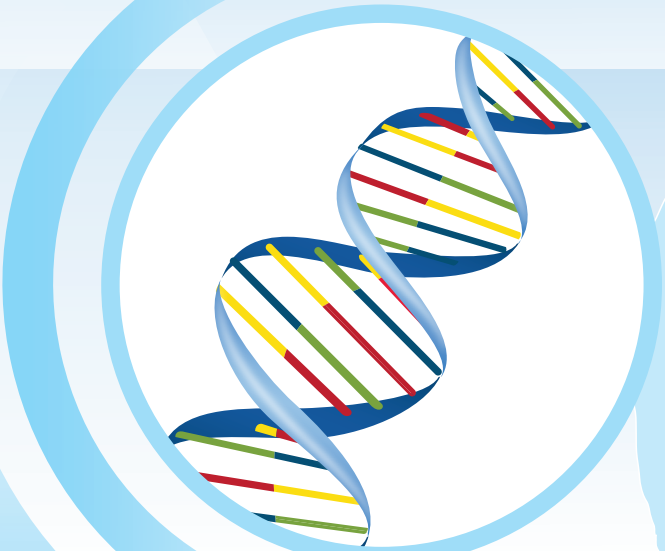

A Guide to

Leukodystrophy gene therapy and other novel treatments

A booklet for patients and families

IMPORTANT

The information in this booklet is for educational purposes. This booklet is not meant to be a substitute for professional health care advice or instructions, nor is it a substitute for medical care. If you have any questions, contact a qualified health care professional.

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HEALTHY BRAINS
HEALTHY LIVES

Table of Contents

Introduction

The purpose of this booklet is to help you understand your child's condition. It will also help you learn about gene therapy and other novel therapies.

Leukodystrophy* is the name of a group of illnesses that affect myelin. **Myelin** is a layer that protects your nerve cells the same way that plastic coating protects electrical wires. It is also a **genetic disease** which means that it can be passed down through generations.

* There are many types of leukodystrophies, but we use the term "leukodystrophy" for simplicity.

This first chapter will review what you need to know to better understand what leukodystrophy is.

How to read this booklet

There is a lot of information in this booklet. **Feel free to read only what is important to you.** Throughout the booklet, you will notice some words in bold. These are medical terms or key messages. We have also included pictures to help explain the information.

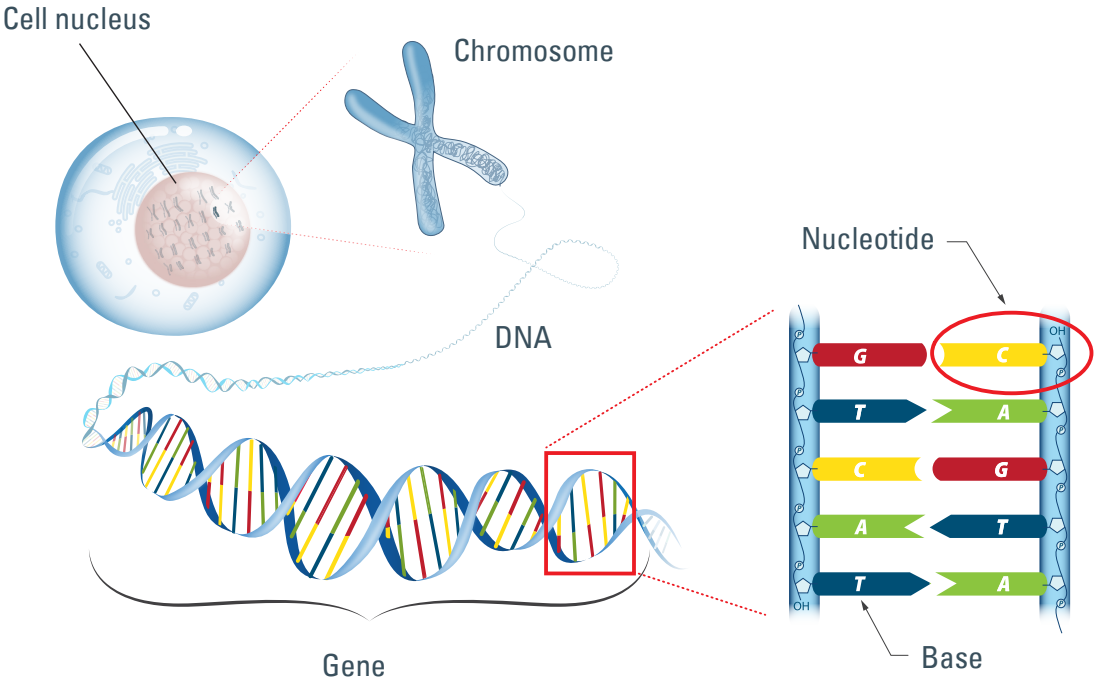
Finally, each chapter ends with a summary to help you remember the most important details.

If there is something that you do not understand, please ask us. We are here to help you care for your child and help you get the information you need to make decisions. This booklet is not a substitute for medical advice.

What is DNA?

To answer that, we need to introduce the cell.

Cells are the smallest units in our bodies that have specialized functions. Our cells store most of our DNA.



DNA has the instructions to determine what each cell will be and what it will do. It is also what makes each cell unique.

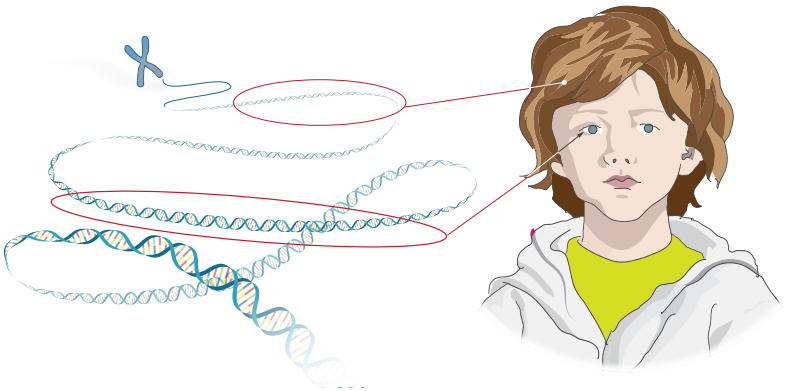
DNA is made of 4 different molecules called bases (or nucleotides). The bases are adenine, thymine, guanine, and cytosine, but are also known by their respective first letter, A, T, G, and C. They are arranged in a string in pairs.

Each combination of A, T, G, and C in a string of DNA is called a sequence.

What are genes?

Genes are small sections of DNA. Genes are like built-in instructions to do a job. **Genes "tell" cells what to do.** The body reads these instructions to know what to do and how to do it. For example, genes can "tell" your body to make your eyes blue, make your hair curly, or give you dimples.

Together, our **genes** make up our **genetic material**. Another word for genetic material is **genome**.



What is genetic variation?

Your genetic material is almost 100% the same as any other person's. But it is not exactly the same. Small genetic differences (variations) between people are what make each person unique.

Genetic variation is a normal part of biology. It affects everything about us: our eye colour, our height, and our health.

But some genetic variation may also be harmful. Genetic diseases happen because one or more variants in a gene cause disease.

Doctors and scientists sometimes call these **gene variants** "disease-causing variants" or "pathogenic variants." You may have also heard the term "mutations" to describe disease-causing variants. In this booklet, we will use the term "variant" to mean "disease-causing variant."

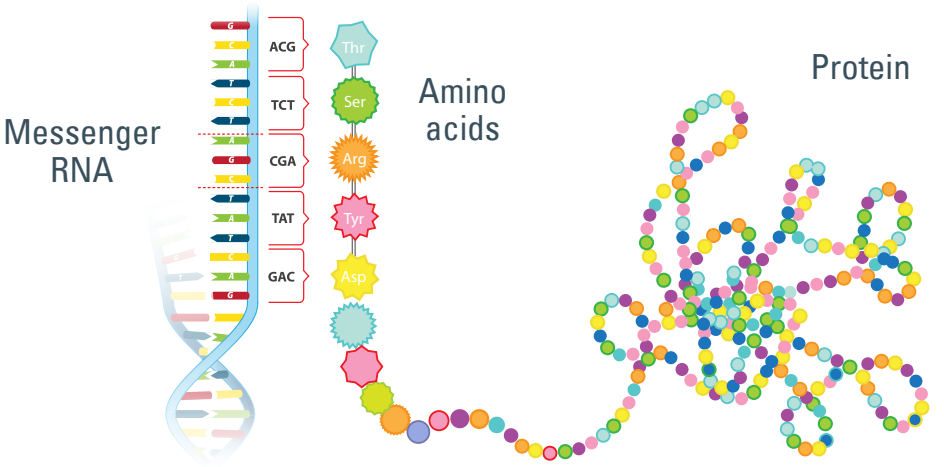
What are proteins?

One category of genes tells the cell how to create proteins. **Proteins** do things like **repair cells, make hormones**, and **react to foreign substances**. But if there is something wrong with a protein, it may cause a problem.

How are proteins made?

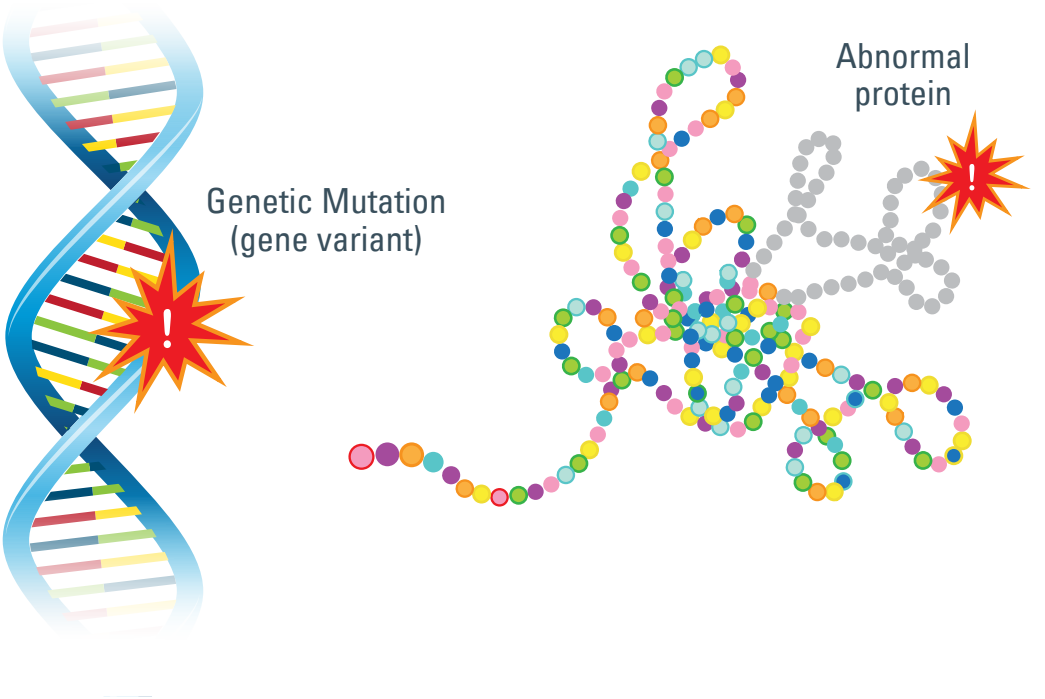
To make a protein

- **The cell** makes a copy of the **DNA gene**, called **messenger RNA** (a type of molecule).
- Next, the cell uses the messenger RNA as instructions to make a **protein.**



- The cell uses **ribosomes** to decode (read) the RNA instructions. Then it uses those instructions to put amino acids in a chain. The protein is the chain of **amino acids**.
- The protein becomes functional when the amino acid chain folds into its three-dimensional (3D) shape.
- The sequence of amino acids and the shape of the protein are important. They determine how the protein functions.

If there is a mistake in the DNA genes (a gene variant), it may cause a mistake in the sequence or shape of a protein. In that case, **the protein may not work correctly**. Most cases of leukodystrophy disease happen because of gene variants that affect how proteins function.



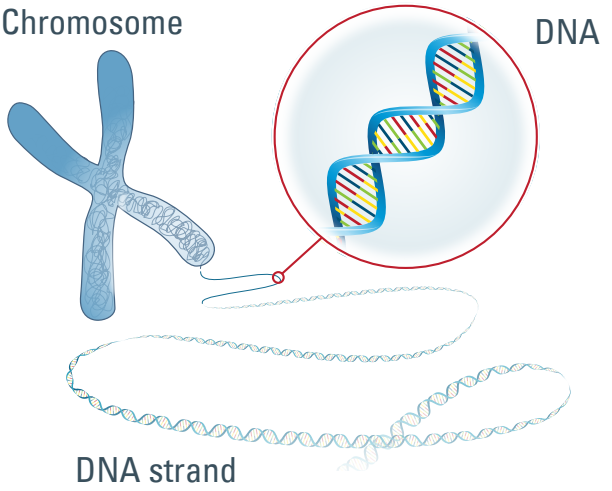
Genes and proteins summary

Our cells have the genetic instructions to perform the tasks required to sustain life.

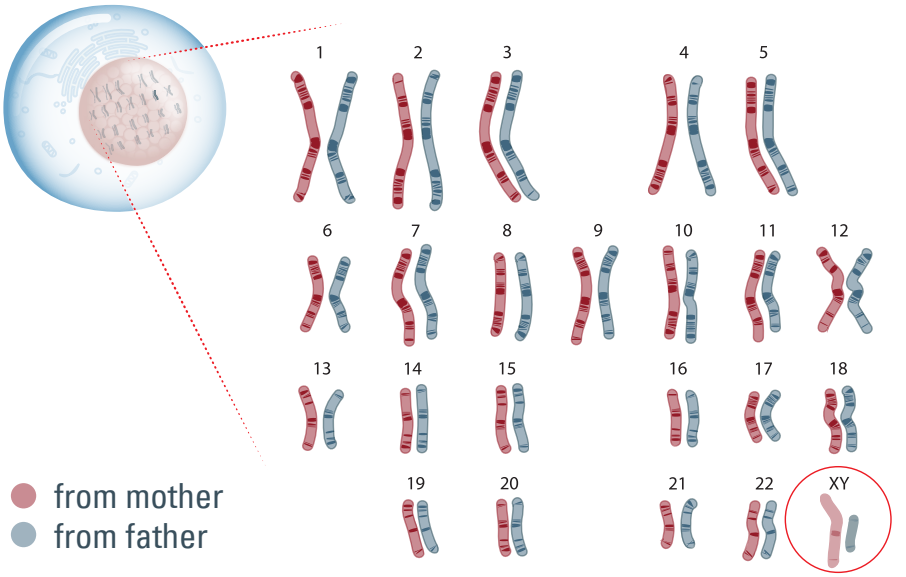
To do this, **the cell uses its genes to make proteins** -- molecules made from amino acids. These proteins do some of the simplest but most essential tasks in our bodies. They can perform even more complex bodily functions (as part of **pathways**). So, genes and their proteins can affect our health.

How is DNA stored in our bodies?

There is so much **DNA** in our bodies that it needs to be stored in a space-saving way. This is where chromosomes come in. **A chromosome is a long string of DNA**, wound around itself like a twisted ladder. **Each string of DNA contains your genes.**



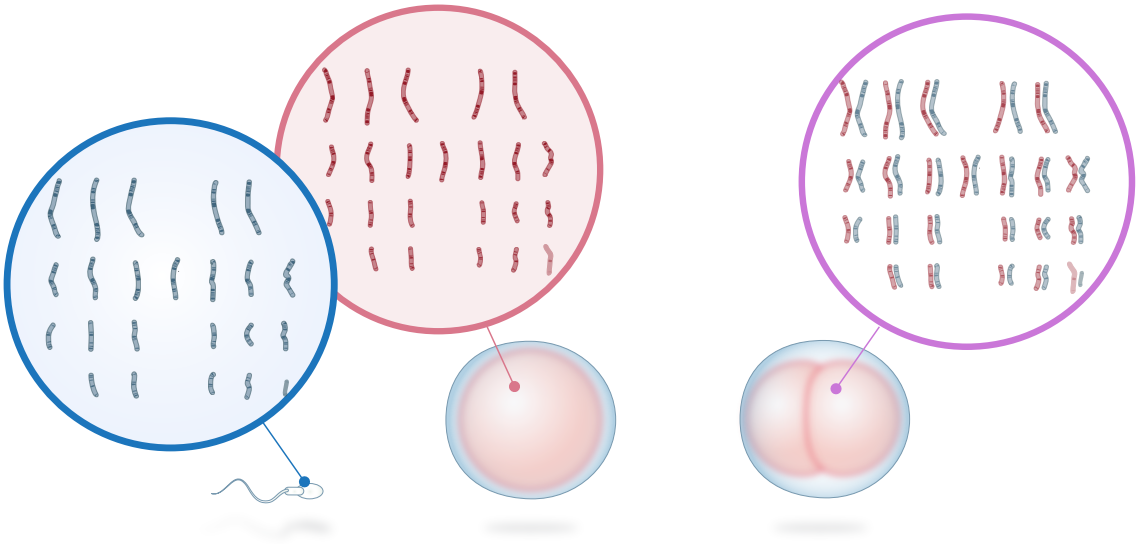
Cells in the human body usually have 2 copies of each chromosome. One is from the biological mother and the other from the biological father.



How are traits passed along in families?

Chromosomes carry genes – the DNA instructions for how cells function. So, **children inherit traits from their family when chromosomes pass from parent to child** during reproduction. This passing on of characteristics through our genes is what makes heredity possible.

Sperm (from the male) and **egg cells** (from the female) have one copy of each of the 23 chromosomes. When sperm and egg cells join during fertilization, they form a specialized cell called a **zygote**. This zygote has its 2 copies of each chromosome – one from the mother, the other from the father. That is why we inherit half our genes from our biological mother, and half from our biological father.



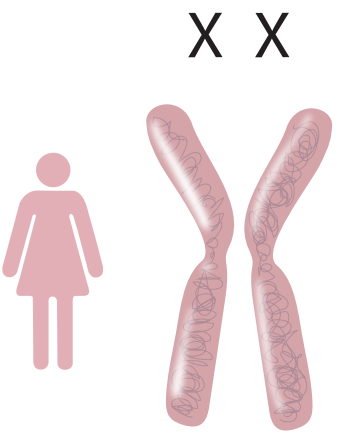
Sperm Egg cell Zygote

What are inheritance patterns?

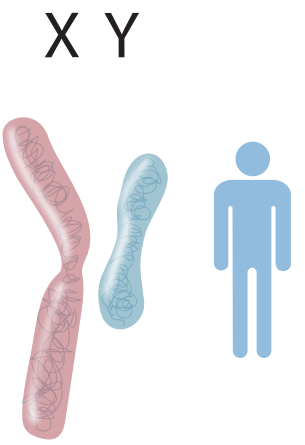
An inheritance pattern is how a trait is passed down through generations. Scientists can map out how it (for example, eye colour) appears in family members over generations. This is called **"mapping."** By studying these patterns, scientists can help explain what gene is responsible for a trait or disease.

To understand inheritance patterns, remember these 4 things about chromosomes:

1. Chromosomes are made of DNA. Genes are small sections of DNA.
2. Each human cell has paired chromosomes: one comes from the sperm, the other from the egg.
3. This means **we have 2 copies of every gene** (except for genes on sex chromosomes for males).
4. In the sex chromosomes, males have **one X** and **one Y.** Females have **two X** chromosomes.



Female sex chromosomes



Male sex chromosomes

Inheritance terminology

Our body also has genes that are responsible for important cellular pathways. In these genes, a variant (mistake) can lead to illness or disease.

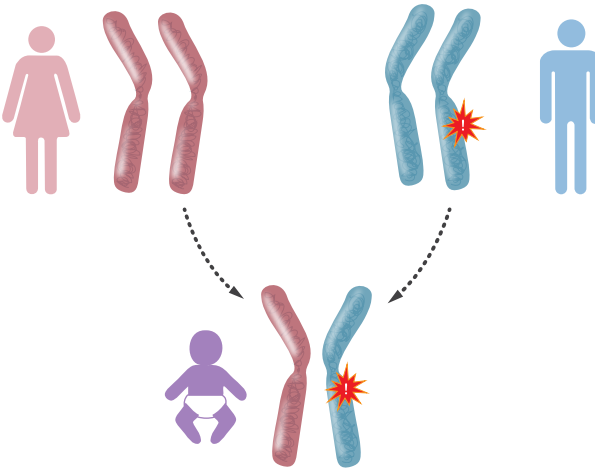
Whether it is a healthy gene or a gene variant, **children get 2 copies of every gene – one from their biological mother and one from their biological father.** Next, we will describe the different ways that genes can come together.

Autosomal

The disease is caused by a gene that is on an autosome (i.e., not on a sex chromosome).

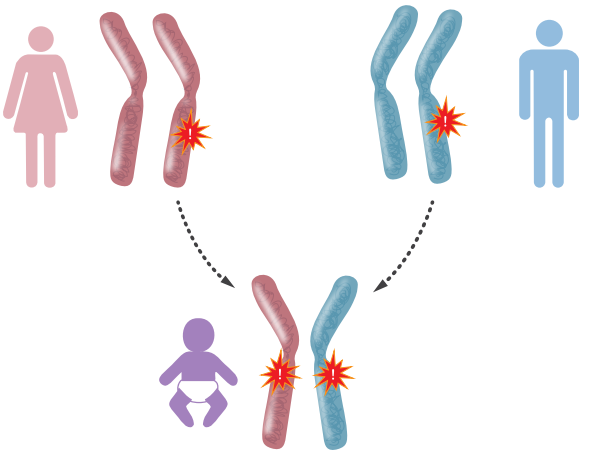
Dominant

You only need to get 1 copy of the gene with a variant to inherit a dominant genetic disease. Autosomal dominant (AD) inheritance happens with just one variant in the gene – one from the father or one from the mother. In this case, at least one parent has the disease because they have the variant copy.



Recessive

You need to get 2 copies of the gene with a gene variant to inherit a recessive genetic disease. Autosomal recessive (AR) inheritance means one variant will be from the father and one will be from the mother. Because both parents also have one normal gene, their health is not usually affected. Most of the time, they don't know that they have a gene variant



X-Linked

The disease is caused by a gene on the X chromosome, making it "linked" to the X chromosome. X-linked (XL) recessive inheritance happens when a child inherits a disease where the variant in the gene is on the X chromosome. This is why it is called X "linked."

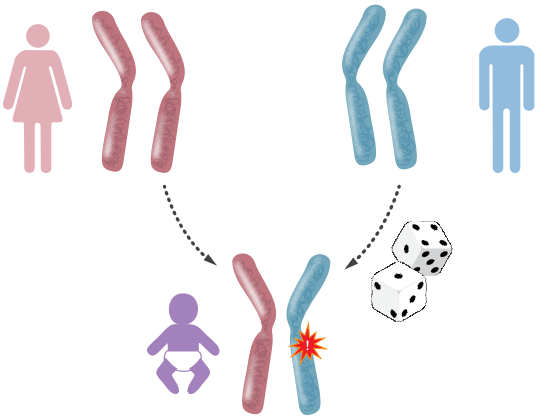
Mitochondrial inheritance

The disease is caused by genes in the mitochondria (not on chromosomes). These diseases have a unique inheritance pattern - mitochondrial diseases are passed down from the mother.

De novo variations

(De novo = new) These variations happen randomly. In this case, the parents do not have a variant in the gene, and do not have the disease. A variation usually occurs in the sperm or egg before or early in reproduction. An affected child can pass the variant to their children since the gene variant is now part of their genetic material.

* There are special cases where one parent may be "**mosaic**" (mixture) for the variant. In this case, some of their cells have the genetic variant while others are healthy. In these situations, the genetic tests may not discover the genetic variant in the parent, but the parent can still transmit the disease to their children.



Now that we know how genes are inherited, we can move on to genetic diseases.

How does genetic variation cause disease?

The last section looked at how genes code for (have instructions for) proteins. Cells use proteins to carry out complex functions in steps called pathways. Problems can happen if:

- There is a mistake in the gene of an essential protein (it has the wrong instructions)
- **The variant in the gene creates a dysfunctional protein or less protein**
- There is a disruption in the pathway
- The body cannot make up for the defective pathway

Genetic diseases happen when a variation in the gene produces a harmful effect.

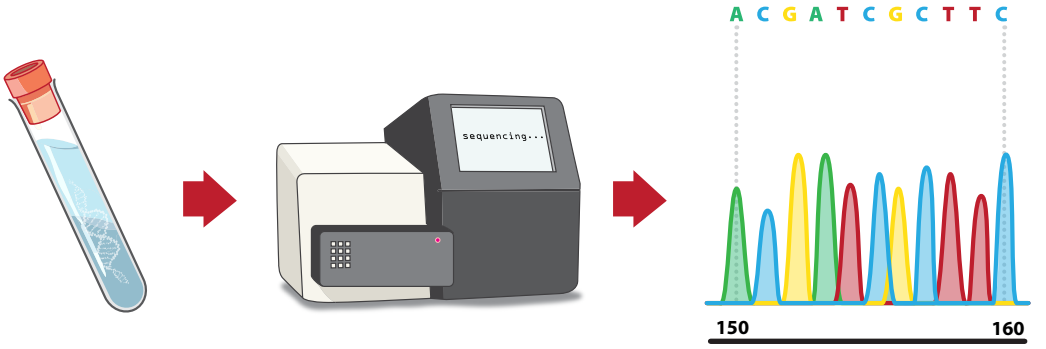
What is gene sequencing?

Gene sequencing is a way of finding out the order of the bases (the As, Ts, Gs, and Cs) in a part of your DNA.

Because everyone's genes are similar, **scientists can identify a specific gene by comparing your unique gene sequence with others that have already been sequenced.**

A person with a genetic disease has at least one gene that is not the same as a healthy person's. Scientists will try to find the difference.

This way, researchers and clinicians can identify genetic variants that may cause disease.



When a doctor asks to have your genes sequenced, this is what happens:

1. Some of your cells are collected (e.g., blood test, cheek swab)
2. Some of your DNA is removed from the cells
3. A computer "reads" the DNA sequence
4. The sequence is analyzed and compared to a reference DNA sequence

What is gene therapy?

One way to treat a genetic disease is to replace the defective gene or to correct the variant that causes the disease. **Gene therapy is one way to do this. Gene therapy corrects cellular dysfunction by targeting genes.**

There are different kinds of gene therapy based on:

- Delivery (how the "therapeutic (healthy) gene" is transported or distributed to cells)
- Mechanism (how it works in cells)

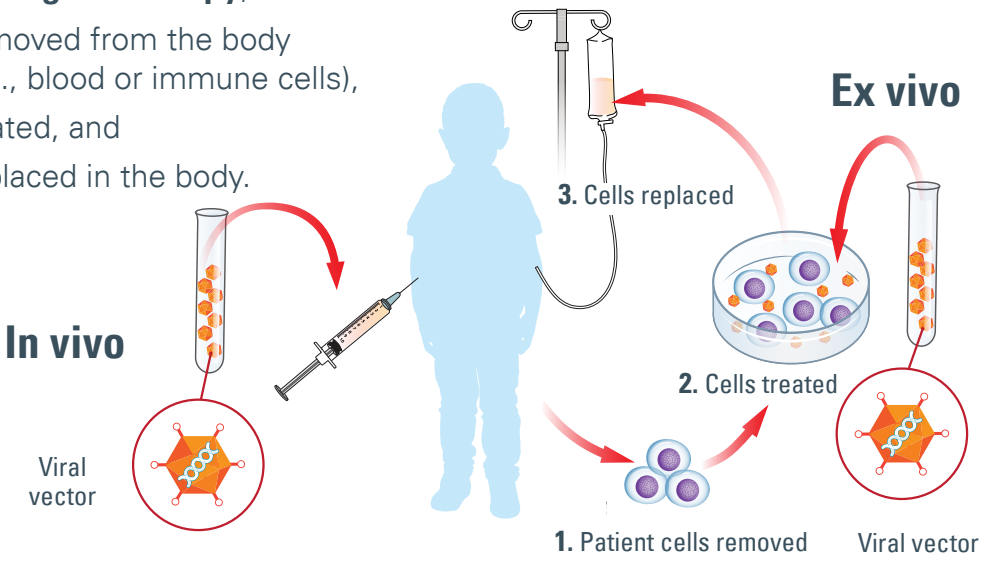
Delivery

In most types of gene therapy, **a piece of artificial genetic material is delivered to a target cell.** The instrument that delivers the genetic material is called a vector. Delivery can be **in vivo** (in the body) or **ex vivo** (out of the body).

In vivo gene therapy is used when it is difficult to work with target cells (e.g., brain, spinal cord, muscle)

In **ex vivo gene therapy**, the cells can be

- Removed from the body (e.g., blood or immune cells),
- Treated, and
- Replaced in the body.



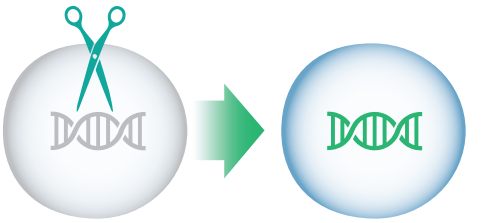
Mechanisms

There are 2 main gene therapy mechanisms.

Gene editing is almost like "rewriting" the "instructions." This method **delivers artificial genetic material to cells to "correct" the gene variant.** The edits fix the variant in the gene sequence, making it the same as in someone who does not have the disease.

Gene addition is different. This therapy **delivers a complete copy (or several copies) of a healthy gene** to cells you want to target. In gene addition, the idea is to encourage the cells to produce a properly functioning protein. In some cases, the added gene may become part of the patient's genetic material. In other cases, it may not.

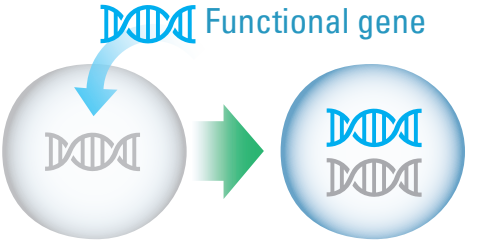
Gene editing



Diseased cell with gene variant

Healthy cell with corrected gene

Gene addition



Diseased cell with gene variant

Healthy cell with both variant and functional genes

Other therapies

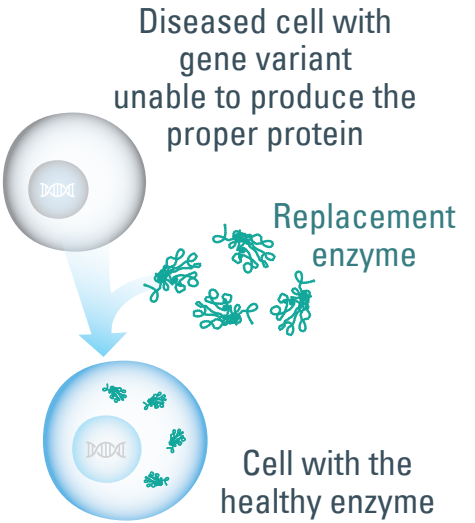
The next 2 therapies may help treat some genetic diseases like leukodystrophy - now or in the future. They are in their own category because they are not gene therapies (they do not target genes directly). Instead, they target RNA or enzymes (a type of protein).

Antisense oligonucleotide (ASO) therapy

Antisense oligonucleotide (ASO) therapy targets the messenger RNA of a gene variant. Depending on the ASO, it can help reduce the amount of a defective protein or correct certain types of problems with protein production.

Enzyme replacement therapy (ERT)

Enzyme replacement therapy targets genetic disorders caused by **enzymes** (a type of protein). In these cases, an enzyme does not work correctly due to a DNA variant. **ERT delivers a healthy replacement enzyme to the cells** that are affected. The new enzyme allows the cells to work correctly. Like ASO, ERT is not gene therapy. But it is another way to treat genetic diseases.



Summary – Leukodystrophy, genetics and gene therapy

- **Genes are like built-in instructions** for our bodies. **One set of genes make proteins**, and these proteins do different jobs.
- As part of pathways (a series of steps), **proteins can do complex things** like repair cells, make hormones, or transport nutrients.
- **Genetic diseases, like leukodystrophy**, happen because of variation(s) in a gene. These genes with variants affect essential cellular pathways.
    - **Because of the variant, the gene does not have the proper instructions to make a protein** (or proteins).
    - Without the proper instructions, cells cannot make the proteins correctly.
    - **When proteins are not made correctly**, they are either harmful or cannot do their job. If the job is an important one, **it can cause disease.**
- Gene therapy may be a way to treat your child's condition now, or in the future.
- **Gene therapy** treats genetic diseases by targeting variants in the genes. **Generally, it can either fix the gene, replace it, or add healthy copies of the gene.**

Other therapies treat the disease by targeting RNA or enzymes (a category of proteins). The next chapters will explain these different types of therapies and how they are delivered.

Gene therapy, ASOs and/or ERT may be available for some types of leukodystrophies, but not all. Speak with your doctor to find out what therapies are available or are in **clinical trials*** for your child's disease.

* Clinical trials are a way of testing new therapies. Trials may offer access to new or improved treatments. Before you or a loved one joins a trial, it is important that you speak with a doctor so that you understand the study.

2. Ex vivo Gene Therapy

Variations in DNA can cause genetic diseases. When a variant in the gene does not have the "instructions" to make the correct protein, the protein pathway is affected. When an important pathway is not working correctly, the cell cannot do its job.

What is the goal of gene therapy?

In Chapter 1, we learned that **gene therapy** is a way to treat genetic diseases. It stops or slows down the damage due to a variant in an important gene.

It does this by targeting the gene to correct its DNA sequence or by adding healthy copies of the gene (ones that have the correct instructions). The goal of gene therapy is to correct the dysfunction in cellular pathways caused by the variant.

How do we know what gene is affected? First, your doctor will sequence your child's DNA.

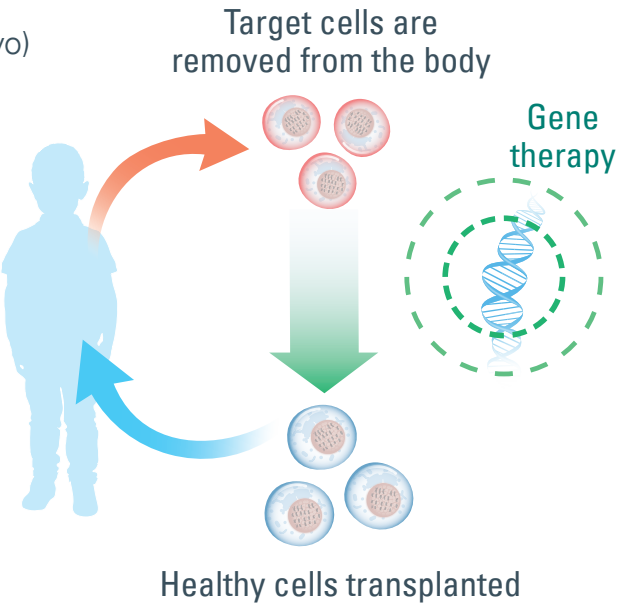
The next question is **how to deliver** the gene therapy.

Gene therapy delivery - in vivo and ex vivo

Gene therapy can correct genetic variants or add copies of the healthy gene in the target cells:

- In the body (in vivo)
  or
- Outside of the body (ex vivo)

This chapter is about ex vivo gene therapy. **In ex vivo gene therapy, target cells are** removed from the body, treated, and transplanted back.



Ex vivo gene therapy mechanisms - gene editing and gene addition

In ex vivo therapy, there are 2 ways to correct genetic information of the target cell:

- The **gene** that causes the disease is **fixed** (i.e., gene editing)
  or
- A properly working copy (or several copies) of the gene is (are) **added** (i.e., gene addition)

If the therapy is successful, **the cell gets the healthy gene**, the **protein works properly,** and the **affected cellular pathway(s) work properly.** Fixing the pathway means that it may be possible to change how the disease progresses. In other words, the damage that happened before the treatment may remain, but the hope is that there will be no more damage, or the damage will occur more slowly.

What type of cells are needed for ex vivo therapy?

Ex vivo gene therapy uses stem cells. These are often taken from the bone marrow. After the cells are treated, they are then transplanted back into the body.

What are stem cells?

Most of the cells in our body have a specific job. For example, nerve cells send and receive information in the brain, while red blood cells transport oxygen throughout the body.

Cells such as nerve cells are specialized or "differentiated." **Differentiated cells have a specific role in the body that does not change.**

Stem cells are unique. They are **undifferentiated** (not specialized) because **they can make more than one type of cell.** For instance, stem cells in the bone marrow can make many different types of blood cells. In addition, stem cells maintain our bodies by generating new cells to replace old ones.

Why are stem cells used in gene therapy?

Stem cells are useful for gene therapy because in theory, **they can become almost any kind of cell.**

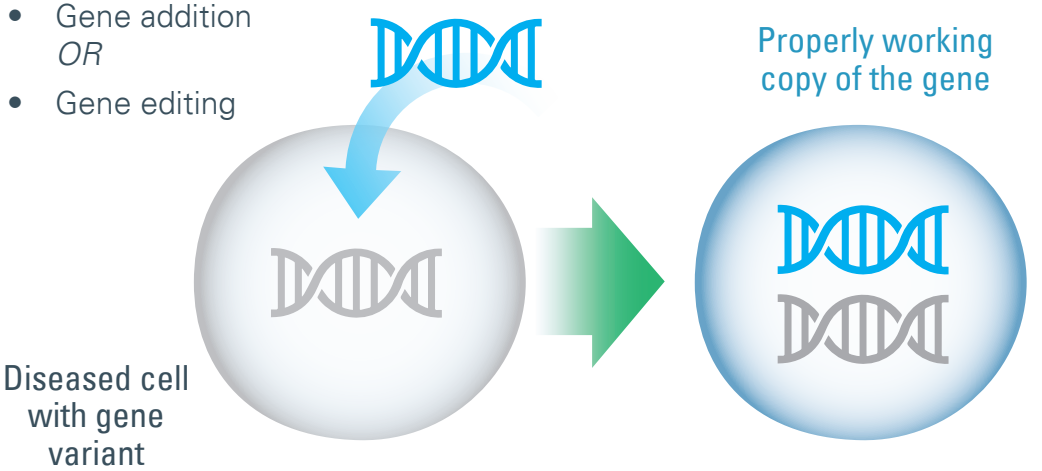
However, in practice, therapeutic stem cells can't replace all types of cells. They only replace the types of cells that occupy the part of the body that they come from. Typically, we are only able to collect stem cells from a few places - bone marrow is the most common source. So, ex vivo gene therapy is only helpful for some genetic diseases.

Using stem cells can help increase the number of treated cells in the body and maintain them over a long time. In ex vivo therapy, the healthy gene uses a viral vector to get inside the cell.

How gene addition and gene editing work

There are at least 2 different mechanisms for ex vivo gene therapy. Cells are usually genetically modified by:

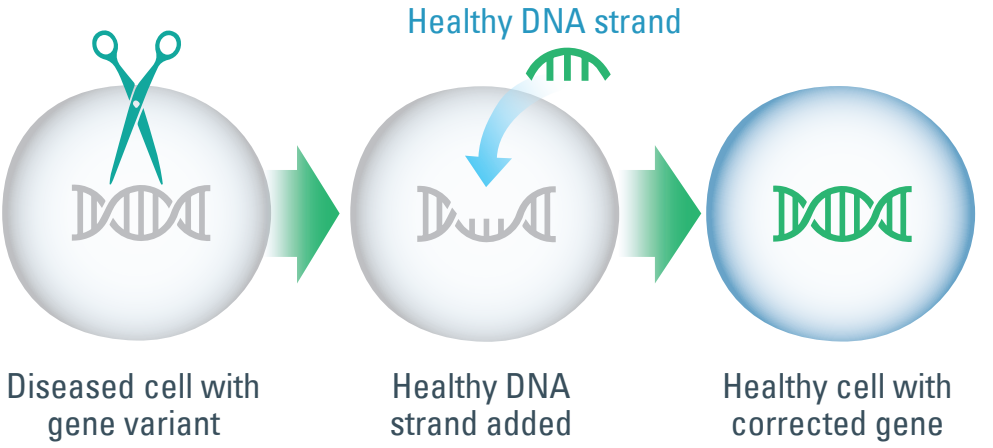
- Gene addition
  OR
- Gene editing



In gene addition, a properly working copy of the gene is inserted into the target cell (or several working copies are inserted).

In most cases, the gene is inserted in the non-coding areas of the cell genome (genetic material). Non-coding areas do not make instructions for proteins. So, inserting them here would mean less chance of disrupting cell functions. But it is still possible to have **"off-target effects."** For example, if the healthy gene is inserted in the wrong location, **this may disrupt cell function.**

In ex vivo gene addition, the goal is to not disrupt cell function.



In gene editing, the defective gene is corrected. There are several advantages to fixing the affected gene this way. For instance, it avoids introducing additional genetic material to the genome. However, it is still possible to have off-target effects. For example, if the editing happens in the wrong location, this may disrupt cell function.

To sum up, in ex vivo gene therapy, the goal is to correct gene function without disrupting other cell functions.

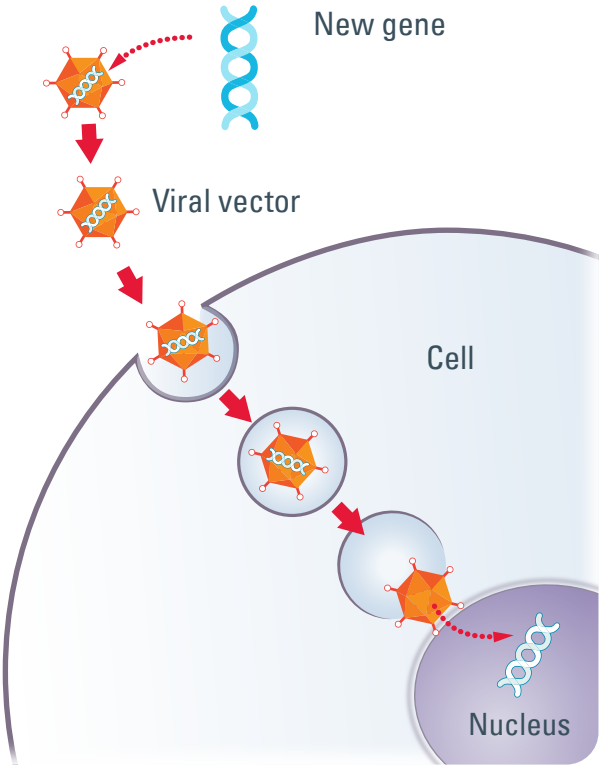
How are genes delivered inside cells?

Adding genes to cells is not as easy as it may sound. To better understand what is involved, let's take another look at the cell.

Cells have a membrane. **This membrane controls what goes in and out of the cell.** For ex vivo gene therapy to work, the modified gene needs to get across this membrane and inside the cells safely.

The question is how to get the gene inside the cell and inside the nucleus of the cell, where the chromosomes are.

Scientists solve this problem by using **viruses.**



Non-disease-causing viruses carry the healthy DNA replacement or modified genes across the membrane and into the cells.

These are called **viral vectors.** Viral vectors are like transport trucks: they **deliver corrected genes into cells.** The corrected genes can make the correct proteins, fix the pathway, and treat the disease.

Why are viruses used in gene addition?

Viruses may seem like an unusual way to cure genetic diseases. But it makes sense if you understand what they do.

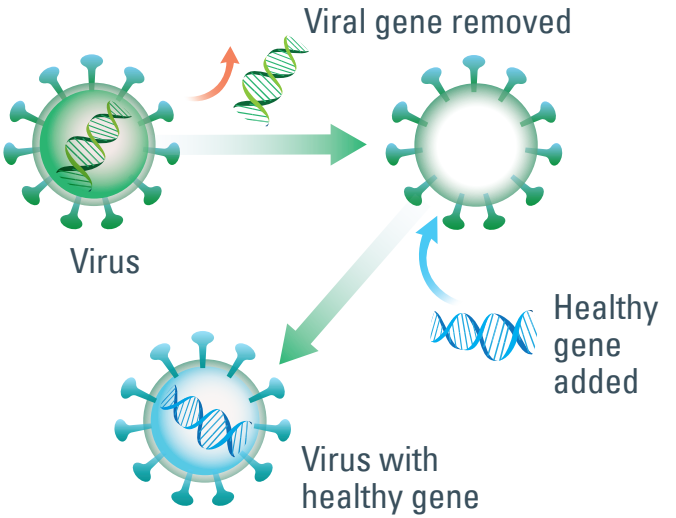
Viruses found in nature are non-living organisms. They are pieces of DNA or RNA. A virus can only make more viruses by infecting a host cell. So, **viruses have become very good at getting inside host cells.** This is why viruses, like colds, can quickly infect a person and then spread to other people.

Scientists take advantage of this natural ability of viruses to get inside cells. First, the virus is made safer by removing all the viral genes that are responsible for causing an illness. Then, they insert the healthy (therapeutic) gene inside the virus.

The modified virus is safe because there are no viral genes. Instead, they have only the healthy gene and can be used to treat cells.

To summarize, the viruses used for gene therapy:

- Do not have viral genes (they are removed)
- Insert a properly working gene inside the same type of cells it would usually infect
- Do not cause a viral infection.



Engineered **viral vectors** are the most common way to deliver the healthy (therapeutic) gene for **ex vivo** gene therapy.

What does ex vivo gene therapy mean for the patient?

Ex vivo gene therapy usually means **staying in the hospital for a few treatment steps.** The first is **to collect the cells** that need to be genetically modified. Depending on the type of cells, your child may need general anesthesia.

Next, your child needs to **get ready to receive the modified cells.** This preparation is a **"conditioning treatment."** It is similar to chemotherapy. If your child needs this, they will get intravenous doses of medication for several days. This step is important because it **lowers the body's immune response (immunosuppression)** for a short while. This weak or low immune response means a better chance the body will take in the corrected cells after transplantation. A strong immune response could reject the modified cells.

NOTE-The immune system

When cells are modified and then put in the body, your child's immune system might see them as "invaders." Our job is to trick the body into accepting the modified cells as part of their body. This is what the conditioning phase does.

The last step transplants the genetically modified cells back into your child. This process is relatively simple if cells are re-introduced into the blood **(intravenous)**. However, this step may be more difficult. It depends on where the target cells are and the type of disease.

We encourage you to speak with your doctor to learn more about the procedure and if it applies to your child.

What are the benefits of ex vivo gene therapy?

One advantage of gene therapies is that they **target the main problem, the affected (variant) gene.** So, if the gene is fixed or replaced, the protein pathways can work correctly and treat the disease. There is also another important advantage. **Patients use their own cells**, so they do not usually have to be immunosuppressed for long.

What are the limitations of ex vivo gene therapy?

Negative side effects can occur from the conditioning treatment. The transplantation of the treated cells can also cause side effects.

The procedure for removing the cells from the body **may involve surgery**.

Ex vivo therapy does not correct the dysfunctional gene in all cells of the body, just the treated cells.

It is most **effective when used early in disease** since we cannot always reverse the damage that the disease has already caused.

Summary of ex vivo gene therapy

- **Ex vivo gene therapy treats genetic disease by fixing gene variants (editing) or adding healthy genes to produce properly working proteins.**
- Properly working proteins can repair the cellular pathways that cause the disease.
- Ex vivo gene therapy has side effects and risks.
- Speak with your child's doctor to learn more about ex vivo gene therapy. Ask if this is an option for your child's illness. Ex vivo gene therapies may be available for some leukodystrophies, but not all.

3. In vivo Gene Therapy

Ex vivo and **in vivo** therapies are 2 ways to treat genetic diseases. They deliver functioning genes to target cells to repair protein pathways.

Ex vivo gene therapy **removes the cells**, treats them, and transplants them back into the body.

In vivo gene therapy **treats the genes while the cells are in the body**. It is used when the cells are difficult to remove, such as brain cells.

This chapter is about in vivo gene therapy:

- Gene editing
- Gene addition

We will focus on gene addition since it is the most common type of in vivo gene therapy.

How do we deliver DNA genes inside cells?

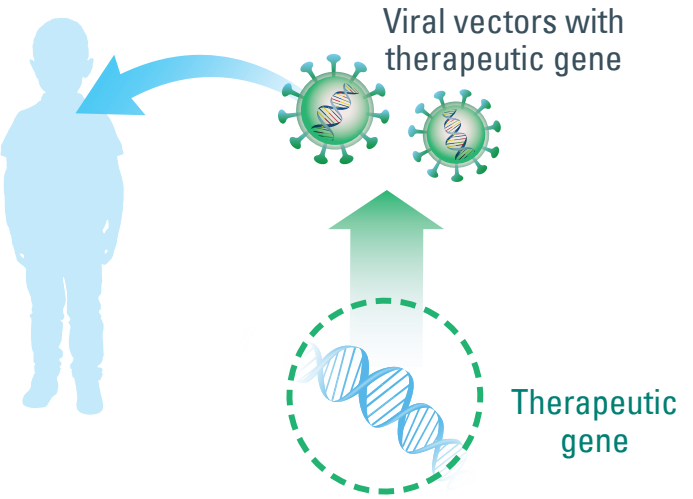
In vivo therapy usually uses viral vectors. A vector is an organism (in this case, a virus) **that can infect another organism**.

The viruses used for in vivo gene therapy must be safe and effective. **Adeno-Associated Virus (AAV)** is the most common one used for in vivo gene therapy. It is important to note that AAV is a small DNA virus that is not known to cause disease. **AAVs is modified** to have the **healthy gene without the viral genes.** So, when the AAVs get into cells, they will not cause infection because they do not have any harmful viral DNA.

How are gene therapies administered?

For in vivo gene therapy to work, **the therapeutic gene has to be delivered to the affected cells.** They must target them as precisely as possible. The best injection site and route will depend on what type of cells need to be treated.

The **main routes** are intravenous, intraventricular, and intrathecal.

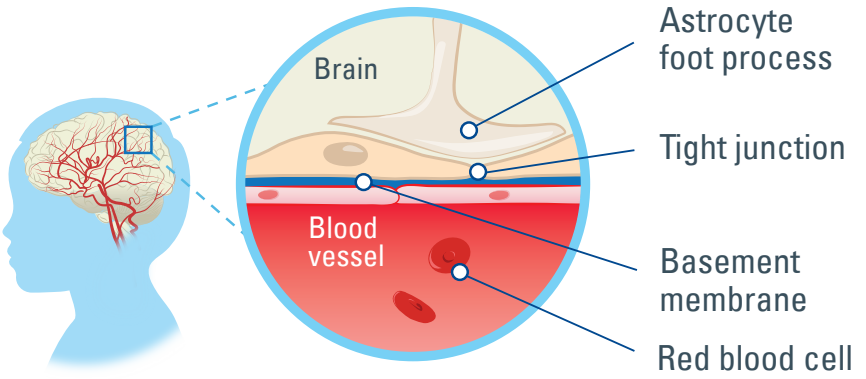


Intravenous injection

Intravenous means delivering fluids through the veins. Because the therapy goes through the blood, it is considered **less invasive** than other methods (for example, through the brain). The downside is that intravenous injection may not reach parts of the body, including the brain, because of the **blood-brain barrier (BBB).**

The blood-brain barrier

The blood-brain barrier protects the brain. It is a thin layer that controls what gets in, so it helps to keep out harmful substances, including most viruses. Unfortunately, the BBB also keeps out some useful medications and therapies.



Intraventricular administration

The cerebrospinal fluid (CSF) is a liquid that surrounds the brain and spinal cord and fills the spaces inside the brain (ventricles). The CSF is always moving, so it can be a useful way to deliver medication that needs to reach the brain.

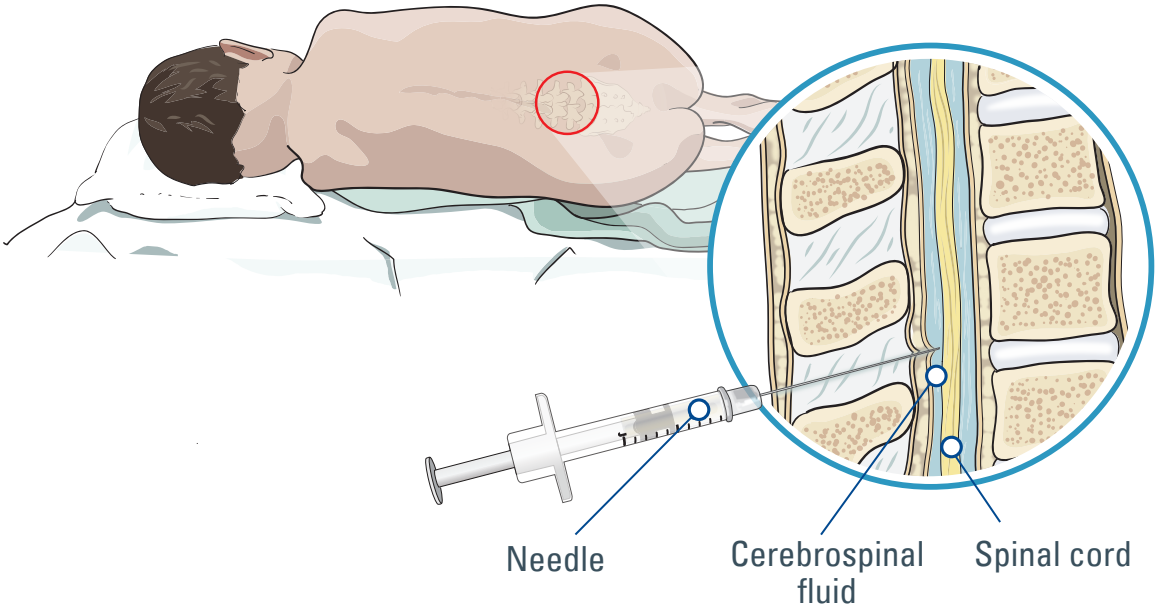
These are the steps for intraventricular injection:

- The medication goes into a syringe.
- Doctors choose the target location and make a small hole in the skull.
- They insert the needle through the brain to reach the CSF in the ventricles.

The treatment reaches the brain and spinal cord because the cerebrospinal fluid moves around them. This is an **invasive** route of administration because it involves opening the skull and injecting inside the brain.

Intrathecal injection

Intrathecal injections deliver treatment to the cerebrospinal fluid (CSF). This method allows treatment to bypass the blood brain barrier, **so the treatment reaches the brain.** The injections are similar to an epidural done for pregnant women during delivery. To perform the injection, a doctor inserts a needle in the lower back, between the vertebrae of the spine.



What are the benefits of in vivo gene therapy?

In vivo gene therapy **targets cells that can't be removed from the body** or are difficult to reach (e.g., brain). It can also **treat many cell types** at one time.

What are the drawbacks of in vivo gene therapy?

In vivo gene therapy has to deliver the corrected gene to as many of the target cells as possible without affecting non-target cells. Sometimes, this means using methods that may have complications (e.g., administration into the brain).

In vivo gene therapy is most effective when used as early in the disease course as possible. Early treatment can slow or stop the harmful effects of the gene variant. But it can't always reverse the earlier effects.

One form of in vivo therapy uses **viral vectors**. This therapy uses a modified virus to deliver healthy genes to cells. However, **sometimes there are side effects, for example, immune response problems.** The risk of off-target effects with AAV vectors is low, but not zero. Because of this, your child will be closely monitored during and after treatment. Children who experience complications may need treatment. In addition, their therapy may not work as well.

Summary of in vivo gene therapy

- **In vivo gene therapy is a way to treat genetic disorders, including disorders of the brain.**
- By targeting the diseases at their source, this treatment can slow or even stop the disease.
- There are side effects and risks linked to **in vivo** gene therapy.

Speak with your doctor to learn more about in vivo gene therapy. Ask if this is an option for your child's illness. In vivo gene therapies may be available for some leukodystrophies, but not all.

4. Antisense Oligonucleotide (ASO) Therapy

Remember that **genetic diseases are caused by variations in DNA that encode essential proteins.** Since RNA is a copy of the original DNA, it is the RNA copy that creates the protein. Variations in DNA can lead to defective proteins:

- DNA variant is copied into RNA
- RNA is copied into protein
- Protein is defective
- Defective proteins disrupt cellular pathways
- Faulty pathways cause disease

The gene therapies we described in chapters 2 and 3 target DNA to correct a variant in the gene.

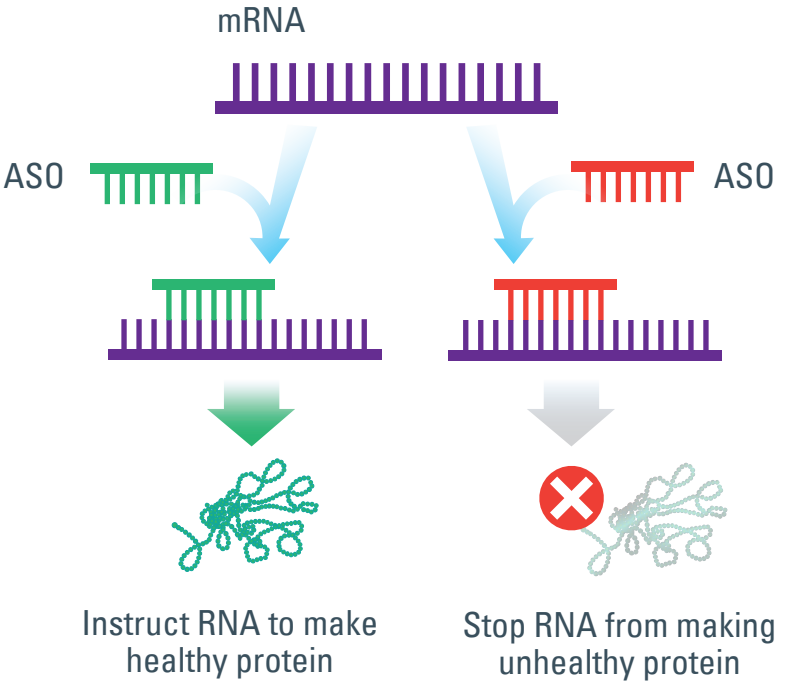
ASO therapy is different. **ASO therapy targets the RNA copy instead of the DNA.**

First let's describe what ASOs are.

What are ASOs?

ASOs are short sequences of nucleotides. These bases are similar but not exactly the same as in DNA. They are adenine (A), cytosine (C), guanine (G) and uracil (U). They attach to RNA in pairs, in a specific way, in a similar fashion to DNA.

ASO therapy makes changes to RNA before it produces a protein. Scientists can design ASO molecules that attach to RNA. In some cases, this attachment limits translation, **reducing the amount of unhealthy protein.** In other cases, the therapy changes how the protein is made.



How do we administer ASO therapy?

The most common method for ASO therapies is **in vivo.** ASOs get inside target cells through the circulatory system or the central nervous system.

Administration to the circulatory system:

- Intravenous injection

Administration to the central nervous system:

- Intraventricular administration
- Intrathecal administration

What are the benefits of ASO therapy?

ASOs do not change the genetic material. It also does not need a viral vector. This means **less chance of immune reactions or off-target effects** that sometimes happen when targeting DNA directly. So, ASOs are considered a safer treatment compared to gene addition or gene editing. ASO therapies often last longer than most drugs, so there are fewer trips to the hospital.

What are the drawbacks of ASO therapy?

Because **ASO therapies last temporarily**, they must be repeated to continue to affect the disease. ASO therapy may be needed long term, possibly for life. They may also use **invasive routes** of administration when targeting the spinal cord or the brain. There may **also be side effects or complications** related to the route of administration.

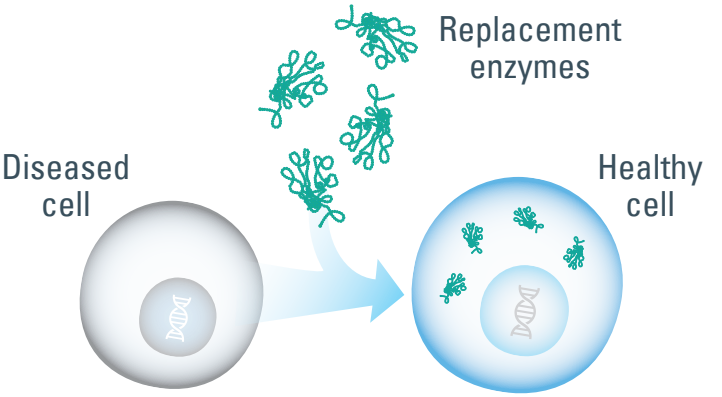
Summary of ASO therapy

- **ASO therapy is a way to treat genetic disorders by targeting RNA instead of DNA.**
- ASO therapies are typically done in vivo.
- They may use invasive routes to administer the treatments (e.g., through the central nervous system).
- ASO therapies may last longer than traditional drug treatments, but they are still temporary. They must be repeated to work. They are needed long term.
- There are side effects and risks associated with ASO therapies.

Speak with your doctor to learn more about ASO therapy. Ask if this is an option for your child's illness. ASO therapies may be available for some leukodystrophies, but not all.

5. Enzyme Replacement Therapy

Enzyme replacement therapy (ERT) is the last therapy we will review in this booklet. As we learned earlier, the goal of gene therapy is to fix defective essential proteins. First, let's take a closer look at an enzyme.



What is an enzyme?

Enzymes are a type of protein that our cells make. Like all proteins, they are encoded (programmed) by our DNA.

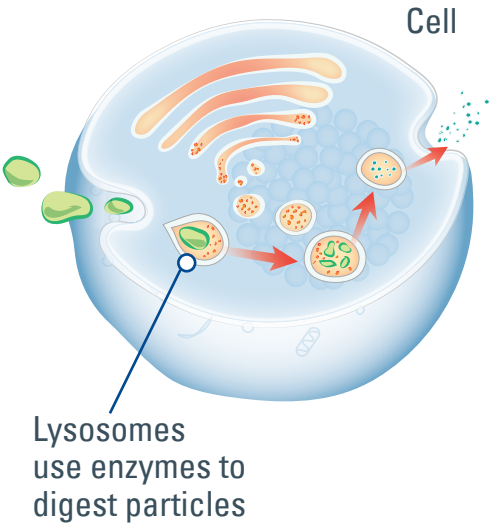
DNA (gene) ⇨ mRNA ⇨ Enzyme (protein)

Enzymes speed up the chemical reactions in our cells - sometimes as much as millions of times faster! There are many different types of enzymes. Some digest food while others help send nerve signals or transcribe DNA. Without enzymes, our cells would not work correctly – they might not even survive!

Enzymes are everywhere in cells, including in the **lysosomes.**

What are lysosomes?

Lysosomes are tiny structures inside a cell that do only one thing - they digest. They use the enzymes stored inside them to break down all sorts of compounds, including sugars, lipids, proteins, DNA, and RNA. These compounds can come from inside the cell (for example, old proteins and waste) or outside the cell (like nutrients from the food we eat).



Did you know?

There are at least 50 different kinds of enzymes in each lysosome!

What happens when a lysosomal enzyme is defective?

Let's review how an enzyme can become defective in the first place. Remember that DNA variants can disrupt the function of proteins (including enzymes). **When lysosomal enzymes do not work correctly, they cannot digest certain compounds.**

When this happens, **waste gets stored** in our cells. Too much **stored waste can harm our cells and organs.** This is what happens in lysosomal storage diseases, or LSDs.

What is enzyme replacement therapy?

Enzyme replacement therapy **(ERT) replaces a defective enzyme with a properly working enzyme**. This way, the enzyme can do its work.

The idea of enzyme replacement therapy is simple

- Deliver enzyme to the body
- Cells take up the enzyme
- Enzymes get where they need to be (e.g., in the lysosomes)

Enzyme replacement therapy

- Helps **reduce the waste** that builds up
- Allows the healthy enzyme to do more than what the non-healthy enzyme can do
- **Lessens some of the symptoms** of the disease

Enzyme replacement therapy has been available for over 15 years. It is now well established for the treatment of some LSDs.

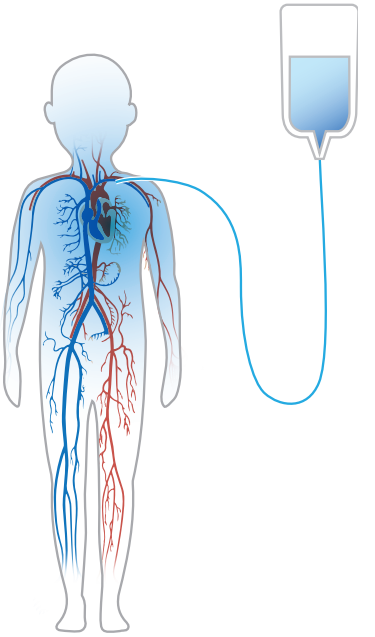
There are 2 main ways to do enzyme replacement therapy treatment (ERT). ERT can be **intravenous (in veins) or intrathecal (in the cerebrospinal fluid).**

Enzymes **only work for a short time** in the body, so they must be given to patients regularly (e.g., once a week or 2 times a month). Treatments are usually in the hospital, but they can also be at home. Your doctor will review each approach to find out what is best for your child.

How is it administered?

Intravenous administration

With the **intravenous** method, the treatment is **injected into the blood system**. Blood circulates to every organ of the body, so this type of injection distributes the treatment widely. It is also considered **less invasive.** Each therapy session lasts between 3 to 4 hours.



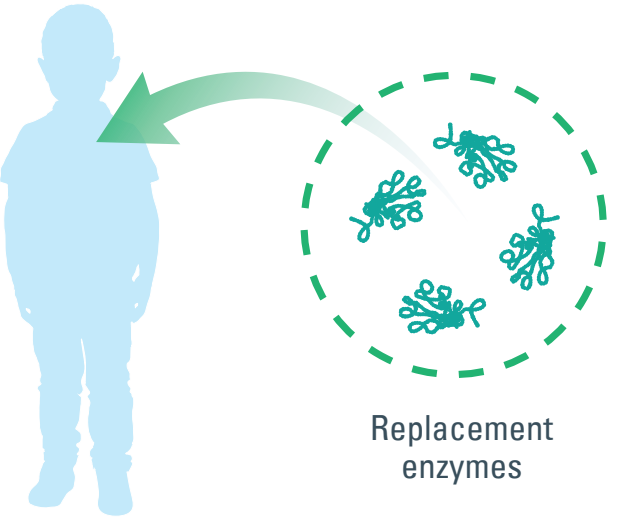
The downside is that **this method does not always work well on the brain. This is because of the blood-brain barrier (BBB).** Remember that the blood-brain barrier protects the brain. Unfortunately, the BBB can keep out medications and therapies. So, intravenous injections can be less effective than therapies that directly target the brain.

Intrathecal administration

Intrathecal injections deliver treatment to the cerebrospinal fluid (CSF) via the spinal canal. This method allows the treatment to bypass the BBB, **so the treatment reaches the brain**. The injections are similar to an epidural done for pregnant women during delivery. To perform this injection, a doctor inserts a needle in the lower back, between the vertebrae of the spinal column. They may use a reservoir under the skin, so they do not have to repeat the injections (lumbar punctures). Because of how CSF circulates, this type of injection **does not always distribute therapies to deeper brain regions.** It depends on the therapy used.

What are the benefits of enzyme replacement therapy?

Enzyme replacement therapy (ERT) **targets the source of the problem: the enzyme.** Other treatments may focus only on symptoms. ERT may slow down or stop the disease from getting worse. Also, patients usually **take less medication** for their symptoms.



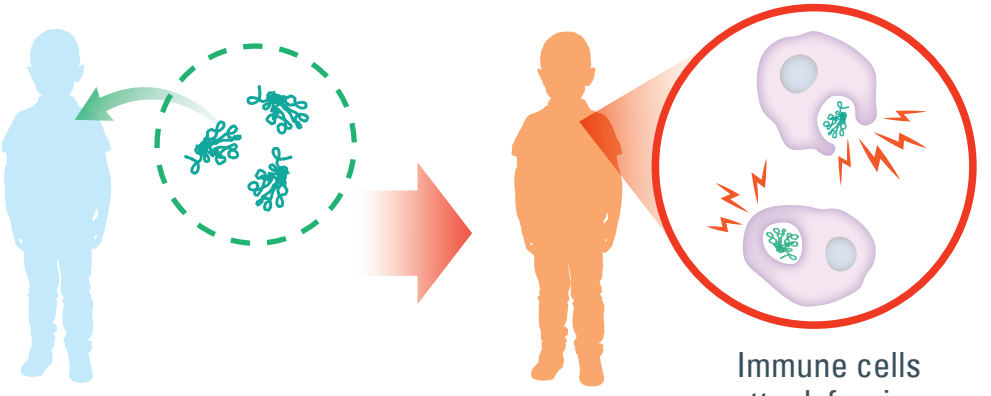
What are the downsides of enzyme replacement therapy?

The blood-brain barrier (BBB)

There are 2 ways to administer enzymes: through the blood or the **CNS** (the brain and spinal cord). Intravenous injections (through the blood) are not as effective on the brain. **Enzymes cannot easily get from the blood to the brain because of the BBB.** That is unfortunate for disorders involving the CNS such as some lysosomal storage disorders. So, doctors may administer the drug to the CSF using a non-intravenous route.

Immune reactions

Enzyme replacement therapy may also produce harmful immune reactions. These reactions are the body's way of defending itself against **foreign substances** (things that do not come from your child's own body or have been changed). **When the body sees something foreign** (like a new enzyme), **it triggers an immune response to get rid of it.** Immune responses can be mild and uncomfortable. But they can also be serious or even dangerous. Because of this possibility, your doctor must monitor treatments.



Immune cells attack foreign substances

Long term treatment

Genetic diseases can cause damage that builds up over time. So, treatments are more effective the earlier they start. Also, **enzymes must be replaced constantly,** so this treatment is ongoing. ERT may mean going to the hospital often if you cannot administer the treatment at home.

Finally, ERT is good at reducing the build-up of compounds, making more healthy enzyme products, and improving symptoms, but **ERT is not a cure.** It does not repair the affected enzymes, which means that **ERT is a life-long treatment.**

Summary of enzyme replacement therapy

ERT

- Is proven to treat specific lysosomal storage diseases.
- **Replaces the defective enzymes.**
- Injects new enzymes into circulatory system or cerebrospinal fluid.
- May reduce the symptoms related to the build-up of toxic compounds.

The drawbacks of enzyme replacement therapy

- Possibly needing to use invasive routes of administration because of the blood-brain barrier.
- Are life-long treatments.
- Can produce harmful immune responses to the therapeutic (properly working) enzyme.
- There are side effects and risks associated with ERT.

Speak with your doctor to learn more about ERT. Ask if this is an option for your child's illness. ERT may be available for some types of leukodystrophies, but not all.

Glossary

Therapies:

- **Antisense oligonucleotide (ASO)** therapy reduces the amount of the defective protein or corrects problems with protein production.
- **Enzyme replacement therapy (ERT)** targets genetic disorders that are caused by gene variants coding for enzymes. It delivers a healthy replacement enzyme to cells.
- **Ex vivo gene therapy** treats (gene editing) or replaces (gene addition) the genes outside of the body.
- **Gene therapy** corrects cellular dysfunction by targeting the gene variant. It can fix the gene or replace it.
- **In vivo gene therapy** treats the genes while the cells are in the body. It is used when the cells are difficult to remove, such as brain cells.

Key parts of the cell:

- **Amino acids:** the smallest part of proteins.
- **ASOs (antisense oligonucleotides):** short sequences of nucleotides (bases A, C, G, and U that attach to RNA in pairs, similar to DNA).
- **Chromosomes:** a string of DNA that stores genetic material that makes us unique.
- **DNA:** what genes are made of. DNA is made of 4 nucleotides (molecules) A, T, C, G.
- **Enzyme:** a type of protein that speeds up chemical reactions.
- **Exons:** coding area of genes (sections of DNA that code for proteins).
- **Genes:** like built-in instructions to do a job. Genes "tell" cells what to do.

- **Genome:** a collection of all your genetic material in the nucleus.
- **Introns:** non-coding area of genes.
- **LSD:** lysosomal storage disease.
- **Lysosomes:** tiny structures inside a cell that digest the waste that can build up in a cell.
- **Membrane:** a layer that controls what goes in and out of the cell.
- **Nucleus:** like a briefcase that holds the genetic material.
- **Proteins:** a combination of amino acids that have specific functions in repairing cells, making hormones, in immunological responses, transporting nutrients, etc.
- **Ribosomes:** the cell's protein-making factories.
- **RNA:** made from DNA via transcription, has different properties in the cell (e.g., it can leave the nucleus).

Making proteins (processes and parts involved)

- **Amino acids:** building blocks of proteins.
- **Cellular pathway:** a series of actions in a cell that lead to a result or event (when a cellular pathway is disrupted, the cell cannot do a task it is usually supposed to do).
- **Ribosomes:** the cell's protein-making factories.
- **Transcription:** copying of DNA to RNA.
- **Translation:** making proteins from the instructions in RNA.

Inheritance terms

- **De novo variations:** random variations (by chance).
- **Disease-causing variant or pathogenic variant:** a gene variant that is harmful (may be known as mutation).
- **Dominant inheritance:** usually only one parent has to "carry" the variant in the gene for the child to have the disease.
- **Gene expression:** in a cell or tissue, gene expression is the amount of RNA or protein the gene produces. When a gene is expressed, we can see features or effects related to the gene.
- **Gene variant:** a mistake in the gene's instructions.
- **Genetic disease:** illness caused by gene variant(s) in DNA.
- **Inheritance pattern:** a way that a trait is passed down through generations.
- **Inherited:** passed down through generations.
- **Mitochondrial inheritance:** diseases caused by variations in genes of the mitochondrial DNA.
- **Recessive inheritance:** usually both parents have to "carry" the variant in the gene for the child to have the disease.
- **Trait:** characteristic feature like eye colour, height, etc.
- **X-linked (XL) recessive inheritance:** a male child inherits the disease from his mother; the affected gene is on the X chromosome.

Delivery mechanisms and terms

- **Blood-brain barrier:** thin layer that controls what gets in the brain to protect it.
- **CNS (central nervous system):** made up of your brain and spinal cord.
- **CSF (cerebrospinal fluid):** a liquid that surrounds the brain and spinal cord.

- **Ex vivo (out of the body):** treats (gene editing) or replaces (gene addition) the genes outside of the body.
- **Gene addition:** working copy(ies) of a gene is (are) inserted into the target cells.
- **Gene editing:** the gene variant (mutated gene) is corrected by making edits.
- **In vivo (in the body):** used when the cells are difficult to remove, such as brain cells. Gene addition and ASO are most common, but gene editing is also possible.
- **Intrathecal:** treatment delivered to the cerebrospinal fluid (CSF) via a lumbar puncture.
- **Intravascular:** directly to the blood circulation.
- **Intraventricular:** directly into the CSF of the ventricles.
- **Off-target effects:** unexpected effects of a treatment that disrupts other cell functions.

Gene therapy terms

- **Adeno-Associated Virus (AAV):** small DNA virus, most common virus used for in vivo gene therapy.
- **Differentiated cells:** cells that have a specific role in the body that can not change.
- **Gene sequence:** order of bases that provide instructions (encode) for proteins.
- **Stem cells:** undifferentiated cells that can become almost any kind of cell.
- **Undifferentiated cells:** cells that are not specialized and can therefore make more than one type of cell (e.g., a progenitor is an undifferentiated cell).

- **Vector:** an organism that can infect another organism. Viruses can be vectors.
- **Viral capsids:** are used to introduce therapeutic genes directly to the body.
- **Viral vectors:** viruses that have had their disease-causing genes replaced by therapeutic genes. Viral vectors deliver corrected genes in both ex vivo and in vivo gene therapy.
- **Virus:** non-living organisms that infect (get inside) host cells and usually cause disease, like colds.

Gene therapy and the conditioning treatment

- **Chronic:** long-lasting.
- **Conditioning treatment:** intravenous medications that lower immune response in preparation to receive the modified cells, like chemotherapy.
- **Foreign substances:** things that do not come from your own body or have been changed before being put back into your body.
- **Immune reactions:** the body's way of defending itself.
- **Immunosuppression:** temporarily lowers the immune system response of the body.
- **Progressive (when speaking about disease):** worsens over time.
- **Therapeutic:** healing, improving a medical condition.

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Notes

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