CYSTIC FIBROSIS

Risk Factors
- Caucasian
- Family history of CF
- Infection
- Exposure to allergens and tobacco

Epidemiology
- Carrier frequency of 1 in 25 for Caucasians
- The most common lethal genetic disease affecting Caucasian populations
- Lower carrier frequency in African Americans, Asians and Hispanics

Pathogenesis
CF is an autosomal recessive genetic disease, caused by mutations to the cystic fibrosis transmembrane conductance regulator (CFTR) gene. An individual must be homozygous for the mutation in order to have the disease. Whilst heterozygotes don’t have the disease, they have a higher incidence of respiratory and pancreatic disease compared to the general population.

If both parents are heterozygotes for the CFTR allele, they have a 25% chance of having an affected child, a 25% chance of having a non-affected child and a 50% chance of having a child who is a carrier for the condition.

The CFTR gene is on the long arm of chromosome 7. There are more than 1800 disease-associated mutations of the CFTR gene which are split into 6 classes:

I. **Defective protein synthesis (10%)** → non-sense, frameshift or premature stop codon → truncated CFTR protein does not reach the cell membrane at all

II. **Abnormal protein folding, processing & trafficking** → mis-sense, phenylalanine deletion at position 508 (ΔF508) → CFTR protein is not fully folded or glycosylated and is instead degraded before it reaches the cell surface

III. **Defective regulation (2-3%)** → mis-sense, G511D → CFTR protein reaches the cell membrane but the channel does not open properly due to defective regulation by ATP, so chloride transport cannot occur

IV. **Decreased conductance (<2%)** → mis-sense, R117H → CFTR protein reaches cell membrane and some is functional, but due to channel narrowing there is reduced chloride transport

V. **Reduced abundance** → splicing defects cause improper processing of mRNA → normal CFTR protein but reduced number

VI. **Altered function in regulation of ion channels** → due to increased turnover of CFTR protein there is defective regulation of other ion channels by CFTR

Class IV and V mutations are associated with a milder disease phenotype, whilst the others are considered severe mutations. Two severe mutations are generally associated with classic CF

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phenotype, characterised by pancreatic insufficiency and GI symptoms. Mild mutations are associated with a milder disease severity.

The manifestations of the disease are influenced not only by the type of mutation but also the presence of environmental factors such as infection and modifier genes. These include genes that play a role in infection and inflammation, such as mannose binding lectin and TGF-b. Patients who also have defects in these genes may have a less adequate immune response and so be more vulnerable to infection. The influence of environment and modifier genes explains why there is more variability in pulmonary manifestations of the disease.

The CFTR protein consists of two transmembrane domains which form a channel for chloride ions to pass through, two cytoplasmic nucleotide domains for ATP to bind and a regulatory “R” domain which has PKC and PKA phosphorylation sites. Increases in cAMP lead to the activation of PKA, which binds to and is phosphorylated by the R domain. Both PKA and PKC bind to the CFTR protein, followed by two molecules of ATP which are hydrolysed to ADP. This triggers the opening of the chloride channel.

The CFTR protein regulates ion transport across cells. In respiratory epithelium, it actively secretes chloride (and inhibits ENaC), which is accompanied by passive sodium secretion into the mucous. This increases total electrolyte concentration in the mucous, resulting in the movement of water out of the cell by osmosis. This hydrates the mucous, enabling it to be cleared from the airways by mucociliary clearance. Defects in the CFTR protein prevent chloride secretion, thereby promoting passive sodium absorption into the epithelial cells. This draws water into the cells by osmosis, making the mucous dehydrated and viscous.

In pancreatic duct cells, CFTR proteins actively secrete chloride, which provides a luminal source of chloride ions which are exchanged for bicarbonate ions by the anion exchanger. Secretion of chloride thereby not only enables the passive movement of water into the lumen, which serves to transport pancreatic enzymes produced by acini, but also enables bicarbonate secretion, which neutralises acidic chyme from the stomach and provides an alkaline pH which enables the activation of trypsin. Defects in the CFTR protein prevent secretion of chloride ions, which prevents the anion exchanger from exchanging chloride for bicarbonate ions and passive secretion of water into the lumen of the pancreatic duct. A reduction in luminal pH favours mucin precipitation leading to viscous mucous. Reduced water secretion prevents enzymes being transported into the GI tract. Enzyme-rich fluid accumulates in the pancreas, leading to autodigestion and cell necrosis. Pancreatitis leads to the destruction of acini and eventually islet cells.

In the sweat ducts, the CFTR protein actively reabsorbs chloride (and activates ENaC), which is followed by passive reabsorption of sodium into the epithelial cells. Defects in the CFTR protein prevent absorption of chloride from sweat, thereby preventing passive absorption of sodium. This results in hypertonic sweat.

**Signs & Symptoms**

- **Productive cough** → lack of CFTR → reduced chloride secretion leads to increased sodium and water absorption → lowers the water content of mucous lining respiratory epithelium, forming
isotonic dehydrated mucous → defective mucociliary action → accumulation of viscid secretions → obstruction of bronchi and bronchioles → triggers cough reflex

- **Recurrent lower respiratory tract infections** → dehydrated mucous → impaired mucociliary clearance → inability to clear pathogens from respiratory tract & increased binding of pathogens to plugged mucins and ability to evade host immune response due to thick mucous

- **Green sputum** → longstanding respiratory infection → inflammation within respiratory tract following infection → degenerating neutrophil myeloperoxidase has a haem pigment which gives it its colour

- **Nasal polyps & sinusitis** → thick mucus in upper respiratory tract → chronic inflammation following infection → hyperplasia of epithelial cells

- **Bronchiectasis** → thick mucus → inability to clear pathogens → chronic infection → immune response unable to destroy pathogen but instead damages lung tissue through neutrophilic proteases, inflammatory cytokines, nitric oxide and oxygen free radicals → damage to muscular and elastic components of bronchial wall → bronchial dilation, permanent distortion of conducting bronchi, diffuse mucosal oedema

- **Lung hyperinflation** → lack of elastin in bronchial walls due to inflammatory mediated damage → bronchi are easily collapsible → cannot oppose elastic recoil of chest wall → on exhalation not all air is breathed out due to collapsing bronchi (air trapping) → on next breath, air is exhaled on top of what is already in the alveoli → leads to hyperinflated lungs

- **Chronic ventilatory failure** → chronic inflammation; malnutrition; air trapping → widespread tissue fibrosis in respiratory tract; weakened respiratory muscles; inability to exhale all air involved in gas exchange → leads to hypercapnia → type II respiratory failure

- **Weight loss & poor growth** → impaired bicarbonate secretion from pancreatic duct cells → increased acidity of GI lumen → less activation of trypsin (prefers alkaline pH) → impaired activation of digestive enzymes → impaired digestion of food in GI tract → malabsorption → malnutrition → weight loss & poor growth

- **Pancreatic insufficiency** → impaired bicarbonate secretion from pancreatic duct cells → impaired water secretion into pancreatic duct → impaired transport of enzymes into GI tract and mucin precipitation due to increased acidity → accumulation of enzyme-rich fluids in pancreas → autodigestion and necrosis of pancreatic acini and fibrosis of parenchyma → loss of pancreatic exocrine function

- **Diabetes mellitus** → chronic pancreatitis → eventual destruction of islet cells (rare effect)

- **Hepatic cirrhosis** → thick mucus → obstruction of bile canaliculi by mucus plugs → ductular proliferation & portal hypertension → increased pressure damages cells resulting in inflammation and regeneration of hepatocytes

- **Neonatal jaundice** → thick mucus → obstruction of small intestine (meconium ileus) → increased reabsorption of bilirubin (NOTE: distal intestinal obstruction may occur in later life, leading to inflammation, scarring and stricture formation)

- **High NaCl in sweat** → lack of CFTR protein → impaired chloride absorption from sweat → reduced passive absorption of sodium from sweat → hypertonic sweat

- **Infertility** → CFTR mutation linked to bilateral absence of the vas deferens and azoospermia in males
Histological Features

Chronic inflammation would be observed in the respiratory tract and pancreas, with the presence of oedema, neutrophils, lymphocytes, fibrosis and vascular congestion. Within the pancreas, leakage of lipase would lead to degradation of TAG into free fatty acids, leading to formation of insoluble salts which may be observed.

Investigations

- **Sweat test** → high levels of NaCl observed due to absence of CFTR protein
- **Genotyping** → patient’s DNA isolated from sample → the strands are separated → amplified via PCR with a mutant and normal CFTR primer → through labelling, you can detect if the patient is homo- or heterozygous for the mutant allele. Also allows you to detect which gene the patient possesses. This will help predict the severity of their condition and help determine the most appropriate treatment.
- **Microbiological cultures** → helps to determine the cause of respiratory tract infections and help determine the best treatment for them
- **Lung function tests** → FEV₁ (volume of air exhaled during first second of a forced expiration) and FVC (maximum volume of air exhaled as forcefully as possible, from maximum inspiration). An FEV₁ of 85% and above indicates normal to near-normal lung function. A FEV₁ to FVC ratio of less than 70% indicates airway obstruction

Differential Diagnosis

In a young child, a failure to thrive may be the first noticeable sign that something is wrong. This may be due to:

- Maternal depression, poor parenting skills, abuse
- CF, coeliac disease, protein intolerance, lactose intolerance, infestation
- Renal failure, congenital heart disease, severe asthma, immune deficiency, other rare conditions (chromosomes or metabolic tests if dysmorphic features present)

Management & Treatment

There are currently no treatments for the ∆F508 mutation. Current treatments manage complications, rather than treat the cause of the disease.

- **Pancreatic enzyme replacement therapy**
  - Treats malabsorption
  - Enzymes are specially coated to prevent premature digestion in the stomach
  - Lipase is the most important enzyme here and helps in the absorption of fat soluble vitamins (A, D, E, K) and prevent steatorrhoea
  - Patients advised to eat a diet high in calories, fat and protein with vitamin supplements
- **Prophylactic antibiotics**
  - *Staphylococcus aureus, haemophilus influenza* and *pseudomonas aeruginosa* are the 3 most common organisms responsible for lung infections in people with CF
  - *Staphylococcus aureus* prophylaxis → flucloxacillin
  - *Pseudomonas aeruginosa* prophylaxis → aminoglycosides or monobactams
Nebulised antibiotics are given so they reach their target site more quickly

- Physiotherapy
  - Coughing and breathing exercises → deep breathing, huffing and coughing to help clear mucous from the airways
  - Postural drainage → changing your position to make it easier to remove mucus from the lungs. Techniques involve leaning or lying down while a physiotherapist or carer uses their hand to vibrate certain sections of the lungs

- Medication
  - Hypertonic saline and bronchodilators help improve clearance of mucus
  - Amiloride blocks sodium reabsorption
  - Stimulate chloride secretion with ATP or UTP to stimulate nucleotide receptors by pathways independent of cAMP
  - Inhalation of human recombinant DNase shown to improve FEV by 20% in some patients → helps to clear DNA from dead inflammatory cells which contributes towards the viscosity of the sputum
  - Treatment of acute infections, suppression of chronic infection and suppression of inflammation
    - Steroids, high dose ibuprofen
  - Non-invasive ventilation
    - Required if lung function is significantly reduced
    - Uses a mask to supply oxygen to the upper respiratory tract, rather than tracheal intubation which has a higher risk of infection
    - Positive pressure causes gas to flow into the lungs until the ventilator breath is terminated. As the airway pressure drops to zero, elastic recoil of the chest accomplishes passive exhalation by pushing the tidal volume out
    - Reduces effort involved in breathing, rests respiratory muscles, reduces respiratory rate, reduces CO₂ levels, increases oxygen levels, corrects pH as alveolar ventilation improves and increases the volume of each breath
  - Prevention of side effects from medication
    - Bisphosphonates → protect against reduced bone density and osteoporosis, a side effect of prednisolone
    - Insulin → may be needed if the patient develops diabetes mellitus
    - Bronchodilators → makes it easier to breathe
    - Vaccinations and flu jabs → to prevent infection
  - Bilateral lung transplant
    - Triple therapy for immunosuppression → tacrolimus, mycophenolate, prednisolone
    - Combination therapy as infection prophylaxis → nystatin, valgancyclovir, co-trimoxazole, colomycin and amphotericin for 4 months → one year after transplant, lower doses of tacrolimus, mycophenolate and prednisolone; infection prophylaxis reduced to co-trimoxazole daily
    - IV methylprednisolone used if rejection occurs
Long Term Effects

90% of children now survive into their teens. The medial survival for those born after 1990 is around 40 years. Cardiorespiratory complications (persistent lung infections and obstructive lung disease) are the most common cause of death in the US.

- **Physical**
  - Pancreatic insufficiency occurs in 85-90% of people with CF
  - Difficulty growing due to malnutrition → delayed sexual maturity → feelings of being different
  - Male infertility → bilateral vas deferens absence found in 95% of males who survive to adulthood
  - Liver involvement occurs in less than 10% of patients
  - 10% develop diabetes mellitus
  - Osteoporosis can occur due to lack of vitamin D absorption following pancreatic insufficiency
  - By age 18, 80% of patients have *P. aeruginosa* infection

- **Psychological**
  - Social isolation → affects cognitive development
  - Children don’t have a normal childhood → lots of time spent in hospital, surrounded by adults, scary environment
  - Cannot join in with others → feeling of being different, abnormal
  - Knowledge of poor life expectancy → depression

- **Social**
  - Poor performance at school
  - Inability to join with friends at school → social relationships suffer

Additional Points

The ΔF508 mutation is the most common in CF, affecting around 70% of people diagnosed with the condition.

Modifier genes include the mannose binding lectin gene. The MBL protein is a member of the collectin family that are produced by the liver. They can opsonise pathogens and are bound by antibodies which then recruit and activate complement. Patients who have defects in the MBL gene have a three times higher risk of end-stage lung disease than those without.

*P. aeruginosa* colonises the lower respiratory tract. Its pili bind ciliated epithelium by sialic acid or mucin. The static mucus creates a hypoxic environment in the airway surface fluid which favours the production of alginate, a mucoid polysaccharide capsule produced by the bacteria. Alginate production allows the formation of a biofilm. This slime layer blocks complement binding and phagocytosis and inactivates IgG, TNF-α and IFN-γ. The bacteria evades host defences and produces elastases and proteases which degrade epithelium and allow it invade deeper into the tissues. This leads to a chronic destructive lung disease.

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A trypsin test is done at birth as part of a routine neonatal screen for several genetic conditions. A high trypsin level may be indicative of cystic fibrosis, due to trypsin release from damaged pancreatic acini cells.