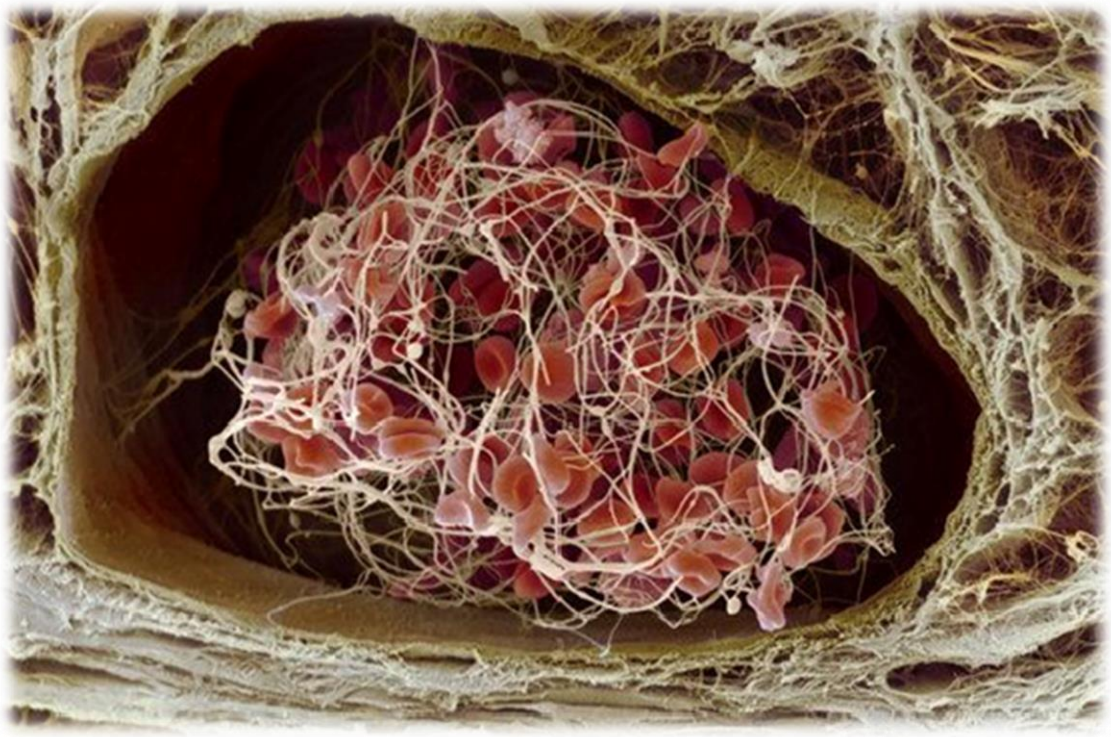
GENE THERAPIES FOR HAEMOPHILIA



After many years of unsuccessful clinical trials, a study carried out on 12 haemophiliacs, using a novel gene therapy developed by the biotechnology company BioMarin Pharmaceuticals, has shown encouraging results for the treatment of type A haemophilia (where the patient has a deficit of coagulation factor VIII). Of the twelve patients in the study, only two were refractory to treatment, and had to continue with the regular administration of factor VIII concentrates.

Gene therapy consists of inserting a gene into the genome or epigenome (extra-chromosomal DNA fragments) of the haemophiliac using a virus as a vector. The haemophilia gene treatments are "all or nothing" therapies. If they do not work, the patient is forced to restart the periodic transfusions of deficient coagulation factor concentrates (factor VIII in type A, or classic haemophilia, and factor IX in type B haemophilia (Christmas disease). There is a rare variant of haemophilia type B called haemophilia B Leyden, which manifests itself during childhood and subsides, partially or totally, from puberty onwards.

The prevalence of haemophilia type A, (classic haemophilia) is estimated at 1 case for every 4,000 or 5,000 male live births; while the prevalence of type B haemophilia (Christmas Disease) is 1 case per 20,000 males.

There has been an awareness of the existence of haemophilia since ancient times. In the Talmud, for example, it was decreed that children whose elder brothers or cousins bled to death after circumcision should not be subjected to such a practice.

The haemophilic trait is transmitted as a recessive inheritance linked to the person's sex (it is encountered on the X chromosome). The disease is suffered by men; the females act as asymptomatic carriers.

Thus, the haemophilia of a grandfather which is not manifested in any of his sons, has a 50% chance of appearing in his male grandchildren.

The daughter of a carrier has a 50% chance of transmitting the disease to her sons.

Haemophilia revealed the early stages of what was later known as a "coagulation cascade":

In "classic haemophilia" or type A, factor VIII (the only factor in the "coagulation cascade" with no protease-like enzyme activity) is absent or only behaves residually. Factor VIII acts as a necessary adjuvant for factor IXa to be able to activate factor X and turn this into factor Xa. The initial "a" refers to its "activated" state; as such, it is able to catalyse the activation of the next factor in the coagulation cascade. [The absence of factor IX is the cause of type B haemophilia or Christmas's disease].

The molecular basis of haemophilia is heterogeneous. More than half a century ago, the geneticist John Burdon Sanderson Haldane (1892-1964) pointed out that serious diseases linked to the X chromosome must always have their origins in the effect of "de novo" mutations (spontaneous and random) given that, if not, these mutations would end up becoming extinct. In fact, almost a third of recently reported cases arise within families who have no history of haemophilia. The difference is important in the case of autosomal recessive genetic defects (not linked to sex) which can spread among the population, since only homozygotes suffer from the disease, while heterozygotes either do not suffer it or only suffer mild symptoms.

The most severely affected haemophiliacs need the intravenous administration of clotting factor concentrates every few days Coagulation factors have very short average lifetimes in the blood, making periodic injections necessary. Despite the treatment, haemophiliacs continue to be at risk from suffering "spontaneous" haemorrhages, after mini-traumatisms, which are imperceptible, even to the patient.

Although the brain is the organ which suffers the most serious haemorrhages, these haemorrhages can irreversibly damage other structures of the body, such as the joints (haemophilic arthropathy) and others, such as osteochondria and synovia (hemarthrosis). It is frequent for haemophiliacs to undergo knee and / or hip prosthetic surgery at an early age, to alleviate the chronic pain suffered by patients.

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Although the brain is the most involved organ, haemorrhages can irreversibly damage other bodily structures, such as the joints (haemophilic arthropathy) and osteochondral and synovial hemarthrosis. It is common for haemophiliacs to undergo knee and / or hip prosthetic surgery at an early age. Chronic pain is common.

Haemophilia seemed to be a genetic disorder suitable for the development of gene therapy. On the one hand, the normal serum levels of the coagulation proteins (factors) have a wide *median*, from 50% to 150%. In this way, even a gene therapy with modest results could be effective. In addition, the genes involved in the two types of haemophilia (A and B) have been known since the early 1980s. The factor VIII protein was purified in 1980, when Gordon A. Vehar, working at the Earl W. Davie laboratory, (part of The Medical School at the University of Washington) at the time, obtained a few milligrams of factor VIII from 25,000 litres of cow's blood. Very soon afterwards, Edward Tuddeham's working group at the Royal Free Hospital in London obtained enough factor VIII for the Californian biotechnology

company Genentech to begin the sequencing of the protein. [Another research team led by David N. Fass at the Mayo Clinic in Rochester, United States, achieved similar results from swine's blood].

The first results with gene therapy in the corrective treatment of haemophilia were obtained about a decade ago at University College, London. An experiment, carried out on ten patients with haemophilia type B, achieved an increase in levels of factor IX of between 2 and 6%. Although this increase was modest, these levels have remained relatively constant since then.

Unexpectedly, a man from Padua, Italy, was found carrying a genetic mutation that causes his cells to synthesise up to 12 times the usual amounts of factor IX (Christmas factor), which became known as "factor IX-R338L"; and, sometimes, "factor IX Padua".

This extraordinary mutation of the gene, once cloned, was inserted into the genome of a virus, which acted as a transporter of the gene to the cells of patients with type B haemophilia.

The advantage of this is that a more powerful gene permitted the use a lower "viral load", and to restrict the possible response of the patient's immune system to the viral capsid.

The first patient treated in this way was a nurse of 23 years of age. Her level of factor IX increased by around 30%, and has remained constant since then. The patient did not need more transfusions of factor IX, nor did she suffer spontaneous haemorrhages.

However, the development of gene therapy for haemophilia type A has been much more problematic.

The viruses used to introduce genes into patients' cells are adenovirus. These cannot transport long genes; and the gene that codes for factor VIII is particularly long. The factor VIII gene consists of 186,000 base pairs, distributed within 26 exons which themselves occupy 70,500 base pairs), meaning that most of the gene is made up of introns. The protein expressed by this gene is formed by 2,351 amino-acids with a total molecular weight of 265,000 Daltons, before processing.

For three decades, the research has been focussed on the elimination of “superfluous” sections of this gene in order to be able to insert it into an adenovirus transporter.

We are faced with the possibility of being able to cure a genetic illness that has affected, like no other, the development of our recent History. For many years it was called "royal haemophilia," because it affected many heirs to the European dynasties of the Bourbons and the Habsburgs.

The clinical curing of haemophilia would be an unprecedented achievement. It would put in place strategies of action which would be extremely useful in the treatment of many other genetic diseases.

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