Learn differently.
Engage differently.
HaemDifferently.

Welcome to a community-built approach to understanding the concepts behind gene therapy research.

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Developed and funded by BioMarin.

Welcome to HaemDifferently

HaemDifferently is an educational platform aiming to open an honest conversation around the topic of gene therapy and to help many people along their journey.

Providing accurate and straightforward information that helps make gene therapy feel more familiar is important to us, but we can't do it alone. We are incredibly grateful to the gene therapy investigators, haemophilia community, advocacy groups and linguistic specialists who have helped inform and shape this educational platform.

Part of doing things differently means listening to your feedback so that, together, we can refine this platform to fit your needs. Feel free to let us know what you like, what can be improved and what you'd like to see in the future at **haemdifferently@bmrn.com**.

Haemdifferently

Contents

The content of this material is not prescriptive and should not replace consultation with a trained healthcare provider. Information regarding gene therapy is provided as a general overview and is not comprehensive.

For more information on any of these topics or to sign up for updates, please visit **HaemDifferently.eu**.

What is a gene?

TAKEAWAYS

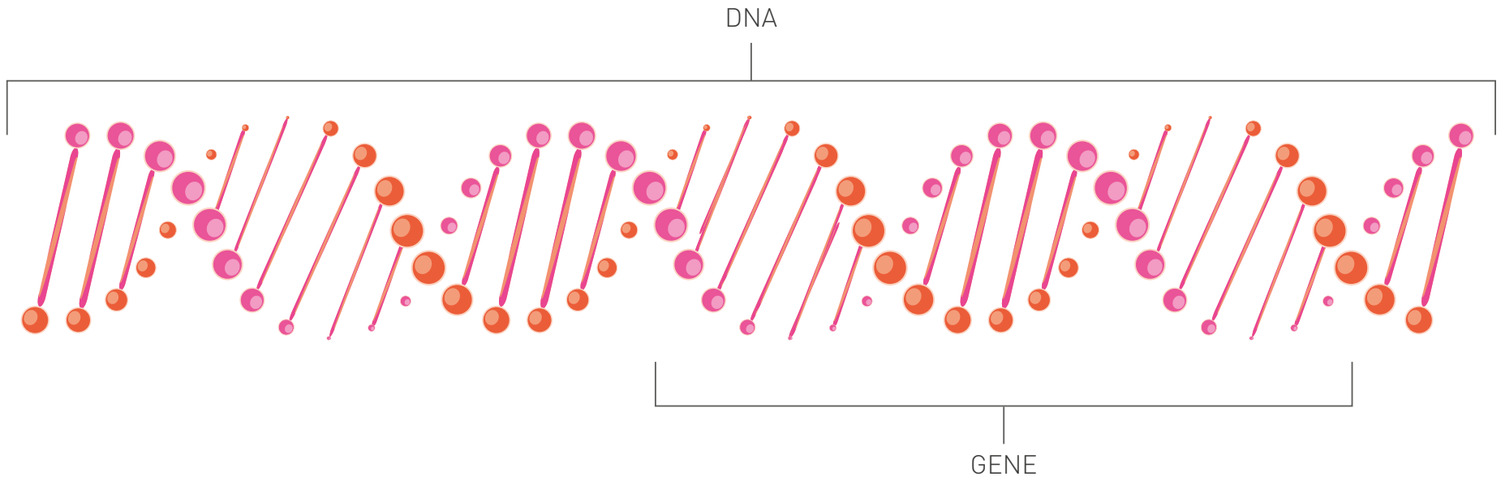
- Genes, located in the chromosomes, are responsible for providing the body with instructions for building proteins that give us our unique traits
- DNA is like the language used for genetic instructions

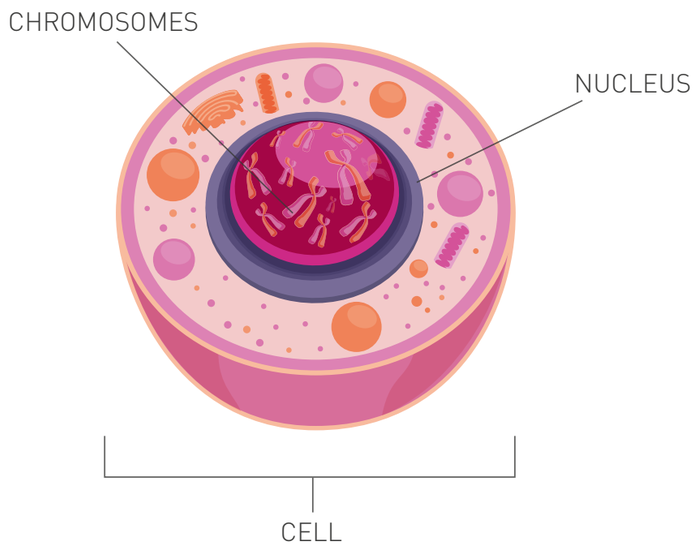
You've probably heard about genes before. But there is so much more to genes than determining the colour of your hair or your eyes. How do genes work in the body?

GENES ARE SEGMENTS OF DNA

The key role of **genes** is to provide the instructions for making **proteins**. Proteins are the building blocks of the body and serve important functions like tissue repair and helping blood to clot.

Think of **DNA** as the language used for your **genetic** instructions. DNA is made up of components called **nucleotide bases**, which are like the letters of a word. To produce a protein with normal function, you must have the correct instructions, encoded in the order of the nucleotide bases.





BUT WHERE ARE OUR GENES?

Our DNA is packaged into **chromosomes** that contain hundreds to thousands of known genes. Chromosomes lie deep within the **nucleus**, a structure acting as the command centre of the **cells** that make up your body.

Need a word defined? **Bolded terms** can be found in the glossary on pages 14–15.

Part of doing things differently means getting your feedback.
We hope you'll get in touch with us at **haemdifferently@bmrn.com** to let us know how we're doing.

What causes genetic conditions?

Did you know there are approximately 20,000 genes in the human **genome**? A **mutation**, or permanent variation, in just one gene can lead to a faulty instruction for the protein and result in a genetic condition. Knowing what causes a genetic condition is the first step in understanding how to treat these conditions.

GENETIC CONDITIONS ARE THE RESULT OF MUTATIONS

Genetic conditions, such as **haemophilia**, are the result of mutations, or variations, in the genetic instruction of a gene. These mutations are most often passed down from biological parents but can sometimes happen spontaneously.

In people with haemophilia A or B, the mutation affects the proteins called **factor VIII** or **factor IX**, respectively. These proteins are critical for blood to clot.

Haemophilia A is more common in males because the gene for factor VIII is located in the X chromosome. Males have only one X chromosome and so one copy of the mutated gene is enough to cause haemophilia.

Females have two X chromosomes. They generally show no signs of haemophilia if just one X chromosome is affected, but they are "carriers" because they can still pass the mutated gene to their children.

MUTATIONS CAN AFFECT YOUR GENETIC INSTRUCTIONS

Like a word can become illegible when there are missing or wrong letters, a mutation can render the genetic instruction missing or incorrect. This may result in a protein that does not work properly or, in some cases, the protein is not produced at all.

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What causes genetic conditions?

THERE ARE THREE TYPES OF GENETIC CONDITIONS

Condition	Cause	Examples
Monogenic condition	Mutation in a single gene	• Cystic fibrosis • Haemophilia • Huntington's disease
Multifactorial inheritance condition (multi-gene disorders)	Multiple small genetic mutations	• Heart disease • Diabetes
Chromosome disorders	Changes to the number or structure of chromosomes	• **Down's syndrome**

TAKEAWAYS

- Genetic conditions such as haemophilia A and B, **Huntington's disease** and **cystic fibrosis** are the result of single-gene mutations

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What is gene therapy?

Simply put, gene therapy attempts to fix a machine that is dysfunctional because of scrambled instructions, by uploading a correct set of instructions.

CONCEPTS OF GENE THERAPY

In gene therapy, genes are used to treat or prevent disease caused by genetic mutations. Gene therapy has been explored for more than 50 years. It has the potential to bring an entirely new treatment option to people with specific genetic conditions and those who support and care for them.

METHODS OF GENE THERAPY BEING EXPLORED

A few different approaches to gene therapy are being explored. Gene therapy may involve attempting to repair or replace a mutated gene, disabling a mutated gene that is causing trouble or introducing a functional copy of the gene into the body to help the body produce the affected protein.

To explore future, current and past research in gene therapy, visit **ClinicalTrials.gov**.

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What is gene therapy?

THREE APPROACHES TO GENE THERAPY ARE BEING EXPLORED

Gene transfer

- A functional gene is inserted into a cell with the intent that it will work in place of the mutated gene

Ex vivo gene therapy

- Affected cells are removed from the body, and the functional genetic material is introduced into the cells in the laboratory. These cells are then delivered back into the patient's body

Gene editing

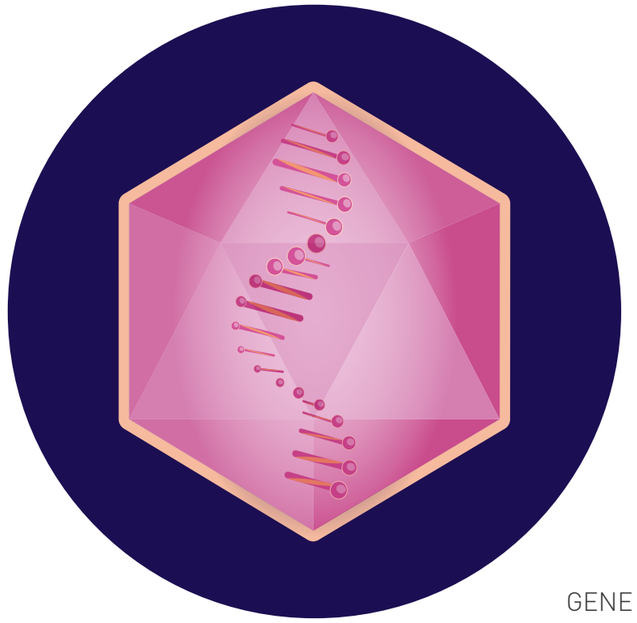
- By making changes to the original DNA, the mutated gene can be repaired, or new DNA can be added in a specific location to replace the mutated gene

In any of these approaches, the original genetic material is not replaced throughout the cells of the body, and the mutated gene might still be passed on to the person's children.

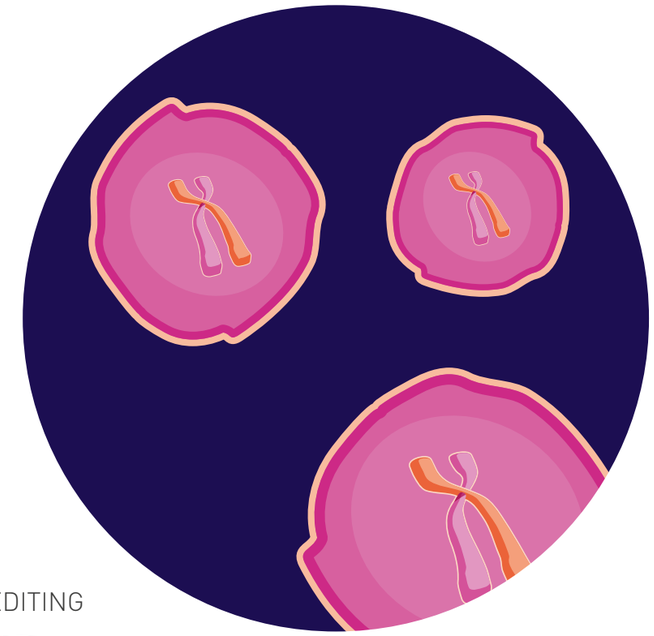
TAKEAWAYS

- Gene therapy attempts to use genes to treat or prevent disease caused by genetic mutations

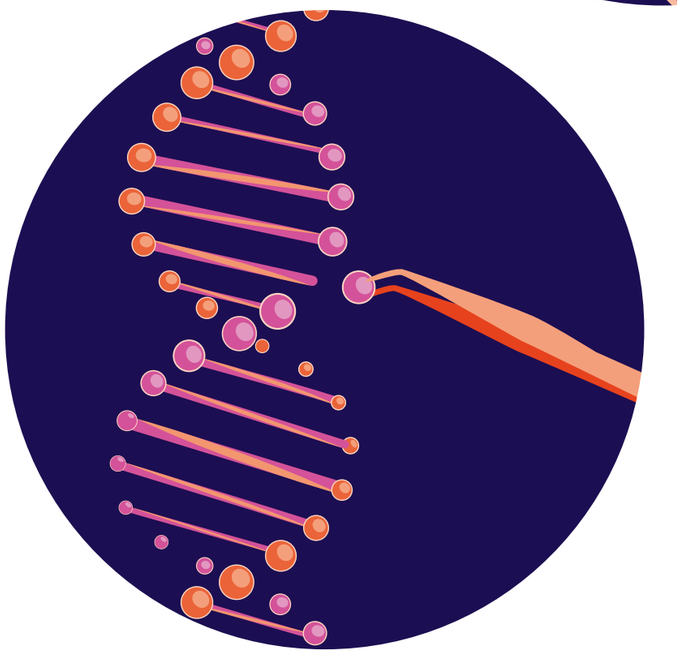
GENE TRANSFER



EX VIVO GENE THERAPY



GENE EDITING



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How does it work and what are the goals of gene therapy?

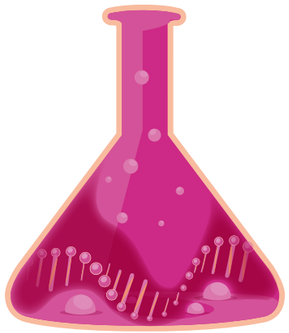
It's not magic – it's science in progress. Gene therapy has the potential to offer a remarkably different approach to the way we've historically managed genetic disease. Many gene therapies are under investigation and some have been approved for use for conditions other than haemophilia A or B.

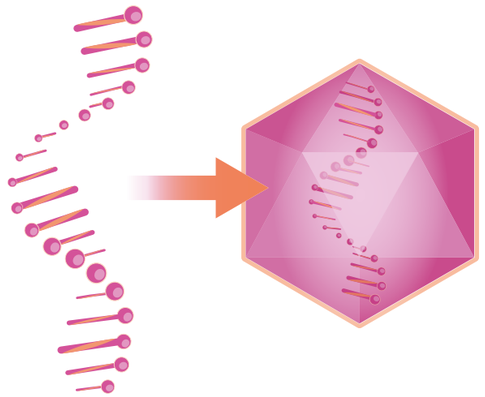
Let's look at gene transfer, as an example...

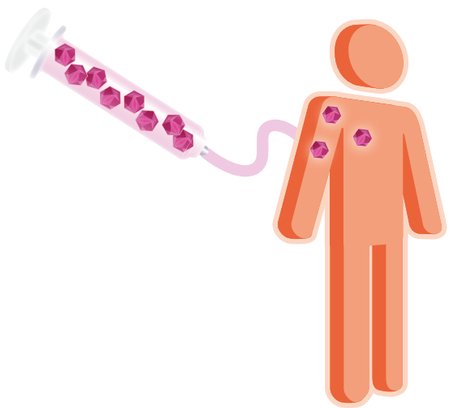
In haemophilia A and B, gene transfer is currently investigated as the method to introduce functioning genes into the body that can instruct the body to produce the needed protein. Think of this like reprogramming a machine by replacing a faulty copy of instructions with a corrected copy, rather than replacing the product the machine makes.

How does gene transfer work and what are the goals?

How does it work and what are the goals of gene therapy?







1 CREATING A FUNCTIONAL GENE

A functional copy of the mutated gene is created in a laboratory.

2 BUILDING A TRANSPORT VEHICLE

To allow the functional gene to be introduced into the body, a transport vehicle is created from a **neutralised virus**. Viruses used in gene transfer include **adenoviruses**, **adeno-associated viruses (AAV)** and **lentiviruses**.

3 DELIVERING THE FUNCTIONAL GENE

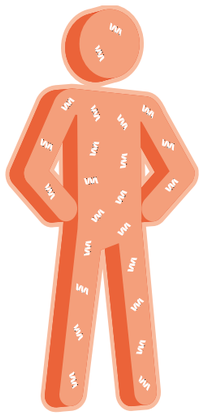
Once the functional gene is placed inside the transport vehicle, it is called a therapeutic **vector**. The therapeutic vector is designed to target the functional gene towards a preferred tissue. In the case of haemophilia, this is the liver. Large numbers of therapeutic vector are administered via an intravenous infusion.

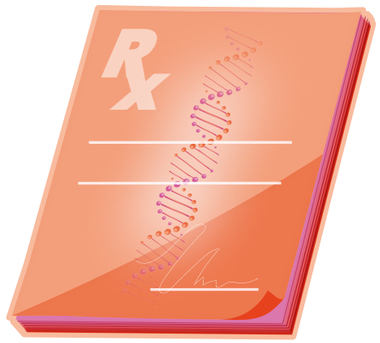
Need a word defined? **Bolded terms** can be found in the glossary on pages 14–15.

Clinical trials are underway to determine the risk and whether, for some people, gene therapy could reduce or eliminate the need for ongoing treatment and the burdens of chronic disease. These trials also investigate the long-term effects of gene therapy, including duration of effect, that have not yet been determined.

TAKEAWAYS

- Gene transfer aims to introduce a functioning gene that can instruct the body to produce the needed protein





4 MAKING PROTEINS

Once introduced into the body, the goal for the new gene is to work in place of the faulty gene and to provide instructions for the body to make the protein it needs.

For example, in haemophilia A or B, the goal is to replace the gene for factor VIII or factor IX, respectively, so the body can produce these factors on its own to restore the natural clotting process.

5 POTENTIALLY REDUCING THE NEED FOR TREATMENT

Research is ongoing to determine if gene therapy may lead to less reliance on currently available medication.

6 POTENTIALLY ELIMINATING OR REDUCING SYMPTOMS

Ongoing studies are also determining if reducing the need for routine treatment may lessen the physical, mental and emotional burden of a disease. Although gene therapy may not be able to address pre-existing damage, it may be able to mitigate progression of any existing damage.

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What are the risks of gene therapy?

TAKEAWAYS

- Like any treatment, gene therapy may have risks. It's important to remember that the long-term effects of gene therapy are also being studied and have not yet been determined

Many forms of gene therapy are being researched only in adults, at least initially, and some gene therapies won't work in patients with certain **antibodies** or other pre existing conditions. Gene therapy also comes with risks. Ongoing clinical trials are being conducted in people across many categories to determine the potential risks of treatment with gene therapy.

SAFETY IS A TOP PRIORITY

Patient safety is the top priority, and many safety precautions are being taken during the development of gene therapy. Clinical trials are closely monitored by authoritative institutions, such as the Food and Drug Administration and the National Institutes of Health in the United States. Ongoing clinical trials and research have identified some risks associated with gene therapy, and further research and experience may uncover additional risks that are currently unknown.

POTENTIAL RISKS

Gene transfer that uses an adeno-associated virus (AAV) vector to deliver the new genetic material may have several risks:

- The body's immune system might recognise the therapeutic vector as an intruder, which may lead to inflammation and other serious risks
- An immune reaction could also make gene therapy work less effectively, or not at all. That is why prospective gene therapy patients undergo a diagnostic blood test to determine whether they have antibodies against a specific virus
- The therapeutic vector may affect other cells that weren't targeted, potentially causing damage or additional illness or disease
- Vector particles can be released from the recipient's body through faeces, urine, saliva and other excreted bodily fluids (called **vector shedding**) and may be passed on to untreated individuals. Clinical trials are currently evaluating this possibility and its medical significance
- Whether gene therapy may have an adverse impact on the health of the organ or tissues targeted is being evaluated withlong-term studies
- Gene therapy may result in too much of the protein being created. The effect of this overproduction, or overexpression, could vary based on the type of protein being created
- For some patients, gene therapy may not work at all, and it is not yet clear how long the effects of gene therapy may last

Gene therapy timeline

50+ YEARS OF RESEARCH

Gene therapy has been explored as a potential treatment approach for well over 50 years. In the past decade, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have approved gene therapies for genetic conditions. Gene therapy is currently being researched in many clinical trials for various genetic disorders, including haemophilia A and B.

1972
Concept of gene therapy considered as a form of treatment in the journal Science

1984
Dr. Gordon Vehar publishes a paper reporting successful factor VIII cloning

1990
First gene therapy trial in humans

1999
Lessons learned regarding risks related to potential for severe immune response in early gene therapy trial with non-adeno-associated virus (AAV) vector

2003
The Human Genome Project is completed

2005
First gene therapy trial in haemophilia B using AAV vector technology

2015
First gene therapy trial in haemophilia A using AAV vector technology

2017
The first gene therapy, for a genetic disease that causes blindness, is approved in the United States

Future
Gene therapy for various genetic conditions is available but research is ongoing to determine the potential risks and benefits of treatment

Glossary

Adeno-associated virus (AAV) – Any of several viruses that enter the nucleus of a host cell but are dependent on coinfection with an adenovirus or herpesvirus for their replication. They infect a wide range of hosts but do not appear to cause disease and are being researched in gene therapy as vectors to introduce genes into cells.

Adenovirus – Causes mild to severe illness, though serious illness is less common. Common symptoms include common cold, sore throat, bronchitis, pneumonia, diarrhoea, conjunctivitis (pink eye) and fever. Adenoviruses are usually spread from an infected person to others through close personal contact, coughing and sneezing, and touching an object or surface with adenoviruses on it, then touching your mouth, nose or eyes before washing your hands.

Antibody – A blood protein created in response to a substance (antigen) not recognised by the body. Antibodies then bind to specific antigens, helping to destroy them. Some antibodies destroy antigens directly; others make it easier for white blood cells to destroy the antigen.

Cell – The fundamental, structural and functional unit of living organisms. In biology, a cell is the smallest unit that can live on its own. All living organisms and the tissues of the body are made of cells. A cell has three main parts: the cell membrane, the nucleus and the cytoplasm.

Chromosome – A structure found in animal cells containing a linear thread of DNA, which transmits genetic information. Humans normally have 46 chromosomes (23 pairs) in each cell. In males and females, 22 of those pairs look the same. The 23rd pair, also called the sex chromosomes, differs between males and females: females have two copies of the X chromosome, while males have one X and one Y chromosome.

Cystic fibrosis – A genetic disease that causes progressive, persistent lung infections that limit the ability to breathe. Genetically, someone must inherit two copies of the cystic fibrosis transmembrane conductance regulator *(CFTR)* gene that contain mutations – one copy from each parent – to have cystic fibrosis.

DNA (deoxyribonucleic acid) – The molecular basis of heredity present in humans and almost all other organisms. Nearly every cell in a person's body contains some DNA. DNA is made up of nucleotide bases, which are like the letters of a word. There are four nucleotide bases responsible for gene construction: adenine (A), guanine (G), cytosine (C) and thymine (T). These nucleotides pair up with each other, A with T, and C with G.

Down's syndrome – A genetic disorder caused when abnormal cell division results in an extra full or partial copy of chromosome 21. Down's syndrome causes lifelong intellectual disability and developmental delays. It is the most common genetic chromosomal disorder and cause of learning disabilities in children.

Ex vivo – From the Latin, meaning "out of the living", ex vivo means something that takes place outside an organism. In science, ex vivo refers to experimentation or measurements done in an external environment on tissue from an organism with minimal alteration of natural conditions.

Factor VIII – The factor VIII gene provides instructions for making a protein called coagulation factor VIII. Coagulation factors are essential for the formation of stable blood clots. Factor VIII is absent or inactive in people with haemophilia A.

Factor IX – The factor IX gene provides instructions for making a protein called coagulation factor IX. Coagulation factors are essential for the formation of blood clots. Factor IX is absent or inactive in people with haemophilia B.

Glossary

Gene – A part of a DNA molecule, usually located on a chromosome, that is the functional unitof inheritance controlling the transmission and expression of one or more traits from parent to child.

Genetics – The study of genes and their heredity. Also, a branch of biology that deals with the heredity and variation of organisms.

Genome – An organism's complete set of genetic material. It contains all the instructions needed for the organism to function. In people, every cell that has a nucleus contains a copy of the entire genome.

Haemophilia – A bleeding disorder that slows the blood clotting process. People with haemophilia experience both spontaneous and prolonged bleeding following an injury. Serious complications can result from bleeding into the joints, muscles, brain or other internal organs. The major types of this condition are haemophilia A (factor VIII deficiency) and haemophilia B (factor IX deficiency).

Huntington's disease – An inherited disease that causes the progressive breakdown (degeneration) of nerve cells in the brain. Huntington's disease has a broad impact on a person's functional abilities and usually results in movement, thinking (cognitive) and psychiatric disorders.

Lentivirus – A retrovirus that causes persistent infection that typically results in chronic, progressive and usually fatal disease in both humans and animals. In humans, human immunodeficiency virus (HIV) may be the most recognisable lentivirus.

Monogenic conditions – Monogenic conditions, such as haemophilia, are caused by a single gene not working properly. The cause of the malfunction may be present in one or both chromosomes inherited from the parents.

Mutation – A change in the structure of a gene that may be passed along to future generations; also called a permanent variant.

Neutralised virus – A neutralised virus is created by removing the inner viral material in a lab, leaving behind an empty protein shell, called a vector.

Nucleotide base – An organic molecule that is the building block of DNA and ribonucleic acid (RNA).

Nucleus – The part of a cell that contains the chromosomes. The nucleus has a membrane around it and is where RNA is made from the DNA in the chromosomes.

Protein – A group of amino acids joined together that perform various biological functions. Examples include enzymes, hormones and antibodies.

Vector – A vector is a means by which a functional gene used in gene therapy is transported to a cell. A vector can be viral or nonviral. This is being investigated in clinical trials.

Vector shedding – The release of the vector/genetic materials through secretions and/or excrement of the patient. Assessment of shedding can be used to understand the potential risk associated with transmission to third parties and the potential risk to the environment.

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