

Symptoms of CF

Digestive -

- Thick mucus clogs the pancreas preventing digestive enzymes from reaching the intestine.
- Results in failure to gain weight, vomiting, pain.
- Now patients take pancreatic digestive enzymes with food which alleviates the lack of enzymes produced by the pancreas.
- Can rarely result in intestinal blockage or rupture.

Pulmonary -

- Now that GI symptoms are controlled with enzymes, pulmonary problems are the leading cause of death in CF patients.
- The respiratory tract is covered by a layer of mucus which traps potential invading microbes. The mucus is then pushed up into the throat by the beating of cilia which line the respiratory tract. The mucus in CF patients is thick and cannot be moved up to the throat by the cilia. It accumulates in the respiratory passages.
- The airways of CF patients become colonized with unusual bacteria such as *Pseudomonas* during the first year of life. These bacteria are close to impossible to eliminate and cause repeated respiratory tract infections throughout life.
- Chronic low level infection plus episodes of acute infection induce immune responses which destroy respiratory tract tissue.

Sweat -

- Sweat is abnormally salty since salt reabsorption during sweat production does not occur.
- This symptom results in the old adage - "Woe to that child which when kissed on the forehead tastes salty. He is bewitched and soon must die."
- Also the basis of the most common test for CF in which a patch of skin is induced to sweat with a drug plus an electrical stimulus. The patch of skin is then covered with gauze for 30 min. After this time, enough sweat has accumulated in the gauze to perform a test to measure salt concentration in the sweat.

Ion Balance -

- CF patients can easily become dehydrated. This may result in cardiac arrhythmias or arrest.

Reproductive -

- Males with the CFTR mutation have a congenital absence of the vas deferens resulting in infertility.
- Females may have excess thick vaginal mucus making it more difficult to conceive.

Average Age of Death

- Males - 31 years
- Females - 28 years

From DNA to membrane protein

- Transcription (DNA to RNA)
- Translation (mRNA to protein) - For proteins which will be secreted or inserted into the cell membrane, translation occurs on ribosomes attached to the endoplasmic reticulum (RER).
- Protein Folding - Occurs in the RER
- Protein Processing - Occurs in the Golgi Apparatus
- Protein Trafficking to the cell membrane - Occurs through vesicles budding off the endoplasmic reticulum

Details of Protein Folding - see additional handout

Details of Protein Processing -

In the Golgi various sugars are added to various sites in the protein. This process is called glycosylation. Often glycosylation is necessary for a protein to be active.

Structure of the CFTR protein

- The CFTR gene was cloned in 1989.
- It encodes a protein 1480 AA long.
- The protein serves as the main chloride ion channel for epithelial cells.

Regions of the CFTR protein

- Transmembrane regions which cross the membrane and hold the ion channel in place within the membrane. (Amino acids 1 - 400 and 850 - 1200). They also form the walls of the ion channel. Note in the figure on the right that the transmembrane regions are actually placed so they form the walls of the ion channel when shown three dimensionally.
- Two nucleotide binding domains (NBDs). (Amino acids 400 - 600 and 1200 - 1400) The chloride ion channel, CFTR, is usually opens when it binds the nucleotide ATP. These areas of the protein are required for the chloride ion channel activation by ATP.
- A regulatory (R domain) (amino acids 600 - 850) which actually opens or closes the ion channel. This domain will become studded with many phosphates which change the conformation of the channel so that it will open.
- Note, there are regions of the protein which bind chaperones (unlabeled). These regions are distributed throughout the protein and ensure that the protein is correctly folded, processed, and trafficked.
- Also note that there are mutations leading to stop codons throughout the protein. These mutations result in a truncated protein being produced. Only amino acids prior to the mutation will be in the protein.

CFTR mutations

To date, more than 700 different mutations in the CFTR gene have been discovered which lead to CF disease. These different mutations occur in different areas of the protein and can lead to different molecular defects. These different molecular defects contribute to the different clinical forms of CF which are observed. Knowing the different molecular defects has allowed researchers to develop treatments which target these specific molecular defects.

Relationship between genotype and phenotype

In this exercise you will see that some mutations lead to less severe disease than other mutations. Thus, genotype (the genetic makeup of an individual) can influence phenotype (the symptoms the individual experiences). However, genetic makeup is only one of many factors which influence severity of disease. Individuals with the same mutation can have very different disease characteristics.

Relationship between CFTR protein levels and disease

Percentage of normal CFTR function	Manifestations of cystic fibrosis
<1	Pancreatic exocrine deficiency Plus manifestations listed below
<4.5	Progressive pulmonary infection Plus manifestations listed below
<5	Positive sweat test result Plus manifestations listed below
<10	Congenital absence of vas deferens
10-49	No known abnormality
50-100	No known abnormality (this range represents the levels of asymptomatic carriers and non-carriers.)

Note that the percentages 4.5 and 5 are quite close. There are a very few patients with normal sweat test but severe pulmonary disease. There are even fewer patients with abnormal sweat chloride ion levels but no pulmonary disease.

Naming Mutations

There is a specific way in which scientists name mutations. First, the amino acids in a protein are numbered sequentially from the amino terminus to the carboxyl terminus. CFTR has 1480 amino acids which are numbered from 1 through 1480.

- The number in you mutation name describes the position of the amino acid which is altered in your mutant CFTR protein.

The letters in the mutation name refer to amino acids. Scientists use a one letter code to represent each of the 20 amino acids. That code is shown below.

- The letter before the number in the mutation name refers to the amino acid which would be at this position in the normal CFTR protein.
- The letter after the number refers to the amino acid which has been put in place of the normal amino acid in the mutant CFTR protein.
- Delta refers to a deletion of an amino acid.
- X refers to a stop codon.

Example

P574H - The proline (P) usually found at position 574 has been replaced by a histidine (H).

Single letter amino acid code

G	Glycine	P	Proline
A	Alanine	V	Valine
L	Leucine	I	Isoleucine
M	Methionine	C	Cysteine
F	Phenylalanine	Y	Tyrosine
W	Tryptophan	H	Histidine
K	Lysine	R	Arginine
Q	Glutamine	N	Asparagine
E	Glutamic Acid	D	Aspartic Acid
S	Serine	T	Threonine
X	Stop		

Classes of mutations

Class I - No synthesis of protein

- Due to mutations causing stop codons which result in truncated proteins
- Accounts for 5% of CFTR mutations (W1282X, R553X, R1162X, G542X)
- mRNAs tend to be unstable
- Results in severe disease (less than 1% normal CFTR protein level is produced)

Class II - Folding/Trafficking Defect

- Most CF mutations fall in this class
- DeltaF508 (deletion of phenylalanine) is the most common CF mutation. The DeltaF508 protein is functional as a chloride channel, but it is not correctly folded and inserted into the membrane, instead it is degraded.
- Only 50% of the normal CFTR synthesized in non-CF individuals is correctly processed and trafficked.
- This type of mutation results in severe disease (less than 1% normal CFTR protein level is produced)

Class III - Block in activation and regulation of the CFTR protein (ion channel)

- CFTR is regulated by cAMP. Some mutations lead to a defect where the CFTR protein is unable to respond to cAMP. This results in the ion channel remaining closed at all times.
- G551D is an example of a Class III mutation
- This type of mutation results in severe disease.

Class IV - Altered Movement of Chloride Ions through the Channel (Conductance)

- This type of mutation results in a change in shape of the CFTR protein such that chloride ions no longer move as efficiently through the channel.
- R117H is an example of a Class IV mutation
- Class IV mutations usually result in less severe disease, and often pancreatic sufficiency

Class V - Reduced synthesis of CFTR

- Less CFTR protein is produced.
- These mutations tend to be less severe since some protein is still produced, allowing some ion transfer
- Individuals with this mutation often retain pancreatic function and are often diagnosed later than early childhood
- A455E is an example of a Class V mutation

Treatments

Symptom specific treatments

Treatments of pulmonary disease

- Albuterol - smooth muscle relaxant commonly used to treat asthma. This helps open the airways making it easier to eliminate mucus.
- Ibuprofen or other non-steroidal anti-inflammatories - reduce pulmonary inflammation.
- Antibiotics - aerosolized tobramycin and other antibiotics are used as maintenance therapy against *Pseudomonas* and other infections. This is administered by nebulizer twice a day. Often intravenous antibiotics are also needed.
- DNase (recombinant) - Dornase alpha - breaks down DNA from dead cells thus thinning mucus.
- Amiloride - blocks the Na⁺ influx usually associated with the lack of Cl⁻ efflux.
- UTP - activates other chloride ion channels.
- Lung transplant

Treatment of pancreatic insufficiency

- Pancreatic enzymes are given orally before each meal or snack. These enzymes help digest the food and allow CF patients to absorb the nutrients they need.

Treatments specific for different classes of mutations

Treatments specific for Class I mutations - No synthesis of protein

- Gentamicin - an aminoglycoside antibiotic (acts as an antibiotic by inhibiting bacterial protein synthesis. Gentamicin suppresses the premature termination caused by the mutations leading to a stop codon in the CFTR gene. Gentamicin thus allows readthrough such that full length mRNA and thus full length protein is produced.

Treatments specific for Class II mutations - Processing and Trafficking defects

- Deoxyspergualin - competes for with Hsp70 for binding to mutant CFTR. Since it is the association of Hsp70 with mutant CFTR that targets it for degradation, deoxyspergualin allows the mutant CFTR protein to be inserted in the cell membrane.

- Butyrate compounds - fatty acids which reduce the levels of Hsp70 (see role of Hsp70 above). In vitro it allows mutant to CFTR to be expressed on the cell surface.
 - Phase I studies show it is safe and does not significantly alter the trafficking of other proteins.
 - Phase I studies also show a change in nasal ion potential, suggesting this drug may improve Cl⁻ balance in patients.
- CPX - 8-cyclopentyl-1,3-dipropylxanthine - adenosine receptor antagonist, binds mutant CFTR. Improves both trafficking and activity of ion channel.
 - Has completed Phase I dose escalation and safety trials. Phase II trial in progress.

Treatments for Class III mutations -

- Genistein - Found in tofu. It is a tyrosine kinase inhibitor. It improves chloride channel activity of DeltaF508, but doesn't improve trafficking. May be effective if used with butyrate compounds
- CPX - 8-cyclopentyl-1,3-dipropylxanthine - adenosine receptor antagonist, binds mutant CFTR. Specifically activates chloride channel. Improves both trafficking and activity of ion channel.
 - Has completed Phase I dose escalation and safety trials. Phase II trial in progress.

Treatments for Class IV mutations - Altered conductance of Cl⁻. Many of the same agents used for Class III mutations are also used for Class IV mutations.

There are no specific treatments for Class V mutations

Gene Therapy

Idea is to deliver a normal copy of the CFTR gene to the airway epithelium (since lung consequences are the most severe).

Why this should work for CF

- Cells which need to take up CFTR gene are easily accessible
- Need to restore only 10% of normal function
- Don't need to eliminate patients own DNA

Procedure

- Isolate normal CFTR gene
- Clone normal CFTR gene into a vector so it can be amplified and maintained in vitro
- Place CFTR gene into appropriate delivery system
- Delivery to airways
- Want DNA to be taken up by epithelial airway cells
- Once DNA is inside cells, want it to be expressed such that normal CFTR protein is produced and chloride channel function is restored

In vitro results show that CFTR gene if taken up into cells can be expressed. Protein is put into the cell membrane and chloride channel function is restored.

Ways to deliver the CFTR gene

Recombinant vectors derived from adenovirus 2 or 5

- effectiveness is limited by low entry rates into cells
- rapid inflammatory response to eliminate virus

AAV - a defective parvovirus. Requires a helper virus to replicate

- Persistence of desired CFTR gene may be increased by possible integration of the virus into the genome.
- Phase 1 trials underway

Cationic liposomes - small droplets surrounded by a cell membrane, essentially a very small empty cell

- Advantages - lower toxicity and lower immunogenicity
- Phase I trials in humans show it is safe
- Gene transfer to nasal epithelium led to improved chloride transport but the effects were transient (2 weeks)

Why have the responses been so limited?

- Airways are highly evolved to resist invasion
- Lots of mucus and clearance by mucociliary escalator
- Apical surface of respiratory epithelium is practically devoid of receptors which internalize molecules after binding

Current directions

- Need to increase expression about 10-fold
- Have found that the ATP/UTP receptor on epithelial cells does internalize molecules after binding. Hoping to generate vectors which express a molecule that binds this ATP/UTP receptor
- Looking at the possibility of delivering CFTR gene via the blood since the basolateral side of the epithelial cell layer is less resistant to invasion.