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Normal CFTR Protein
Key points

- Normal cystic fibrosis transmembrane conductance regulator (CFTR) protein channels transport ions, such as chloride and bicarbonate, through the apical membrane of epithelial cells to regulate fluid and electrolyte balance in epithelial tissues throughout the body.\(^1\)\(^2\)
- The CFTR gene, located on chromosome 7, encodes the CFTR protein.\(^1\)
- The presence of CFTR mutations on both alleles generally results in cystic fibrosis.\(^2\)

References:

Key points

- The CFTR gene encodes a CFTR protein channel that is made up of 1480 amino acids organized into 5 functional domains:\(^1,2\):
  - 2 membrane-spanning domains (MSD1 and MSD2)\(^*,3\)
  - 2 nucleotide-binding domains (NBD1 and NBD2)
  - 1 regulatory domain (R)

\(\star\)MSDs are also known as transmembrane domains (TMD1 and TMD2).\(^2,3\)

References:
The CFTR protein reaches the apical epithelial cell membrane as part of a multistep process. As with all proteins, this process involves:

- **Synthesis** (transcription and translation)
- **Folding and processing**
- **Trafficking to its destination** (e.g., apical cell membrane)
- **Turnover**

### CFTR protein synthesis

- In the nucleus, the CFTR gene is transcribed into mRNA
- Introns (noncoding sequences) are then removed from mRNA during a process called splicing
- The protein is synthesized in the cytoplasm and enters the endoplasmic reticulum (ER) during synthesis

### CFTR protein folding and processing

- Immature CFTR protein is folded and processed in the ER
- Any protein that does not fold properly is degraded

### CFTR protein trafficking

- CFTR protein is transported to the Golgi apparatus for final processing, then mature protein is trafficked to the cell surface

### CFTR protein function

- At the cell surface, CFTR proteins function as channels that transport chloride and bicarbonate ions

### CFTR protein turnover

- CFTR channels have a limited lifespan and are eventually removed in a process called turnover

When protein synthesis, folding and processing, and trafficking occur properly, fully functional CFTR proteins reach the cell surface in sufficient quantity to maintain adequate ion transport.

### References:

Key points

- CFTR quantity is determined by\textsuperscript{1,2}:
  - CFTR synthesis: CFTR gene transcription, proper splicing, and mRNA translation
  - CFTR processing and trafficking: maturation of the CFTR protein and its delivery to the cell surface
  - CFTR surface stability: amount of time a CFTR channel is at the cell surface before being removed and recycled

References:
Key points

- CFTR proteins function as channels that transport ions through the apical cell membrane of epithelial cells
- CFTR protein function is determined by channel-open probability (gating) and conductance
  - Channel-open probability: the fraction of time that a single CFTR protein channel is open and transporting ions
  - Based on in vitro experimentation, normal CFTR channels have channel-open probability of ~40%
  - Channel conductance: rate at which ions move through open channels

References:
Key points

• Total CFTR activity can be defined as total ion transport mediated by CFTR protein channels at the cell surface. Total CFTR activity is determined by:
  – CFTR quantity: the number of CFTR channels at the cell surface
  – CFTR function: the functional ability of each channel to open and transport ions

References:
Key points

- The CFTR gene is expressed in epithelial tissue of multiple organs throughout the body. The cystic fibrosis transmembrane conductance regulator, or CFTR, channel plays an important role in maintaining electrolytes and fluid balance in many organ systems.
  - The regulated transport of electrolytes and fluid is necessary for the proper function of the airway, pancreas, gastrointestinal tract, and sweat glands, among others.
  - In individuals without CF, normal expression of CFTR protein and normal CFTR activity contribute to the proper function of these organs.

References:
CFTR Mutations
Key points

Approximately 2000 mutations in the CFTR gene have been identified to date, although the majority are extremely rare.\(^1^\)\(^\text{1-15}\)

- **F508del** is the most common CFTR mutation worldwide.
  - Up to 91% of patients with CF have an F508del mutation on at least one allele, based on individual country registries.\(^3^\)\(^\text{3-15}\)
  - This frequency varies among countries and ethnic groups.
- Although occurring at a much lower frequency than F508del, the following 11 mutations occur at a frequency of >1% globally:
- Another 3 mutations occur at a frequency >1% in Canada, Europe, and Australia, but not in the United States:
  - 711+1G->T, 2183AA->G, and R1162X.
- Frequency and mutational diversity vary among countries.
- About 23 mutations in total occur at a frequency >0.1%.

Not all CFTR mutations lead to CF. To date, only 127 CFTR mutations have been confirmed as CF-causing.\(^1\)

References:

The mutational diversity of cystic fibrosis

- Cystic fibrosis (CF) is a common autosomal recessive disorder affecting approximately 70,000 people worldwide.
- The majority of people with CF are of Caucasian descent, therefore the disease is most prevalent in North American, European, and Australasian populations, however, CF can affect all races and ethnicities, including African, Latin American, and Middle Eastern populations.
- The prevalence of CF and spectrum of CFTR mutations vary considerably among populations and regions of the world.

### Examples of birth prevalence by country

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References:

Key points

- Cystic fibrosis (CF) is a common autosomal recessive disorder affecting approximately 70,000 people worldwide. The majority of people with CF are of Caucasian descent, therefore the disease is most prevalent in North American, European, and Australasian populations. However, CF can affect all races and ethnicities, including African, Latin American, and Middle Eastern populations.
- The prevalence of CF and spectrum of CFTR mutations vary considerably among populations and regions of the world.

References:
**Key points**

- Different mutations in the CFTR gene can cause disruptions at various stages of CFTR protein synthesis or in several aspects of CFTR protein function. They can result in less CFTR protein at the cell surface, virtual absence of CFTR protein, or dysfunctional CFTR protein at the cell surface.

- Traditionally, the CFTR class system groups CFTR mutations by the primary molecular defect in the CFTR protein. Although each mutation is categorized by a single defect, an individual mutation can result in multiple defects, spanning multiple classes.

  - **Class I**: defective synthesis of full-length CFTR protein. Premature stop codon prevents full translation of mRNA, resulting in truncated CFTR protein. Few to no mature CFTR proteins are formed.
  - **Class II**: defective CFTR protein processing and trafficking. Defective post-translational processing and transport reduce quantity of CFTR protein delivered to cell surface.
  - **Class III**: defective CFTR channel gating. CFTR is at the cell surface but has reduction in channel-open probability.
  - **Class IV**: defective CFTR channel conductance. CFTR is at the cell surface but has impaired movement of ions through channel.
  - **Class V**: reduced synthesis of CFTR protein. A splicing defect reduces quantity of properly processed CFTR mRNA transcripts, decreasing quantity of CFTR protein at the cell surface.
  - **Class VI**: reduced stability of CFTR protein. Accelerated turnover of CFTR protein at the cell surface reduces quantity.

**References:**
Key points

- Loss of CFTR protein activity is the underlying cause of CF.\(^1,2\)
  - Individual CFTR mutations can lead to decreased quantity or function (and sometimes both) of CFTR proteins at the epithelial cell surface
  - These defects in CFTR proteins limit ion transport through the apical cell membrane
- Defective ion transport in the lungs, pancreas, gastrointestinal (GI) system, sinuses, sweat glands, and reproductive system leads to the symptoms of CF.\(^1,5\)
  - The resulting imbalance of fluid and electrolytes causes thick, sticky mucus (in lungs, sinuses) or viscous secretions (in pancreas, GI tract, reproductive tract) to accumulate, which interferes with the proper function of these organs
  - Defective chloride ion transport in the sweat gland leads to high salt concentration in sweat, but does not impact the morphology of the gland

References:

Key points

- **Total CFTR activity** is a function of how each CFTR mutation affects:
  - **CFTR quantity**: the number of CFTR channels at the cell surface
  - **CFTR function**: the functional ability of each channel to open and transport ions

- Individual CFTR mutations can decrease the **quantity** or **function** (and sometimes both) of CFTR proteins at the cell surface. These defects in CFTR protein cause a reduction in total CFTR activity

References:
Some CFTR mutations result in little to no CFTR activity

Key points

Some CFTR mutations reduce CFTR protein quantity or function at the cell surface to such an extent that the result is little to no CFTR activity.1-4

- CFTR mutations that cause a defect in protein synthesis due to nonsense mutations (e.g., G542X) or a splicing abnormality that results in a premature stop codon (e.g., 621+1G->T) result in few to no CFTR proteins at the cell surface
- CFTR mutations that affect processing (e.g., F508del) result in few to no CFTR proteins delivered to the cell surface
- CFTR mutations that produce CFTR proteins with decreased stability (e.g., 4326delTC) result in functional CFTR channels that rapidly degrade at the cell surface, leaving few to no CFTR proteins at the cell surface
- CFTR mutations that severely affect gating (e.g., G551D) or conductance (e.g., R334W) result in a normal quantity of CFTR channels at the cell surface that have little to no function

References:
Key points

- Some CFTR mutations result in a limited reduction in CFTR protein quantity or function at the cell surface that can produce residual or partial CFTR activity:
  - Some CFTR mutations that cause a defect in mRNA splicing (e.g., 2789+5G->A) can result in reduced protein synthesis but delivery of some functional CFTR proteins to the cell surface
  - CFTR mutations that reduce conductance and/or gating (e.g., R117H) can result in a normal quantity of CFTR channels at the cell surface that have some level of function and ion transport

References:
Key point

- The potential clinical impact of an individual CFTR mutation may be related to the amount of total CFTR ion transport activity. **Total CFTR activity** is associated with the extent of CF manifestations and phenotypic variability.¹⁻⁵

References:
Key points

- The degree to which a CFTR mutation reduces CFTR quantity or function (or both) at the cell surface determines the total CFTR activity of the cell\(^1\,^2\).
- Total CFTR activity can be defined as total ion transport mediated by CFTR protein channels at the cell surface\(^1\).
- The potential clinical impact of an individual CFTR mutation may be related to the amount of total CFTR ion transport activity. Total CFTR activity is associated with the extent of CF manifestations and phenotypic variability\(^2\,^6\).

References:
Genotype to Phenotype
Key points

- Total CFTR activity, which is mainly determined by CFTR genotype, is one of a few factors that influence the phenotype of an individual and determines if he or she will develop CF disease and to what degree.\(^1\,^3\)
  - The mutations present on both CFTR alleles determine CFTR protein production and activity
- Individuals with 2 normal (wild-type) CFTR alleles produce CFTR proteins of normal quantity and function, and therefore, sufficient activity\(^1\,^4\,^5\)
  - These individuals neither have nor are carriers of CF
- Carriers of CF have 1 normal CFTR allele, which produces normal CFTR protein, and 1 mutated CFTR allele, which produces defective CFTR protein with reduced quantity or function\(^1\,^4\,^5\)
  - In this case, there is sufficient functional CFTR protein, and hence CFTR activity, to result in a non-CF phenotype
  - Nonetheless, some carriers may have increased risk for certain pulmonary conditions (e.g., asthma)
- People who have CF-causing mutations on both alleles produce defective CFTR proteins that are defective in quantity or function (and sometimes both), leading to a reduction of total CFTR activity and a CF phenotype\(^1\,^3\)

Other factors that may contribute to a cystic fibrosis phenotype include:

- Modifier genes: may affect lung function and disease course\(^1\,^6\)
  - Examples include key factors of the immune system, mannose-binding lectin 2 (MBL2) and transforming growth factor beta 1 (TGF-ß1)
- Environmental factors: can also significantly affect phenotype. Examples may include\(^1\,^2\,^6\,^8\):
  - Level of care/socioeconomic status
  - Nutritional status
  - Exposure to cigarette smoke and other pollutants
  - Age at onset of lung infection

References:
Key points

- **CFTR mutations** on both alleles contribute to the quantity of functional CFTR proteins and respective levels of total CFTR activity\(^1^,^4\)
  - CFTR mutations can result in either little to no CFTR protein activity or in residual CFTR protein activity
  - The extent to which the combination of CFTR alleles affects CFTR protein activity (i.e., normal, residual, little to no) influences the phenotype of an individual person

References:
Key points

- **G542X** is an example of a mutation resulting in little to no CFTR activity. In combination with another allele that produces little to no CFTR activity, **G542X** usually results in a CF phenotype\(^1\)\(^-\)\(^5\)
  - The CFTR2 database is the primary source for this composite CF phenotype of patients who have a **G542X** mutation on 1 allele and a pancreatic insufficient mutation on the second allele. These patients exhibit the following characteristics\(^1\):
    - Elevated sweat chloride (average): 102 mmol/L
    - Lung function decline over time
    - Pseudomonas colonization: 56% of patients
    - Pancreatic insufficiency: 96% of patients

References:

Key points

• **3849+10kbC→T** is an example of a mutation resulting in residual CFTR activity. In combination with another allele that produces little to no total CFTR activity, 3849+10kbC→T can result in CF symptoms that may emerge later in life1-5.

• The CFTR2 database is the primary source for this composite CF phenotype of patients who have a 3849+10kbC→T mutation on 1 allele and a pancreatic insufficient mutation on the second allele2:
  - Elevated sweat chloride (average): 67 mmol/L
  - Late onset of CF lung disease compared to homozygous F508del patients2
  - Pseudomonas colonization: 57% of patients
  - Pancreatic insufficiency: 32% of patients

References:
Key points

- Cystic fibrosis, a systemic, multiorgan disease, is caused by loss of CFTR protein-mediated ion transport (activity)1-5
  - Defective ion transport leads to an imbalance of fluid and electrolytes causing thick, sticky mucus and viscous secretions to accumulate in different organs
  - This interferes with the proper function of the lungs, pancreas, gastrointestinal system, sinuses, and reproductive system
  - In the sweat glands, loss of CFTR activity restricts reabsorption of chloride in the duct, limiting the amount of salt that can be reabsorbed
- Symptoms of CF manifest throughout life with great variability among patients, though lung disease is the primary cause of mortality1,5-7

References:
Summary

- Normal CFTR protein channels transport ions through the apical epithelial cell membrane
- Total CFTR activity equates to the total ion transport mediated by CFTR protein channels at the cell surface
- Loss of CFTR protein activity is the underlying cause of CF
- 127 CFTR mutations are currently known to be CF-causing—most are extremely rare
- Individual CFTR mutations can reduce cell-surface quantity or function (or both) of CFTR protein, resulting in:
  - **Little to no** CFTR activity
  - **Residual** or partial CFTR activity
- Multiple factors influence CF clinical phenotype
  - Total CFTR activity, which is mainly determined by CFTR genotype
  - CFTR mutations on both alleles, which contribute to the expression of functional CFTR proteins and corresponding CFTR activity
  - Degree of reduction in total CFTR activity, which determines extent of CF manifestations
  - Modifier genes and environmental factors
- Loss of CFTR activity causes clinical manifestations of CF, affecting the proper function of the lungs, pancreas, gastrointestinal system, sinuses, and reproductive system