is a major concern, reducing their efficacy.

Recently, recombinant factor VIII and factor IX have become commercially available which partly alleviate this problem and reduced the risk of viral transmission since the source is non-human. In the U.S. 60% of replacement therapy is now supplied by recombinant technology. However, factor VIII is not stable in such concentrations and human serum albumin is added as a stabilizer reintroducing some risk of viral transmission. There is currently a recombinant factor IX therapy that does not require stabilization with serum albumin.

The levels of replacement factor required are heavily dependent on the patient’s baseline levels and the severity of the bleeding episode. The half-life of factor VIII in plasma is approximately 8 hrs, which means that repeated doses or continuous infusion (in the case of surgery or life threatening bleeding) may be required.

Gene therapy is considered the next frontier of hemophilia treatment. An endogenous source of factor VIII or factor IX to maintain close to normal factor levels would prevent the occurrence of any severe bleeding episodes. Current problems with therapies being studied include low expression levels, but new approaches are being investigated to alleviate this obstacle.

**Prognosis**

There is currently no cure for hemophilia but the effective treatments whose safety from infection has been greatly reduced has brought the life expectancy and level of disability of hemophiliacs to levels close to the normal population. The long-term symptoms of hemophilia have been greatly reduced by the advent of relatively safe replacement therapy. Hemarthroses, or bleeding into the joints, will cause major disability if untreated. The irritation to the joints causes synovitis lasting well beyond the bleeding episode. The resulting roughness and loss of cartilage within the joints elicits more bleeding, which continues a spiraling cycle of arthropathy limiting motion. Similarly hemorrhaging into muscle and severe bruising can be treated and often prevented by proper replacement therapy. Trauma to the head or abdomen and surgical disease are not necessarily death sentences for hemophiliacs today. An especially high dose has made elective surgery and survival through trauma an option for those even with severe disease. There is especially hope that hemophilia may be one of the first diseases to be cured by gene therapy, eliminating the need for risky replacement therapy and the
nuisance of multiple transfusions per day. This would greatly improve both the life expectancy and quality of life experienced by patients with this disease.

Risks For Other Family Members

Due to the X-linked recessive inheritance pattern of both hemophilia A and hemophilia B, they both display identical risks for family members. For an affected son, the father will not be a carrier of the disease nor will he have the disease. The mother of an affected son who also has one other affected relative in the maternal line is an obligate carrier. In some cases, the mother may not be a carrier and the affected son may have a de novo mutation. If no other relative is affected, the mother may be a carrier of a de novo mutation, which may be due to a germline mutation, a somatic mutation or germline mosaicism. It is also possible that the mother is a carrier who inherited the mutation from her mother who has a de novo mutation, or the mother is a carrier of a mutation that has been passed through the family only through female carriers without producing affected male family members.

The siblings of an affected male being at risk for having the disorder is dependant on if the mother is a carrier. If she is a carrier, each male sibling has a 50% chance of having the disease, and each female sibling has a 50% chance of being a carrier. The female offspring of an affected male will be carriers for the disease and pass it on to their sons whereas the sons of an affected male will not inherit the mutation. Other relatives that may be at risk include maternal aunts who may be carriers and their offspring may be at risk of being carriers or being affected.

References


