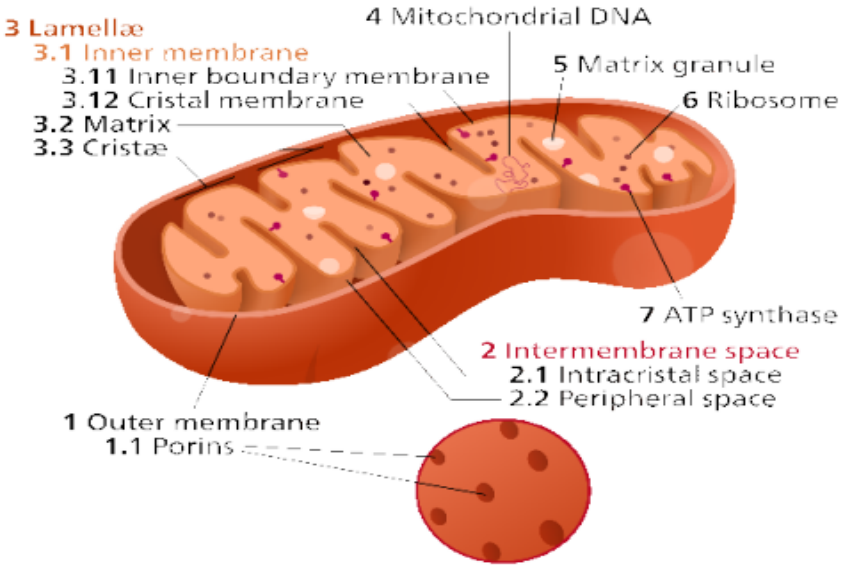
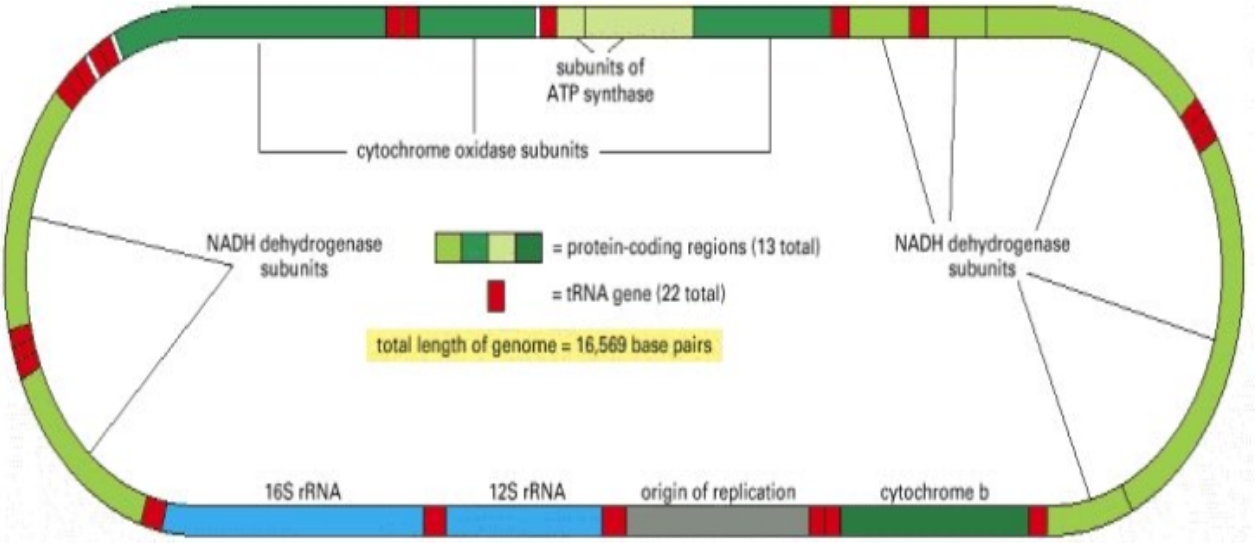
MITOCHONDRIAL INHERITANCE

- Our bodies are made up of millions of cells.
- Each cell contains a complete copy of a person's genetic book of life.
- Chromosomes can be thought of as being made up of strings of genes (DNA that codes for proteins) with non-coding DNA between them.
- The chromosomes, including the genes, are made up of a chemical substance called DNA (DeoxyriboNucleic Acid).
- Chromosomes are found in the nucleus of all body cells except for red blood cells which have no nucleus and therefore do not contain chromosomes.
- Another place in the cell where DNA is found is in very small compartments called mitochondria (the energy centres of the cell) that are found scattered outside the nucleus.
- The DNA in mitochondria is much smaller and has very little non-coding DNA.
- Mitochondria are found randomly scattered outside the nucleus but still within the cell.
- The DNA within the mitochondria is arranged as one long circle.
- The role of mitochondria in each of the cells of the body is mainly to manufacture energy for the cell and therefore the rest of the body.
- It is important to remember that **while each cell will always have only one nucleus, the number of mitochondria can vary from one cell to another**





The genome contains 2 rRNA genes, 22 tRNA genes, and 13 protein-coding sequences.

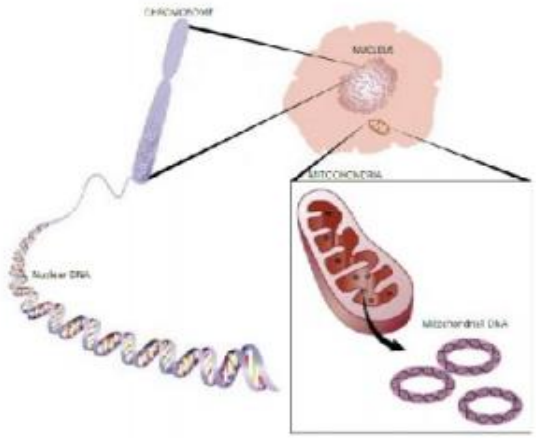
The DNAs of many other animal mitochondrial genomes have also been completely sequenced.

Most of these animal mitochondrial DNAs encode precisely the same genes as humans, with the gene order being identical for animals that range from mammals to fish.

MITOCHONDRIAL DNA

- The cells in the body, especially in **organs such as the brain, heart, muscle, kidneys and liver, cannot function normally unless they are receiving a constant supply of energy**. The cell's energy source is a **chemical called ATP** (adenosine triphosphate) that is used to drive the various reactions essential for the body to function, grow and develop.
- A number of **biochemical reactions that occur in an ordered sequence within the mitochondria are responsible for this process of ATP production**. These reactions are under the control of special proteins called enzymes. The genes found within the mitochondria contain the information that codes for the production of some of these important enzymes.
- The **biochemical processes which occur in the mitochondria and produce energy make up the mitochondrial respiratory chain**. This **'chain' is made up of five components called complexes 1, 2, 3, 4 and 5.**
- Each of these complexes is made up of a number of proteins. The instructions for these proteins to be produced by the cells are contained in a number of different genes.

- There are **many different genes needed to produce the components of the mitochondrial respiratory chain. Some of these genes are found in the mitochondria and others are in the nucleus. A variation in a gene (either in the nucleus or mitochondria) that creates a fault is called a pathogenic variant or mutation.**
- A mitochondrial DNA mutation can result in biochemical problems **due to absence of enzymes involved in the respiratory chain**, or **enzymes that are impaired and do not work properly**.
- This **leads to a reduction in the supply of ATP**, and may result in problems with the body's functions.



WHAT DOES IT MEAN IF YOU HAVE A MITOCHONDRIAL DNA GENE MUTATION?

- The number of mitochondria in every cell of a person's body varies from a few to hundreds.
- All of **these mitochondria, and therefore the DNA within the mitochondria, descend from the small number of mitochondria present in the original egg cell at the time of that person's conception.**
- **The sperm contributes very few mitochondria to the baby.**
- An individual's mitochondria are generally only inherited from his or her mother.
- **A variation (mutation) in one of the mitochondrial genes that makes it faulty, can therefore be passed by the mother to a child via her egg cells** .
- This pattern of inheritance is therefore often referred to as **maternal inheritance.**
- The **egg cell contains many mitochondria**.

- If a particular gene in every mitochondria in an egg cell has a mutation and is therefore sending the incorrect instructions, the disruption to energy production would be so severe that the early embryo would probably not survive.

- The fact that a **person survives to birth** and is affected with a mitochondrial condition means that they must **have inherited two types of mitochondria** from his or her mother **: some containing the working copy of the gene, and some containing the mutation.**

- The working **copy of the mitochondrial gene will still be able to send the right instructions**, but the **total amount of energy produced may be impacted and may result in a mitochondrial condition.**

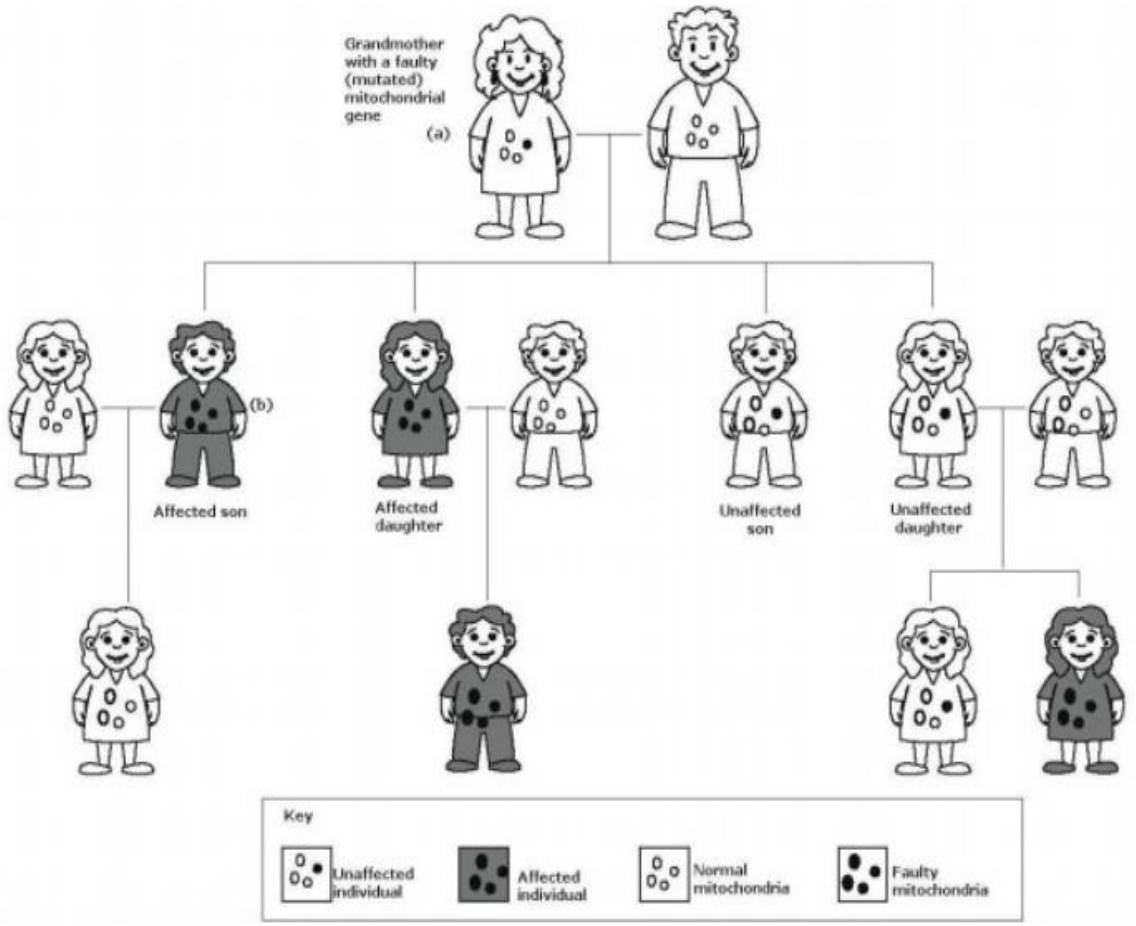
- In some cases, the variation in the mitochondrial gene occurs for the **first time in the egg** or at the time of **fertilisation of the egg.**

- This is a **new or spontaneous change** that has occurred to make the particular mitochondrial gene faulty.

- In this case the affected person is the first in the family to be affected by the condition and the condition is described as **sporadic.**

- If **the affected person is female**, she **may pass on the mitochondrial gene mutation to her children**.

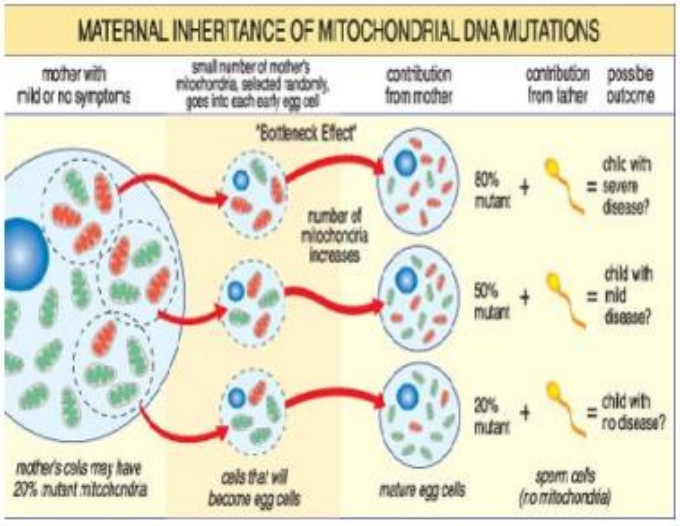
- Usually, however, the mitochondrial mutation is inherited from a mother whose own cells, including her egg cells, contain both working and faulty copies of this mitochondrial gene.

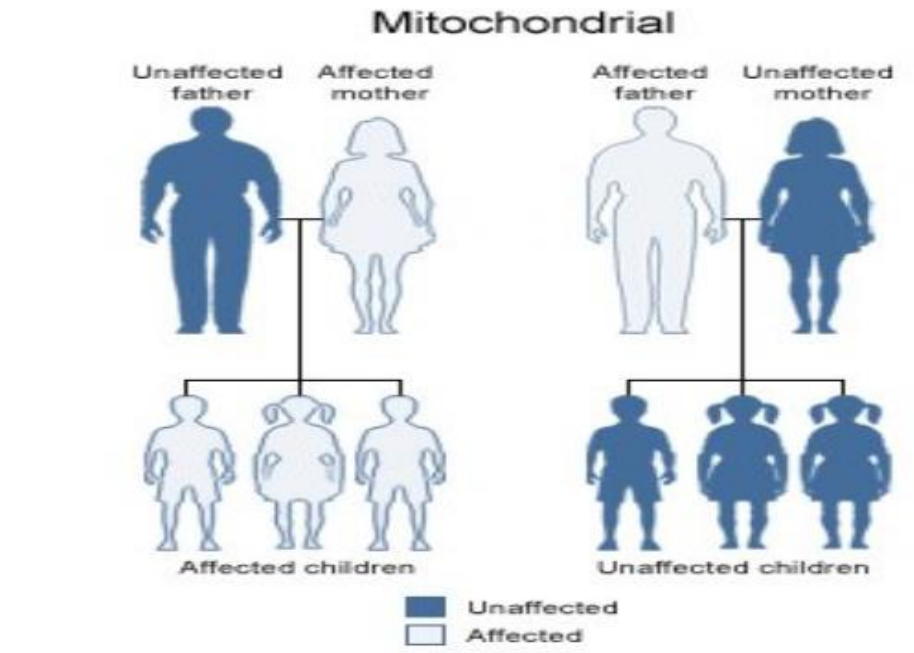


In Figure :

- **The grandmother has one or more faulty mitochondrial** genes but is **not affected because she has enough working copies** to enable **most of the mitochondria in her cells to work properly.**
- While **she has passed on these faulty mitochondrial genes to her children**, through **her egg, not all are affected by the condition.**
- One of the reasons for this is thought to be the **threshold effect of mitochondrial faulty genes.**
- Due to the way **that mitochondria are randomly distributed into the egg cells when the eggs are forming in the ovary, each individual egg cell's mitochondrial make-up may vary from mostly correct to mostly faulty**.
- Therefore, **all of the children of this grandmother, regardless of the sex of the child, would inherit some faulty mitochondria** .

- The **child would only develop symptoms, however, if the proportion of mitochondria with the faulty gene reached a critical level** in enough cells, which would interfere **with energy production in the body organ that is vulnerable to the condition .**
- It is **only when there are so many copies of the faulty mitochondrial genes present in the cells** that the **working copies are unable to provide enough working gene product**, that the person will have the condition
- The **number of mitochondria that are faulty may also vary from one cell to the next, and so symptoms of the condition will not occur unless there are enough cells, with enough faulty mitochondria** (i.e. exceed the critical level).
- So even though **two of the grandmother's unaffected children** in Figure 12.2 **have inherited the faulty mitochondrial genes, they have more working copies than faulty copies.**
- While **the father (b) in the family shown in Figure 12.2 is affected, his children are not at risk for inheriting the condition** as the **vast majority of the mitochondria are passed to children from their mother through the eggs.**
- **<u>The grandmother's daughters, however, are at risk of having a child affected with the mitochondrial genetic condition, regardless of whether they themselves are affected.</u>**





Mitochondrial Disorders

- ✓ Many of the disorders caused by **mutations in mtDNA affect tissues** that have a **high energy demand, such as the central nervous system, the heart, and muscle**.
- ✓ Therefore, mitochondrial disorders often involve the neuromuscular system and may include encephalopathy, myopathy, ataxia, retinal degeneration, and loss of function of the external ocular muscles.
- ✓ **Nuclear DNA codes for many of the mitochondrial proteins**.
- ✓ Therefore**, mutations in nuclear genes can also affect mitochondrial function** and cause disease, such as **Friedreich's ataxia-progressive damage of nervous system-autosomal recessive disorder-FXN gene** .

While these disorders are inherited in Mendelian patterns (not maternally, like mutations in mitochondrial DNA), they exhibit symptoms similar to the disorders caused by mtDNA mutations.

Some examples of conditions caused by mtDNA mutations include

1. LHON (**L**eber's **H**ereditary **O**ptic **N**europathy) and
2. MELAS (**M**itochondrial**E**ncephalomyopathy, **L**actic **A**cidosis, and **S**troke-like episodes).

LHON (Leber's Hereditary Optic Neuropathy)

- Individuals with LHON **experience fast, sudden, painless loss of vision in both eyes in their late teens or early 20s**.
- **Males are more commonly affected than females; however, women tend to develop the disorder later in life and be more severely affected.**
- Some individuals with LHON (**usually women) may also have a multiple sclerosis-like condition.**
- An estimated 1 in 10,000 individuals carry a LHON mutation. However, due to the reduced penetrance of the condition, 1 in 14,000 adult males have visual loss due to LHON.
- Approximately **95% of patients have one of three common mtDNA mutations**; testing is available for these **three mutations-MTND4*LHON11778G-A>R340H,. MTND1*LHON3460G-A>A52T, MTND6*LHON14484 G-A>R340H.**
- However, LHON mutations **exhibit reduced penetrance**: **males who carry a mutation have a 40% chance of developing symptoms in their lifetime and women a 10% chance.**

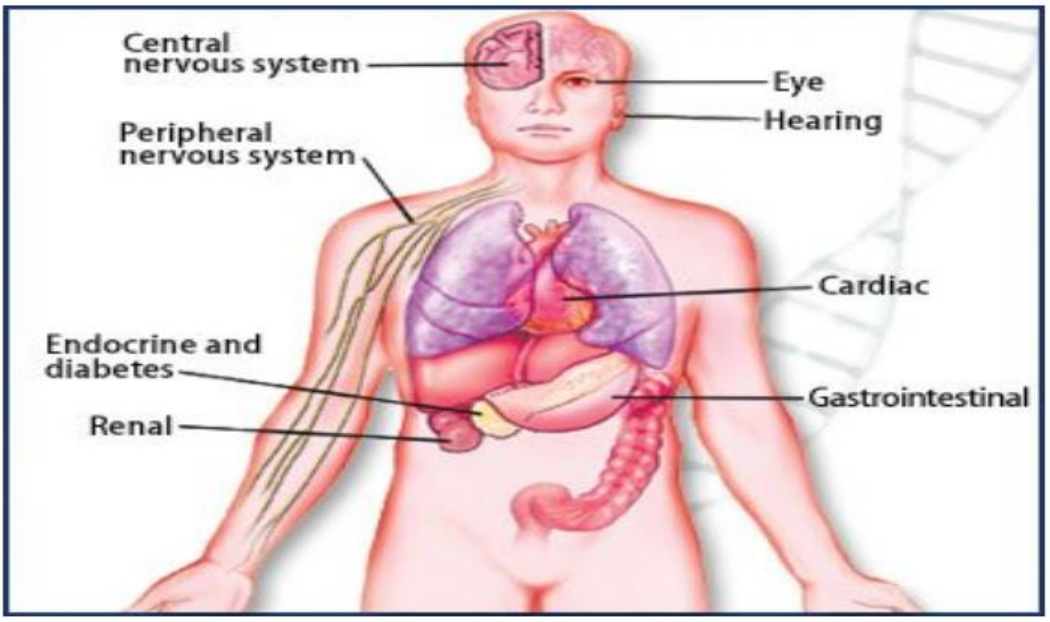
LHON is inherited in a **maternal pattern due to the mutations in mtDNA**; an individual must be **homoplasmic for the LHON mutation**, or have a very high percentage of mutated mtDNA, in order to be at risk for developing the condition.

MELAS (Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes)

- MELAS affects many systems of the body, with typical onset in the first decade of life. The major clinical features, like the name suggests, include:
  - Encephalomyopathy with **seizures and/or dementia**
  - Mitochondrial myopathy, as seen by lactic acidosis and/or ragged red fibers (RRF) on muscle biopsy
  - Stroke-like episodes, usually **before the age of 40**
- However, there are a wide range of clinical symptoms in addition to these major three, **including diabetes mellitus, cardiomyopathy, migraines, and deafness.**
- Approximately 1.6 per 10,000 individuals are affected with MELAS.

- The most common mutation, **present in 80% of MELAS patients**, is in the **mtDNA gene MTTL1-Mt encoded tRNA leucine.** Clinical testing is available for this gene.

Different systems that may be affected in a mitochondrial disorder



Symptoms Associated with Systems Affected by Mitochondrial Disorders

- **Central nervous system**
  - Encephalopathy
  - Stroke-like episodes
  - Seizures and dementia
  - Psychosis and depression
  - Ataxia
  - Migraine headaches
- **Eye**
  - External ophthalmoplegia
  - Ptosis
  - Cataract
  - Pigmentary retinopathy
  - Optic atrophy
- **Hearing**
  - Bilateral sensorineural deafness
- **Heart**
  - Cardiomyopathy
  - Heart block
  - Pre-excitation syndrome
- **Renal**
  - Renal tubular defects
- **Endocrine**
  - Hypoparathyroidism
  - Hypothyroidism
  - Gonadal failure
- **Intestinal**
  - Dysphagia
  - Constipation
  - Hepatic failure
- **Peripheral nervous system**
  - Myopathy
  - Axonal sensorimotor neuropathy

MUTATIONS AND ITS TYPES

What is a gene mutation and how do mutations occur?

- A **gene mutation** is a permanent alteration in the DNA sequence that makes up a gene, such that the sequence differs from what is found in most people.
- Mutations range in **size**; they can affect anywhere from a single DNA building block (base pair) to a large segment of a chromosome that includes multiple genes.

Gene mutations can be classified in two major ways:

- **Hereditary mutations** are inherited from a parent and are present throughout a person's life in virtually every cell in the body. These mutations are also called **germline mutations** because they are present in the **parent's egg or sperm cells**, which are also called **germ cells**. When an egg and a sperm cell unite, the resulting fertilized egg cell receives DNA from both parents. If this DNA has a mutation, the child that grows from the fertilized egg will have the mutation in each of his or her cells.
- **Acquired (or somatic) mutations** occur at **some time during a person's life and are present only in certain cells**, not in every cell in the body. These **changes can be caused by environmental factors such as ultraviolet radiation from the sun, or can occur if a mistake is made as DNA copies itself during cell division**. Acquired mutations **in somatic cells (cells other than sperm and egg cells) cannot be passed on to the next generation**.

de novo (new) mutations

- Genetic changes that are described as de novo (new) mutations can be either hereditary or somatic.
- In some cases, the mutation occurs in a person's egg or sperm cell but is not present in any of the person's other cells. In other cases, the mutation occurs in the fertilized egg shortly after the egg and sperm cells unite. (It is often impossible to tell exactly when a de novo mutation happened.)
- As the fertilized egg divides, each resulting cell in the growing embryo will have the mutation.
- De novo mutations may explain genetic disorders in which an affected child has a mutation in every cell in the body but the parents do not, and there is no family history of the disorder.

Mosaicism

- Somatic **mutations that happen in a single cell early in embryonic development** can lead to a situation called mosaicism.
- These genetic changes are not present in a parent's egg or sperm cells, or in the fertilized egg, but happen a bit later when the embryo includes several cells.
- As all the **cells divide during growth and development**, cells that arise from the cell with the altered gene will have the mutation, while other cells will not.
- Depending on the mutation and how many cells are affected, mosaicism may or may not cause health problems.

- **POLYMORPHISMS-Genetic Variation**
- Most disease-causing gene mutations are uncommon in the general population. However, other genetic changes occur more frequently.

- **Genetic alterations that occur in more than 1 percent of the population are called polymorphisms**. They are common enough to be considered a normal variation in the DNA.
- Polymorphisms are responsible for many of the normal differences between people such as eye color, hair color, and blood type.
- Although many polymorphisms have no negative effects on a person's health, some of these variations may influence the risk of developing certain disorder

- Do all gene mutations affect health and development?
- No; only a small percentage of mutations cause genetic disorders—most have no impact on health or development. For example, some mutations alter a gene's DNA sequence but do not change the function of the protein made by the gene(synonyms mutations).
- Often, gene mutations that could cause a genetic disorder are repaired by certain enzymes before the gene is expressed and an altered protein is produced.
- Each **cell has a number of pathways through which enzymes recognize and repair mistakes in DNA.**

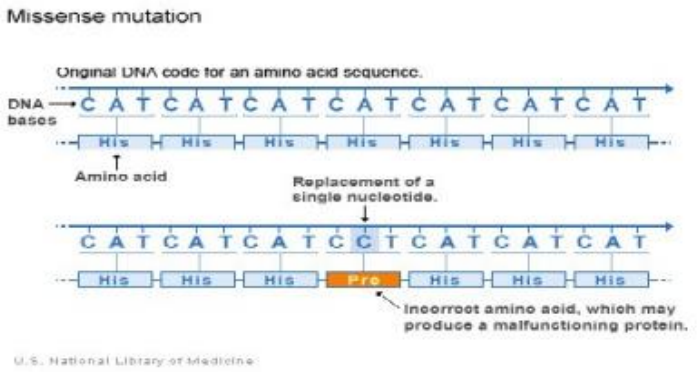
- Because **DNA can be damaged or mutated** in many ways, DNA repair is an important process by which the body protects itself from disease.
- A **very small percentage of all mutations actually have a positive effect**. These mutations lead to **new versions of proteins that help an individual better adapt to changes in his or her environment**.
- For example, a **beneficial mutation** could result in a protein that protects an individual and **future generations from a new strain of bacteria.**
- Because a **person's genetic code can have a large number of mutations with no effect on health, diagnosing genetic conditions can be difficult**.
- Sometimes, **genes thought to be related to a particular genetic condition have mutations, but whether these changes are involved in development of the condition has not been determined**; these genetic changes **are known as variants of unknown significance (VOUS).**
- Sometimes, **no mutations are found in suspected disease-related genes, but mutations are found in other genes whose relationship to a particular genetic condition** is unknown. It is difficult to know whether these variants are involved in the disease.

- **What kinds of gene mutations are possible?**

- The **DNA sequence of a gene can be altered in a number of ways. Gene mutations** have varying effects on health, depending on where they occur and whether they alter the function of essential proteins. The types of mutations include:

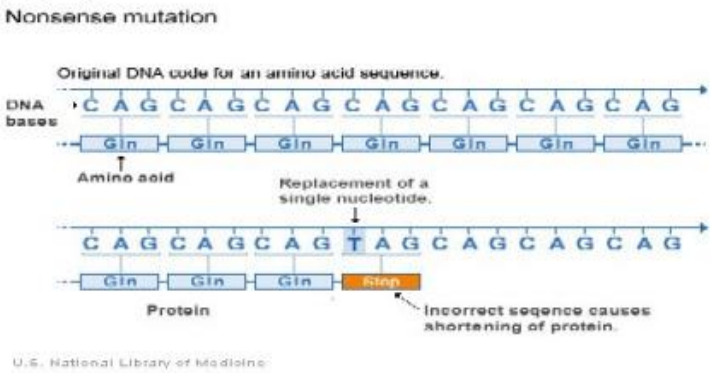
- **Missense mutation**:

- This type of **mutation is a change in one DNA base pair** that results in the substitution of one amino acid for another in the protein made by a gene.



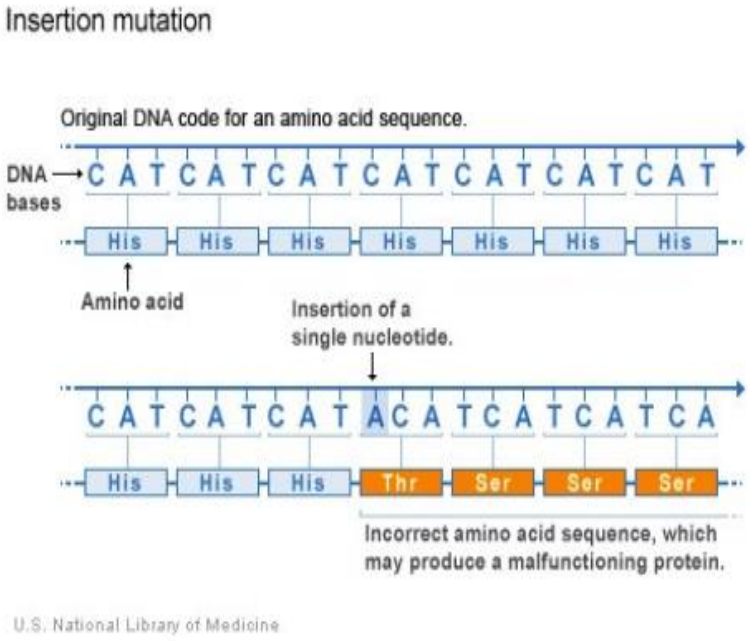
Nonsense mutation

- A nonsense mutation is also a change in one DNA base pair.
- Instead of substituting one amino acid for another, however, the altered
- DNA sequence prematurely signals the cell to stop building a protein.
- This type of mutation results in a shortened protein thatmay function improperly or not at all.



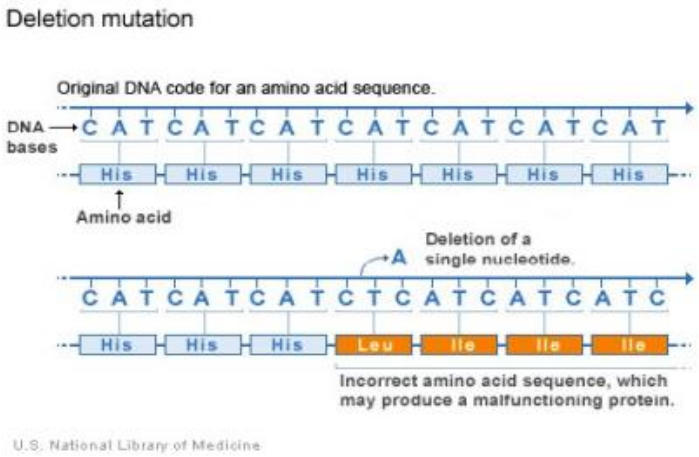
Insertion

An insertion changes the number of DNA bases in a gene by adding a piece of DNA. As a result, the protein made by the gene may not function properly



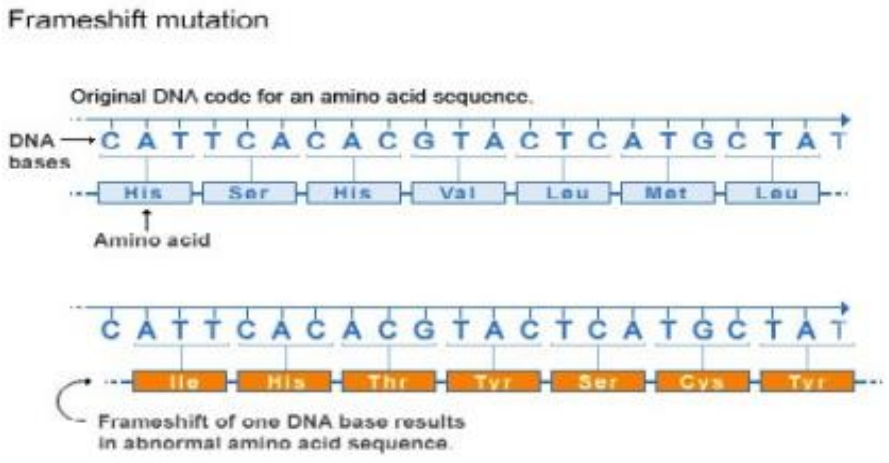
Deletion

A deletion changes the number of DNA bases by removing a piece of DNA. Small deletions may remove one or a few base pairs within a gene, while larger deletions can remove an entire gene or several neighboring genes. The deleted DNA may alter the function of the resulting protein(s)



Frameshift mutation

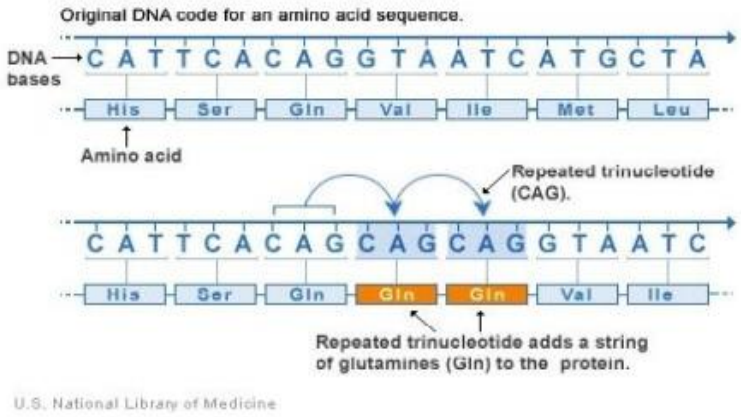
This type of mutation occurs when the addition or loss of DNA bases changes a gene's reading frame. A reading frame consists of groups of 3 bases that each code for one amino acid. A frameshift mutation shifts the grouping of these bases and changes the code for amino acids. The resulting protein is usually nonfunctional. Insertions, deletions, and duplications can all be frameshift mutations.



Repeat expansion

Nucleotide repeats are short DNA sequences that are repeated a number of times in a row. For example, a trinucleotide repeat is made up of 3-base-pair sequences, and a tetranucleotide repeat is made up of 4-base-pair sequences. A repeat expansion is a mutation that increases the number of times that the short DNA sequence is repeated. This type of mutation can cause the resulting protein to function improperly.

Repeat expansion mutation



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Can a change in the number of genes affect health and development

- People have two copies of most genes, one copy inherited from each parent. In some cases, however, the number of copies **varies—meaning that a person can be born with one, three, or more copies of particular genes**. Less commonly, one or more genes may be entirely missing. This type of genetic difference **is known as copy number variation (CNV).**
- Copy number variation results from **insertions, deletions, and duplications of large segments of DNA**. These segments are big enough to include whole genes.
- **Variation in gene copy number can influence the activity of genes and ultimately affect many body functions.**

- Researchers were surprised to **learn that copy number variation accounts for a significant amount of genetic difference between people**.
- More than **10 percent of human DNA appears to contain these differences in gene copy number.**
- While much **of this variation does not affect health or development,** some differences likely influence a **person's risk of disease and response to certain drugs.**
- Future research will focus on the consequences of copy number variation in different parts of the genome and study the contribution of these variations to many types of disease

Types of DNA Mutations and Their Impact

Class of Mutation	Type of Mutation	Description	Human Disease(s) Linked to This Mutation
Point mutation	Substitution	One base is incorrectly added during replication and replaces the pair in the corresponding position on the complementary strand	Sickle-cell anemia
	Insertion	One or more extra nucleotides are inserted into replicating DNA, often resulting in a frameshift	One form of beta-thalassemia
	Deletion	One or more nucleotides is "skipped" during replication or otherwise excised, often resulting in a frameshift	Cystic fibrosis
Chromosomal mutation	Inversion	One region of a chromosome is flipped and reinserted	Opitz-Kaveggia syndrome
	Deletion	A region of a chromosome is lost, resulting in the absence of all the genes in that area	Cri du chat syndrome
	Duplication	A region of a chromosome is repeated, resulting in an increase in dosage from the genes in that region	Some cancers
	Translocation	A region from one chromosome is aberrantly attached to another chromosome	One form of leukemia
Copy number variation	Gene amplification	The number of tandem copies of a locus is increased	Some breast cancers
	Expanding trinucleotide repeat	The normal number of repeated trinucleotide sequences is expanded	Fragile X syndrome, Huntington's disease

How Mutations Occur

Mutations and the Environment

- **DNA interacts with the environment**, and sometimes that interaction can be detrimental to genetic information.
- In fact, every time you go outside, you put your DNA in danger, because **ultraviolet (UV) light from the Sun** can induce mutations in your skin cells.
- One type of **UV-generated mutation** involves the **hydrolysis of a cytosine base to a hydrate form**, causing the **base to mispair with adenine during the next round of replication** and ultimately be replaced by **thymine.**
- Indeed, researchers have found an extremely high rate of occurrence of this **UV-induced C-to-T fingerprint-type mutation** in genes associated **with basal cell carcinoma**, a form of skin cancer .
- UV light can also cause covalent bonds to form between adjacent pyrimidine bases on a DNA strand, which results in **the formation of pyrimidine dimers**.
- **Repair machinery exists to cope with these mutations**, but it is somewhat prone to error, which means that **some dimers** go unrepaired.
- Furthermore, some people have an inherited genetic disorder called **xeroderma pigmentosum (XP)**, which **involves mutations in the genes that code for the proteins** involved in repairing **UV-light damage.**
- In **people with XP, exposure to UV light triggers a high frequency** of mutations in skin cells, which in turn results in a **high occurrence of skin cancer**. As a result, such individuals are unable to go outdoors during daylight hours.
- In addition to **ultraviolet light, organisms are exposed to more energetic ionizing radiation in the form of cosmic rays, gamma rays, and X-rays.**
- **Ionizing radiation** induces **double-stranded breaks in DNA**, and the resulting repair can likewise introduce mutations if carried out imperfectly.
- Unlike **UV light, however, these forms of radiation penetrate tissue well,** so they can cause **mutations anywhere in the body**.

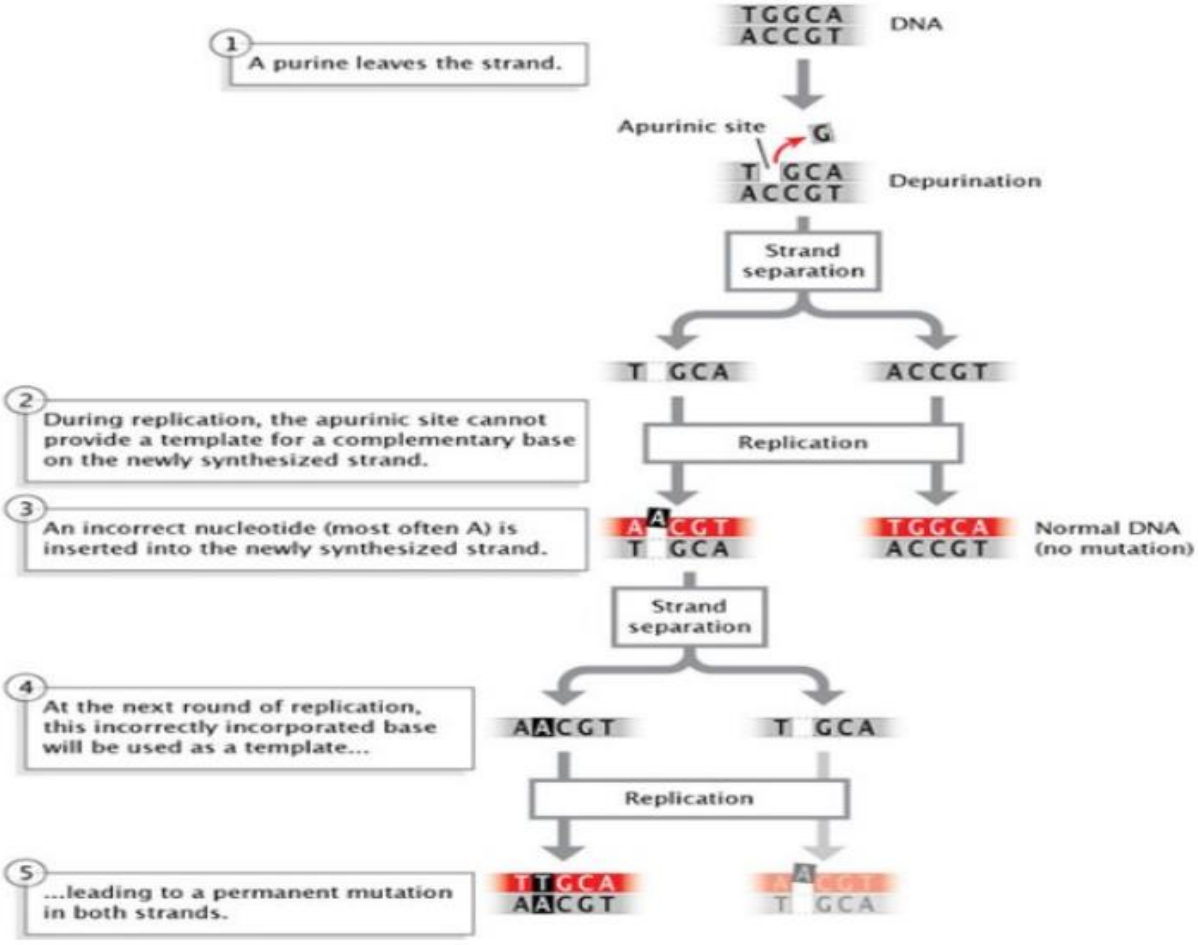
Mutations Caused by Chemicals

- **Oxidizing agents**, commonly known as **free radicals**, are substances that can chemically modify nucleotides in ways that **alter their base-pairing capacities**.
- For instance, **dioxin intercalates between base pairs**, disrupting the **integrity of the DNA helix** and predisposing that **site to insertions or deletions**.

- Similarly, **benzo[a]pyrene,** a **known carcinogen** and a component of cigarette smoke, has been demonstrated to induce lesions at guanine bases in the tumor suppressor gene P_{53} **at codons 157, 248, and 273.** These codons are the major mutational hot spots seen in clinical studies of human lung cancers.
- Mutations such as these that are fairly specific to particular mutagens are called **signature mutations.**
- A variety of chemicals beyond those mentioned here are known to induce such mutations.

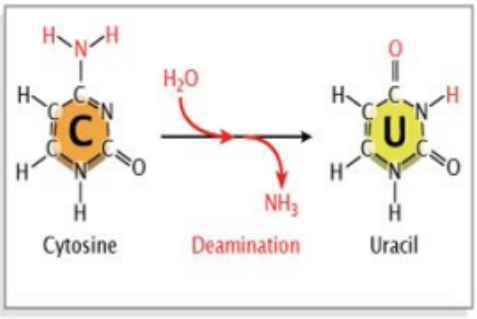
Spontaneous Mutations

- Mutations can also occur spontaneously. For instance, depurination (Figure), in which a purine base is lost from a nucleotide through hydrolysis even though the sugar-phosphate backbone is unaltered, can occur without an explicit insult from the environment.
- If uncorrected by DNA repair enzymes, depurination may result in the incorporation of an incorrect base during the next round of replication.



- **Depurination** is a spontaneous mutation that occurs when a nucleotide loses a purine base.
- During replication, two strands of DNA separate. If a nucleotide on one strand has lost a purine base, the apurinic site on this strand cannot provide a template for a complementary base on the newly-synthesized strand.
- An incorrect nucleotide (most often adenine) is inserted into the newly-synthesized strand, across from the empty apurinic site on the template strand. The result is a normal double-stranded DNA molecule that does not contain a mutation, and a mutant double-stranded DNA molecule. When the mutant DNA undergoes a second round of replication, the incorrectly incorporated base (adenine) acquired during the previous replication round is used as a template for synthesizing a new DNA strand. The two resulting double-stranded DNA molecules each contain a permanent mutation in both of their strands.

- **Deamination**, or the removal of an amine group from a base, may also occur. Deamination of cytosine converts it to uracil, which will pair with adenine instead of guanine at the next replication, resulting in a base substitution. Repair enzymes can recognize uracil as not belonging in DNA, and they will normally repair such a lesion. However, if the cytosine residue in question is methylated (a common modification involved in gene regulation), deamination will instead result in conversion to thymine. Because thymine is a normal component of DNA, this change will go unrecognized by repair enzymes



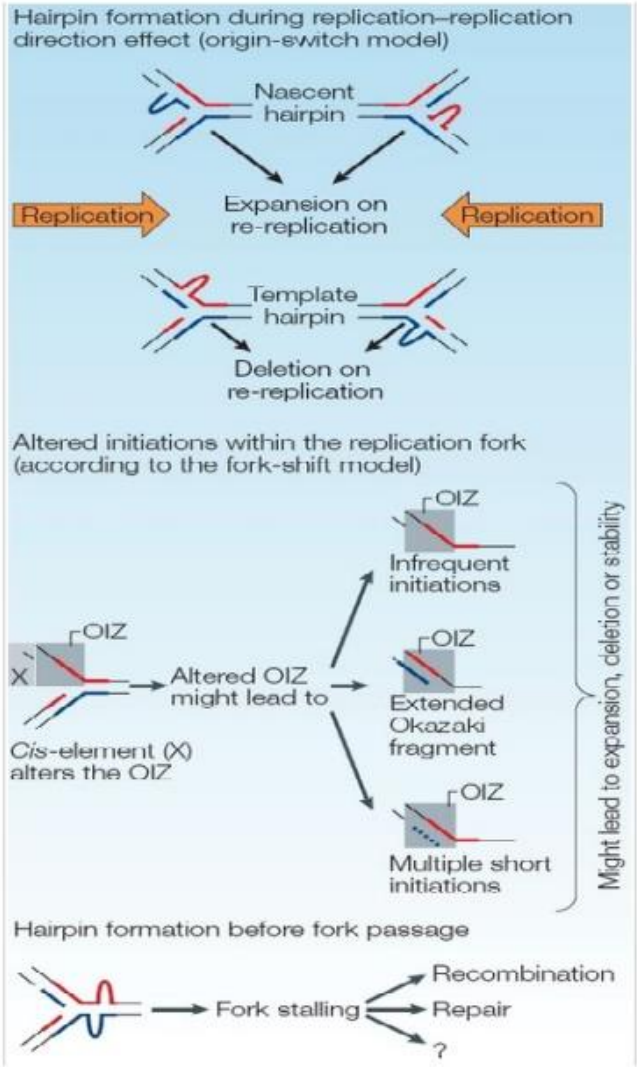
The nitrogenous base cytosine is converted to uracil after the loss of an amine group. Because uracil forms base-pairs with adenine, while cytosine forms base-pairs with guanine, the conversion of cytosine to uracil causes base substitutions in DNA.

Errors During DNA Replication

- Errors that occur during DNA replication play an important role in some mutations, **especially trinucleotide repeat (TNR) expansions**. It is thought that the ability of repeat **sequences to form secondary structures**, such as intrastrand hairpins, during replication might contribute to **slippage of DNA polymerase**, causing this enzyme to **slide back and repeat replication of the previous segment** (Figure).

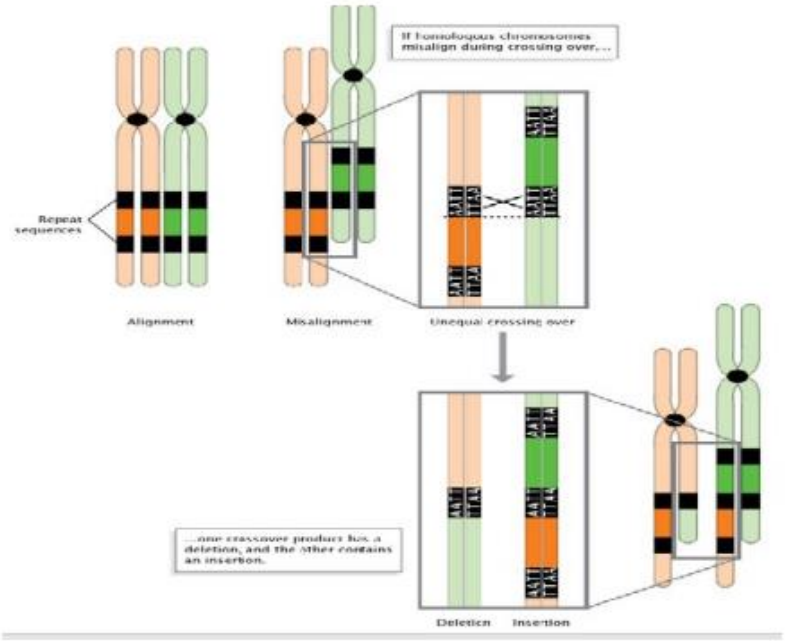
- Supporting **this hypothesis, lagging-strand** synthesis has been shown to be particularly **sensitive to repeat expansion**. For instance, **the secondary structure of some TNR DNA has been shown to inhibit an enzyme (FEN1) necessary for proper resolution of the Okazaki fragments** generated during lagging-strand replication; as a result, FEN1 mutant yeast cells demonstrate increased expansion of CAG repeats.

As previously mentioned, repeats also occur in nonmitotic tissue, and CAG repeats have further been shown to accumulate in mice defective for individual DNA repair pathways, suggesting that multiple repair mechanisms must be operative in repeat expansion in nonproliferating cells (Pearson *et al.*, 2005). In agreement with this hypothesis, studies have revealed increased repeat instability following induction of double-stranded breaks and UV-induced lesions, which are corrected by nucleotide excision repair.



MUTATIONS DUE TO UNEQUAL CROSSING-OVER DURING MEIOSIS

- Mutations can result from a number of events**, including unequal crossing-over during meiosis** (Figure).
- In addition, some areas of the genome simply seem to be more prone to mutation than others.These "hot spots" are often a **result of the DNA sequence itself being more accessible to mutagens.** Hot spots include areas of the **genome with highly repetitive sequences**, such as **trinucleotide repeats**, in which a sequence of three nucleotides is repeated many times.
- During **DNA replication, these repeat regions** are often altered because the **polymerase can "slip" as it disassociates** and **reassociates with the DNA strand.**
- To better understand a **polymerase slip, imagine you are reading a page of text that is a repeat of a simple sequence**. Say that the whole page is just copies of the word "And" ("And And And..."). **Now, imagine that while reading the page, you briefly glance away and then look back at the text.** It's quite **likely that you will have lost your place**. As a result**, you may read the wrong number of copies from the page.** Similarly, DNA **polymerase sometimes slips and makes mistakes when reading repeats**.



When homologous chromosomes misalign during meiosis, unequal crossing-over occurs. The result is the deletion of a DNA sequence in one chromosome, and the insertion of a DNA sequence in the other chromosome.

MOLECULAR BASIS OF CYSTIC FIBROSIS

CYSTIC FIBROSIS

- ✓ **Cystic fibrosis (CF)**,also known as **mucoviscidosis** is a genetic disorder that affects mostly the lungs but also the pancreas, liver, kidneys and intestine.
- ✓ The **main signs and symptoms of cystic fibrosis** are salty-tasting skin, poor growth, and poor weight gain despite normal food intake,accumulation of thick, sticky mucus, frequent chest infections, and coughing or shortness of breath
- ✓ Cystic fibrosis is an **inherited disease characterized by the buildup of thick, sticky mucus** that can damage many of the body's organs.
- ✓ The disorder's most common signs and symptoms include **progressive damage to the respiratory system** and **chronic digestive system problems.**
- ✓ **The features of the disorder and their severity varies among affected individuals.**

Mucus

- ✓ **Mucus is a slippery substance that lubricates and protects the linings of the airways, digestive system, reproductive system, and other organs and tissues.**
- ✓ **In people with cystic fibrosis, the body produces mucus that is abnormally thick and sticky.**
- ✓ **This abnormal mucus can clog the airways, leading to severe problems with breathing and bacterial infections in the lungs.**
- ✓ **These infections cause chronic coughing, wheezing, and inflammation.**
- ✓ **Over time, mucus buildup and infections result in permanent lung damage, including the formation of scar tissue (fibrosis) and cysts in the lungs.**
- ✓ **Most people with cystic fibrosis also have digestive problems.**
- ✓ **Some affected babies have meconium ileus, a blockage of the intestine that occurs shortly after birth.**
- ✓ **Other digestive problems result from a buildup of thick, sticky mucus in the pancreas. The pancreas is an organ that produces insulin (a hormone that helps control blood sugar levels).**
- ✓ **It also makes enzymes that help digest food. In people with cystic fibrosis, mucus blocks the ducts of the pancreas, reducing the production of insulin and preventing digestive enzymes from reaching the intestines to aid digestion.**

- ✓ **Problems with digestion can lead to diarrhea, malnutrition, poor growth, and weight loss.**
- ✓ **In adolescence or adulthood, a shortage of insulin can cause a form of diabetes known as cystic fibrosis-related diabetes mellitus (CFRDM).**
- ✓ **Cystic fibrosis used to be considered a fatal disease of childhood. With improved treatments and better ways to manage the disease, many people with cystic fibrosis now live well into adulthood.**
- ✓ **Adults with cystic fibrosis experience health problems affecting the respiratory, digestive, and reproductive systems.**
- ✓ **Most men with cystic fibrosis have congenital bilateral absence of the vas deferens (CBAVD), a condition in which the tubes that carry sperm (the vas deferens) are blocked by mucus and do not develop properly.**
- ✓ **Men with CBAVD are unable to father children (infertile) unless they undergo fertility treatment.**
- ✓ **Women with cystic fibrosis may experience complications in pregnancy.**
- ✓ **In rare cases, cystic fibrosis can manifest itself as a coagulation disorder. Vitamin K is normally absorbed from breast milk, formula, and later, solid foods.**
- ✓ **This absorption is impaired in some cystic fibrosis patients. Young children are especially sensitive to vitamin K malabsorptive disorders because only a very small amount of vitamin K crosses the placenta, leaving the child with very low reserves and limited ability to absorb vitamin K from dietary sources after birth.**
- ✓ **Because factors II, VII, IX, and X (clotting factors) are vitamin K–dependent, low levels of vitamin K can result in coagulation problems.**
- ✓ **Consequently, when a child presents with unexplained bruising, a coagulation evaluation may be warranted to determine whether there is an underlying disease**

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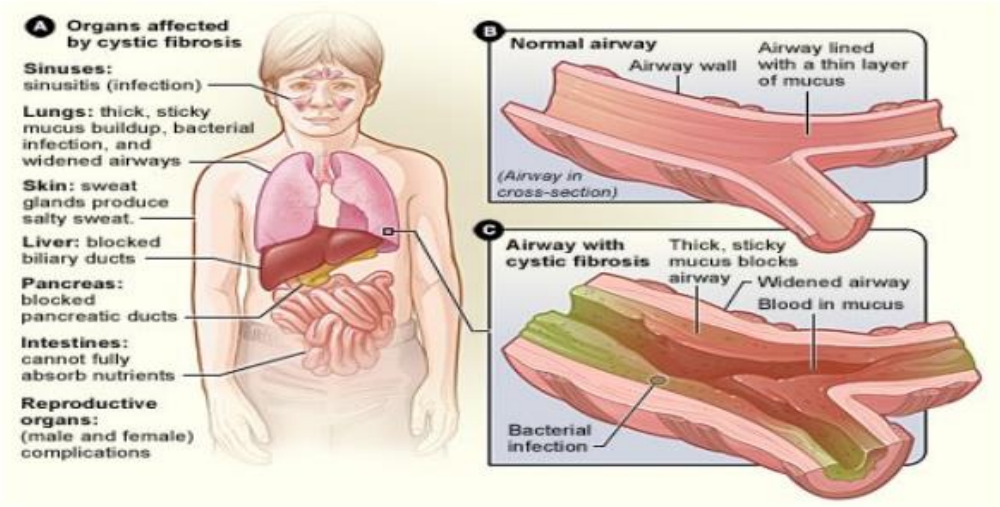
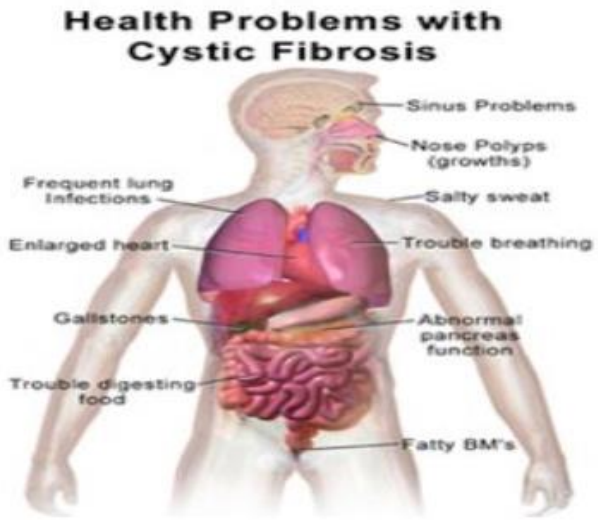


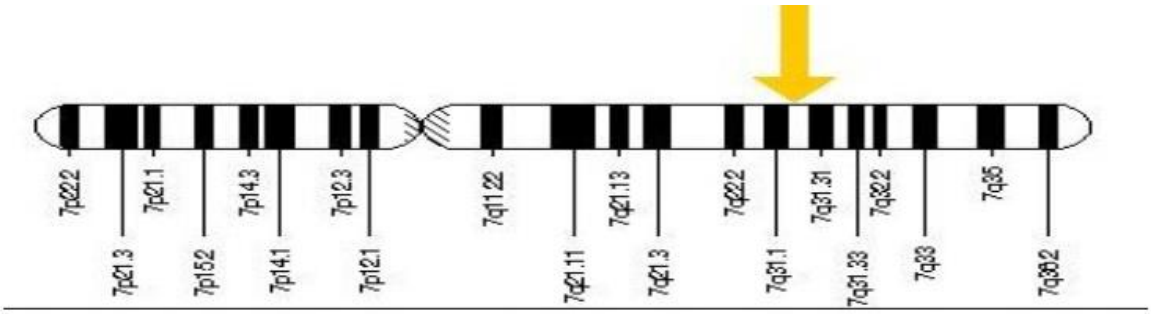


What genes are related to cystic fibrosis?

- ✓ Mutations in the **CFTR (CFTR gene-"cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7")**gene cause cystic fibrosis.

Where is the CFTR gene located?

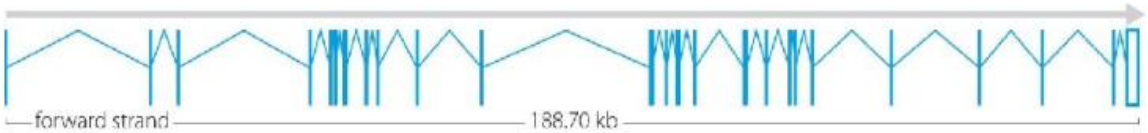
Cytogenetic Location: 7q31.2



- ✓ Molecular Location on chromosome 7: base pairs 117,470,771 to 117,668,664

The CFTR gene is located on the long (q) arm of chromosome 7 at position 31.2.

- ✓ More precisely, the CFTR gene is located from base pair 117,470,771 to base pair 117,668,664 on chromosome 7.



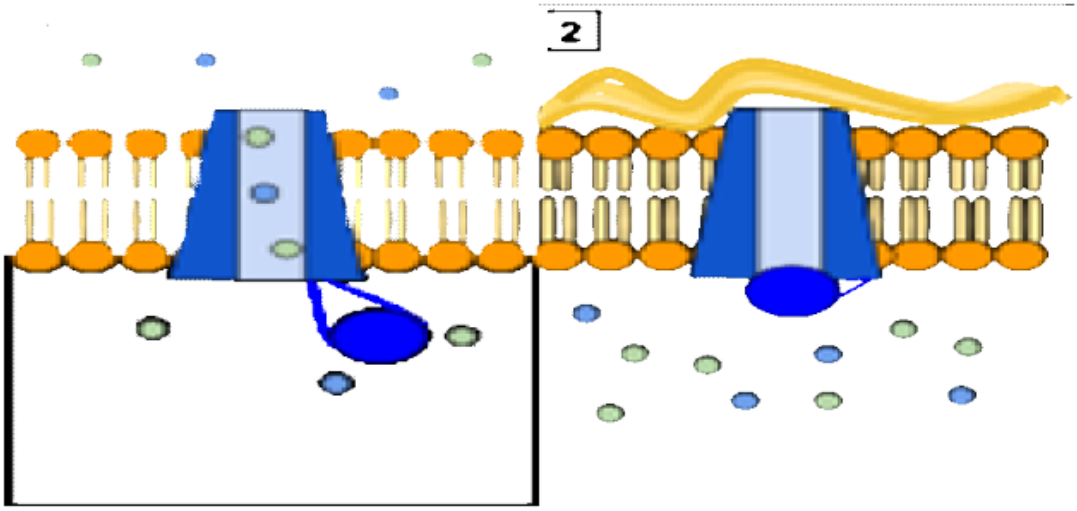
Structure of the *CFTR* gene

An alternative graphical display of Human Genome Project data, as displayed by the ENSEMBL genome browser. Some closely spaced exons appear as a single bar. The arrow shows the 5' → 3' direction of the sense strand.

What is the normal function of the CFTR gene?

- ✓ The CFTR gene provides instructions for making a protein called the **cystic fibrosis transmembrane conductance regulator**.
- ✓ This protein functions as a channel across the membrane of cells that produce mucus, sweat, saliva, tears, and digestive enzymes.
- ✓ The channel transports negatively charged particles called chloride ions into and out of cells.
- ✓ The transport of chloride ions helps control the movement of water in tissues, which is necessary for the production of thin, freely flowing mucus.
- ✓ Mucus is a slippery substance that lubricates and protects the lining of the airways, digestive system, reproductive system, and other organs and tissues.

- ✓ The CFTR protein **also regulates the function of other channels, such as those that transport positively charged particles called sodium ions across cell membranes.**
- ✓ These channels are necessary for the normal function of organs such as the lungs and pancreas.
- ✓ Protein CFTR possesses two ATP-hydrolyzing domains, which allows the protein to use energy in the form of ATP.
- ✓ It also contains two domains comprising 6 alpha helices, which allow the protein to cross the cell membrane.
- ✓ A regulatory binding site on the protein allows activation by phosphorylation, mainly by cAMP-dependent protein kinase.
- ✓ The carboxyl terminal of the protein is anchored to the cytoskeleton by a PDZ domain interaction.

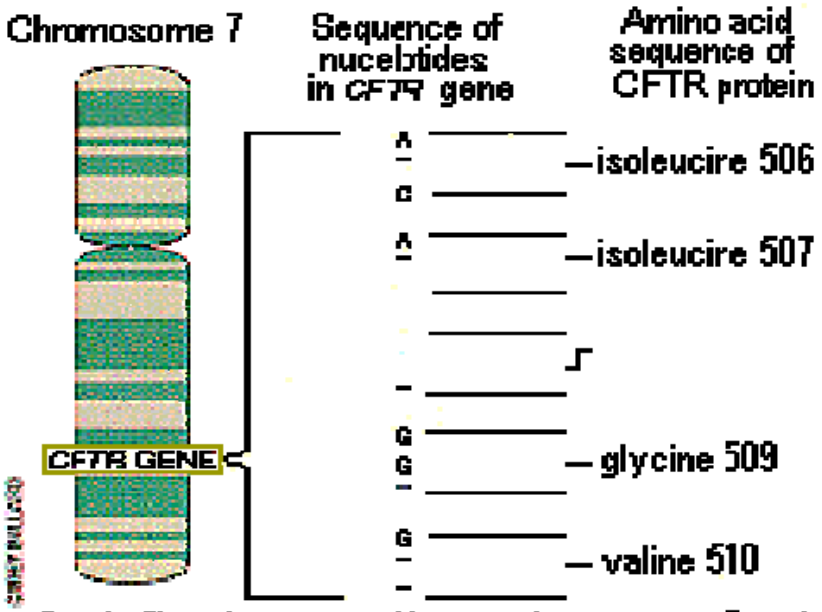


The CFTR protein is a channel protein that controls the flow of **H_2O and Cl- ions** in and out of cells inside the lungs. When the CFTR protein is working correctly, **as shown in Panel 1,** ions freely flow in and out of the cells. However, when the CFTR protein is malfunctioning as in **Panel 2, these ions cannot flow out of the cell due to a blocked channel.** This causes cystic fibrosis, characterized by the buildup of thick mucus in the lungs.

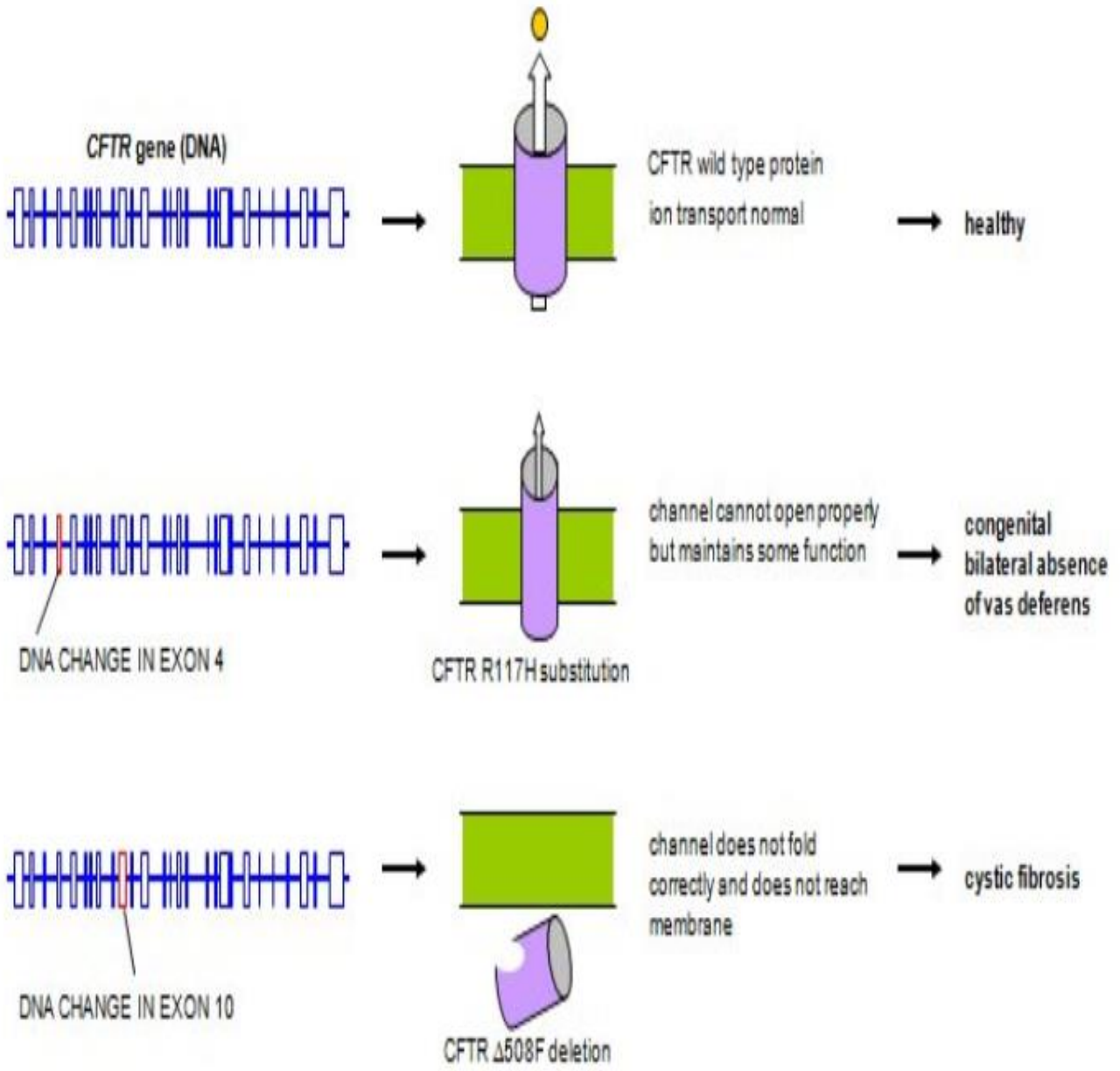
How are changes in the CFTR gene related to CF?

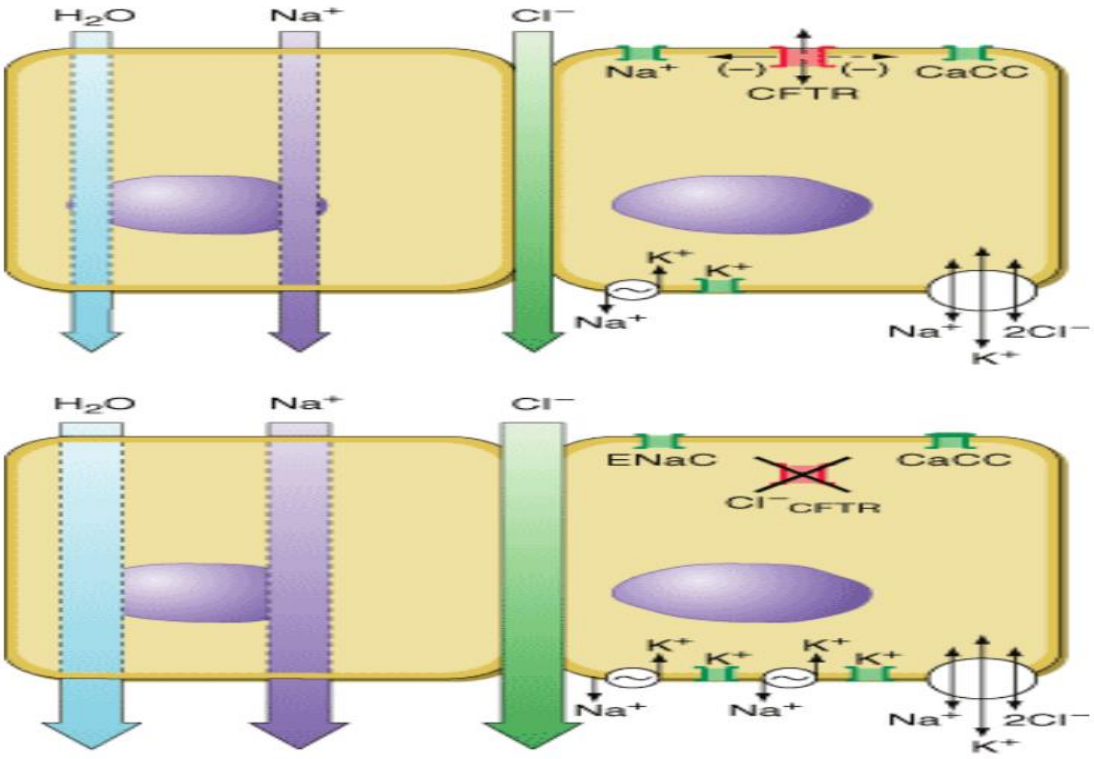
- ✓ More than **1,000 mutations in the CFTR gene** have been identified in people with cystic fibrosis.

- ✓ Most of these mutations change single protein building blocks (amino acids) in the CFTR protein or delete a small amount of DNA from the CFTR gene.
- ✓ The most common mutation, called **delta F508**, is a deletion of one amino acid at position 508 in the CFTR protein.
- ✓ The resulting **abnormal channel breaks down shortly after it is made, so it never reaches the cell membrane to transport chloride ions**.
- ✓ Disease-causing mutations in the **CFTR gene alter the production, structure, or stability of the chloride channel.**
- ✓ All of these changes prevent the channel from functioning properly, which impairs the transport of chloride ions and the movement of water into and out of cells.
- ✓ As a result, **cells that line the passageways of the lungs, pancreas, and other organs produce mucus that is abnormally thick and sticky**.
- ✓ The abnormal **mucus obstructs the airways and glands, leading to the characteristic signs and symptoms of cystic fibrosis.**

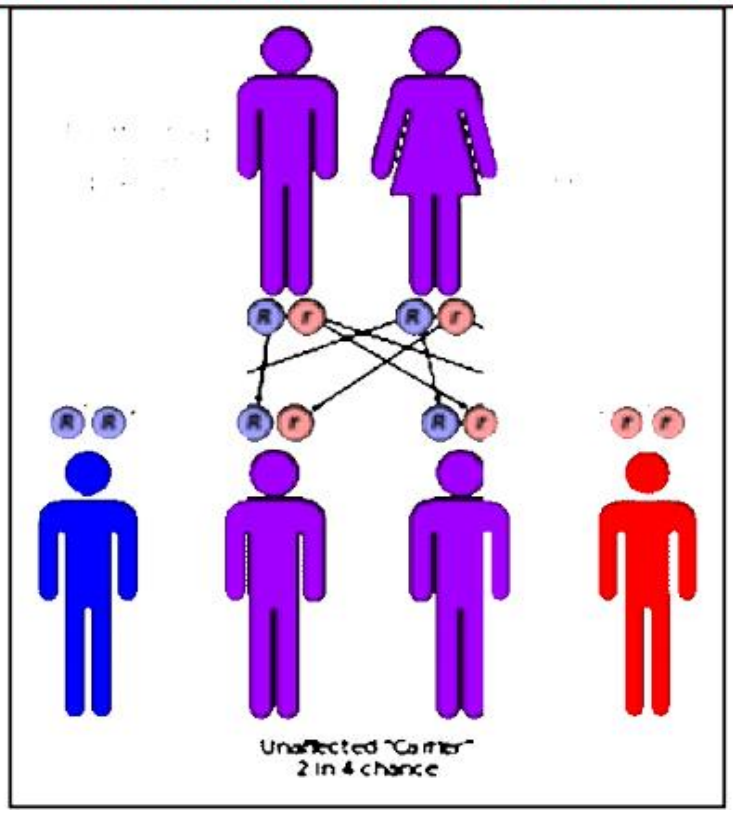


Cystic fibrosis gene resides on chromosome 7 and normally gives rise to a protein called the cystic fibrosis transmembrane conductance regulator (CFTR). The defect that most often leads to the disease is the deletion of three nucleotides from the gene (red letters above); this alteration, known as the ΔF508 mutation, results in the loss of one amino acid – phenylalanine at position 508 – in the CFTR protein. Phenylalanine is lost because the protein-making machinery of the cell now sees ATT (an alternative way to encode isoleucine) at the gene region coding for the protein's 507th amino acid, followed by the GGT sequence for the glycine that normally follows phenylalanine.


CFTR gene (DNA)
CFTR wild type protein
ion transport normal
healthy
DNA CHANGE IN EXON 4
CFTR R117H substitution
channel cannot open properly
but maintains some function
congenital
bilateral absence
of vas deferens
DNA CHANGE IN EXON 10
CFTR Δ508F deletion
channel does not fold
correctly and does not reach
membrane
cystic fibrosis



Cystic fibrosis has an autosomal recessive pattern of inheritance



Diagnosis and monitoring

Cystic fibrosis may be diagnosed by many different methods including newborn screening, sweat testing, and genetic testing.

sweat testing

- ✓ The most commonly used form of testing is the sweat test.
- ✓ Sweat-testing involves application of a medication that stimulates sweating (pilocarpine).
- ✓ To deliver the medication through the skin, iontophoresis is used to, whereby one electrode is placed onto the applied medication and an electric current is passed to a separate electrode on the skin.
- ✓ The resultant sweat is then collected on filter paper or in a capillary tube and analyzed for abnormal amounts of sodium and chloride.
- ✓ People with CF have increased amounts of sodium and chloride in their sweat. In contrast, people with **CF have less thiocyanate** and **hypothiocyanite** in their **saliva and mucus**.
- ✓ The newborn screen initially measures for raised blood concentration of immuno reactive trypsinogen.
- ✓ Trypsinogen levels can be increased in individuals who have a single mutated copy of the *CFTR* gene (carriers) or, in rare instances, in individuals with two normal copies of the *CFTR* gene.
- ✓ Due to these false positives, CF screening in newborns can be controversial.

Prenatal diagnosis

- ✓ Couples who are pregnant or planning a pregnancy can have themselves tested for the CFTR gene mutations to determine the risk that their child will be born with cystic fibrosis.
- ✓ Testing is typically performed first on one or both parents and, if the risk of CF is high, testing on the fetus is performed.
- ✓ The American College of Obstetricians and Gynecologists (ACOG) recommends testing for couples who have a personal or close family history of CF, and they recommend that **carrier testing be offered to all Caucasian couples and be made available to couples of other ethnic backgrounds.**

- ✓ Because development of CF in the fetus requires each parent to pass on a mutated copy of the CFTR gene and because CF testing is expensive, testing is often performed initially on one parent. If testing shows that parent is a CFTR gene mutation carrier, the other parent is tested to calculate the risk that their children will have CF
- ✓ CF can result from more than a thousand different mutations, and as of 2006 it is not possible to test for each one.
- ✓ Testing analyzes the blood for the most common mutations such as ΔF508—most commercially available tests look for 32 or fewer different mutations.
- ✓ If a family has a known uncommon mutation, specific screening for that mutation can be performed. Because not all known mutations are found on current tests, a negative screen does not guarantee that a child will not have CF.
- ✓ During pregnancy, testing can be performed on the placenta (chorionic villus sampling) or the fluid around the fetus (amniocentesis)
- ✓ However, chorionic villus sampling has a risk of fetal death of 1 in 100 and amniocentesis of 1 in 200; a recent study has indicated this may be much lower, approximately 1 in 1,600.
- ✓ Economically, for carrier couples of cystic fibrosis, when comparing preimplantation genetic diagnosis (PGD) with natural conception (NC) followed by prenatal testing and abortion of affected pregnancies, PGD provides net economic benefits up to a maternal age of approximately 40 years, after which NC, prenatal testing and abortion has higher economic benefit.

Management/Treatment

- ✓ While there are no cures for cystic fibrosis, there are several treatment methods. The management of cystic fibrosis has improved significantly over the past 70 years.
- ✓ While infants born with cystic fibrosis 70 years ago would have been unlikely to live beyond their first year, infants today are likely to live well into adulthood.
- ✓ Recent advances in the treatment of cystic fibrosis have meant that an individual with cystic fibrosis can live a fuller life less encumbered by their condition.

- ✓ The cornerstones of management are proactive treatment of airway infection, and encouragement of good nutrition and an active lifestyle.
- ✓ Pulmonary rehabilitation as a management of cystic fibrosis continues throughout a person's life, and is aimed at maximizing organ function, and therefore quality of life.
- ✓ At best, current treatments delay the decline in organ function. Because of the wide variation in disease symptoms, treatment typically occurs at specialist multidisciplinary centers, and is tailored to the individual.
- ✓ Targets for therapy are the lungs, gastrointestinal tract (including pancreatic enzyme supplements), the reproductive organs (including assisted reproductive technology (ART)) and psychological support.

Antibiotics

- ✓ Many people with CF are on one or more antibiotics at all times, even when healthy, to prophylactically suppress infection.
- ✓ Antibiotics are absolutely necessary whenever pneumonia is suspected or there has been a noticeable decline in lung function, and are usually chosen based on the results of a sputum analysis and the person's past response.
- ✓ This prolonged therapy often necessitates hospitalization and insertion of a more permanent IV such as a peripherally inserted central catheter (PICC line) or Port-a-Cath.
- ✓ Inhaled therapy with antibiotics such as tobramycin, colistin, and aztreonam is often given for months at a time to improve lung function by impeding the growth of colonized bacteria.

- ✓ Inhaled antibiotic therapy helps lung function by fighting infection, but also has significant drawbacks like development of antibiotic resistance, tinnitus and changes in the voice.

- ✓ Oral antibiotics such as ciprofloxacin or azithromycin are given to help prevent infection or to control ongoing infection.
- ✓ The aminoglycoside antibiotics (e.g. tobramycin) used can cause hearing loss, damage to the balance system in the inner ear or kidney problems with long-term use.
- ✓ To prevent these side-effects, the amount of antibiotics in the blood are routinely measured and adjusted accordingly.

Other treatments for lung disease

- ✓ Several mechanical techniques are used to dislodge sputum and encourage its expectoration. In the hospital setting, chest physiotherapy (CPT) is utilized; a respiratory therapist percusses an individual's chest with his or her hands several times a day, to loosen up secretions.
- ✓ Devices that recreate this percussive therapy include the ThAIRapy Vest and the intrapulmonary percussive ventilator (IPV).
- ✓ Newer methods such as Biphasic Cuirass Ventilation, and associated clearance mode available in such devices, integrate a cough assistance phase, as well as a vibration phase for dislodging secretions. These are portable and adapted for home use.
- ✓ Aerosolized medications that help loosen secretions include dornase alfa and hypertonic saline.
- ✓ Dornase is a recombinant human deoxyribonuclease, which breaks down DNA in the sputum, thus decreasing its viscosity.
- ✓ Denufosol is an investigational drug that opens an alternative chloride channel, helping to liquefy mucus.
- ✓ It is unclear if inhaled corticosteroids are useful.
- ✓ As lung disease worsens, mechanical breathing support may become necessary. Individuals with CF may need to wear special masks at night that help push air into their lungs.
- ✓ These machines, known as bilevel positive airway pressure (BiPAP) ventilators, help prevent low blood oxygen levels during sleep.
- ✓ BiPAP may also be used during physical therapy to improve sputum clearance.

- ✓ During severe illness, a tube may be placed in the throat (a procedure known as a tracheostomy) to enable breathing supported by a ventilator.
- ✓ For children, preliminary studies show massage therapy may help people and their families quality of life.